

Research Article

Prevalence of urinary tract infections and antibiotic effectiveness against uropathogens among females of different ages in Dera Ismail Khan, Khyber Pakhtunkhwa-Pakistan

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Abstract

Infections of Urinary system are predominant worldwide and the incidence of these infections is more serious in developing countries like Pakistan. The incidence of UTIs, causative organisms and resistance developed in pathogen due to continuous usage of conventional antibiotic was investigated in one of the remote area of Pakistan. The urine samples of females were collected from three groups on the basis of age and primed to screen out positive samples having infection and pus cells. Moreover, the pus cells containing urine samples were then cultured and biochemical tests were completed to characterize the causative pathogens. Afterwards, the resistance against different antibiotics was studied by smearing antibiotics on specified culture media. The percentage of positive samples was recorded high in females of young age (15 to 30 years). Several strains of bacteria were identified for example *E. coli*, *Klebsella* spp: *Pseudomonas aeruginosa* and *enterococcus* ssp. The lavish use of orthodox antibiotics including Ampicillin, Tetracycline, Ciprofloxacin and Amoxicillin showed resistance against pathogen. Ceftriaxone, Amikacin, Imipenem and Fosfomycin meaningfully demonstrated their efficacy to control the bacterial species responsible for urinary tract infections in female of district Dera Ismail Khan, KP, Pakistan. The careful use of specific antibiotics can successfully control the Urinary Tract Infections in female.

Keywords: Antibiotics; Culturing, Urinary tract infection; Uropathogens

Introduction

Dera Ismail Khan (DIK) is considered as under developed area of Pakistan where

many communities particularly women are facing sever health issues due to unhygienic conditions and unavailability of advance

medical facilities [1]. Urinary tract infection (UTI) is a provocative response of pathogenic microorganisms attack on human body [2]. In the world, 150 million people are affected from urinary tract infections (UTIs). About 900,000 cases of hospital linked UTI reported every year [3]. Moreover, in United State, the most prevalent hospital acquired infection is UTI [4, 5].

Mis-treatment of UTIs can lead to serious problems such as disorders of the urinary tract, hypertension [6], uremia and early delivery and even abortions in pregnant women [7]. The severity of these infections depends on factors such as bacterial virulence, host characteristics, host performance, sexual activity and contraceptive procedures [8]. UTI becomes more sever for heart transplant patient as well as for pregnant women [9].

The UTIs are more prevalent in female as only 20% male and more than 80% females are susceptible [10]. Every second woman has to face this problem once during her life and it is due to unique structure of her urinary tract [11, 12]. Pathogens easily enter into urinary tract from vagina due to small sized urethra in female [13]. The major symptoms of UTIs include elevated body temperature, increased frequency of urination, supra pubic pain, altered urine configuration and decreased mental stability [14]. A lot of pathogenic bacteria are symptomless resident of female urinary tract so these bacteria mainly cause UTIs in any type of systemic disease. [15]. In the symptomatic infection colonization symptoms such as: microbial attack and inflammation in the urinary tract are detected [16].

Most of the uncomplicated UTIs result due to *Escherichia coli* along with *Staphylococcus saprophyticus*, *Klebseilla* spp; and *Proteus mirabilis* [17]. The lavish use of antibiotics has created the antibiotic resistance over the globe [18]. Every pathogen causes infection displays particular symptoms like Cystitis,

medical term for inflammation of the bladder caused by *Escherichia coli* [11]. Besides, some common microorganisms responsible for UTI include *Klebsiella pneumonia* *Proteus vulgaris*, *Staphylococcus epidermium*, *Enterobacter*, *Citrobacter* and *Pseudomonas aeruginosa* [19]. Moreover, some bacteria which are Gram-negative bacteria rods (*Klebsiella*, *Proteus* and *Enterobacter*) are lesser pathogenic in UTI as compared to Gram-positive cocci (*Staphylococcus saprophyticus*) which are responsible for 5-10% acute UTIs among school girls [13].

