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Original Article

Prevalence and Phenotypic Detection of Carbapenem and Multi Drug Resistant of *E. coli* in Urinary Tract Infection Patients in District Swat

Uzma Noor[1], Muhammad Suliman[1], Husna Shams[2], Amir Sultan[3*], Shah Hassan Khan[4] and Shazia[1]

[1]Upper Swat College of Nursing Swat, Pakistan
[2]Microbiology Department, Sarhad University Peshawar, Pakistan
[3]Nursing Department, Saidu Group of Teaching Hospital Swat, Pakistan
[4]Provincial TB Program, Khyber Pakhtunkhwa, Pakistan

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***Corresponding Author:**

Amir Sultan
Nursing Department, Saidu Group of Teaching Hospital Swat, Pakistan
nursingwithamir@gmail.com

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ABSTRACT

Among prevalent infectious diseases, the most frequently occurring infections are the Urinary Tract Infections (UTIs) which predominantly occur in the community as well as in the hospital settings and are one of the main cause of morbidity and mortality worldwide. **Objectives:** To determine the frequency of MBL *E. coli* species in urine samples, antibiotic susceptibility pattern and the prevalence of MDR for *E. coli*. **Methods:** There were 200 urine samples obtained from Anwar clinical laboratory Saidu Sharif, District Swat, Pakistan. Samples of urine were obtained and then cultivated in selective media i.e. Cysteine Lactose Electrolyte Deficient Agar (CLED) and MacConkey Agar Plates. In traditional morphological and biochemical studies, isolates were identified. **Results:** Total 58 (29.6%) positive isolates were recovered from male while 116 (70.3%) urine specimens were positive from female patients, A total isolated bacteria were MBL positive including 36 (31.3%) isolated *E. coli*, Furthermore, in the total isolated species were identified as MDR positive in which 80 (69.5%) were *E. coli*, The most potent antibiotics found against bacteria were the highest for Meropenum (78.2%), Imepenum (73.9%), and Amikacin (26.0%) Cefuroxime (21.7%), respectively and Cefaclor (19.1%) were most sensitive while antibiotic mostly resistant showed. **Conclusion:** This study concludes that, the most prominent bacterial isolate in the urine samples was *E. coli* 115 (69.6%), Carbapenem resistance is frequently observed isolates of *E. coli*, which indicate that MBL phenotype should be regularly determined in clinical settings to prevent emerging Carbapenem resistance.

INTRODUCTION

Each year, approximately 150 million infections are caused by urinary tract pathogens throughout the world. Among nosocomial infections, UTI's are the most recurrent, with a prevalence of 35%. Most UTI's are caused by *E. coli*, which occurs commonly in less severe UTI's [1]. After respiratory infections, this is the most common condition. The kidneys, ureters, bladder, and urethra are collectively referred to as the urinary tract. Escherichia coli is the bacteria mostly involved in causing UTI's Almost 35% of healthy women are suffering from UTI symptoms, and about 5% of women are suffering from painful urination (dysuria) each year. In women, the frequency of UTIs is greater than in men [2]. Carbapenemase production in *Enterobacteriaceae* is a dominant issue affecting human health throughout the world. Carbapenemases can degrade β-lactam antimicrobial agents as well as carbapenems [3]. Metallo β lactamases (MBL's) include a wide range of β-lactamases not merely resistant to cephalosporin but to carbapenems as well. In *Enterobacteriaceae*, *the* ESBL phenotype is mostly observed, such as in *Morganella* and *E. coli* [4]. Recently, this pathogen has been frequently isolated in hospital settings across the globe, and MDR is emerging. These strains pose a challenge for clinicians and microbiologists

