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Original Article

Gender Differences in Relation to Antimicrobial Resistance in *Escherichia coli* among Urinary Tract Infections in Patients from Kerala

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Abstract

Introduction: Urinary tract infections (UTIs) are one of the most common infections requiring medical care. Due to anatomical and physiological variations, the incidence of UTI is more common in females. Among the UTI samples, *Escherichia coli* is the most commonly isolated organism. Antibiotics are prescribed and the dose and duration of antibiotic usage depend on several factors. An estimation of the effectiveness of antibiotics among the male and female patients in the general population can help in prescribing the right antibiotic for the patient.

Aim: The aim of this study was to assess the usefulness of antibiotics against *E. coli* based on CLSI guidelines using Kirby–Bauer method and to estimate the prevalence of extended-spectrum beta-lactamase producing *E. coli*.

Materials and Methods: A total of 1349 Clean Catch Midstream Urine Samples (CCMSUs) were selected during the study period. After exclusions, totally 445 samples of monobacterial growth of more than 10^5 cfu/mL were considered for the study. Among these 179 isolates were *E. coli* and these isolates were considered for the present study. One hundred and twenty-one isolates were from female patients, whereas 78 isolates were from male patients. All samples were cultured on culture enriched and selective media by semi-quantitative method. Antibiotic susceptibility testing of all isolates was performed by Kirby–Bauer's disk diffusion method and interpretation of the results was done based on CLSI 2021. Bacterial suspension was made and compared to 0.5 McFarland turbidity standards in peptone water. Antibiotic disks (HiMedia Laboratories Pvt. Ltd. Mumbai) used were ampicillin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), cotrimonazole (25/1.25 μg), amoxicillin–clavulanic acid (20/10 μg), piperacillin/tazobactam (100/10 μg), norfloxacin (10 μg), amikacin (30 μg), ceftazidime (30 μg), ceftazidime + clavulanic acid (20/10 μg), cefuroxime (30 μg), cefotaxime (30 μg), imipenem (10 μg), aztreonam (30 μg), tetracycline (30 μg), nitrofurantoin (300 μg), tobramycin (10 μg), colistin (10 μg), etrapenem (10 μg), doripenem (10 μg), and meropenem (10 μg). Extended-spectrum β-lactamase (ESBL) producing *E. coli* were was confirmed phenotypically using combined disk diffusion method.

Results: The *E. coli* isolates from male patients were more resistant to antibiotics ciprofloxacin, gentamicin, nitrofurantoin tobramycin, and piperacillin-tazobactum. Ciprofloxacin was resistant in 66.7% of isolates from males and 50.4% in females with a statistically significant $P = 0.046$. Gentamicin resistance was at 38.5% of males and 21.5% in isolates from female patients with $P = 0.015$. Nitrofurantoin showed a resistance of 15.4% in males compared to 5.8% in females with $P = 0.045$. Tobramycin was resistant in 38.5% of male isolates compared to 10.7% from female patients with a highly significant $P < 0.001$. Piperacillin-Tazobactam showed a resistance percentage of 16.7% in males compared to 6.6% in *E. coli* isolates from female patients with $P = 0.033$. Ninety-five isolates (53.07%) of isolates of *E. coli* were ESBL producers.

Conclusions: Every health-care institutions must develop its own antimicrobial policy and this policy need to be reassessed at least once in 6 months to know the pattern of emerging resistance as well as to decide about the use of antibiotic recycling for the better usage of available antibiotics for treatment of UTI.

Key words: Adherence, Antibiotics, Enterobacteriaceae, *Escherichia coli*, Extended-spectrum β-lactamase, Pili, Uncomplicated Urinary Tract Infection, Uropathogenic *E. coli*, Uropathogens

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INTRODUCTION

One of the most important events in history of mankind is the discovery of antibiotics. Countless number of lives has been saved over the decades. Antibiotics are being used in treatment of variety of infections. This ranges from prophylactic antibiotics before elective or

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emergency surgeries to life-threatening infections. Over a period of time, the pathogenic bacteria have an ability to exhibit resistance to the antimicrobials and are termed as antimicrobial resistance (AMR). Due to the AMR, the effective usage of antibiotics is coming down leading to increase in economic burden to the patient as well as limiting the therapeutic options available to the treating physician.

