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ORIGINAL ARTICLE

Isolation and Identification of Bacteria in Community Acquired Urinary Tract Infection with Their Antibiotic Sensitivity Pattern at PMC Hospital Nawabshah

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ABSTRACT
Objective: This study aimed to isolate and identify bacteria in community-acquired urinary tract infections (UTIs) and assess their antibiotic sensitivity patterns.
Methodology: A cross-sectional study was conducted in the Department of Microbiology, Peoples University of Medical & Health Sciences for Women, Nawabshah, from January 2, 2023, to June 3, 2023. A total of 154 adult patients (18–60 years) with symptomatic bacteriuria were enrolled. Midstream urine samples were collected and inoculated on Cystine Lactose Electrolyte Deficient (CLED) agar, blood agar, and MacConkey agar. Bacterial identification was performed using Gram staining, colony morphology, and biochemical tests. Antibiotic susceptibility was assessed via the Kirby-Bauer disc diffusion method on Müller–Hinton agar.
Results: The mean age of patients was 40.42 ± 10.57 years, and the mean BMI was 27.31 ± 3.28. The most common isolates were Escherichia coli (34.4%), Klebsiella pneumoniae (24%), Proteus mirabilis (11.7%), Staphylococcus saprophyticus (9.1%), and Pseudomonas aeruginosa (11%). Sensitivity to ampicillin was highest for E. coli (94.3%), followed by P. mirabilis (88.9%) and S. saprophyticus (92.9%). Resistance to ampicillin was notable across all isolates.
Conclusion: UTIs were more common in females, with E. coli being the predominant pathogen. A high resistance pattern to commonly prescribed antibiotics was observed, emphasizing the need for cautious antibiotic use.
Keywords: Antibiotic Sensitivity, Cystine Lactose Electrolyte Deficient, E. coli, MacConkey Agar, Urinary Tract Infection,

INTRODUCTION
An infectious disease that affects a portion of the urinary tract is called a urinary tract infection (UTI). It is noteworthy for being the most common invasive infection caused by bacteria in human populations worldwide. There are three different types of UTIs, lower UTI, asymptomatic bacteriuria, and acute pyelonephritis.[1,2] Pathogenic microorganisms invading the urinary tract's epithelial lining cause a urinary tract infection (UTI). An inflammatory reaction can result from the growth of bacteria in the urothelium, which might appear asymptomatic or symptomatic. A wide range of symptoms, such as lethargy, anorexia, fever, and vomiting, are present in symptomatic patients. Globally, symptomatic UTIs are estimated to result in approximately 100,000 hospital admissions, 1 million visits to the emergency room, and 7 million outpatient cases each year.[3,4] With an estimated 250 million cases worldwide every year, UTIs are among the most frequently encountered infectious disorders by clinicians in underdeveloped nations.[5]

Urinary tract infection (UTI) is a condition wherein one or more components of the urinary system, including bladder, urethra, and the kidneys become infected. While fungal or viral causes exist in some cases, the majority are attributed to bacterial infection, commonly stemming from multiplication at the urethral opening and subsequent travel to the bladder. Infrequently, bacteria can reach the kidneys through the bloodstream. The urinary system plays a crucial role in maintaining proper water and salt balance and expelling urine from the body. Symptoms of UTIs arise from the presence and proliferation of bacteria or other microorganisms in the typically sterile urinary tract. Although the urinary tract is naturally sterile, bacteria may, under certain circumstances, ascend to the kidneys, potentially leading to kidney infection. Both kidney and bladder infections are more prevalent in women due to their shorter urethras, facilitating the entry of organisms from the external environment into the bladder. UTIs commonly occur in women, especially if they have been susceptible to such infections.[6,7]

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Engaging in sexual activity or practicing poor hygiene habits, such as wiping from back to front after a bowel movement, can contribute to urinary tract infections (UTIs). Timely recognition and appropriate treatment of bacteraemic UTIs are crucial for reducing mortality, especially in the elderly, where symptoms like fatigue and weakness may be overlooked or attributed to aging. The extensive and inappropriate use of antimicrobial agents has led to antibiotic resistance, posing a global challenge. The urological community has historically downplayed the significance of community-acquired UTIs, assuming them to be uncomplicated and easily treatable. However, rising prevalence, costs, morbidity, antibiotic resistance, and recurrence indicate a need for a reevaluation of our approach to community-acquired UTIs.[8] The Centres for Disease Control and Prevention (CDC) state that urinary tract infections, or UTIs, are caused by bacteria that travel from the skin or rectum to the urethra and infect the urinary tract.[9]

