ORIGINAL ARTICLE

Study of Urinary Tract Pathogens and Determination of Their Susceptibility to Antibiotics in the Residents of Lahore

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Abstract

Background
Urinary tract infection (UTI) is second most common and a serious medical condition affecting millions people every year. They are the infections of urinary tract i.e. urethra, bladder, urinary ducts and kidneys. Most of these infections are bacterial, particularly gram negative pathogen. We aim this study to identify the common pathogens leading to UTI in our local population and their antibiotic susceptibility.

Methodology
This study was carried out on the residents of different area in Lahore city between Jan and June 2016, and all research work was conducted in The Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL). About 250 samples of the all age groups were collected and investigation was made utilizing two main instruments, questionnaire and urine testing to identify microorganisms responsible for urinary tract infections to explore sensitivity patterns of identified microorganisms to certain antibiotics used in the treatment of UTI.

Results
Of all collected samples (n=250) 109 (43.6%) samples showed no growth on culture plates. Approximately 56% (n=141) were positive samples. Among the positive cases gram negative bacterial pathogens were accountable for about 86% of urinary tract infections in contrast to gram positive bacterial pathogens. *E. coli* were major uro-pathogen 39.72% trailed by *Klebsiella spp.* 16% and *Staphylococcus spp.* 12%. The prevalence of *Candida spp.* 20% was also very high in males and females. With respect to the antibiotic sensitivity test, the results revealed that antibiotics like Nitrofurantoin, Piperacillin-Tazobactam and Imipenem for gram negative bacteria and tetracycline against gram positive bacteria, showed a low resistance rate in this study over commonly used antibiotics.

Conclusion
Urine culture is an important diagnostic tool to confirm urinary tract infections. Rules must be put on the consumption of antibiotics to limit their abuse and misuse. Pragmatic antimicrobial choice in management of urinary tract infections should be founded on facts of local occurrence of causal uro-pathogens in addition to corresponding antibiotic sensitivities than on international guiding principle.

Key words
Urinary tract infections, Microorganisms, susceptibility.

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Introduction
Urinary tract infections are chief health problems affected lots of peoples for long time. They rank second of greatest communal sort of infections in the human body.[1] Urinary tract infections occur in both sexes i.e. males and females. Urinary tract infections are instigated by bacterial pathogens which characteristically grow at the neck of urethral duct and move to the urinary bladder. Maximum contagions are initiated by backward mounting of microbes from the fecal flora of anus through the urethral tube to urinary bladder and kidneys particularly in the women that possess a smaller and broader urethral tube.[2] In men, the micro-organisms often invent from the sub prepucial pouch. Urinary tract infections don't at all times cause sign and symptoms, but when they cause they can contain i.e. wanting to pass urine more over and over again and immediately, if only a small number of drops, scorching pain or a 'scalding' feeling when passing urine, urine that look like bright ruddy or cola colored i.e. a mark bloody urine. Other sign and symptoms of urinary tract infections may be a sensation that the urinary bladder is still full after passing urine, cloudy urine, robust odor in urine, pain above the pubic bone in men and pelvic pain in women.

The bacterial strains that cause Urinary tract infections (UTIs) take account of *Escherichia coli* which are accountable for greatest uncomplicated cystitis issues in females, particularly in younger ladies. *Escherichia coli* are commonly an inoffensive microbe originating in guts. If it reaches to the vagina, it can inhabit urinary bladder, creating infections. *Staphylococcus*

saprophyticus are responsible for 5 – 15 percent of Urinary tract infections (UTIs) typically in younger females.[3] The doubtful bacteria *Streptococcus sp*. and *Pseudomonas sp*. are the most frequently causative micro-organisms of urinary UTIs.[4] *Klebsiella, Enterococci* species of bacterial pathogens and *Proteus organism* reason for other bacterial pathogens that responsible for UTIs. They usually found in urinary system in older females. *Lactobacillus, Corynebacterium* and *Streptococcus mileri* probably will be cause of the pathogenesis of urinary tract infections.[5] Rare microorganism causes of urinary tract infections include *ureaplasma urealyticum* and *Mycoplasma hominis* that are usually inoffensive organisms. The objectives of antimicrobial treatment were to eliminate the bacteriological progression in the urinary system using antibiotics that is effective, harmless, and asking price. The tenacity of contagion is reliant on sensitivity of the bacterial pathogens to concentrated amounts of the antibiotics attained in urine.