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because of their increased propensity to cause not only nosocomial infections but also community-acquired infections [5]. Recent research has shown that uropathogenicity is the main cause of UTI's in *E. coli*, although *K. pneumoniae* is also a common cause of UTI's. The phenotypic tests comprise the modified Hodge test (MHT) and the process to inactivate carbapenems. The MHT senses the development of Carbapenemase in Enterobacteriaceae isolates and is easy, cheap, and highly accessible in routine clinical microbiology settings according to the Clinical and Laboratory Standards Institute (CLSI) [6]. A variety of hospitals reported UTI's in admitted as well as non-admitted patients. Nosocomial UTI's are frequently reported with a prevalence of 25–50% among hospitals, leading to economic loss and affecting human health [7]. The carbapenems group of drugs is very effective in the treatment of bacterial infections and can also save lives in the event of an MDR infection, in particular health-related infections. The resistance to carbapenems is also a significant problem for the healthcare system, restricting the antibiotic choices available to treat these infections. Carbapenemase production is recorded globally at higher rates in gram-negative bacilli [8]. Production of antimicrobial resistance has been increasing rapidly in recent times, and the future public health problems this may cause need concerted interventions worldwide in many health sectors. As a result, there have been several deaths in Europe, and the European Centre for Disease Prevention and Control (ECDC) estimated that 25,000 people could die per year from antimicrobial resistance-related infections [9]. Carbapenems are considered to be one of the most effective medicines for the treatment of bacterial infections, and a major public health issue is the rise and spread of resistance to these antibiotics [10]. A study was conducted where a total of 200 gram-negative bacilli (GNB) samples were collected. Isolates that display intermediate or prone areas, i.e., 16 mm or 21 mm, on disc diffusion were included in the study. (MHT) examined these isolates afterwards. Of the 200 isolates, 138 (69 %) were positive for Carbapenemase development by Modified Hodge. Of the 138 positive MHT species, the frequency is *E. coli* [11]. The MHT senses the development of Carbapenemase in *Enterobacteriaceae* isolates and is easy, cheap, and highly accessible in routine clinical microbiology settings according to (CLSI) [6]. A study was conducted on patients suffering from UTIs. Among 1074 isolates, the most highly reported microorganism was *E. coli*, isolated in 559 cases and exhibiting 14.5% MDR. *K. pneumoniae* was present in 168 samples and showed the highest MDR rate of 54.2%, followed by *P. aeruginosa*, which was isolated in 97 samples with a 38.1% MDR [12]. The aim of the current research work was prevalence and phenotypic detection of Carbapenem and multi drug resistant bacteria in UTI patients in District Swat. The study will identify the prevalence of MDR among isolated uropathogens i.e. *K. pneumoniae*, Citrobacter and *E. coli*.

METHODS

A descriptive, cross-sectional study design was used in this study. The samples of urine were collected from Anwar clinical laboratory in Saidu Sharif, District Swat, Pakistan, and included males and females of different age groups. A total of 200 urine samples were collected randomly. The patients previously not administered with antibiotics were included in the study, while others were excluded from the study. The urine samples were collected in sterile bottles from patients expected to have UTI's and were cultured on Cystine-Lactose-Electrolyte-Deficient Agar (CLED) and MacConkey agar plates. The samples were then incubated at 37°C for 24 hours. Nutrient agar was used for sub-culturing of samples a standard Gram staining procedure was used for morphological identification of bacterial isolates [13]. To identify *E. coli* morphologically and biochemically, different identification tests were used. *E. coli* species appeared in the form of opaque yellow colonies on CLED agar plates [14]. Four different biochemical tests were performed for the identification of microorganisms, i.e., urea, citrate, Triple Sugar Iron (TSI), and Sulfur Indole Motility Media (SIM) [15]. The carbapenemase enzyme production in *E. coli is* identified by modified Hodge test MHT. 0.5 McFarland dilution of the test microorganism was inoculated in 5ml of distilled water and the inoculum turbidity was adjusted with 0.5 McFarland. Test microorganism was streaked on MHA plates and was allowed to dry for 3-5 minutes. A 10µg Imepenum or Meropenum antibiotic disc was placed in the center of agar plate and then subjected to incubation at 37oC for 24 hours [11]. To determine MDR in isolated bacteria, different antibiotic discs were inoculated on MHA to build a uniform bacterial lawn with the aid of a sterile swab. Using sterile forceps at similar distances, antibiotic discs were placed above the surface (not less than 22 mm). The plates were set at 37°C for 24 hours in the incubator. The resistance of bacteria to a specific antibiotic has been demonstrated by the inhibition zone around antibiotic discs. The inhibition zone was calculated in mm according to the guidelines of CLSI, 2019. The antibiotic sensitivity test was conducted through "Kirby Bauer disc diffusion" method to determine the susceptibility pattern as per the CLSI guidelines, 2019 according to the procedure followed by [16]. The discs were placed on the test organism and control strains as controls on different agar plates. The diameter of the test organism's inhibition zone was measured with a scale. The filter paper discs with a proven antibiotic concentration

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were placed on Muller-Hinton Agar (MHA) plates after inoculating MHA with a pure colony of microorganism. Antibiotics placed on MHA inhibit bacterial growth in an area where drug concentration is sufficient to kill bacteria or prevent their spread. The inhibition zone of the test organism is calculated after the formation of a clear visible zone.

