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ORIGINAL RESEARCH

A Cross-Sectional Study to Evaluate Antimicrobial Susceptibility of Uropathogens from South Punjab, Pakistan

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Background: Urinary tract infections (UTIs) are a common infection caused by uropathogenic bacteria. Drug resistance against common antibiotics is a leading cause of treatment failure in UTIs.

Objective: This study was conducted to check the prevalence of antimicrobial susceptibility against uropathogens and identify the best treatment option against UTIs.

Methods: In this cross-sectional study, urine samples (n = 1000) were collected and cultured for pure bacterial growth by using cysteine–lactose–electrolyte-deficient (CLED) media. After physical and biochemical characterization, antibacterial susceptibility was performed by the Kirby–Bauer disk diffusion method.

Results: Uropathogenic bacteria were successfully isolated in 57% (n = 572) of total tested samples (n = 1000). *Escherichia coli* 51.2% (n = 293/572), *Klebsiella* species 15.4% (n = 88/572), *Enterococcus* species 15.4% (n = 88/572), *Pseudomonas* species 9.4% (n = 54/572), *Staphylococcus aureus* 3.2% (n = 18/572), coagulase-negative *Staphylococci* (CoNS) 3.0% (n = 17/572) and *Proteus* species 2.4% (n = 14/572) were the most prevalent organism in UTIs. Prevalence of Gram-negative rods (GNRs) was 78.5% (n = 449/572) among UTI patients as compared to Gram-positive cocci (GPCs) 21.5% (n = 123/572). *Escherichia coli* 65.3% (n = 293/449), *Klebsiella* species 19.6% (n = 88/449), *Pseudomonas* species 12.0% (54/449) and *Proteus* species 3.1% (n = 14/449) were the most prevalent GNRs in UTIs, while *Enterococcus* species 71.5% (n = 88/123), *Staphylococcus aureus* 14.6% (n = 18/123) and coagulase-negative *Staphylococci* (CoNS) 13.8% (17/123) were the most prevalent GPCs in UTIs. The majority of isolated uropathogens showed resistance against routinely used antibiotics. However, teicoplanin and linezolid were the most effective drugs against GPCs and piperacillin/tazobactam, meropenem and imipenem were the most effective drugs against GNRs. Nitrofurantoin and fosfomycin were shown to be most effective against both GNRs and GPCs.

Conclusion: In conclusion, *Escherichia coli* (GNRs) and *Enterococcus* species (GPCs) are the most prevalent organisms among UTIs patients, which are shown to be antibiotic-resistant to the most commonly used antibiotics. However, nitrofurantoin and fosfomycin are the most effective drugs against uropathogens in UTIs.

Keywords: uropathogens, UTIs, biochemical analysis, antibacterial drugs

Introduction

Urinary tract infections (UTIs) are the most common community or hospital-acquired infection caused by bacteria, which affects 150 million people worldwide every year.[1,2] Normally, all ages and populations can be affected by UTIs. However, certain factors including age, gender, genetic factor, race, and sexual activity are risk factors for UTIs.[3] Females are more at risk for UTIs due to their anatomical positions of the urethra, so almost 35% of females suffer from symptomatic UTIs in their lifespan.[4,5] In addition, vaginal normal flora, pregnancy, sexual intercourse, and obstruction

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of the urethra may increase the risk for UTIs in females.[6] UTIs can be either symptomatic or asymptomatic. However, symptomatic UTIs have a high threshold for bacteriuria (100,000 CFU/mL urine) and sites of infections characterized UTIs into pyelonephritis, cystitis, urethritis, or prostatitis.[7,8] UTIs are mainly caused by enteric microflora, for example, *Escherichia coli* (*E. coli*) is the most prevalent cause of UTIs (75–85%).[7,9] *Enterobacteriaceae, Pseudomonas* species, *Enterococcus* species, *Staphylococcus aureus* (*S. aureus*), and Coagulase Negative *Staphylococci* (CoNS) are also associated with UTIs.[10] Mostly, a single bacterial species is responsible for UTI rather than more bacterial strains or species.[11] Excessive use of antibiotics, false diagnosis, deficiency of productive research, lack of awareness, and self-medication may cause bacterial resistance due to the development of new genetic variants, resulting from treatment failure either in developed or under developing countries and leading to increased morbidity.[12,13] In uropathogenic bacteria, the abundance of virulence genes is coding to different virulence factors, eg FimH: adherence factor produced by uropathogenic *Escherichia coli* (UPEC)[14] and associated underlying mechanisms making it difficult to treat these infectious diseases.[15,16] Bacteria-harboring genetic variants, eg *hlyA, Utah, cnf1, ibeA* and *cdtB* in UPEC leading to antibiotic resistance have been increased remarkably and globally become a serious challenge for medical treatment.[17,18] Extended-spectrum β-lactamases (ESBLs), *Klebsiella pneumoniae* carbapenemase (KPC)[19] and Metallo-β-lactamases are produced by corresponding genes present in the uropathogenic bacterial chromosome that contribute to antibiotic resistance.[20] It usually occurs in a normal health care setting in which antibiotics are administered without antibiotic susceptibility testing. In Pakistan, this is a leading risk factor in the development of multidrug-resistant bacteria in UTIs.

