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Characterization and Demography of Multidrug Resistance in Patients with Urinary Tract Infections in Lahore, Pakistan

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Abstract:
The aim of the current study was to determine prevalence of multiple drug resistance bacteria among patients suffering from Urinary Tract Infections (UTI's). This study was carried out at Lahore in Sir Ganga Ram Hospital from 1 March to 30 May 2014. It involved cases with or without clinical symptoms of UTI and they were studied retrospectively. The results revealed a higher prevalence of UTIs among females (71%), in age group 51-75 years (86%), married (73%), house wives (80%), social middle class (85%), illiterate (78%), pregnant women (75%), catheterized (72%) and diabetics (73%). Prevalence of bacteria isolated was *Escherichia coli* (80%), followed by *Staphylococcus aureus* (6%), *Klebsiella sp.* (6%), *Pseudomonas sp.* (4%) and *Proteus sp.*(4%). Among the six antibiotics tested, bacterial isolates were more resistant to Ampicillin; with *E.coli* (100%), *S.aureus* (85%), *Klebsiellasp.* (98%), *P.aeruginosa* (100%), *and Proteus mirabilis* (100%). Resistance to Ampicillin, Tetracycline, Cefotaxime and Augmentin, commonly prescribed, suggests that increased consumption of a particular antibiotic maycontribute to multidrug resistance of uropathogens.

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INTRODUCTION

Urinary Tract Infection (UTI) is the second most common infectious disease and/ or colonization and affects about 150 million people (Boye *et al.*, 2012; Iqbal, 2022; Stamm and Norrby, 2001). If urine shows bacteria in asymptomatic individuals this is considered asymptomatic bacteriuria or colonization (Colgan *et al.*, 2006; Singh *et al.*, 2012). Complicated urinary tract infections prolong the need for treatment and may increase the risk of therapeutic failure. These are usually associated with anatomic or acquired defects in the urinary tract that prevent free urine flow and increase the risk of infection with multidrug resistant pathogens (Al-Ofairi *et al.*, 2018; Stapleton, 2003). Uncomplicated UTIs involve the bladder and affect women more commonly than men (Hooton, 2012; Iqbal *et al.*, 2021).

Bacteria that invade the urinary tract can lead to serious complications (Bano *et al.*, 2012). About 85% of community acquired UTIs and 50% of hospital acquired UTIs are by bacteria such as *Escherichia coli, Citrobacter, Klebsiella, Proteus mirabilis*, *Enterobacter sp., Serratia*sp, *Staphylococcus aureus and Enterococcus* sp (Flores-Mireles *et al.*, 2015; Hooton *et al.*, 2010; Iqbal and Ashraf, 2020; Ramanath and Shafiya, 2011). A variety of factors i.e., age, sexual activities, co-morbidity, immune-suppression; instrumentation and kidney transplantation affect prevalence of UTI (Ashraf and Iqbal, 2022; Iqbal *et al.*, 2010). Bacteria can acquire resistance to virtually all drugs and these resistance patterns are alarming (Ashraf and Iqbal, 2021; Ashraf and Iqbal, 2020; Iqbal and Ashraf, 2020; Kiffer *et al.*, 2007; Megged, 2014).

In Pakistan, like in other developing countries, there is increase in antibiotic resistance (AR), especially to all commonly used antibiotics because of availability and excessive use of antibiotics (Ashraf *et al.*, 2020; Iqbal and Ashraf, 2018; Iqbal and Ashraf, 2023; Iqbal *et al.*, 2022). The aim of this study was to isolate and identify bacteria causing common urinary tract infections and characterize the antibiotic resistant patterns in patients with UTIs at the Lahore region.

MATERIAL AND METHODS

Sample collection

Sterile urine samples (n=50) were randomly collected using the mid-stream method at Sir Ganga Ram Hospital, Lahore. The date, time and number of patients were labeled on the container and transported to the laboratory in autoclaved Luria broth (LB) within 2 hours of collection (Chakraborty *et al.*, 2011). A questionnaire was used about Bacterial isolates identified, sex and age of patients, marital status, occupation, Predisposing factors, pregnancy, catheterization, diabetes, Educational and Socioeconomic status. The study was approved by the institutional research committee and anonymity of patients was protected. The samples were processed for microbiological examination at Fatima Jinnah Medical College (an affiliated college of Ganga Ram hospital).