RESULTS

A total of 200 urine samples were obtained from Anwar clinical laboratory in Saidu Sharif, District Swat Pakistan. Samples were collected from children and adults of both genders. Of the 200 samples, (82%) n = 165 were positive, while (18%) n = 35 were negative. In 165 positive samples, the number of female positive samples was higher (67%) compared to male positive (33%). In the urine samples, E. coli was recovered in the majority (69.6%), followed by K. pneumoniae (18.7%) and Citrobacter (11.5%) (table 1).

Variables	Characteristic	Frequency (n=200)
Total samples	Positive	165 (82%)
	Negative	35 (18%)
Gender	Male +	58 (33%)
	Female +	116 (67%)
Isolates	E. coli	115 (69.6%)
	K. pneumoniae,	31 (18.7%)
	Citrobacter	19 (11.5%)

Table 1: Distribution of isolates and their gender wise frequency

The total samples of E.coli n=115 were analyzed through Metallo β Lactamase which showed (31.3%) n=36 were positive while the majority of the MBL was negative (68.6%) (table 2).

Bacterial species	MBL positive	MBL negative	Total
Escherichia coli	36 (31.3%)	79 (68.6)	115

Table 2: Metallo β Lactamase (MBL) findings of samples

Imepenum (73.9%) shows sensitivity, respectively, followed by Amikacin (69.5%), ampicillin (69.6%), and co-trimaxazole. The isolated bacterial species were tested for antibiotic sensitivity profiles. The most potent antibiotics found against bacteria were the highest for Meropenum (78.2%) and (60.8%) and Nitrofurantoin (60%), Ampicillin + Salbactum (57.3%), Fusidicacid (53.9%), Penicillin (51.3%) Piperacillin + Tazobactam (50.4%), Linezolid (51.3%), Ceftazidime (48.6%), Amoxicillin + Clavulanic acid (43.4%), Cefepime (39.1%), Fosfomycin and Gentamicin (30.4%). Norfloxacin and Penicillin (29.5%), Cefadroxil and Amikacin (26.0%), Cefuroxime (21.7%), and Cefaclor (19.1%) were the most sensitive, while antibiotics were mostly resistant as shown in table 3.

S. No	Antibiotics	*E. coli*	
		Sensitivity	Resistant
1.	Co-trimaxazole	70 (60.8%)	45 (39.2%)
2.	Fosfomycin	35 (30.5%)	80 (69.5%)
3.	Amoxicillin + Clavulanic acid	50 (43.5%)	65 (56.5%)
4.	Cefadroxil	30 (26%)	85 (74%)
5.	Cefaclor	22 (19.2%)	93 (80.8%)
6.	Cefexime	19 (16.5%)	96 (83.5%)
7.	Gentamicin	35 (30.5%)	80 (69.5%)
8.	Amikacin	80 (69.5%)	35 (30.5%)
9.	Ciprofloxacin	30 (26%)	85 (74%)
10.	Norfloxacin	34 (29.5%)	81 (70.5%)
11.	Nitrofurantoin	69 (60%)	46 (40%)
12.	Ceftazidime	56 (48%)	59 (52%)
13.	Ampicillin + Salbactum	66 (57%)	49 (43%)
14.	Cefepime	45 (39.2%)	70 (60.8%)
15.	Piperacillin +Tazobactam	58 (50.5%)	57 (49.5%)
16.	Imipenem	85 (73.9%)	30 (26.1%)
17.	Meropenem	90 (78.2%)	25 (21.7%)
18.	Ampicillin	79 (68.6%)	36 (31.3%)
19.	Cefuroxime	25 (21.7%)	90 (79.2%)
20.	Linezolid	59 (51.3%)	56 (48.6%)
21.	Pencillin	34 (29.5%)	81 (70.43%)
22.	Fusidic acid	62 (53.9%)	53 (46.0%)

Table 3: Susceptibility pattern of E.coli

A total of 115 isolates were MDR positive, of which 80 (69.5%) isolates were E. coli, while 35(30.4%) were MDR negative (see table 4).

