Original Research Article

ANTIMICROBIAL PROFILES OF UROPATHOGENS IN A TERTIARY CARE HOSPITAL OF NORTHWESTERN INDIA

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Absract

Background: Urinary tract infections (UTIs) are extensively studied due to their high prevalence and potential severity, particularly concerning antimicrobial properties.. This study aimed to evaluate the prevalence of common uropathogens and their antimicrobial resistance.

Method: A prospective cross-sectional study was conducted on a total of 16,077 urine samples at a tertiary care hospital in Patiala, Punjab, from January 2022 to December 2022 using standardized microbiological methods. The isolated microorganisms underwent antibiotic resistance testing using the Kirby-Bauer disk diffusion method.

Results: A total of 16,077 urine samples were analyzed, among gram negative microorganism Esch.coli (n=1962) was the most prevalent microorganism, followed by Klebsiellapneumoniae (n=722), Pseudomonas aeruginosa (n=572), Acinetobacter Baumanni Complex(451), Citrobacter species (n=132), Proteus spp. (n=96), and Enterobacter spp. (n=12). Among gram positive Enterococcus spp. (n=786) was most prevalent followed by Staphylococcus aureus (n=420), MRSA (n=30), Antibiotic profile data revealed that gram negative have high resistance rates towards Ampicillin, Ciprofloxacinand Amoxicillin-clavulanateand among gram positive resistance rate was higher in Ampicillin, Ciprofloxacin Amoxicillin-clavulanate, Gentamicin, Levofloxacin, Erythromycin and Netilmicin.

Conclusion: Esch. coli and Enterococcus spp. emerged as the predominant uropathogen, exhibiting a high level of antibiotic resistance against various antibiotics.

Keywords: Antibiotic resistance; Urinary tract infections; Uropathogens; gram negative;gram positive; Healthcare-associated infections

Introduction:

Urinary tract infection (UTI) stands as the most prevalent bacterial infection, contributing to 25.0% of all reported infections [1]. It constitutes a significant cause of morbidity and ranks as the second most frequent reason for hospital visits. Approximately 35% of healthy women experience UTI symptoms at some point in their lives [2]. Esch.coli, a part of the normal bowel flora, is the primary causative agent, accounting for over 75% of UTI cases [3]. Esch.coliis increasingly implicated in both community-acquired and hospital-acquired infections, facilitated by its ability to bind selectively to uroepithelial cells using P fimbrine [4]. UTIs present with symptoms such as fatigue, dysuria, urgency of urine, and urinary tract irritation. Prolonged hospital stay and indiscriminate antibiotic usage are the risk factors [5]. Other pathogens such as Gram-negative Enterobacteriaceae, Gram-positive Enterococcus faecalis, and Staphylococcus saprophyticus contribute to the remaining UTI cases. Although UTI affects both genders, it is more prevalent in women due to their shorter urethra and its proximity to the anus, with exceptions observed in older men above the age of 60 with prostatic hypertrophy [6].

UTIs associated with Esch. coli lead to significant morbidity and long-term consequences, affecting approximately 10% of individuals globally [7]. These infections can manifest as asymptomatic or symptomatic, posing a substantial health burden. However, the effectiveness of antibiotics in controlling these infections is diminishing due to the widespread resistance of bacterial uropathogens to various antibiotics. Early detection of antibiotic resistance and the formulation of appropriate antibiotic policies are crucial for effective management in tertiary care settings [8].

Given the geographic variability in the prevalence of resistance strains, understanding the current levels of antimicrobial resistance among common urinary pathogens is essential [9]. Establishing institutional antibiotic policies can assist clinicians in selecting the most appropriate treatment strategies [10]. Therefore, this study aimed to investigate the prevalence and antibiotic sensitivity patterns of uropathogens in urine samples, with the findings intended to inform antibiotic policies and control measures.

