Article

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Prevalence and Resistance Patterns of Urinary Tract Infection in Al-Madinah Al-Munawarah, Saudi Arabia: A Retrospective Study

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Background: Urinary tract infections (UTIs) are among the most common infections and can cause numerous complications of the renal system. This study aimed to assess the prevalence of uropathogens and their antibiotic susceptibility patterns in Al-Madinah Al-Munawarah, Saudi Arabia.

Methods: Data was collected from patients with UTIs presented at King Fahad General Hospital in Al-Madinah Al-Munawarah, Saudi Arabia. In this retrospective cross-sectional study, UTI microbial-causing agents and antimicrobial resistance profiles identified using automated systems, Phoenix and VITEK2, were collected between July 2022 and June 2023. In addition, minimal demographic data, including date of collection and sex and age of patients were collected and analyzed using Chi-square test.

Results: The study included 1394 patients positive for UTI, comprising 50.57% males and 49.43% females (chi-square goodness-of-fit, $p > 0.999$). Microbial identification and antimicrobial susceptibility tests were performed on UTI-positive cultures. Among UTIs, mono-infection, caused by a single pathogen, was the most prevalent, accounting for 88.16% of cases, whereas poly-infection (caused by multiple pathogens) presented at 11.9%. The most prevalent UTIs' pathogens were *E. coli* (30.59%), followed by *Klebsiella pneumoniae* (21.40%), *Enterococcus faecalis* (8.46%), *Pseudomonas aeruginosa* (7.81%), *Streptococcus agalactiae* (6.35%), *Enterococcus faecium* (3.01%), *Proteus mirabilis* (3.01%), *Enterobacter cloacae* (2.52%), *Candida* sp. (2.44%), *Acinetobacter calcoaceticus-baumannii* (1.95%), *Staphylococcus aureus* (1.79%), and *Enterobacter aerogenes* (1.30%). The most dominant pathogens that coexisted with other uropathogens to cause UTIs were *K. pneumoniae* and *P. mirabilis* (9.32%, chi-square 5.550, $p = 0.018$), *K. pneumoniae* and *P. aeruginosa* (8.07%, chi-square 6.285, $p = 0.012$), *K. pneumoniae* and *E. faecalis* (7.45%, chi-square 5.785, $p = 0.016$), *Candida* sp. and *Enterococcus faecium* (4.97%, chi-square 9.176, $p = 0.002$, and *Candida* sp. and *Acinetobacter calcoaceticus-baumannii* (3.11%, chi-square 4.312, p=0.038)). Among the uropathogens, gram-negative pathogens showed resistance to most of the tested antimicrobials (ampicillins, cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole, aztreonam, and nitrofurantoin). High rates of resistance were identified to cephalosporins, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole.

Conclusion: This study reported UT mono-infection and poly-infection in Al-Madinah Al-Munawarah, Saudi Arabia, with a predominant representation from gram-negative bacteria, *Enterobacteriaceae*. Most of the UT microbial strains showed a highly resistant profile.

Keywords: antimicrobial susceptibility test; bacteria; mono-infection; UTI; poly-infection; prevalence; uropathogens

Introduction

One of the most prevalent bacterial diseases is urinary tract infections (UTIs), and females are more likely than males to have this condition [1]. Sixty percent of females experience UTIs at least once in their lifetime, and 30–40% experience recurrent UTIs [2]. In Saudi Arabia, UTIs are the second leading cause of infection, predominantly in females [3]. The prevalence of UTI among pregnant females was 16% [4]. Catheter-associated urinary tract infections (CAUTIs) are the most common nosocomial infections representing a percentage of 7%. There are several risk factors for CAUTIs in patients in the intensive care unit (ICU), such as the duration of catheter use, female sex, pregnancy, and poor nutrition [5]. In addition, the prevalence of UTIs depends on sex and varies geographically and across cities in Saudi Arabia. For example, the prevalence rate among males was more than half in Jeddah (57.9%) and Aseer

(59.9%), whereas the rate among females was 42.1% and 40.1%, respectively [6,7]. However, in Al-Baha region, the prevalence was higher in females with a percentage reaching 68.64%, compared to males (31.36%) [8].

UTIs are associated with severe morbidity and a declining quality of life, with symptoms ranging from mild bladder pain and dysuria to severe bladder complications [9]. Although numerous therapeutic approaches, including antibiotic treatment, probiotics, bioactive natural foods, and essential personal hygiene, have been deployed to treat and prevent chronic and recurrent UTIs, they have been ineffective [10]. Urinalysis is the most commonly performed diagnostic test in the world. The gold standard method for diagnosing UTI is urine culture from a midstream urine sample, followed by standard antimicrobial susceptibility testing (AST) using automated or manual approaches [11].

Various UTI classification schemes have been deployed, the most common of which was developed by the Centers for Disease Control and Prevention (CDC) [12]. The recommendations of the European Association of Urology (EAU) divide UTIs into five categories [13]. Uncomplicated UTIs are limited to non-pregnant females and are defined as acute, sporadic, or recurrent lower (uncomplicated cystitis) and upper (uncomplicated pyelonephritis) UTIs, with no known related anatomical and functional abnormalities within the urinary tract or comorbidities. The second category is complicated UTIs (all UTIs that are not classified as uncomplicated). Specifically, it refers to UTI in patients with a higher risk of a complicated course, such as males, pregnant females, individuals with relevant urogenital structural or functional abnormalities, individuals using indwelling urinary catheters, patients with renal diseases, and individuals with additional concurrent immunocompromised conditions such as diabetes. Third, there were recurrent UTIs (recurrences of uncomplicated and complicated UTIs with a frequency of at least three infections per year or two in the previous six months). Fourth, catheter-associated UTIs (CAUTIs or catheter-associated tract infections) occur in individuals whose urinary tracts have been catheterized or were catheterized within the last 48 h. The fifth condition is urosepsis, which is characterized by life-threatening organ failure caused by an unbalanced host response to an infection that initiates in the urine tract and/or male genital organs [13].

