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Hubza Ruatt Khan
Department of Microbiology and Molecular Genetics, The Women University, Multan, Pakistan., hubzakhan12345@gmail.com

Mehvish Javeed
Department of Microbiology and Molecular Genetics, The Women University, Multan, Pakistan., mehvishjaved15@yahoo.com

Asghar Javed
Department of Pathology, Nishtar Medical University, Multan, Pakistan, asgharmicro@hotmail.com

Nisma Farooq
Department of Microbiology and Molecular Genetics, The Women University, Multan, Pakistan., nismaansari@yahoo.com

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PREVALENCE AND MULTIDRUG RESISTANCE PATTERN OF *E. COLI* AMONG URINARY TRACT INFECTION PATIENTS IN TERTIARY CARE HOSPITAL OF MULTAN

HUBZA RUATT KHAN[1], MEHVISH JAVEED[1], ASGHAR JAVED[2] and NISMA FAROOQ[1]

[1] *Department of Microbiology and Molecular Genetics, The Women University, Multan, Pakistan.*
[2]*Department of Pathology, Nishtar Medical University, Multan, Pakistan.*

Corresponding author's email: hubzakhan12345@gmail.com

ABSTRACT

Urinary Tract Infection is alarming problem worldwide due to the intensity of antimicrobial resistance. *Escherichia coli* is the most predominant organism in UTI**.** This study was planned to evaluate demographic parameters, the prevalence of *E. coli*, and antimicrobial resistance patterns among *E. coli* isolates from UTI patients in Nishtar Hospital of Multan from January to June 2018. A total of 350 mid-stream urine samples were collected from different patients having age group from 25 to 60 years and processed by standard laboratory procedures. Out of 350 samples, 100 samples were observed as critical bacteremia. *Escherichia coli* and *Klebsiella pneumonia* were the most persistent (47 % and 19 % individually) among the Gram-negative pathogens followed by *S. aureus* (14 %), Enterobacter spp. (11 %) and Candida (9 %) respectively. The incidence of UTI was found higher in 25-35 age groups. The prevalence of UTI with *E. coli* as an infectious agent was 72 % in females, and 28 % in males. The prevalence of *E. coli* was more in rural areas (70 %) than in urban areas (26 %). The antimicrobial testing against *E. coli* showed the highest resistance to amoxicillin (65.9 %) and ciprofloxacin (38.2 %), whereas highly sensitive rate observed against Fosfomycin (FOS) (95.7 %), Gentamicin (GEN) (89.3 %), and Nitrofurantoin (NIT) (85 %) respectively. The increased resistance against ampicillin and ciprofloxacin was observed in Multan have a great emerging problem so there is a need for effective prevention strategies for the *E. coli* drug resistance and successful surveillance required to be improved.

Keywords: Urinary Tract Infection, prevalence, escherichia coli, resistance, antibiotics.

INTRODUCTION

Urinary tract infection is the most familiar microbial disease especially present in hospitals and communities affecting people of all age groups (Rosen et al., 2007). The occurrence of urinary tract disease depends upon little variables that give the proximity of minute organisms (more than 105/ ml) in urine samples (Mihankhah et al., 2017). Every year, 150 million individuals are influenced by UTI around the world (Shabbir, 2017). UTI is considered as the second most common cause of contagions (Klumpp et al., 2006). The prevalence of UTI is found in both genders while the frequency is higher in females than in males and there is a 50 to 70% chance that every woman will at least acquire one UTI during her lifetime (Wiedemann et al., 2014) While UTI is not common in men but it can be very severe when it happens (Elahe, 2015). The UTIs is affected by other risk factors like age, gender, sexual behavior, immunity, diabetes, and neurological diseases (Hsueh, 2011).

The most causative agent of the UTI is *E. coli* and this bacterium is responsible for different cases of these infections (Daza et al., 2001). Among the uropathogenic and nosocomial UTI, *E. coli* is the most predominant pathogen (Shatalov, 2015). Moreover, *E. coli* is the

cause of emergency clinically gained UTI (De Cueto & Aliaga, 2016). The microbial spectrum that is often isolated from urine samples is *Escherichia coli*, *Staphylococcus aureus, Enterococcus, Proteus sp., Candida albicans,* and *Klebsiella pneumonia, Pseudomonas aeruginosa* (Bronsema et al., 1993).

