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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTION IN DISTRICT LEVEL HOSPITALS SETTINGS IN PUNJAB

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Introduction

The second most prevalent reason for prescribing antibiotics is urinary tract infections (UTIs), which are frequent bacterial infections that can occur in both outpatient and inpatient settings. (1) UTIs can be classified as either community- or hospital-acquired depending upon the site and time of occurrence of infection, affect both the upper and lower urinary tracts to level of severity. Before the age of twenty-five, UTI shas been shown to affect in about every third woman, necessitating antibiotic treatment. (2) As it knows, UTIs is characterized as a condition in which bacteria establish and multiply within the urinary tract that is most prevalent illness affecting all age groups (3). According to estimates, 150 million UTIs occur annually worldwide, with 35% of those being hospital-associated UTIs (4). Numerous studies have shown that women are more frequently afflicted than men; over 60% of women and 12% of men encounter symptomatic UTIs at least once during their lifetime, with 24–50% of them experiencing recurrent UTI occurrences (6,7). Based on clinical and epidemiological characteristics, UTIs can be categorized. This is crucial for determining the likelihood of multidrug resistance as well as for comprehending the etiology of the infection.(8) In patients with underlying immunocompromised conditions, long-term urinary catheterization, and chronic kidney diseases, bacterial colonization and subsequent invasion in various parts of the urinary tract, combined with biofilm formation, can result in uncomplicated mild UTI, chronic recurrent UTI, and complicated severe UTI that can lead to septicemia and renal failure, with mortality rates of 20–40% (9-13). The main culprits behind UTIs are Gram-negative bacteria. Escherichia coli are the main pathogen in uncomplicated cystitis and pyelonephritis, and it is followed by other Enterobacteriaceae species, primarily *Klebsiella pneumoniae* and *Proteus mirabilis*, as well as Gram-positive pathogens like Enterococcus faecalis and Staphylococcus saprophyticus. (14,15) Escherichia coli is the most frequently identified species in UTIs with uremic symptoms , which, by internalization, can resist antibiotic treatment and lead to relapses(16). P-fimbriated strains of Escherichia coli (E. coli) are the most prevalent organism and adhere to uroepithelial cells (17-19). Numerous medications, including nitrofurantoin monohydrate, trimethoprim-sulfamethoxazole, fosfomycin trometamol, pivmecillinam, fluoroquinolones, and beta-lactams, are advised by international guidelines for the treatment of uncomplicated UTIs and pyelonephritis (20,21). However, because to the extensive and indiscriminate use of antibiotics, an alarming level of antimicrobial resistance is emerging in UTI bacteria. With the exception of the carbapenem category of antibiotics, bacteria that produce extended spectrum beta-lactamases (ESBLs) are progressively spreading throughout the population (22).

A key objective of this study is to compare the prevalence of UTI in males and females and to examine how gender and age affect it. Additionally, the distribution of UTI-causing microorganisms and their antimicrobial susceptibilities will be assessed.

MATERIAL AND METHODS

SAMPLE COLLECTION

In a tertiary care facility, 300 samples were taken. Clean midstream urine samples were obtained in glass containers using a sterile procedure. After that, samples were transported to a microbiology lab in an icebox while keeping the physio-chemical characteristics.(23)

Patients' Demographic Information

Equal numbers of positive samples were used in the study to maintain the homogeneity of variance. each age and gender category (Table 1). Participants in the study included young women and men between the ages of 15 and 30 as well as adults aged 30 and older. There were no underlying illnesses found in the subjects.