The two most frequent causal agents of UTI are *Klebsiella pneumoniae* and *Escherichia coli*, which are transferred from the gastrointestinal tract (GIT) to the urethral opening and proliferate. The external genitalia, vagina, genital tract, and GIT are the main reservoirs of *K. pneumoniae* and *E. coli*. Approximately 75–95% of all cases of uncomplicated UTI are community-acquired by uropathogenic (UPEC) *E. coli* and *Klebsiella* spp. [14]. Uropathogenic *E. coli* has been reported to be the predominant infectious agent in complicated and uncomplicated UTIs. The gram-positive bacterium *Staphylococcus aureus* is often a component of the body's microbiota and is regularly found in the skin and upper respiratory tract [15]. However, it has rarely been identified as a UTI-causing pathogen [16]. *Staphylococcus saprophyticus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* are other bacteria isolated from UTIs [17]. In complicated infections, *Enterococcus* spp. and *Candida* spp. are significantly more prevalent [18].

When one of the uropathogens is present in midstream urine at titers greater than 10^5 colony-forming units (CFU)/mL, the UTI is defined as a mono-infection [19]. However, the etiology of polyinfection UTI is consistent with the presence of numerous uropathogens in midstream urine at titers >100,000 CFU/mL. Patients who are older adults, immunocompromised, have indwelling catheters, are HIV-positive, have cancer, or have diabetes are more likely to develop polymicrobial infections. In contrast, young and sexually active females have a lower incidence of polymicrobial UTIs [20]. Polymicrobial microbiota of the gastrointestinal and reproductive tracts is a significant source of infection that causes UTI [20]. In a rat model, transurethral inoculation of *P. mirabilis* or *S. saprophyticus* caused ascending pyelonephritis more frequently when the two organisms were inoculated together than when they were inoculated separately, indicating a synergistic virulence between the two species [21]. *Pseudomonas aeruginosa* and *E. faecalis* coinfections causing pyelonephritis developed more rapidly in mice than when each species was introduced alone [22]. Additionally, pyelonephritis developed more rapidly when *P. aeruginosa* and *E. faecalis* were co-infected than when they were introduced separately [22]. It has been discovered that the presence of Group B Streptococci (*S. agalactiae*) can modify host immunity and alter host susceptibility to persistent with high titer of UPEC infection of the bladder and kidneys, as shown by co-infection experiments with GBS and UPEC in a mouse UTI model [23].

The increasing emergence of drug-resistant microbial strains in UTIs is a health concern. Treating gram-negative bacteria is challenging because of the emergence of antibiotic resistance in this group, and the likelihood of recurrence within 6 months increases with UPEC infection [18]. Determining the prevalence of UTIs, the most common UTI-causing agents, and antimicrobial resistance profiles in Saudi Arabia is essential. This study conducted a retrospective study aimed to determine the prevalence of UTI mono-infections and UTI polymicrobial infections, the most predominant microbial agents, and the resistance profile using data from King Fahad Hospital, Madinah Al-Munawarah, from 2022 to 2023.

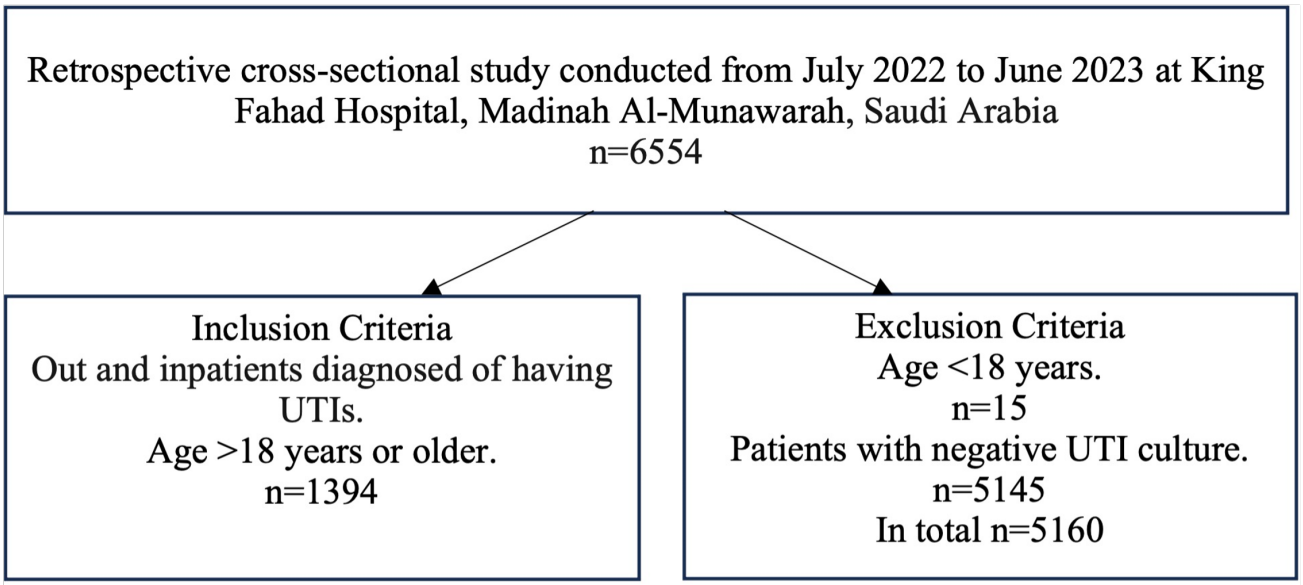

Fig. 1. Flowchart of inclusion and exclusion criteria for the study. n stands for the number of cases. UTIs, urinary tract infections.

Materials and Methods

Study Design

This retrospective cross-sectional study was conducted between July 2022 and June 2023 at King Fahad Hospital in Madinah Al-Munawarah, Saudi Arabia. Patients with suspected UTIs Both outpatients and inpatients diagnosed with UTIs were included in the study. The study included all patients aged ≥18 years with confirmed UTI. 6554 patients suspected to have UTI presented in King Fahad General Hospital in Al-Madinah Al-Munawarah, Saudi Arabia. Negative urine cultures (5160) and patients younger than 18 years were excluded from the study. Data from patients with positive UTI cultures (total 1394) (bacterial counts in the urine sample culture exceeding 10^5 CFU/mL) were included in this study. Data on patient sex, date of sample collection, microbial-causing agents, and antimicrobial resistance profiles were also collected. All information collected from patients with negative UTI cultures (bacterial counts $<10^5$ CFU/mL) were excluded (Fig. 1). All the protocols and procedures of this study were approved by the General Directorate of Health Affairs in Al-Madinah Al-Munawarah (IRB23-121) and the Scientific Research Ethics Committee at the College of Applied Medical Sciences (2024/185/104 MLT). A signed informed consent was obtained to reuse and collect the data by the legally authorized representative (the Ethics Committee of King Salman bin Abdulaziz Medical City (General Directorate of Health Affairs in Al-Madinah Al-Munawarah-IRB23-121)).