In developing countries, antibiotic resistance is the major public health issue to treat such kind of infections because of the fast spread of mutant strains antibiotics fail to work against these drug resistance mutated pathogens. Thus, it complicates the treatment of UTI (Kibret and Abera, 2011). UTIs influenced the nature of infected people and it becomes the reason for increasing monetary and common well-being loads. Patients with UTI are treated with anti-infectious agents. Anti-toxins that are used as medicine can be a result of the transformation of the microbial flora of the vagina and gastrointestinal tract. It involves the improvement of multidrug-safe microorganisms (Kostakioti et al., 2012). The cure for UTIs is getting to be hard because of the development of the anti-toxin barrier mechanism (Hooton, 2012). Increased resistance to antimicrobial agents makes it difficult to manage the UTIs (Mandal et al., 2012). In *E. coli*, the emerging resistance may be due to the chromosomal transformation, transportation of the resistant genes that make it resistant to generally used drug and it becomes MDR (Dever and Dermody, 1991).

To overcome the problem of emerging MDR uropathogens, the epidemiological surveillance data can be obtained from different hospitals and analyzed truly. The anti-microbial agents should be prescribed after cultural investigation according to the guidelines "Infectious Diseases Society of America" (Khawcharoenporn et al., 2013).

Thus, the present study aimed to isolate the most infectious agent of UTI according to their demographic parameters like age, gender, location and check the susceptibility pattern of UTI's causative agent to different anti-microbial agents among patients that referred to different wards of Nishtar Medical Hospital and University, Multan.

MATERIAL AND METHODS

Study Area and Period

A cross-sectional study was conducted at Nishtar Medical University and Hospital, Multan for a period of six months (from January 2018 to June 2018).

Ethical Approval

The study plan was approved from Ethical committee of Nishtar Medical University and Hospital, Multan, prior to execution of the research.

Demographic Parameters of the Study

During this time of six months, samples were obtained from patients with their consent having symptoms of UTI. Demographic parameters such as age, gender, residency was also collected from all the patients of gynaecology, general medicine, urology, and surgery ward.

Sample Collection and Nature of the Sample

A total of 350 mid-stream urine samples were collected in sterilized plastic containers and transported to the laboratory immediately to avoid contamination and further processing to determine the presence of *E. coli* in urine samples. The nature of the urine samples was different from patient-to-patient situation according to their diseases like diabetes, renal diseases, pregnancy, catheterization, and surgical site infection. They were all susceptible to urinary tract infection. The results were recorded according to standard protocols (Aghazadeh et al., 2015)

Isolation and Identification of E. coli

For isolation of bacteria, culturing of about 0.001 mL of urine samples on the Cystine Lactose-Electrolyte-Deficient Agar (CLED agar) was done with the help of a calibrated loop. The samples were incubated overnight at 37 °C. The selection of media was based on the desired organism which could be capable to grow on it (Kiffer et al., 2007; Al-Benwan et al., 2010).

After culturing, the Gram staining technique was used to differentiate between Gram-Negative and Gram-Positive Bacteria. Further biochemical testing such as TSI citrate, indole, motility, catalase and oxidase tests were performed to identify the presence of desired bacteria in the dissimilar bacterial colony (Holt et al., 1994; Washington, 2012).

Antimicrobial Susceptibility Testing

After the identification of *E. coli*, antimicrobial susceptibility testing was performed on Muller Hinton Agar with the help of Kirby–Bauer disc diffusion method (Hudzicki 2009). The antibiotics such as Amikacin (AK), Tazobactam/Piperacillin (TPZ), Nitrofurantoin (NIT), Colistin (CT/CST), Ciprofloxacin (CIP), Imipenem (IMP), Amoxicillin (AMX/AML), Fosfomycin (FOS), Gentamicin (GEN) and Tetracycline (TCN/TET) were used to obtain profiles of antimicrobial susceptibility. The results were interpreted according to the guidelines of CLSI criteria (Clinical and Laboratory Standards Institute) (Balouiri et al., 2016).

