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ORIGINAL ARTICLE

A Study on Escherichia Coli Isolated from Urogenital Tract Infections, Emphasizing their Occurrence and Antibiograms

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ABSTRACT

Background: Urinary tract infections (UTIs), which are among the most prevalent infections both in the general population and in health care facilities, are ranked second only to upper espiratory tract infections in terms of the prevalence of the disease.

Methodology: Samples for the current investigation were gathered from severalprivate and public hospitals. Based on colony morphology, these samples were identified via Gramstaining and biochemical analysis. Following CLSI 2019 standards, these isolates were nexttested for antibiotic susceptibility using Kirby-disc Bauer's diffusion assay.

Results: For the presence of Escherichia coli, 48(26.6%) of the 180 sample stested negative, while 132(73.3%) test edpositive. Escherichia coli isolates were found to be highly resistant to ciprofloxacin, Amoxicillin-Clavulanic acid, Gentamicin, Ofloxacin, Piperacillin-Tazobactam, Meropenem, Imipenem, Tobramycin, Amikacin, andColistin, according to the antimicrobial susceptibility pattern. Imepenem, meropenem, amikacin,gentamicin, amoxicillin-clavulanic acid, cefixime, cefotaxime, ciprofloxacin, and cefoperazone were all highly effective against the isolates.

Clinical Implication: The clinical implication of the study is to isolate the E.coli from community-acquired UTI and also to enlighten the antimicrobial resistance pattern regarding E. coli.

Conclusion: Antimicrobial susceptibility testing results should be heeded when prescribing treatment for urinary tract infections to avoid the possibility of Escherichia coli'sgrowingresistancetoseveralantimicrobialmedications.

Keywords: Urinary tract infections (UTIs), Antimicrobial Activities, Healthcare, Antibiotics

INTRODUCTION

Urinary tract infections (UTI) are one of the commonest infections acquired in the community as well as in health care settings, therefore considered as 2nd rank disease after upper respiratory tract infection1 and also comes under the category of nosocomial infections that occurs frequently[2]. Human beings of all ages are affected by UTIs. UTIs are generally self-limiting in those individuals which are not facing any anatomical or functional abnormalities, but have an affinity to relapse[3].

About 80 % of cases of uncomplicated UTI are caused by Escherichia coli in outpatients whileother microbial pathogen contributes about 5 to 15 % in causing urinary tract infection[4]. The common pathogens causing UTI are Escherichia coli, Staphylococcus saprophyticus, Staphylococcus aureus, Proteus sp., Klebsiella pneumonia, Pseudomonas aeroginosa and enterococci[5].

A number of virulence factorsencode the strains of uropathogenic E. coli (UPEC) thatpermits the pathogen to colonize the urinary tract. Virulence factors of E. coli can be divided into two groups that might have been significantlyinvolved in causing UTIs: (i) association of virulence factors with bacterial cell surface and (ii) secretion and spread of virulence factors to the site of action[6].Different types of adhesive organelles (fimbriae) are included in surface virulence factors of UPEC which helpsin the attachment of bacterial to the host tissues within the urinary tract. Adhesins of UPEC can play role as a virulent in different ways: (i) directly provoking host and signaling pathways of the bacterial cell, (ii) enabling the distribution of other microbial products to host tissues, and (iii) stimulatinginvasion of bacteria[6].

The management of Urinary tract infectiondiffers according to the age, gender, underlying disease, causative agent and whether there is involvement of lower or upper urinary tract. Trimethoprim/sulphamethoxazole is the drug of choice for the cure of urinary tract infection in health care settings where the prevalence rate of antimicrobial resistance is <10-20 per cent and ciprofloxacin is the drug of choice in case of theantimicrobial resistance is >20 per cent, according to the Infectious Diseases Society of America (IDSA) standards. The other means used in the management of UTI include fluoroquinolones, cephalosporins and other β-lactams with or without β-lactamase inhibitors, nitrofurantoin[7].

The aim of this study is to find out the commonly occurring microbial pathogens specifically E.coli that are responsible for UTI[4] and to isolate the E.coli from community acquired UTI and also to enlighten the antimicrobial resistance pattern regarding E. coli.