Material and MethodsStudy settings

This study was carried out on the residents of different area in Lahore and all research work was conducted in The Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL).

Sources of data

Over the course of study Patient's information such as age, sex, clinical presentation, existence or nonexistence of urinary catheter was noted. Brief history of patients was gained about UTIs sign and symptoms.

Sample Size

Overall 250 urine samples were collected, about 120 males and 130 females and stayed in the age of 01 to above 50 year old. There were higher numbers of samples collected from the all age groups excluding neonates and infants because of samples collection is much difficult in sterile conditions.

Collection of urine specimen and transportation

The samples were taken into clean, broad necked, leakage resistant, plastic containers. Mid-stream urine specimens were taken from the males and females residing in the Lahore. Each of the people was instructed verbally on mode of collection of mid-stream urine that was during forceful urination after the 10-20 ml has been voided. Participants were adequately educated on precautions to prevent contamination of specimen. All the study participants were fully informed of the purpose of study and consented to provide specimens before starting the study. Urine samples were transported immediately without any prolonged delay in Microbiology laboratory in sterilized conditions.[6]

Sample Preparation and Microscopy

Urine samples were prepared for microscopy according to standard method. The urine specimens were homogenized and aliquots rotated (centrifuged) at 5000 rpm for 5 minutes. The deposited materials were inspected by individually 10X then 40X objective lens. Specimens' with $\geq$10WBC/mm^3 were considered as pyuria.[7] Media used were Nutrient Agar (NA) and CLED Agar as termed by Cheesbrough.[8]

Sample culturing

The collected urine samples were processed for culture and report according to standard method procedure and protocol. For the visual examination and calibrated Loop Streak method for urine culture performed 1 ul wire loop was treated by heat and immersed in urine specimens. Wire loop was at that time streaked on dishes of CLED Agar.[8] Bacteria were reported in colony-forming units (CFU). Less than 10^4/ml organisms were not considered significant, 10^5/ml were significant bacteriuria.

Identification of Uro-pathogens

All the microbial isolated organisms were categorized according to form and structure of colonies, cell morphology**,** Gram stain, Catalase test, and DNase and oxidase tests, Germ tube test. On basis of the respective results of primary identification test the gram negative isolates were exposed to predictable biochemical tests i.e. citrate utilization test, triple sugar iron reaction indole production, urease production, coagulase, Bile solubility, litmus milk decolorization test and motility test.[9] Antibiotic treatment was also performed for GBS.

Pathogen sensitivity test

Entirely isolated micro-organisms were tested against various antibiotics in laboratory via disc diffusion assay method on Muller Hinton Agar by improved Kirby-Bauer method.[10] The zone size and susceptibility testing was don according to CLSI book Performance Standards for Antimicrobial Susceptibility Testing 27th Edition.

Statistical analysis

All data were statistically analyzed by taking due care for completeness, consistency, coding and sorting using SPSS statistical package, version 22.0

Results

Isolated uro-pathogens in male and female participants

The occurrence of urinary tract infections isolated microorganisms in relative to sex is revealed in Table 2. *E. coli* was furthermost recurrently isolated uro-pathogen in both genders though prevalence of *Klebsiella* was higher in males than females. More isolates of *Staphylococcus species* and *Group B Streptococcus* (GBS) were recovered more from females than males. One case of *Neisseria gonorrhea* was positive in males. *Candida* was most prevalent after *E. coli* in both sexes but its percentage is higher in males. There is no prominent sex difference for the remaining isolates i.e. *Proteus, Pseudomonas* and *Citrobector.*