Statistical Analysis

All the categorical data was cleaned in MS Excel and all analysis were carried out with the help of XL-stat software 2010. All analysis was performed to investigate the prevalence of *E. coli* as an infectious agent of UTI patients in Multan.

RESULTS

Prevalence of E. coli and other Isolates of Bacteria in UTI Patients

A total of 350 urine samples were analyzed from different patients of Nishtar Medical University and Hospital during the period of six months from January 2018 to June 2018. Out of 350 urine samples, 100 (28.57 %) of samples showed bacteremia. As shown in Table 1, the prevalence of *E. coli* was observed high 47(47 %) out of 100 growing cultures (N=100) as compared to other isolates such as *K. pneumonia* 19 (19 %), *S. aureus* 14(14%), Enterobacter 11(11 %) and Candida 9 (9 %). N showed the total number of growing cultures.

Table 1: Distribution of isolated strains from UTI patients (N=100).

Isolates	Percentage of Isolates
E.coli	47 %
K. pneumonia	19 %
S. aureus	14 %
Enterobacter	11 %
Candida	09 %
Total	100 %

Prevalence of E. coli in Demographic Parameters

The data were categorized according to age, gender, marital status, and types of residency. Patients from different departments of Gynaecology, General Medicine, Urology, and Surgery were analyzed. The prevalence of UTIs with positive culture was observed in these departments. The maximum number of cases was found in Gynecology 39 (39 %). followed by General Medicine 32 (32 %), Urology 18 (18 %), and surgery 11(11 %) in UTI (Table 2).

For age distribution, patients were divided into different categories of age: 25-35, 35 - 45, 45 -55 and > 55 years. Out of 100 positive cases, 72 were females and 28 were males.

Table 2: Prevalence of UTI in different wards (N=100).

Departments	%age
Gynecology	39%
General Medicine	32%
Urology	18%
Surgery	11%
Total	100%

The highest occurrence of UTI patients was found in the age group of 25-35 followed by 35 - 45, 45 - 55 and > 55 years respectively. In both females and males, the prevalence of UTI was observed higher in the age group of 25-35 followed by 35 - 45, 45-55 and > 55 years. The females (52.7 %) were more infected in their reproductive age group 25 - 35 as compared to males (50 %) (Table 3).

Table 3: Prevalence of UTI in different age groups in both males and female (N=100).

Ages	Male	% age	Female	% age
25-35	14	50%	38	52.7%
35-45	10	35.7%	13	18.0%
45-55	3	10.7%	11	15.2%
>55	1	3.57%	10	13.8%

In demographic parameters, the prevalence of *E. coli* as an infectious agent of UTI was analyzed. Out of 100 positive samples, 47 samples were found with *E. coli*. About 13 (28 %) were males and 34 (72 %) were females found to be infected with *E. coli* as the main cause of UTI. A high prevalence of *E. coli* was observed in married patients 28 (60 %) rather than single patients 19 (40 %). In the same way, the occurrence of *E. coli* was relatively high in rural areas 33(70 %) than the urban areas 12(26 %) as described in Table 4.This difference may be due to the use of unhygienic food, less access to health care, and other socioeconomic factors.

Table 4: Demographic distribution of subjects in UTI patients with E. coli positive.

Gender	Number of Patients	Total *E.coli* Positive Cases	Prevalence % (*E. coli* +)
Males	13	47	28%
Females	34	47	72%
Marital status			
Single	19	47	40%
Married	28	47	60%
Types of Residency			
Urban	12	47	26%
Rural	33	47	70%