It is an alarming condition for health care to overcome multidrug resistance bacteria associated with bacterial infections including UTIs.[21] It has become important to get information about the antibacterial resistance and sensitivity to overcome this challenge.[22] However, overall epidemiological information on the incidence of UTIs and antibiotics susceptibility against these pathogens was lacking in South Punjab, a province of 5 million people. Six hundred patients out of thousand patients enrolled in 4 months at MIKD with chronic kidney disease (CKD) were also suffering from UTIs, indicating a very high incidence and prevalence of UTIs likely due to multidrug-resistant bacteria and lack of availability of effective drugs.

Continuous surveillance is required on the use of antibiotics, drug resistance, and susceptibility in UTIs to control the antibiotic resistance and to find the most susceptible drugs against uropathogens.[21] We should also need to identify the mutant variants leading to antibiotic resistance by using molecular techniques.[23] In the most recent study conducted in Pakistan in a far province, *Escherichia coli* was found to be the most prevalent isolates, and fosfomycin and imipenem were the most susceptible drugs against uropathogens.[24] Although multiple studies have also been conducted on antibiotic resistance and sensitivity in South Punjab, the remarkable last study conducted in this region to find multidrug resistance and sensitivity survey in uropathogens was six years ago.[25] However, this study has shown very limited epidemiological information on multidrug resistance and susceptibility in UTIs in this region. In South Punjab, medical practitioners do not have a piece of good information over the prevalence of antibiotic resistance and susceptibility in uropathogenic associated with UTIs. Recommendations of antibiotics without antibiotic sensitivity testing are likely to further aggravate the situation. Thus, an urgent study was needed and designed for the epidemiological surveillance of uropathogens associated with UTIs along with the information of antibiotics sensitivity or resistance in this region. Another perspective of this study is to find out the most susceptible drugs against different uropathogens causing UTIs, which will help to reduce the economic burden to treat infectious diseases.

Materials and Methods

Study Design and Ethical Approvals

A cross-sectional study was designed and conducted at Multan Institute of Kidney Diseases (MIKD) Hospital, Multan, Pakistan and Institute of Molecular Biology and Biotechnology (IMBB), Bahauddin Zakariya University (BZU), Multan, Pakistan, from September 2020 to December 2020. All ethical approvals were duly obtained from the Institutional Review Board (IRB) of IMBB with approval number 334/A. Informed consent was obtained from all the participants, and the study was performed as per the Declaration of Helsinki.

Sample Collection

MIKD hospital is a dedicated hospital for kidney diseases. One thousand urine samples (n = 1000) were collected from the patients with urinary tract dysfunction without any age limit, gender, or other discrimination. UTis patients were characterized by physical urine examination, i.e putrid or foul odor, smoky or milky color and microscopic urine examination, ie bacteriuria, hematuria, pyuria in this study. All UTI patients with bacteriuria and pyuria[26] visited "MIKD" Hospital, and urine samples were taken in sterile urine culture and sensitivity (c/s) container having boric acid.[27] Samples were transported immediately to the laboratory on ice and processed within two hours for further analysis.

Inoculation

Samples were inoculated onto Cysteine Lactose Electrolyte Deficient (CLED, Oxoid, Basingstoke Hampshire, United Kingdom) media, selective and differential media.[28] A sterile wire loop with 0.01 µL was used for the midstream urine sample and 0.1 µL for the percutaneous nephrolithotomy (PCNL) urine sample. After inoculation, media plates were incubated into an incubator at 37°C.[29] After 24 hours of incubation, pure growth was considered for further gram staining and biochemical analysis with antimicrobial susceptibility testing.

Gram Staining

A commercially prepared gram stain (Oxoid, Basingstoke Hampshire, United Kingdom) was used to differentiate between gram-positive and negative bacteria, either rods or cocci, as previously described.[30–32] After confirming the bacterial nature, the biochemical tests were performed for identification and characterization of isolates along with antimicrobial testing.

Biochemical Identification

Biochemical analysis was performed to distinguish the bacterial strains. Different types of biochemical analysis including triple sugar iron, motility, indole, sulfide, urease, citrate, and oxidase assays were used for gram-negative rods (GNRs), while catalase, coagulase, and bile esculin assays were used for the gram-positive cocci (GPCs) bacteria.[29]

Antimicrobial Susceptibility

Pure colonies were used to make inoculum (0.5 McFarland for gram-negative and 1.0 McFarland for gram-positive bacteria). Samples were further cultured on Muller Hinton agar (MHA, Oxoid, Basingstoke Hampshire, United Kingdom) with a cotton swab. Different antibiotics were dispensed on MHA after lawning. Antibacterial activity of the below-mentioned antibiotic disks (Oxoid, Basingstoke Hampshire, United Kingdom) was done by the Kirby–Bauer disk diffusion technique.[29,33] Antibiotics resistant (R) and sensitive (S) isolates were identified according to the guidelines of Clinical and Laboratory Standards Institute (CLSI)[34] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[35] According to CLSI and EUCAST, each bacterial strain has its specific antibiotics recommendations. Antibiotics recommended to use against *Enterobacteriaceae* include ampicillin (AMP), augmentin (AMC), piperacillin/tazobactam (TZP), cefotaxime (CTX), ceftazidime (CAZ), meropenem (MEM), imipenem (IPM), gentamicin (G), amikacin (AK), nalidixic acid (NA), norfloxacin (NOR), ciprofloxacin (CIP), co-trimoxazole (SXT), nitrofurantoin (F), sulbactam/cefoperazone (SCF) and fosfomycin (FOS). Antibiotics are recommended for *Pseudomonas* species include piperacillin/tazobactam (TZP), ceftazidime (CAZ), cefepime (FEP), colistin (CT), meropenem (MEM), imipenem (IPM), gentamicin (G), amikacin (AK), norfloxacin (NOR), ciprofloxacin (CIP) and sulzone (SCF). Antibiotics are recommended for *Staphylococcus aureus* (*S. aureus*) and *Enterococcus* species include penicillin (P), ampicillin (AMP), augmentin (AMC), linezolid (LZD), teicoplanin (TEC), gentamicin (G), amikacin (AK), ciprofloxacin (CIP), levofloxacin (LFX), nitrofurantoin (F) and fosfomycin (FOS). Antibiotics are recommended for *S. aureus*, not against *Enterococcus* species, including fusidic acid (FD), tetracycline (TE), co-trimoxazole (SXT) and cefoxitin (FOX).[34,35]