S No	Bacterial species	MDR positive	MDR negative	Total
Escherichia coli	36 (31.3%)	79 (68.6)	115	

Table 4: MDR results of samples

DISCUSSION

UTIs are the most common infectious disease associated with the multiplication of microorganisms in the urinary tract. This research has been carried out to isolate and identify this research has been carried out to isolate and identify *E. coli* in the urine samples and also to find antibiotic susceptibility, MDR and MHT detected in the clinical isolates. UTIs are mostly (about 95%) caused by a single bacterial species, *E. coli*. Other bacteria that cause UTIs include *Proteus, Staphylococcus, Mycoplasma, Chlamydia, Klebsiella, Pseudomonas, Enterobacter Serratia,* and *Neisseria* spp. Each year, approximately 35% of healthy women are reported to have UTI symptoms, and approximately 5% of women experience painful urination (dysuria).The prevalence of UTI is higher in women compared to men [2]. In this study, we found a high incidence of *E. coli* (50.8%). Our results for *E. coli* are in agreement with the findings of a study that reported a high incidence of *E. coli* (50%). In this study, we found an elevated incidence of *Enterobacteriaceae* isolates [16]. In the finding of a study, 339 (77.1 %) were *E. coli*, 56 (12.7%)

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were *K. pneumoniae*, and 14 (3.2%) were strains of *P. mirabilis* and accounted for 11% (2.5%), while Sub Enterica had 9(2.0%), and *C. freundii. E. cloacae* Enterobacteriaceae resistance rates to cephalosporin are ranged from 47.5% for Cefepime to 63.2% for cefuroxime [17]. Cefoxitin was more active, with a resistance rate of 22.7% among nine cephalosporin's tested. This group of bacteria had a cumulative tolerance rate of 31.6% and 32.7%, respectively, to aminoglycosides, gentamicin, and tobramycin. The prevalence of MDRs among *Enterobacteriaceae* isolates was documented in patients having symptomatic treatment. Our findings showed that the rate of susceptibility of clinical bacterial isolates was the highest for Meropenum (78.2%) Imepenum (73.9%), respectively, followed by Amikacin (69.5%), Ampicillin (69.6%), Co-trimaxazole (60.8%), Nitrofurantoin (60%), Ampicillin + Salbactum (57.3%), Fusidic acid (53.9%), Penicillin (51.3%). Piperacillin + Tazobactam (50.4%), Linezolid (51.3%), Ceftazidime (48.6%) Amoxicillin + Clavulanic acid (43.4%) Cefepime (39.1%), Fosfomycin and Gentamicin (30.4%), Norfloxacin and Penicillin (29.5%), Cefadroxil and Amikacin (26.0%) Cefuroxime (21.7%), respectively and Cefaclor (19.1%) were the most sensitive, while antibiotics were mostly resistant, as shown in previous studies. The major MDR isolates were found to be *K. pneumonia* (95.6%) and *E. coli* (92.9%). Although the rate of MDR is different in different areas, similar groups of bacteria were found in Bahirdar, Ethiopia (*E. coli* at 94.6 percent and *K. pneumoniae* at 80%) [18], Nepal (*E. coli* at 74 percent and *K. pneumoniae* at 44 percent) and Dakar (*E. coli* and *K. pneumoniae* at 89 percent) [19]. The prevailing MDR was uropathogens. In addition, these bacteria are often difficult to treat because of their intrinsic resistance to several groups due to the predominant MDR uropathogens [20].

CONCLUSIONS

The study results established the sensitivity and resistance of the identified organisms to the drugs. In the current study, the most prominent bacterial isolate in the urine samples was E. coli 115 (69.6%). Culture sensitivity revealed that the most effective antibiotics were Meropenum and Imepenum while resistance was observed against Cefixime, Ampicillin, Cefaclor, and Ceftriaxone. MHT was detected in any isolate, whereas E. coli, MHT production was tested to be positive.

Conflicts of Interest

The authors declare no conflict of interest

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