antibiotics

MDPI

Proceeding Paper

Epidemiological study of antimicrobial resistance pattern of suspected urinary tract infection in a super surgical, super specialty hospital in Northern India

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Abstract: Background: This study aimed to assess the antimicrobial resistance profiles of urinary tract infection (UTI) collected from individuals of various age groups, both male and female. Methods: This study analyzed 266 urine samples from diverse individuals. Midstream urine samples were collected, transported, and processed on CLED medium within two hours. Bacterial identification was done based on colony morphology, Gram staining, and biochemical characteristics. The VITEK 2 Compact system (Biomerieux, France) was used for antimicrobial susceptibility testing, ESBL detection, and Carbapenemase detection. Results: Out of 61 significant UTIs in both male and female patients, 78.69% were caused by gram-negative bacteria, 11.48% by gram-positive bacteria, and 9.84% by Candida species. *Escherichia coli* (37.70%), *Klebsiella pneumoniae* (26.22%), and *Pseudomonas aeruginosa* (11.47%) were the most common uropathogens. Tetracycline (88.89%) and ceftriaxone (77.14%) showed high resistance, while amikacin (89.36%) and colistin (97.30%) were the most effective against both gram-negative and gram-positive uropathogens. Colistin susceptibility was exclusively observed in cases of multidrug resistance (MDR) and pandrug resistance (PDR). Conclusions: This study indicates that gram-negative bacteria, particularly *E. coli*, are responsible for a higher number of UTIs compared to gram-positive uropathogens. Amikacin and colistin were identified as the most effective antibiotics against both gram-negative and gram-positive uropathogens.

Keywords: Urinary tract infection; uropathogens; antibiotic susceptibility; antimicrobial resistance; gram-positive bacteria; gram-negative bacteria

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1. Background

Urinary tract infections (UTIs) are incredibly prevalent, affecting people of all ages and genders worldwide, with millions of cases reported annually [1]. Among UTI cases, Escherichia coli stands out as the leading cause, responsible for approximately 90% of community-reported cases and 50% of hospital-reported cases [2,3]. In addition to *E. coli*, various other pathogens, such as *Klebsiella* species, *Proteus* species, *Acinetobacter* species, *Pseudomonas* species, *Staphylococcus* species, *Enterococcus* species, and *Streptococcus* species, are also identified as uropathogens [4,5]. Frequent UTI symptoms that patients commonly encounter include dysuria, polyuria, and cystitis [6]. Failing to address UTIs can result in severe health complications, including kidney damage, renal scarring, and renal failure [7]. This issue affects a significant portion of adult females, with approximately 40-50% experiencing UTIs [6]. The increased incidence of UTIs in adult females can be attributed to factors like sexual activity and pregnancy [8]. In women, the prevalence of UTIs tends to rise by 5% over a decade, while the occurrence of UTIs during pregnancy sees an approximate 7% increase [6]. In males, the frequency of UTI cases tends to rise

with age, often due to declining immunity. Additionally, common factors contributing to UTIs in males encompass issues like urethral structure, bladder neck obstructions, bladder stones, bladder tumors, bladder diverticula, enlarged prostate gland and prostate cancer [9].

UTIs are also relatively common in children and infants under the age of approximately 2 years, and can be acquired both in community settings and hospitals [10]. Up to the age of 7 years, approximately 5% of girls and 2% of boy's experience at least one UTI incident [11,12]. Diagnosing UTIs in younger children can be challenging, as they may be unable to articulate their symptoms. Conversely, older children may express urinary discomfort, including a burning sensation during urination, loss of bladder control, increased urination frequency, and malodorous urine, among other complaints [13]. Notably, a higher number of complicated UTI cases are observed in males, while uncomplicated UTIs are typically treated without the need for culture testing and sensitivity analysis [14]. In many instances, UTIs are often characterized as re-infections because the condition re-emerges after several weeks of antibiotic treatment. The primary instigator of these infections is the initial bacterial attachment, which subsequently leads to the formation of a biofilm, offering resistance against the host's immune responses [15]. Numerous studies have reported multiple antimicrobial resistances among uropathogens, with concerns escalating globally, particularly regarding multidrug resistance (MDR) and extended-spectrum beta-lactamases (ESBLs) [6,15].