Quality Control

American Type Culture Collection (ATCC) strains (Manassas, Virginia, near Washington DC, USA) of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 8427) and *Staphylococcus aureus* (ATCC 25923) were used as a control to check the growth-supporting ability of prepared media (CLED and MHA agar) throughout the study.[35] *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used as quality control along with samples during gram staining. Biochemical tests were performed including catalase test, coagulase test, indole test, triple sugar iron test, citrate test, urease test and oxidase test. Accuracy and reproducibility of biochemical test results were confirmed by using ATCC strains (*Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus mirabilis* (ATCC 35659)) as positive and negative quality control. Different antibiotics were used against different ATCC strains to check the accuracy and reproducibility of antimicrobial sensitivity technique and the results were interpreted according to CLSI guidelines.[36]

Statistics

A *chi*-square test was performed to analyze the data using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). *p*-value ≤0.05 was considered to be significant statistically.

Results

Prevalence and Distribution of Bacterial Isolates

In this study, one thousand (n = 1000) urine samples were collected and analyzed from the patients with suspected urinary tract infections (UTIs). All of these samples were inoculated onto CLED plates, and only six hundred (n = 600) patients were positive for bacterial infection. Twenty-eight out of the six hundred (n = 28) samples were rejected due to contamination of skin normal microflora (*Staphylococcus epidermidis*). Uropathogens were detected and successfully isolated from the remaining 57% (n = 572) samples and further analyzed for identification and characterization. *Escherichia coli* 51.2% (n = 293/572), *Klebsiella* species 15.4% (n = 88/572), *Enterococcus* species 15.4% (88/572), *Pseudomonas* species 9.4% (n = 54/572), *Staphylococcus aureus* 3.2% (n = 18/572), Coagulase Negative *Staphylococci* (CoNS) 3.0% (n = 17/572) and *Proteus* species 2.5% (n = 14/572) were most prevalent uropathogens in analyzed samples (Figure 1).

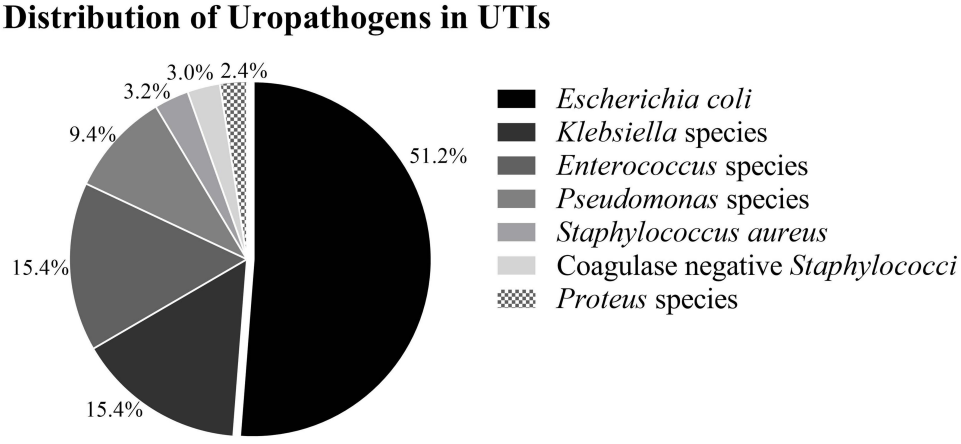

Figure 1 Distribution and prevalence of bacterial isolates in UTIs. Distribution of most prevalent (%) uropathogenic bacteria among the total number of isolates (n=572) from UTIs. *Escherichia coli* were the most prevalent (51.2%) among the UTIs pathogens followed by *Klebsiella* species (15.4%), *Enterococcus* species (15.4%), *Pseudomonas* species (9.4%), *Staphylococcus aureus* (3.2%), Coagulase-negative *Staphylococci* (CoNS) (3.0%) and *Proteus* species (2.4%) Bacterial isolates presented were significantly associated with UTI (*p*-value was < 0.000).