Material and Methods:

A retrospective cross-sectional study was conducted in the Department of Microbiology in a tertiary care hospital in Patiala from January 2022 to December 2022. A total of 16,077 urine samples were processed, including 577 samples from the ICU, 7500 samples from the outpatient unit, and 8000 samples from the inpatient unit. The conventional loop method, a semi-quantitative technique, was used to cultivate urine. The organisms isolated from urine culture were identified by standard methods1. The antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines8. Antibiotics used for gram negative organism were Ampicillin (10µg), Amikacin (30µg), Gentamic in (10µg), Ciprofloxacin (5µg), Levofloxac in (5µg) Ofloxacin (5µg), Norfloxacin (10µg), Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefepime (30µg), Piperacillin- Tazobactum (100/10µg), Nitrofurantoin (300µg), Cotrimoxazole (25µg), Imipenem (10µg), Meropenem (10µg. For gram positive microorganism Ampicillin (10µg), Amoxycillin (30 µg), Amoxy-Clav (20/10µg), Erythromycin (15µg), Clindamycin (2µg), Netilmicin (30µg), Linezolid (30µg) and Vancomycin (30µg) antibiotics were used.If an isolate was discovered to be resistant to three or more antibiotics from various classes or groups of antibiotics, it was deemed multidrug resistant (MDR). Bacterial isolates were identified using standard microbiological protocols, and antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Various antibiotics were tested, and zone sizes were interpreted based on established criteria.

Results: The findings presents the distribution of various bacterial species isolated from clinical samples, highlighting their prevalence and contribution to microbial infections. Understanding the prevalence of these organisms is essential for effective clinical management and antimicrobial stewardship programs. Table 1 in the current data indicated that among the 16,077 urine samples analyzed, a notable 32.23% (5183 samples) were culture positive. Among gram negative microorganism the study identified Esch. colias the most prevalent organism with 1962 isolates (37.86%), followed by the second most prevalent Klebsiella pneumonia with 722 isolates (13.93%,), Pseudomonas aeruginosa with 572 isolates (11.03%), Acinetobacterbaumanni complex with 451 isolates (8.70%,), and among gram positive microorganism Enterococcus spp., with 786 isolates (15.17%) was found to be most prevalent followed by Staphylococcus aureus with 420 isolates (8.10%). The less prevalent organisms isolated from urine samples were Citrobactersppwith 132 isolates (2.55%), followed by Proteus spp. with 96 isolates (1.85%), andEnterobacterspp 12 isolates (0.23%) among gram negative microorganism and MRSA with 30 isolates (0.58%) among gram positive microorganism as shown in Table 1 and Figure 1.

Organism	Gram staining	Number
E. Coli	Gram negative microorganism	1962
Kiebsiella Pneumonia	Gram negative microorganism	722
Pseudomonas Aerogenosa	Gram negative microorganism	572
AcinetobacterBaumanni Complex	Gram negative microorganism	451
Citrobacter Species	Gram negative microorganism	132
Proteus Spp	Gram negative microorganism	96
EnterobacterSpp	Gram negative microorganism	12
Enterococcus Spp	Gram positive microorganism	786
Staphylococcus Aureus	Gram positive microorganism	420
MRSA	Gram positive microorganism	30
Total		**5183**

