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Original Article

Comparison of Age, Gender, and Bacteria Isolated with Antibiotics Resistance Patterns on Urine Cultures

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ABSTRACT

Objective: To compare age, gender, and bacteria isolated from urine cultures with antibiotic resistance patterns.

Study Design: Cross-sectional study.

Place and Duration of Study: Surgery and Pathology Department Combined Military Hospital, Bannu Pakistan, from Jan 2023 to Apr 2024.

Methodology: Three hundred and eighty-six patients aged 1 to 80 years with urinary tract infection symptoms were included. Patients having recurrent urinary tract infections, immunocompromised patients already taking antibiotics, and those with multiple co-morbid conditions were excluded. Urine cultures were evaluated, and the sensitivity/resistance pattern was compared with gender, age, and bacteria isolated in positive cases.

Results: Forty-six samples were culture positive (11.9%). *Escherichia coli* was the most frequently isolated organism, obtained from 32 samples (69.6%), while *Pseudomonas aeruginosa* was isolated from 8 samples (17.4%), *Klebsiella pneumoniae* from 4(8.7%), *Enterobacter* from 1(2.2%) and *Serratia liquefaciens* from 1(2.2%) sample. Resistance to Ampicillin was found in 30 samples (88.2%), Amoxicillin/Clavulanic acid 24(85.7%), Ciprofloxacin 23(74.2%), Ceftriaxone 18(69.2%), Levofloxacin 10(66.7%), Co-trimoxazole 21 samples (65.6%), Cefepime 9(36%), Gentamicin 7(33.3%), Meropenem 10(27%), Piperacillin/Tazobactum 3(14.3%), Fosfomycin 4(13.8%), Amikacin 4(10.5%), and Nitrofurantoin 3 samples (7.5%) respectively. No statistically significant difference was found for gender, age, or type of bacteria with antibiotic sensitivity/resistance profiles (p>0.05).

Conclusion: Urinary tract bacteria resist most prescribed antibiotics, including Ampicillin, Amoxicillin/Clavulanic acid, Ciprofloxacin, Levofloxacin, Ceftriaxone, and Co-trimoxazole.

Keywords: Antibiotics, Antibiotics Resistance, Drug Resistance, Urinary Tract Infections.

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INTRODUCTION

Urinary tract infections rank among the most prevalent infections worldwide, varying from mild to potentially life-threatening.[1] It is estimated that every year, nearly 150 million people suffer from urinary tract infections all over the world.[2] They include infection of the kidneys (pyelonephritis), urinary bladder (cystitis), and urethra (urethritis).[3] Urinary tract infections are linked with substantial mortality and morbidity and affect the quality of life of patients as well.[4] They are the most common cause of prescription of antibiotics by doctors and are so common that both males and females have been documented to suffer once from such infections in their lives. It has been estimated that urinary tract infections are causing an economic burden of more than 5 billion dollars annually in the United States alone.[6] Prompt diagnosis and appropriate treatment are essential for preventing life-threatening conditions, such as urosepsis.[5]

Anatomical or functional causes of urinary tract infections involving bacteria ascending the urethra into the bladder.[5] Several predisposing factors are associated with an increased susceptibility to urinary tract infections. These include urinary retention, vesicoureteral reflux, family history of urinary tract infections, prostate hyperplasia in males, and vulvovaginal atrophy in females.[6] Urine culture is the gold standard for confirmation of urinary tract infections.[7] It involves collecting a clean catch urine sample used to grow colonies on culture media, and antibiotic sensitivity is subsequently tested. However, it is associated with cost and time consumed until the urine culture report is available.[7] Newer techniques such as flow cytometers, mass spectrometry, and multiplex PCR panels have been developed, but they are not readily available in clinical practice.[8]

Patients with urinary tract infections are treated with empirical antibiotics based on an antibiogram till

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a urine culture report becomes available.[9] This may result in injudicious use of antibiotics. Over the last decade, multi-drug resistance has increased exponentially, likely due to the misuse of antibiotics.[2] With ever-increasing antibiotic resistance worldwide, a grave global health issue, it is essential to understand antibiotic resistance profiles of different organisms causing urinary tract infections in relation to demographic profiles in our population. This led us to our aim to compare age, gender, and bacteria isolated from urine cultures with antibiotic sensitivity and resistance patterns in our setup.

