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Prevalence of UTI Causing Pathogens among different Subgroups of the Local Population, Pakistan

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Abstract

The Urinary Tract Infection (UTI) is one of the most frequent and contagious infection, caused by range of pathogens, affecting an estimated 150 million people around the globe every year. The current study has investigated the prevalence of UTI among the local population of Pakistan. The urine samples of 200 patients were collected based on different UTI related symptoms and the infection was detected through quantitative urine culture and microscopic enumeration of urine leukocytes. Then UTI causing pathogens were isolated from positive samples and their sensitivity against antibiotics was checked through antimicrobial susceptibility method. The E.coli was the most predominant uropathogen found among all the positive samples followed by Klebsiella, Staphylococcus, Pseudomonas, Acinetobacter, Proteus species, and Enterococcus. The comparative analysis of UTI between both males and females showed that UTI is highly prevalent among female patients as compared to males especially in adult age ranges from 21-30 years due to hormonal changes and pregnancy. All the isolated pathogens were differentially sensitive against conventional antibiotics but they showed highest sensitivity against imipenem while highest resistivity against ampicillin. This study has also shown that the excessive use of antibiotics leads to the drug resistance and increases the chance of any bacterial infection.

KEYWORDS
Urinary tract infection (UTI), Uropathogens, Antibiotic susceptibility

INTRODUCTION

The urinary tract infection (UTI) can develop in any part of urinary tract posing a growing global issue due to their increasing prevalence in every region of the world [1,2]. Adult females are more susceptible as compared to the adult males, primarily due to anatomical differences and large bacterial load in the genitourinary tract [3,4]. Based on their symptoms, UTI categorized into two main types i.e. uncomplicated and complicated [5].

Uncomplicated UTIs typically occur in individuals who are otherwise healthy and lack significant structural abnormalities in their urinary tract [6]. In contrast, complicated UTIs are often associated with factors comprising urinary tract health, such as urinary obstruction, neurological disease-related urinary retention, renal failure, immunosuppression, and renal transplantation [7]. Uncomplicated UTIs

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can further be categorized by the affected region of the urinary tract: lower UTIs, known as cystitis [8], and upper UTIs, known as pyelonephritis [9]. While most cases are not inherently dangerous, kidney involvement in pyelonephritis carries a high risk of permanent tissue damage and bacteremia [10,11].

Bacteria are the primary cause of UTIs, accounting for over 95% of cases. Among them, *E. coli* is the most common frequent infecting agent. While bacteria naturally live and colonize around the urethral opening in both genders, they are typically flushed out during micturition[12]. Most infections occur when bacteria from the fecal flora ascend back to bladder and kidneys. Numerous risk factors increase susceptibility to UTIs, including gender, sexual activity, previous UTIs, diabetes, vaginal infections, genetic predisposition, and obesity [13]. The widespread concern with UTIs stems from their microbial origins and the emergence of drug-resistant strains due to antibiotic overuse. Catheters are another significant source of urinary tract infections, contributing to bacteriuria. Hospitalized patients with catheters face a 5% daily risk of developing a catheter-associated UTI (CAUTI) [14].

Some children experience recurring UTIs, defined as multiple uncomplicated infections within six months of the first one. Around 25% women with a history of UTI fall into this category [15]. Untreated recurrent UTIs can lead to serious complications and even pose a mortality risk. This type of UTI is often classified as a relapse. Differentiating between relapse and reinfection based solely on symptoms can be challenging. Relapses occur when the same microorganism responsible for the initial infection persists in the urine. Conversely, reinfections involve a new bacterial strain invading the urinary tract [16].

Antibiotics are the mainstay of treatment for UTIs, but overuse and misuse can lead to antibiotic resistance in the responsible microorganisms. One study conducted at the University Hospital of Campania "Luigi Vanvitelli" investigated this phenomenon by analyzing urine samples from 1745 patients. It found that *E. coli* was the most frequently isolated pathogen and demonstrated the highest resistant to ampicillin [17]. Another study, this time in UK (United Kingdom), examined the frequency of UTIs across different age groups and identified potential inefficiencies in primary care as a contributing factor [18]. The aim of current study is to investigate the leading causes of UTIs, their prevalence, and the antibiotics resistance patterns of causative agents among the local population. We will analyze infection rates across different age groups and genders.