The footing of suitable treatment for UTIs is the assortment of a decent and low-cost antibiotic but major infection caused by *E. coli* is main encounter to a lot of usual anti-bacterial [20]. During last thirty years, it has been reported that the excessive use of antimicrobial agents or antibiotics built resistance among pathogen which cause UTIs [21, 22]. Antibiotic resistance in pathogens is an alarming threat to public health as about 30% of urinary isolates of *E coli* are now resistant to trimethoprim [23]. Besides heavy use of antibiotics, the appearance of resistant bacterial species as a result of genetic mutation and increased population ultimately results in high ingestion of antibiotics [24]. Alterations in the resistance of uropathogens highlight the urgent need of the overall investigation to yield an updated data that can be useful to control the infection in a better way [25, 26].

Materials and Methods

Collection area and sample handling

The Dera Ismail Khan (DIK), Khyber Pakhtunkhwa (KP), Pakistan acquiring land area of 7,326 km² (2,829 m²) and total population of 1627132 was selected for the sample collection.

A total of 150 urine samples were aseptically collected from the female patients suffering from any type of urinary disorder during their routine checkup in District Teaching Hospital

D. I. Khan from January to March 2019. These samples were taken from 3 different age groups of female i.e. Group I (15-30 years), Group II (31-45 years) and Group III (46-60 years). Four ml urine sample was taken from each patient using clean catch midstream method and transported to the laboratory of Microbiology, Gomal Medical College, D. I. Khan within 2-3 hours [27].

Procedures

Sample screening

Every sample was cultured by using a sterilized wire loop on a petri dish of MacConkey and blood agars, and then incubated at 37°C. Twenty-four hours post incubation a cultured plate with 10⁵ colonies/ml of the culture was taken as positive urine sample [28]. Each positive sample was then centrifuged @ 300 revolutions per minutes for ten minutes. Thin smear was prepared from the pellet for microscopic study. A sample containing 10-20 pus cells was taken as UTI positive [20].

Biochemical tests

Every positive sample was re-cultured and Gram staining was done [29]. Catalase test was performed to ratify *E. coli*, *Enterobacter* and *Klebsiella spp* [30]. Indole test is used to confirm the presence of indole in culture [31]. Capsule staining was also done to identify capsulated bacteria [32]. *Klebsiella* found as capsulated whereas *E. coli* and *Enterobacter* were found non capsulated. The flagella test was done to recognize the *Enterobacter* [33]. Finally, coagulase test was done to confirm the presence of *Staphylococcus aureus* as uropathogens [34].

Calculation of antibiotic resistance

Antibiotic sensitivity was calculated by Kirby Bauer disc diffusion method on Muller Hinton Agar medium [16]. Reading (Sensitivity or Resistance) was taken by measuring the diameters of zone of inhibition of bacterial growth as recommended by disc manufacturer [35]. Ampicillin (AMP), Tetracycline (TET), Ceftriaxone (CRO),

Nitrofurantoin (F), Amikacin (AK), Cefixime (CFM), Imipenem (IM), Ciproflaxacin (CIP), Cloramphenicol (C), Ofloxacin (OFX), Fosfomycin (FOS) and Amoxicillin (AMX) antibiotics were tested to calculate antibiotic sensitivity. The commercially available discs (Oxide-UK) were used in this test.

Ethical certificate

After getting a satisfactory level willingness between patient and owners the urine sample was collected. It was committed with patients that data of this research will be published without any identity of the patient. All cost of the tests was paid by the researcher only.

Data analysis

Fishers of Variance Technique was used to analyze statistically and means values of all treatments were compared with standard error [36].

Results

UTI prevalence on the basis of pus cell count

The pus cells were counted from each urine samples all three groups and the result indicated that all age groups contain pus cells but Group I was found with highest amount of pus cells as compared to Group II and Group III as shown in (Fig. 1).

