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Etiology and Antibiotic Resistant Pattern of Urinary Tract Bacterial Pathogen in District Mardan

Muhammad Karim[1]*, Shahzar khan[2], Irfan Ullah[3], Asim Gamaryani[4], Muhammad Hasnain[5], Syed Hamza Abbas[2], Humaira Nawaz[2], Zunaira Saeed[6], Inam ur Rahman Zafar[2], syed yawar saeed[2] and Muhammad Usman Ali Malik[3]

[1]*Institute of Microbiology and Molecular Genetics, University of the Punjab Lahore, Pakistan*

[2]*Department of Microbiology, Quaid-i-Azam University Islamabad Pakistan*

[3]*Department of Microbiology, Abdul Wali khan University Mardan, Pakistan*

[4]*School of Health and society, University of Wollongong Australia*

[5]*Department of Pharmacy, University of Peshawar, Pakistan*

[6]*Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan*

***Corresponding author:** Muhammad Karim, Institute of Microbiology and Molecular Genetics, University of the Punjab Lahore, Pakistan

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ABSTRACT

Urinary tract infection an infection that affects the urinary tract. Infection of the upper urinary tract is called pyelonephritis (kidney infection) while infection of lower urinary tract is called the cystitis (bladder infection). Pain with urination and frequent urination are the symptoms of cystitis. While fever and flank pain are the symptoms of pyelonephritis in addition to the symptoms of cystitis. Women gets UTI most frequently than men. About 10% females experience a urinary tract infection annually and more than 50% experience 1 urinary tract infection in their lifetime and 2 to 8% children which are under the age of 10 years. Gram negative bacteria are the main cause of urinary tract infection in frequent urinary tract infections. Less commonly gram positive bacteria also cause urinary tract infection. Sample collection was held from patients with different ages and gender at Mardan Medical complex, Bacha Khan medical complex and Benazir Bhattu hospital Mardan in sterile containers. The pathogens were confirmed through gram staining and biochemical tests. Sensitivity was performed on MHA media through Kirby Bauer method, 5 different Uropathogens were isolated from 55 samples, in which 12 samples were Positive for *E. coli*, 7 for *Pseudomonas aeruginosa*, 5 for *Enterobactor spp*., 2 for *Staphylococcus aureus,* and 3 for E. faecalis. The frequency of urinary tract infection was high in Female compared to Male. The frequency of urinary tract infection was higher in age greater than 10 years (12.72%), age less than 50 years (32.72%) and age greater than 50 years (7.72%).

Keywords: UTI; Uropathogens; Antibiotics; Mardan

Introduction

UTI is an abbreviation for Urinary Tract Infection. Bacteria are rarely originating in urine (germs). Urine is a byproduct of our kidneys' purification system. When your kidneys eliminate waste and extra water from your blood, urine is created. In normal circumstances, urine is not polluted. However, bacteria can get into the urinary system from external of the body, causing problems like infection and irritation. (D Prakash [1]). The urinary tract includes kidneys, ureters, bladder and urethra. kidney serve as your body's filters, drawing water and waste from your blood. This trash turns into pee, ureters Urine travels from your kidneys to your bladder through narrow tubes called ureters, bladder The bladder is a sac-like receptacle that holds your pee until it leaves the body and urethra urethral stricture

is a thick scar that develops along the urethra, the tube that exits the body through the penis to carry urine from the bladder.

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting 150 million people worldwide each year. Clinically, UTIs are categorized as uncomplicated or complicated (O Amali [2]). The increased rates of antibiotic resistance make treating a urinary tract infection (UTI), which is prevalent, more challenging. It has been expected that 25–35% of women identified with a UTI will suffer a reoccurrence UTIs are responsible for w10.5 million physician office and emergency department visits annually in the United States within 6 months, Approximately 60% of women will have more than one UTI in their lifetime. UTIs account for more than 10.5 million physician office and emergency department visits in the United States each year. (Hoberman, et al. [3]).

