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Investigation of bacteria liable for Urinary Tract Infections and their antibiogram analysis

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Abstract

Urinary tract infection mainly occurs by microorganism when they overcome the human immune system. These microbes have caused the high rate of morbidity and motility effect all over the world to every age and gender. Lower urinary tract infection is more common among females than males. A prospective observational study was conducted in Pathology Department at Lady Reading Hospital Medical Teaching Institution, Peshawar, Pakistan in a 4th quarter (October, November, December) of the year 2017. Mid-stream urine samples were collected from 1357 patients from all hospital-admitted and outdoor patients. They were cultured on CLED agar, after isolation, uropathogens was identified through biochemical tests like oxidase, catalase and TSI tests. Only 205 samples were reported positive, with the prevalence of 15.10%. Females (51.70%) were more prone to UTI then males (48.29%). *E.coli* was most frequently isolated uropathogen with frequency of 90.24%. Other isolates were *Pseudomonas aeruginosa* (4.8%), *Enterococcus faecium* (2.92%), *Enterobacter species* (1.46%) and *Proteus mirabilis* (0.49%). Thirteen antibiotics were used to test the susceptibility of isolated microbes through Kirby Bauer Disc Diffusion method. Carbapenem drug like Imipenem (89.7%) was most effective against gram-negative microbes belonging to *Enterobacteriaceae* family. Other antibiotics like, Piperacillin + Tazobactam (84.86%), Cefoperazone /Sulbactam (84.3%), Amikacin (84.3%), Fosfomycin (83.7%) and Nitrofurantoin (77.2%) also showed sensitivity. Fluoroquinolones Drugs like Ciprofloxacin (82.1%) and Levofloxacin (81.6%) showed high resistivity followed by Co-trimoxazole (80.54%) and Cephalosporine drugs. For Gram positive *Enterococcus spp*, Glycopeptide antibiotics and protein synthesis inhibitor drugs had shown 100% sensitivity, but carbapenem and fluoroquinolones were highly resistant.

Keywords: Enterobacteriaceae Family, Urinary Tract Infection (UTI), Fluoroquinolones Drug

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1. INTRODUCTION

Infection that occurs in urinary tract is called Urinary Tract Infection (UTI) that is mainly caused by microorganisms (those organisms which are so small that can't be seen without help of the microscope). It is the most common infection all over the world. Bacterial biofilms is the common cause of UTI.

They cause regular urinary tract infection, responsible for recurrence and relapses. A high percentage of all nosocomial UTI infections caused by microbial biofilms developed catheters. In hospitalized patients, the most common sources of bacteria causing infection are Gram-negative. Whenever bacteria enter urinary tract, immediately eliminated by the body, they manifest. But sometimes they overcome human's body immune system and cause disease [1,2,3].

Urinary tract infections (UTI) are the foremost healthcare-acquired infection, and are related to high morbidity and mortality[4,5] .There are two types of urinary tract infection; upper urinary tract infection and lower urinary tract infection. Lower tract is consisting of urethra and urinary bladder; while upper tract is consist of kidneys. Lower urinary tract infection is more prevalent then upper tract infection. As the urethra is in close proximity to anus, so intestinal bacteria are mostly predominating among microbes causing urinary tract infection. Bacterial infection of urethra is urethritis; infection of the urinary bladder is cystitis, and infection of ureters is called urethritis, while most dangerous infections are of kidney known as pyelonephritis[6,7,8]. It is one of the most common bacterial infections in women, reported nearly 25% of all infections. During the lifetime, around 50-60% of women will develop UTIs[9]. The common cause of frequent infection in women is due to rarely draining and unrestrained bladder[10].

Risk factors associated with recurrent UTI are hormonal deficiency in post menopause, secretory type of blood groups and controlled diabetes mellitus. Extra-urogenital risk factors with more severe outcomes are pregnancy, male gender, badly controlled diabetes mellitus, and premature or new-born babies. Patients with Ureteral obstruction i.e. stone, stricture, Transient short-term urinary tract catheter, Controlled neurogenic bladder dysfunction and Urological surgery are sustainable to urological risk associated with urinary tract infection[11,12].