METHODOLOGY

The cross-sectional study was conducted at Combined Military Hospital, Bannu Pakistan, from January 2023 to April 2024. Ethical committee approval was sought via letter ERC/2023/17 dated 12 Oct 2023. The sample size was determined using the WHO sample size calculator. Three hundred and eighty-six patients were included in the study, diagnosed clinically as having symptoms of urinary tract infection, by non-probability convenient sampling.

Inclusion Criteria: All patients aged 1 to 80 years who presented in the Surgical Outpatient Department clinically with symptoms of urinary tract infection (painful micturition, flank pain, frequency, pain in suprapubic region, visible hematuria or pyuria) were included.

Exclusion Criteria: Patients having recurrent urinary tract infections, immunocompromised, already taking antibiotics, and those with multiple co-morbid conditions were excluded.

The data obtained were ensured to be confidential. Informed consent was obtained before enrolling in the study.

The urine culture procedure was done according to predefined standard operation procedures at the Pathology Department of the hospital. Clean-catch midstream urine samples were collected from patients with symptoms of urinary tract infections in sterile wide-mouth containers, and sample entries were made on the computer. These were then delivered to the pathology laboratory within one hour. With the help of the bacteruritest strip (MAST), ten microliters of urine were inoculated on the cysteine-, lactose-, and electrolyte-deficient (CLED) agar media plate, and ten microliters were inoculated on both the Blood and CLED agar plate. This was followed by incubation at 35°C aerobically for 24–48 hours in an incubator. The colonies were calculated, and concentrations were determined using colony-forming units per ml. The standard for determining positive urine culture was pure culture or <2 bacterial species produced ≥104 colony-forming units per ml.

After 48 hours of incubation, bacterial identification was performed by morphology and culture/colony characteristics, and the Kirby Bauer disc diffusion method carried out antimicrobial sensitivity testing. Susceptibility testing was applied on Mueller Hinton agar to identify bacteria, whether lactose fermenter, non-lactose fermenter, or gram-positive cocci. API 10S was also applied to the sample to identify Gram-Negative Rods. The antibiotic discs were applied in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines 2022. For gram-negative bacilli, antibiotic discs applied included Ampicillin, Cefepime, Ceftriaxone, Ciprofloxacin, Levofloxacin, Sulfamethoxazole–Trimethoprim, Meropenem, Amikacin, Gentamicin, Piperacillin–Tazobactam, Nitrofurantoin and Fosfo-mycin.

Using the disc diffusion method, the zone of inhibition of every antimicrobial agent was measured and classified into sensitive, intermediate, or resistant, as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2022. The quality control of the antibiotic sensitivity testing process was ensured by using Staphylococcus aureus ATCC 25923, E. coli ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 strains.

Data collected included gender, age group, culture positive or negative, type of bacteria isolated, and antibiotic sensitivity/resistance. Collected data was analyzed using Statistical Package for Social Sciences (SPSS) version 26.0. Quantitative variables like age were depicted as mean and standard deviation. Qualitative data, such as gender, type of bacteria isolated, and antibiotics sensitivity/resistance, was presented as frequency and percentage and compared using the Chi-Square test. The p-value of ≤0.05 was taken as significant.

RESULTS

Three hundred and eighty-six patients were included in the study, of which 238(61.7%) were males and 148(38.3%) were females. Their ages ranged from 1 year to 80 years. Urine samples were analyzed. A total of 46 samples (11.9%) were culture-positive, 284(73.6%) were culture-negative, and 56(14.5%) revealed mixed growth.

Gender-wise, the distribution of culture-positive samples was 27 males (58.7%) and 19 females (41.3%). *Escherichia coli* was the most frequently isolated organism, obtained from 32 samples (69.6%), while *Pseudomonas aeruginosa* was isolated from 8 samples (17.4%), *Klebsiella pneumoniae* from 4(8.7%), *Enterobacter* from 1(2.2%) and *Serratia liquefaciens* from 1(2.2%) sample. *Escherichia coli* was the most frequently isolated organism among males and females, found in 18 and 14 samples, respectively, while *Pseudomonas aeruginosa* was found in 6 males and two females, respectively (*p*-value 0.52). *Escherichia coli* was the most frequently isolated organism in all age groups as well (isolated in 3 samples in 1-20 years old, 7 in 21-40 years, 10 in 41-60 years, 12 in 61-80 years old), while *Pseudomonas aeruginosa* was found in 7 patients belonging to 21-40 years old group (*p*-value 0.04).

