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Original Article

Antibacterial Susceptibility Pattern of Gram Negative Bacteria Isolated from Patients with Urinary Tract Infection

Hina Mukhtar[1*]

[1]Department of Biosciences, Kohsar University Murree, Pakistan

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***Corresponding Author:**
Hina Mukhtar
Department of Biosciences, Kohsar University Murree, Pakistan
hinamukhtar90@hotmail.com

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ABSTRACT

In clinical setting, urinary tract infections (UTIs) rank second among infectious presentations. Each year, around 150 million people receive a UTI diagnosis worldwide. **Objectives:** To determine the antibacterial sensitivity of meropenem, imipenem and ciprofloxacin against bacteria isolated from patients presented with Urinary Tract Infection. **Methods:** This Descriptive type Cross-sectional study was carried out at Clinical Microbiology Laboratory, Pathology Department of King Edward Medical University. A total of 100 patients were included that had Urinary Tract Infections. Urine culture was done using standardized Cysteine lactose electrolyte deficient (CLED) agar. Gram reaction, morphology, and biochemical properties were used to identify bacterial pathogens. Antibiotic sensitivity of the selected Gram negative bacteria was observed by using the Kirby Bauer method for antibiotic susceptibility. **Results:** The bacteria isolated were *E. coli* (62%), *Klebsiella* spp. (11%), *Candida* spp. (8%), *Pseudomonas aeruginosa* (7%), *Proteus* spp. (4%), *Staphylococcus* spp. (3%), *Staphylococcus saprophyticus* (3%) and *Enterobacter* spp. (2%). Sensitivity pattern for Ciprofloxacin was 20 (23.3%) sensitive and 66 (76.7%) resistant. Similarly 87.2% were sensitive to Meropenem and 12.8% were resistant. While for Imipenem 95.3% were sensitive and 4.7% were resistant. **Conclusions:** It was concluded that UTI most commonly caused by *E. coli* which is a gram negative bacteria followed by *Klebsiella*, *Pseudomonas*, *Proteus* and *Enterobacter*. Other microorganisms such as *Staphylococcus* spp., *Staphylococcus saprophyticus* and *Candida* spp. were also isolated in least amount. Antimicrobial susceptibility showed that Gram negative isolates of uropathogens were highly resistant against Ciprofloxacin and showed sensitivity against Imipenem and Meropenem.

INTRODUCTION

Infection of the urinary tract (UTI) is a general term that refers to both asymptomatic microbial colonization of the urine and symptomatic infection, in which microbial invasion and inflammation of the urinary tract organs, such as the kidneys, ureters, bladder, prostate, and urethra, occur [1]. The second most frequent infectious presenting in community practice is a urinary tract infection (UTI). The worldwide economy loses close to 6 billion US dollars each year due to the estimated 150 million UTI diagnoses [2]. Urinary Tract Infections were the cause of 100,000 hospitalizations and nearly 7 million office visits, according to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey. However, because urinary tract infections are not recognized as diseases in the United States, it is challenging to determine their frequency and prevalence [3]. According to estimates, urinary tract infections affect 18 people per 1000 each year, making it one of the most prevalent infections in the general population [4]. Urinary tract infections, which make up approximately 25% of all infections in non-institutionalized older people, are the second-most prevalent type of infection [5]. Nearly 10% of people may encounter a urinary tract infection at some point in their lives, making them one of the most prevalent infectious disorders [6]. Similar to most epithelia, the bladder wall is covered in a range of cationic antimicrobial peptides such defensins and cathelicidin that damage bacterial cell walls [7]. Uropathogenic *Escherichia coli*

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invades superficial umbrella cells during cystitis and multiplies quickly to create intracellular bacterial communities, which compromise innate defenses [8]. Gram-negative pathogens like *Escherichia coli* are more prevalent than other gram-negative pathogens including *Klebsiella pneumonia*, *Enterobacter* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, gram-positive *Staphylococcus saprophyticus*, and *Candida albicans* in urinary tract infections [9]. *Escherichia coli* is the most common pathogen isolate which is responsible for urinary tract infection [10]. Acute cystitis may be accompanied by dysuria (painful urination), urgency, hesitation, polyuria, and incomplete voiding. Hematuria, suprapubic or low back discomfort, and urinary incontinence could also be present. Fever, pain in the costovertebral angle, nausea, and vomiting are signs of pyelonephritis. Reduced functional capacity, which is shown in dementia patients, cardiovascular accidents, neurological abnormalities, and faecal incontinence are risk factors [11]. Multidrug resistance of antimicrobial classes is common among the uropathogenic bacteria like *E. coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [12]. This study aim to determine the antibacterial sensitivity of meropenem, imipenem and ciprofloxacin against bacteria isolated from patients with Urinary Tract Infection.