The microorganism that is frequently involving UTI infection is Escherichia coli also known as E.coli, and is responsible for almost 80% of UTI[13,14]. Recurrent UTIs are generally caused by reinfection of the same pathogen10. After *E.coli* other microbes that cause UTIs are *Klebsiella pneumoniae, Enterococcus* spp, *Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter* spp*, Staphylococcus aureus, Enterobacter* spp, and *Streptococcus* spp[15]. *Escherichia coli* is small, motile, Gram-negative rod shape, a facultative anaerobe that can ferment lactose. It is non-fastidious, enteric bacteria; commonly reside in the digestive tract. It belongs to family *Enterobacteriaceae. E.coli* is an obligate parasite, and cause hospital-acquire infections[16]. Pseudomonas aeruginosa is small Gram negative bacillus with single polar flagella, non-spore former and produce noticeable blue-green pigment. Its metabolism occurs aerobically and is non-lactose fermenter, which differentiates them from other members of family *Enterobacteriaceae*. It is an opportunistic pathogen that causes infection when host immune system is compromised. *Pseudomonas* usually causes skin and respiratory diseases, but it is also involve in urinary tract infection[17]. Enterococcus is a genus of Gram positive bacteria. They are cocci in shape; usually occur in various lengths of chains. They are non-motile, non-spore former and encapsulated. They are facultative anaerobes with lactose fermenting property. It grows on a temperature between 0-44 C^0 (32-112 F^0) and are opportunistic microbes18. Enterobacter are motile, rod shaped, gram negative and facultative anaerobes that can ferment lactose. They are opportunistic organism. They are usually present in the intestine, but they also cause UTI in humans16. Proteus spp are rod shape Gram negative bacteria. They are motile, facultative anaerobes and are non-lactose fermenter. *Proteus* spp. can metabolize urea and neutralize the acidity of urine through which it colonizes and can effectively infect urinary tract. They are non-coliforms that is they do not exist in gastrointestinal tract19. *Klebsiella* also belongs to family *Enterobacteriaceae*. It is gram-negative, rod shape, non-motile. It is facultative anaerobe and can ferment glucose. It causes hospital-acquired nosocomial infection, and is opportunistic pathogen[20].

Most of the organisms belong to UTI developed high resistance to broad-spectrum antibiotics, especially to extended-spectrum β-lactams, carbapenems (imipenem, ertapenem, meropenem and doripenem), fluoroquinolones and trimethoprim/ sulphamethoxazole (TMP/SMX), and it is becoming a critical problem in almost every country. For MDR uropathogens, polymyxin (colistin or polymyxin B), fosfomycin, trigecycline, nitrofurantoin, linezolid, and daptomycin can be considered as for treatment of uncomplicated and complicated urinary tract infection in some countries[21,22,23].

The aim of this study was to identify all the isolated etiological agents from urine samples to species level by performing the different biochemical tests. Gender wise prevalence was also identified. Susceptibility test was also determined by using the CLSI 2015 protocol.

2. MATERIALS AND METHODS

2.1 Study area

This prospective study was conducted in Pathology Department at Lady Reading Hospital Medical Teaching Institution, Peshawar, Pakistan in a 4th quarter (October, November, December) of the year 2017. It is tertiary care hospital run by government. Patients from all over Khyber Pakhtunkhwa (KPK), province of Pakistan attend this hospital for convalescence. This study was based upon the analysis of urine samples from the patients with a grievance of urinary tract infection. All urine samples from hospital-admitted and outdoor patients were collected and cultured for microbial examination.

2.2 Sample collection

Mid-stream urine was collected in clean and sterile plastic bottles. Patients were instructed either verbally or the procedure from urine collecting was labeled on the bottle. For children, urine bags were used from collecting urine sample. Samples were processed soon after collection without any delay.