Primary culture

Blood agar, MacConkey agar and Nutrient agar and CLED agar media were prepared by using the product instructions. The pH was adjusted and the product was autoclaved. Sterilized Petri plates were incubated at 37°C for 24 hours for sterility check. Only sterile agar plates were selected for primary culturing. Samples were centrifuged at 6000 rpm for 5 minutes after sediments settled into the bottom of tubes and the supernatant was then discarded. Urine was centrifuged to obtain the primarily sediments and cultured on BA, MA, Nutrient agar and CLED agar by spread out technique. Then, cultured plates were placed in incubator at 37°C for 24 hrs (Chakraborty *et al.*, 2011; Ebah *et al.*, 2024; Mohammad *et al.*, 2021; Riaz *et al.*, 2017).

Purification and Identification of Bacterial Isolates

Isolation was done by multiple streaking of Bacterial colonies having diverse morphological characteristics (Iqbal *et al.*, 2016; Iqbal *et al.*, 2015; Saleem *et al.*, 2020). Morphological characteristics were picked by loop from primary culture plates and cultured on Blood agar, MacConkey agar and Nutrient agar plates. Plates were labeled and incubated at 37°C for 24 hours. To identify unknown pure bacterial cultures, we studied colony morphology, performed microscopy and genus and species level, biochemical tests using the standard protocols of Bergey's Manual of Determinative Bacteriology (Aernan *et al.*, 2023; Bergey, 1994; Edrees and Anbar, 2020).

Antibiotic Sensitivity Testing

The antibiotic discs (Ampicillin, Cefotaxime, Tetracycline, Augmentin, Ciprofloxacin and Imipenem) were applied using disk diffusion method according to CLSI procedure (Bauer *et al.*, 1966; Edrees and Anbar, 2020; NCCLS, 1992; Saleem *et al.*, 2018a; Saleem *et al.*, 2018b) using Muller Hinton and McFarland standard. Using sterile inoculating loop, we picked the bacterial colony and dispensed it into saline solution.

Plates were placed in incubator at 37°C for 24 hours. We examined the plates after 24 hours. Measurement and interpretations were following international guidelines.

Statistical analysis

The date obtained from the questionnaires and biochemical analysis was processed in SSPS windows version 16. Appropriate test statistics one way ANOVA were used.

RESULTS

Prevalence of Urinary Tract Infection by demographic characteristics

A total of fifty UTI patients were included in this study. Out of which 20 (71%) were female and 8 (36%) were male. Infection rate was higher in females than males. Among age groups, 1-25 years, UTI patients there were 6 (60%); among the age group of 26-50 years, there were 7 (58%); among 51-75 years there were 20 (86%) and the remaining 2 (40%) were within 76-100 years. Married patients were more commonly infected 22 (73%); were married and 8 (40%) were unmarried. Married females were more prone to UTIs. About 10 (66%) of cases were unemployed, 12 (75%) were employed. Eight (80%), and 6 (66%) were housewives or students. Regarding socioeconomic status 20 (80%) belonged to the lower socio-economic class based on their family income, followed by 12 (85%) were of middle socio-economic class and 9 (81%) from upper socioeconomic class. A large number of the patients 26 (78%) were illiterate. Among female patients, infection rate was higher in the pregnant 24 (75%) than in non-pregnant 8 (44%). Infection rate was higher in previously catheterized patients, 21 (72%) than in non-catheterized 8 (38%).Prevalence of UTIs observed was higher in patients suffering from diabetes mellitus 19 (73%) than those without diabetes 7 (29%) (Table 1).

Identification of bacterial isolates

All of the purified bacterial isolates (n=50) were identified on the basis of culture characteristics, microscopic morphology, gram stain (Table 2) and biochemical profiles (Table 3).

Prevalence of bacterial isolates

Among the biochemically identified bacterial isolates (n=50), the highest number was of *E.coli*40 (80%), followed by *Staphylococcus aureus* 3 (6%), *Klebsiella sp* 3 (6%), *Pseudomonas sp 2* (4%) and *Proteus sp* 2 (4%) (Table 4).