Antimicrobial Screening of Isolated Strains of E. coli

In this present study, only the antibiotic susceptibility pattern of *E. coli* was analyzed. Ten antibiotic discs amikacin (AK), tazobactam/piperacillin (TPZ), nitrofurantoin (NIT), colistin (CT), imipenem (IMP), ciprofloxacin (CIP), amoxicillin (AML), fosfomycin (FOS), gentamicin (GEN) and tetracycline (TET) were used. According to their zone of inhibition, all the drugs have different resistant, intermediate and sensitive patterns. The antimicrobial susceptibility profile of *E. coli* against these drugs is presented in Table 5. In the present study, the percentages of isolated *E. coli* were mostly resistant to these drugs amoxicillin (AMX/AML) 65.9 %, ciprofloxacin (CIP) 38.2 %, colistin (CT/CST) 27.6 %

respectively and these drugs were not considered to be very effective against UTI treatment. *E. coli* have been found highly sensitive to Fosfomycin (FOS) 95.7 %, Gentamicin (GEN) 89.3 %, Nitrofurantoin (NIT) 85 %, Tazobactam/Piperacillin (TPZ) 78.7 %, Amikacin (AK) 74.4 %, Tetracycline (TCN/TET) 70.2 %, and very less sensitive to imipenem (IMP) 68 % (Figure 1 and Table 5).

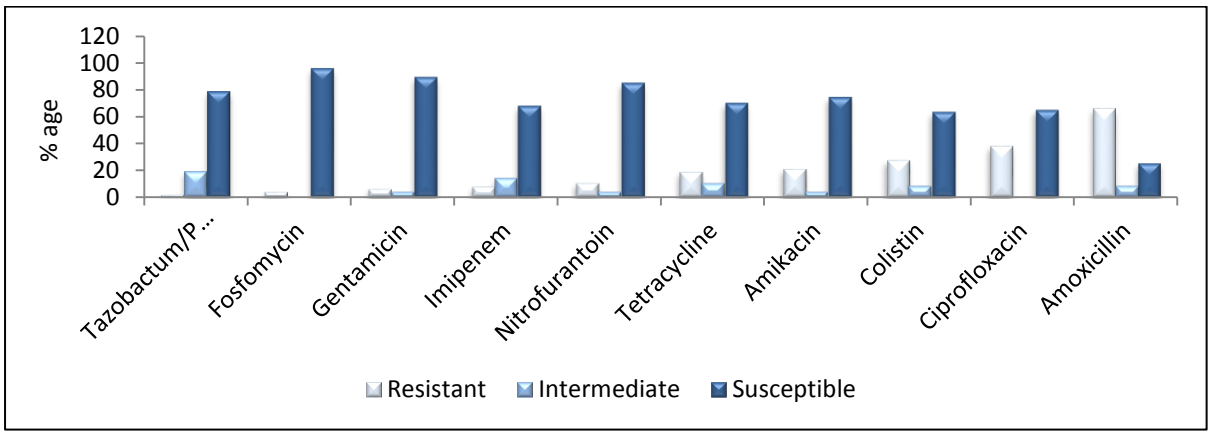

Figure 1: Resistance-wise distribution (%) of *E. coli* isolates with different antibiotics as resistant, intermediate and sensitive.

Table 5: Profile of antimicrobial susceptibility testing.

Antibiotics	Total isolates (*E. coli*)	Resistant (%)	Intermediate (%)	Sensitive (%)
Amikacin (AK)	47	10 (21.2)	2 (4.2)	35 (74.4)
Tazobactam/Piperacilin (TPZ)	47	1 (2.1)	9 (19.1)	37 (78.7)
Nitrofurantoin (NIT)	47	5 (10.6)	2 (4.2)	40 (85)
C0listin (CT/CST)	47	13 (27.6)	4 (8.5)	30 (63.8)
Ciprofloxacin (CIP)	47	18 (38.2)	0 (0.0)	31(65.9)
Imipenem (IMP)	47	8 (17.0)	7 (14.8)	32 (68.0)
Amoxicillin (AMX/AML)	47	31 (65.9)	4 (8.5)	12 (25.5)
Fosfomycin (FOS)	47	2 (4.2)	0 (0.0)	45 (95.7)
Gentamicin (GEN)	47	3 (6.3)	2 (4.2)	42 (89.3)
Tetracycline (TCN/TET)	47	9 (19.1)	5 (10.6)	33 (70.2)