METHODOLOGY

Study Method: Population: Sample Collection: A total number of 180specimens of urine were collected from patients having symptoms of Urinary tract infections from different private and government hospitals of District Faisalabad. Midstream urine samples were gathered by clean catch method and collection of samples was done in sterilized containers and transportation of the samples to laboratory was doneinstantly[8].

Isolation of Escherichia coli: Escherichia coliwas isolated using standard media includingMacConkey agar as selective mediumandCystein Lactose Electrolyte Deficient (CLED) agar for primary isolations and quantification of microorganism by streak plate method. Urine samples were streaked via streak plate method in a biosafety cabinet. Incubation was done of all the readyto use culture plates at 35- 37°C for a period of 18-24 hours former to use for sterile testing[9]

Identification of Escherichia coli: After 24 hours of incubation, the plates were observed for the growth of the uropathogens. If growth will be shown on culture plates, they will be promptly further processed via standard microbiological methods for the biochemical tests of Escherichia coli including gram staining, colony morphology, motility and pigment production[10]. Biochemical tests were done for the identification of Escherichia coli. Following biochemical tests were done to identify E. coli: catalase test, indole production, cytochrome c oxidase negativity, and, glucuronidase positivity[11].

Gram staining: Gram staining was done to differentiate gram positive and gram negative organism and was done according to standard method. For gram staining, firstly smear of bacteria was

prepared on glass slide and heat gently to fix. After gram staining of the smear, observation was done via 40X and 100X lense of microscope[12].

Motility Test: The motility of bacteria is checked by using the wet preparations. Motility test helps to identify the bacteria and to differentiate between motile and non-motile organisms.Escherichia coliis a motile bacteria so to confirm the motility of this bacteria, motility test was performed.

Biochemical Tests: For further confirmation of Escherichia coli, numerous biochemical tests were performed such as catalase test, indole production, cytochrome c oxidase negativity.

Catalase Test: Presence of catalase enzyme (i.e.Escherichia coli) was confirmed by catalase test. If catalase enzyme is present then it react with Hydrogen Peroxide and produce bubbles.

Indole Test: Indole test is used to identifythe organisms capable of tryptophan production. When these tryptophan producers are incubated in a medium containing tryptophan (aminoacid), they degrade it and covert it into a compound named, indole[13]. This conversion is indicated when is addition of Kovac's or Ehrlich's reagentwas done in the medium and reaction of 4 (p)-dimethylaminobenzaldehyde take place with indoleandyields a red colouredcompound.Indole test plays a significant role in the differentiation ofEnterobacteriaceae and other genera.

Methyl Red Test: Some bacteria have the ability to ferment the glucose with mixed acid that can be used to differentiate bacteria by using methyl red test. MR-VP broth is used that contains glucose peptone and phosphate buffer that supports the growth of microorganisms. MR indicator notices the medium pH[14].

Voges-Proskauer Test: Voges-Proskauer is a best biochemical test used for the confirmation of enterobacericeae.The test is performed by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth which has been inoculated with bacteria. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result[15].

Oxidase Test: The oxidase test is a biochemical reaction that examines for the existence of an enzyme cytochrome oxidase which is also called indophenol oxidase. An oxidized colored product produces from the reduced colorless reagent in the existence of a bacterium whichcomprisesof the enzyme cytochrome oxidase.Toconfirmcytochrome c oxidase negativityforEscherichia coli, oxidase test was performed[16].