Table 1 Prevalence of Gram-Negative Rods (GNRs) and Gram-Positive Cocci (GPCs) in UTIs

UTIs Isolates		Frequency (n)	Percentage	*p*-value
Gram Negative Rods (GNRs), n = 449/572 (78.5%)	*Escherichia coli*	293	65.3% (293/449)	0.000
	Klebsiella species	88	19.6% (88/449)	
	Pseudomonas species	54	12.0% (54/449)	
	Proteus species	14	3.1% (14/449)	
Gram Positive Cocci (GPCs), n = 123/572 (21.5%)	*Enterococcus* species	88	71.5% (88/123)	0.000
	Staphylococcus aureus	18	14.6% (18/123)	
	Coagulase Negative *Staphylococci* (CoNS)	17	13.8% (17/123)	
	Total	572		

Notes: The frequency of UTI-associated GNRs and GPCs were presented in this table with percentages. *Escherichia coli* was the most prevalent GNRs bacteria followed by *Klebsiella* species, *Pseudomonas* species, and *Proteus* species, while *Enterococcus* species was most common among uropathogens the GPCs, followed by *Staphylococcus aureus* and coagulase negative *Staphylococci* (CoNS). Prevalent bacterial isolates presented in this table were significantly associated with the incidence of UTIs (*p*-value <0.000).

Prevalence of Gram-Negative Rods (GNRs) and Gram-Positive Cocci (GPCs)

Gram-negative rods (GNRs) were more prevalent in UTIs patients. Prevalence of Gram-negative rods (GNRs) was 78.5% (n = 449) in UTIs patients as compared to Gram-positive cocci (GPCs) bacteria 21.5% (n = 123). *Escherichia coli, Klebsiella* species, *Pseudomonas* species, and *Proteus* species were the most prevalent uropathogens among GNRs, while *Enterococcus* species, *S. aureus*, and Coagulase Negative *Staphylococci* (CoNS) were the most prevalent uropathogens among GPCs.

The prevalence of the *Escherichia coli, Klebsiella* species, *Pseudomonas* species, and *Proteus* species was 65.3% (n = 293/449), 19.6% (n = 88/449), 12.0% (n = 54/449), and 3.1% (n = 14/449), respectively, among GNRs, while the prevalence of *Enterococcus* species, *Staphylococcus aureus*, and Coagulase Negative *Staphylococci* (CoNS) was 71.5% (n=88/123), 14.6% (n=18/123), and 13.8% (n=17/123), respectively, among GPCs (Table 1). The GNRs and GPCs bacteria were significantly associated with UTIs (*p*-value <0.000).

Effectiveness of Antibiotics Against *Enterobacteriaceae*

In this study, the majority of bacteria isolated from urine samples of the patients positive for UTIs belong to the family *Enterobacteriaceae,* including *Escherichia coli, Klebsiella* species, and *Proteus* species. These isolates have been shown to be resistant to the most commonly used antibiotics against UTIs. Antibiotics were shown resistance from higher to lower level include AMP > NA > AMC > CTX > NOR > CAZ > SXT >CIP, while certain antibiotics were also shown to have a great susceptibility against *Enterobacteriaceae* which included FOS > F > IPM > MEM > AK > TZP > SCF > G (Figure 2).

Effectiveness of Antibiotics Against *Pseudomonas* Species

Pseudomonas species (n = 54) were the second most important organism after *Enterobacteriaceae* among GNRs. Different antibiotics were tested against *Pseudomonas* species to check their effectiveness. CT was the most sensitive drug followed by TZP > AK > MEM > IPM > SCF, respectively, that can be used as a choice of treatment if UTIs occurred by *Pseudomonas* species. NOR was the most resistant drug followed by G > CIP > CAZ > FEP that was ineffective in treating UTIs (Figure 3).

Effectiveness of Antibiotics Against *Enterococcus* Species

Enterococcus species (n = 88) was the most prevalent organism among GPCs bacteria isolated from UTIs patients followed by *Staphylococcus aureus* and CoNS. The commonly recommended antibiotics against these bacteria were shown to have a great resistance (AK > CIP > G), while LZD was the most sensitive drug followed by TEC > F > SCF > FOS > AMP > AMC, which can be used in UTIs caused by *Enterococcus* species (Figure 4).