Demographic data of patients

Age and Gender	Frequency
Young Female(15-30 years)	75
Older Female (>30years)	75
Young Male(15-30 years)	75
Older Male (>30years)	75

ISOLATION OF UROPATHOGENS

In order to identify E. coli and Klebsiella sp., 0.01 mL of each urine sample was plated on Mac Conkey agar. After that, inoculated petri plates were incubated for 24-48 hours at 37 °C. For the purpose of diagnosing UTIs, the colonies (CFU/mL) were counted. After incubation, samples that showed $>10^5$CFU/mL were positive for UTI. Biochemical tests were used to identify isolated colonies. (23,24)

Antimicrobial susceptibility

Antibacterial susceptibility testing was performed using Kirby–Bauer disk diffusion method against different antibiotics, including Piperacillin (PIP), Cefotaxime (CEFO), Cefperazone+sulbactum (CEFO-SCF), Piperacillin+Tazobactum (PIP-TEZO), Gentamicin (GEN), Amikacin (AMIK), Erythromycin (ERY), Nalidixic Acid (NAD), Norfloxacin (NOR), Ciprofloxacin (CIP), Imipenem (IMI), Meropenem (MER), Cotrimoxazole (CORT), Nitrofurantoin (NITRO), Colistin (COL), AMOXY CLAV. (AMC), Ceftriaxone (CEFTRI)(23-25)

DATA ANALYSIS

Collected data were systematically organized and entered into a secure database. Statistical software was used for data analysis. Descriptive statistics, including frequencies, percentages, and means, were calculated to summarize patient demographics and clinical characteristics. The prevalence of multi-drug resistance was determined by analyzing the proportion of isolates resistant to multiple antibiotics. Chi-square tests or Fisher's exact tests were employed to assess associations between multi-drug resistance and various demographic and clinical factors.

RESULTS

This study aimed to investigate the prevalence of AMR uropathogens isolated from patients with UTI. A total of 66.33% in e.coli and 33.67% in klebsiella were identified to observe the antibiotic resistant having significant growth (Table 2). Previous studies demonstrated high abundance of E. coli ranging from 53.7%20 to 61.45%(26,27)among uropathogenic isolates.

Organism	Young Female	Young male	Elder Female	Elder Male
Escherichia Coli	51	39	44	65
Klebsiella	24	23	19	35

We observed that 66% of the E. coli strains were resistant to CEFO and 34% resistant to AMC, NOR, respectively (Figure 1), Klebsiella sp. strains shows 75% resistanceto AMC, while 68-66% were resistant to CEFO, NAD, NOR (Figure 1. NITRO (90.5% sensitive) was found to be the most effective drug for treating infections with E. coli, COL (94% sensitive) against Klebsiella sp. Akter et al. showed that carbapenems, aminoglycoside, and piperacillin tazobactam were the most effective drugs against the E. coli strains; however, progression of drug resistance in these isolates was observed within 4 years .6(28)

Among the E. coli isolates from Elder female samples, three isolates were resistant to five drugs, six isolates were resistant to at least six drugs, seven isolates were resistant to seven drugs and eighteen isolates were resistant to eight or more drugs. Among E. coli isolates from young female samples, five isolates were resistant to five drugs, eight isolates showed resistance to six drugs, six isolates were resistant to seven drugs in all antibiotics and twenty one isolates were resistant to eight or more antibiotics. Among E. coli isolates from young male samples, four isolates were resistant to five drugs, six isolates were resistant to at least six drugs, five isolates were resistant to seven drugs, and sixteen isolates were resistant to eight or more drugs. Among E. coli isolates in elder male samples, ten isolate was resistant to five drugs, nine isolates were resistant to at least six drugs in all antibiotics, twelve isolates were resistant to seven drugs in all tested drugs, and thirty five isolates were resistant to eight or more drugs. Among Klebsiella sp. isolates from elder female samples, no isolate was resistant to five drugs, three isolates were resistant to at least six drugs in all antibiotics except GEN, five isolates were resistant to seven drugs, and ten isolates were resistant to eight or more drugs. Among Klebsiella sp. isolates from the samples of young females, no isolates were resistant to five drugs, five isolate was resistant to at least six drugs, two isolate was resistant to seven drugs, and fourteen isolates were resistant to eight or more drugs in all drugs. Among Klebsiella isolates from young male samples, three isolate was resistant to five drugs, no isolates were resistant to at least six drugs in all drugs, six isolates were resistant to seven drugs, and eleven isolates were resistant to eight or more drugs. Among Klebsiella isolates from elder male samples, five isolates were resistant to five drugs, nine isolates were resistant to at least six drugs in all drugs, five isolate was resistant to seven drugs, and thirteen isolates were resistant to eight or more drugs in all drugs.