Although the exposure of humans to antibiotic resistant strains is uniform, there are definite anatomical, socioeconomic, biological, and cultural differences among the members of different gender. For example, urinary tract infections (UTIs) are more common in women and if left untreated can result in serious health complications. From the literature, it is learnt that about 15% of all prescription antibiotics are used to treat UTI.[1,2] The most important element in decision making is the choice of antibiotic that is effective as well as cost efficient. Wide-spectrum antibiotics are commonly employed to treat UTIs when, instead, a narrow-spectrum antibiotic could have been sufficient for an effective treatment.[3] The effect of blanket use and misuse of antibiotics has led to emergence of resistance.[4-6] Apart from the above-mentioned factors, patient compliance to the prescription and over the counter pharmacy plays a major role in increasing the resistance to commonly used oral antimicrobial agents.

Gender-Wise Differences in Pathogenesis

Due to age and gender, UTIs tend to exhibit different epidemiological and etiological properties.[7] Isolates obtained from males and females might have different resistance profiles. Anatomical factors and alterations play an important role in the pathogenesis of UTI in women. The shortness of the urethra and its close relationship to the anus allows the bacteria to ascend easily into the urinary tract in females. The fecal-perineal-urethral contamination is the most probable explanation for infections caused by enteric bacteria, as demonstrated by experiments evaluating the genotype of *Escherichia coli* strains causing UTI in women.[8,9] Lactobacilli are the dominant bacteria in the vaginal biota and are responsible for maintaining the acidic pH by producing hydrogen peroxide.[10] Incomplete treatment and recurrent infections may shift the local predominance of flora from lactobacilli to coliform Uropathogens or a mix of both leading to loss of protective effects conferred by lactobacillus.[11] An alteration in vaginal flora is also observed during the postmenopausal period as the estrogen stimulating the proliferation of lactobacilli is lacking. Studies suggest that a family history of UTI in a first-degree relative increase the risk of recurrent UTI and pyelonephritis in women.[12] In premenopausal women, 90% of the vaginal flora is *Lactobacilli*, which protect the system against colonization with uropathogens such as *E. coli*. After menopause, the decrease in estrogen levels leads to thinning of vaginal epithelium and decrease amount of glycogen. The end result is a hostile environment to growth of Lactobacilli and reduced ability of the epithelium to resist bacterial colonization.[13]

There are evidences to suggest that human P and ABO blood group antigens play a role in susceptibility to UTI. Individuals with the P1 antigen are more predisposed to invasion by P fimbriated microorganisms.[14] Apart from the above-mentioned factors, the pregnancy and sexual intercourse have been documented as risk factors for women developing UTIs.

As far as the males are concerned, the common risk factors for development of UTI are Diabetes, Urolithiasis, Benign hypertrophy of Prostrate (BPH), an abnormal narrowing of the urethra, incontinence of urine, use of immunosuppressive agents, or history of instrumentation of the urinary tract. Men's risk for UTI increases with age, men are more likely more susceptible to UTIs after 50 years of age, when they are more likely to develop prostate problems. Enlarged prostate gland in BPH impedes and slows the flow of urine, thus raising the risk of infection. It has been observed that men who are not circumcised tend be more prone to developing UTIs, because these bacterial build up much more easily in the folds of the extra skin on the penis, thereby making them more susceptible to developing UTIs.[15]