Gram-positive and gram-negative bacteria, are commonly responsible for UTI. It is also caused by a variety of fungi. Escherichia coli is the most common causative agent of both complex and simple UTI. *Klebsiella pneumonia, Proteus mirabilis, E. coli Streptococcus saprophyticus, group B Streptococcus (GBS), Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis* and *Candida spp*. are some of the causative agents involved with UTI. Each phase in the pathophysiology of UTIs is launched by a critical event called adhesion. (Camacho et al., 2004) Staphylococcus a significant contributor to urinary tract infections is *saprophyticus* (UTI) in young, sexually active girls, the organism was the next most common cause of UTI. The second most typical culprit behind acute UTIs was *S. saprophyticus*. (Sabrina 2010). *Pseudomonas aeruginosa* is an opportunistic human pathogen that, especially in people with impaired immune systems, can lead to serious acute and chronic infections. These infections are challenging to treat because *P. aeruginosa* has a high inherent drug resistance and can acquire additional resistances while receiving antibiotic therapy. With nonresistant strains, the death rate for individuals with UTIs caused by *P. aeruginosa* might reach up to 23%. (Lacovelli, et al. [4]). *Staphylococcus aureus* is a common component of the body's microbiota. In the general population, *Staphylococcus aureus* is a very uncommon cause of urinary tract infections. Although staphylococcal bacteremia that develops elsewhere frequently leads to the isolation of *S. aureus* from urine samples (e.g., in cases of endocarditis). Most *S. aureus* bacteriuria infections are not accompanied by signs of a urinary tract infection. Because long-term urine catheterization almost always goes hand in hand with bacteriuria (Olowe [5]).

E. coli is the most dominant gram-negative bacterium in the human intestinal tract, *E. coli*, is not virulent in this environment. Most women who experience their first UTI go on to experience a second one within six months, and the chance of developing a third UTI is 3%. because Escherichia coli accounts for 90% of UTls in individuals who are ambulatory. *E. coli* might be the cause of both the first and second UTls. However, molecular bio- logic techniques, such as Southern hybridization and pulsed- field gel electrophoresis (PFGE), can distinguish genotypes (Conway, et al. [6]). The bacteria known as Klebsiella pneumoniae typically reside in your intestines and feces. If these bacteria are in your intestines or stool, they are not harmful. However, they can result in serious infections if they move to another area of your body. K. pneumoniae can cause a UTI if it enters the urinary tract. (Dias, et al. [7]).

High levels of -haemolysin (HlyA) are secreted by UPEC, and these molecules oligomerize and integrate in the cholesterol-rich micro domains of the host cell membrane in a Ca+-dependent manner62,63. This causes the umbrella cells to develop pores and encourages their lysis, which makes it easier for the bacteria to absorb nutrients and iron. (N. J. S. W. Iduoriyekemwen and A. E. Sadoh 2012). The uropathogens *P. mirabilis, S. saprophyticus, K. pneumoniae*, and *P. aureginosa* all encode urease, which is crucial for colonization and persistence during *P. mirabilis* and *S. saprophyticus* UTIs. This enzyme catalysis the hydrolysis of urea to carbon dioxide and ammonia, resulting in elevated urine pH and the production of calcium crystals (apatite) and magnesium ammonium phosphate ammo precipitates (struvite) in urine and on catheters importantly, the accumulation of ammonia becomes toxic for the uroepithilial cells, inducing direct tissue damage. There is hardly much iron in the bladder environment. Therefore, uropathogens use siderophore systems for iron (Fe3+) scavenging in order to proliferate in human urine. These systems are made up of siderophore assembly machinery, a siderophore that binds iron, and a membrane receptor that internalizes the iron bound.

Materials and Methods

Data Collection

The data was collected from Bacha khan medical complex, Mardan medical complex and Benazir bhattu hospital Mardan from patients of different age and gender referred by their physicians for culture. The samples were collected and labeled carefully and brought to the Microbiology laboratory.

Microscopy

First the samples were processed for examination under microscope. Microscopy was done carefully using 10x lenses for focusing and 40x for examination. during examination different cells, casts and crystals were observed.

Sterilization

The media and equipment sterilized for 15 minutes in autoclave, the glassmaterial sterilized before the use in hot air oven for 1 hour.

Culture

All the samples were inoculated on cysteine lactose electrolyte deficient (CLED is a valuable and growth non inhibitory medium which is used for the identification and differentiation of microorganisms that cause urinary tract infections.it contains cysteine and lactose and is electrolyte deficient which prevents the swarming of

protius species) and Macconkey agar (it is a solid, selective and differential media that is to cultivate or grow only gram negative microorganisms) using sterile wire loop. Streaking method was used. The plates were incubated for 18 to 24 hours at 34C0 in incubator. After 18 to 24 hours of incubation observable colonies were processed for identification like morphology and different biochemical tests.

Gram Staining

Separate colonies were taken from the plate inoculated (using normal saline) on the slide in circular motion of about 1cm in diameter through a sterile wire loop and then heated to fix the cells.