METHODS

This Descriptive type Cross-sectional study was carried out for 4 months duration at Clinical Microbiology Laboratory, Pathology Department of King Edward Medical University, Mayo Hospital, Lahore. A total of 100 patients were included that had Urinary Tract Infections. A brief history and biodata was taken from all patients. Clean Catch or early morning Mid stream urine samples were collected from all patients. Mid stream urine was collected in a sterilized wide mouthed container after necessary precautions. Only Mid stream urine samples received at Microbiology Lab Pathology dept. King Edward Medical University were included. Samples other than mid stream urine e.g. Catheter tips and supra pubic puncture sample and sample having mix growth of bacteria on culture plates were excluded. CLED agar, Muller Hinton agar, Triple sugar iron agar, Urease test medium, Oxidase test medium, Indole test medium and Citrate test medium were obtained from Oxoid Ltd., England. Commercially prepared Crystal violet solution, Iodine solution, Acetone-alcohol decolorizer solution and Safranine solution were used. Drug discs of Ciprofloxacin (5ug), Meropenem (10ug) and Imipenem (10ug) were obtained from Oxoid Ltd., UK. A commercially available reagent strip (DIRUI, Jilin, China) was used to detect pH, proteins, and sugar in urine after a physical examination for color. Then, using a microscope, it was possible to identify casts, red blood cells (RBCs), epithelium cells, pus cells, and RBCs. A calibrated loop was used to cultivate urine. For the development of microorganisms in the case of pyurea, urine was cultured for an entire day on standardized Cysteine lactose electrolyte deficient (CLED) agar. Gram reaction, appearance, and biochemical characteristics were used to identify bacterial pathogens. A drop of distilled water was applied to a spotless glass slide for Gram staining. Using a sterile loop in a drop of water on a slide, bacteria were cultured for 24 hours. On the slide, smear was equally distributed before air drying. Heat removes smears. Smear was cleaned with distilled water, dyed with crystal violet solution for 1 minute, and then submerged in iodine solution for 1 minute. After being cleaned with water, the smear was soaked in 95% alcohol to get rid of any remaining discoloration. The smear was then water washed, air dried, and counter dyed with safranine for 30 or 1 minute. seen using an oil immersion lens and a microscope. Biochemical tests like Urease, Oxidase, Indole and Citrate utilization test for Gram negative bacteria and Coagulase test for Gram positive isolates were done for further confirmation. Antibiotic sensitivity of the selected Gram negative bacterias was observed by using the Kirby Bauer method for antibiotic susceptibility. Muller Hinton agar plates were prepared and a single colony of selected strain was taken and swabbed on to agar surface by means of a sterile cotton swab. The antibiotic discs were then aseptically applied on the surface of agar plate at well spaced intervals (30 mm apart). The plates were incubated for 24 hours. According to the disc manufacturer's recommendations, the sizes of the zones that impede bacterial growth were used to determine whether a disc was "Sensitive" or "Resistant." Imipenem (10 ug), Meropenem (10 ug), and Ciprofloxacin (5 ug) drug discs were commercially manufactured and used in accordance with the National Committee for Clinical Laboratory Standards' standard disc diffusion procedure (NCCLS). According to the NCCLS criteria, the diameter of the zone of growth inhibition was measured and classified as either sensitive or resistant [13]. Statistical analysis was performed using SPSS, version 13.

RESULTS

A total of 100 urine samples were collected from which, 54% are females and 46% are males. The 52% patients were from 21-40 years age group, 28% were from 41-60 years, 15% were from 1-20 years and 5% were from 61-80 years age group as shown in table 1.