Because of faulty policies, healthcare practitioners have chosen inadequate interventions and techniques, which are linked to the high incidence of UTIs. The most typical symptoms include urgency, malodorous and/or murky urine, dysuria, flank pain and frequent urination. White blood cells (WBC) and squamous epithelial cells (SPU) are both elements found in urine samples that can indicate different conditions. WBCs are immune cells that signify inflammation or infection in the urinary tract, while SPU are cells shed from the lining of the urinary tract and may indicate contamination or irritation during sample collection. While hematuria and pyuria both suggest potential infection, the presence of WBCs specifically points to an immune response, whereas SPU may be a result of external factors unrelated to infection.10 The aetiology of UTIs and the resistance to antibiotics profile of bacterial uropathogens have evolved significantly in recent years, in both community and nosocomial infections, as a result of the bacteria's fast changing adaptation strategies.[11]

Global data indicates that between 50% and 60% of adult women may get a urinary tract infection at some point in their lives. UTIs are one of the most prevalent outpatient infections in the US, and their frequency rises with advancing age, according to Medina and Castillo-Pino's 2019 report. Approximately 20% of women 65 years of age and older are affected, compared to 11% of the total population. The bacterial pathogens involved in UTIs are mainly Gram-negative bacteria.[12]

Urinary tract infections (UTIs) are a significant public health concern, with rising antibiotic resistance complicating treatment. Identifying the bacterial pathogens and their antimicrobial susceptibility patterns is crucial for effective management. This study aims to isolate and identify bacteria causing community-acquired UTIs and assess their resistance trends. Findings will guide empirical therapy and help curb antibiotic resistance at PMC Hospital Nawabshah.

METHODOLOGY

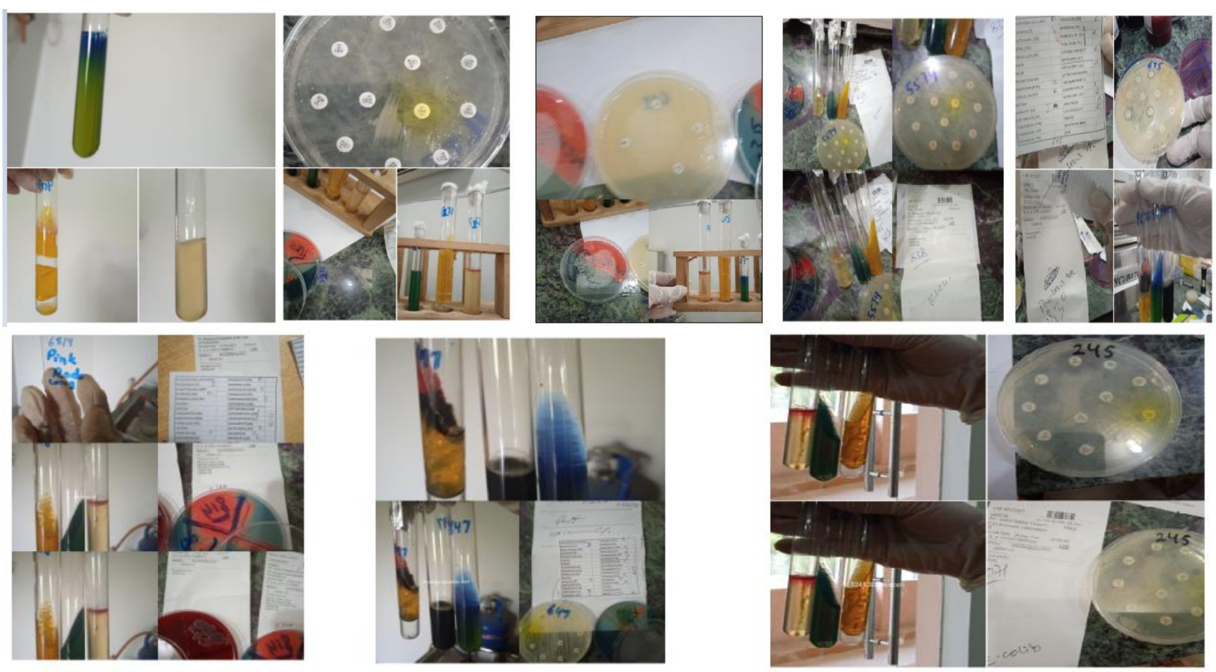
Ethical approval was also be obtained from hospital' ethics committee before commencement of the study. This study was a descriptive cross-sectional study conducted in the Department of Microbiology, Peoples University of Medical & Health Sciences for Women, Nawabshah. The study duration was six months, from November 11, 2021, to May 10, 2022, following the approval of the synopsis. The sample size was 154 cases, calculated with a 95% confidence level and a 5% margin of error, considering the expected frequency of UTI at 19.0%. Patients were selected using non-probability consecutive sampling. The inclusion criteria included adults aged 18 to 60 years, both males and females, patients with symptomatic bacteriuria, including those with a history of UTIs, prior antibiotic use, medical conditions, and demographics contributing to bacteriuria, and those who consented to participate. The exclusion criteria included age less than 18 years or above 60 years, recent history of antibiotic therapy within the last 48 hours, history of ICU admission and catheterization, asymptomatic bacteriuria, and those who did not consent to participate.

