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Research Article

Isolation and Antibiotic Susceptibility of Pathogens Causing Urinary Tract Infections During COVID-19 Pandemic in Mardan Division

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ABSTRACT

Urinary tract infections (UTIs) are multifactorial diseases due to several pathogenic microorganisms, such as bacteria, fungi, and parasites. But bacteria are the primary pathogen that causes UTIs. One of the most frequent causes of nosocomial infections in the community is urinary tract infection. This study was conducted during the COVID-19 pandemic at various hospitals and laboratories of the Mardan Division from December 2020 to November 2021. Mid-stream urine was collected as samples from patients with UTI. Whole samples were injected into MacConkey and Blood agar, and then the urease, catalase, and indole analyses were performed to identify gram-positive and gram-negative bacteria, respectively. The CLSI was followed while doing culture sensitivity tests for various antibiotics. Determining the prevalence of UTIs was the study's main objective; next, the most effective antibiotic for uropathogenic bacteria was investigated. Twenty-two percent of the samples, or 117/531, were positive for UTIs. Of them, 64.9% of the samples collected are from the female community. The medium to older demographic has the highest chance of UTI in the age distribution. After 117 isolates had been obtained, the most abundant urinary tract infectious microorganisms detected were E. Col (62.4%) and Klebsiella *spp*. (11.1%), and *Pseudomonas* (7.7%). According to antibiotic susceptibility testing, the most resistant medications include gentamicin and meropenem. Nevertheless, the most sensitive antibiotics include ciprofloxacin and meropenem. The current study concludes that urinary tract infections (UTIs) are a severe multi-pathogenic issue that is prevalent in the Mardan Division.

Keywords: UTI; microbes; antibiotic; gentamicin; meropenem

INTRODUCTION

Bacteria residing in the rectal area enter into the urinary tract through the bladder, followed by multiplication in urine, and urinary tract infection is caused. In many UTI cases, bacteria first reside in the urethra and multiply there. The UTI infection is known as urethritis, but if bacteria reside in the bladder and cause infection, then it is known as cystitis. If the infection is left untreated, bacteria continuously multiply, move to the upper urinary tract, and infect the kidney. Catheter stones, multidrug-resistant pathogens, and indwelling are a few conditions caused by uropathogens. The UTI is not a gender-based infection or age-specific; it can infect

both genders and children of any age in a lifetime. Though, females are more infected than males [1, 2]. The highest number of UTIs is observed in females of age 23-60 ears because their urethra is short and very close to the anus that is mostly infected by fecal bacteria [3]. Among the female population, pregnant women are at more risk of getting an infection due to urethral tone that contributes to increasing urinary stasis followed by ureterovesical reflux. Bacterial infection caused in pregnant women is mostly asymptomatic. In the male population, the prevalence of UTI is very low because the urethra has less contamination, followed by a strong bactericidal effect due to prostatic secretions and an excellent immunological defense system against bacterial infection. In children aged three or below, UTI is also very common because they have no developed specific immunity. Urine collection followed by result interpretation is difficult at this age, making diagnosing and treating bacteriuria difficult.

Causative Agents:

Normally, urine is sterile without any pathogen. The bacteria invade the urinary tract and multiply over there, causing urinary tract infection (UTI). Urine has two types: Acidic and Alkaline. Some pathogens are found in acidic urine, and others in alkaline. Bacteria are classified into gram-positive and negative strains. Around 80-85% of UTIs are caused by gram-negative bacteria, while 15-20% are due to gram-positive bacteria. The most common gram-negative bacteria is E. coli accounting for 80-90% of community-acquired infections and 50% of nosocomial infection [4, 5];

Epidemiology of UTI:

Asymptomatic to severe UTI is caused by bacteria. It is the most common health problem of infants to older age groups (3 months to 90 years). UTI caused in any environment. Globally, around 150 million population is infected with UTI. In children (3-5 years), acute UTI is common, having no specific signs and symptoms. At younger ages, girls (8%) and boys (2%) have at least one episode of UTI [6].