Prevalence of Gram positive and negative isolates of UTIs

Most of the cases of urinary tract infections were because of

Table 1. Isolated uropathogens in male and female participants

Organisms Isolated	Males	(n,%)	Females	(n,%)
E. coli	37	(50%)	19	(28%)
Klebsiella	13	(18%)	10	(15%)
Staphylococcus aureus	03	(4%)	06	(9%)
Staphylococcus epidermidis	03	(4%)	03	(5%)
Staphylococcus saprophyticus	01	(1%)	01	(1%)
Pseudomonas	03	(4%)	02	(3%)
Proteus	01	(1%)	01	(1%)
Group B Streptococcus (GBS)	02	(3%)	04	(6%)
Neisseria gonorrhea	01	(1%)	00	(0%)
Citrobector	01	(1%)	01	(1%)
Candida	09	(12%)	20	(30%)
Total	74	(100%)	67	(100%)

gram negative *bacilli* 85.93 percent. Gram negative *bacilli* cases were higher in males than females. However the gram positive *cocci* were less prevalent than gram negative *bacilli.* The prevalence of gram positive *cocci* was higher female than males.

Percentage susceptibility in Gram negative uro-pathogens
Isolated organisms for *Escherichia coli* presented resistance to frequently used antimicrobials. The highest sensitivity was shown by Gentamicin, Imipenem, Cefotaxime and Nitrofurantoin. *Klebsiella* isolates were only sensitive to Imipenum. *Pseudomonas* was sensitive to Amikacin, Cefoperazone, Piperacillin-tazobactam. *Proteus* isolates were highly sensitive to Cefotaxime, Tazocin, and Cefoperazone. Isolates of *Citrobacteria* were sensitive to orally administered antibiotics Amoxicillin-Clavulanic acid and Nitrofurantoin. It was also sensitive to injectable antibiotic Imipenem. *N. gonorrhea* was not sensitive to any tested antibiotic.

Percentage susceptibility in Gram positive uro-pathogens
Staphylococcus aureus was among highly susceptible to ciprofloxacin, tetracycline and Gentamicin. *Staphylococcus epidermydous* was 100% sensitive to Novobiocin and highly susceptible to Nitrofuratoin. *Staphylococcus saprophyticus* was sensitive to Cefotaxime. *Group B Streptococcus* (GBS*)* showed high susceptibility to Tetracycline, Nitrofuratoin and Novobiocine.

Discussion
Urinary tract infections mean different types of medical illnesses extending in sternness from symptomatic to asymptomatic renal infection with resulting sepsis.[11]

The uro-pathogens recognized in our research are alike to numerous readings conducted in different parts of the world either in local area or universally. Pathological microbes were isolated from 141 positive samples which in the vast majority of cases were of *E. coli* (39.72%) followed in order by *Candida* fungus (20.57%), *Klebsiella* (16.31%) *staphylococcus* (12.06 %), *Group B Streptococcus* (4.25 %), *Pseudomonas* (3.55 %), *Proteus* (1.42 %), *Citrobector* (1.42%) and *Neisseria gonorrhea* (0.71%) were among the most common urinary pathogenic bacteria. That was as to other readings where it places the most common uro-pathogen causing urinary tract infections as studied by Mehar where 62.6% cultures grew *E. coli*.[12] These consequences remained also alike that a research directed by Dilnawaz in 2005 in India that showed first two common uro-pathogens was *Escherichia coli* and *Klebsiella pneumonia;* both microorganisms were different with respect individual percentage from previous studies.