Isolation and Identification of Uropathogens

Each patient provided a clean-catch midstream sample of urine (MSU) collected in sterile leak-proof vials. All the participants were instructed to clean the urethral area to prevent specimen contamination. The MSU was placed in a clean, sterile urine container with a wide aperture. In patients with urinary catheters, urine samples were collected with a syringe from brand-new catheters and placed into a sterile specimen tube. Each sample of the uncentrifuged, uniformly mixed MSU samples was inoculated on blood and cystine lactose electrolyte deficient (CLED) agar media for overnight aerobic incubation at 37 °C [24]. The bacterial isolates were identified following the protocol of the hospital's microbiology department, which included Gram staining and biochemical tests such as urease testing, oxidase testing, and indole production for gram-negative isolates, and catalase and coagulase testing for gram-positive cocci. A positive UTI was defined as the presence of 10^5 CFU/mL in the culture of an appropriately collected MSU [24]. Automated identification systems, Phoenix (BD, Sparks, MD, USA) and VITEK 2 (bioMérieux, Marcy l'Etoile, France), were used to confirm isolate identification. Mono-infection was assigned based on the identification of a single microorganism in the urine culture, whereas polymicrobial infection was assigned based on the identification of two or more microorganisms from at least three different samples. *S. aureus* (ATCC29213), *S. pneumoniae* (ATCC49619), *E. faecalis* (ATCC29212), *E. coli* (ATCC25922), *K. pneumoniae* (ATCC700603), *P. aeruginosa* (ATCC27853) were included as positive control for the identification system, Phoenix. For VITEK 2, the positive controls strains were *Stenotrophomonas maltophillia* (ATCC17666), *S. aureus* (ATCC29213), *S. saprophyticus* (ATCCBAA-750), *E. faecalis* (ATCC29212), *E. coli* (ATCC25922), and *P. aeruginosa* (ATCC27853).

Antimicrobial Susceptibility Testing (AST)

In accordance with the manufacturer's recommendations, antimicrobial susceptibility testing (AST) was performed using the automated systems Phoneix (BD, USA) and VITEK 2 (bioMérieux, USA). Briefly, 0.5 McFarland bacterial solution was diluted to 1.5×10^7 CFU/mL in 0.45% saline. The instruments were loaded, sealed, and incubated for the analysis. For VITEK 2, GN-91 and GN-92 cards were used for gram-negative bacteria, and GB-87, GB-580, and GB-68 cards were used for *Staphylococci*, *Enterococci*, *S. agalactiae,* and *S. pneumoniae*, respectively. GB and GN were used for Gram-positive and -negative AST tests in Phoneix, respectively. A SMIC-ID-11 card was used for *S. pneumoniae*. For gram-negative bacteria, 22 antibiotics were assayed: amoxicillin+clavulanic acid, amikacin, ampicillin, ceftazidime, cephalexin, ciprofloxacin, cefuroxime, cefazolin, cefepime, cefoxitin, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, trimethoprim/sulfamethoxazole, tigecycline, and piperacillin+tazobactam. The following antibiotics were assessed for gram-positive bacteria: amoxicillin+clavulanic acid, amikacin, penicillin, ampicillin, gentamicin, gentamicin, imipenem, levofloxacin, meropenem, colistin, aztreonam, ceftazidime, cephalexin, ciprofloxacin, ceftriaxone, cefotaxime, cefuroxime, cefazolin, cefepime, cefoxitin, trimethoprim/sulfamethoxazole, tigecycline, piperacillin+tazobactam, erythromycin, vancomycin, oxacillin, and linezolid. AST results have been reported to be sensitive or resistant. *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *E. coli* ATCC 35218, and *S. pneumoniae* ATCC 49619 were included in the AST.

Statistical Analysis

The prevalence of UT mono-infections, UT poly-infections, and UTI by sex and age were represented as numbers and percentages. Chi-square goodness-of-fit test was used to assess whether the population study had the same number of male and female and age groups (Table 1) and the same number of mono-infection, di-infection, and poly-infection (Table 2). The association between poly-infection and uropathogens was assessed using Pearson's chi-squared test. p values of <0.05 were considered statistically significant. Data were analyzed using SPSS version 23 (IBM SPSS Statistics, Armonk, NY, USA).

Results

Prevalence of UTIs

A total of 1394 patients with positive urine cultures were enrolled in this study. Samples were clean catch midstream urine, collected from patients presented at King Fahad Hospital in Madinah Al-Munawarah. The patients' sex was equally represented in UTI patients as male and female percentages were 50.57% (705 out of 1394) and 49.42% (689 out of 1394), respectively (chi-square test, $p > 0.999$). The different age groups were equally presented among UTI patients as the percentages in the age group 18–50-year-old and >50-year-old were 49.49% (690 out 1394) and 50.50% (704 out of 1394) (chi-square test $p = 0.759$) (Table 1). Mono-infection (UTI caused by a single microbe) was the most common with a parentage of 88.16% (1229 out of 1394). Among the total UTI-positive cases, 11.55%, (161 out of 1394) were caused by two microbes and only four UIT-positive cases were caused by *Candida sp*., *K. pneumoniae*, and *P. aeruginosa*. (4/1394, 0.29%) (Table 2).

Table 1. Demographical characteristics of positive UTI patients.

Characteristics	Number of cases (%)	Chi-square	*p*-value
Age	18–50-year-old = 690 (49.50%) >50-year-old = 704 (50.50%)	0.937	0.759
Gender	Male = 705 (50.57%) Female = 689 (49.43%)	96.96	>0.999
Total	1394		

Table 2. The prevalence of urinary tract infection by mono-infection, di-infection, and poly-infection.

