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Antimicrobial Resistance Patterns of Urine Culture in Tertiary Care Teaching Hospital Islamabad, Pakistan

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ABSTRACT

Background: Urine cultures are performed in laboratories to identify any bacteria or other microorganisms present in urine samples. The purpose of this study was to investigate the microorganisms growing resistant to antibiotics in the urine culture and their antibiotics susceptibility testing.

Methods: For this investigation, the Microbiology Department's hospital management information systems records provided the culture and sensitivity data of the uropathogens found in urine samples. Urine samples collected midstream were prepared for microscopy and culture, and the organisms were identified using globally accepted methods. The Kirby-Bauer disk diffusion method was used to determine antibiotic susceptibility in accordance with hospital clinical and laboratory standards as well as international protocols. In this investigation, the information and data were assessed using descriptive statistics.

Results: A total number of 207 urine culture data were extracted from the hospital management information system records of which, 116 samples showed positive bacterial growth. Candida had positive growth in 16.4%. Out of positive samples gender-wise, females were in the majority 66.4% and males were 33.6%. *Escherichia coli* bacteria frequently isolated in positive samples 58/116 (50%), whereas, *Enterococcus spp* were positive in 17 patients out of 116 (14.7 %). Gram-negative rods were positive in 16 patients out of 116 (13.8%), *Klebsiella spp* were positive in 4 patients out of 116(3.4%) and *Pseudomonas aeruginosa* were least positive in only 2 patients out of 116 positive samples (1.7 %).

Conclusion: In our study according to gender distribution female frequency was higher than males. *Escherichia coli* bacteria were the most common isolate in positive samples, whereas, *Enterococcus spp* were observed. Vancomycin was most sensitive in all urine cultures against all organisms and Erythromycin was most resistant in urine culture. Antimicrobial resistance is a serious subject on national and international levels that needs continued surveillance and resolute management.

Keywords: *E. coli*, Antimicrobial Resistance, Urine Culture, Uropathogens, Antibiotic Susceptibility Testing

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INTRODUCTION

When bacteria, viruses, fungi, and parasites learn to grow and adapt to the presence of medications that once affected them, it's known as antimicrobial resistance (AMR).[1, 2] AMR is regarded as posing a serious threat to public health systems everywhere, not only in developing nations.[3] The fact that antibiotics are no longer effective in treating infectious diseases portends a future in healthcare that is uncertain.[4] AMR infection results in severe sicknesses, extended hospital stays, higher healthcare expenses, higher second-line medication costs, and treatment failures. For example, it has been estimated that antimicrobial resistance is associated with over nine billion euros annually in just Europe.[2] Furthermore, excluding the roughly $35 billion in lost productivity each year, the Centers for

Disease Control and Prevention (CDC) estimates that antimicrobial resistance adds a 20-billion-dollar surplus to direct healthcare costs in the US.[5] Antimicrobial resistance (AMR) poses a threat to public health due to its emergence and spread. In order to address AMR, the World Health Organization (WHO) presented a global action plan at the World Health Assembly in 2015.[6] The Ministry of National Health Services of Pakistan released its national action plan (NAP) for antimicrobial resistance in May 2017.[7] In Pakistan, national and provincial strategies have been launched based on the NAP.[8] As previously indicated, Pakistan has recently created a national action plan (NAP) with five strategic and operational work plan components that are in line with the goals of the GAP to detect, prevent, and control infectious diseases as well as to fight antimicrobial resistance (AMR).[9] The National Institute of Health (NIH), working with various partners, decided to be the custodian of AMR surveillance in Pakistan by joining the Global Antimicrobial Surveillance System (GLASS) as one of the governments of Pakistan's ongoing initiatives to combat AMR.[10] Before this, a few other Pakistani organizations, such as the Pakistan Antimicrobial Resistance Network (PARN) and the Antibiotic Stewardship Initiative in Pakistan (ASIP), both operated under the auspices of the Medical Microbiology and Infectious Diseases Society of Pakistan (MMIDSP), were addressing AMR on a small scale.[5, 11] Following national and international efforts to reduce antibiotic resistance (AMR), the Pakistan Global Antibiotic Resistance Partnership (GARP) was also formed. However, there are worries that the NAP's implementation to combat AMR in Pakistan has been insufficient thus far, and there are still serious worries about the country's AMR prevalence.[7]

