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Isolation, Identification, and Characterization of *Escherichia coli* from Urinary Tract Infection Patients Using Conventional and Advanced Diagnostic Technique

Muhammad Saqib Khalil[1], Zarak Khan[2], Hafsa[3], Zainab Liaqat[4], Sulha Syed[5], Naila Gulfham*[6]

[1, 2,3,4,5] Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology

[6] Jinnah College for Women University of Peshawar. (Corresponding Author)

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Abstract

The main aim of this study was the isolation, identification and detection of *Escherichia coli* (*E. coli*) among Urinary Tract Infection (UTI) Patients. Data was collected from UTI patient visiting Out Patients Department (OPD), Hayatabad Medical Complex (HMC). A total of 60 patients, both male and female, were included in the study. All the samples were cultured on microbiological medium. For isolation, CLED agar media was utilized while for purification the study used MacConkey agar media. Identification of *E. coli* was done on the basis of morphological characteristics, gram staining and biochemical tests i.e. Indole, Urease, Catalase, Oxidase, Citrate, and Triple Sugar Iron (TSI), Methyl red, Eosin Methylene Blue Agar media tests were performed. ESBL detection was done according to Double Disc Synergy test. The results of the study indicated that out of 60 samples, 42 (70 percent) patients were *E. coli* positive. *E. coli* was positive and higher among married patients as compare to unmarried patients. ESBL was positive among 33 patients while negative among 09 patients. The *E. coli* was resistant to ATM among 14.29 percent while it was sensitive to ATM for the remaining 85.21 percent samples. The *E. coli* was resistant to AMC among 21.43 percent and sensitive to AMC for 78.57 percent sample. The lowest resistant was shown against ATM with 12.12 percent while AMC are 100 percent sensitive against positive *E. coli*. CFM are lowest second number resistant showing 27.27 percent resistant followed by NOR and CIP which are 33.33 percent and 39.39 percent resistant, respectively. NOR, CIP, CAZ, CTX and AMC are 100 percent resistant against2 negative *E. coli*, while CFM are 88.89 percentresistant to negative *E. coli*. ATM is less resistant antibiotic having only 22.22 percent resistivity.

Key Words: *Escherichia coli* (*E. coli*), ESBL, CLED, MacConkey, Susceptibility, UTI

Introduction

A urinary tract infection (UTI) is the colonization of a pathogen in the kidney, ureter, bladder, or urethra (Byron, 2019). UTIs are defined as the presence of microbial pathogens in the urinary tract and are typically categorized by the site of infection, such as the bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria), as well as whether they are asymptomatic or symptomatic (Byron, 2019) "Uncomplicated" UTIs are those that develop in a healthy genitourinary tract without any prior instrumentation, whereas "complicated" infections are those that are diagnosed in genitourinary tracts with structural or functional abnormalities, including instrumentation like indwelling urethral catheters, and are frequently asymptomatic (Wagenlehner et al., 2020).

More than 95.0% of UTI cases are caused by bacteria, however fungi and viruses can also contribute to a small number of UTI cases (Flores-Mireles et al., 2019; Wagenlehneret al., 2020). Based on age and related morbidities, the causal agent may greatly vary. The urinary tract can be infected by a wide variety of species, although gram negative bacteria are the most prevalent. UTI bacteriology can be predicted extremely well. Although a variety of organisms can cause UTIs, like the gram negative facultative anaerobic, Uro pathogenic *E. coli* (UPEC) is responsible for the vast majority of infections in all populations. For many years, *E. coli* has been identified as the etiologic agent in roughly 75.0 to 90.0% of infections, keeping the pathogens linked to uncomplicated UTIs constant (Bhardwaj, 2019; Flores-Mireles et al., 2019). Other Enterobacteria, particularly *Klebsiella species*., *Proteus mirabilis*, and *Pseudomonas* a*eruginosa*, are the remaining gram-negative urine pathogens (Johnson *et al*., 2021). The most frequently identified gram positive organisms are coagulase negative *Staphylococci* (CoNS) like *Staphylococcus saprophyticus* and *Enterococci* (Shen, 2021).

Escherichia coli is the main pathogen in nosocomial UTI, but other gram-negative pathogens like *Pseudomonas* species, *Enterobacter* species, *Serratia* species, *Citrobacter* Species, and urease-producing *Klebsiella* species, *Proteus* species, *Corynebacterium urealyticum* species, and *Providencia* species are also involved (Bhardwaj, 2019; Nimer, 2022; Yan, et al., 2023). Due to the inability of antibiotics to pierce the biofilm established around and within the infectious stones, they are frequently involved in nosocomial UTI. Epidemiology of UTI

The first description of *E. coli* was made in 1885 by paediatrician and scientist Theodor Von Escherich, who in a series of ground breaking studies of infants' intestinal flora discovered a typical microbial resident of healthy individuals that he named Bacterium coli commune. In 1956, his name was adopted to name these bacteria to honour his research efforts (Sharma, 2012). The *E. coli* species consists of motile, gram-negative, rod-shaped, non-spore-forming bacteria. According to phylogenetic study, the majority of *E. coli* strains belong to the A, B1, B2, and D phylogenetic groups. Extra intestinal infections caused by *E. coli* are typically caused by strains from groups B2 and, to a lesser extent, D. Group A and B1 strains are the majorityof commensal bacteria and are largely devoid of virulence factors (Johnson & Russo, 2002). *E. coli* strains that cause diarrhoea or extra intestinal pathogenic *E. coli* (ExPEC) are two general categories for pathogenic strains. Diarrheagenic *E. coli* is the term used to describe *E. coli* that causes intestinal illness. Rarely do diarrheagenic *E. coli* strains invade the gastrointestinal epithelium; instead, they mostly cause pathophysiological alterations in the intestinal cells. *E. coli* pathotypes that cause diarrhoea include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E. coli* (DAEC). Atypical EPEC (a-EPEC) and typical EPEC (t-EPEC) are two recent subtypes of EPEC. While a number of diarrheagenic *E. coli* pathotypes can cause gastroenteritis, they hardly ever spread illness outside the gastrointestinal system (Manges et al., 2019).