UTI infections	Number of cases (%)	Chi-square test	*p*-value
UT-mono-infection	1229 (88.16%)	1266.24	<0.01
UT-di-infection	161 (11.55%)		
UT-poly-infection	4 (0.29%)		
Total	1394		

Predominant UTI-Causing Agents

Escherichia coli was the most prevalent bacterial uropathogens causing mono-infection with a percentage of 30.59% (376/1229), followed by *K. pneumoniae* (21.40%, 263/1229), *E. faecalis* (8.46%, 104/1229), *P. aeruginosa* (7.81%, 96/1229), *S. agalactiae* (6.35%, 78/1229), *E. faecium* (3.01%, 37/1229), *P. mirabilis* (3.01%, 37/1229), *Enterobacter cloacae* (2.52%, 31/1229), *Candida* sp. (1.95%, 24/1229), *Acinetobacter calcoaceticus-baumannii* (1.95%, 24/1229), *S. aureus* (1.79%, 22/1229), and *E. aerogenes* (1.30%,16/1229). Other microbes were uncommonly present as their prevalence percentage was <1% (Fig. 2 and **Supplementary Table 1**).

The analysis identified 11.55% (161/1394) distinct combinations of microbes (two microbes) that co-occurred to cause UTIs. *Klebsiella pneumoniae* was the most synergistic microbe that coexists with other uropathogens to cause UTIs. The most prevalent co-infections were *K. pneumoniae* and *P. mirabilis* (9.32%, chi-square 5.550, $p = 0.018$), followed by *K. pneumoniae* and *P. aeruginosa* (8.07%, chi-square 6.285, $p = 0.012$). Subsequently, co-infection of *K. pneumoniae* and *E. faecalis* (7.45%, chi-square 5.785, $p = 0.016$), and *Candida* sp. and *Entero-*

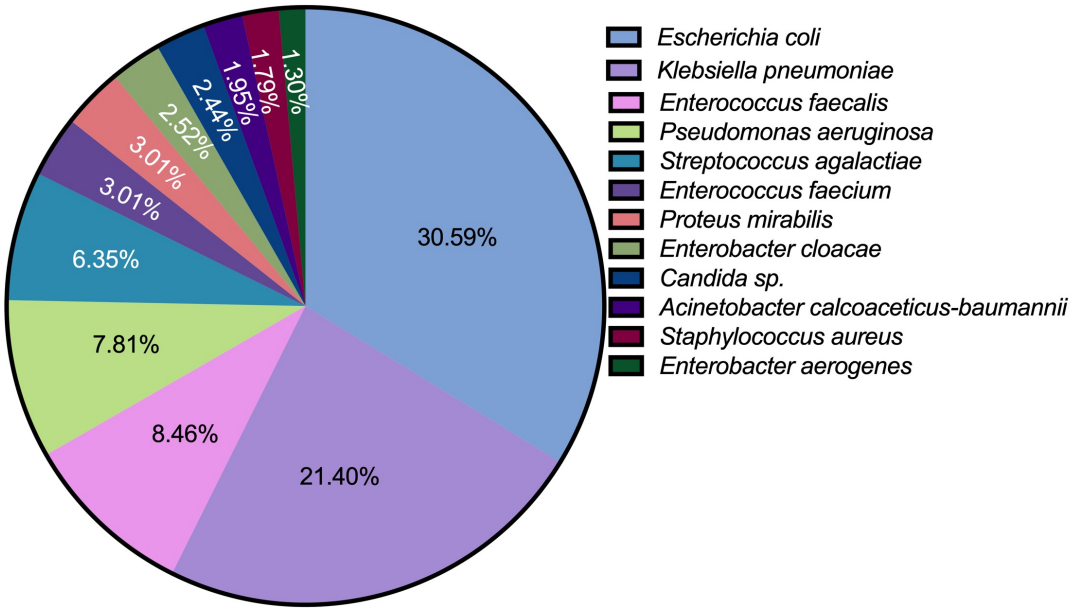

Fig. 2. Prevalence of the most common pathogens causing urinary tract mono-infection. The most common pathogens presented in this graph have a prevalence percentage of >1%.