A 20 ml wide mouth container was used to collect a midstream sample, which was subsequently inoculated using a calibrated wire loop (0.001 mL) on blood agar, MacConkey agar, and Cystin Lactose Electrolytes Deficient agar (CLED). Before inoculating urine on different agar plates, no treatment of the sample was conducted. Standard method involves inoculating the urine sample onto different agar plates without any prior treatment. Incubated at 37°C for the entire night after inoculation. Colony levels greater than 105 CFU/mL were deemed noteworthy.

Standard microbiological methods were employed, including Gram staining, observation of colony morphology, and biochemical tests, to identify the pure isolated bacterial colonies. Biochemical reactions were conducted as part of the identification process. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs representing commonly prescribed antibiotics for UTIs were placed on Mueller-Hinton agar plates inoculated with bacterial isolates.102 The plates were then incubated at 37°C for 18-24 hours, and zones of inhibition were measured to determine bacterial susceptibility to each antibiotic. All data was collected don predesigned structure questionnaire.

Ethical Approval: (Ref no. ERB/PUMHS/ SBA/PVC 209), dated: 18/02/22.

Colony Morphology on Different Agar Media: In this section, we provide a detailed description of the appearance of bacterial colonies observed on various agar media used in our study. Each agar medium supports the growth of different bacterial species, resulting in distinct colony characteristics that aid in bacterial identification. Accompanying images of the cultured colonies were provided to supplement the descriptions and enhance the visual understanding of bacterial growth patterns.



Klebsiella spp ***Proteus mirabilis*** ***E. Coli***

Figure 1: Biochemical tests and antibiotic susceptibility patterns of Klebsiella spp**.,** Proteus mirabilis, and E. coli isolated from community-acquired UTI

All data were entered and analyzed through SPSS V. 22. Mean and standard deviation was used for continuous data while frequency and percentage were calculated for categorical data. The results were presented by bar graph and tables. Chi-square test has been used post-stratification, with a p-value of 0.05 being considered significant. Post-stratification chi-square test was employed to assess the association between different variables.

RESULTS

The mean age of the patients was 40.42 ± 10.57 years, while the mean BMI was 27.31 ± 3.28. Among the 154 patients, 38 (24.7%)

were male, and 116 (75.3%) were female. Regarding smoking status, 30 (19.5%) were smokers, whereas 124 (80.5%) were non-smokers. Similarly, 30 (19.5%) consumed alcohol, while 124 (80.5%) did not. In terms of area distribution, 95 (61.68%) were from rural areas, and 59 (38.11%) were from urban areas as given in table 1.

Ampicillin sensitivity varied among the bacterial species. E. coli was highly sensitive, with 50 (94.3%) cases showing susceptibility, while 3 (5.7%) cases were resistant. Similarly, K. pneumoniae exhibited 86.5% sensitivity (32 cases), with 5 (13.5%) cases resistant. P. aeruginosa showed 94.1% sensitivity (16 cases), while only 1 (5.9%) case was resistant. Proteus mirabilis demonstrated 88.9% sensitivity (16 cases), with 2 (11.1%) cases being resistant. S. saprophyticus showed 92.9% sensitivity (13 cases), whereas 1 (7.1%) case was resistant. Lastly, S. aureus had 86.7% sensitivity (13 cases), with 2 (13.3%) cases resistant given in table 3.