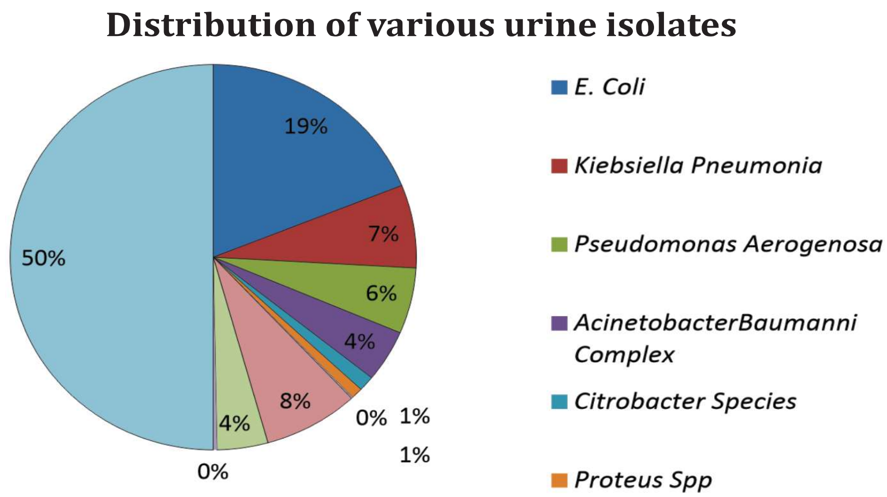

Figure 1: Distribution of various urine isolates Among the total cases, 5,233 (32.55%) were from male patients, and 10,844 (67.45%) were from female patients (Figure 2).

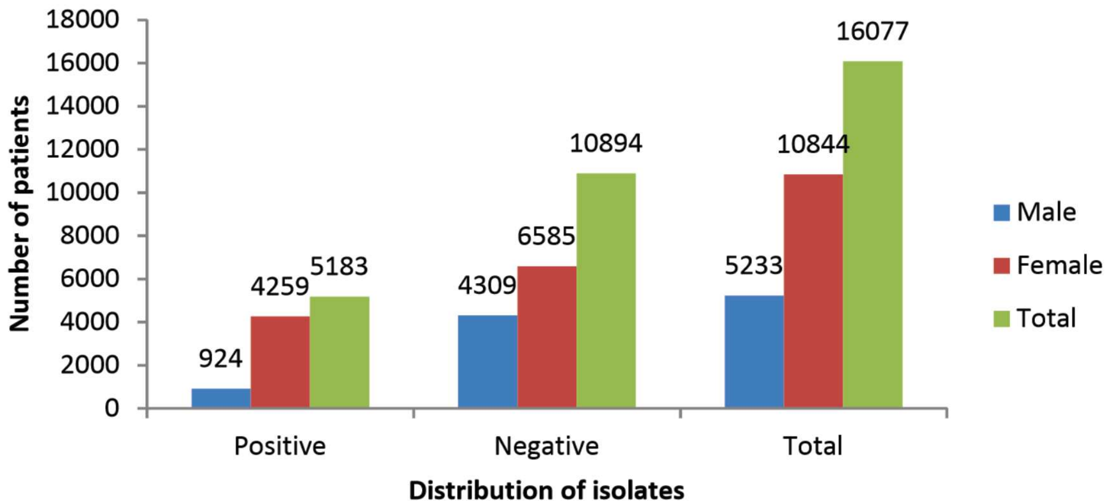

Figure 2: Gender wise distribution of culture positive cases among the study population (n=16,077) Out of 5183 culture positive cases , urinary tract infection was more common in the age group 20-40 years (n=2825) 55%, followed by 40- 60 years (n=1268) 24.5%, above 60 years (n=951) 18.3% and the age group 0-20 years (n=139) 2.7% . Additionally, females tend to have a higher prevalence of UTIs across all age groups compared to males (Figure 3).

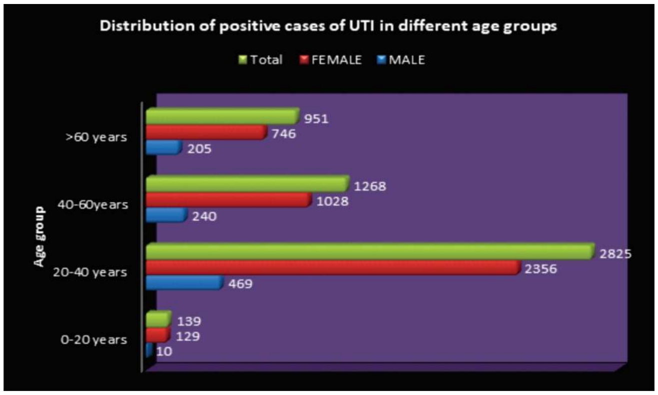

Figure 3: Distribution of positive cases of UTI in different age groups (n=5183)

Table 2 presents data on the antimicrobial sensitivity patterns of Gram-negative isolates obtained from urine samples, categorized by different antibiotics and bacterial species. Among gram negative bacteria, Esch.coli and Klebsiellapneumoniae showed high sensitivity towards Imipenem (100%), Meropenem, Nitrofurantoin (83%) and Amikacin (80%). However, it displayed lower sensitivity to Ampicillin, Ciprofloxacin and Amoxicillin-clavulanate, with sensitivities ranging from 7.2% and 20%.