1. Crystal violet was applied to the heat fixed smear of bacterial culture for about 1-2 minutes.
2. Gram iodine was added to bind crystal violet and traps it to bacterial cells.
3. Acetone was used for decolourization.
4. Gram safranin was applied for counter staining, side was air dried and then was examined under microscope for differentiating the bacterial species into two large groups (gram positive and gram negative).

Morphology of the Culture

After potential growth we first look physical observation. Like color of colony, size of colony, shape of colony and swarming of colony.

Antibiotics Sensitivity

The antibiotics sensitivity pattern was done through disk diffusion method (also called Kirby Bauer method) in which separate bacterial colonies was inoculated through solid Muller Hinton agar plate (The Mueller-Hinton agar is a non-selective, non-differential medium capable of growing a wide range of non-fastidious organisms. It is considered a 'loose' agar, which helps to mediate the rate of diffusion of the antimicrobial more effectively than other types of media). the inoculation was performed through by cotton swab stick. Isolated colonies of urinary tract pathogens mixed in the normal saline and lawn was prepared on surface of Muller Hinton agar with sterilize cotton swab.

Totally eight (8) antibiotics was applied for urinary tract pathogens. Seven antibiotics were applied at each standard plate and were incubated at 35c0 for 22-24 hours in standard incubator. After 22-24 hours' incubation the zone of inhibition were measured and understood according to CLSI (clinical and liberty standard institutes). The tested antibiotics include Meropenem, Amikacin, Ciprofloxacin, Augmentin, Ceftriaxone, Ceftaizdime, Vancomycin. Special arrangements of antibiotics were performed for the ESBL (extended spectrum beta lactamase) phenomena (Figure 1).

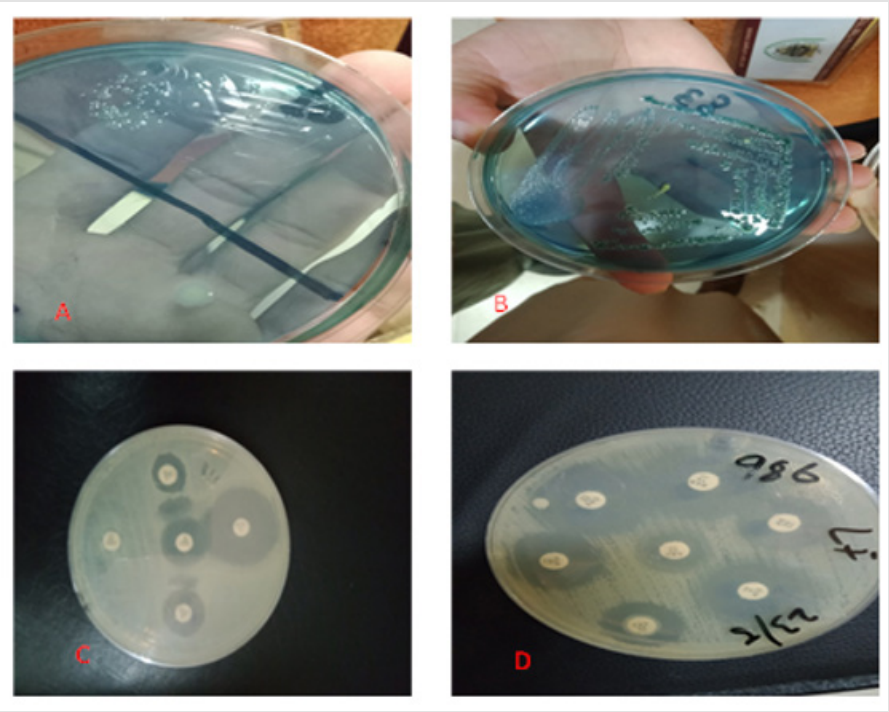

Figure 1: Culture and antibiotic resistant pattern of Uro pathogens.

Results

29 different pathogens were isolated from total of 55 clinical samples encompassing 21.81% *E. coli*, 9.09%Enterobactor Species, 3.63% *Staphylococcus aureus*, 12.72% *Pseudomonas aeruginosa*, and 5.45% *E. faecalis* shown in the (Table 1). The percentage of urinary tract infection was higher in female than male that is 29.09 % in female and 23.63% was in male that is shown in the (Table 1). Percentage of pseudomonas in female 5.45% and in male was 7.27%, *E. coli* in male is 7.27% and in female 14.54%, *E faecalis* in male is 1.81% and in female is 3.63%, *enterobacter* in male 5.54% and in female 3.63%, *Staphylococcus aureus* in male 1.81% and in female 1.81% that is shown in the (Table 1). The percentage of the patient that is less than 10 years old 12.72%, less than 50 year old is 32.72% and in patient greater than 50 year old is 7.27% shown in the (Table 2).