Antibiotic Microbial Resistance Profile in Indian Scenario

The recent literature on urinary tract infections in India highlight the fact that the 60–80% of infections are seen in the female population. Among the female gender, the most common organism causing UTI is *E. coli* that the antibiotic sensitivity pattern in the recent years shows that the antibiotic resistance of *E. coli* to cephalosporins and fluroquinolones is a Pan-India phenomenon. The fluroquinolones, namely, ciprofloxacin, ofloxacin, and levofloxacin are not effective in 50–70% of the Indian isolates. In the same way, the antibiotics belonging to the cephalosporins are ineffective in 60–70% of *E. coli* isolates.[16-21] The sensitivity to antibiotics varies considerably depending on the local prescribing pattern. In a study done by the author in 2018, the antibiotic sensitivity pattern of *E. coli* showed marked difference between the extended-spectrum β-lactamase (ESBL) and non-ESBL isolates.[18] The ESBL isolates exhibited a remarkable lowering of sensitivity to cephalosporins, fluroquinolones, aminoglycosides, and tetracycline. The National Center for Disease Control under the Ministry of Health and Family Welfare, Government of India has published an important document named National Treatment Guidelines for Antimicrobial Use in Infectious Diseases in the year 2016.

In this guideline, the empiric therapy is with nitrofurantoin or cotrimoxazole or ciprofloxacin along with a comment to modify antibiotics based on culture and sensitivity report.

The guidelines issued by Indian Council of Medical Research (ICMR) in the year 2019 for treatment "Guidelines for antimicrobial use in common syndromes" emphasises *E. coli* as the predominant cause of Urinary Tract Infections and has suggested nitrofurantoin as a drug of choice in empirical therapy and fosfomycin as a go to drug in gram negative multidrug resistant organisms.

MATERIALS AND METHODS

The study was conducted at Azeezia institute of Medical Sciences and Research, Kollam district of Kerala State during the period of January 2021 to January 2022 to assess the antibiotic sensitivity pattern of uropathogenic *E. coli* among the isolates from urinary samples of males and female patients collected in patients reporting to the hospital with complaints suggestive of urinary tract infections.

All non-repetitive midstream urine samples obtained during the study period were included in the study. A total of 1349 Clean Catch Midstream Urine Samples (CCMSUs) were received in the microbiology laboratory during the study period. These samples were subjected to wet mount examination and were inoculated to 5% sheep blood agar and MacConkey agar plates. The plates were kept for incubation at 37°C for 18–24 h. After 24 h of incubation at 37°C 322 samples did not show any evidence of bacterial growth and were reported as no growth. One hundred and six samples showed presence of more than three types of bacterial growth which also corroborated with the wet mount findings and were not processed further. Three hundred and fifty-four samples showed $<10^4$ cfu/mL and in the absence of clinical history of catheterization or antibiotic intake which were reported as insignificant growths. One hundred and twenty-two samples grew Gram-positive yeast such as organisms (candida sp) or Gram-positive spore bearing bacilli and were excluded from the study. After exclusions, totally 445 samples of monobacterial growth of more than 10^5 cfu/mL were considered for the study. Eighty-nine samples grew Gram-positive organisms such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp., *and Enterococcus* sp. Three hundred and fifty-six isolates were Gram-negative bacilli out of which 177 isolates were *Klebsiella pneumoniae*, *Citrobacter* sp., *Enterobacter, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa,* and *Acinetobacter* sp. One hundred and seventy-nine isolates were *E. coli* and these isolates were considered for the present study. Among these 179 isolates of Uropathogenic *E. coli,* 121 isolates were from female patients, whereas 78 isolates were from male patients.