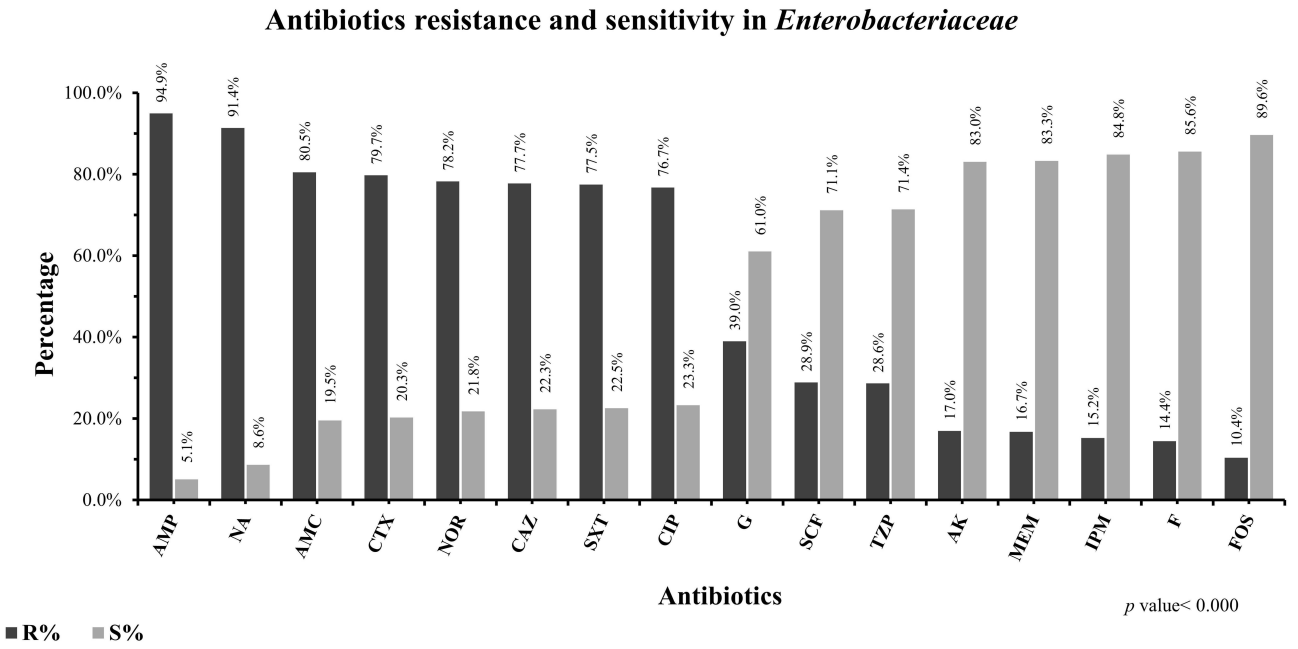

Figure 2 The pattern of antibiotics resistance and susceptibility against *Enterobacteriaceae*. Frequency of antimicrobial resistance and susceptibility of antibiotics including ampicillin (AMP), nalidixic acid (NA), augmentin (AMC), cefotaxime (CTX), norfloxacin (NOR), ceftazidime (CAZ), co-trimoxazole (SXT), ciprofloxacin (CIP), gentamicin (G), sulbactam/cefoperazone (SCF), piperacillin/tazobactam (TZP), meropenem (MEM), imipenem (IPM), amikacin (AK), nitrofurantoin (F), fosfomycin (FOS) were presented in percentages against *Enterobacteriaceae*. Resistance (black bars) and susceptibility (grey bars) of all antibiotics mentioned in this graph were significantly associated with UTIs caused by bacterial isolates belonging to *Enterobacteriaceae* such as *Escherichia coli, Klebsiella* species, and *Proteus* species (*p*-value was < 0.000).

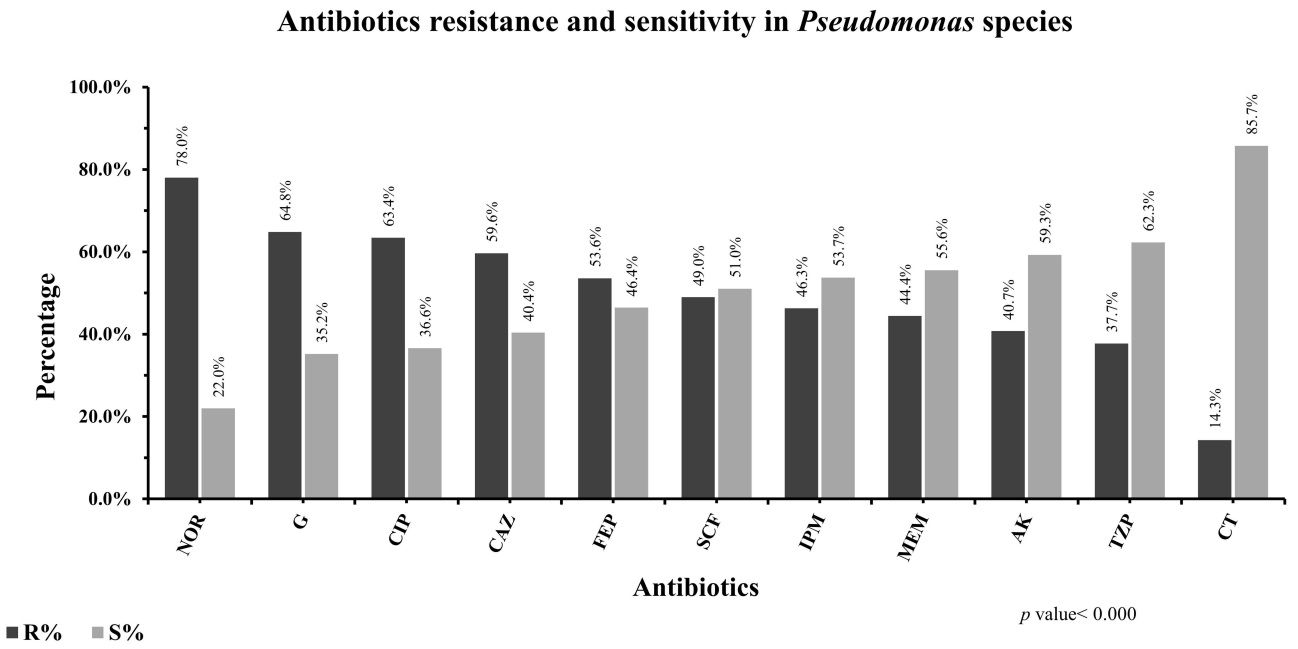

Figure 3 The pattern of antibiotics effectiveness against *Pseudomonas* species in UTIs. Frequency of antimicrobial resistance and susceptibility of antibiotics including norfloxacin (NOR), gentamicin (G), ciprofloxacin (CIP), ceftazidime (CAZ), cefepime (FEP), sulbactam/cefoperazone (SCF), imipenem (IPM), meropenem (MEM), amikacin (AK), piperacillin/tazobactam (TZP), colistin (CT) were presented in percentage against *Pseudomonas* species. Resistance (black bars) and susceptibility (grey bars) of these antibiotics mentioned in this graph were significantly associated with UTIs associated caused by *Pseudomonas* species (*p*-value was < 0.000).

