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COMPARATIVE ANTIMICROBIAL STUDY OF *E. COLI* CAUSING URINARY TRACT INFECTIONS (UTIS)

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ABSTRACT

The study was conducted to isolate and determine the antibiotic sensitivity in *E. coli* from urinary tract infections in Lahore. Urine samples (n=35) were collected from patients with signs and symptoms of Urinary tract infections. Bacteria were isolated and identified by conventional biochemical profile. Antibiotic sensitivity pattern of *E. coli* against different antibiotic was determined.15 samples showed bacterial growth and they were included for the study of antibiotic sensitivity. Among the samples processed the isolation of bacteria, 4 (25 %) from males and 11 (68.75%) from females showed growth of bacteria. The isolated bacteria were *Escherichia coli.*

It was found that amikacin is most effective antimicrobial drug followed by tazobactam and meropenem in urinary tract infections caused by *E. coli.*

Keywords: Urinary tract infection, Prevalence, *E. coli*, Antibiotic sensitivity

INTRODUCTION

UTI or Urinary tract infection is an infection that can happen anywhere along the urinary tract. Urinary tract infections have different names, depending on which part of the urinary tract is infected (Zieve *et al.*, 2012). UTIs are classified as uncomplicated or complicated (Stamm and Norrby, 2001

The urinary tract is normally sterile, bacteria generally ascend from the perianal area may cause UTIs. Bacteria in the urinary tract may remain asymptomatic or cause irritative symptoms such as frequency and urogency. If untreated, the infection may ascend to the upper urinary tract and produce fever, chills,

and flank pain. Bacterial entry into the blood stream is associated with severe morbidity, including sepsis and death. Most infections are caused by retrograde ascent of bacteria from the fecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra and are more readily transversed by microorganisms. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (Mahmood, 2009).

Infections of the urinary tract are common. Women have a higher risk of developing a UTI than men; approximately 50% to 70% of women will have UTI during their lifetimes, and 20% to 30% of women will have recurrent infection. Pregnant women are at increased risk for UTI (starting in week 6 through week 24), because uterus sits directly on top of the bladder and displaces it. Shift in the position of the urinary tract and hormonal changes during pregnancy make it easier for bacteria to travel up the urethras to the kidneys (Gupta *et al.,* 2001).

The bacterial strains that cause UTIs include: *Escherichia coli (E. coli)* is responsible for most uncomplicated cystitis cases in women, especially in younger women. *E. coli* is generally a harmless microorganism originating in the intestines. If it spreads to the vaginal opening, it may invade and colonize the bladder, causing an infection. The spread of *E. coli* to the vaginal opening most commonly occurs when women or girls wipe themselves from back to front after urinating, or after sexual activity. *Staphylococcus saprophyticus* accounts for 5 - 15% of UTIs, mostly in younger women. *Klebsiella Enterococci* bacteria and *Proteus mirabilis* account for most of remaining bacterial organisms that cause UTIs. They are generally found in UTIs in older women. Rare bacterial causes of UTIs include *Urea plasmaurealyticum* and *Mycoplasma hominis*, which are generally harmless organisms (Simon and Zieve, 2011).

E. coli can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults. *Escherichia coli* is a Gram negative rod in the family *Enterobacteriaceae*. Most *E. coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism distinguished from normal flora by their possession of virulence factors such as exotoxins. The specific virulence factors can

be used, together with the type of disease, to separate these organisms into pathotypes (Stapleton, 2003).

The goals in the treatment of urinary tract infection are to prevent or treat systemic symptoms, to relieve symptoms, to eradicate sequestered infection, to eliminate uropathogenic bacterial strains from fecal or vaginal reservoirs, and to prevent long-term sequelae all at minimal cost, with the lowest rate of side effects, and with the least selection of an antibiotic resistant flora. A steady increase of resistance patterns to antimicrobials has been documented of the past years. The pathogens and resistance patterns may have changed over the years; thus, local studies of a large scale are needed as a guide in the management of complicated and uncomplicated urinary tract infection (Annabelle *et al.,* 1999).

Antibiotic resistance may develop in uropathogen due to frequent misuse of antibiotics. Antibiotics are usually prescribed empirically before the laboratory results of urine culture are available. To ensure appropriate therapy current knowledge of the organisms that cause UTI and there antibiotic susceptibility is mandatory. Multidrug resistant pathogens travel not only locally but also globally and newly introduced pathogens spreading rapidly in susceptible host (Tambekar, *et al*., 2006).