To get beyond the urinary system's regular defences, bacteria that cause UTI in otherwise healthy hosts frequently exhibit specific qualities called virulence factors.

Specialised virulence factors in UPEC strains allow them to colonise and infiltrate the host, interfere with the host's defence system, damage host tissues, and/or incite a harmful host inflammatory response (Johnson, 1991). The capacity of UPEC to grow extra intestinally may allow them to spread and cause other illnesses besides UTIs. The virulence of *E. coli*is the consequence of the

interaction of various characteristics, or virulence factors (VFs), which serve to distinguish potentially pathogenic intestine strains from non-pathogenic intestinal strains (Johnson, 1991). The presence and actual expression of the virulence genes in each strain as well as the host's environmental factorsinfluence how virulent each strain is in a particular infection. An essential first step in the infection process is frequently bacterial adhesion to host mucosal surfaces. In the case of urinary tract infections (UTIs), this is particularly true (Kaipainen et al., 2002). It has been demonstrated that some uropathogenic *E. coli* strains express flagella. Flagella are complicated organelles that can be up to 15 m long and help with bacterial movement. The harmful activity could be a factor in the kidney damage caused by pyelonephritis. A bacterial siderophore called aerobactin has recently been linked to E. coli strains that cause cystitis and pyelonephritis (Brock et al., 1991). E. with low complement levels, thanks to an iron sequestration and transport system (Brock et al., 1991). *E.coli* can flourish in conditions where there is a lack of iron, such as diluted urine and serum because it encourages bacterial growth in the limitingiron concentrations encountered during infection. Urease has been identified as a significant virulence factor for UTI. The urease of Proteus *mirabilis* and *Klebsiella spp*. has also been linked to uropathogenic factors that encourage kidney stone development and chronic growth. It has been demonstrated that other Enterobacteriaceae, including as *Klebsiella spp*., *Proteus spp*., and *Providencia stuartii*, express fimbriae that are crucial foruroepithelial adhesion and attachment to urinary catheters (Armbruster et al., 2017). *Staphylococcus saprophyticus* is known to grow persistently and become invasive in the bladder due to adhesion to the uroepithelial cell and the generation of urease (Armbruster et al., 2017; Flores-Mireles et al., 2019).

Organisms that are common commensals in the distal urethra and nearby areas usually cause UTI. Ascension is the most typical method of infection. Uropathogens are a natural component of the faecal flora. Females with these bacteria rise to the introitus vagina, which serve as a reservoir for a number of uropathogens, from the perianal region (Leimbach et al., 2013). Strongly reliant on sexual activity, colonisation spreads to the periurethral region, urethra, and bladder. One of the crucial elements that aids UPEC colonisation of the urinary tract and encourages UPEC invasion of urothelial cells is its capacity to bind host tissue. This property also enables the bacteria to tolerate the bulk flowof urine (Kaper et al., 2004). UPEC preferentially colonises the bladder after it enters the urinary tract and causes cystitis; but, it can also ascend through the ureters and enter to the kidney, where it causes pyelonephritis. In order to avoid these innate immune reactions, UPEC have developed a variety of tactics, which has improved the viruses' ability to colonise and remain in the urinary system (Johnson & Russo, 2002; Kaper et al., 2004).

Depending on the patient's age, sex, underlying disease, infectious agent, and whether the issue is with the upper or lower urinary tract, the treatment for UTI may vary. Becauseof worries about contracting an infection with a resistant organism, UTIs are frequently treated with several broad spectrum antibiotics when one with a narrow spectrum of activity may be appropriate (Sharma, 2012). The antimicrobial drugs trimethoprim, sulfamethoxazole, trimethoprim, beta lactamase, fluoroquinolone, nitrofurantoin, and fosfomycin tromethamine are the ones that are most frequently used to treat uncomplicated urinary tract infections. These medications are mostly used because of their tolerability, range of potential uropathogen targets, and favourable pharmacokinetic profile (Weese *et al*., 2011).

One of nature's never-ending processes is drug resistance, in which organisms get tolerant to novel environmental conditions. It can be brought on by a factor that already existed in the organisms or come from a component that was acquired (Mazzariol et al., 2017). A significant source of antibiotic resistance genes in humans is the gastrointestinal tract, which helps to maintain and spread resistance in the environment. E. coli is said to be the primary antimicrobial resistance carrier, and enteric bacteria found in faecal flora are frequently observed to be extremely resistant (Mohammadi et al., 2010). Antimicrobial resistance, which was initially noticed in *E. coli* in 1940, is today acknowledged as a growing global issue (Singh et al., 2019). Antimicrobial resistance in UTI is becoming more common and varies by geography and regional area. Both uropathogens that cause community and nosocomially acquired UTIs have a steadily rising antibiotic resistance (Sharma, 2012). It has been shown that the causative E. coli increases resistance to ampicillin (30.0-40.0%), cephalothin (20.0-30.0%), and cotrimoxazole (15.0-20.0%) evenin women with acute, uncomplicated UTI (Sharma, 2012; Soltani et al., 2020; Warren et al., 1999). The majority of the isolates exhibit resistance to tetracycline, gentamycin, fluoroquinolones, cefazolin (Jafri et al., 2014).