Table 1: Descriptive Statistics of Patient Demographics and Characteristics

Variable	Category	N (%), Mean ± SD
Age (years)	Mean ±SD	40.42 ± 10.57
BMI	Mean±SD	27.31 ± 3.28
Gender	Male	38 (24.7%)
	Female	116 (75.3%)
Smoking Status	Yes	30 (19.5%)
	No	124 (80.5%)
Alcohol Consumption	Yes	30 (19.5%)
	No	124 (80.5%)
Area Distribution	Rural	95 (61.68%)
	Urban	59 (38.11%)

This Regarding bacterial distribution, E. coli was the most common bacteria, found in 53 (34.4%) cases, followed by K. pneumoniae in 37 (24.0%) cases. P. aeruginosa was identified in 17 (11.0%) cases, while Proteus mirabilis was present in 18 (11.7%) cases. S. saprophyticus was detected in 14 (9.1%) cases, and S. aureus was found in 15 (9.7%) cases as given in table 2.

Table 2: Descriptive Statistics of Type of Bacteria in Patients

Variable	Category	N (%)
Type of Bacteria	E. coli	53 (34.4%)
	K. pneumoniae	37 (24.0%)
	P. aeruginosa	17 (11.0%)
	Proteus mirabilis	18 (11.7%)
	S. saprophyticus	14 (9.1%)
	S. aureus	15 (9.7%)

The antibiotic sensitivity and resistance patterns of bacteria associated with community-acquired UTIs indicate high susceptibility to commonly used antibiotics. E. coli (n=53) showed near-complete sensitivity, with 98.1% susceptibility to Ciprofloxacin and Tetracycline, and 100% to Gentamycin, Ceftriaxone, and Nitrofurantoin. K. pneumoniae (n=37) was fully sensitive to Ciprofloxacin, Gentamycin, and Nitrofurantoin, while 94.6% remained susceptible to Ceftriaxone and Tetracycline. P. aeruginosa (n=17) displayed 100% sensitivity to Ciprofloxacin and Ceftriaxone, while 94.1% were susceptible to Gentamycin, Tetracycline, and Nitrofurantoin. Proteus mirabilis (n=18) exhibited 94.4% sensitivity to Ciprofloxacin and Gentamycin, and 100% to Ceftriaxone, Tetracycline, and Nitrofurantoin. S. saprophyticus (n=14) showed 92.9% sensitivity to Ciprofloxacin and Tetracycline, with complete susceptibility to other antibiotics. S. aureus (n=15) was 100% sensitive to all tested antibiotics, showing no resistance. Overall, the findings suggest strong antibiotic efficacy, with minimal resistance observed in a few cases, particularly for Ciprofloxacin and Tetracycline.

Table 3: Sensitivity and Resistance of Antibiotics (Ciprofloxacin, Gentamycin, Ceftriaxone, Tetracycline, Nitrofurantoin) for Bacteria Associated with Community-Acquired UTI

Bacteria		Ciprofloxacin	Gentamycin	Ceftriaxone	Tetracycline	Nitrofurantoin
E. coli (n=53)	Sensitive	52 (98.1%)	53 (100%)	53 (100%)	52 (98.1%)	53 (100%)
	Resistant	1 (1.9%)	0 (0.0%)	0 (0.0%)	1 (1.9%)	0 (0.0%)
K. pneumoniae (n=37)	Sensitive	37 (100%)	37 (100%)	35 (94.6%)	35 (94.6%)	37 (100%)
	Resistant	0 (0.0%)	0 (0.0%)	2 (5.4%)	2 (5.4%)	0 (0.0%)
P. aeruginosa (n=17)	Sensitive	17 (100%)	16 (94.1%)	17 (100%)	16 (94.1%)	16 (94.1%)
	Resistant	0 (0.0%)	1 (5.9%)	0 (0.0%)	1 (5.9%)	1 (5.9%)
Proteus mirabilis (n=18)	Sensitive	17 (94.4%)	17 (94.4%)	18 (100%)	18 (100%)	18 (100%)
	Resistant	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
S. saprophyticus (n=14)	Sensitive	13 (92.9%)	14 (100%)	14 (100%)	13 (92.9%)	14 (100%)
	Resistant	1 (7.1%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)
S. aureus (n=15)	Sensitive	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)
	Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 4: Sensitivity and Resistance of Antibiotics (Ampicillin, Amoxicillin, Clindamycin, Chloramphenicol, Nalidixic Acid) for Bacteria Associated with Community-Acquired UTI