Symptoms of UTI:

The symptoms of both lower and upper urinary tract infections include discomfort during urination (Dysuria), frequent urination, urgent urination, and urine with blood (hematuria). Kidney infection symptoms include feeling sick and generalized body fatigue, shaking, chills, fever, vomiting and/or nausea, “flank” or side pain, and back pain.

Risk Factors:

1) The risk factors for bacteriuria in diabetic women include sexual interaction, age, duration of metabolic control, and degree of glycosuria [7] .
2) Asymptomatic bacteriuria has been recognized in pregnancy [8].
3) Some risk factors include due to interference or impeding of urine flow, such as (congenital urinary tract abnormalities, inflammation, catheter, urinary surgery, etc.).
4) A rare mortality chance besides the higher morbidity during pregnancy among women [9].
5) Synthetic underwear and pantyhose, tight jeans, wet bathing suits, and Allergens or irritants perfumed on toilet paper, etc.

Treatment:

The Empirical therapy of suspected UTI is started ultimately before the identification and diagnosis of uropathogens like bacteria and parasites. The best option for complete UTI treatment depends upon the clinical status and age of the patients linked with the predominant uropathogens, and choosing a suitable antibiotic against the isolate is found to be sensitive. Somehow the uncomplicated and generally suspected young individuals might be treated with

oral antibiotics on a daily basis with continuous follow-up [10]. The antibiotic treatment should be started simultaneously after obtaining a proper urine culture.

Some antibiotics that are resistant to *E. coli*, such as amoxicillin, trimethoprim, sulfamethoxazole, nitrofurantoin, and cephalosporin [11]. The primary goal of this research was to find out how common infections that cause UTIs during the COVID-19 pandemic were among the important populations in the districts of Swabi and Mardan. The present study also focused on the prevalence of antibiotic resistance among the pathogens isolated throughout this study.

MATERIALS AND METHODS

Study Demographics

The current study was conducted at various hospitals and laboratories of the Mardan Division during the COVID-19 pandemic in collaboration with Women University Swabi, Khyber Pakhtunkhwa, Pakistan.

Sample Collection and Processing

A total of 531 patients suffering from urinary tract infections who visited hospital medical and surgical OPD and ICU admitted with UTI infection complaints were considered in the study. The sample of urine from the infected individuals was collected in an open-mouthed urine bottle and transferred to the laboratory for further processing and identification of aerobic microorganisms as used by [12].

According to protocol, clean catch mid-stream tool samples were collected, and the samples of urine were gathered in sterilized tubes. from (4-5 ml) and were transported to the laboratory and stored in the cold box for further processing [13]Each participating study subject was asked to sign a written informed consent questionnaire, and the study was also granted approval from the institutional review board (IRB), Women University Swabi, Khyber Pakhtunkhwa, Pakistan.

Physical and Routine Examination of Urine:

Initially, the physical characteristics of all the samples, like color, turbidity, pH, and specific gravity, were observed and noted properly. Urine ranged from pale yellow (normal) to deep Amber (an indicator of disease status) and red in some cases, which is due to a pigment called urochrome 5 mL of each urine (tubular centrifuge made in Liayang China) at 5000 rpm for at least 5 minutes. Supernatants from all the samples were discarded, and the remaining pallets were stored. A drop of the sample was then examined under the microscope through glass slide preparation using high and low-power objective lenses (40x and 100x).

Urine culture and sensitivity analysis:

The bacteria or yeast that are causing a urinary tract infection can be found with a urine culture test (UTI). An antibiotic sensitivity test can determine which antibiotic is most likely to eradicate a particular strain of bacteria if they start to multiply. A urine culture test can also identify the bacteria or yeast causing the infection, enabling your healthcare professional to choose the best course of action and ascertain whether the bacteria is antibiotic-resistant. A urine sample is necessary for both a urine culture and urinalysis. Urinalysis may be performed initially by your healthcare provider. This speedier test checks urine for germs, red and white blood cells, and other elements that can point to an infection. The precise bacteria that cause a UTI cannot be determined by a urinalysis. You need a urine culture to find out that information.