MDR bacteria are those that exhibit structural independence and resistance to a widearray of antibiotics (at least two or more drugs). The growing MDR organism and its implications in developing countries are currently a major worry for medical and clinical professionals. These disorders increase the difficulty of the treatment and many even endanger the lives of the individual patients. The first bacterium to exhibit multidrug resistance was Shigella (Mahony et al., 2020). MDR has been demonstrated in *E. coli*, *Salmonella enterica serovar typhimurium, Shigella dysenteriae, Enterococcus faecium, Staphylococcus aureus, Mycobacterium tuberculosis, Haemophilus influenza, Pseudomans aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Stenotrophomonas maltophilia, Xanthomonas* and *Burkholderia* (Baral et al., 2012; Tenney et al., 2018; Walker et al., 2016). The frequency of antibiotic resistance in microorganisms has been caused by a variety of circumstances such as high prevalence ofinfectious disorders leads to widespread use of antibiotics, Physician shortage, selective prescribing results from financial restrictions and pressure from pharmaceutical corporations promotional efforts, lack of support for laboratories in rural regions and the challenges in disseminating facts about antibiotic resistance (Mazzariol et al., 2017; Mohammadi et al., 2010).

Methodology

This research work has been carried out in order to assess *E. coli* prevalence in UTI patients in HMC Peshawar

Study Design

This was a Cross–Sectional Study design which is more suitable for the assessment of disease prevalence in a population at one particular time

Study Settings

Samples for this research work were collected from patients visiting Out Patient Department (OPD) of Hayatabad Medical Complex (HMC). Laboratory tests were performed in the Department of Microbiology.

Study Duration

The study duration was 45 days.

Sample Size

Total sample size for the study was 60 participants including half male and half female patients.

Inclusion Criteria

In this study those patients were included who visited HMC as Out Patient Department (OPD) patients and have UTI. Both male and female were included in the study.

Exclusion Criteria

All those patients were excluded who have no symptoms of UTI. Those who refused to participate in the study were also excluded.

Data Collection Procedure

The study was carried out in the HMC OPD where potential participants were identified using inclusion and exclusion criteria. History, chief complaints, sign and symptoms were recorded in a predesigned questionnaire.

Test Performed

Isolation and Purification

The samples were inoculated on CLED Agar media plate and incubated at 37 °C for 24 hours. The colonies were then purified by sub culturing on MacConkey agar plate.

Identification of Isolates

Identification of the desired microbes was done by noting the morphological characteristics of the colonies through gram staining. In morphological characteristics, we noted the shape, colour and movement of the microbes.

Gram Staining

Gram staining techniques are used for both gram positive and gram negative bacteria. As we know that *E. coli* is a gram negative bacterium. The gram staining standard procedure consist of preparing the smear by mixing the desired bacterial colony from petri- dish on slide using the sterile loop and then adding a drop of distilled water. The smear was fixed on slide through heat. The crystal violet dye was then dropped on the cell of bacteria for 40 second. The stain was washed gently with tap water. Gram iodine was used on Bacteria and wait for 40 seconds. After that the slide was washed with tap water. After applying the iodine, a drop of decolourizer containing 95 percent alcohol was dropped on the said organism and wait for 10 seconds. The slide was washed again by tap water. In the last step, counter stain with safranin dye was applied on organism for few seconds and then washed with tap water. The slide was then examined under a microscope with the oil immersion lenses at a magnification of 100X. Pink color represents Gram negative bacteria while purple color represents Gram positive bacteria.

Biochemical Characterization

The confirmation of the desired organism was done through different biochemical tests including Indole, urease, methyl red, triple sugar iron, citrate, oxidase, catalase and Eosin Methyl Blue Agar.

Indole Test

The Indole test was performed to analyse the ability of bacteria that divide amino acid present in the tryptophan in to the compound Indole. Indole is basically the last resulting product of tryptophan hydrolysis. The Kovacs reagent was used for the detection of Indole production. When Kovacs reagent reacts with Indole, it produces a pink compound.In Indole test, the procedure started with preparing tryptophan broth through autoclave. The said broth was then pouring into test tubes. The bacterial culture was inoculated through sterile loop in broth with labeling the name of inoculated organism and incubated these test tubes at 37 °C for 24 hours. After incubation, 2-3 drops of Indole reagent were added in the test tubes and then noted the formation of color ring on the surface of broth. The formation of pink ring confirms the presence of *E. coli* bacteria.

Urease Test

Many bacteria especially those that causes Urinary tract infection (UTI) contain a urease enzyme that split urea in the presence of water into Carbon Dioxide and Ammonia. When ammonia reacts with water and carbon dioxide it produces Ammonium carbonate which made the media acidic. The indicator phenol changes color from orange yellow to bright pink. According to standard procedure, the process of urease test started by preparing urease media through autoclave and the media was poured in the test tubes for further Process. The test tubes were then kept tilled for the formation of slant. After formation of slant, the desired organisms were then inoculated on the slant. After that the test tubes were incubated at 37 °C for 24 hours. After incubation, the test tubes were inspected for color change. Thepresence of *E. coli* can be confirmed if the color remains unchanged (yellow).

Triple Sugar Iron (TSI) Test

The TSI agar test was performed to separate between the various *enterobacteriaceae* genera which are gram negative bacilli that can ferment glucose to produce acid. This set them apart from other gram negative intestinal bacteria. This distinction is because of differences in carbohydrate fermentation patterns and the production of hydrogen sulphide by various intestinal organisms. The release of gas and a visible change in the color of pH is used as an indicator. The phenol red indicated the presence of carbohydrate fermentation. The creation of black precipitate in the butt of the tube designates the existence of hydrogen sulphide. The process of TSI starts by preparing TSI media through autoclave and the media is poured in the test tubes for further process. The test tubes are then keep tilled for the formation of slant. After formation of slant, the desired organisms are then inoculated on the slant. After that the test tubes are incubated at 37 °C for 24 hours. After incubation, the test tubes inspected for color change, H_2S and gas production. The presence of *E. coli* can be confirmed if the color remains unchanged (yellow), no H_2S produce, and if gas produced at the butt.