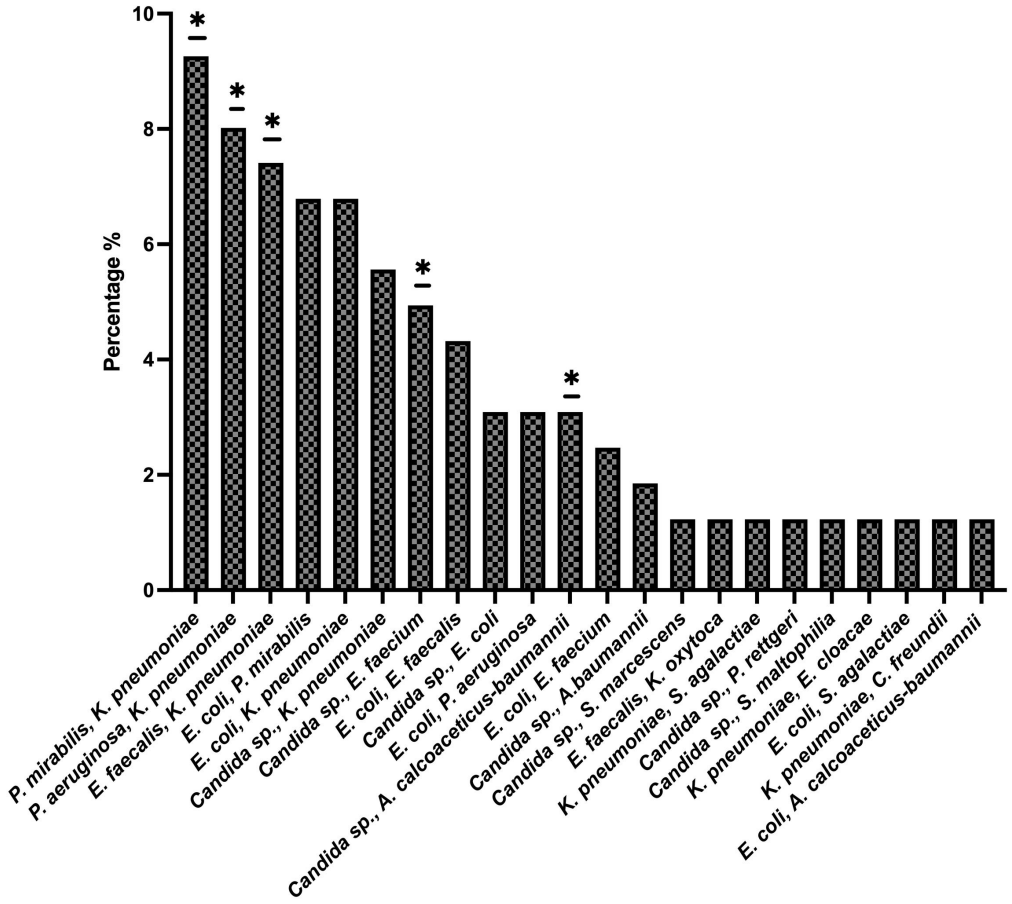

Fig. 3. The prevalence of urinary tract di-infections in this study. The most common pathogens causing urinary tract di-infections presented in this graph have a prevalence percentage of >2%. The symbol * stands for significant at $p < 0.05$, using Pearson's chi-square test.

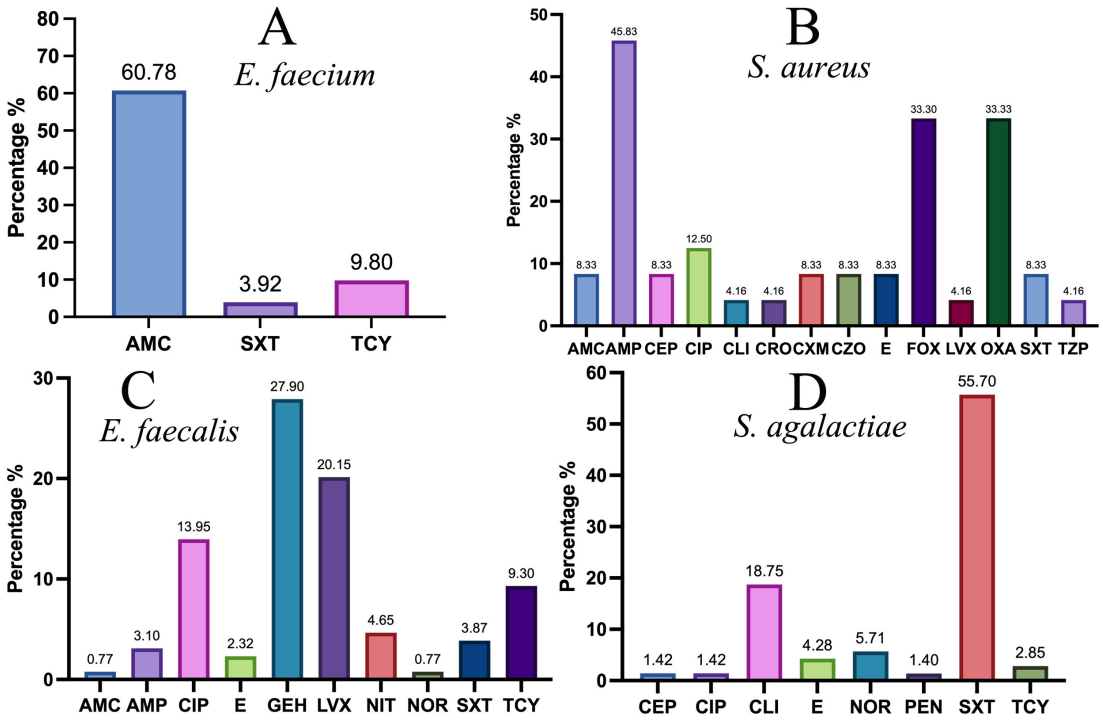

Fig. 4. Prevalence of resistance among the most common Gram-positive uropathogens. Resistance percentage of (A) *E. faecium*, (B) *S. aureus*, (C) *E. faecalis*, (D) *S. agalactiae*. The resistance percentage was calculated by the number of resistant isolates of specific pathogen divided by the total number of the pathogen. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CEP, cephalothin; CIP, ciprofloxacin; CLI, clindamycin; CRO, ceftriaxone; CXM, cefuroxime; CZO, cefazolin; E, erythromycin; FOX, cefoxitin; LVX, levofloxacin; OXA, oxacillin; PEN, penicillin G; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin+tazobactam; NIT, nitrofurantoin; TCY, tetracycline; NOR, norfloxacin.

coccus faecium (4.97%, chi-square 9.176, $p = 0.002$), and *Candida* sp. and *A. calcoaceticus-baumannii* (3.11%, chi-square 4.312, $p = 0.038$). Other coinfections occurred at a percentage less than 2%, as shown in Fig. 3 and **Supplementary Tables 2,3**. Only four UTI cases were caused by the same three organisms: *Candida sp*., *K. pneumoniae*, and *P. aeruginosa*.

Antibiotic Resistance Profile of Uropathogens

Antimicrobial Profile of UTI Gram-Positive Uropathogens

Among the most prevalent UTI-causing Gram-positive pathogens, *S. agalactiae, E. faecalis*, and *E. faecium* were resistant to several antimicrobial classes (Fig. 4A–D). The resistance rate of *E. faecium* to the beta lactamase inhibitor amoxicillin/clavulanic acid (AMC) was 60.78% , *E. faecium* also exhibited resistance to tetracycline (TCY) at 9.80% and trimethoprim/sulfamethoxazole (SXT) at 3.92% (Fig. 4A). *Staphylococcus aureus* had the highest resistance prevalence to ampicillin (AMP) as the percentage reached 45.83% (Fig. 4B). *Staphylococcus aureus* also showed 33.30% resistance to the second-generation cephalosporin cefoxitin (FOX) (Fig. 4B).

The prevalence of resistance to the first-generation cephalosporins, cephalothin (CEP), and ceftriaxone (CXM) and erythromycin (E) was 8.33% among *S. aureus* isolates (Fig. 4B). The resistance rate of *E. faecalis* to high-level gentamycin (GEN) was 27.90%, followed by resistance to two quinolone antibiotics: levofloxacin (LVX) (20.15%) and ciprofloxacin (CIP) (13.95%) (Fig. 4C). When SXT and clindamycin (CLI) were tested against *S. agalactiae*, the resistance rate to SXT and CLI reached 55.70% and 18.75%, respectively (Fig. 4D).