Multiple antimicrobial resistances and sensitivity among gram-negative organisms have been a long term and well-recognized problem with urinary tract infections. Resistance has been observed in genera *Escherichia.* Penicillin G, Vancomycine, Meropenum, Amikacin, Tazobactom, Kenamycine is routinely and effectively used for the treatment of uncomplicated lower urinary tract infection (Bauer *et al*., 1966).

This study was carried out to investigate the pathogen prevalence and antimicrobial susceptibility patterns of the most prevalent pathogen isolated from urine samples of some patients. Analysis of this antimicrobial susceptibility data provides information for selection of antimicrobials for treatment of UTIs in patients.

Objectives:

The objective of our study was

1. To isolate and identify *E. coli* from UTI patients.
2. To evaluate the antibiotic sensitivity of narrow spectrum group against *E. coli* causing UTI.

MATERIALS AND METHODS

Place of work:

The research work was conducted in Microbiology Laboratory, Institute of

Molecular Biology and Biotechnology, The University of Lahore.

Sample collection:

Urine samples were collected in sterile containers and brought to the microbiology laboratory, Institute of Molecular Biology and Biotechnology, The University of Lahore.

Isolation of Bacteria:

Media was prepared according to the manufacturer manual and after checking the sterility the samples were inoculated on the medium.

- ***CLED Agar***

18.1g of CLED agar was dissolved in 500 ml distilled water. Media of pH was within range 7.3-7.5 at room temperature. Autoclaved at (121°C for 15 minutes). Dispense aseptically 15ml in sterile Petri dishes. Store at 2-8°C (Cheesbrough, 2000).

- ***Urine culture***

Gently swirled the urine to ensure even distribution of any organisms before culturing. Hold the loop in a vertical position, inserted the loop just below the surface of the urine. Streaked the loop full of urine down the center of the CLED agar plate. Without changing or reflaming the loop, streaked many times at right angles over the original streak covering the entire plate. Did not re-streak over already streaked area.Incubated the plates at 35℃ in air incubator.Added a label to one of the incubated plates indicating the number of hours (Cheesbrough, 2000).

Identification of E.coli:

Colony characteristics

Colour, shape and texture of colonies were observed.

Gram staining

A smear of bacteria was prepared by placing a drop of distilled water on a clean slide to perform Gram staining. Using sterile technique, a smear of the bacterial culture was prepared by placing a drop of sterile water on a clean glass slide and then transferring bacterial culture to the drop of water with a sterile cooled platinum loop. Bacterial culture was mixed and spread by circular motion of inoculating loop. Smear was allowed to air-dry and then heat fixed by gliding slide 2-3 times over Bunsen's burner flame. The smear was stained with crystal violet (primary) for one minute and washed with tap water. Gram's Iodine (mordant) was added for one minute and washed with tap water. The slides were decolorized with 95% ethanol. Safranin (counter) solution was added for a half minute and washed with tap water. Stained slides were examined under oil immersion objective (100X) of the bright field compound microscope. Staining

characters and microscopic morphological appearance of individual bacteria were recorded (Cheesbrough, 2000).

Biochemical identification:

Different biochemical tests were performed to confirm the *E.coli* causing UTI.

Indole production test

The test organism was inoculated in a bottle containing 3ml of sterile peptone water Incubated at 35-37℃ up to 48 h. Then added 0.5 ml of kovac's reagent (p-dimethylaminobenzaldehyde). Shaked gently examine for a red colour in the surface layer within 10 minutes (Faddin and Jean, 1980)

Motility test

To test for motility, used a sterile needle to pick a well-isolated colony and stabed the medium to within 1 cm of the bottom of the tube. Be sure to keep the needle in the same line it entered as it is removed from the medium. Incubated at 35°C for 18 hours or until growth is evident. A positive motility test was indicated by a red turbid area extending away from the line of inoculation. A negative test was indicated by red growth along the inoculation line (Difco, 1998).

Triple sugar iron agar test

Suspended 60 g of the medium in one liter of purified water. Heated with frequent agitation and boiled for one minute to completely dissolve the medium. Dispensed into tubes and autoclaved at 121°C for 15 minutes. After autoclaving, allowed medium to solidify in a slanted position (Sulkin and willett, 1940).