Consequently, effective management of urinary tract infections and the establishment of antibiotic guidelines are of paramount importance in combatting antibiotic resistance and multidrug resistance. In this context, clinical microbiologists play a crucial role in identifying pathogenic organisms and collaborating with physicians to develop personalized antibiotic therapies for each patient, thus reducing the inappropriate use of antibiotics, dosing errors, and potential drug interactions. Ultimately, this approach enhances the quality of care provided to patients. Therefore, the primary goal of this study is to assess the antimicrobial susceptibility patterns in positive UTI samples isolated from both male and female patients.

2. Methods

2.1. Study Population

The study took place from January to July 2023 at the Microbiology Department of Sanar International Hospitals in Gurgaon, India. 266 samples were collected from diverse urine samples, with 161 from male patients and 105 from female patients, both from the outpatient and inpatient departments.

2.2. Sample Collection

Clean-catch midstream urine (MSU) samples were collected from suspected UTI patients using 20 ml sterile screw-capped containers (BD Urine Collection Kit). To prevent bacterial growth, samples were transferred to a container where boric acid was added (0.2 mg). Aseptic sample collection instruction was provided to patients for proper urethral sample collection [16].

2.3. Sample Processing

A 4 mm nichrome wire inoculating loop was used to inoculate urine samples on Cysteine lactose electrolyte deficient (CLED) agar medium, with an inoculum size of 0.01 ml. The culture plates were then incubated at 37℃ for 24 to 48 hours. After incubation, plates were checked for clear, distinct bacterial growth. If no colonies were visible, the incubation extended by 24 hours. To confirm positive urine cultures, we relied on colony counts exceeding $>10^3$ to 10^5 CFU/ml, indicating significant bacteriuria.

2.4. Identification and Sensitivity

Positive bacterial isolates were identified by their colony morphology, Gram staining, and biochemical characteristics. Gram-negative isolates were identified using the VITEK 2 Compact machine with the GN ID-card, while Gram-positive isolates and yeast-like cells like Candida were identified using GP ID-card and YST ID-card in the VITEK 2 Compact machine, respectively.

Antimicrobial susceptibility testing was conducted using the widely recognized VITEK 2 Compact system. To maintain consistency, we prepared a McFarland standard inoculum with an OD of 0.5, which was assessed with a Vitek Densicheck display base machine (Ref. No- 422220) following established standards. The testing procedure followed the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) in 2022. Vitek cards were used for antimicrobial susceptibility testing, including AST-N405, AST-406, and AST-407 for gram-negative bacteria, AST-P628 for gram-positive bacteria, and AST-YS08 for Candida. The VITEK 2 Compact system was used to detect ESBL production, which was confirmed through a synergistic assay between AMC and a 3rd or 4th generation cephalosporin.

3. Results

Over a span of 7 months, 266 urine samples from individuals suspected of urinary tract infections (UTIs) were investigated. Among these samples, 161 (60.53%) were from male patients, and 105 (39.47%) were from female patients. The analysis revealed 61 (22.93%) significant UTI cases in both male and female patients. When examining positive bacterial isolates, a higher occurrence was observed in female patients, with 34 (55.74%) cases, compared to 27 (44.26%) in male patients. The positive isolates were categorized into four age groups: 1-16, 17-35, 36-60, and over 60 years. The percentage of UTI occurrence in female samples was highest (83.33%) in the 1-16 age group and lowest (37.50%) in the 36-60 age group, indicating a decrease in UTI occurrence with increasing age. Conversely, for male-positive samples, the UTI occurrence was highest (62.50%) in the 36-60 age group and lowest (16.67%) in the 1-16 age group. This suggests that the trend of UTI occurrence in male samples generally increased with age, except for the 1-16 age group, but decreased in the over 60 age group (29.41%). A total of 61 uropathogenic microorganisms were identified, comprising 48 (78.69%) gram-negative bacteria, 7 (11.48%) gram-positive bacteria, and 6 (9.84%) *Candida* species. Among the isolated uropathogens, *Escherichia coli* emerged as the most prevalent, accounting for 37.70% of the cases. The second most frequently isolated microorganism was *Klebsiella pneumoniae* (26.22%), followed by *Pseudomonas aeruginosa* (11.47%), *Enterococcus faecalis* (4.91%), *Candida albicans* (4.91%), *Candida tropicalis* (4.91%), *Citrobacter koseri* (3.27%), *Staphylococcus haemolyticus* (3.27%), *Enterococcus gallinarum* (1.63%), and *Staphylococcus hominis* (1.63%), as illustrated in Figure 1.