Methyl Red Test

The methyl red test used to check the organism‟s ability which produce as well as maintain sufficient amount of stable acid like succinic acid, lactic acid, acetic acid and formic acid with carbon dioxide and water which is the end product from the glucose fermentation followed by bacteria. Actually these acids are responsible to lowers the pH of the medium which is nearly neutral. The methyl red indicator pH ranges from 4.4-6.0. As the acid production lower the pH of medium so the methyl red indicator changes it color to red showing positive test but if there is no or low production of acids or production of other neutral end products remain unchanged the pH of the medium while the indicator shows yellow color which is negative test. The process was started by preparing the MRVP broth by using autoclave. The broth was then pouring into test tubes for further process. The bacterial culture was than inoculated through sterile loop in broth and labeled the name of inoculated organism and incubated at 37 °C for 24 hours. After incubation few drops of methyl red reagent were added in the test tube which changes the color of the broth into red from yellow confirming gram negative bacteria.

Citrate Utilization Test

This test depends on the usage of citrate application an energy source. Citrate positive bacteria change the color of the media from green to blue whereas citrate negative bacteria did not change the color and remains green. According to standard procedure, the process of citrate utilization test initiated by preparing Simmons‟s citrate media through autoclave and the media was poured in the test tubes. The test tubes were then kept tilled for the formation of slant. After formation of slant, the desired organisms are then inoculated on the slant surface for the confirmation. After that the test tubes were incubated at 37 °C for24 hours for further process. After incubation, the test tubes were inspected for color change. The presence of *E. coli* can be confirmed if the color remains unchanged (yellow) which indicates that this test was negative for *E. coli* confirmation.

Catalase Test

Catalase is a bacterial enzyme that catalysis the breakdown of hydrogen per oxide into water and oxygen. Catalase positive bacteria produce bubble from hydrogen per oxide while catalase negative bacteria do not produce bubble from hydrogen per oxide. The catalase test was started by taking a sterile glass slide and placing a small amount of colony of the desired bacteria on it. 2-3 drops of 3% hydrogen per oxide are then added on colony placed on glass slide. The formation of bubble confirms positive result indicating that catalase was a positive test for *E. coli* confirmation.

Oxidase Test

The oxidase test was used to identify those bacteria which produce cytochrome oxidase. The cytochrome oxidase is an electron transport chain enzyme found in bacteria. The cytochrome oxidase enzyme converts tetra methyl-p-phenylenediamine to indophenols as a resulting product with purple color. In the absence of this enzyme the reagent remains Reduce and remains colorless. The process of Oxidase test was started by taking filter paper and places it in sterile Petri dish. Few drops of oxidase reagent were then added on filter paper and the colonies of desired bacteria are smeared on filter paper by using sterile loop. In the last it was examined for the color change within few seconds. If the color remains unchanged, it indicated negative result for *E. coli* confirmation.

Eosin Methylene Blue Agar Test

Eosin Methylene blue agar media is a selective stain for gram negative bacteria which consists of dyes that are toxic to gram positive bacteria. It is blend on two stains eosin methylene blue in the ratio of 6:1. It distinguishes between the organisms that ferment lactose by showing color. On EMBA *E. coli* give a distinctive metallic green sheen due to the met achromatic property of the dye. According to standard procedure, the process was started by preparing Eosin Methylene blue agar media plates by using autoclave. After that the bacterial culture was to inoculate through sterile loop on media plates with labeling the name of inoculated organism and incubated these plates at 37 °C for 24 hours. After incubation, the plates were to be inspected for color change. The presence of green sheen confirms the presence of *E. coli* bacteria.

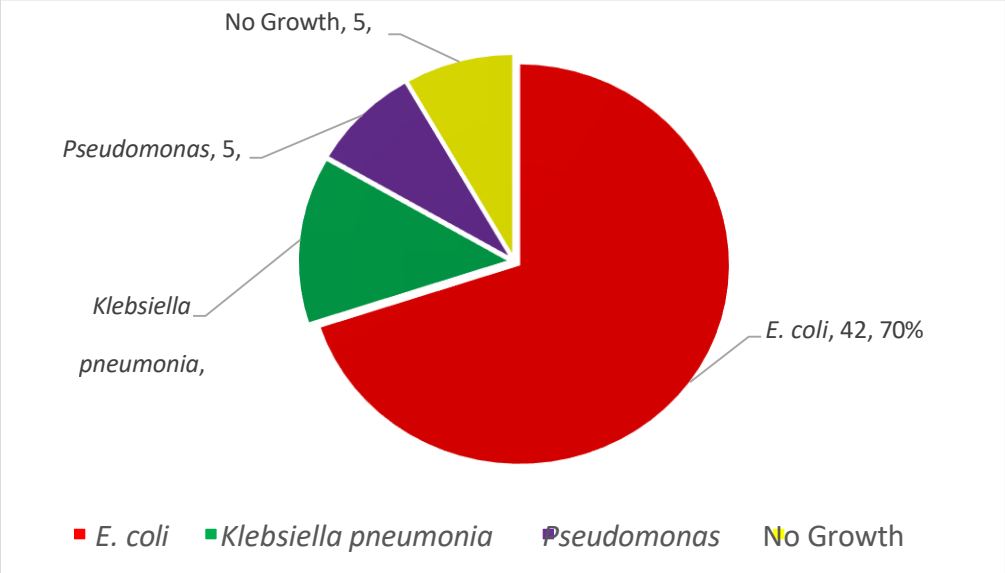
Determination of Antibiotic Susceptibility Profile

In order to check the antibiotic susceptibility profile of bacteria, Kirby-Bauer disc diffusion method was implemented. A Mueller Hinton agar plats were prepared and colonies were inoculated by rotating the plats in clock wise and anti-clock wise direction to make the bacterial lawn over the surface of Mueller Hinton agar plats. Using a sterile needle, the antibiotic discs were placed about 25 mm apart on the surface of MHA plates. Aztreonam (ATM), Ceftazidime (CAZ), Cefotaxime (CTX), Amoxocillin (AMC), Cefixime (CFM), Norploxacin (NOR) and Ciprofloxacin (CIP) were used to determine the antibiotic susceptibility profile of *E. coli*. The Clinical and Laboratory Standard Institute (CLSI, 2015) guidelines were followed to interpret the results.