Table 1: Frequency distribution of uropathogens.

Uropathogen	Male	Female	Total
Pseudomonas	04	03	7
E Faecalis	01	02	3
E. Coli	04	08	12
Enterobacter spp.	03	02	5
Staph aureus	01	01	2
Total	13	16	29

Table 2: Age wise distribution of UTI.

Uropathogen	<10	<50	>50	Total
Pseudomonas	03	03	01	7
E. faecalis	01	02	00	03
E. Coli	02	08	02	12
Enterobacter spp.	00	04	01	5
Staph Aureus	01	01	00	2
Total	07	18	04	29

Antibiotic Sensitivity Pattern

Ceftriaxone, meropenem and amikacin was the most sensitive antibiotics to Gram negative rods (Sensitivity of Ceftriaxone is 83.33%, Sensitivity of meropenem is 79.16% and sensitivity of amikacin 70.83). Ciprofloxacin, ceftazidim and meropenem was most sensitive antimicrobial Drugs to Gram positive rods. (Sensitivity of Nitrofurantoin= 90.47%z, Sensitivity of Vancomycin = 76.19%), Linezolid and erythromycin also shows sensitivity greater than 50% while sensitivity of Penicillin G and Ciprofloxacin was <50%. Antibiotic sensitivity pattern of gram negative uropathogen (Tables 3 & 4).

Table 3: Antibiotic resistant and sensitivity pattern of different uro pathogens.

Antibiotic name	*Pseudomonas*		*E. coli*		*Enterobacter*	
	S	R	S	R	S	R
Amoxicillin	03	05	05	03	04	04
Vancomycin	02	06	03	05	06	02
Ciprofloxacin	04	04	03	05	06	02
Cefotaxime	05	03	05	03	04	04
Ceftazidime	04	04	03	05	03	05
Ceftriaxone	07	01	08	00	05	03
Meropenem	06	02	06	02	07	01
Amikacin	08	00	04	04	05	03

Table 4: Antibiotic sensitivity pattern of gram positive uropathogen.

Antibiotic name	*Staphylococcus aureus*		*E faculis*	
	R	S	R	S
Amoxicillin	06	02	04	04
Vancomycin	03	05	03	05
sCiprofloxacin	01	07	00	08
Cefotaxime	00	08	02	06
Ceftazidime	04	04	03	05
Ceftriaxone	05	03	03	05
Meropenem	01	07	02	06
Amikacin	04	04	03	05

Discussion

Urinary tract infection is the infection of urinary tract and its caused by different species of bacteria like *E. coli*, *klebsiella pneumonia, porteus, S. aureus, pseudomonas* and etc. urinary tract infection can be upper and lower and can be complicated and uncomplicated. Gram negative uropathogens are the most prominent bacteria involved in the urinary tract infection. Urinary tract infection is higher in female than male. UTI was significantly more prevalent among female (30.6%), compare to male (8.8%). And female were 3.4 time more prone than male (Okojie R O [8]), and our study shows that The percentage of urinary tract infection was higher in female than male that is 29.09 % in female and 23.63% was in male. The ratio of urinary tract infection is higher in patients >10 years and <50 years (47.6%). This is compared with another study in which it was higher in the same aged patients which is 33%. (kaslsoom, et al. [9]). The rise of resistance to broad spectrum such as carbapenem, extended spectrum beta lactamase and fluoroquinolones lead to the difficulties worldwide.

The growing resistance of Escherichia coli to antimicrobial such as nitrofurantoin is of major worry and demands the necessity for proper antimicrobial usage toward the suggested procedures. As there is no strict adherence to antimicrobial stewardship, the physician mostly recommends broad-spectrum antibiotics that cooperate to increased antibiotic resistance Scientists have commended good quality development plans to confirm suitable antimicrobial use according to the planned guidelines, particularly in a developing countries. The increased resistance of common microorganisms causing UTI is of great worry for the physician to provide broad-spectrum substitutes can predispose the patients to their awful effects. Appropriate regulation on antimicrobial treatment according to good guidelines will help in the transport of top-quality patient care and stop the progress of MDR organisms [10].

Conclusion

Our study exposes that the frequency of urinary tract infection is higher in female then male. *E. coli* is the most prominent bacteria involved in urinary tract infection in both male and female. Gram negative pathogens show highest sensitivity towards ceftriaxone, Amikacin and Meropenem, while gram positive shows highest sensitivity towards Ciprofloxacin, Ceftazidim and Meropenem.

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