Antimicrobial sensitivity testing:

Antimicrobial sensitivity was determined by the disk diffusion method. The antibiotic discs such as Meropenem (10µg), Penicillin-G (10µg), Amikacin (30µg), Vancomycin (10µg) and Tazobactam (5µg) were placed on the surface of Nutrient Agar plates. The Nutrient agar plates were incubated for 18 to 24 hours at 37ºC. The zone of inhibition was measured (Bauer *et al*., 1966).

Antimicrobial assay

Preparation of agar plates for sensitivity

Medium was best prepared from ready to use dehydrated powder, available from most suppliers of culture media. The medium was usually used at a concentration of 2.8g in 100ml distilled water. After that sterilized by autoclaving at 121°C for 15 minutes.Dispensed aseptically in the required amount. Dated the medium and gave it a batch number. Stored in a cool dark place. The pH of medium was within the range 7.2-7.6 at room temperature (Cheesbrough, 2000).

Disk diffusion method

The method is used for antimicrobial sensitivity testing.

Inoculation of sensitivity plates

Each sensitivity plate inoculated by dipping the sterile cotton swabs in the saline suspension of the organism under test and a uniform thin layer of organism will be formed. The plates were not inverted during drying. When the plates were dry no more than 6 discs, preferably 5, of Meropenem, Penicillin G, Amikacin, Vancomycin, Tazobactam were placed on each plate. The discs were not arranged in a circular fashion so that the disc centers are equidistant 24 and each disc center is approximately 15-20 mm from the edge of the plate. Contact with the agar of each antibiotic disc was ensured by gently pressing on each disc with the tips of a pair of sterile forceps. The plates were placed immediately in an incubator with a temperature strictly controlled at 37°C and incubated for 24-72 hours (Barrett *et al.,* 1968).

Results Interpretations:

After overnight incubation at 37°Cin incubator a ruler used on the underside of the plate to measure the diameter of each zone of inhibition in mm. By using the Interpretative Chart, the zone sizes of each antimicrobial disc interpreted and reported the organism as Resistant, and Sensitive.

RESULTS

Sample collection

A total of 35 urine samples were collected from patients of different hospitals of Lahore city suspected of having urinary tract infection, out of which total 35 samples showed bacterial growth. Of the total samples the bacteria isolated consisting of 4 (25 %) from males and 11 (68.75 %) from females.

Isolation and screening

CLED media was used for isolation and screening of urine samples. Yellow colonies of *E.coli* were observed (Figure 3.1).

Identification

E.coli was identified using Bergey's manual of determinative Bacteriology. All bacterial isolates were identified on the basis of Gram staining. It showed negative rods in pink colour.

Biochemical testing

Triple sugar iron agar test and indole motility test was performed for identification.

Indole motility

Indole production showed red colour in the surface layer and motility showed in the lower region (Figure 3.2).

Triple sugar iron test

Triple sugar iron test showed yellow (alkaline) slope and yellow (acid) butt indicating presence of *E.coli* (Figure 3.3).

Antimicrobial Sensitivity testing:

Antimicrobial sensitivity was determined by the disk diffusion method, according to National Committee for Clinical Laboratory Standards document (Bauer*et al.*, 1966) (Figure 3.4 and Table 3.1).

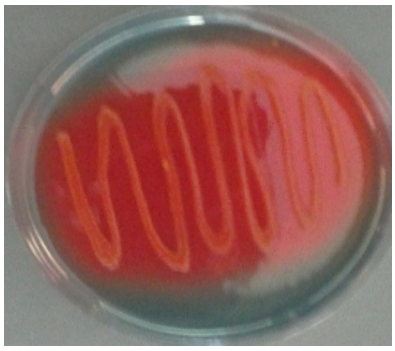

Fig. 3.1: Yellow coloured colonies of *E.coli* on CLED agar

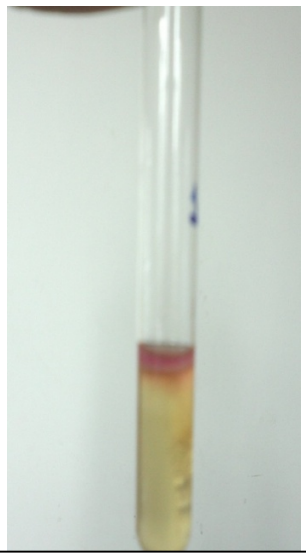

Fig. 3.2: Indole motility test showing presence of *E.coli*

Fig.3.3: Triple sugar iron test showing presence of *E.coli.*

Table 3.1: Percent sensitivity against each antibiotic

Number of antibiotics against which sensitivity was observed	% sensitivity against each antibiotic	Measurement of antibiotic zones (µm)
Tazobactam 11	30.55	21
Meropenem 09	25	23
Penicillin G 0	0	0
Vancomycine 0	0	0
Amikacine 32	88.88	23