Table 2: Antimicrobial Sensitivity Pattern of Gram Negative Isolates from Urine samples

Antibiotic	*Esch. coli*	*Kiebsiella Pneumonia*	*Pseudomonas Aerogenosa*	*Acinetobacter Baumanni Complex*	*Citrobacter Species*	*Proteus Spp*	*Enterobacter Spp*
Ampicillin	141 (7.2%)	43 (5.9%)	291 (51%)	nil	17 (13%)	4 (5.2%)	5 (44%)
Amoxy–clav	372 (19%)	238 (33%)	297 (52%)	nil	19 (15%)	10 (10.5%)	nil
Amikacin	1569 (80%)	599 (83%)	160 (28%)	293 (65%)	81 (62%)	92 (96%)	nil
Gentamicin	902 (46%)	353 (49%)	228 (40%)	nil	67 (51%)	85 (89%)	9 (78%)
Ciprofloxacin	392(20%)	180(25%)	200 (35%)	nil	76 (58%)	81 (85%)	9 (75%)
Levofloxacin	1020 (52%)	231 (32%)	211 (37%)	nil	79 (60%)	43 (45%)	4 (38%)
Ofloxacin	1079 (55%)	209 (29%)	286 (50%)	nil	80 (61%)	39 (41%)	4 (38%)
Norfloxacin	824 (42%)	325 (45%)	314 (55%)	nil	75 (57%)	55 (58%)	5 (41%)
Cefatzidime	1039 (53%)	361 (50%)	280 (49%)	nil	72 (55%)	57 (60%)	4 (38%)
Cefuroxime	961 (49%)	339 (47%)	291 (51%)	202 (45%)	79 (60%)	51 (54%)	6 (45%)
Cefotaxime	1059 (54%)	368 (51%)	303 (53%)	189 (42%)	76 (58%)	47 (49%)	7 (58%)
Ceftrioxane	981 (50%)	353 (49%)	308 (54%)	193 (43%)	85 (65%)	52 (55%)	6 (45%)
Cefepime	nd	nd	572 (100%)	nd	nd	nd	6 (45%)
Piperacillin+ Tazobactem	1138 (58%)	563 (78%)	480 (84%)	nd	112 (85%)	81 (85%)	8 (65%)
Nitrofurantoin	1628 (83%)	577 (80%)	Nd	nd	116 (88%)	nd	nd
Cotrimoxazole	412 (21%)	223 (31%)	Nil	nd	39 (30%)	9 (10.2%)	nil
Imipenem	1962 (100%)	722 (100%)	572 (100%)	451 (100%)	132 (100%)	96 (100%)	11 (89%)
Meropenem	1962 (100%)	722 (100%)	573 (100%)	452 (100%)	133 (100%)	97 (100%)	10 (85%)

In contrast, Pseudomonas aeruginosa showed lower sensitivity to some antibiotics like Amikacin (28%), Ciprofloxacin (35%), Levofloxacin (37%),Gentamicin (40%) and Cefatzidime (49%). It showed high sensitive towards Imipenem (100%), Meropenem (100%) and Piperacillin-tazobactam (84%). Acinetobacterbaumannii complex displays mixed sensitivity patterns, with notable susceptibility to Amikacin, Ciprofloxacin, Piperacillin-tazobactam, Imipenem, and Meropenem ranging from 40% to 65%. Citrobacterspp. and Enterobacter spp. show moderate to high sensitivity to Piperacillin-tazobactam, Nitrofurantoin, Imipenem, and Meropenem ranging from 80% to 100%, while resistance is observed against Ampicillin and Amoxicillin-clavulanate, with variable susceptibility to certain Cephalosporins.Proteus spp. exhibits moderate to high sensitivity to Amikacin, Ciprofloxacin, Gentamicin, Piperacillin-tazobactam, Imipenem, and Meropenem. However, resistance rates against Ampicillin, Amoxicillin-clavulanateand Cotrimoxazole are significant. The overall moderate sensitivity of all gram negative isolates was shown towards Norfloxacin, Cefuroxime, cefotaxime and ceftrioxane ranging from 40% to 60%.