MATERIALS AND METHODS

Urine samples were collected from 200 patients presenting with symptoms suggestive of urinary tract infection (UTI). These symptoms include urinary frequency, back pain, burning sensation, supra pubic pain, irritation during urination, fever and sudden urge to urinate. The participants were categorized into seven age groups: <10 years (7 males, 9 females), 10-20 years (6 males, 7 females), 21-30 years (10 males, 47 females), 31-40 years (7 males, 22 females), 41-50 years (11 males, 10 females), 51-60 years (11 males, 18 females), >60 years (21 males, 14 females). Detailed information on their sex, age, symptoms, laboratory diagnosis and antimicrobial susceptibility was collected through medical questionnaire. The comparative analysis of UTI infection was performed between

different age groups and gender among the local population of Pakistan.

Sample Collection and Urine Culture

Urine samples were collected aseptically at midstream in 100 mL sterile containers, labelled with patient's name, age, and gender. A quantitative urine culture determined the presence of UTI. Briefly, we prepared nutrient agar, poured it onto petri plates, and streaked 10 μL of each urine sample onto the agar plates. Following incubation at 37 °C for 18-24 hours, colonies were examined according to established guidelines. Positive UTI diagnosis was based on a colony forming unit (CFU), a cutoff point of 10^5 CFU per mL [19] was taken into account.

Isolation and Examination of Pathogens

UTI-causing pathogens were isolated from the urine samples by using 3.62% cysteine lactose-electrolyte-deficient media plates [20]. Ten microliters of urine were then spread onto the plates and incubated at 35 °C ± 1°C for 24 hours. Distinct colony characteristics, including colony size, color, and shape, helped in initial organism identification. Following observation of growth and preliminary identification based on colony morphology, standard biochemical tests, including sugar fermentation, urease, methyl red test, indole production, citrate utilization and coagulase, were performed for definitive microbial identification.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. The inoculum of isolated pathogens from urine samples was prepared and spread onto Mueller Hinton (MH) II agar plates using sterile swabs. Then sterile swab was used to streak the inoculum over the agar plates and incubated the plates for at least 5 minutes for equal distribution of inoculum over the plates. Then antibiotic discs were containing Norfloxacin (NOR), Amikacin (AK), Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Erythromycin (E), Imipenem (IPM), Fosfomycin (FF), Urixin (PI), Gentamicin (CN), and Nitrofurantoin (F) were applied using sterile forceps. Plates were incubated at 35 °C ± 2 for 18 hours. After incubation, the clear zones of inhibition around the disc were measured in order to determine the level of antibiotic resistance in pathogens.

Statistical Analysis

The significance of results was determined through ANOVA. The study was performed in triplicates for the proper findings. The resistance or susceptibility of the organism were assessed to each drug and recorded in the form of different charts with p-value <0.05. For each drug, recording sheets were used for indications whether the size of zone is intermediate sensitive (IS), sensitive (S) or resistant (R).

RESULTS

Comparative Analysis among Different Age Groups and Gender

Among females, UTI prevalence peaked in the 21-30 age group, followed by a gradual decline in older age groups (31-40, 41-50, 51-60, >60). Interestingly, the lowest prevalence was observed in the youngest (<10 years) and teenage (10-20 years) groups (Figure 1).

Males, in contrast, displayed a relatively steady increase in UTI with age, reaching the highest rates in the >60 age group. Lower prevalence was observed in younger groups, with the lowest occurring in the youngest (<10 years) and teenage (10-20 years) groups (Figure 1). Notably, statistical analysis revealed no significant difference between male UTI prevalence across age groups, as indicated by the "ns" symbol in Figure 1.