Types of Uropathogens

Pus cell containing urine samples were further analyzed to investigate different types of bacteria. On the basis of biochemical tests *E. coli*, *Klebsiella spp.*, *Enterobacter*, *Staphylococcus aureus* were characterized. Among the characterized pathogens the percentage of *E. coli* was the highest followed by *Klebsiella pneumoniae*. The prevalence of *Staphylococcus aureus* was recorded only in Group I as shown in (Fig. 2).

Antibiotic susceptibility patterns of Bacteria

Resistance patterns against various antibiotics

E. coli showed the highest value of resistance against all antibiotics particularly

Ceftriaxone (CRO) and Ceftriaxone (CRO) while *Staphylococcus aureus* exhibited the least resistance against Amikacin (AK) and Fosfomycin (FOS) antibiotics as mentioned in (Table 1).

Sensitivity of uropathogens against antibiotic

All pathogens considered in present study showed significantly different susceptibility pattern against antibiotic treatments (Uropathogens × antibiotic). The data revealed that *Staphylococcus aureus* expressed maximum sensitivity against

Amoxicillin (AMI) and Amikacin (AK) while *E. coli* showed minimum sensitivity against Ciprofloxacin (CIP) and Ceftriaxone (CRO).

Moreover, *Klebsiella pneumoniae* was found most sensitive and *E. coli* was found least sensitive against all antibiotics among all types of bacteria. Furthermore, all the pathogens showed significantly high sensitivity against Fosfomycin (FOS) and least sensitivity against Ciprofloxacin (CIP) antibiotic (Table 2).