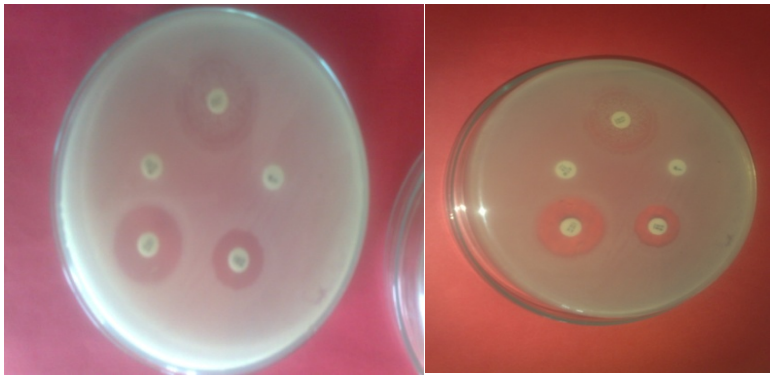

Fig.3.4: Nutrient agar plates showing zone of antibiotic senstivity patterns. *Escherichia coli* had highest sensitivity 88.88% to(amikacine) 30.55% to (tazobactam) 25% to (meropenem) and recorded 0% to (pencilline and vancomycine).

DISCUSSION

Urinary tract infection (UTI) is a general term referring to the infection anywhere in the urinary tract. This is among the most common serious bacterial infections in infants and children (Wald, 2004). Urinary tract infections are one of the most common types of bacterial infections in human beings occurring both in the community and the health care settings and rank high amongst the most common reasons that compel an individual to seek medical attention *E.coli* is the most common harmless microorganism causing UTIs (Kolawale *et al.,* 2009).

CLED media is used to isolate urinary pathogens because it gives consistent results and allow both gram negative and gram positive pathogens (Hassan *et al.,* 2011).

In present study the results showed that 15 (42.84%) out of 35 patients were suffering from UTIs. Similar results were reported by Zaid *et al.,* (2011) where out of 174 urine samples, 68 (59%) were caused by *E.coli*. According to Aiyegoro *et al.,* (2007) 36 (11.96%) out of the 301 patients studies had UTI. According to Jambo *et al.,* (2011) total of 56 samples, 26% were found to be *E.coli*.

In present study bacteria isolates were 4 (25%) from males and 11 (68.75%) from females. According to Zaid *et al.,* (2011) the urinary tract infections were found most frequently in female (63%) than male (37%). Similar results were reported by Aiyegoro, *et al.,* (2007) bacteria isolates 28 (22.4%) from females and 8 (4.56%) from males. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (Mahmood, 2009).

Disk diffusion method was performed to check antibiotic sensitivity of *E.coli* isolates. Amikacin showed (88.88%) sensitivity in

Escherichia coli, meropenem (25%), tazobactam (30.55%), penicillin G and vancomycin were resistant to all gram negative rods bacteria. Similar results were reported by Zaid *et al.*, (2011) that isolated uropathogens showed sensitivity to meropenem (52%), tazobactum (54%), amikacine (71%) and resistant to vancomycine (90%), gentamycine (98%). According to Aiyegoro *et al.*, (2007) meropenem showed (50%), sensitivity in *E.coli* tazobactam (68%), amikacine (80%) and resistance topencilin G, amoxicillin and vancomycine as more than 60% of the isolates. Similar results showed by Jambo*et al.*, (2011) amikacine (65%), meropenum (37%), tazobactam (21%), and resistance to penicillin (85%) and vancomycine (95%). These all antibiotics are specific for gram negative rods. Penicillin, vancomycine, tazobactam and meropenem are inhibitors of bacterial cell wall synthesis. While amikacin are inhibitor of protein synthesis (Cheesbrough, 2000).

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