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Isolation and identification of *Escherichia coli* from urine samples and their antibiotic susceptibility pattern

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Abstract
Escherichia coli cause Urinary tract infection (UTIs) throughout the world, so to improve infection control measurement knowledge about the distribution and frequency of Urinary tract infection (UTI) is very important. A total of 70 patients were examined of different ages and sexes suffering from UTIs. The isolated bacteria were recognized by conventional biochemical test and the antimicrobial susceptibility tests are performed on Mueller-Hinton agar using disk diffusion (Kirby Bauer's) technique. Out of them UTI's prevalence in female was 68% and male 32%, the maximum UTIs prevalence rate within the age of 21-40 was (30%), followed by above 80 (07%). *E. coli* was sensitive to antibiotics (TZP, FOS, F, IMP, AK and SCF) in males 61-80 (09 patients), followed by age 01-20 (09 patients) in the female. However, *E. coli* show resistant to antibiotic found in male between the age of 61-80 (09 patients), followed by 41-60 (04 patients), while in female, consequently, from the age of 21-40 (15 patients) were reported, followed by 01-20 (09 patients) and 41-60 (06 patients). The sensitivity of antibiotic is TZP, FOS, F, IMP, AK and SCF 6/14 (40%), while resistivity antibiotics to *E. coli* are as follow, AM, AMC, CFX, SXT, DO, CIP, CEF and CRO 8/14 (60%). The emergence of antibacterial-resistant bacteria is also contributed by inappropriate antibacterial treatment and abuse of antibiotics. The aim of this research work was to isolate the bacteria from urinary tract infection to determine its prevalence and the antibiotics susceptibility patterns of the *E. coli* Bacteria.

Keywords: UTI, resistance, prevalence, antibiotic. *E. coli*

Introduction
Urinary tract infection (UTI) can be defined as the process by which microorganisms' growing actively within the urinary tract continuously [1]. UTIs is one of the most serious and contagious infectious in human population all around the globe [2] and because of its complications, it targets a variety of fragile human body organs such as bladder, ureters, and urethra [3]. The infection in the urinary tract is 14 times more common in women because of the shorter size of the female urethra and near to anus, hence UTI developed up to 40% in women at least once at some stage in their lives and the majority of these women experience frequent UTI. Alternately, prostate gland has bactericidal substance and Zn that play a crucial role in the killing of microorganism like *E. coli*, so such types of infection is inhabit in men [4,7, 2].

The most common bacteria that cause UTI in human are, *E. coli*, *S. marcescence*, *E. faecalis*, *S. saprophyticus*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* and *S. aureus* [8, 9, 7, 5]. The main cause for 85% community-acquired urinary tract infection (UTIs) and 50% hospital-acquired urinary tract infections (UTIs) is *E. coli* bacteria [10, 11]. There are different factors that affect the prevalence of urinary tract infections (UTIs) like gender, age, urological instruments, and immunosuppression [12, 13]. The empirical usage of ciprofloxacin and cephalosporins has been threatened by the emergence of broad-spectrum beta-lactamases [14, 15]. Although, microorganisms use a diverse mechanism to get hold of drug resistance *viz.* horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in diverse chromosomal locus [16, 17]. However, antibiotic resistances are diverse to respective communities [18]. Therefore, in Pakistan, the resistance to antibiotics becomes more exacerbated due to the excess uses and misuses of antibiotics [12, 19]. The lack of national systematic surveillance of antibiotic drugs resistance, non-evidence base prescriptions and unsatisfactory data is existed to measure the problem [20].

Moreover, for improving the efficiency of empirical treatment the recognition of UTI causing organism and its resistance to usually recommend antibiotic in clinical setups is very supportive and crucial [21]. Therefore, the aim of this study was to isolate and determine the etiological agents from clinical samples and evaluate their susceptibility to normally used antibiotics. It may help the physicians to select suitable antibiotics for the control and prevention of urinary tract infection.

Materials and Methods

Study area

The present study was conducted at Tehsil Head Quarter (THQ) hospital Matta, Swat, Khyber Pakhtunkhwa, Pakistan during the period of January-February 2015.

Sampling

The samples were collected from the infected UTIs patients (70n), who were registered in this research and the urine samples were taken aseptically for microbial culture. The sterile containers were properly labelled with the name, age and sex of the patients. The samples were cultured immediately after collection.