Resistance to Ampicillin was found in 30 samples (88.2%), Amoxicillin/Clavulanic acid 24(85.7%), Ciprofloxacin 23(74.2%), Ceftriaxone 18(69.2%), Levofloxacin 10(66.7%), Co-trimoxazole 21 samples (65.6%), Cefepime 9(36%), Gentamicin 7(33.3%), Meropenem 10(27%), Tazobactum-piperacillin 3(14.3%), Fosfomycin 4(13.8%), Amikacin 4(10.5%), and Nitrofurantoin 3 samples (7.5%) respectively (Figure).

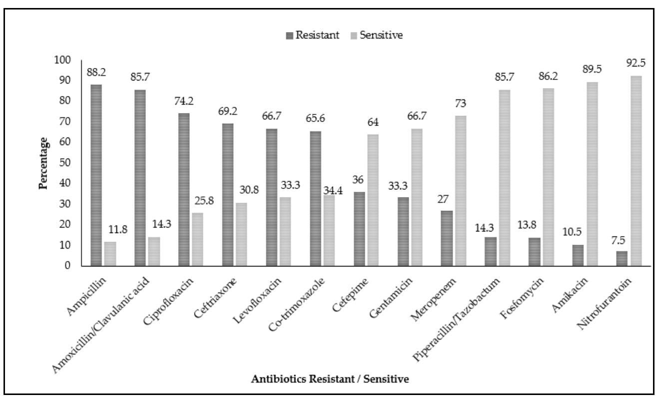

Figure: Antibiotics Sensitivity and Resistance Percentages in Positive Urine Cultures (n=46)

Antibiotic sensitivity/resistance profiles were compared with gender. No statistically significant difference between males and females was found in different antibiotic sensitivity/resistance profiles, except Ciprofloxacin (*p*-value 0.028) (Table-I).

Antibiotic sensitivity/resistance profiles were compared with age (Table-II). No statistically significant difference was found in different age groups' antibiotic sensitivity/resistance profiles. Antibiotic sensitivity/resistance profiles of different isolated bacteria were compared. No statistically significant difference between different bacteria was found in antibiotic sensitivity/resistance profiles, except for Amikacin (*p*-value 0.021) (Table-III).

DISCUSSION

Urinary tract infections are the most prevalent and are treated mainly using empirical antibiotics.[9] As antibiotic resistance is increasing, antibiotic stewardship is needed. The correct choice of antibiotic is essential for this purpose, and urine culture is considered a gold standard investigation for confirmation of urinary tract pathogens, along with their sensitivity and resistance profiles.[7]

In our study, 11.9% of samples were culture-positive, which is in accordance with other studies.[10] Mixed growth was found in 14.5% of samples, much lower than in other studies. This may be because of adherence to a strict policy regarding obtaining and transporting the samples, which is an essential component of the test.[10] *Escherichia coli* was the most frequently isolated organism in our study in all age groups, as well as both genders. This finding is similar to other studies on the same topic,[11] with *Escherichia coli* being the most frequently obtained bacteria, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Enterococcus faecium*.[3]

Resistance to antibiotics of urinary tract pathogens is increasing, and studies have identified increasing numbers of multi-drug resistant uropathogenic *Escherichia coli* isolates.[12] We found resistance to some of the frequently prescribed anti-microbial agents, including Ampicillin, Amoxicillin/ Clavulanic acid, Ciprofloxacin, Ceftriaxone, Levofloxacin and Co-trimoxazole in more than 60% of positive cases. This speaks of the grave nature of the problem of antibiotic resistance. Studies have found a high resistance rate to Ampicillin, Co-trimoxazole,[13] Ciprofloxacin,[14] and Ceftriaxone.[15] The resistance in *Escherichia coli* to Ceftriaxone and Ciprofloxacin, being isolated in Pakistan, has been determined to be more than 70% by Bullen *et al*.[16] We found low resistance to Cefepime, Gentamicin, Meropenem, Piperacillin/ Tazobactum, Fosfomycin, Amikacin, and Nitrofurantoin. This is similar to other studies on this topic.[13-15] Most studies have found low resistance to Carbapenems and Nitrofurantoin.[17,18] However, they have found that resistance to anti-microbial agents increases with time.[18]

Table-I: Comparison of Antibiotic Sensitivity with Gender (n=46)