Processing of Samples

All samples were cultured on culture enriched and selective media by semi-quantitative method. Samples were inoculated on 5% Sheep Blood Agar plate and MacConkey agar plate by streaking using sterile calibrated wire loop and incubated aerobically for 18–24 h at 37°C. Samples which showed monobacterial significant grown ($>10^5$ CFU/mL) were included in this study. Isolation and identification of isolates were based on their morphology in Gram staining, cultural characteristics, and biochemical reactions.[22] Antibiotic susceptibility testing of all isolates was performed by Kirby–Bauer's disk diffusion method and interpretation of the results was done based on CLSI 2021. Bacterial suspension was made and compared to 0.5 McFarland turbidity standards in peptone water. Antibiotic disks (HiMedia Laboratories Pvt. Ltd. Mumbai) used were ampicillin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), cotrimoxazole (25/1.25 μg), amoxicillin–clavulanic acid (20/10 μg), piperacillin/tazobactum (100/10 μg), norfloxacin (10 μg), amikacin (30 μg), ceftazidime (30 μg), ceftazidime + clavulanic acid (20/10 μg), cefuroxime (30 μg), cefotaxime (30 μg), imipenem (10 μg), aztreonam (30 μg), tetracycline (30 μg), nitrofurantoin (300 μg), tobramycin (10 μg), colistin (10 μg), etrapenem (10 μg), doripenem (10 μg), and meropenem (10 μg).

Procedure

Antibiotic sensitivity testing was done using Kirby–Bauer method. Disks were applied aseptically. Gap of 24 mm center-center was ensured as per CLSI guidelines. Plates were incubated at 35 ± 2°C and examined after a minimum of 16–18 h.

Screening for ESBL Isolates

Screening of ESBL producing *E. coli,* according to CLSI guidelines, strains showing zone of inhibition of ≤22 mm for ceftazidime, and/or ≤17 mm for cefpodoxime and/or ≤ 27 mm for cefotaxime were considered for confirmation test for ESBL. ESBL producing *E. coli* isolates were the sub-cultured into sterile Nutrient Agar plates and incubated for 24–48 h. The isolated single colonies were used for further comparative studies. ESBL production among potential ESBL producing isolates was confirmed phenotypically using combined disk diffusion method. Comparison of the zone of inhibition was made for the ceftazidime (30 μg) versus that of the ceftazidime disk in combination with clavulanic acid (30/10 μg), placed 25 mm apart (center to center). A difference in the inhibition zone diameter of ≥5 mm for a combination disk versus ceftazidime disk alone confirmed ESBL production (phenotypic confirmatory disk diffusion test).[18]

RESULTS

The antibiotic sensitivity pattern of *E. coli* showed a marked difference in resistance percentage between the isolates from males and females. The *E. coli* isolates from male patients were more resistant to antibiotics such as ciprofloxacin, gentamicin, nitrofurantoin tobramycin, and piperacillin tazobactum. Ciprofloxacin was resistant in 66.7% of isolates from males and 50.4% in females with a statistically significant $P = 0.046$. Gentamicin resistance was at 38.5% of males and 21.5% in isolates from female patients with $P = 0.015$. Nitrofurantoin showed a resistance of 15.4% in males compared to 5.8% in females with $P = 0.045$. In the same manner, tobramycin showed a resistance percentage of 38.5% in males compared to 10.7% in *E. coli* isolates from female patients with a highly significant $P < 0.001$. Piperacillin-Tazobactam showed a resistance percentage of 16.7% in males compared to 6.6% in *E. coli* isolates from female patients with $P = 0.033$. The resistance percentage of antibiotics such as amoxicillin-clavulanic acid, amikacin, ampicillin, ceftazidime, cefotaxime, imipenem, and cotrimoxazole was similar in males and females. In our study, 95 isolates (53.07%) of isolates of *E. coli* were ESBL producers.

DISCUSSION

Urinary tract infections are one of the most common infections in clinical practice. In our study, total of 1349 samples were collected, out of which 106 samples were identified to have more than three bacterial species. This may be due to errors of sample collection or improper instructions being given to patients during sample collection. Although the percentage of such samples when you look at the total number may be less there is a chance of missing out true cystitis and the probability of patient coming back with a repeat sample is comparatively less in a community set up. Three hundred and fifty-four samples showed no evidence of UTI as per the current diagnostic guidelines. Three hundred and twenty-two samples showed no bacterial growth despite the clinical suspicion of UTI. Both these categories account for nearly 50% of samples. This may be due to the fact that some of the organisms were not cultivable by culture or the more practical explanation that would be the delay in transport of samples to the laboratory leading to reduction in bacterial numbers due to acidity of urine. Although the above said factors are speculative and questionable, the mere numbers highlight the importance of training and repetitive emphasis to the clinicians, nurses, laboratory technicians, and other ancillary staff regarding importance of proper instructions being given to the patient regarding sample collection, specimen handling, transport, and early processing of samples to achieve a microbiological report which coincides with the clinical judgment and the one which will be finally be useful for treatment of UTI.