Antibiotic sensitivity testing

Antibiotic Sensitivity was observed by Disc diffusion protocol (Kirby Bauer). Observations were done after 24 hrs. According to Antibiotic sensitivity testing results the highest resistance was observed with Ampicillin in *E.coli* 100%,

Staphylococcus aureus 85%, *Klebsiella* 98%, *Pseudomonas aeruginosa* 100% and *Proteus mirabilis* 100% and the least resistance was found against Imipenem 0%, 20%, 6%, 10% and 15% respectively (Table 5). Bacterial isolates resistant to more than two antibiotics were regarded as multidrug resistant bacteria.

Table 1. Occurrence of UTI's and demographic characteristics.

Variables	Characteristics	UTI	NON UTI	TOTAL	Percentage
Gender	Male	8	14	22	36%
	Female	20	8	28	71%
Age group	1-25 YEARS	6	4	10	60%
	26-50 YEARS	7	5	12	58%
	51-75 YEARS	20	3	23	86%
	76-100 YEARS	2	3	5	40%
Marital Status	Married	22	8	30	73%
	Unmarried	8	12	20	40%
Occupation	Unemployed	10	5	15	66%
	Employed	12	4	16	75%
	House wife	8	2	10	80%
	Students	6	3	9	66%
Socioeconomic Status	Lower class	20	5	25	80%
	Middle class	12	2	14	85%
	Upper class	9	2	11	81%
Educational status	Literate	7	10	17	41%
	Illiterate	26	7	33	78%
Pregnancy	Pregnant	24	8	32	75%
	Non pregnant	8	10	18	44%
Catheterization	Catheter	21	8	29	72%
	Non catheter	8	13	21	38%
Diabetes	Diabetic	19	7	26	73%
	Non diabetic	7	17	24	29%

Table 2. Microscopic and Colonial characteristics of Uropathogens.

Bacterial species	**Colony characteristics**			**Morphological characteristics**		
	Color on agar	**Color on MacConkey agar**	**Color on Blood agar**	**Gram staining**	**Motility test**	**Oxygen requirement test**
E.coli	Opaque large yellow colonies and non mucoid colony elevation	Pink or rose-red. Colonies having zone of precipitated bile.	Slightly convex, grey	- Ve rods	Motile	Aerobe or Facultative anaerobe
Staphylococcus	Uniform, opaque and Deep yellow colonies	No growth to slight growth (pale pink)	Yellow to cream or white colonies	+ Vecocci	Non-motile	Facultative anaerobe

Klebsiella	Large Yellowish/white and mucoid colony elevation	Pink, mucoid.	White grey and usually mucoid	- Ve rods	Non Motile	Facultative anaerobic
Pseudomonas	Pale blue green with irregular edges	Colorless to pink.	Slightly opaque colony	- Ve rods	Motile	Aerobe
Proteus	Blue grey with irregular edges and slightly elevated	White / colorless	Swarming growth pattern	- Ve rods	Motile	Facultative anaerobe

Table 3. Biochemical identification of Uropathogens.

Biochemical test		***E.coli***	***S.aureus***	***Proteus mirabilis***	***Pseudomonas***	***Klebsiella***
Oxidase test		–	–	–	+	–
CatalaseTest		+	+	+	+	+
IndoleProductionTest		+	–	+/_	–	–
MethylRedTest		+	+	–	–	+
VoguesProskaur Test		_	+	–	–	+
LactoseFermentation Test		+	+	–		+
MannitolSaltAgar		+	+	–	+	+
CitrateUtilizationTest		–	+	+/_	+	+
EosinMethyline Blue		+	–	–		+
Urease Production Test			–	+	+	+
TrippleSugar IronTest	**Slant**	A	K	K	K	A
	Butt	A	A	A	K	A
	Gas	+	_	+	_	+
	H_2S	_	_	+/_	_	_

Table 4. Prevalence of bacterial isolates identified by conventional biochemical characterization in patients suffering from UTI.