A significant percentage of the samples tested in standard diagnostic labs are urine samples. While there are many quick diagnostic techniques available, such as automated assays, Gram stain, dipstick, and wet mount microscopy, quantitative urine culture is the gold standard for urinary tract infection (UTI) diagnosis.[12] Standard laboratory procedures can take up to 18 hours to grow bacteria on culture media.[13, 14] This means that during the first 24 to 48 hours following presentation, the diagnosis is unclear, delaying treatment. Urine culture is also a costly process that requires a well-stocked microbiology lab with knowledgeable technicians. Six Conversely, reagent strip testing of urine samples is a technique intended to enable early infection detection in the ER and an earlier start of the treatment. The purpose of the 7 Reagent strips is to test for infection markers. Leukocyte esterase and nitrite, two markers, have been combined onto a single dipstick to screen urine samples for UTIs.[15, 16] The purpose of this study was to investigate the microorganisms growing resistant to antibiotics in the urine culture and their antibiotics susceptibility testing.

MATERIAL AND METHODS

In the pathology department's microbiology lab at the Dr. Akbar Niazi Teaching Hospital in Islamabad, a retrospective study was conducted on the patterns of antimicrobial resistance in urine samples over six months (June 2021 to November 2021). The Dr. Akbar Niazi Teaching Hospital in Islamabad's Microbiology Laboratory's pathology department's records provided the culture and sensitivity reports. The interpretation of the microorganisms isolated from different cultures is resistant or sensitive against antimicrobial drugs for this purpose clinical samples of urine for culture and sensitivity were provided by Dr. Akbar Niazi Teaching Hospital, Islamabad. Ethical clearance of the study was obtained from the Institutional Review Board (IRB)of Islamabad Medical and Dental College (IMDC) (IRB letter No. 51/IMDC/IRB-2021) before the study conductance. Permission was obtained from the Director, Dr. Akbar Niazi Teaching Hospital for the collection of data from the Microbiology laboratory of the pathology department of Dr. Akbar Niazi Teaching Hospital, Islamabad.

To prevent commensal overgrowth or the loss of pathogen viability, properly collected specimens should be sent to the laboratory as soon as possible during office hours. To prevent contamination from native plants, specimens should be collected from the appropriate anatomic sites using the right methods. If at all possible, specimens have to be obtained before antibiotics are administered. Antibiotic use should be disclosed on the request form if it is administered. The specimens that are collected ought to be sufficiently large and stored in suitable containers. A completed request form must be sent with each specimen.

Fill a sterile container with 2–10 milliliters of midstream urine. Urine collected early in the morning is best so the organism has time to grow in the bladder. If it's not feasible, three hours must pass since the last urination. After pulling back the foreskin, male patients should wash their glans penis with regular soap and water. Female patients should also clean their vulva and labial folds. Urine should be collected midstream and put straight into a sterile bottle. The first portion of the urine should be discarded. The specimen should reach the laboratory within 1 hour after collection. In case of delay, store at 4◦C for not more than 24 hours. Use alcohol to cleanse the skin surrounding the catheter site. Remove the 5 cm distal tip catheter aseptically, clip it into a sterile container, and send it straight to the laboratory.

Urine tested positive for both culture and sensitivity. Urine samples collected midstream were placed in sterile containers. The samples were cultivated for the entire night at 37°C on blood agar and MacConkey

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media with a standard loop. Conventional biochemical techniques and Gram-staining were used to identify the isolates and standard CLSI guidelines followed.
The Statistical Package for social science 2021 (SPSS), a computer program, was used to conduct the statistical analysis.

RESULTS

In our study, a total of 207 urine samples were included of which, 91 samples showed no growth. In 207 samples 116 samples showed positive bacterial growth. Candida had positive growth in 16.4%. Out of the positive samples, females (66.4%) frequency was higher than males (33.6%) (Table 1). The distribution of participants in our study according to age is shown in (Table 2). *Escherichia coli* bacteria isolated frequently with 50%, whereas, *Enterococcus spp* was 17/116 (14.7 %). Other Gram-Negative Rods were 16/116 (13.8%), *Klebsiella spp* were 4/116(3.4%%) and *Pseudomonas aeruginosa* was the least microorganism 2/116 (1.7 %) (Table 3). The overall percentage (%) of resistance and sensitivity of various antibiotics and Growth patterns in different organisms in urine specimens (Table 4).

Table 1: Frequency of patients urine culture according to gender-wise.

Gender	Percentage (Frequency) % (n)
Male	33.6 (39)
Female	66.4 (77)
Total	100 (116)

Table 2: Frequency of patients urine culture according to age-wise.

Age (years)	Frequency (n)
<20	16
21-30	12
31-40	10
41-50	19
51-60	17
>60	42

Table 3: The Growth patterns of different organisms in urine specimen.