The prevalence of the urinary tract infections pathogens in relative to sex is presented in Table 2 and 3 are steady with reports from Abubakar.[13] Most of the urinary tract infection cases were because of gram negative *bacilli* 85.93 percent. Gram negative *bacilli* cases were higher in males than females. However the gram positive *cocc*i were less prevalent than gram negative *bacilli.* The prevalence of gram positive *cocci* was

Table: 2. Percentage susceptibility in Gram negative uro-pathogens

Organisms	Antibiotics									
	AML	AK	CIP	CTX	CPZ	GEN	IPM	NA	NIT	PTZ
E. coli	20	52	28	70.0	10	90	88	35	77	69
Klebsiella	21	65	15	68	35	20	90	25	61	66
Pseudomonasaeruginosa	45	85	11	NA	75	69	57	5	26	81
Proteus	61	78	31	95	84	63	75	65	59	85
Citrobacteria	74	56	19	53	44	59	73	24	89	55
N. gonorrhea	65	20	12	14	16	22	69	25	20	58

E: Escherichia, N: Neisseria

AML-Amoxicillin-Clavulanic acid, AK-Amikacin, CIP-Ciprofloxacin, CTX-Cefotaxime, CPZ-Cefoperazone, GEN-Gentamicin, IPM-Imipenum, NA-Nalidixic, NIT-Nitrofuratoin, PTZ-Piperacillin-Tazobactam.

Table 3. Percentage susceptibility in Gram positive uro-pathogens

Organisms	Antibiotics										
	AML	CIP	CTX	CPZ	E	GEN	NA	NIT	P	TC	NV
S. aureus	63	76	58	54	48	80	35	63	29	77	67
S. epidermydous	29	18	52	41	56	19	44	83	24	67	100
GBS	38	18	8	NA	48	12	15	71	00.0	95	97

S: Staphylococcus, GBS: Group B Streptococcus
AML-amoxicillin-Clavulanic acid, CIP-Ciprofloxacin, CTX-Cefotaxime, CPZ-Cefoperazone, GEN-Gentamicin, E-Erythromycin, NA-Nalidixic, NIT-Nitrofurantoin, P-Penicillin, TC-Tetracycline, NV-Novobiocine.

higher female than males.
Consequently that higher prevalence of *E. coli* might be due to fecal contamination, the predilection of the organisms from the toilets and the shortness of the female urethra in females. That occurrence was also reported in previous research don by Australian Smith[6]. There is a conceivable relation among UTIs prevalence amongst the population and the standard of personal hygiene and the condition of toilet facilities. It is previously reported by Fihn.[14]

Many people inspected valued the toilets were bad. Bad, in that situation suggests that there was no suitable water supply for cleaning and flushing toilets on public places congestive areas frequently. According to this case population were at the risk of becoming infected during passing urine, that was observed by visiting different areas in Lahore and through by asking questions from population. Sexual activity was another factor that predisposes people to UTIs. For example, *Staphylococcus aureus* (12.06 %), which was a member of skin flora might stay on the skin and get transmitted during sexual intercourse. If demographic situation of the cases examined by age then it was observed that both the extremes of age groups have risk of UTIs. These results are similar to Handley.[15] There were many cases of UTIs caused by *candida spp.* (20.57%). *Candida* urinary tract infection was typically presented in the patients with immune-suppressant.[7] These results of antibiotic sensitivity shown in Tables 4 and 5 resemble to the research of Dr. Alka Nerurkar.[16]

The implication of that was the opportunity of easy access of self-medication, exploitation of medicine leading progress to resistance against pathogens. It is recommended to establish good management of drugs in order to prevent their easy accessibility in public sector without recommendation. Public awareness about the potential risk factors of UTIs is important to reduce the risk of the disease. In this study, all participants of different age groups and children above one year were included, where as participants with known T.B, on urinary catheters and children below one year old were excluded.

Conclusion

There is an emerging resistance of commonly isolated bacteria to commonly used antibiotics, which can be ascribed to inappropriate antibiotic administration. Pragmatic antibiotic choice in dealing of urinary tract infections must be grounded on the information of indigenous prevalence of contributing uro-pathogens and their respective antibiotic sensitivities rather than on universal guidelines. *Candida* was most prevalent after *E. coli* in both sexes but their percentage is higher in males because female cases do not report well to hospital due to ethical issues in under study area. Undiscriminating recommendation and usage of antibiotics necessity be disheartened in both public and clinical trials by incessant public alertness and teaching on coherent antimicrobials usage and their choice of antimicrobials must be on the basis of culture and sensitivity test.

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