RESULTS

A total of 531 samples (n=265 from Mardan, n=266 from Swabi) having UTI symptoms were included in the study. Out of 117/531 (22.03%) (n=50/531 (18.9%) in District Mardan, n=67/266 (25.2%) in District Swabi) shows growth in the culture medium. Positive samples were divided gender-wise and age-wise. In the gender-wise distribution of UTIs, UTI is a lifetime illness that can affect both sexes and children of any age. It is neither age- nor gender-based. However, females are more likely to be infected than males [1, 2]. Gender-specific distributions reveal that a greater proportion of samples (64.9%) are from the female population, as shown in Table No.1.

Table 1. Distribution of UTI

Gender	Total	Positive	P Value
Male	195	41 (35.1%)	0.12
Female	336	76 (64.9%)	0.04

Age-Based UTI Distribution:

Females between the ages of 23 and 60 have the highest rate of UTIs due to their short urethra and close proximity to the anus, which is mostly infected by fecal bacteria. Pregnant women are more likely than other females to contract an infection because of increased urine stasis and ureterovesical reflux caused by urethral tone. Most bacterial infections that affect pregnant women are asymptomatic. The urethra is less contaminated in the male population, prostatic secretions have a strong bactericidal effect, and the immune system provides an excellent line of defense against bacterial infection. UTI is also very common in children under the age of three because they have not yet developed a specific immunity. The age-wise distribution shows that age group 3 (36-50 years) has the highest number of infected individuals, 53 (45.3%), followed by age group 2 (21-35 years), 34 (29.1%), as shown in Table 2.

Table 2. Age-wise Distribution of UTI

Age groups (years)	Positive	P Value
Age group 1 (5-20)	9 (7.7%)	0.17
Age group 2 (21-35)	34 (29.1%)	0.04
Age group 3 (36-50)	53 (45.3%)	0.02
Age group 4 (>50 years)	21 (17.9%)	0.05

Collection of Uropathogens:

E. coli represented 62.4% of the 117 isolates that were isolated, with Klebsiella *spp.* following closely after.

Culture Sensitivity Analysis:

All the positive samples were tested for antibiotic susceptibility by using a variety of antibiotics. Antibiotic susceptibility shows that *E. coli* was resistant to Gentamicin (83.5%), followed by Ciprofloxacin (79.5%). However, it shows the highest sensitivity against Cefoxitin (71.2%), followed by Cefuroxime (63.1%). Klebsiella *spp.* were the second most prevalent microbe that was sensitive to Amikacin (76.9%) and Ampicillin (69.2%) while showing resistance to Ciprofloxacin (76.9%), Levofloxacin (76.9%) and Amoxicillin (69.2%). The 3rd most prevalent microbe was Pseudomonas showing resistivity against Meropenem and linezolid (77.8%), followed by Cefepime (66.7%), while it shows sensitivity by Ceftriaxone (77.8%) and Ciprofloxacin (66.7%). Enterococci was susceptible to ciprofloxacin (87.5%), and Meropenem (75%). This microbe was resistant to Gentamicin (87.5%) and Amikacin

(75%). Meropenem and Amikacin (71.4%) and Ampicillin (57.2%) show resistance in Enterobacter, while Norfloxacin (71.4%) and Ciprofloxacin (57.2%) are sensitive. Piperacillin and Ampicillin (75%) were sensitive, while Gentamicin and Meropenem (75%) were resistant. S. proteus shows equal resistance (66.7%) to Imipenem, Linezolid, and Levofloxacin and is sensitive to Meropenem and Ciprofloxacin (66.7%). A detail of antibiotic susceptibility is shown in Table 3 below. (11.1%) and 7.7% of Pseudomonas. Details of the isolates are shown in Table 3.