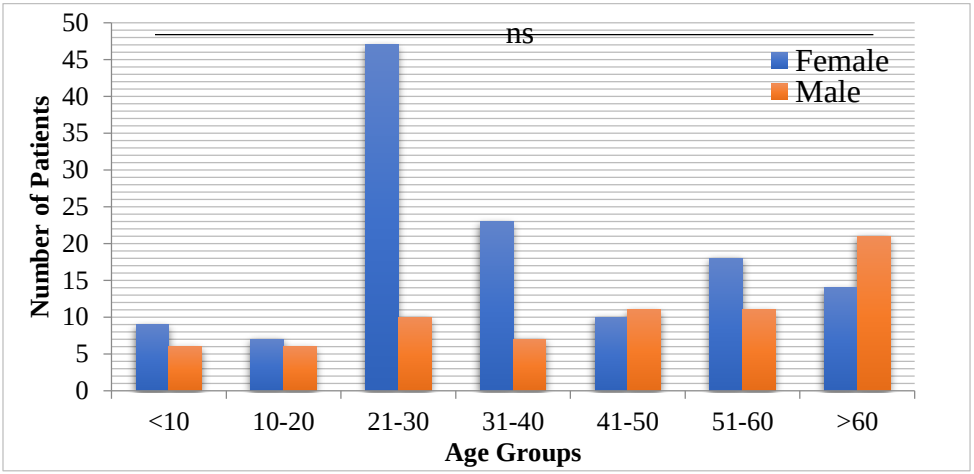

Figure 1. Prevalence of UTI among the different age groups of both male and female ($p < 0.05$)

Comparative Prevalence of Bacterial Species among UTI Patients

Various microorganisms may be present in the urine samples, their proportions often differ significantly. This study analyzed the prevalence of UTI causing bacteria among participating patients. The *E. coli* emerged as the most predominant pathogen, accounting for 56% of isolates, followed by *Klebsiella* (19%) and *Staphylococcus* (12%). On the other hand, the lower prevalence was observed for *Pseudomonas* (5%), *Acinetobacter* (4%) and *Proteus species* (3%), and *Enterococcus* (1%).

Interestingly, all identified pathogens, except *Pseudomonas*, displayed a higher prevalence in female patients compared to males. The comparative prevalence of these UTI pathogens, highlighting the dominance of *E.coli*,*Klebsiella* and *Staphylococcus* and the substantially lower prevalence of *Pseudomonas*, *Acinetobacter* and *Proteus species* (Figure 2).

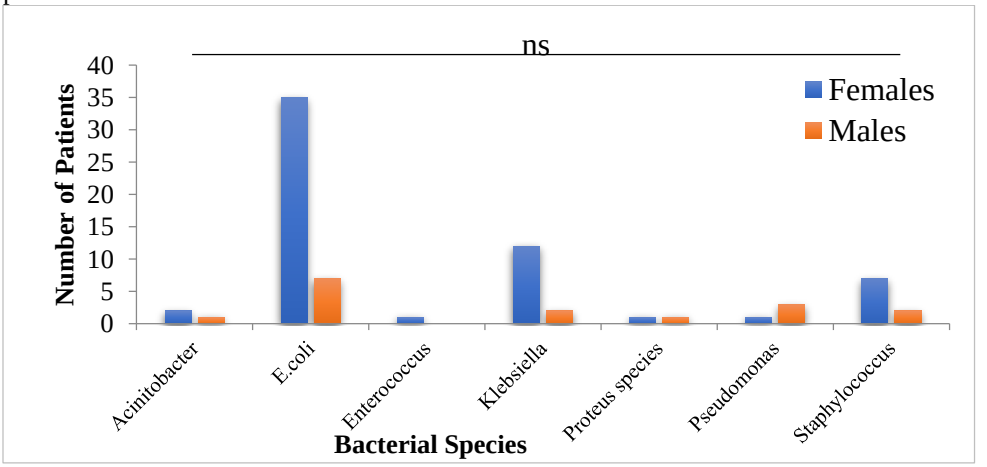

Figure 2. Comparative prevalence of different UTI causing pathogens between males and females ($p < 0.05$)