Antimicrobial Profile of UTI Gram-Negative Uropathogens

AST data for the most common gram-negative uropathogens, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *A. calcoaceticus-baumannii*, *E. cloacae*, and *E. aerogenes* are included (Fig. 5A–G). The resistance rate of AMC among *E. coli* isolates was the highest at 56.74%, followed by CEP (56.28%), CIP (53.95%), SXT (47.67%), ceftazidime (CAZ) (47.67%), cefepime (FEP) (46.74%), and AMP (38.60%) (Fig. 5A).

The resistance rate of *E. aerogenes* was the highest for AMC, with a percentage reaching 70.59%, followed by CEP (64.77%), CIP (52.94%), and FOX (35.21%) (Fig. 5B). Among *K. pneumoniae* isolates, the highest rate of resistance was against CEP (63.72%), followed by ceftazidime (CAZ) (59.59%), cefepime (FEP) (58.99%),

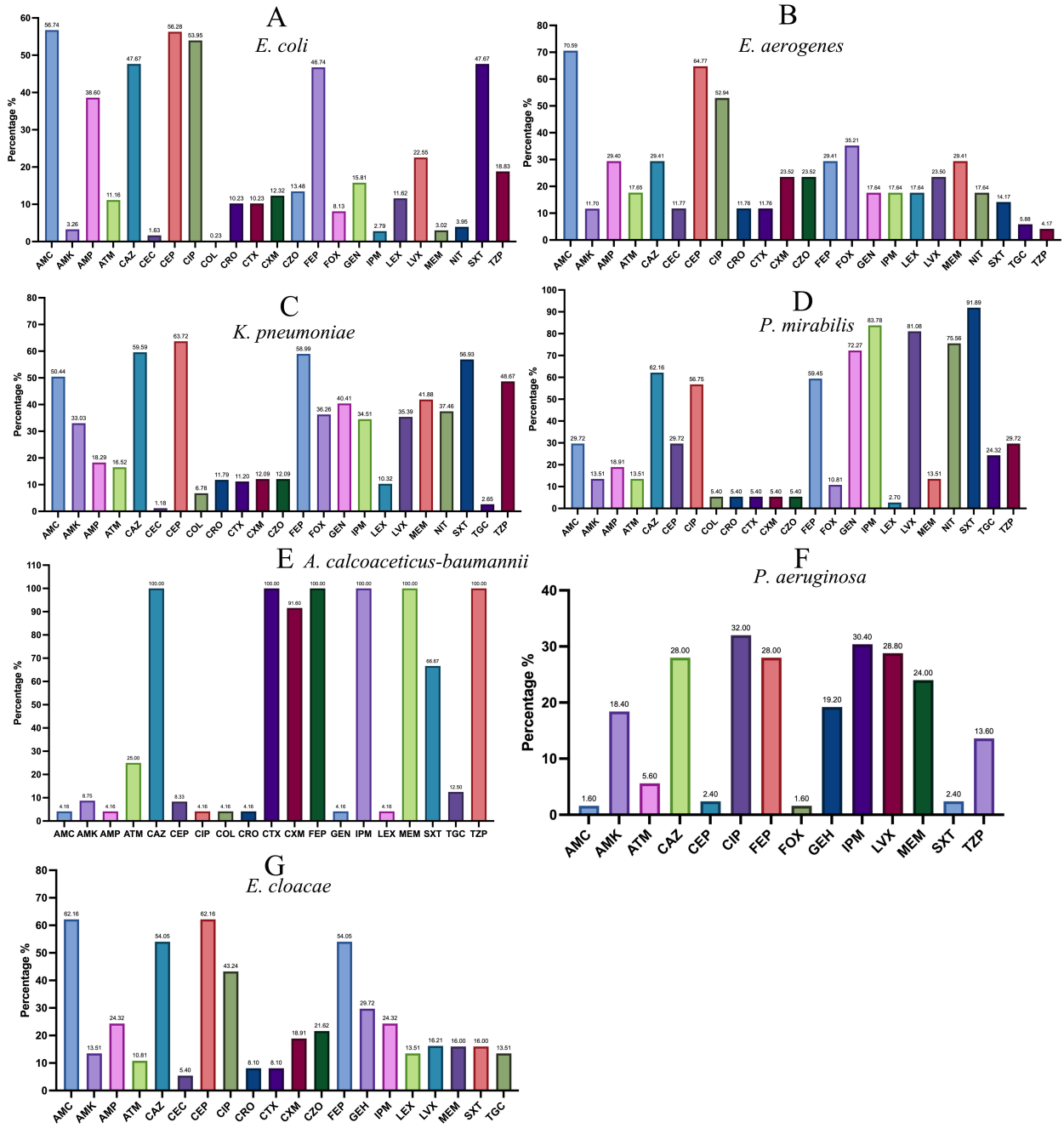

Fig. 5. Prevalence of resistance among the most common gram-negative-UTI pathogens. (A) *E. coli*, (B) *E. aerogenes*, (C) *K. pneumoniae*, (D) *P. mirabilis*, (E) *A. calcoaceticus-baumannii*, (F) *P. aeruginosa*, (G) *E. cloacae*. The resistance percentage was calculated by the number of resistant isolates of specific pathogen divided by the total number of the pathogen. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CEC, cefaclor; CEP, cephalothin; CIP, ciprofloxacin; COL, colistin; CRO, ceftriaxone; CXM, cefuroxime; CZO, cefazolin; CTX, cefotaxime; FOX, cefoxitin; IPM, imipenem; GEN, gentamicin; GEH, high level gentamicin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin+azobactam; MEM, meropenem; NIT, nitrofurantoin; TGC, tigecycline.

SXT (56.93%), AMC (50.44%), piperacillin+tazobactam (TZP) (48.67%), meropenem (MEM) (41.88%), gentamicin (GEN) (40.01%), nitrofurantoin (NIT) (37.46%), FOX (36.26%), levofloxacin (LVX) (35.39%), imipenem (IPM) (34.31%), and amikacin (AMK) (33.03%) (Fig. 5C).