Table 3: Antimicrobial Sensitivity Pattern of Gram positive Isolates from Urine samples.

Antibiotic	***Enterococcus Spp***	***Staphylococcus Aureus***	***MRSA***
Ampicillin	440 (56%)	235 (56%)	nil
Amoxy–clav	354 (45%)	218 (52%)	8 (28%)
Amikacin	252 (32%)	201 (48%)	23 (78%)
Gentamicin	385 (49%)	nil	6 (20%)
Ciprofloxacin	110 (14%)	nil	nil
Netilmicin	432 (55%)	210 (50%)	10 (35%)
Erythromycin	463 (59%)	201 (48%)	9 (30%)
Cefepime	Nd	nd	nd
Nitrofurantoin	628 (80%)	252 (60%)	21 (70%)
Clindamycin	526 (67%)	247 (59%)	nd
Linzeolid	754 (98%)	390 (93%)	25 (85%)
Vancomycin	786 (96%)	399 (95%)	26 (88%)

Table 3 provides the antimicrobial sensitivity pattern of Gram-positive bacterial isolates obtained from urine samples. Enterococcus spp. exhibits moderate (40% to 60%) to high (80% to 100%) sensitivity to several antibiotics, with notable susceptibility to Amikacin, Amoxy-clav, Erythromycin, Netilmicin, Gentamicin, Levofloxacin, Nitrofurantoin, Linzeolid, Vancomycinand Teicoplanin. Resistance is observed against Ampicillin and Ciprofloxacin. Staphylococcus aureus shows high sensitivity to Nitrofurantoin (60%), Linzeolid (93%)and Vancomycin. (95%), Complete resistance was observed against Ciprofloxacin, Gentamicin while moderate resistance was observed against Erythromycin (48%) and Amikacin (48%). MRSA (Methicillin-Resistant Staphylococcus aureus) exhibits reduced sensitivity to many antibiotics compared to Staphylococcus aureus. It showed susceptibility to Amikacin (78%), Nitrofurantoin (70%), Linzeolid (85%), and Vancomycin (88%). Resistance rates of gram positive microorganism against Ampicillin, ciprofloxacin, Amoxicillin-clavulanate, Gentamicin, Levofloxacin, Erythromycin and Netilmicin are high.

Discussion:

In the present study, bacterial isolates obtained from urine samples were analyzed to investigate their antibiotic susceptibility patterns in Northwest India. Our findings are consistent with previous research, which consistently identifies Escherichia coli as the predominant pathogen responsible for urinary tract infections (UTIs) in both hospital settings and the general population [11]. In a study conducted in eastern Nepal also reported a high incidence of Esch. coli (53.57%), Klebsiella spp(14.29%) and Enterococcus (11.90%) [12]. Moreover, a study conducted in southern India found similar results, with Esch. coli being the most prevalent pathogen causing UTIs, followed by Klebsiella pneumoniae and Enterococcus species [13]. In North India, a study conducted in Delhi reported Escherichia coli as the predominant pathogen causing UTIs, followed by Klebsiella pneumoniae and Enterococcus species [14]. Similarly, a study in Punjab found Esch. coli to be the most common pathogen associated with UTIs, corroborating our findings [15].