Table 1. Antibiotic susceptibility of bacterial isolated from UTI patients ($p < 0.05$). Norfloxacin (NOR), Amikacin (AK), Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Erythromycin (E), Imipenem (IPM), Fosfomycin (FF), Urixin (PI), Gentamicin (CN), and Nitrofurantoin (F)

Antibiotics		Bacterial Isolates (%, n)						
		Acinetobacter	*E. coli*	*Enterococcus*	*Klebsiella*	*Proteus Species*	*Pseudomonas*	*Staphylococcus*
NOR	R	33.3%±0.01	77.2%±0.02	100%±0.01	81.8%±0.01	0%±0.01	75%±0.04	71.4%±0.03
	S	33.3%±0.04	13.6%±0.04	0%±0.01	9.0%±0.02	50%±0.02	25%±0.03	0%±0.01
	IS	33.3%±0.01	9.0%±0.03	0%±0.01	9.9%±0.01	50%±0.04	0%±0.04	28.5%±0.03
AMP	R	100%±0.03	84.3%±0.04	100%±0.02	91.6%±0.04	100%±0.03	100%±0.02	75%±0.02
	S	0%±0.01	9.3%±0.03	0%±0.01	0%±0.01	0%±0.01	0%±0.02	0%±0.01
	IS	0%±0.02	6.2%±0.04	0%±0.01	8.3%±0.04	0%±0.01	0%±0.01	25%±0.03
AK	R	33.3%±0.02	0%±0.01	0%±0.01	14.2%±0.02	50%±0.02	0%±0.01	0%±0.01
	S	66.6%±0.01	83.7%±0.01	100%±0.02	71.4%±0.02	50%±0.04	50%±0.04	66.6%±0.03
	IS	0%±0.02	16.2%±0.01	0%±0.01	14.2%±0.02	0%±0.01	50%±0.02	33.3%±0.03
CRO	R	66.6%±0.04	65.8%±0.01	0%±0.01	42.8%±0.01	50%±0.04	75%±0.03	71.2%±0.04
	S	33.3%±0.01	30.7%±0.02	0%±0.01	28.5%±0.04	0%±0.01	25%±0.03	14.2%±0.03
	IS	0%±0.03	3.8%±0.03	100%±0.02	28.5%±0.03	50%±0.04	0%±0.02	14.2%±0.02
CIP	R	33.3%±0.02	83.3%±0.02	100%±0.03	83.3%±0.03	50%±0.03	66.6%±0.04	100%±0.01
	S	33.3%±0.01	5.5%±0.03	0%±0.01	8.3%±0.02	0%±0.01	33.3%±0.01	0%±0.01
	IS	33.3%±0.01	11.1%±0.04	0%±0.01	8.3%±0.04	50%±0.02	0%±0.01	0%±0.01
E	R	100%±0.04	93.3%±0.01	0% ±0.01	78.5%±0.01	0%±0.01	66.6%±0.03	85.7%±0.01
	S	0%±0.03	0%±0.01	100%±0.03	7.1%±0.04	100%±0.01	0%±0.01	14.9%±0.02
	IS	0%±0.01	6.6%±0.01	0%±0.03	14.9%±0.03	0%±0.01	33.3%±0.03	0%±0.01
CN	R	33.3%±0.04	28.5%±0.03	0%±0.01	30.77%±0.04	0%±0.01	25%±0.02	22.2%±0.02
	S	66.6%±0.03	50%±0.01	100%±0.01	30.7%±0.04	50%±0.04	50%±0.04	33.3%±0.03
	IS	0%±0.01	21.4%±0.02	0%±0.01	38.6%±0.02	50%±0.04	25%±0.02	44.5%±0.03
IPM	R	0%±0.01	0%±0.01	0%±0.03	0%±0.01	0%±0.01	0%±0.01	0%±0.01
	S	100%±0.02	100%±0.01	100%±0.02	100%±0.01	100%±0.01	100%±0.01	87.5%±0.03
	IS	0%±0.01	0%±0.01	0%±0.02	0%±0.01	0%±0.01	0%±0.01	12.5%±0.04
F	R	33.3%±0.03	0%±0.01	100%±0.03	27.7%±0.01	0%±0.01	33.3%±0.01	16.7%±0.01
	S	0%±0.01	84.2%±0.03	0%±0.02	45.6%±0.01	50%±0.02	33.3%±0.04	83.3%±0.03
	IS	66.6%±0.03	15.7%±0.01	0%±0.01	27.7%±0.01	50%±0.04	33.3%±0.03	0%±0.01
FF	R	0%±0.01	15.7%±0.03	100%±0.01	45.5%±0.04	0%±0.01	33.3%±0.02	16.6%±0.02
	S	66.6%±0.04	84.2%±0.01	0%±0.04	45.5%±0.04	100%±0.01	66.6%±0.04	50%±0.03
	IS	33.3%±0.03	0%±0.01	0%±0.02	9.1%±0.03	0%±0.02	0%±0.01	33.3%±0.04
PI	R	33.3%±0.03	91.6%±0.02	0%±0.01	60%±0.02	100%±0.01	50%±0.04	100%±0.02
	S	33.3%±0.02	8.3%±0.04	0%±0.01	10%±0.03	0%±0.01	0%±0.01	0%±0.01
	IS	33.3%±0.04	0%±0.01	100%±0.02	30%±0.02	0%±0.01	50%±0.02	0%±0.01