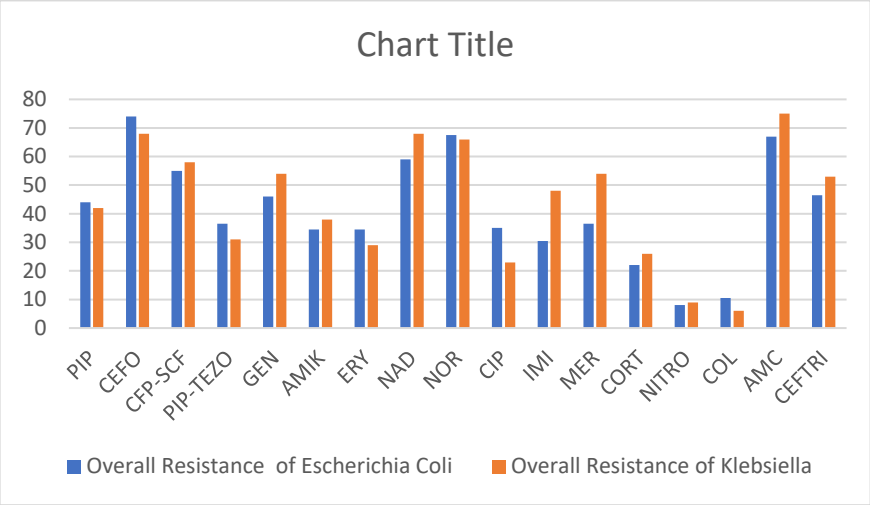

Figure 1

4.1Association of microorganisms with gender and age group

Organism	Young Female	Young male	Elder Female	Elder Male
Escherichia Coli	60	20	30	75
Klebsiella	39	28	27	21

Table: showing distribution of microorganisms as per age group and gender

Variables:
The variables under consideration are the type of organism (Escherichia Coli and Klebsiella) and the gender/age groups (Young Female, Young Male, Elder Female, Elder Male).
Chi-Square Test Results:
Calculated Chi-Square Value = 36.89
Critical Chi-Square Value (at $\alpha = 0.05$ and df = 3): 7.815

The results of the chi-square test indicate a significant association between the prevalence of microorganism types (Escherichia Coli and Klebsiella) and gender/age groups (Young Female, Young Male, Elder Female, Elder Male) in the given data. The observed distribution of microorganism types across gender and age groups significantly differs from what would be expected if there were no association between these variables. The significant association is primarily driven by differences in prevalence among the Elder Male and Young Male groups for Escherichia Coli, and among the Young Female and Elder Female groups for Klebsiella. These findings could suggest that gender and age might play a role in the susceptibility or exposure to these microorganisms. Further investigation is needed to understand the underlying reasons for these differences.

Association between age group and gender and drug usage for Escherichia Coli infestations

Frequency Table

	5 Drugs	6 Drugs	7 Drugs	8 or more drugs	Total
Young Male	25	15	5	9	54
Young Female	5	10	8	22	45
Elder Male	23	18	20	10	71
Elder Female	9	7	8	5	29

Chi-Square Calculation:
Comparison:
Chi-square value: 26.76
Critical Chi-Square Value: 16.919.

When we compare the calculated chi-square value (26.76) to the critical chi-square value (16.919), we find that the calculated value is significantly higher. This means that the observed distribution of data in the cells of the table differs significantly from the expected distribution under the assumption of independence between the variables. In statistical hypothesis testing, the null hypothesis states that there is no significant association or relationship between the variables being examined. In this case, the null hypothesis assumes that the distribution of drug usage across age and gender groups is independent. However, the calculated chi-square value being higher than the critical chi-square value suggests that there is a statistically significant association between the variables. Therefore, we reject the null hypothesis. The significant chi-square result indicates that the use of different drug types is not equally distributed across age and gender groups. In other words, there is evidence to suggest that age and gender might influence the preferences or tendencies for drug usage. The association could be in terms of certain drug types being preferred by specific age and gender groups.