2.3 Sample size

A total 1357 specimens were received in a 4th quarter of the year 2017, out of which 205 were positive. Sixty three samples were positive in the month of October, seventy one in November and also seventy one in December. Samples were collected from patients of all ages, including children, young and old age patients who were either admitted in hospital or outdoor patients. Males and females were also included. Soon after sample collection, each bottle was labeled with the patient's MR number before further process. Then the samples were processed at room temperature.

2.4 Culturing

Cysteine Lactose Electrolytes Deficient (CLED) agar is an enrich media, which was used as a growth medium for the isolation of microbes, especially in urine. Inoculation loop was first sterilized and then was loaded with sample. After that, sample was inoculated through streaking over the CLED agar and was incubated at 35 C^0 for 18 hours. The morphological characteristics of the colonies, including shape, color, odor, and either lactose fermenter or non-lactose fermenter were observed.

2.5 Gram staining

It is a process through which gram-positive and gram-negative bacteria are differentiated from each other. In the end of the process gram-negative bacteria were stained pink/red, while gram-positive bacteria were stained purple.

2.6 Biochemical tests

Oxidase, Catalase and Triple sugar iron tests were performed for the identification of the isolated uropathogens.

2.7 Antibiotic susceptibility test

The sensitivity of different drugs upon different pathogen is determined through Kirby Bauer's disc diffusion method. The antibiotics that were used during antibiogram analysis for *E.coli, enterobacter* species and *proteus mirabilis* were Cefoperazone/Sulbactem (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), Piperacillin/Tazobactam (100 µg), Fosfomycin (200 µg), Amikacin (30 µg), Nitrofurantoin (300 µg), Co-trimoxazole (1.25/23.75 µg). The antibiotics that were used during antibiogram analysis for Gram positive enterococcus faecium were Imipenem (10 µg), Levofloxacin (5µg), Ciprofloxacin (5 µg), Piperacillin/Tazobactam (100 µg), Nitrofurantoin (300 µg), Vancomycin (30 µg), Linezolid (30 µg) and Co-amoxiclav (20 µg).

2. RESULTS AND DISCUSSIONS

All the urine samples were cultured only on CLED agar in petri plates. Isolates were identified through their morphological characteristics (Table 1). For further differentiation, biochemical tests were performed, which included oxidase test, catalase test and TSL test (Table 2).

Table 1. Morphological characterization of isolated uropathogen

Morphology	E.coli	Pseudomonas aeruginosa	Enterococcus faecium	Enterobacter species	Proteus mirabilis
Shape	Round	Irregular	Round	Round	Round
Size	Large	Small	Smaller than *E.coli*	Medium	Small
Color	Pink	Pale yellow	Pink	Pink to whitish blue	Yellow-green to blue
Margin	Entire	Irregular	Entire	Rough	Entire
Surface	Matte	Rough	Matte	Matte	Matte
Opacity	Opaque	Opaque	Opaque	Opaque	Translucent
Elevation	Raised	Raised	Flat	Raised	Convex
Texture	Buttery	Mucoid	Buttery	Extremely mucoid	Buttery

Out of 1357 urine samples, only 205 were positive for the UTI with the prevalence of 15.10%, and the number of negative patients were 1152 with the prevalence of 84.89% (as shown in Table 3). This study is in disagreement with the study performed by Hasan et al.[24] which was conducted in an Indian hospital. Total 5073 urine samples were tested among which 2436 were positive with the prevalence of 48.01%. This difference is due to geographical distribution.