The antibiotic susceptibility results revealed that tetracycline displayed the highest resistance (88.89%) among all the uropathogens, followed by ceftriaxone (77.14%). Conversely, colistin (97.30%) and amikacin (89.36%) exhibited the highest sensitivity for both groups. Detailed antibiotic susceptibility data are presented in Figure 2.

Escherichia coli exhibited remarkable resistance to ceftazidime (75.00%) and ciprofloxacin (73.91%), while it displayed its highest sensitivity to tigecycline, colistin, and nitrofurantoin (100.00%) (Figure 3). In the case of *Klebsiella pneumoniae*, resistance was prevalent against ceftazidime (100.00%) and cefuroxime (83.33%), but susceptibility was observed towards amikacin (93.33%), colistin (92.86%), and fosfomycin (71.43%). *Pseudomonas aeruginosa*, on the other hand, showed a high susceptibility to nitrofurantoin, colistin, and aztreonam (100.00%), but proved resistant to ceftazidime and levofloxacin (60.00%) (Figure 3). *Enterococcus faecalis* exhibited an extensive resistance to tetracycline (100.00%), but complete susceptibility to nitrofurantoin, vancomycin, and linezolid (100.00%) (Figure 3).

Notably, *Candida albicans* and *Candida tropicalis* demonstrated complete sensitivity to all tested antibiotics (amphotericin B, caspofungin, flucytosine, fluconazole, micafungin, vancomycin).

In terms of resistance mechanisms, 30.43% of *Escherichia coli* and 18.75% of *Klebsiella pneumoniae* among the total isolates were extended spectrum β-lactamase producers. Additionally, 17.39% of *Escherichia coli* and 56.25% of *Klebsiella pneumoniae* were identified as carbapenemase producers.

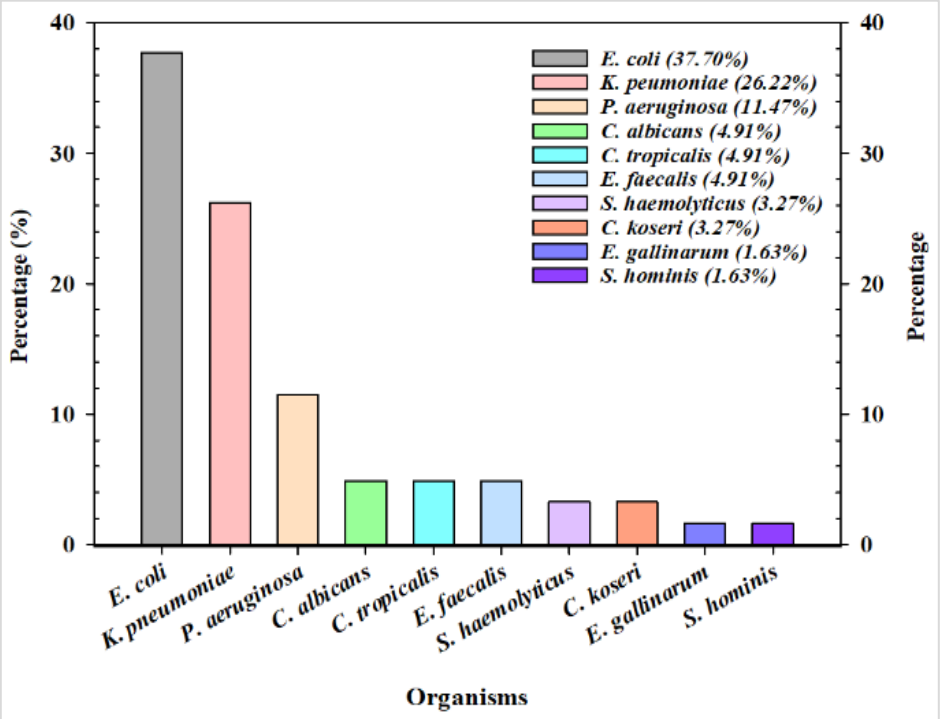

Figure 1. Occurrence of positive organisms in urine samples.