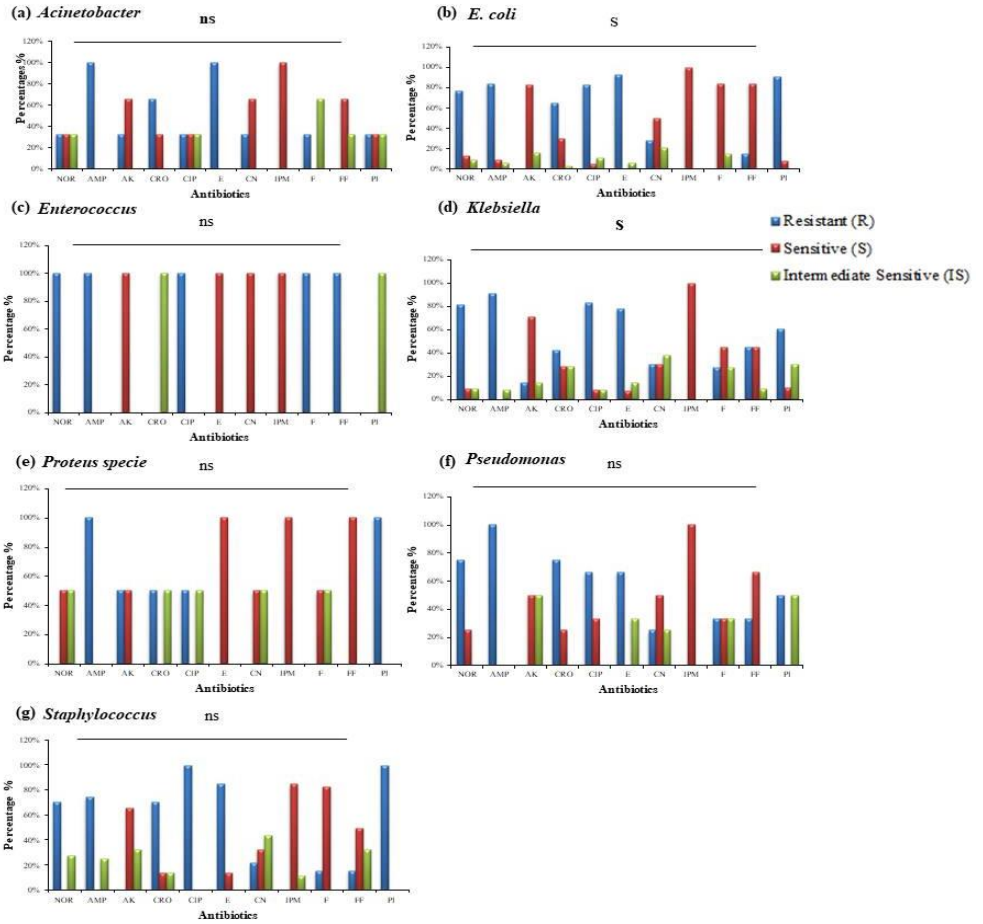

Figure 3. Graphical representation of antibiotic susceptibility of isolated UTI causing pathogens. (a) *Acinetobacter* (b) *E. coli* (c) *Enterococcus* (d) *Klebsiella* (e) *Proteus species* (f) *Pseudomonas* (g) *Staphylococcus*, blue bars represents the percentage of drug-resistive pathogens, red shows drug-sensitive pathogen, green shows intermediate sensitive microorganisms

Antibiotic Susceptibility

The emergence of drug resistance among urinary isolates against commonly used antibiotics was observed as shown in Figure 3. The imipenem showed highest antimicrobial activity against all urinary isolates while ampicillin showed least sensitivity against microorganisms and almost all pathogens were resistant to ampicillin. In this study, *E.coli*, *Klebsiella*, *Enterococcus*, *Acinetobacter*, *Proteus species*, and *Staphylococcus* showed the highest sensitivity (100%) to imipenem. The *Staphylococcus* was 87.5% sensitive to imipenem. These microorganisms showed very less or approximately no sensitivity against other antibiotics i.e. ampicillin, ciproxin and norfloxacin. The other antibiotics showed variety of results with different microorganisms. The *Acinetobacter* showed 33.3% resistivity against norfloxacin, amikacin,

ciprofloxacin, gentamicin, nitrofurantoin, and urixin, 100% to ampicillin and erythromycin, 66.6% to ceftriaxone. The *E. coli* exhibited high resistant to erythromycin (93%), urixin (91%), ampicillin (84.3%), ciprofloxacin (83.3%), norfloxacin (77.27%), ceftriaxone (65.3%), and gentamicin (28.57%). Similarly, *Enterococcus* displayed complete resistance (100%) to several antibiotics (norfloxacin, ciprofloxacin, ampicillin, nitrofurantoin, and fosfomycin) while remaining entirely susceptible to amikacin, erythromycin, and gentamicin. *Klebsiella* resistant varied across several antibiotics: ampicillin (91.67%), ciprofloxacin (83%), norfloxacin (81.82%), erythromycin (78.5%), urixin (60%), fosfomycin (45%), ceftriaxone (42%), gentamicin (30.7%), nitrofurantoin (27.2%), and amikacin (14%). *Proteus* species were entirely resistant to ampicillin and urixin, with 50% resistance to amikacin, ceftriaxone, and ciprofloxacin. *Pseudomonas* exhibited the diverse resistance profile, showing varying degrees of resistance against different antibiotics. Notably, *Staphylococcus* demonstrated high resistance to ciprofloxacin and urixin (100%), and significant resistance to erythromycin (85%), norfloxacin (71-75%), ampicillin, ceftriaxone. Notably, gentamicin, nitrofurantoin, and fosfomycin showed lower resistance rates in *S. aureus* (Table 1). These findings strongly suggest that frequent antibiotic use diminishes their effectiveness against pathogenic microorganisms.

DISCUSSION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections globally, affecting individuals of all ages. However, females are significantly more susceptible than males, particularly during adulthood. The nature of UTIs can vary depending on the causative pathogen, manifesting as either complicated or uncomplicated, symptomatic or asymptomatic [22]. This study sought to investigate the leading causes of urinary tract infections, acknowledging the diverse range of microorganisms capable of infecting the urinary tract. Notably, numerous studies across various regions have identified *E. coli* as the predominant culprit.