Triple Sugar Iron (TSI) Test: It is carried out to detect those organisms capable of fermenting carbohydrates along with hydrogen sulphide gas production. Three sugars were used in this test i.e. lactose, sucrose and glucose and for the detection of H_2S, iron is also added into this medium. Triple sugar iron is a semisolid media having a slant and a butt. Phenol red is used as an indicator and when the sugars are fermented, acid is produced in the medium due to which phenol red changes its color to yellow. Similarly, ferrous sulphate present in the medium turns the medium black in the presence of H_2S gas [17]

Antibiotic Susceptibility Testing: Antibiotic susceptibility pattern of Escherichia coli isolates was performed by Kirby-Bauer disc diffusion method according to Clinical Laboratory standards Institute (CLSI) guidelines, formerly known as National Committee for Clinical Laboratory Standards (NCCLS)[18]**.** The sensitivity was monitored by zone of inhibition around the disc. Mueller-Hinton Agar (MHA) was used for disc diffusion method of antimicrobial susceptibility testing[19]. According to CLSI guidelines, antibiotic susceptibility testing was done for positive clinical isolates of Escherichia coli by usingAmoxicillin-Clavulanic acid, Piperacillin-Tazobactam, Cefixime, Cefoperazone, Cefotaxime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Nalidixic acid, Ciprofloxacin, Levofloxacin, Ofloxacin, Fosfomycin and Colistin. Mueller-Hinton Agar was used for performing AST. MH agar must be stored at 4-20℃ instead of placing at room temperature. Agar plates streaked with organism were autoclaved for 48 hours. Zones of inhibition should be measured after 18 hours and 48 hours [20]

McFarland Standard Turbidity Standard: For performing disc diffusion test, 0.5 McFarland Standard Turbidity Standard was prepared to adjust the test bacterial suspensions so that the bacterial number remains within the given range to avoid too thick or too dilute suspensions which can lead to false results. McFarland Standard Turbidity Standard was prepared in a screw capped test tube by stirring sulphuric acid and barium chloride which will precipitate as barium sulphate. This prepared chemical solution was the stored at room temperature. The test suspension were then compared with this standard to adjust their turbidity. The prepared 0.5 McFarland turbidity standard shows that cell density in prepared bacterial suspension is approximately 1.5x 10^8 CFU/ml[21].

Kirby-Bauer Disk Diffusion Technique: Mueller Hinton agar medium was assembled and then placed in autoclave at 121℃ for 15 minutes. Medium's pH was kept between 7.2-7.4.

RESULTS

Occurrence of Escherichia coli: A total of 180 specimens of urine were collected from patients with underlying urinary tract infections from different private and government hospitals of District Faisalabad. Out of one hundred and eighty samples, 132 (73.3 %) were positive and 48 (26.6 %) were negative for the existence of Escherichia coli.

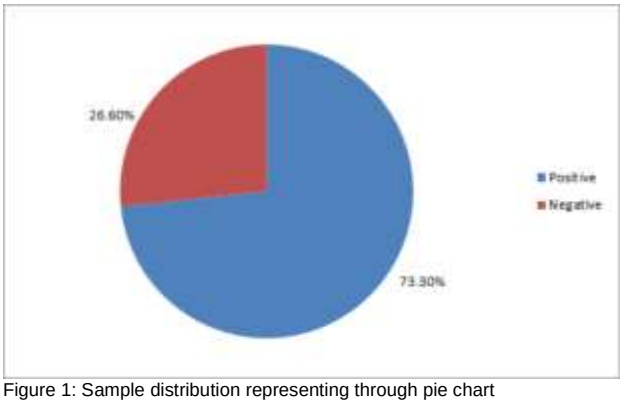

Figure 1: Sample distribution representing through pie chart

Gender based occurrence of UTI: Out of 132 confirmed isolates of Escherichia coli, 33 (25 %) were isolated form males, while 99 (75 %) were from females. Gender based comparison of UTI occurrence depicted that UTI were more affected in females as compared to males as shown in Figure 3.2.