	Male Sensitive	Male Resistant	Female Sensitive	Female Resistant	*p*-value
Ampicillin (n=34)	3(8.82%)	16(47.06%)	1(2.94%)	14(41.18%)	0.412
Cefepime (n=25)	7(28%)	6(24%)	9(36%)	3(12%)	0.128
Ceftriaxone (n=26)	4(15.38%)	12(46.15%)	4(15.38%)	6(23.08%)	0.42
Ciprofloxacin (n=31)	2(6.45%)	16(51.61%)	6(19.35%)	7(22.58%)	0.028
Co-trimoxazole (n=32)	6(18.75%)	10(31.25%)	5(15.63%)	11(34.38%)	0.710
Gentamicin (n=21)	9(42.86%)	5(23.81%)	5(23.81%)	2(9.52%)	0.743
Piperacillin/Tazobactum (n=21)	10(47.61%)	2(9.52%)	8(38.10%)	1(4.76%)	0.719
Amikacin (n=38)	19(50%)	3(7.89%)	15(39.47%)	1(2.63%)	0.464
Amoxicillin/Clavulanic acid (n=28)	2(7.14%)	14(50%)	2(7.14%)	10(35.71%)	0.755
Meropenem (n=37)	17(45.95%)	4(10.81%)	10(27.03%)	6(16.22%)	0.211
Nitrofurantoin (n=40)	22(55%)	2(5%)	15(37.5%)	1(2.5%)	0.806
Levofloxacin (n=15)	1(6.67%)	5(33.33%)	4(26.67%)	5(33.33%)	0.264
Fosfomycin (n=29)	16(55.17%)	2(6.90%)	9(31.03%)	2(6.90%)	0.592

Table-II: Comparison of Antibiotic Sensitivity with Age (n=46)

	Age 1-20 Sensitive	Age 1-20 Resistant	Age 21-40 Sensitive	Age 21-40 Resistant	Age 41-60 Sensitive	Age 41-60 Resistant	Age 61-80 Sensitive	Age 61-80 Resistant	*p*-value
Ampicillin (n=34)	0 (0%)	4(11.76%)	2(5.88%)	10(29.41%)	1(2.94%)	6(17.64%)	1(2.94%)	10(29.41%)	0.818
Cefepime (n=25)	0 (0%)	2(8%)	7 (28%)	4(16%)	4(16%)	2(8%)	5(20%)	1(4%)	0.482
Ceftriaxone (n=26)	1(3.85%)	2(7.69%)	5(19.23%)	5(19.23%)	0(0%)	5(19.23%)	2(7.69%)	6(23.08%)	0.252
Ciprofloxacin (n=31)	2(6.45%)	1(3.23%)	1(3.23%)	9(29.03%)	2(6.45%)	6(19.35%)	3(9.68%)	7(22.58%)	0.260
Co-trimoxazole (n=32)	2(6.25%)	3(9.38%)	4(12.5%)	7(21.88%)	3(9.38%)	3(9.38%)	2(6.25%)	8(25%)	0.647
Gentamicin (n=21)	1(4.76%)	1(4.76%)	8(38.10%)	1(4.76%)	2(9.52%)	2(9.52%)	3(14.29%)	3(14.29%)	0.321
Piperacillin/Tazobactum (n=21)	3(14.29%)	0(0%)	5(23.81%)	0(0%)	4(19.05%)	2(9.52%)	6(28.57%)	1(4.76%)	0.375
Amikacin (n=38)	4(10.53%)	0(0%)	11(28.95%)	1(2.63%)	8(21.05%)	1(2.63%)	11(28.95%)	2(5.26%)	0.835
Amoxicillin/Clavulanic acid (n=28)	0(0%)	5(17.86%)	0(0%)	9(32.14%)	2(7.14%)	5(17.86%)	2(7.14%)	5(17.86%)	0.198
Meropenem (n=37)	5(13.51%)	0(0%)	7(18.92%)	4(10.81%)	7(18.92%)	2(5.41%)	8(21.62%)	4(10.81%)	0.443
Nitrofurantoin (n=40)	5(12.5%)	0(0%)	10(25%)	1(2.5%)	10(25%)	1(2.5%)	12(30%)	1(2.5%)	0.922
Levofloxacin (n=15)	2(13.33%)	1(6.67%)	0(0%)	2(13.33%)	0(0%)	4(26.67%)	3(20%)	3(20%)	0.154
Fosfomycin (n=29)	2(6.90%)	0(0%)	4(13.79%)	2(6.90%)	8(27.59%)	0(0%)	11(37.93%)	2(6.90%)	0.314