Culturing

20µl of the specimen is inoculated through an incinerated wire loop on CLED-agar. The inoculation was performed through the streaking method inside a flow hood. After inoculation incubates the plates for 24 hours at 37°c in an inverted position. After 24 hours of incubation, if more than one type of organism was isolated, results were recorded as "mixed culture," and are excluded from this study. Equal to 10^5 CFU/ml of a single potential microorganism is interpreted as positive.

Gram staining

A gram stain was performed to confirm and identify the pathogen and bacteria were searches in pus cells in urine. While, the Gram-negative rods were identified by gram staining, and such type of culture was then inoculated by streaking to MacConkey agar for subculturing. MacConkey agar is used to isolate and differentiate the Gram-negative enteric bacilli [22].

Biochemical test

According to the microscopic examination and culture characteristics, selected colonies were identified and differentiated. For further confirmation, the IMViC tests can be used to differentiate *E. coli* from other enteric bacilli [23]. The IMViC series includes four tests, a) Indole production, b) Methyl red test, c) Voges-Proskauer test and d) Citrate utilization [24, 23].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests are performed on Mueller-Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique.

Disk diffusion technique

The disk diffusion technique of antimicrobial susceptibility testing involves the assignment of antibiotic disks onto MHA (Mueller-Hinton agar) plates. MHA (Mueller Hinton Agar) is recommended by Bauer, Kirby and Tuck for performing antibiotic susceptibility tests using a single disk of high concentration. This medium has been nominated by the "Clinical and Laboratory Standard Institute" (CLSI) for several reasons [25].

Transfer of inoculums

Select four-five well-grown colonies of the same morphology from MacConkey Agar plate culture. By touching the top of each colony with a wire loop and the growth is transferred into Mueller-Hinton Agar plate. Now spread the culture by streaking the sterile cotton swab three to four times over the entire surface of the agar, performing the streaking each time rotate the plate about 60° to ensure an even distribution of the inoculums. Allow the plates 3-5 minutes, to dry the surface of agar before applying the antibiotics disks. Moreover, through Forcep Place the proper antimicrobial-impregnated disks on the surface of the MHA agar culture plate and inverted for overnight at 37 ^{0}C.

Reading Plates and Interpreting Results

After 16-18 hours of incubation hold the Petri dish a few inches above on a black, nonreflecting background and lightened with reflected light, zones of complete growth inhibition around each of the disks are carefully examined with the scale on every Petri plate.

Results

A total of 70 samples of urine were cultured on CLED agar, equal or more than 10^5 CFU/ml of a single potential pathogen was interpreted as positive. Out of these samples, 65 give positive growth on CLED agar. The gram staining 58 isolates were identified as gram-negative and such types of culture was grown on selective media, while the rest of the samples were termed as "mixed culture," and excluded from this study. The colonies grown on MacConkey agar were then confirmed by biochemical tests i.e. (IMViC), of them 53 out of 58 were gram-negative isolates give positive results for the indole, methyl-red test and give a negative result for Voges-Proskauer and Citrate Utilization Test which confirms that the tested organism is *E coli*.

Fig 1: Indole positive *E. coli* (left side); Citrate utilization negative for *E. coli*.

Prevalence rate

The UTI's prevalence rate was high in females which were 68% as compared to males which were 32%. In age group 1-20 the prevalence rate of UTI was less in males which is 02% and in females, it was high i.e., 17%, and total of which is 19% which was 11% less than the age groups of 21-40 which are 30%. In the age group 21-40 the prevalence rate of UTI in males was 02% which was less than the female's prevalence rate which was 28%. In this age group, the prevalence rate of urinary tract infection was high from the female isolates of the entire age groups. In the age group of 41-60, 11% of females have a urinary tract infection and 08% of males have urinary tract infections. In the age group 41-60 the prevalence rate of urinary tract infection was 11% less than the age group of 21-40. In the age group of 61-80, the males have 17% of urinary tract infection and females have 08% 0f urinary tract infection, which was 09% high from females of this age group. The age group Above 80 have a prevalence rate of 07%, which was less as compared to the entire age groups (Table 1).