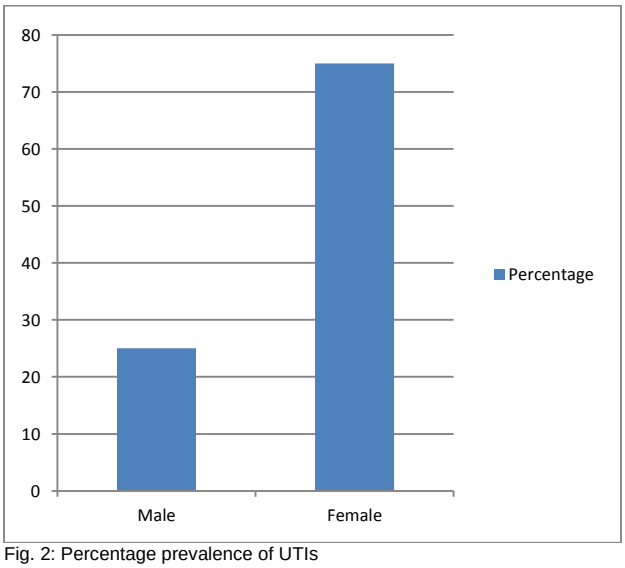

Fig. 2: Percentage prevalence of UTIs

Age based occurrence of UTI: Out of 132 isolates of Escherichia coli, frequency in number (n) and percentage (%) was calculated by dividing the affected population in 9 age groups. Frequency distribution between age groups shown in Figure (3.3).

Table 1: Frequency distribution of UTI between Age groups

Age Group	Frequency (n)	Frequency (%)
1-10	21	15.9
11-20	0	0
21-30	12	9
31-40	6	4.5
41-50	14	10.6
51-60	25	18.9
61-70	27	20.4
71-80	13	9.8
81-90	14	10.6

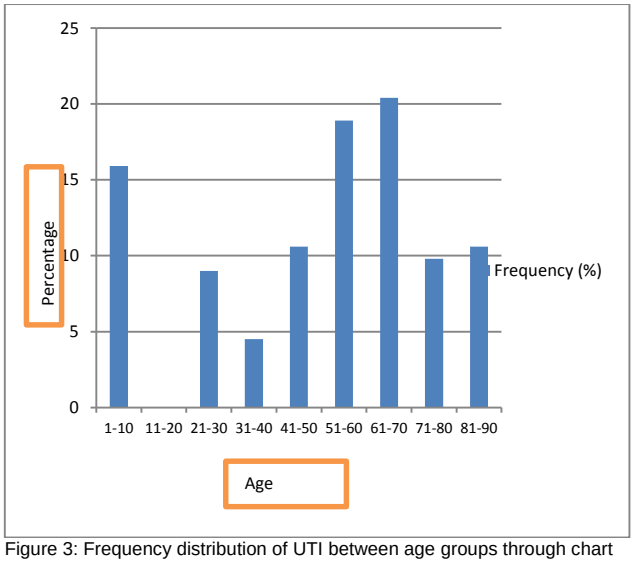

Figure 3: Frequency distribution of UTI between age groups through chart

Colony Morphology: MacConkey agar and CLED agar were used for the isolation and identification of Escherichia coli. Isolates of Escherichia coli ferments lactose, producing smooth pink colonies on MacConkey agar and yellow colonies on CLED agar.

Fig 4: Escherichia coli smooth pink colored colonies on MacConkey agar and opaque yellow colonies on CLED agar

Microscopic Characteristics: Escherichia coliis a gram negative usually motile rod that presents positive motility test underneath microscope were observed (Fig. 3.5).

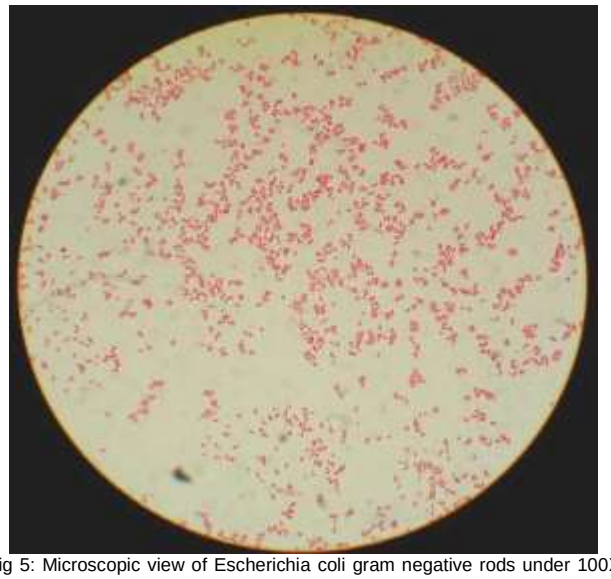

Fig 5: Microscopic view of Escherichia coli gram negative rods under 100X lenses

Biochemical Analysis: Biochemical tests (catalase, indole, oxidase, methyl red,voges-proskauer and triple sugar iron tests) were performed for the confirmation of E. coli (Table 3.1).Catalase along with indole and methyl redwere positive while oxidase test and voges-proskauerwas negative.