This study has identified the major causative agent among the population based on different age groups and genders. The urine samples of 200 patients were collected and examined through different techniques. The prevalence of UTI was found to be higher in females. The positive samples were analyzed on the basis of age, gender, and pathogens. Based on these results, a comparative analysis was conducted, followed by antimicrobial susceptibility testing on the isolated pathogens. The results of this study revealed the higher proportion of UTIs in females, particularly within the age group 21-30. This may be attributed to several factors, including hormonal change, sexual activity and pregnancy. Notably, the prevalence of UTIs dropped in older females, with the lowest frequency observed in the 10-20 age group. Generally, the chances of UTI increase with age and sexual activity in females. While, 60 above age group i showed more positive results. This aligns with the observation that 59 out of 75 patients diagnosed with UTI were females, likely due to increased availability of femle patients with UTI symptoms. Anatomical factors also play a role, with the shorter urethra in females, along with its proximity to the genitourinary tract, can facilitate bacterial entry during intercourse and pregnancy. Additionally, the shorter urethra and its nearness to the anus increase

the risk of bacterial ascension into the urinary tract [23]. During pregnancy, hormonal changes can further elevate the risks of pyelonephritis, particularly towards the end of second trimester and beginning of the third [24].

In our study population, *E.coli* was the most predominant uropathogen, because it was resistant to many antibiotics ranges from 4% to 90%. The *E.coli* has destructive components, for example, the sort of fimbria which elevates imposing to the epithelium of vagina and urethra and upgrades the capacity to bring about cystitis [25]. The different variables enhance rigidity to host defense mechanism and serum bactericidal action. An experiment conducted in Kohat Teaching Hospital of Kohat district, Khyber Pakhtunkhwa, Pakistan. They collected urine samples of 500 patients including both males and females and investigated the prevalence of UTI among them. The proportion was high among females as compared to males. The reported results have shown the prevalence rate of 11.6% with frequency of UTI 8.9% in males and 13.8% in females. The most common isolated pathogen was *E. coli, Klebsiella pneumoniae,* and *Proteus species* [26]. This study has shown that the *E. coli* can be a major causative agent of UTI.

A study conducted in Lahore, Pakistan, and investigated the prevalence of UTI among pregnant women. They found a high rate infection, with 65 out of 80 (81%) diagnosed with UTI. pregnant women, had urinary tract infection which reflects the prevalence of UTIs. Notably, Tthe majority of these patients were aged 24-35 years and in their third trimester. The most common isolated uropathogen was *E. coli* followed by *Klebsiella, Pseudomonas, Streptococcus, Staphylococcus, Enterococcus* and *Proteus species* [27]. This further support the evidence of *E. coli* as a leading cause of UTIs in our study population. While the study provides valuable insights into local UTI patterns, it also has limitations. Bacterial species identified relied solely on colony characteristics like size, shape and color which may no always be definitive. Implementing techniques like 16srRNA sequencing in future studies could offer more accurate species-level identification and contribute to a deeper understanding of local UTI etiology and antibiotic resistance profiles.

CONCLUSION

The UTIs are among the most widespread bacterial infections, which affecting either the upper or lower sections of urinary tract. This study, after statistical analysis, concludes that women, face a significantly higher risk of UTIs compared to men. In females, hormonal changes play a crucial role in age-related UTI occurrence. Among the uropathogens tested for susceptibility, *E.Coli* emerged as the most prevalent causative agent in both genders. Other isolated etiological agents included *Klebsiella, Staphylococcus*, *Pseudomonas, Acinetobacter*, *Proteus specie* and *Enterococcus*. Notably, while the majority of tested antibiotics displayed limited effectiveness, Imipenem demonstrated high sensitivity against several uropathogens, followed by Amikacin. These study suggest that Imipenem could be a suitable antibiotic for UTI treatment. It exhibited the highest sensitive not only in *E.Coli* but also in other uropathogens.

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