Table-III: Comparison of Antibiotic Sensitivity with Isolated Bacteria (n=46)

	E Coli Sensitive	E Coli Resistant	P. aeruginosa Sensitive	P. aeruginosa Resistant	Klebsiella Sensitive	Klebsiella Resistant	*p*-value
Ampicillin (n=34)	2(5.88%)	20(58.82%)	0(0%)	7(20.59%)	0(0%)	3(8.82%)	0.605
Cefepime (n=25)	11(44%)	5(20%)	3(12%)	3(12%)	1(4%)	0(0%)	0.789
Ceftriaxone (n=26)	3(11.54%)	15(57.69%)	0(0%)	4(15.38%)	2(7.69%)	1(3.84%)	0.092
Ciprofloxacin (n=31)	6(19.35%)	16(51.61%)	0(0%)	5(16.13%)	1(3.23%)	1(3.23%)	0.231
Co-trimoxazole (n=32)	7(21.88%)	14(43.75%)	0(0%)	7(21.88%)	1(3.13%)	2(6.25%)	0.859
Gentamicin (n=21)	9(42.86%)	5(23.81%)	5(23.81%)	0(0%)	0(0%)	1(4.76%)	0.088
Piperacillin/Tazobactum (n=21)	10(47.62%)	2(9.52%)	5(23.81%)	0(0%)	2(9.52%)	0(0%)	0.117
Amikacin (n=38)	24(63.16%)	3(7.89%)	6(15.79%)	0(0%)	4(10.53%)	0(0%)	0.021
Amoxicillin/Clavulanic acid (n=28)	3(10.71%)	15(53.57%)	0(0%)	5(17.86%)	1(3.57%)	3(10.71%)	0.692
Meropenem (n=37)	19(51.35%)	7(18.92%)	5(13.51%)	1(2.70%)	3(8.11%)	1(2.70%)	0.386
Nitrofurantoin (n=40)	28(70%)	2(5%)	6(15%)	0(0%)	2(5%)	1(2.5%)	0.323
Levofloxacin (n=15)	4(26.67%)	8(53.33%)	0(0%)	2(13.33%)	1(6.67%)	0(0%)	0.223
Fosfomycin (n=29)	19(65.52%)	3(10.34%)	3(10.34%)	0(0%)	2(6.90%)	1(3.45%)	0.659

We found no statistically significant association of gender or age with antibiotic sensitivity patterns. Other studies have found increased resistance to Amikacin, Colistin, and Nitrofurantoin with increasing age.[3,19] Similar studies have found increased resistance to Amikacin, Colistin, and Nitrofurantoin among males.[19] Sensitivity and resistance patterns were similar among different organisms isolated in our study. Other studies have documented similar results, as well.[20]

The alarming levels of antibiotic resistance among common urinary pathogens advocate for much more emphasis on antibiotic stewardship and sensitization of all clinicians regarding this threat. Emphasis needs to be given on the correct diagnosis, right drug, correct dose, right duration, and de-escalation (the five D's).[21]

It is recommended that antibiotic therapy should only be started after a detailed evaluation of the patient, keeping in view local antimicrobial sensitivity profiles and avoiding the prescription of antimicrobial agents in asymptomatic patients. Nitrofurantoin or Fosfomycin may be used as first-line agents in uncomplicated urinary tract infections, except in pregnant and elderly patients.[15-18] Quinolones should not be prescribed. In contrast, Carbapenems may be used in severe cases.[15,16-18] Curbing the spread of antibiotic-resistant pathogens responsible for urinary tract infections requires increased awareness and adjustments of antibiotic prescribing practices by local antibiotics sensitivity data.

CONCLUSION

Urinary tract bacteria are resistant to most commonly prescribed antibiotics, including Ampicillin, Amoxicillin/ Clavulanic acid, Ciprofloxacin, Levofloxacin, Ceftriaxone, and Co-trimoxazole.

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Authors' Contribution

The following authors have made substantial contributions to the manuscript as under:

MA & AHM: Conception, study design, drafting the manuscript, approval of the final version to be published.

MRS: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree 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.

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