Table 2. Biochemical identification of isolated uropathogens

Tests		E.coli	Pseudomonas aeruginosa	Enterococcus faecium	Enterobacter species	Proteus mirabilis
Oxidase		-	+	-	-	-
Catalase		+	+	-	+	+
Triple sugar test	**Slop**	Yellow (acid)	Red (alkaline)	Yellow (acid)	Yellow (acid)	Yellow (acid)
	Butt	Yellow (acid)	Red (alkaline)	Yellow (acid)	Yellow (acid)	Black –red (alkaline)
	Gas	+	-/+	-	+	+
	H2S	-	-	-	-	+

Table 3. Total isolated organisms

Positive Bacterial Isolates	Number of positive samples	Percentage of positive samples
E.coli	185	90.24%
Pseudomonas aeruginosa	10	4.8%
Enterococcus faecium	6	2.92%
Enterobacter species	3	1.46%
Proteus mirabilis	1	0.49
Total	205	15.10%

The data in Table 4 indicated the gender wise distribution of UTI among male and female positive patients. The 51.70% of females were reported as positive; while 48.29% of males as positive, out of total 205 patients. These results are in agreement with the finding of Hasan et al.[24], in which high difference was observed among females (70.5%) and males (29.5%) positive patients. This difference in the ratio among females and males indicates that they are always more prone to UTI due to their physiological structure of the urinary system.

In this study, 90.24% of *E.coli* was predominantly isolated organism that causes UTI, which is quite similar to 81.7% of *E.coli* observed by Ipek et al. [25] in Istanbul, Turkey; but lower from the results obtained by Malik et al.[26] who isolated only 17.6% of *E.coli* in his report in Peshawar, Pakistan. This indicates that *E.coli* became the main cause of infection, and it may be due to development of high resistance towards antibiotics. Similar study was carried out in United States, and it showed 79% of *E.coli* among all uropathogens27. Other isolates were Gram negative *P. aeruginosa* 4.85%, *Enterococcus faecium* 2.92%, *Enterobacter* species 1.46% and *Proteus mirabilis* 0.49% as shown in Table 3.

Table 4. Gender wise distribution of isolated organisms from urine samples.

Isolates	No of positive samples	Females	Males	Females (%)	Males (%)
E.coli	185	98	87	52.97	47.02
Pseudomonas aeruginosa	10	2	8	20	80
Enterococcus faecium	6	4	2	66.66	33.33
Enterobacter species	3	2	1	66.66	33.33
Proteus mirabilis	1	0	1	0	100
Total	**205**	**106**	**99**	**51.70**	**48.29**

In the current study, it was also observed that the most sensitive antibiotic was carbapenem (imipenem) drug about 89.7% for *Enterobacteriaceae* family (Table 5), which was identical to the study carried by Jones et al.[28] which had 89.7% to 92.1% of imipenem sensitive strains. After imipenem some other drugs like Piperacillin + Tazobactam (84.86%), Amikacin (84.3%), and Nitrofurantoin (77.2%) also showed sensitivity. It is totally in disagreement with the study occurred in North America in which they were highly resistant to penicillin with 79.8% 29.

Fluroquinoles drugs like Ciprofloxacin (82.1%) showed high resistivity followed by Co-trimoxazole (80.54%). Similar results were also reported in a study performed by Edlin et al. [27] that Co-trimoxazole (Trimethoprim/ sulfamethoxazole (TMP/SMX) had shown highest (24%) resistivity against E.coli. In one of the study[30], trimethoprim (17.5%) and ciprofloxacin had shown highest resistance, which is analogue to this study.

Gram-positive bacteria *Enterococcus faecium* exhibited 100% sensitivity to teicoplanin and vancomycin drugs while they were resistant to Penicillin, carbapenem and Fluroquinole class drugs (Table 6). These results were in agreement with a study 24 in which gram positive were sensitive to vancomycin, teicoplanin. Tantry and Rahiman[31] also reported the same results that *Enterococcus* spp were highly sensitive to glycopeptide drugs.