As it has been noted in various other studies, the most common single bacteria causing UTI in our study were *E. coli*. About 80% of UTI were due to Gram-negative bacteria, out of which 50.28% was due to *E. coli*. Adult women had a higher prevalence of UTI than men may be due to anatomical and physiological factors. *E. coli* belonging to the Enterobacteriaceae family is known to colonize the uroepithelium with adhesion, pili, fimbriae, and P1 blood group phenotype receptor.[23] In our study, 53.07% of *E. coli* isolates were ESBL producers. This higher percentage reflects on the emergence of resistance in the geographical area and local antibiotic usage patterns. It is to be emphasized that the ESBL producing bacteria may not be detectable by routine disk diffusion antibiotic susceptibility testing. This may lead to emergence of resistance as well as treatment failures in the community. The net effect of these factors would be the resultant increase in cost of treatment to the patient.

The National Treatment Guidelines for antimicrobial use in infectious diseases was developed by the National Center for Disease Control under the aegis of Ministry of Health and Family Welfare Government of India. This guideline dictates the use of Nitrofurantoin 100mg BD for 7 days or cotrimoxazole 960 mg BD for 3–5 days or ciprofloxacin 500 mg BD for 3–5 days as presumptive treatment of Urinary Tract Infections. Alternatively, the guidelines also suggest Cefuroxime 250 mg BD for 3–5 days. The empiric therapy prescribed correlates with our findings, where 5.8% of isolates from female patients show resistance to nitrofurantoin, and in case of male patients, 15.4% exhibit antimicrobial resistance (AMR) to nitrofurantoin. As far as cotrimoxazole is concerned, 48.8% isolates from female patients and 51.3% of male patients exhibit resistance. Ciprofloxacin shows resistance of 50.4% in females compared to 66.7% in males. These levels of antibiotic resistance dictate the use of nitrofurantoin as a first-line treatment of UTI. Nitrofurantoin also known as urinary antiseptic has no role in treatment of other infections. Its efficiency in achieving a higher concentration in urine and the ease of administration being an oral antibiotic makes it an ideal choice as a drug for first-line treatment for UTI. However, in India, it would be ideal to rely on culture and sensitivity reports in view of higher antimicrobial resistance pattern, the gradually increasing ESBL produced among Enterobacteriaceae, the reduced compliance of patients to follow-up for treatment, and the socioeconomic factors dictating a fewer number of female patients approaching medical care.

In our study, the higher number of UTI were seen among the female patients which are attributable to the anatomical structure of urinary tract in relation to the gastro intestinal system, the knowledge, attitude, and practice of genital hygiene among Indian women and the natural ability of the Enterobacteriaceae family to adhere to uroepithelium. The antimicrobial resistance of the commonly used antibiotics such as ciprofloxacin, cotrimoxazole, and nitrofurantoin is comparatively less among *E. coli* isolates when compared to males. This may be due to the fact that majority of samples collected were of acute uncomplicated cystitis among female patients, where, as in males, the infections also included the ones accompanied by Benign prostatic hypertrophy, renal stones, and neurogenic bladder. It is worthwhile to be noted that every health-care institution must develop its own antimicrobial treatment policy based on the culture and sensitivity report prevailing in the past 6 months and these policies need to be reassessed at least once in 6 months to know the pattern of emerging resistance as well as to decide about the use of antibiotic recycling for the better usage of available antibiotics for treatment of UTI.

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