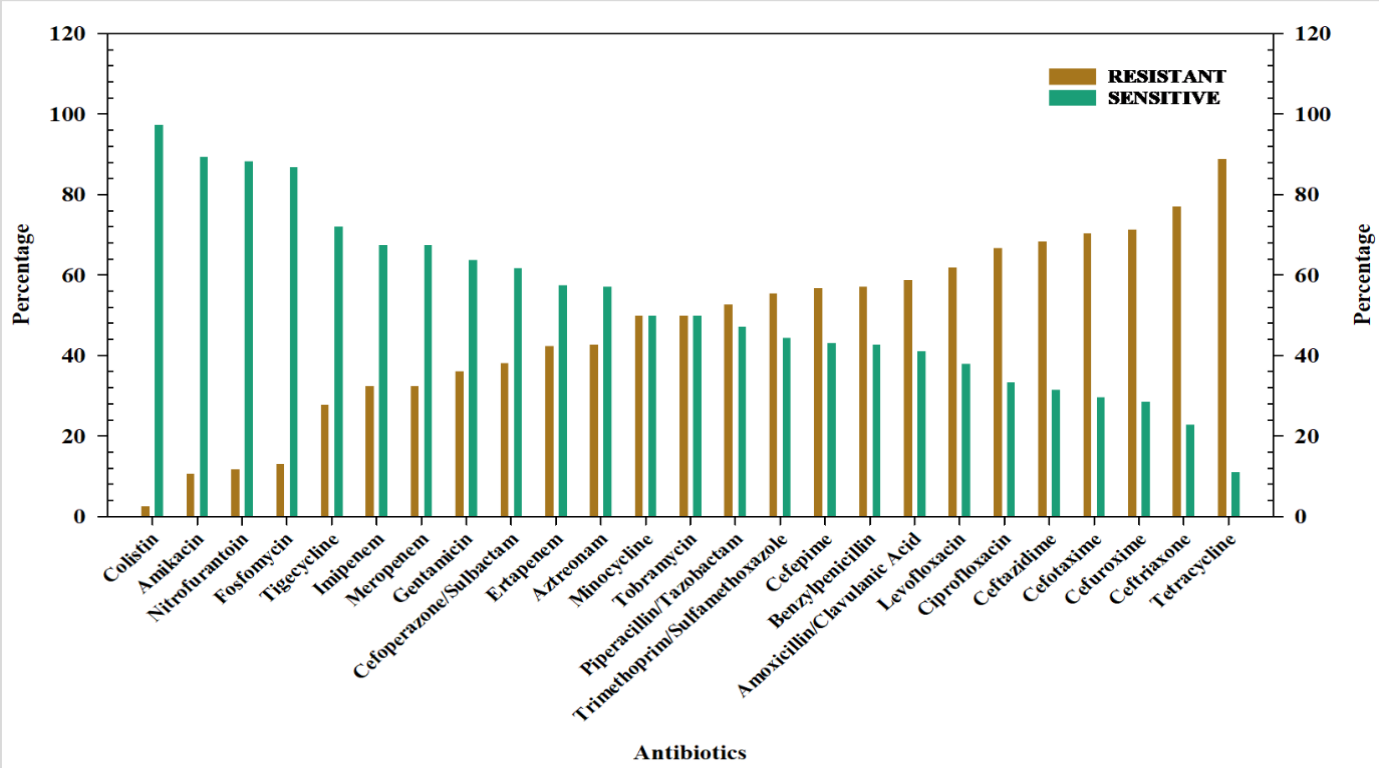

Figure 2. Overall, antibiotic susceptibility pattern observed in positive urine samples.