Results

Isolation of Uropathogenic Microorganism

In order to examine the presence of *E. coli* among UTI patients, the study collected data from 60 patients. The results of isolated bacteria from urine are given in Figure 1. The results indicated that out of 60 samples, 55 (91.67 percent) samples have shown growth while 05 (8.33 percent) shown no growth. *E. coli* was present in 42 patients which are 70 percent. In the remaining sample, 08



(13.33 percent) and 05 (8.33 percent) samples included *Klebsiella pneumonia* and *Pseudomonas*, respectively.

Fig.1. Isolation of different uropathogenic organism from urine sample

Gender wise classification of sample patients indicated that 34 (57 percent) of patients were female while the remaining 26 (43 percent) are male patients (Figure 2). Marital status wise categorizations of patients are given in Figure 4.3. The results show that out of 60 participants, 20 (33 percent) are unmarried and 40 (67 percent) are married.

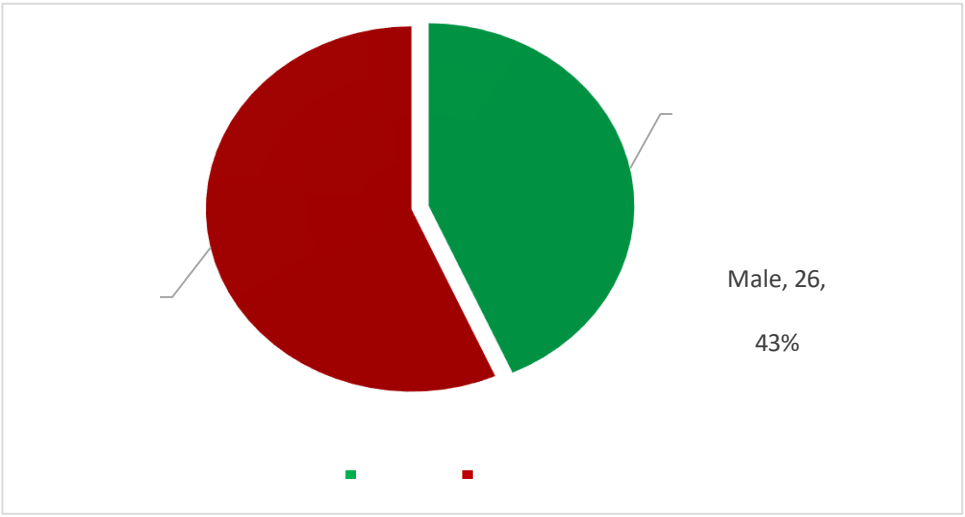


Fig. 2. Gender wise sample categories

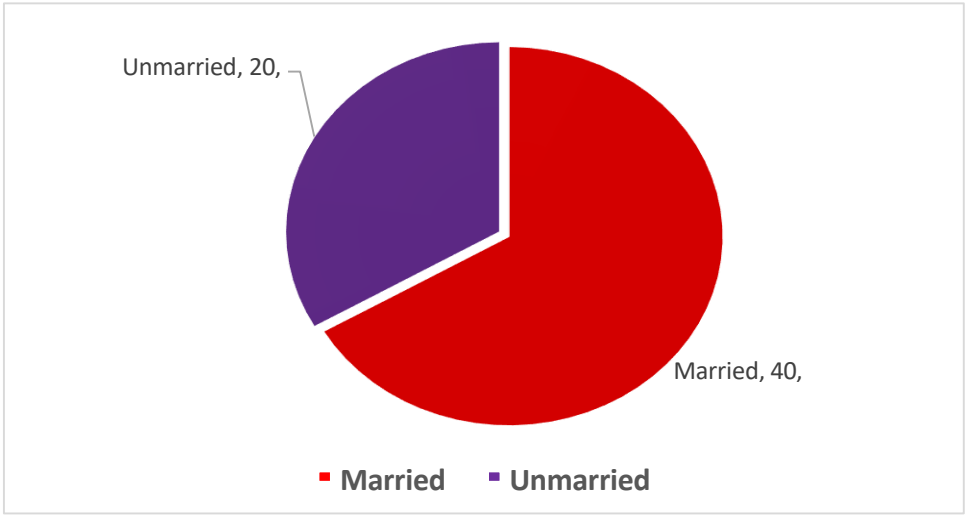


Fig. 3. Marital status of sample patient

The study used 42 samples having *E. coli* bacteria for further analysis. Among 42, sample, 21 (50 percent) of *E. coli* patients were from male and female each (Figure 3).