Effectiveness of Antibiotics Against *Staphylococcus* Species

Staphylococcus species were also identified to cause UTIs in our population with less frequency as compared to others. Different antibiotics were tested to check their effectiveness against these pathogens to cure the UTIs. CIP

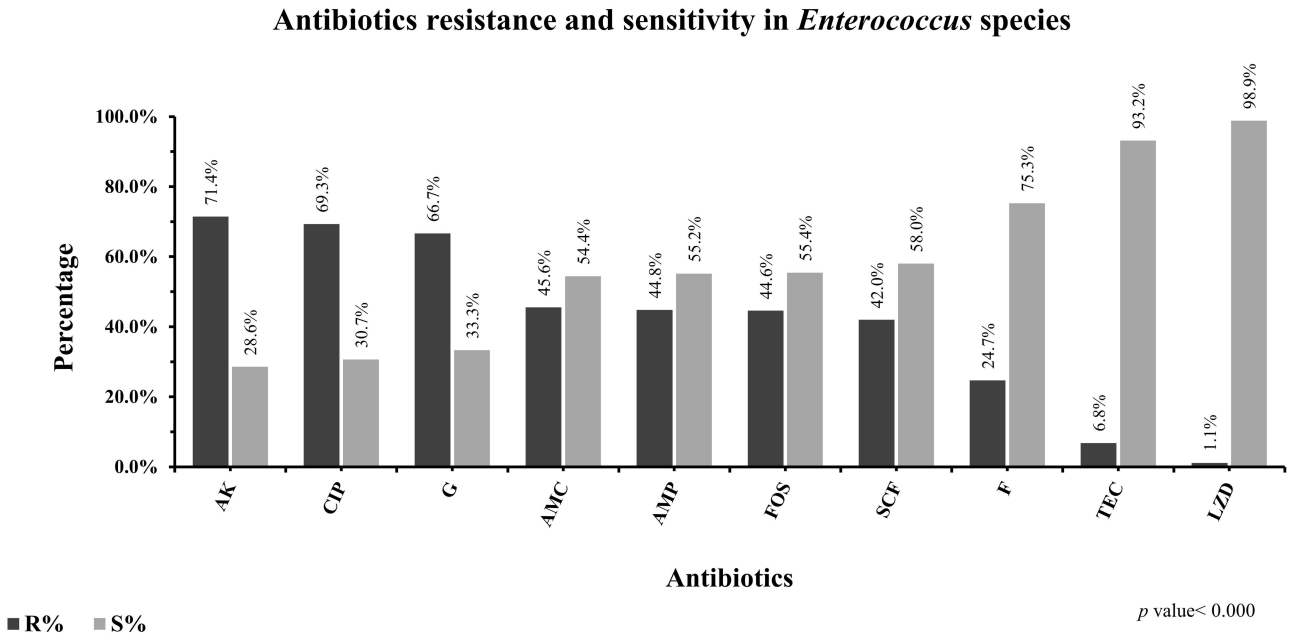

Figure 4 The pattern of antibiotics effectiveness against *Enterococcus* species in UTIs. Frequency of antimicrobial resistance and susceptibility of antibiotics including amikacin (AK), ciprofloxacin (CIP), gentamicin (G), augmentin (AMC), ampicillin (AMP), fosfomycin (FOS), sulbactam/cefoperazone (SCF), nitrofurantoin (F), teicoplanin (TEC), linezolid (LZD) were presented in percentage against *Enterococcus* species. Resistance (black bars) and susceptibility (grey bars) of all antibiotics mentioned in this graph were significantly associated with UTIs caused by *Enterococcus* species (*p*-value was < 0.000).

was the most resistant drug in the case of *Staphylococci* species, followed by LFX > P > TE > AMC > FOX, respectively. TEC and LZD were shown to have a great susceptibility followed to F > AK > FD > SXT > G (Figure 5).