Table 1: Prevalence rate of UTI due to *E. coli* percentage wise in male and female

Age	No of male patients	No of female patients	Prevalence rate age wise %age
01-20	01(02%)	09(17%)	19%
21-40	01(02%)	15(28%)	30%
41-60	04(08%)	06(11%)	19%
61-80	09(17%)	05(08%)	25%
Above 80	02(05%)	01(02%)	07%
Total	17(32%)	36(68%)	100%

Therefore, *E. coli* sensitivity to antibiotics was checked in the male population among different ages. The most abundant sensitivity was found at the age of 61-80 (09 patient), followed by 41-60 (04 patient), while the minimum sensitivity was found in the age of 01-20 and 21-40 (01 patients each), followed by above 80 (02 patient) (Table 2).

Table 2. *E. coli* sensitive to antibiotics in males

Age	No of male patients	Antibiotics
01-20	01	TZP, FOS, F, IMP, AK, SCF
21-40	01	TZP, FOS, F, IMP, AK, SCF
41-60	04	TZP, FOS, F, IMP, AK, SCF
61-80	09	TZP, FOS, F, IMP, AK, SCF
Above 80	02	TZP, FOS, F, IMP, AK, SCF
Total	17	

Consequently, *E. coli* sensitivity to antibiotics was checked into the female population among different ages. The most abundant sensitivity was found at the age of 21-40 (15 patient), followed by 01-20 (15 patient), while the minimum sensitivity was found in the age of above 80 (01 patient) followed by 61-80 (05 patient) and 41-60 (06 patient) (Table 3).

Table 3: *E. coli* sensitive to antibiotics in females

Age	No of female patients	Antibiotics
01-20	09	TZP, FOS, F, IMP, AK, SCF
21-40	15	TZP, FOS, F, IMP, AK, SCF
41-60	06	TZP, FOS, F, IMP, AK, SCF
61-80	05	TZP, FOS, F, IMP, AK, SCF
Above 80	01	TZP, FOS, F, IMP, AK, SCF
Total	36	

The antibiotics to which *E. coli* was sensitive in males and females: Peracillin+Tazobactam, Cefoperazone+sulbactam, Amikacin, Fosfomycin, Nitrofurantoin, Imipenem.

However, *E. coli* show resistant to antibiotic found in male, accordingly, from the age of 61-80 (09 patients), followed by 41-60 (04 patients), while less resistance shown in the age of 01-20 and 21-40 (01 patients each), followed by above 80 (02 patients) (Table 4).

Table 4: *E. coli* resistant to antibiotics in males

Age	No of male patients	Antibiotics
01-20	01	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
21-40	01	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
41-60	04	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
61-80	09	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
Above 80	02	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
Total	17	

Nevertheless, *E. coli* show resistant to antibiotic found in female, consequently, from the age of 21-40 (15 patients) were reported, followed by 01-20 (09 patients) and 41-60 (06 patients), while less resistance shown in the age of above 80 (01 patient), followed by 61-80 (05 patients) (Table 5).

Table 5: *E. coli* resistant to antibiotics in females

Age	No of female patients	Antibiotics
01-20	09	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
21-40	15	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
41-60	06	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
61-80	05	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
Above 80	01	AM,AMC,CFX,SXT,DO,CIP,CEF,CRO
Total	36	

The antibiotics to which *E. coli* was resistive in males and females: Amoxycillin+clavulanic Acid, Ampicillin, Ciprofloxacin, Cefixime, Cefuroxime, Ceftriaxone, CoTrimaxazole, Doxycyclin. Moreover, the overall sensitivity and resistivity antibiotics to *E. coli* antibiotics are as followed TZP, FOS, F, IMP, AK, SCF, AM, AMC, CFX, SXT, DO, CIP, CRF and CRO. The sensitivity of antibiotic is TZP, FOS, F, IMP, AK and SCF 6/14 (40%), while resistivity antibiotics to *E. coli* are as follow, AM, AMC, CFX, SXT, DO, CIP, CEF and CRO 8/14 (60%), (Table 6).