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Demographic characteristics	n (%)
Gender	
Male	46 (46%)
Female	54 (54%)
Age group	
1-20	15 (15%)
21-40	52 (52%)
41-60	28 (28%)
61-80	5 (5%)

Table 1: Gender and age wise frequency of UTI

Over the study period, total samples cultured on CLED medium, of these the isolated causative pathogens were *E. coli* (62%), *Klebsiella* spp. (11%), *Candida* spp. (8%), *Pseudomonas aeruginosa* (7%), *Proteus* spp. (4%), *Staphylococcus* spp. (3%), *Staphylococcus saprophyticus* (3%) and *Enterobacter* spp. (2%) (Table 2).

Growth on culture	n (%)
E. coli	62 (62 %)
Klebsiella spp.	11 (11 %)
Pseudomonas aeruginosa	8 (8 %)
Candida spp.	7 (7 %)
Proteus spp.	4 (4 %)
Staph. spp.	3 (3 %)
Staph. Saprophyticus	3 (3 %)
Enterobacter spp.	2 (2 %)
Total	100 (100&)

Table 2: Frequency of different Microbes on Culture.

E. coli was the most common uropathogen detected equally in females (31%) and in males (31%). *Klebsiella* species, *Candida* species, *Pseudomonas aeruginosa*, *Proteus* species, *Staphylococcus* species, *Staphylococcus saprophyticus* , *Enterobacter* species were also detected in decreasing frequencies. *Candida* species and *Staphylococcus saprophyticus* were detected only in females, while on the other hand *Staphylococcus* aureus was detected only in males as shown in figure 1.

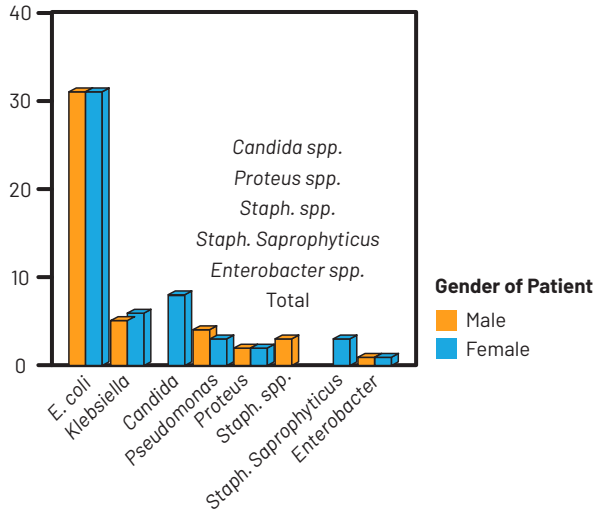

Figure 1: Frequency of microbes in male and female patients

In second part of the study the sensitivity pattern of gram negative uropathogens against Ciprofloxacin, Meropenem and Imipenem were detected. Out of 100 cultural growths, Gram negative isolates were 86%. Which showed, sensitivity pattern for Ciprofloxacin was 20 (23.3%) sensitive and 66 (76.7%) resistant. Out of 86% gram negative isolates 12 *E. coli*, 4 *Klebsiella* spp., 3 *Pseudomonas aeruginosa* and 1 *Proteus* spp. were sensitive to Ciprofloxacin while 50 *E. coli*, 7 *Klebsiella* spp., 4 *Pseudomonas aeruginosa*, 3 *Proteus* spp. and 2 *Enterobacter* spp. showed resistance (table 3). The p value for Ciprofloxacin was 0.44 which is > 0.05, it is not significant. It showed, increasing resistance pattern of uropathogens against Ciprofloxacin. Similarly, out of 86% gram negative isolates sensitivity pattern for Meropenem and Imipenem were showed in table no. 8 and 9, according to which 87.2% were sensitive to Meropenem and 12.8% were resistant. While for Imipenem 95.3% were sensitive and 4.7% were resistant. Only 1 *E. coli* isolate showed resistant to meropenem while 7 isolates of *Klebsiella* spp. showed resistance. In (table 11) only 3 *E. coli* isolates and 1 *Enterobacter* spp. showed resistance against Imipenem (table 3). The p value for Imipenem and Meropenem was < 0.05, which is significant.