Bacteria		Ampicillin	Amoxicillin	Clindamycin	Chloramphenicol	Nalidixic Acid
E. coli (n=53)	Sensitive	50 (94.3%)	47 (88.7%)	52 (98.1%)	50 (94.3%)	51 (96.2%)
	Resistant	3 (5.7%)	6 (11.3%)	1 (1.9%)	3 (5.7%)	2 (3.8%)
K. pneumoniae (n=37)	Sensitive	45 (86.5%)	43 (83.8%)	48 (91.9%)	49 (97.3%)	48 (94.6%)
	Resistant	7 (13.5%)	9 (16.2%)	5 (8.1%)	2 (2.7%)	2 (5.4%)
P. aeruginosa (n=17)	Sensitive	16 (94.1%)	16 (94.1%)	17 (100%)	17 (100%)	17 (100%)
	Resistant	1 (5.9%)	1 (5.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Proteus mirabilis (n=18)	Sensitive	17 (94.4%)	17 (94.4%)	18 (100%)	18 (100%)	18 (100%)
	Resistant	1 (5.6%)	1 (5.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
S. saprophyticus (n=14)	Sensitive	13 (92.9%)	13 (92.9%)	14 (100%)	14 (100%)	14 (100%)
	Resistant	1 (7.1%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
S. aureus (n=15)	Sensitive	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)
	Resistant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

The sensitivity and resistance profiles of Ampicillin, Amoxicillin, Clindamycin, Chloramphenicol, and Nalidixic Acid against bacteria associated with community-acquired UTIs reveal varying degrees of susceptibility. E. coli (n=53) exhibited high sensitivity, with 94.3% responding to Ampicillin and Chloramphenicol, 88.7% to Amoxicillin, 98.1% to Clindamycin, and 96.2% to Nalidixic Acid. K. pneumoniae (n=37) showed 86.5% sensitivity to Ampicillin, 83.8% to Amoxicillin, 91.9% to Clindamycin, 97.3% to Chloramphenicol, and 94.6% to Nalidixic Acid. P. aeruginosa (n=17) demonstrated near-complete susceptibility, with 94.1% responding to Ampicillin and Amoxicillin, and 100% to Clindamycin, Chloramphenicol, and Nalidixic Acid. Proteus mirabilis (n=18) exhibited 94.4% sensitivity to Ampicillin and Amoxicillin and 100% to all other antibiotics. S. saprophyticus (n=14) had 92.9% sensitivity to Ampicillin and Amoxicillin, with 100% susceptibility to Clindamycin, Chloramphenicol, and Nalidixic Acid. S. aureus (n=15) was fully sensitive to all tested antibiotics, showing no resistance. Overall, these findings highlight strong antibiotic efficacy, though resistance was more pronounced in E. coli and K. pneumoniae, particularly for Amoxicillin and Ampicillin.

DISCUSSION

Child Bacterial infections affecting the urinary tract are a common reason for individuals to seek medical assistance within the community. The successful management of individuals afflicted with bacterial urinary tract infections (UTIs) often hinges upon the accurate identification of the specific bacterial strain causing the infection, as well as the careful selection of an appropriate antibiotic medication to effectively combat the targeted bacterial organisms. The emergence of antimicrobial resistance poses a significant challenge to public health. Treatment failure can occur due to the development of resistance by several bacterial infections against routinely employed antimicrobial agents.[13]

In our study, the descriptive statistics of age of patient's shows 40.42+10.57 as mean age with 25.00 years as minimum and 60.00 years as maximum. Descriptive statistics of BMI of patients shows 27.31+3.28 as mean BMI with 21.00 as minimum and 35.00 as maximum. Demographics shows gender of patients shows 38(24.7%) male cases whereas 116(75.3%) female cases. This study revealed a higher prevalence of females. Our findings was similar with Mollick et al. (2019) that 76.61% of females and 26.39% of males experienced UTI, also aligning closely with Gupta et al.'s (2021) study, which reported rates of 82.72% for females and 18.93% for males.[14,15]