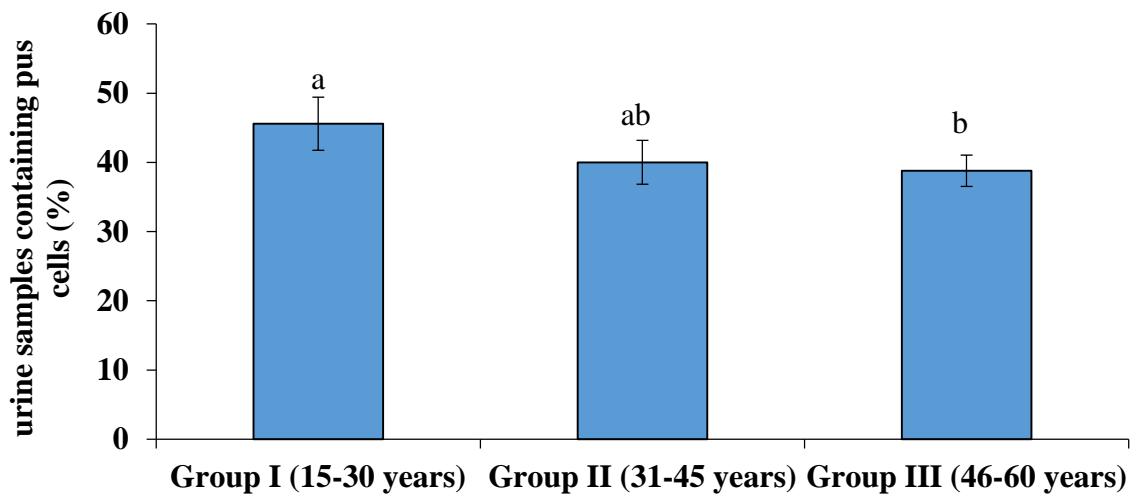


Figure 1: UTI prevalence on the basis of pus cell count

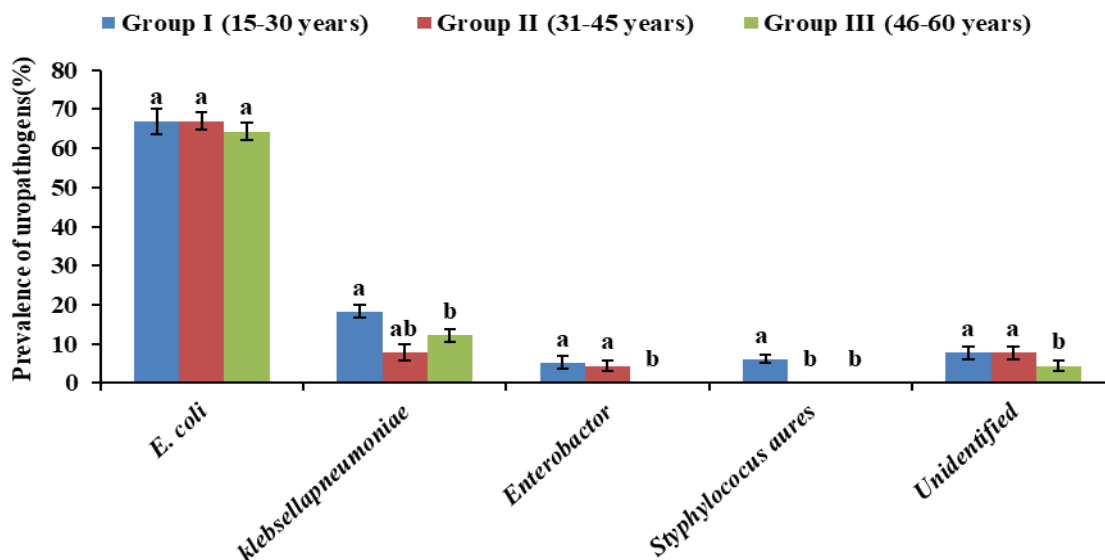


Figure 2: Prevalence of different types bacteria in Urine samples

Table 1. The resistance values of different bacteria against various antibiotics

Bacteria	Antibiotics												Mean
	AMP	T	CRO	F	AK	CFM	IM	CIP	C	OFX	F0s	AMI	
<i>E.coli</i>	85.2c	14.8v	97.2 a	18.2 u	32.3 opq	85.2 c	19.8 tu	94.9 ab	35.5 mn	80.4 de	11.1 w	83.0 c	54.8 A
<i>Klebsiela</i>	40.9k	59.1h	42.0 k	6.0 x	25.0 s	40.9 k	20.0 tu	77.9 e	40.0 kl	22.1 t	37.8 lm	40.0 kl	37.6 D
<i>Enterobacter</i>	40.0kl	60.0h	30.0 pqr	28.0 r	32.9 no	40.0 kl	37.9 l m	94.1 b	51.6 j	27.9 r	29.5 qr	55.6 i	44.0 B
<i>Staphylococcus aureus</i>	35.0n	65.0g	32.2 op	65.7 fg	4.0 xy	35.0 n	32.2 op	67.8 f	42.0 k	65.9 fg	2.0 y	68.0 f	42.9 C
Mean	50.3C	49.8C D	50.4 C	29.5 F	23.3 H	50.3 C	27.5 G	83.7 A	42.3 E	49.1 D	20.1 I	61.7 B	

Ampicillin = AMP, Tetracycline =T, Ceftriaxone = CRO, Nitrofurantoin=F, Amikacin =AK. Cefixime = CFM, Imipenem =IM, Ciprofloxacin = CIP, Chloramphenicol= C, Ofloxacin= OFX, Fosfomycin= F0s, Amoxicillin=AMI

Table 2. Represents the sensitivity percentage of uropathogens against antibiotic

Uropathogens	Antibiotics												Mean
	AMP	T	CRO	F	AK	CFM	IM	CIP	C	OFX	F0s	AMI	
<i>E.coli</i>	88.6 b	11.4 st	2.8 u	81.8 c	68.5 ghi	14.8 rs	80.0 0 cd	5.1 u	64.5 ijk	19.6 qr	88.9 b	17.00 qrs	45.3 C
<i>Klebsiela</i>	77.9 cde	22.2 pq	58.0 01	94.0 ab	75.0 0 def	59.1 kl	80.0 0 cd	27.9 op	58.0 01	78.9 cd	64.5 ijk	60.00 kl	63.0 A
<i>Enterobacter</i>	65.1 ghijk	36.9 n	70.0 0 fghi	72.0 0 efg	71.1 ghijj	60.00 kl	62.1 jkl	5.9 tu	48.4 m	71.1 fgh	70.5 fghi	44.4 m	56.1 B
<i>Staphylococcus aureus</i>	65.00 ghijk	35.0 0 n	67.8 ghij	34.3 n	96.0 a	65.00 ghijk	67.8 ghij	32.2 no	58.0 01	34.1 n	98.0 a	32.00 no	57.1 B
Mean	74.2 BC	26.4 H	49.7 F	70.6 D	76.7 B	49.8 F	72.5 CD	17.8 I	57.2 E	51.0 F	80.5 A	38.4 G	