Proteus mirabilis was found to be highly resistant against SXT (91.89%), followed by IPM (83.78%), LVX (81.08%), NIT (75.56%), GEN (72.27%), CAZ (62.16%), FEP (59.45%), and CIP (56.75%) (Fig. 5D). *Acinetobacter calcoaceticus-baumannii* complex was highly resistant to six antibiotics, including CAZ, CTX, FEP, IPM, TZP, and

MEM (100%). They also showed high resistance to SXT (66.67%) (Fig. 5E). *Pseudomonas aeruginosa* showed resistance to CIP (32.00%), IPM (30.40%), LVX (28.80%), FEP (28.00%), CAZ (28.00%), and MEM (24.00%) but at lower rate compared to other Gram-negative uropathogens (Fig. 5F). *Enterobacter cloacae* exhibited high resistance to AMC and CEP as the percentage reached 62.16% (Fig. 5G). Resistance to CAZ and FEP was 54.05%. CIP resistance rate of *E. cloacae* approached 43.24% (Fig. 5G).

Discussion

This retrospective study was conducted to determine the prevalence, etiology, and patterns of antibiotic susceptibility of uropathogens isolated from patients in Al-Madinah Al-Munawarah, Saudi Arabia, between January July 2022 and June 2023. In this study, the prevalence of UTIs was equally presented between males and females (50.57% male and 49.42% female), which is not in line with a previous study [25]. Other studies have reported that the prevalence and incidence of UTI are higher in females than in males due to several clinical factors, including anatomic differences and hormonal fluctuations [26]. The less acidic pH of the vaginal surface in females, proximity of the urethra to the anus, width and length of the urethra, sexual behavior, and poor hygiene practices are specific factors that have been suggested to explain the high prevalence of UTIs among females [27,28]. However, a study conducted in 2012 targeting lower urinary tract infection prevalence in adults reported a higher UTI incidence rate in older males (age range: 51–60 years) with a rate of 54.28%, whereas a lower rate was documented in younger males (age range: 21–30 years) with a percentage of 10% [26]. These disparities may be explained by the sample size, sexual behavior, awareness, hygienic practices, educational attainment, and community traditions [29]. It can also be associated with other health conditions such as diabetes, renal diseases, and urological conditions, or as a result of catheterization due to surgical needs [26].

Herein, UTI-positive cultures showed two types of infections: mono-infection (88.16%) and poly-infection (11.55%). Polymicrobial infections are common in the UT with a high rate reaching 39% [30]. They are commonly found in patients with diabetes, HIV, cancer, indwelling catheter users; the older adults; and immunocompromised individuals. However, young sexually active females are less likely to develop polymicrobial UTIs [30]. The highly polymicrobial microbiota of the GI and reproductive tracts has been proposed as a significant inoculation source, leading to dual-species or polymicrobial UTIs [20]. Another factor associated with polymicrobial UTI is CAUTI biofilms, which are also frequently found to be polymicrobial [31,32]. However, diagnostic laboratories do not consider assessing the antimicrobial susceptibility of polymicrobial infections, which can partially explain the failure of antimicrobial treatment and recurrent infections.

In this study, the most common isolates from the urine samples of our patients were gram-negative bacteria, belonging to *Enterobacteriaceae*, which represented the most prevalent family causing UTIs (66.57%). *Escherichia coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *E. cloacae*, and *E. aerogenes* were the predominant gram-negative bacterial uropathogens that caused UTI. Our results are in line with several reports from Saudi Arabia and worldwide [17,33–36], supporting the dominance of gram-negative bacteria as the causative agents of UTIs. Gram-positive bacteria can also cause UTIs. However, their prevalence is lower than that of gram-negative bacteria. In this study, the predominant gram-positive uropathogens were *E. faecalis*, *S. agalactiae*, *E. faecium*, and *S. aureus*. Numerous studies in Saudi Arabia, Bangladesh, and Pakistan have reported *Enterococcus* spp. as the most frequently isolated gram-positive uropathogenic bacteria, consistent with our findings [37–39]. However, other uropathogens have also been reported as the most common gram-positive bacteria, including *S. aureus* and *S. agalactiae* [17,40]. These discrepancies could be ascribed to variations in the inclusion criteria, sample size, sensitivity of bacterial identification systems, or methodology used for data collection. Another uropathogen identified among UTIs was *Candida* sp., accounting for 2.44 UTI cases. In recent studies, *Candida*-causing UTIs have become more common, particularly in patients who are hospitalized [41]. The risk factors associated with *Candida* spp. with UTIs are numerous, including prolonged hospital stay, admission to intensive care units, female sex, diabetes mellitus, immunosuppressive medications, recent use of broad-spectrum antibiotics, prior surgical procedures (both urological and non-urological), radiation therapy, genitourinary tuberculosis, neutropenia, urinary tract instrumentation, transplantation, abnormalities of the urinary tract, and catheterization [42].

In polymicrobial infections, uropathogens coexist and act synergistically to cause UTIs. In this relationship, one microorganism can predispose the host to allow the colonization and invasion of other pathogens by creating a niche or weakening the host's immune system. The most frequent distinct combinations of microbes causing UT poly-infections mainly involved *K. pneumoniae* with *P. mirabilis*, *P. aeruginosa*, or *E. faecalis*. Additionally, *Candida* sp., *E. faecium* or *A. calcoaceticus-baumannii* coexisted and caused UTI. Polymicrobial UTIs caused by *P. mirabilis* and *K. pneumoniae* have been proposed to result from the development of catheter biofilms or bacterial resistance to antibiotics [43]. *P. mirabilis* is one of the most prevalent microorganisms found during polymicrobial urine colonization and infection [44] and can produce more urease enzymes when co-cultured with other uropathogens [45,46]. *P. aeruginosa* is a common constituent of polymicrobial biofilms and is a potent biofilm-former in polymicrobial infection [47], which is significant for its interaction with other microbes during CAUTI. Additionally, *P. aerug-*

inosa was found to increase its virulence when the peptidoglycan layer was detected in other bacterial cell walls [48]. CAUTIs are among the most common biofilm-associated diseases, and *Enterococcus* spp. largely contribute to them. Relevant features of *Enterococcus* spp. that could explain their role in polymicrobial UTIs include the generation of lactic acid through acidification, attachment of fibrinogen and collagen to promote biofilm formation [49], synthesis of serine proteases and gelatinases [50], and inhibition of natural inflammatory responses [51].