Table 6: Overall resistive and sensitive antibiotics

Total antibiotic used	Sensitive *E. coli* to antibiotics	Resistant *E.* coli to antibiotics
TZP, FOS, F, IMP, AK, SCF, AM, AMC, CFX, SXT, DO, CIP, CEF, CRO	TZP, FOS, F, IMP, AK, SCF	AM, AMC, CFX, SXT, DO, CIP, CEF, CRO
14	6	8
100%	40%	60%

Discussion

From the present study, we investigate the prevalence of *E. coli* in UTI, as well as their antibiotic sensitivity pattern. The most common causative agent of UTIs in the current study was *E. coli* (91%). Bashir *et al.*, [26] examined 67% prevalence of *E. coli*, while Khan *et al.*, [27] reported uro-pathogens 45.6% prevalence of *E. coli*. Although, from the present research *E. coli* were (91%) reported. Additionally, both of the study areas patients were relatively same to each other. However, enteric bacilli are the most common pathogen isolated from UTIs infection accounting 94.4% of total isolated bacteria by Amin *et al.*, [28]. From the present study, *E. coli* was also the most predominant pathogen of UTIs. Zhanel *et al.*, [29] and Christensen *et al.*, [30] examined *E. coli* and their existence as gender wise, age, environmental factors, host factor and socioeconomic factors accordingly. Even though from the present study similarities and dissimilarities in the type and distribution of UTI's pathogens may result from different environmental factors and host factors, and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices respectively.

Gupta *et al.*, [31] reported a total of 65 patients including children, adults and women were clinically suspected patients of having a urinary tract infection, for 53 patients *E. coli* was the causative agent of UTI. As UTI affects all the age groups, and both the males and females, but many scientists proved that, UTIs is the most frequent bacterial infection in female Islam *et al.*, [32]. However, the present study also supports this likeliness as 68% positive female patients, where the women are more susceptible to UTIs than men as the urethra is much shorter and closure to the anus in females, 19% positive patients were found below 20 years, where situations may be due to the reduced personal hygiene practices. After isolation of *E. coli* and their confirmation through biochemical tests, we performed an antibiotic sensitivity test. Thus we determined that it show resistance to the common antimicrobial drugs like Amoxycillin+clavulanic Acid, Ampicillin, Ciprofloxacin, Cefixime, Cefuroxime, Ceftriaxone, Co-Trimoxazole, Doxycyclin and show sensitivity to Peracillin+Tazobactam, Cefoperazone+sulbactam, Amikacin, Fosfomycin, Nitrofurantoin, Imipenem. There are earlier studies Amin *et al.*, [28] isolated *E. coli* from UTI showed the extreme degree of resistance to ampicillin and tetracycline while showed sensitivity to gentamicin, ceftriaxone, amikacin and ciprofloxacin. Bashir *et al.*, [26] reported that *E. coli* resistance to ampicillin, Ciprofloxacin, Nitrofurantoin, Co-Trimoxazole, Amikacin. Niranjan *et al.*, [33] express the resistance of *E. coli* in India, that the resistance to ampicillin, amoxicillin-clavulanic acid, cefuroxime, ceftriaxone and co-trimoxazole and were sensitive to amikacin, piperacillin-tazobactam, nitrofurantoin and imipenem. Although, from the present study the following antibiotic Peracillin+Tazobactam, Cefoperazone+sulbactam, Amikacin, Fosfomycin, Nitrofurantoin, Imipenem were used. In both research study, the same antibiotic was used and found good results. Antibiotic resistance is a severe and developing phenomenon in up-to-date medicine and has appeared as one of the prominent public health concerns of the 21st century. During the past decade in our area, uncontrolled usage of these common antibiotics influenced the increase of resistance property among the causative agents. The emergence of antibacterial-resistant bacteria is also contributed by inappropriate antibacterial treatment and abuse of antibiotics. Consumption of antibacterial without a doctor's prescription is an example of antibiotic abuse. High resistance against these commonly used antibacterials is definitely worrisome. Thus these drugs must no longer be recommended as initial empirical therapy but unluckily these useless antibiotics are yet being recommended as first-line drugs in the developing countries.

Conclusion

It is concluded that the most common isolated bacteria from urinary tract infections (UTI's) was *E. coli* (91%). UTIs are the most frequent bacterial infection in female i.e., 68%. The

prevalence rate of UTIs was highest for the age group 20-40. Most of the strains were resistant to Amoxycillin+clavulanic Acid, Ampicillin, Ciprofloxacin, Cefixime, Cefuroxime, Ceftriaxone, Co-Trimoxazole, Doxycyclin and effective antimicrobial agents were Peracillin+Tazobactam, Cefoperazone+sulbactam, Amikacin, Fosfomycin, Nitrofurantoin, Imipenem.

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