Ampicillin = AMP, Tetracycline =T, Ceftriaxone = CRO, Nitrofurantoin=F, Amikacin =AK. Cefixime = CFM, Imipenem =IM, Ciprofloxacin = CIP, Chloramphenicol= C, Ofloxacin= OFX, Fosfomycin= F0s, Amoxicillin=AMI

Discussion

Urinary tract infections (UTIs) are more prevalent in women than man (8:1) and around 50 to 60% female get UTIs once in their life [37]. Like the findings of Prakash [38] the females of different ages showed the different pattern of UTI and maximum prevalence of uropathogens was found in early aged females [39]. Saber [40] documented that UTIs prevail more prominently in women during their reproductive phase (15-44 years). More than 50 to 60% women susceptible to get UTIs once till the age of 24 years [41], While, [16]

documented the maximum infection in middle aged females.

The uropathogens responsible for UTI were identified as *E. coli*, *Klebsiela spp.*, *Enterobacter*, *Staphylococcus aureus* and some unidentified pathogens out of which *E. coli* is the predominant pathogen prevail in communities [42-44]. As experienced in this study the *E. Coli* is most common factor [45-47] isolated from samples of all infected females independent to their age collected during study. The findings documented [48] showed that *E. coli* was a major causative agent for urinary tract infections around the

world while findings of [13] described that *Klebsiella* stood 2nd for occurrence of UTI. *E. coli* declared as major causative agent because it developed high resistance against conventional antibiotics like AMP [20]. Similarly, high magnitude of resistance against TET was observed by *E. coli* [49, 50]. In Bangladesh, the management of UTIs seemed difficult because of establishment of resistance in *E. coli* against conventional antibiotics [51]. The antibiotic effectiveness of Ceftriaxone [13] and CFM [15] was tested against *E. coli*. The results were in lined with the findings of the present study as *E. coli* developed high resistance against both antibiotics and thus these both are ineffective to control the infections caused by this pathogen [13]. Alternatively, AK [52] and IM [35, 53] were proved as an effective antibiotic against gram negative *E. coli*. Less susceptibility of CIP against Uropathogens was also recorded [32, 54]. Among all pathogens, *E. coli* indicated the highest resistance against CIP [16]. Furthermore, OFX was proved its effectiveness against *E. coli* as compared to *Klebseilla* [13]. Similarly, FOS was also recommended as the most influential antimicrobial against *E. coli* [55].

Conclusion

Urinary tract infection was prevailed in females residing in hard areas of Pakistan like Dera Ismail Khan. Among all the pathogens, *E. coli* was found most common causing organism of urinary tract infection as it developed resistance against antibiotic particularly Ciprofloxacin and Ciprofloxacin nevertheless, among all conventional antibiotics Fosfomycin was found most effective antibiotic to control uropathogens. As UTI is an alarming disease and may lead to further urinary and reproductive tract infection and complications. Therefore, it is need of the time to further work on it and sort out solution of not only antibiotic resistance but safe and sound way of treatment as well.

In this regard further studies are required on this topic.

Authors' contributions

Conceived and designed the experiments: A Khan, AU Rehman & MI Malik, Performed the experiments: F Ramzan, Habibullah & SN Malik, Analyzed the data: SA Rustam & G Jilani, Contributed reagents / materials / analysis tools: M Zeeshan & A Tariq.

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