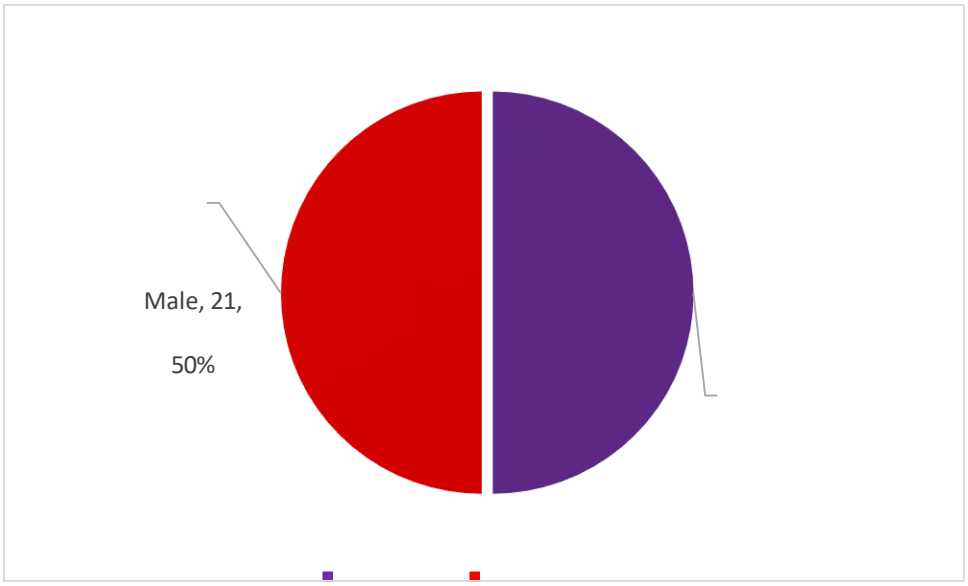


Fig. 4. Gender wise distribution of *E. coli* among UTI patients

The marital status of patients having *E. coli* bacteria indicated that 15 (36 percent) patients were unmarried while 27 (64 percent) were married (Figure 5).

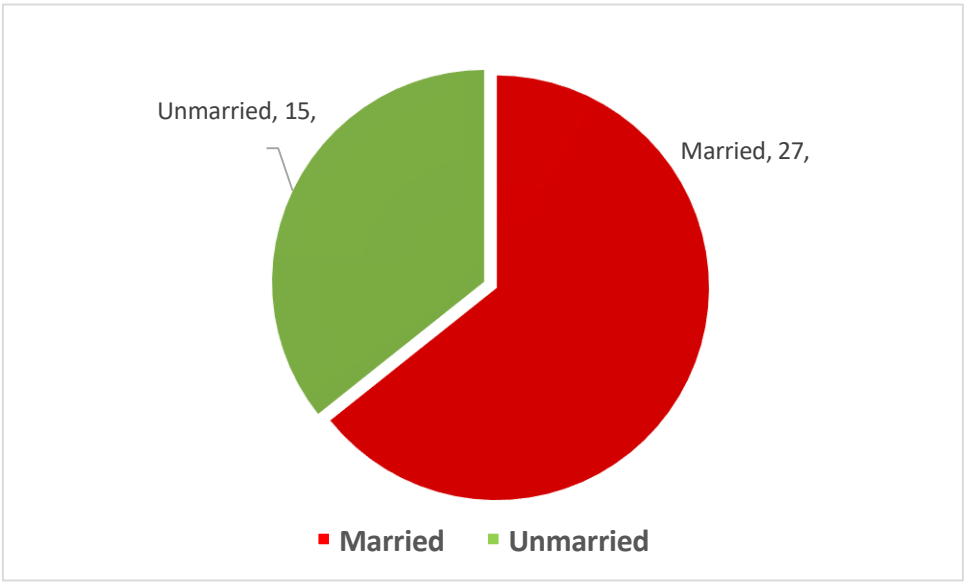


Fig. 5. Marital status wise distribution of *E. coli* among UTI patients

Morphological Identification of UroPathogenic Microorganism

The results of morphological identification of Uropathogenic Microorganism are presented in Table 1. The study used CLED agar and MacConkey agar as growth medium. The colonial characteristics were checked to identify and separate various gram negative uropathogenic microorganism i.e. *E. coli*, *Klebsiella pneumonia* and *Pseudomonas*. The microscopic characteristics were checked by using Gram staining. On CLED medium the *E. coli* shown yellowish opaque colonies while it shown pink lactose positive clear colonies on MacConkey medium. The *K. pneumonia* displayed mucoid yellow to white-blue colonies on CLED medium and on MacConkey medium it displayed Mucoid pink lactose positive colonies. The *Pseudomonas* was identified by displaying green colonies with typical matted surface and rough periphery blue medium on CLED agar while on MacConkey medium it shown brown lactose negative colonies. The microscopic characteristics have shown Gram negative pink rod shape for all three Uropathogenic isolates.

Table 1. Morphological characteristics of causative agents of UTI

Bacterial Strain	**Growth Medium**	**Colonial Characteristics**	**Microscopic Characteristics**
E. coli	CLED Agar	Yellow Opaque Colonies with yellowish medium	Gram negative pink rod shape
	MacConkey Agar	Pink Colonies Lactose Positive	
Klebsiella pneumonia	CLED Agar	Yellow to White-blue colonies often mucoid	
	MacConkey Agar	Mucoid pink colonies Lactose Positive	
Pseudomonas	CLED Agar	Green Colonies with typical Matted surface and rough periphery blue medium	
	MacConkey Agar	Brown Colonies Lactose Negative	