Table 5. Antibiotic susceptibility results for Enterobacteriaceae (E.coli, Enterobacter species and Proteus mirabilis)

ANTIBIOTICS	**Symbol**	**Disc Conc. µg/ disc**	**Interpretive Criteria**			**No. Of Sample Processed =189**		
			S	**I**	**R**	**S%**	**R%**	**I%**
Cephalosporine Family								
Cefoperazone Sulbactem	CFP	30µg	>=21	16-20	<=15	159(84.1)	21(11.1)	9(4.76)
Ceftazidime	CAZ	30µg	>=21	18-20	<=17	55(29.1)	121(64.0)	13(6.8)
Cefotaxime	CTX	30µg	>=26	23-25	<=22	52(27.5)	132(69.8)	5(2.64)
Cefepime	FEP	30µg	>=25	19-24	<=18	56(29.6)	123(65.0)	10(5.2)
Ceftriaxone	CRO	30µg	>=23	20-22	<=19	49(25.9)	137(72.4)	3(1.58)
Carbapenem Family								
Imipenem	IMP	10µg	>=23	20-22	<=19	169(89.4)	11(5.8)	9(4.76)
Fluroquinole Family								
Levofloxacin	LEV	5µg	>=19	14-18	<=15	32(16.9)	154(81.4)	3(1.5)
Ciprofloxacin	CIP	5µg	>=41	28-40	<=27	31(16.4)	155(82.0)	2(1.05)
Penicillin Family								
Piperacillin/	TZP	100µg	>=21	18-20	<=17	161(85.1)	19(10.05)	9(4.76)
Miscellaneous								
Fosfomycin	FOS	200µg	>=16	11-15	<=12	158(83.5)	20(10.5)	11(5.8)
Aminoglycosides Family								
Amikacin	AMI	30µg	>=17	15-16	<=14	158(83.5)	24(12.6)	7(3.70)
Macrolide Family								
Nitrofurantoin	NIT/F	300µg	>=17	15-16	<=14	144(76.1)	27(14.2)	18(9.5)
Folate-antagonist anti-infection								
Co-trimoxazole	SXT/ COT	23.75µg	>=16	11-15	<=10	24(12.69)	152(80.4)	13(6.87)

Table 6. Antibiotic susceptibility results for *Enterococcus faecium*

ANTIBIOTICS	Symbol	Disc Conc. µg/ disc	Interpretive Criteria			No. Of Sample Processed =6		
			S	**I**	**R**	**S%**	**R%**	**I%**
Carbapenem Family								
Imipenem / Sulbactem	IMP	10µg	>=23	20-22	<=19	0(0%)	6(100%)	0(0%)
Fluroquinole Family								
Levofloxacin	LEV	5µg	5µg	>=19	14-18	<=15	0(0%)	6(100%)
Ciprofloxacin	CIP	5µg	>=41	28-40	<=27	0(0%)	6(100%)	0(0%)
Penicillin Family								
Piperacillin/ Tazobactam	TZP	100/10µg	>=21	18-20	<=17	0(0%)	6(100%)	0(0%)
Macrolide Family								
Nitrofurantoin	NIT/F	300µg	>=17	15-16	<=14	2(33.3%)	2(33.3%)	2(33.3%)
Glycopeptide antibiotic Family								
Teicoplanin	TEP	30 µg	>=17	15-16	<=14	6(100%)	0(0%)	0(0%)
Protein synthesis inhibitor								
Linezolid	Lzd	30 µg	>=23	21-22	<=20	6(100%)	0(0%)	0(0%)

4. CONCLUSIONS

In this observational prospective study, the susceptibility and resistance profile was recorded for all the isolated uropathogens. Females were more susceptible to uropathogen then males. *E.coli* was the most isolated microbe with a percentage of 90.24%. *Pseudomonas aeruginosa, Enterococcus faecium, Enterobacter* species and *Proteus mirabilis* were only isolated from the UTI patients. Most of *Enterobacteriaceae* family was sensitive to antibiotic that was carbapenem (imipenem) drug about 89.7% and was the best choice of drug. Fluroquinoles drugs like Ciprofloxacin (82.1%) and Levofloxacin (81.6%) showed high resistivity. Co-trimoxazole and Cephalosporine drugs exhibited least resistivity, and they should be avoided as used for empirical therapy. For Gram positive Enterococcus spp, Glycopeptide antibiotics were sensitive while carbapenem and fluroquinoles were highly resistant.

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CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this article.

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