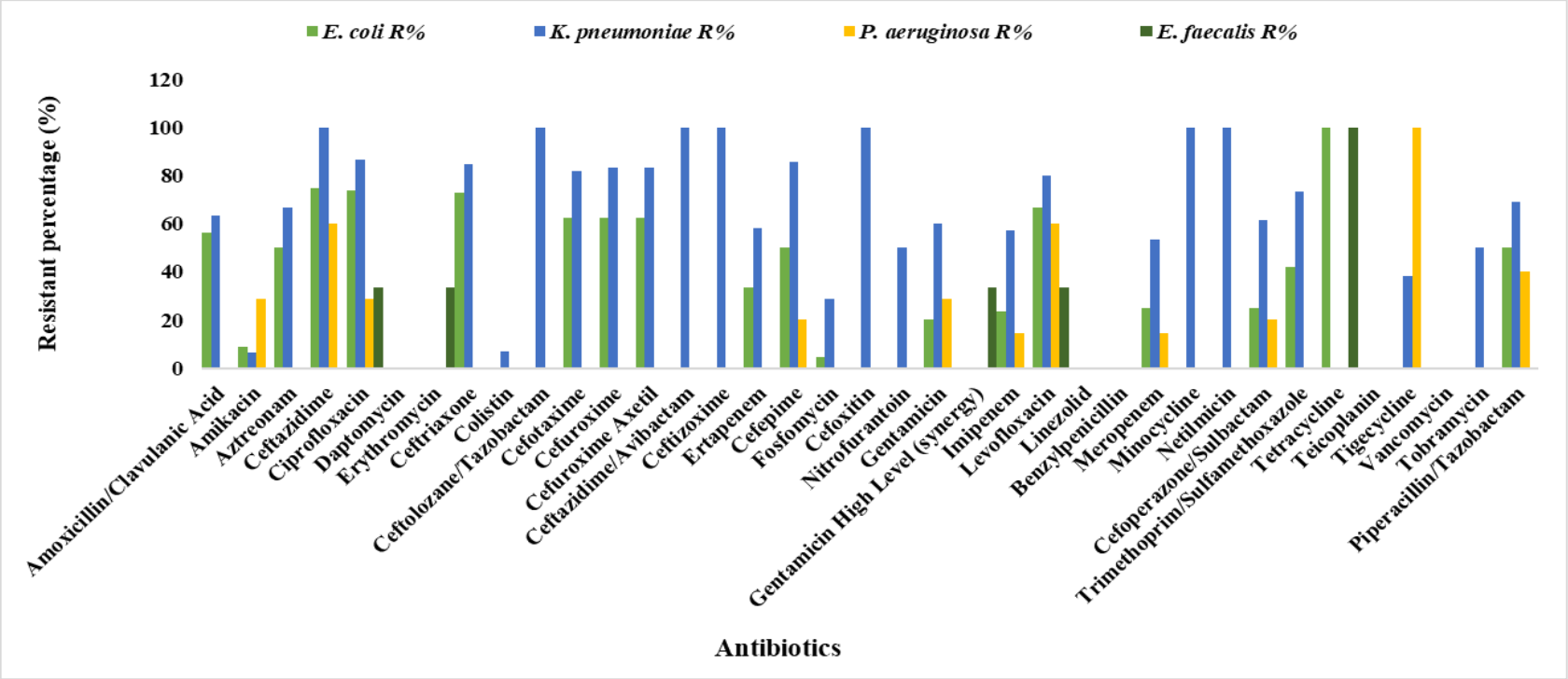

Figure 3. Antibiotic resistance pattern against organisms.