Fig. 6. *E. coli* on CLED agar

Fig. 7. *E. coli* colonies on MacConkey agar

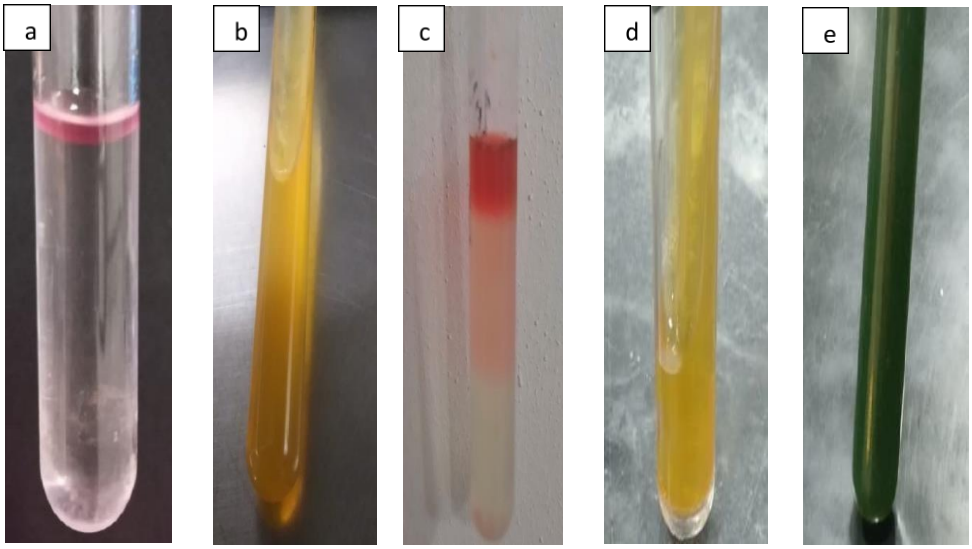
Fig. 8. Microscopic identification of *E. coli*

Biochemical Identification for E. coli

The results of various biochemical tests are presented in Table 2. The *E. coli* is Indole positive by forming pink ring on the top of broth. It is Urease and Oxidase negative bynot changing its color from yellow. The EMB Agar biochemical test shows that the *E. coli* was detected by forming shiny green metallic sheet. On the basis of Catalase, the *E. coli* was confirmed by forming bubbles while from the Methyl Red test, the *E. coli* was confirmed by changing colour from yellow to red. The citrate test confirmed that this test is negative for *E. coli* because the colour was not changed and remained green. The Triple sugar Iron test result confirmed the presence of *E. coli* bacteria as the slant and butt wasyellow while there was a production of gas but no H_2S was produced.

Table 2. Biochemical characteristics of E. coli

Bacterial Strain		***E. coli***
Indole		Positive, Pink ring
Urease		Negative, No colour change
Oxidase		Negative, No colour change
Catalase		Positive, Bubble formation
Citrate		Negative, No colour change
EMB Agar		Green Metallic sheet
Methyl Agar		Positive, Red colour
Triple Sugar Iron	**Slant**	Yellow
	Butt	Yellow
	Gas	Negative
	$\boldsymbol{H2S}$	Negative



a. Indol **b.** Urease **c.** M. red **d.** TSI **e.** Citrate

f. Oxidase **g.** Catalase

h. EMB

Fig. 9. Biochemical identification of *E. coli*

Antibiotic Susceptibility Profile of E. coli

In order to check the susceptibility of antibiotic for *E. coli*, the study used Aztreonam (ATM), Ceftazidime (CAZ), Cefotaxime (CTX), Amoxicillin (AMC), Cefixime (CFM), Norploxacin (NOR) and Ciprofloxacin (CIP). The results are presented in Figure 10. The *E. coli* was resistant to ATM among 14.29 percent and sensitive to ATM for the remaining 85.21 percent sample. The *E. coli* was resistant to NOR among 52.38 percent and sensitive to NOR for 47.62 percent patients. The *E. coli* was resistant to CFM among 40.48 percent and sensitive to CFM for the remaining 59.52 percent sample. The *E. coli* was resistant to CIP among 52.38 percent and sensitive to CIP for the remaining 47.62 percent sample. The *E. coli* was resistant to CAZ among 45.24 percent and sensitive to CAZ for the remaining 54.76 percent sample. The *E. coli* was resistant to CTX among 47.62 percent and sensitive to CTX for the remaining 52.38 percent sample.

The *E. coli* was sensitive to AMC among 78.57 percent and resistant to AMC for 21.43 percent sample.