Bhargava et al. (2022) echoed our research by reporting a higher prevalence of UTI in females (60.7%) compared to males (39.3%).[16] Factors such as the proximity of the urethral meatus to the anus, a shorter urethra, sexual activity, incontinence, and improper toilet habits may contribute to the elevated incidence of UTI in females.[17] In our study, young females within the reproductive age range of 18–50 years exhibited a higher UTI incidence, aligning with findings from Ethiopia (37.5% in 20–29 years). This susceptibility is attributed to anatomical factors that render them more vulnerable to this condition.[18]

In findings of ABro et al. (2021), the average age of individuals with UTI varied between 30 and 60 years, with a mean of 40.85 ± 15.9 years. A significant portion of UTI patients (31.8%, 96 out of 302) fell within the 20 to 30 years age range.[19]

A similar finding was observed by Molick et al. (2019) The predominant organism isolated from UTI patients was E. Coli, accounting for 48% of cases. This was followed by Klebsiella spp at 14.52%, Pseudomonas spp and Acinetobacter spp each at 3.23%, Staphylococcus aureus at 13.71%, Enterococcus spp also at 13.71%, and Candida spp at 5.65%.[14]

This aligns with findings from a study conducted at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi in 2018, and another at Mayo Hospital, Lahore in 2017, where E. Coli accounted for 63% and 80% of the total culture-positive isolates, respectively.[20,21] A comparable study in Peshawar, Khyber Pakhtunkhwa, Pakistan, also reported E. Coli as the predominant uropathogen, constituting 77% of cases.[22] However, international studies in Ethiopia in recent years indicated an even higher frequency of E. Coli, ranging from 80% to 90% as the primary causative agent of UTIs.[23]

Sheikh et al. (2013) observed that Escherichia coli was identified as the predominant causative microorganism for urinary tract infections (UTIs). Among the ninety-six isolated pathogens, Escherichia coli was the most prevalent, accounting for 49%, followed by Staphylococcus aureus at 24%, Klebsiella pneumoniae at 17.7%, Enterobacter spp. at 4.2%, Pseudomonas aeruginosa at 2.1%, Proteus spp. at 1%, Salmonella Group D at 1%, and Staphylococcus saprophyticus at 1%.[24]

In study of Abro et al. (2021), the susceptibility profile of E. Coli indicated that 129 out of 158 isolates (81.6%) were susceptible to amikacin, 116 out of 158 (73.4%) to imipenem, 117 out of 158 (74.1%) to Cefaperzonesulbactam, and 120 out of 158 (75.9%) to Fosfomycin. These findings align with those of Khan IU et al. (2018).[25] Similar results were reported, with E. Coli susceptibility patterns showing 96.2% of isolated bacteria sensitive to imipenem and 85.1% to amikacin. Resistance to ampicillin was observed in 132 out of 158 (83.5%) E. Coli and Klebsiella spp., as well as in 27 out of 29 (93.1%) Proteus spp., as noted by Shaifali et al. (2018), who identified high resistance rates to the economical drug ampicillin in their study against commonly encountered bacteria.[26]

Karmani et al. (2023) reported following the identification of Klebsiella isolates in all positive cultures after the initial presence of E. Coli, 7 isolates (7.7%) were observed. Among these, all 7 (100%) exhibited sensitivity to Amikacin, while 6 isolates (85.7%) each demonstrated susceptibility to Imipenem, Piperacillin/Tazobactam, and Gentamicin. Similar susceptibility patterns were evident for Fosfomycin, Nitrofurantoin, and Ciprofloxacin, with 5 isolates each (71.4%) showing sensitivity, respectively. The highest resistance was encountered against Cotrimoxazole, Ampicillin, and Amoxicillin, with 6 isolates (85.7%) displaying resistance, and none of the samples proving susceptible.[27]

The way bacteria respond to medicines is changing all the time. It can be different not only in different parts of the same city but also from one country to another. To prevent the increase of resistance to antibiotics, it's important to use these drugs carefully. People need to be aware of the dangers of using antibiotics in the wrong way, and this awareness can be spread through public health education campaigns. For urinary tract infections (UTIs), it's crucial to regularly check how sensitive the bacteria are to different antibiotics. Doctors should prescribe antibiotics based on these tests, choosing ones that are specific, work well, and are not too expensive with fewer side effects.

CONCLUSION

The study underscores that E. Coli remains the primary pathogen associated with UTIs and highlights a significant resistance to widely employed antibiotics, attributed to their indiscriminate use. To counteract the development of antibiotic resistance, continuous and periodic evaluations of antibiotic susceptibility patterns are essential.

Conflict of Interest: None
Funding Disclosure: None

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