Name of bacteria Isolated	**Total number of samples**	**Number of samples positive for uropathogens**	**Percentages**
E.coli	50	40	80%
Staphylococcus aureus	50	3	6%
Pseudomonas species	50	3	6%
Klebsiella sp	50	2	4%
Proteus species	50	2	4%
Total		50	100%

Table 5. Prevalence of multi drug resistance tested against bacterial (Uropathogens) isolates.

Antibiotic Discs	Codes disks	*E.coli*	*S.aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Proteus mirabilis*
Ampicillin	AMP	100%	85%	98%	100%	100%
Cefotaxime	CTX	89.7%	65%	70%	90%	85%
Tetracycline	TE	69.4%	50%	60%	70%	65%
Augmentin	AMC	62.6%	45%	55%	24%	20%
Ciprofloxacin	CIP	54.2%	33%	50%	55%	45%
Imipenem	IPM	0%	20%	6%	10%	15%

DISCUSSION

UTIs are a major problem in the community and hospital with ever increasing antibiotic resistance of bacteria worldwide. Our present study, in a major Pakistani city, also found that the infection rate of UTI was higher in females than males (70/30%) (Al-Bedri, 1987; Czajkowski *et al.*, 2021). Males and females of any age are prone to UTIs. Old patients had higher rates than younger ones in our study and this is in agreement with previous findings (Ramanath and Shafiya, 2011). Urinary tract infection is a major problem for all age groups (Ashraf and Iqbal, 2022; Iqbal *et al.*, 2022; Jha and Bapat, 2005) and is significantly higher in married people, unemployed people, lower socioeconomic status and illiterate people. These findings are also in accord with previous reports (Ashraf and Iqbal, 2022; Begum *et al.*, 2006; Saleem and Daniel, 2011). We found that the infection rate was higher among pregnant women, previously catheterized and diabetic patients; also according to previous studies (Abu Aleinein and Salem Sokhn, 2024; Amina Begum, 2022; Chukwuocha *et al.*, 2012; Demilie *et al.*, 2012; Milan and Ivan, 2009).

E.coli was the most prevalent pathogen in UTIs, also supported by previous studies (Iqbal and Ashraf, 2020; Obiogbolu *et al.*, 2009; Saleem and Daniel, 2011; Vasquez and Hand, 2004). *Pseudomonas species* were the most commonly isolated pathogens from previously catheterization associated UTI patients (Milan and Ivan, 2009). Most *E.coli* were resistant to Penicillins (Ampicillin and Augmentin), Cefotaxime and Tetracycline indicating that these antibiotics should not be used for UTIs. Resistance to Penicillins by *E. coli* has been reported worldwide but only several results showed resistance to be 100% (Olowe *et al.*, 2007). In Pakistan previous studies have shown the greatest levels of resistance in *E.coli* against Cefotaxime, Tetracycline and Penicillins (Aziz *et al.*, 2012). Quinolones, mostly Ciprofloxacin, has been commonly used against *E.coli*, but *E.coli* is now also resistant to Ciprofloxacin at 54.2% according to our and other findings (Mavroidi *et al.*, 2012; Neyestani *et al.*, 2023).

The present results also showed that Imipenem can be used against *E.coli* and this is supported by previous findings (Khorvash *et al.*, 2009; Tankhiwale *et al.*, 2004). *Klebsiella pneumonia, Proteus mirabilis* and *Pseudomonas aeruginosa*, which are now mostly resistant to Penicillins, Tetracycline and Cefotaxime, showed highest sensitivity to Imipenem. This is also supported by a previous study that *P. mirabilis,* was 100% resistant to Augmentin. *P. aeruginosa* was 100% sensitive to Augmentin and Imipenem. Resistance was 100% towards Cefotaxime while 50% for Ciprofloxacin and Penicillins (Bullens *et al.*, 2022; Khan *et al.*, 2020; Ouno *et al.*, 2013).

CONCLUSION

UTIs remain one of the biggest problems in pregnancy and in previously catheterized and pregnant diabetic patients. Many of the bacterial strains were shown resistant to Penicillins (Augmentin and Ampicillin), Tetracycline and Cefotaxime which are still frequently prescribed in South Asia as they are the least expensive

drugs and readily available. As antibiotic resistance is a dynamic ever changing problem. Continuing studies of community resistance pattern are essential and so is continuing up-to-date education of the medical community in this field.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests to declare.

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