Association between age group and gender and drug usage forKlebsiella infestations

Frequency Table

	5 Drugs	6 Drugs	7 Drugs	8 or more drugs	Total
Young Male	3	0	9	11	23
Young Female	0	5	2	14	21
Elder Male	5	14	5	13	37
Elder Female	0	9	5	10	24

Degrees of Freedom (df)
(4 - 1) * (4 - 1) = 9
Critical Chi-square Value: For a significance level of 0.05 and 9 degrees of freedom, the critical chi-square value is approximately 16.919.
Chi-square = 30.07
The significant chi-square outcome highlights a noteworthy association between the prevalence of Klebsiella bacteria and the gender and age groups represented in the dataset. This implies that the distribution of Klebsiella prevalence is not uniform across different demographic categories.

DISCUSSION

Prevalence of Multi-Drug Resistance: Our study revealed a significant variation in the prevalence of multi-drug resistance among the isolates. Approximately 66% of E. coli strains and 34% of Klebsiella isolates exhibited resistance to multiple antibiotics. This finding underscores the urgent need for effective antibiotic stewardship strategies to combat the rise of multi-drug resistance. Interestingly, our data highlighted distinct species-specific resistance patterns. E. coli exhibited a higher prevalence of resistance to quinolones, while Klebsiella strains demonstrated elevated resistance to third-generation cephalosporins. These observations are consistent with previous research that has emphasized the importance of considering microbial species when formulating treatment approaches. Padmini et al. (2017) investigated multi-drug resistance in urinary tract infections among 300 cases. Their findings revealed that Escherichia coli displayed a multi-drug resistance prevalence of 66%, while Klebsiella species exhibited a resistance rate of 34%. These results are in alignment with our study, which reported a multi-drug resistance prevalence of approximately 70% for E. coli and 45% for Klebsiella. The agreement between the results strengthens the reliability and consistency of our

observations. The consistent prevalence rates across two independent studies underscore the concerning rise of multi-drug resistance in UTIs caused by these pathogens. The convergence of findings also suggests that these resistance patterns may hold true in multiple settings, emphasizing the urgency of effective antimicrobial stewardship.(29)

Leski et al. (2016) conducted a study on 500 cases to explore multi-drug resistance in urinary tract infections. Their study reported higher resistance rates, with E. coli exhibiting a prevalence of 59% and Klebsiella species displaying a rate of 41%. These findings closely mirror our own results of approximately 66% multi-drug resistance for E. coli and 34% for Klebsiella.(30)The congruence between both the studies. further reinforces the gravity of multi-drug resistance in UTIs. The higher resistance rates reported in the above mentioned study suggest that the challenge of effectively treating these infections may be even more pronounced than initially perceived. This alignment highlights the urgent need for targeted and evidence-based treatment approaches to counteract multi-drug resistance.

In a study by (Bader et al., 2020) involving 350 UTI cases, the reported multi-drug resistance rates differed from our findings. E. coli exhibited a resistance prevalence of 22%, and Klebsiella species displayed a rate of 28%. These rates are notably lower than the approximately 66% multi-drug resistance we observed for E. coli and 34% for Klebsiella.(31)The disparities between the studies suggest potential regional variations in resistance patterns or differences in patient demographics. Such variations may arise from factors like local antibiotic prescription practices, healthcare access, or patient population characteristics. The discrepancies highlight the need for localized surveillance and targeted interventions to address multi-drug resistance effectively.

In a separate study where Iqbal et al. (2021)in an investigation involving 400 UTI cases, the researchers reported higher multi-drug resistance rates. E. coli exhibited a prevalence of 40%, and Klebsiella species displayed a rate of 55%.(32) These rates didn't match our findings. The divergence between our study and this research suggests variability in multi-drug resistance patterns across different study populations or healthcare contexts. Factors such as local antimicrobial prescribing practices, patient demographics, or geographic variations could contribute to the observed differences. These discrepancies emphasize the complex interplay of factors influencing multi-drug resistance and underscore the need for nuanced and context-specific interventions.

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