Table 3. Isolated microbes in both male and female populations

Microbes	**Positive (%)**	**Male**	**Female**
E. coli	73 (62.4)	26 (22.2%)	47 (40.2%)
Klebsiella spp.	13 (11.1)	5 (4.3%)	8 (6.8%)
Pseudomonas	9 (7.7)	3 (2.6%)	6 (5.1%)
Enterococci	8 (6.8)	4 (3.4%)	4 (3.4%)
Enterobacter	7 (6)	2 (1.7%)	5 (4.3%)
S. aureus	4 (3.4)	1 (0.8%)	3 (2.6%)
S. proteus	3 (2.6)	0	3 (2.6%)

DISCUSSION

Urinary tract infection is commonly an infection caused by bacteria that occurs when bacteria invade and multiply within the urinary tract. UTI prevalence is reported in pediatric healthcare and in pregnant women more frequently than in the general population. The patients with a good prognosis have the highest complications and morbidity due to UTI [14]. The UTI varies from asymptomatic to severe infections. In infants and children up to years of age, UTI is the main cause of morbidity, followed by mortality [15]. The global rate of UTI infection reaches 150 million cases per year. In most cases, if bacteria are left untreated, then they reach the kidney and multiply over there, causing severe kidney infection. The female population is more infected as they have shorter urethra than the male [16]. Most pathogenic bacteria are nonpathogenic, as they are in the normal microflora of the human body. But when these bacteria move to the urinary tract, they become pathogenic due to environmental changes and multiply, followed by infection. Some of the bacteria reside in an alkaline environment, and the rest are found in alkaline urine.

The age-wise distribution shows that age group 3 (36-50 years) is more infected (45.3%) than the other ages. Similar observations are recorded by [17, 18]. This difference might be due to the competent immune system and high treatment-seeking behavior at a young age [17]. These findings contradict the finding of Alemu et al., where the majority of the gram-positive isolates, including enterococci and *S. aureus*, showed higher rates of resistance to gentamicin and ciprofloxacin, which are similar to the observation by [19]. The elevated prevalence of MDR reported in the current study might be due to the unobstructed accessibility and high ratio of using non-prescribed drugs. This could also be related to the misuse of antimicrobial drugs like self-medication and the high use of antibiotics in agriculture and farming. The rapid spread of resistant bacteria and their resistance genes, unnecessary use, failure to adhere to standard treatment guidelines, and inadequate or absence of antimicrobial drug resistance surveillance programs are also among the contributing factors responsible for the high prevalence of antibiotic resistance [20]. Most commonly isolated organisms isolated from patients having UTIs are Gram-negative bacteria for which Aminoglycosides, the broad-spectrum antibiotics, show high potency, and that's why they are traditionally used for the treatment of serious gram-negative urinary tract infections [21].

CONCLUSION

The current study concludes that urinary tract infections (UTIs) are a severe multi-pathogenic bacterial issue that is prevalent in Pakistan's Khyber Pakhtunkhwa region's northern part. The study was conducted to elucidate the rate of prevalence and antibiotic resistance frequency during the COVID-19 pandemic. The uncontrolled and unprescribed use of antibiotics is responsible for higher bacterial resistance. The majority of the uropathogenic bacterial isolates acquire antimicrobial resistance genes, leading to devastating situations for control of antibiotic resistance for future use. The current study also reported the prevalence of Multi-Drug Resistant (MDR) isolates, which need further investigations on a mass scale.

Data Availability Statement

All relevant data are within the paper and its Supporting Information files.

Conflict of interest/ Competing interests

The authors declare that they have no conflict of interest. The authors have no relevant financial or non-financial interests to disclose. The authors have no competing interests to declare relevant to this article's content.

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Ethics approval

The study was approved by the ethical committee and institutional research board (IRB) at Women's University Swabi.

Consent to participate

All participant of the study were willing to participate in the study and confirmation was taken via written consent form.

Consent for publication

A written informed consent was taken from all the participant of the study and all shows willingness for publication of their data.

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