Growth on culture	Sensitivity pattern of ciprofloxacin		Sensitivity pattern of meropenem		Sensitivity pattern of imipenem	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
E. coli	12	50	61	1	59	3
Klebsiella spp.	4	7	4	7	11	0
Pseudomonas aeruginosa	3	4	6	1	7	0
Proteus spp.	1	3	3	1	4	0
Enterobacter spp.	0	2	1	1	1	1
Total	20	66	75	11	82	4

Table 3: Sensitivity pattern of ciprofloxacin, meropenem, and imipenem in gram negative isolates

DISCUSSION

In terms of bacteriology, a UTI occurs when pathogenic germs are found in the urine, urethra, bladder, kidney, or prostate. When a correctly taken midstream clean catch sample grows more than 105 organisms per ml, infection is usually the cause [14]. Because females have shorter urethras than males, germs can more easily travel up the urinary canal in females [15]. According to our study's findings, 54% more women than men were infected, and this is a common pattern that UTIs all across the world follow. The Enterobacteriaceae were the pathogens most frequently found to be responsible for 84.3% of UTIs[16]. In this Research UTI causing microbial agents were isolated.The results also revealed that gram negative isolates were the major cause of UTI. Among them most

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prevalent strain *E. coli* detected to be 62.0% and the second most prevalent strain was *Klebsiella* spp. 11%, while *Pseudomonas aeruginosa*, *Proteus* and *Enterobacter* spp. were least prevalent in causing UTI. *Candida* spp., *Staphylococcus* spp. and *Staphylococcus saprophyticus* also isolated in 8%, 3% and 3% respectivly. Amin *et al*. and Ziad Daoud showed similar percentage of uropathogens isolates [17, 18]. In this study, 86% gram negative isolates were isolated out of which 20(23.3%)were sensitive and 66 (76.7%) were resistant (Graph no.8). It showed high resistance pattern for uropathogens. The right antibiotic should be chosen based on factors such regional patterns of antibiotic resistance, pharmacokinetics, once-daily versus multiple-daily dosing, impact on the body's normal vaginal and intestinal flora, and antibiotic safety [19]. *E. coli* bacteria that are resistant to antibiotics are on the rise globally [20]. Despite improvements in the detection and treatment of infectious infections, the persistent growth of antibiotic-resistant strains in microorganisms continues to provide a challenge to antimicrobial chemotherapy. Millions of people around the world now live in harsher conditions as a result of these [21]. Third generation Cephalosporins and Quinolones are substantially less effective against isolates of *E. coli* in our country due to multidrug resistance [22]. Consuming quinolones has been linked to people in hospitals developing more resistance [23]. This has also been shown in other parts of the world, particularly Spain, where 22% of the *E. coli* isolates tested positive for ciprofloxacin resistance [21]. In both wealthy and developing nations, including Pakistan, antibiotic resistance has become a significant issue. In a developing nation like Pakistan, the price of efficient wide spectrum antibiotics is extremely exorbitant and out of the grasp of the average middle class individual. This has made things more difficult and contributed to the emergence of diseases with increased resistance. People who have common infections that were once easily treatable with straightforward medications may experience severe danger and suffering as a result. The free availability of medicines over the counter, the prescription of antibiotics without considering susceptibility, and other variables may be the main causes of the rising degree of resistance to commonly used antibiotics in these isolates in this region. The resistance genes carried by these pathogens need to be further studied at the molecular level, and the sequences can be compared to those of genes reported from other parts of the world.

CONCLUSIONS

The microbiological causative agents of UTI's are Gram negative & positive bacteria and fungai. It was also observed that UTI most commonly caused by *E. coli* which is a gram negative bacteria followed by *Klebsiella*, *Pseudomonas*, *Proteus* and *Enterobacter*. Gram positive bacteria like *Staphylococcus* spp., *Staphylococcus saprophyticus* and *Candida* spp. were also isolated in least amount. Urinary Tract Infections are most frequent in females as compared to males. Antimicrobial susceptibility showed that Gram negative isolates of uropathogens were highly resistant against Ciprofloxacin and showed sensitivity against Imipenem and Meropenem. It showed that resistance against Ciprofloxacin increased among gram negative uropathogens.

Conflicts of Interest

The authors declare no conflict of interest

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