Isolated bacteria	Percentage
Escherichia coli	50%
Pseudomonas aeruginosa	2%
Candida albicans	16%
Enterococcus spp	15%
Klebsiella spp	3%
Other Gram negative rods	14%

Table 4: Sensitivity of different antibiotics.

Antibiotics	Sensitive
Amphiciline	5%
Amoxiciline	5%
Augmentin	10%
Tazobactam	63%
Imipenam	64%
Cefuroxime	26%
Cefaxime	6%
Cefracidine	24%
Ciprofloxacine	38%
Levofloxacin	26%
Amikacin	23%
Gentamicine	74%
Trimethaprime	50%
Pencillin	19%
Vancomycin	92%
Linzolid	100%
Clindamicine	0%
Erythromycin	0%
Pepraciline	0%
Minocyclin	61%
Fosfomycin	86%
Nitrofurantine	88%
Cefipime	13%
Cefoperazone	64%
Meropenem	80%
Ceftriaxone	70%
Aztreunam	40%
Cephalotin	0%
Cefotaxime	36%

DISCUSSION

As in nearly every study we reviewed, *E. coli* was the most frequently isolated bacteria from urine specimens in our investigation. Globally, *E. coli* is becoming more and more of an MDR pathogen. This will have disastrous effects because antimicrobial resistance (AMR) is linked to poor patient outcomes and higher medical expenses.[17] WHO initiated the creation of the Global Antimicrobial Resistance Surveillance System (GLASS) in October 2015 to track AMR globally.[18] The most frequent uropathogen-producing bacteria is *E. coli*. Penicillin, cephalosporins, and aztreonam are examples of β lactam antibiotics that are rendered ineffective by the bacterial enzyme ESBL. Critically high prevalence of *E. coli* that produces ESBLs has been reported worldwide; in Asia, this prevalence is higher than in Europe.[19] In addition, ESBL-producing *E. coli* carry additional antimicrobial resistance genes that make them resistant to trimethoprim, aminoglycosides, cotrimoxazole, and other clinically significant substitutes. This raises the likelihood of therapeutic failure and drastically decreases therapeutic options. An additional concerning factor is the rise in E. Col production, particularly in infections linked to

healthcare facilities, which makes carbapenems ineffective.[20] The following medications have been shown to cause resistance when used empirically to treat UTIs: ciprofloxacin 74.3%, cotrimoxazole 82.1%, amoxicillin-clavulanic acid 87.5%, cefuroxime 88.9%, and nitrofurantoin 31.7%. There was more than 85% resistance to third- and fourth-generation cephalosporins. Overall resistance is significantly higher than previously reported. Pseudomonas, *E. coli*, and *Klebsiella spp* showed notable resistance to ceftazidime and ciprofloxacin. Compared to previous reports, the overall resistance in our study is significantly higher.[21] *E. coli* resistance to fluoroquinolones ranging from 5 to 64.7%, third-generation cephalosporins from 15 to 87%, cotrimoxazole 4.2 to 88.2%, and ESBL positivity from 17.4-66.2% from various Iranian regions.[22] According to Pakistan, resistance to aminopenicillins was 97%, carbapenems was 10%, third-generation cephalosporins was 86%, and fluoroquinolones was 59%. Globally, AMR exhibits significant regional variation.[23] This makes it necessary for antimicrobials to be used and disposed of properly in both the food industry and the health sector (animal health and agriculture). The single-center experience was the main focus of this study. It was impossible to distinguish between asymptomatic bacteriuria and a genuine infection because patient records were unavailable. It was also impossible to distinguish between samples from inpatients and outpatients. HAUTI data frequently overstates AMR.[24]

CONCLUSION

In our study according to gender distribution female frequency was higher than males out of a total of 116 positive cultures 77(66.4) were females and 39(33.6) were female. *Escherichia coli* bacteria were the most common isolate in positive samples 58/116 (50%), whereas, *Enterococcus spp* were positive in 17 patients out of 116 (14.7 %). Gram-negative rods were positive in 16 patients out of 116 (13.8%), *Klebsiella spp* were positive in 4 patients out of 116(3.4%) and *Pseudomonas aeruginosa* were least positive in only 2 patients out of 116 positive samples (1.7 %). Vancomycin was most sensitive in all urine cultures against all organisms and Erythromycin was most resistant in urine culture. Measures to be taken to minimize community antibiotic exposure on a national level in Pakistan. Antimicrobial resistance is a serious subject on national and international levels that needs continued surveillance and resolute management.

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