The present study unveiled that middle-aged female patients demonstrated elevated rates of Esch. coli infections in the urinary tract compared to male patients. This observation mirrors findings from Mumbai, where a notable proportion of middle-aged female patients were diagnosed with bacterial infections compared to their male counterparts. Similar patterns were identified in studies conducted at Aligarh University and in Bangladesh [16-18].

Additionally, research conducted in other regions has also reported similar trends in gender-specific prevalence of Esch. coli infections. A study conducted in Karachi, Pakistan, found a higher incidence of urinary tract infections caused by Esch. coli among middle-aged women as compared to men [19]. Similarly, in a study conducted in Sri Lanka reported a higher prevalence of Esch. coli urinary tract infections among females, particularly in the middle-aged group [20]. Furthermore, a study in Nigeria found a higher prevalence of Esch. coli urinary tract infections in women aged 40-60 years compared to men in the same age group [21].

These studies collectively reinforce the observed gender-specific differences in the prevalence of Esch. coli infections in the urinary tract, particularly among middle-aged women, and highlight the need for targeted interventions in this demographic group.

The antimicrobial sensitivity profiles among Gram-negative bacteria provide crucial insights into the evolving landscape of antimicrobial resistance (AMR) and therapeutic options. In concordance with previous studies, Esch.coliand Klebsiellapneumoniae exhibited pronounced susceptibility to carbapenems like Imipenem and Meropenem, underlining the continued efficacy of these agents against Enterobacteriaceae [22, 23]. Similar findings regarding the high rates of antibiotic resistance in Esch.coli have been reported in various regions of India, reflecting a concerning trend of increasing antimicrobial resistance among bacterial pathogens nationwide. For instance, a study conducted in Pondicherry, South India, highlighted the prevalence of multidrug-resistant Escherichia coli isolates [24]. However, the documented decline in susceptibility to conventional antibiotics like Ampicillin, Ciprofloxacin, and Amoxicillin-clavulanate underscores the pressing need for judicious antimicrobial prescribing practices to mitigate the development of resistance [25, 26]. A study by Kapoor et al. demonstrated high rates of resistance among Gram-negative bacteria, including Esch. coliand Klebsiella pneumoniae, to commonly prescribed antibiotics such as Ampicillin, Ciprofloxacin, and third-generation cephalosporins [27]. Similarly, a retrospective analysis by Mendiratta et al. revealed increasing resistance trends among Pseudomonas aeruginosa isolates, emphasizing the need for judicious antimicrobial prescribing practices and infection control measures conversely, the variable susceptibility patterns observed in Pseudomonas aeruginosa underscore its adaptability, necessitating tailored treatment strategies guided by species-specific susceptibility data [28, 29]. Furthermore, the emergence of mixed sensitivity profiles in Acinetobacter baumannii complex emphasizes the multifaceted challenges associated with managing infections caused by this pathogen, urging the exploration of alternative treatment modalities [30, 31].

Among gram positive organism, the present study has corroborated with global trends, demonstrating moderate to high sensitivity of Enterococcus spp. to various antibiotics, including Ciprofloxacin, Levofloxacin, and Nitrofurantoin [32, 33]. Similarly, Staphylococcus aureus exhibited susceptibility to certain antibiotics, notably Piperacillin-tazobactam and carbapenems, consistent with international findings [34, 35, 36]. Nonetheless, the emergence of Methicillin-Resistant Staphylococcus aureus (MRSA) poses significant challenges, with reduced sensitivity to multiple antibiotics, necessitating the exploration of alternative treatment modalities [37].

Conclusion: Our study reaffirms that Esch. coli remain the predominant pathogen responsible for infections across both urban and rural populations. Notably, we identified substantial rates of resistance to commonly prescribed antibiotics among urinary pathogens, signaling a worrisome trend of antimicrobial resistance (AMR) in community settings throughout India. The findings emphasize the urgent need for ongoing surveillance of culture and sensitivity patterns, alongside concerted efforts in community awareness programs. These initiatives are essential for informing and guiding appropriate treatment strategies amidst the escalating challenge of bacterial resistance to multiple drugs.

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