4. Discussion

The investigation aimed to assess the rising trend of antimicrobial resistance among uropathogens and enhance effective treatment strategies. Antimicrobial resistance has been on the rise globally over time [17]. In our study, the overall UTI occurrence was 22.93%, with some studies reporting lower (e.g., 13.9%, 17%, 17.26%) and others reporting higher (e.g., 23.7%, 32.7%, 53.82%) rates [17–22]. We observed a higher UTI occurrence in females (55.74%) compared to males (44.26%), consistent with other studies [23,24]. Among males, UTI cases increased with age, possibly due to weakened immunity, prostate issues, or prostate enlargement [11,17]. The age-wise distribution of susceptible UTI patients in females ranged from >60 years (35.29%), 17-35 years (32.35%), 36-60 years (17.64%), to 1-16 years (14.70%). For males, the distribution was 17-35 years (40.74%), 36-60 years (37.03%), >60 years (18.51%), and 1-16 years (3.70%). In Ethiopia, a similar study found the highest UTI prevalence in the >45 age group (23.4%), while a Ugandan study reported the highest occurrence in the 20-29 age group (32.6), differing from our findings [25,26]. Another study in 2021 reported the highest frequency in the 21-30 age group (22.8%) [27]. We found a 78.69% occurrence of gram-negative bacteria and 11.48% of gram-positive bacterial isolates, differing from other studies [28,29]. *Escherichia coli* was the predominant uropathogen (37.70%), followed by *Klebsiella pneumoniae* (26.22%), and *Pseudomonas aeruginosa* (11.47%) (Figure 1). While previous research has explored the prevalence of *E. coli* as a uropathogen and reported higher rates, such as 50.09% [30], 50% [28], and 42.7% [21], it's important to note *that E. coli* and *K. pneumoniae* collectively account for over 80% of UTI cases globally, occurring in both community and hospital-acquired infections [31,32]. Tetracycline exhibited the highest resistance (88.89%), while colistin showed the highest sensitivity (97.30%) [14,33,34]. Notably, the treatment of uncomplicated UTIs without culture and susceptibility reports has contributed to increased antimicrobial resistance [35]. Among *Escherichia coli* isolates, 75.00% were resistant to ceftazidime, while nitrofurantoin, tigecycline, and colistin were highly effective (100.00%). In a study conducted in Northeast Ethiopia by Adugna and collaborators, they also found that tetracycline exhibited the highest resistance rate at 65.70%, while colistin was identified as the most effective drug with a 100.00% success rate against *E. coli* [21]. *Klebsiella pneumoniae* showed 100.00% resistance to ceftazidime but high sensitivity to Amikacin (93.33%), colistin (92.86%), and fosfomycin (71.43%) which is comparable to other studies also [36,37]. *Pseudomonas aeruginosa* was highly resistant to ceftazidime and levofloxacin (60.00%) but sensitive to nitrofurantoin, colistin, and aztreonam (100.00%). Linezolid and nitrofurantoin (100.0%) were effective against *enterococcus faecalis*, while tetracycline exhibited 100.00% resistance.

The majority of organisms showed resistance specially to the beta-lactam antibiotics, but on the other hand, recent drugs like amikacin, colistin, nitrofurantoin, and fosfomycin proved effective in fighting uropathogens.

5. Conclusion

Our findings reveal a captivating picture of urinary tract infections, where Gram-negative organisms, with *E. coli* at the forefront, take center stage as the most prevalent culprits. In the dynamic landscape of antibiotic susceptibility, tetracycline consistently presents a formidable opponent, while on the opposite end of the spectrum, colistin and amikacin emerge as the champions, demonstrating their unparalleled effectiveness. The escalating challenge of antimicrobial resistance finds its roots in various factors, including the intricate battle against UTIs in cancer patients and the complexities of treating intricate urinary tract infections. Equally noteworthy is the practice of addressing uncomplicated UTIs without the invaluable guidance of culture and susceptibility reports, inadvertently contributing to the formidable rise in resistance. Delving deeper, a constellation of risk factors comes into play, with patient age, gender, diabetes, catheter use, immune

compromise, and prior antibiotic history all influencing the heightened susceptibility to urinary tract infections. Amid this intricate tapestry of factors, bacteriological culture stands as a beacon of diagnostic significance. It not only confirms the presence of infections but also plays a pivotal role in the crucial identification of the responsible microorganisms.

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Institutional Review Board Statement: The present study protocol was reviewed and approved by Medical Superintendent, Sanar International Hospitals Gurugram (Ref. No: SIH010622/SR01) Consent for inclusion of the samples for the study was taken by the patients at the time of sample collection.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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