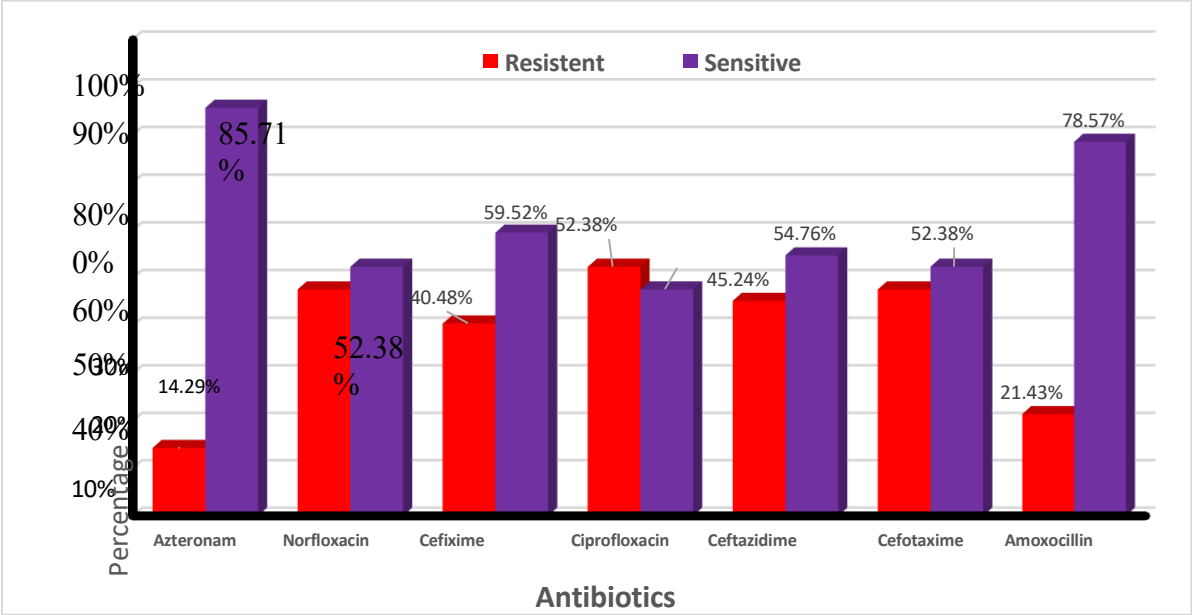


Fig. 10. Antibiotic susceptibility profile of *E. coli*

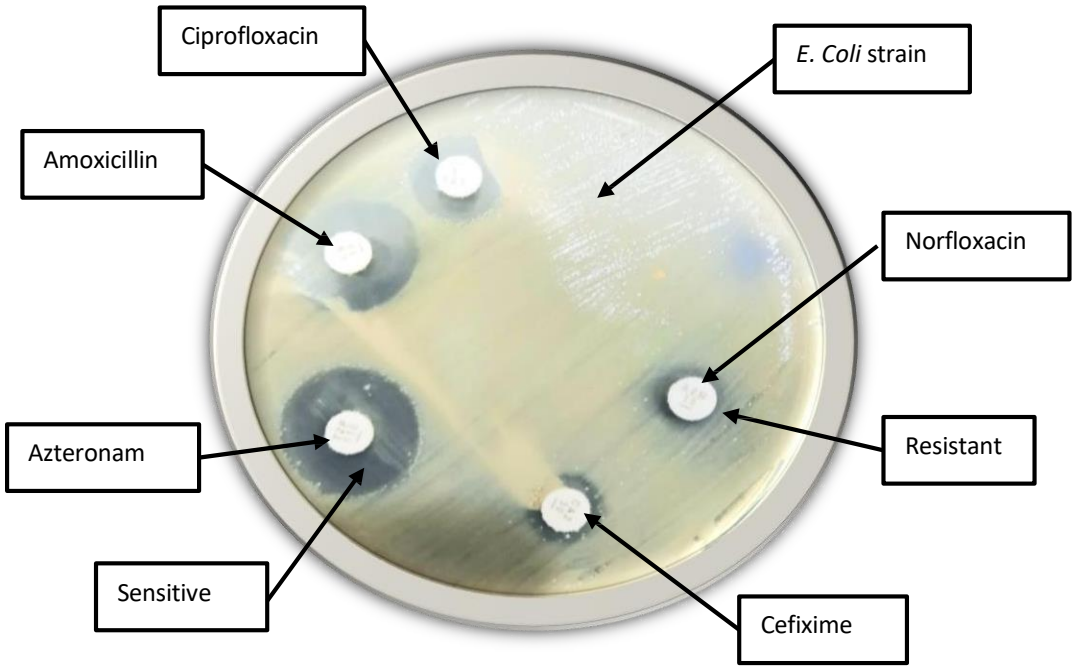


Fig. 11. Antibiotic susceptibility profile of *E. coli*

Discussion

This study was conducted in Out-Patient Department of Hayatabad Medical complex, Peshawar. Those patients reporting with sign and symptoms of UTI were recruited for the study. Total 60 patients which included 34 male (57 percent) and 26 female (43 percent), were identified. Out of 60 participants 40 were married while only 20 were unmarried patients. Urine samples were collected in order to examine the presence of *E. coli* among UTI patient. The results indicated that out of 60 samples, 55 (91.67 percent) samples have shown growth while 05 (8.33 percent) shown no growth. Furthermore, *E. coli* was present in 42 patients which are 70 percent while 08 (13.33 percent) and 05 (8.33 percent) samples included *K. pneumonia* and *Pseudomonas*, respectively. These results were in agreement with a study reported by Tanvir et al. (2012), Riaz et al. (2012) and Habeeb et al. (2013).

The susceptibility of antibiotic towards *E. coli* was checked against these antibiotics including aztreonam, norfloxacin, cefixime, ciprofloxacin, ceftazidime, cefotaxime and amoxocillin. In our study, *E. coli* was resistant to aztreonam among 14.29 percent and sensitive to aztreonam for the remaining 85.71. These results are in line with another study conducted in Lahore, Pakistan by Tanvir et al. (2012) which reported 32.9 percentresistance and 67.1 percent sensitivity to aztreonam. The *E. coli* resistance to Ciprofloxacin and norfloxacin was 52.38 percent and 47.62 percent respectively. More recently fluoroquinolones have been extensively used all over the world to treat UTI, therefore the emergence of resistant bacteria isolates has been frequently observed. Consistently gradual increase in *E. coli* resistance to the drug ciprofloxacin was observed from the year 1995 (0.7 percent) to 2001 (2.5 percent) by Bolon et al. (2004). Worldwide, resistance to ciprofloxacin in Italy, Portugal, Netherlands, and Germany was 24.3 percent, 25.8 percent, 6.8 percent and 15.2 percent, respectively Oteo et al. (2005). In this study it was observed that the resistance to ciprofloxacin and norfloxacin was 52.38 percent and 47.62 percent, which is

alarmingly high and is in accordance with another study reporting 65 percent resistance to ciprofloxacin in Pakistan Jafri et al. ` (2014). In the earliest years, E. coli was 100 percent susceptible to the fluoroquinolones. In1996, Egri- Okwaji had reported 100 percent susceptibility profile for *E. coli* isolates to the drug of loxacin. In case of Ethiopia only 24 percent of 189 *E. coli* isolates were resistant to the drug ciprofloxacin Desenclos et al. (1988). In the current study, the *E. coli* resistance to the cephalosporin drugs such as cefixime, ceftazidime and cefotaxime was 40.48 percent, 45.24 percent and 47.62 percent, respectively which are much lower as compared to the study conducted by Iqbal et al. (2021) with 93 percent resistance for cefixime and more than 80 percent resistance of *E. coli* to cefotaxime and ceftazidime.

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