In this study, *E. coli* was identified as a significant contributor to polymicrobial UTIs co-occurring with *P. mirabilis*, *K. pneumoniae*, *E. faecalis*, *Candida* spp., *P. aeruginosa*, and *E. faecium*. Comparison between *E. coli* isolates from monomicrobial cultures to those in polymicrobial UTIs, *E. coli* isolates from polymicrobial infection was statistically more invasive *in vitro* epithelial cell infection assays [52]. According to recent research, *E. coli* infections in polymicrobial UTIs are highly invasive and resistant to eradication due to antimicrobial resistance [53]. Current diagnostic procedures in clinical microbiology for identifying polymicrobial UTIs can be better developed [52]. Hence, reassessment of the current procedures and development of innovative protocols to identify polymicrobial UT infections are warranted.

The emergence of antibiotic resistance in uropathogens is a global public health concern. In our study, all isolates were multi-drug resistant (resistant to three or more drugs). Over 50% of *E. coli* isolates resisted AMC, CEP, CIP, and SXT. These results are consistent with other reports [37,54], that revealed that the *E. coli* resistance rate to these antibiotics ranged from 50–70%. *Enterobacter* spp., specifically *E. aerogenes* and *E. cloacae* resisted almost all the antibiotics tested in our study. Over the past 30 years, *E. aerogenes* and *E. cloacae* have been documented in multiple hospital-acquired outbreaks in Europe, particularly in France [55]. The multi-drug resistance of *Enterobacter* spp. is attributed to their efflux pump mechanism [56]. Despite its higher resistance rate, *K. pneumoniae* exhibited a resistance pattern broadly similar to that of *E. coli*. Remarkably, *K. pneumoniae* isolates (>50% of the isolates) showed resistance to more antibiotics than *E. coli*. Our findings align with those of previous reports [57], highlighting the need for urgent intervention strategies, such as the discovery of novel antibiotics, to overcome the significant increase in drug resistance among *K. pneumoniae*. A previous study has indicated that MEM was the most effective antibiotic, with a sensitivity rate of 100%, whereas NIT was the least effective antibiotic, with a resistance rate of 80% [40] against *Proteus* spp. However, our study showed an increased resistance to both antibiotics. Additionally, a previous study has reported a high sensitivity rate to IPM (90%); in contrast, our findings indicated a high resistance rate to IPM (83.78%) against *Proteus* spp. Additionally, *P. mirabilis* showed high resistance (>50% of the isolates) to other antibiotics such as SXT, LVX, GEN, CAZ, FEP, and CIP, similar to previous findings [58]. Compared with *Proteus* spp., *Acinetobacter* spp. showed high resistance rates to almost all tested antibiotics, parallel to that reported in Northern Saudi Arabia [38]. Among gram-positive bacteria, the emergence of antibiotic resistance was documented in this study, similar to other studies [38]. *Enterococcus* spp. and *E. cloacae* are resistant to numerous antibiotic treatment approaches, such as the beta-lactamase inhibitors AMC, GEN, LVX, and CIP. *Staphylococcus* spp. displayed resistance to ampicillin, second-generation cephalosporins, cefoxitin, erythromycin, and oxacillin, in parallel with the resistance rates in previously published data [30,33]. In this study, *Streptococci* spp. exhibited a high resistance rate against one of the most essential treatment options, SXT and clindamycin, as the resistance rate reached 55.70%, which is significantly higher than the 25% reported in Hai'l, Saudi Arabia [17]. In conclusion, the resistance rates among gram-negative and positive bacteria that cause UTIs are alarming, highlighting the urgent need to develop novel strategies to control the emergence and transmission of antibiotic resistance.

The scope of this study, which must fully account for potential UTI patterns or capture more significant population trends, is a notable limitation. Furthermore, despite the importance of these findings, the country's vast geographic expanse and varied environments may limit their applicability to Saudi Arabia. Additionally, given the specific nature of this retrospective study, we had limited control over the data collected from the patients. Therefore, our study did not capture baseline characteristics, such as race and underlying medical conditions. Furthermore, given the critical importance of carefully examining the antibiotic sensitivity patterns of all isolated bacteria, the problem of antibiotic shortage presents a significant challenge. Therefore, antibiotic availability is crucial for obtaining a complete understanding of antibiograms in a particular community. Physicians must consider the local epidemiological trends and antimicrobial resistance patterns of common uropathogens before starting any empirical antibacterial therapy to optimize therapy and reduce the risk of multidrug-resistant uropathogenic infections. It would also be beneficial to broaden the conclusions of this study by examining the prevalence of UTIs and antimicrobial trends within specific risk groups. It is also important to reconsider polymicrobial UTIs in diagnostic laboratories and develop and implement novel protocols to identify and assess the antimicrobial patterns of such infections.

Conclusion

In this retrospective study, we examined uropathogenic etiology, prevalence, and trends in antibiotic sensitivity. This study may elucidate the best

antimicrobial choices for treating urinary tract infections. Rather than applying general guidelines, we strongly suggest that any empirical antibiotic selection should consider local epidemiological trends and resistance patterns of the most common uropathogens.

Availability of Data and Materials

The data are available upon request in accordance with confidentiality and privacy regulations from the corresponding author.

Author Contributions

Conceptional design of the project and generating figures and tables were performed by AAA. Acquisition of data, data analysis, and interpretation of data were performed by RAA, ESA, WMA, ZIA, MAA. Writing and editing of the manuscript were conducted by RAA, ESA, WMA, ZIA. MAA and AAA reviewed the manuscript. All authors have read and agreed to the published version of the manuscript. All authors have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of King Salman bin Abdulaziz Medical City (General Directorate of Health Affairs in Al-Madinah Al-Munawarah (IRB23-121) and the Scientific Research Ethics Committee at the College of Applied Medical Sciences (2024/185/104 MLT). As the study relied on previously examined laboratory culture data without any direct involvement of patients, a signed informed consent was obtained to reuse and collect the data by the legally authorized representative (the Ethics Committee of King Salman bin Abdulaziz Medical City (General Directorate of Health Affairs in Al-Madinah Al-Munawarah-IRB23-121). Patients' identities remained confidential, and numerical codes were used to represent the cases. All methods used in this study were conducted in compliance with the relevant guidelines and regulations pertaining to studies involving human subjects.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436183.80.

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