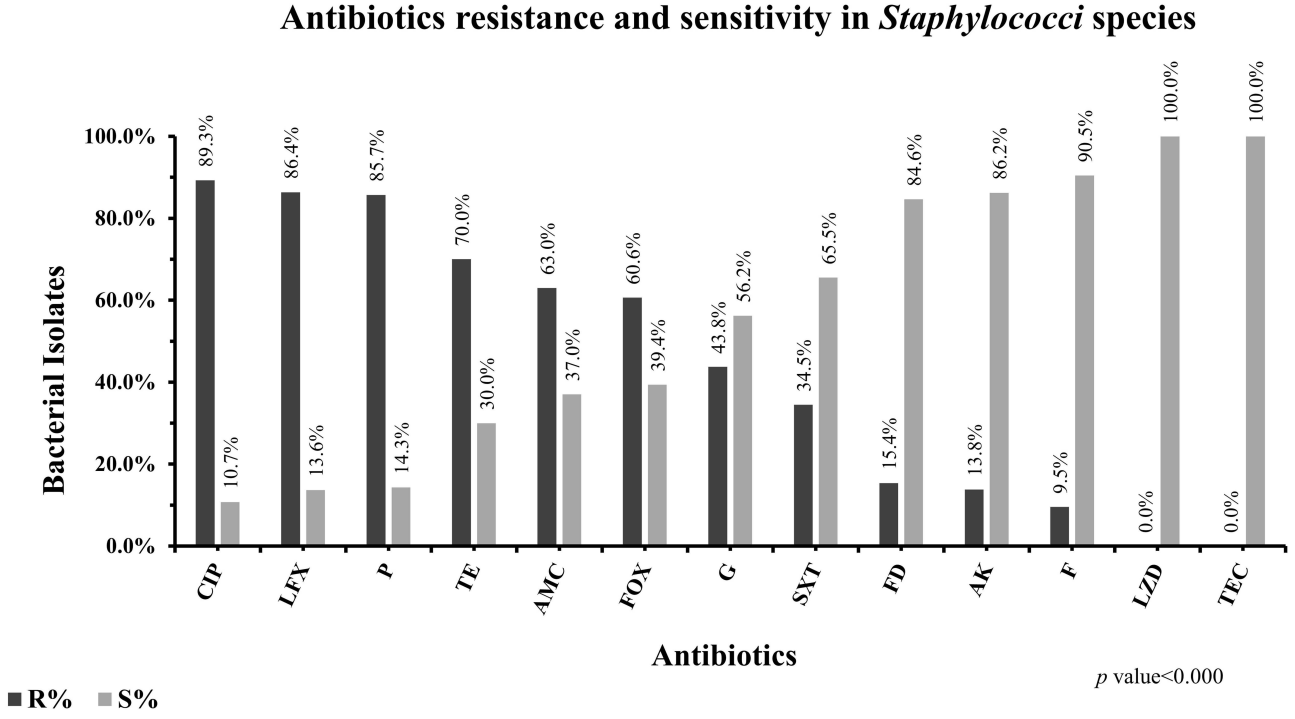

Figure 5 The pattern of antibiotics effectiveness against *Staphylococci* species in UTIs. Frequency of antimicrobial susceptibility of antibiotics including ciprofloxacin (CIP), levofloxacin (LFX), penicillin (P), tetracycline (TE), augmentin (AMC), fosfomycin (FOS), gentamicin (G), co-trimoxazole (SXT), fusidic acid (FD), amikacin (AK), nitrofurantoin (F), linezolid (LZD), teicoplanin (TEC) was presented in percentage against *Staphylococci* species. Resistance (black bars) and susceptibility (grey bars) of all antibiotics mentioned in this graph were significantly associated with UTIs caused by *Staphylococci* species (*p*-value was < 0.000).

Discussion

Urinary tract infections (UTIs) are common infections in both Indoor Patients (IPD) and Outdoor Patients (OPD) settings throughout the world. Here, we studied UTIs in both IPD and OPD settings and further antibiotic sensitivity was analyzed. *Escherichia coli* (65.3%) were the predominant isolates among the gram-negative bacteria, while *Enterococcus* species (71.5%) were the predominant isolates among the gram-positive bacteria. *Klebsiella* species, *Proteus* species, *Pseudomonas* species, and *S. aureus* were other uropathogens that can cause UTIs. Previously, Gupta et al 2002,[37] and Haque et al 2015[7] also reported similar uropathogens associated with UTIs. Similar to the previous study,[5,38] *Escherichia coli* was the most prevalent uropathogen in this study associated with UTIs in the South Punjab region of Pakistan.

Drug resistance patterns among uropathogens have been increased and become a major challenge in clinical practices to treat UTIs. AMP, AMC, CTX, CAZ, CIP, LFX, NA, and SXT are commonly used drugs to overcome the UTIs caused by gram-positive and negative bacteria in developing countries like Pakistan.[39] Unfortunately, all these antibiotics were identified as ineffective against uropathogens in our setting. It is an alarming condition for physicians to use antibiotics as an effective therapeutic option to control UTIs.[40]

In this study, *Enterobacteriaceae* found highly resistant to AMP (94.9%), NA (91.4%), AMC (80.5%), CTX (79.7%), NOR (78.2%), SXT (77.5%), CAZ (77.7%) and CIP (76.7%) and less resistant to G (39.0%), SCF (28.9%), TZP (28.6%), AK (17%), MEM (16.7%), IMP (15.2%), F (14.4%) and FOS (10.4%). Falagas et al, 2010[41] also reported high resistance of TZP, CTX, CAZ, IMP, G, CIP, SXT, while Woldemariam et al, 2019[42] reported less resistance of AMP, AMC. CTX, CAZ, G, AK, CIP, F against *Enterobacteriaceae*, both studies showed a different pattern of antibiotic resistance. The difference in drug resistance patterns in similar bacteria in different populations is likely due to different prevention and treatment strategies against UTIs in different geographic regions. However, certain antibiotics also showed similar resistance patterns in different geographic regions indicating the involvement of common mechanisms involved in drug resistance. For example, in our study, FOS and SXT showed 10.4% and 77.5% resistance, respectively, against *Enterobacteriaceae*, in a previous study conducted in Greece, a similar resistance pattern of these drugs, FOS (2%) and SXT (87) in UTIs, has been also reported,[41] which indicates the involvement of common mechanisms involved in drug resistance in these uropathogens. However, some studies conducted in our region[39] reported different drug resistance patterns including CTX, CAZ, MEM, IMP, G, AK, NOR, F, FOS against *Enterobacteriaceae*, which were also indicating the different treatment strategies of UTIs in the same regions or misuse of drugs and self-medication by the population; however, this study also reported antibiotic resistance pattern of AMC, CIP, and NA similar to our study.