DISCUSSION

In therapeutic management of UTIs, the advanced knowledge on the prevalence and antibiotic susceptibility pattern of pathogenic bacteria is very necessary which serves as an infectious agent of UTIs. The number of virulence factors such as capsule, lipopolysaccharide, flagella, fimbriae, toxins, and iron scavenger receptors associated with different types of uropathogenic *Escherichia coli* (UPEC) is concerned to cause UTIs (Karam et al., 2019). The present study showed that the prevalence of Gram-Negative bacteria 77 % was high as compared to other Gram-Positive bacteria (14 %). *E. coli* was the most dominant among the Gram-Negative bacteria 47 % than the *K. pneumonia* 19 %. The results of this study were supported by the previous study of (Elmanana` et al., 2018) which showed that *E. coli* was in higher number as compared to other isolates. Other previous studies revealed the higher frequency of *E. coli* (i.e. 70 %) followed by *K. pneumonia* 11 % (Noor et al., 2013). This shows the most powerful evidence that *E. coli* is the

most common and predominant pathogen in UTIs, similar findings have been observed in the past (Sabir et al., 2014).

In our study, the UTI patients associated with the Gynecology department were higher in number. It was concluded that women were greater in number as compared to men associated with other departments. The chances of UTI increase during pregnancy. This study supported by the previous study by Pallet (Pallet and Hand, 2010). This study shows that the prevalence of UTI was very high in Females 52.7 % in their reproductive age group 25-35 as compared to males 50 %. A previous study, Bari et al, (2017) revealed higher number of female patients in their reproductive age are being affected by UTI (Bari et al., 2017). In our study, the prevalence of *E. coli* in UTI patients was very high in females about 72 % as compared to males 28 %. A number of studies reported the prevalence of *E. coli* in UTI patients very high in females than in males (Okonko et al., 2009; Martin et al., 2019). This may happen due to the colonization of the vagina with coliforms with the help of vaginal microflora which can be associated with UTIs (Hooton and Stamm, 1997). Furthermore, females have the short urethral tube so that bacteria can easily access to the bladder to cause infections. These factors of causing UTI are accelerated by low soci-economic status, sexual behavior, and the use of unhygienic food (Nas et al., 2019).

The location and socioeconomic status may contribute to the development of UTI with *E. coli* because several UTI patients belonged to rural areas (70 %) and less from urban areas (26 %). This happens due to less access to pure water, the use of unhygienic food, and poor living condition. Present study findings are in line with Dhodhi et al., (2014). Resistance to antibiotics is increasing day by day and it leads to becoming a world-wide problem (Sefton, 2000). According to this study, the prevalence of higher resistance rate was observed to Amoxicillin (65.9 %) and ciprofloxacin (38.2 %) which cannot be used for the treatment of UTI in that particular area of study. Higher sensitivity rates were detected to antibiotics such as Fosfomycin (95.7 %), Gentamicin (89.3 %), Nitrofurantoin (85 %) and Tazobactum (78.7 %). According to the findings, which have been reported in previous studies carried out in Ethiopia, Iran respectively (Moges, et al., 2002; Farajnia et al., 2009; Beyene and Tsegaye, 2011) and in Kosovo (Raka et al., 2004), Ciprofloxacin has great susceptibility against *E. coli* but in our study, *E. coli* have greater resistance against this antibiotic. This increased resistance in strains may be due to the geographical changes in Multan because most of the people belong to less developed areas and excessive use of antibiotics without any microbial investigation might be the reason for resistance against antibiotics.

It should be needed to minimize the rising resistance in *E. coli* from UTI patients by increasing knowledge about some necessary measurements by the healthcare professionals such as antibiotics should be prescribed after microbial investigation and there should be the presence of infection control strategies to decrease the hazard of future resistance to antimicrobial agents.

CONCLUSION

Studies revealed the re-emergence resistance of *E. coli* against several antibiotics makes it difficult to treat patients with UTI. It is very difficult to treat these infections in developing countries due to the financial burden on the health care system. Proper guidelines for the regular surveillance for sensitivity pattern of antibiotics are very necessary for the management of UTIs. Therefore, routine sensitivity screening of antibiotics should be carried out before the prescription is recommended. This will help in designing the emerging multidrug

resistance problem to the frequently used antibiotics.

CONFLICT OF INTEREST

The authors declare have no any conflict of interest. They have not competing professionally and financially with other parties.

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