Mehrishi et al, 2019[43] reported less resistance of AMP, TZP, CTX, CAZ, IMP and NOR, while MEM, CIP, SXT showed more resistance against *Enterobacteriaceae*. This difference likely came from the frequency of use of certain antibiotics. However, the antibiotic resistance pattern of F, AK, G in this population was similar to our study. Similar to our data, other researchers reported ceftazidime resistance up to 100% in India,[44] while in other countries, Malaysia and China ceftazidime resistance has been reported at 11% and 28%, respectively.[45,46] Variation in findings with different studies conducted by different authors in different countries is due to epidemiological variation, various treatment strategies against UTIs, samples numbers, and awareness about the misuse of antibiotics among the population.

Pseudomonas species is an important bacterium that contributes to hospital-acquired UTIs and other infections. Similar to the previous report,[39] we found TZP, CT, MEM, IPM, AK, SCF sensitive, while CAZ, FEP, G, NOR, CIP were resistant against *Pseudomonas* species Similarly, another study[47] also reported some resistance patterns (MEM, IPM, TZP, G) in *Pseudomonas* species; however, the sample size in this study was very low.

Among gram-positive bacteria, *Enterococcus* species were the most prevalent organism associated with UTIs in our population. We found G, AK, CIP resistant, while AMP, AMC, LZD, TEC, F, SCF, FOS were the most sensitive drugs against *Enterococcus* species. Similarly, Woldemariam et al, 2019[42] also reported that F was the most sensitive drug against *Enterococcus* species in Ethiopian UTI patients, most likely due to having the same treatment strategy and similar mechanism to develop antibiotics resistance in *Enterococcus* species. On the other hand, Muhammad et al, 2020[39] reported resistance of VA, CIP, FOS in *Enterococcus* species along with AMC and LZD in our populations likely due to different antibiotics strategies in a different province of our country. Pouladfar et al, 2017[47] reported antibiotic resistance

patterns including AMP, AMC, CIP, and F in *Enterococcus* species in the Iranian population, while LZD and VA were the most sensitive drugs against *Enterococcus* species in this study.

In this study, we reported that *Staphylococcus* species were shown to have a high level of resistance to P, AMC, FOX, TE, CIP and LFX antibiotics that cannot be used to treat this infection, while FD, LZD, TEC, G, AK, SXT and F were reported to be more sensitive drugs against *Staphylococcus* species. These findings were also confirmed in different studies in different geographic regions including Ethiopia, Pakistan, and Iran,[39,42,47] respectively, which confirmed the P, AMC, FOX, TE, CIP, LFX more resistant and G, SXT, F more sensitive drugs against *Staphylococcus* species in UTIs.

Ciprofloxacin was considered the most effective drug against uropathogens; however, it lost effectiveness in the past few years likely due to irrational use or self-medication, which leads to the development of resistance against CIP along with other drugs including 1st, 2nd, and 3rd generations of cephalosporins.[48,49] Our study demonstrated that nitrofurantoin and fosfomycin were the most effective drugs against both gram-negative and positive bacteria. Other studies also supported our finding that nitrofurantoin and fosfomycin were good alternative treatment options for UTIs.[41–43,50] Similar to previous studies,[51,52] we also reported that F, FOS, TEC, and LZD were the most effective drugs against gram-positive bacteria, while F, FOS, TZP, MEM, and IPM were the most effective drugs against gram-negative bacteria. Fosfomycin was identified as an effective drug against *Enterobacteriaceae* and *Enterococcus* species causing UTIs.

In conclusion, drug resistance against uropathogens is an evolving process that is increasing gradually. Ampicillin, augmentin, cefotaxime, ceftazidime, ciprofloxacin, levofloxacin, nalidixic acid and co-trimoxazole are used as a choice of drugs to overcome the UTIs caused by gram-positive and negative bacteria in developing countries like Pakistan. Unfortunately, most of the antibiotics used to treat UTIs showed a high level of antibiotic resistance in our setting due to overuse and/or misuse of these antibiotics, prolonged stay in the hospital, no proper monitoring, lack of testing and awareness in the population, which is an alarming situation to treat UTIs. Our study advocates that nitrofurantoin and fosfomycin were the most effective drugs against both gram-negative and positive bacteria that can be a better option against UTIs. In addition to nitrofurantoin and fosfomycin, teicoplanin and linezolid were identified as effective drugs against gram-positive bacteria and piperacillin/tazobactam, meropenem, and imipenem against gram-negative bacteria. Thus, continuous investigation and monitoring are required to identify the drug effectiveness and resistance against uropathogens to treat UTIs. However, authorities should take actions to prevent the overuse or self-medication of these highly susceptible drugs in UTIs to avoid the development of resistance.

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Disclosure

No potential conflict of interest in this work was reported by the authors.

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