Table 2: Results of Biochemical Tests Performed

Biochemical Test	Result
Catalase	Positive
Indole	Positive
Methyl red	Positive
Voges-Proskauer	Negative
Oxidase	Negative
Triple sugar iron	Positive

Fig 6: Sample collection for the isolation of Escherichia coli from UTI patients

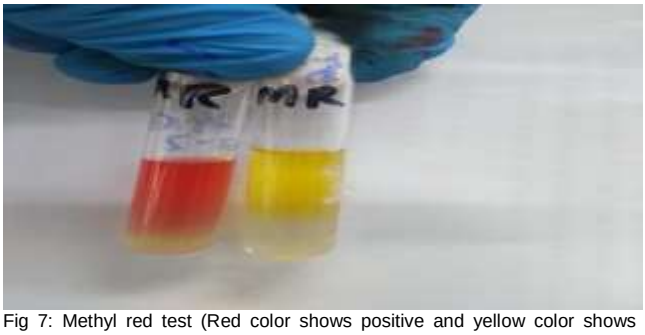

Fig 7: Methyl red test (Red color shows positive and yellow color shows negative)

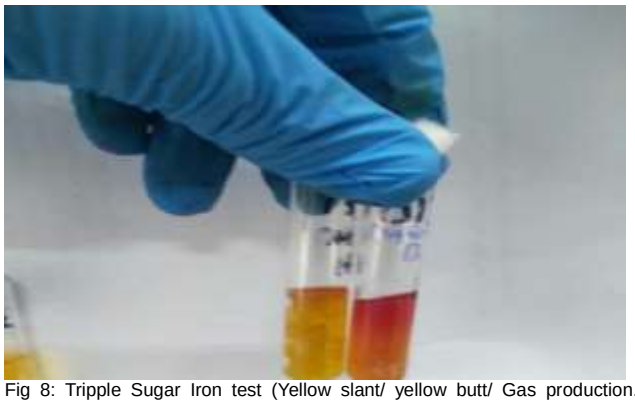

Fig 8: Tripple Sugar Iron test (Yellow slant/ yellow butt/ Gas production. shows positive test for Escherichia coli)

Fig 9: Catalase Test (Active bubbles indicates positive catalase test

Antibiotic Susceptibility testing: Antimicrobial susceptibility testing was done on Mueller- Hinton agar using disk diffusion (Kirby Bauer's) method for checking susceptibility towards different drugs of Escherichia coli isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the following antimicrobial drugs: Gentamicin, Tobramycin, Amikacin, Colistin, Imepenem, Meropenem, Cefixime, Cefoperazone, Cefotaxime, Ciprofloxacin, Levofloxacin, Ofloxacin, Amoxicillin+ Clavulanic acid and Piperacillin + Tazobactam[22].

Table 3: Antibiogram Resistance Pattern of Escherichia coli

Antimicrobial agents (µg)	Susceptible isolates (%)	Resistant isolates (%)
Gentamicin (10 µg)	67.3	32.7
Tobramycin (30 µg)	64.7	35.3
Amikacin (30 µg)	21	79
Colistin (10 µg)	91	9
Imepenem (10 µg)	91.6	8.4
Meropenem (10 µg)	91	9
Cefixime (30 µg)	39	61
Cefoperazone (30 µg)	36.6	63.4
Cefotaxime (30 µg)	23.6	76.4
Ciprofloxacin (5 µg)	27.3	72.7
Levofloxacin (5 µg)	33	67
Ofloxacin (5 µg)	32.8	67.2
Amoxicillin+ Clavulanic acid (20,10 µg)	28.7	71.3
Piperacillin + Tazobactam (100, 10 µg)	84.1	15.9

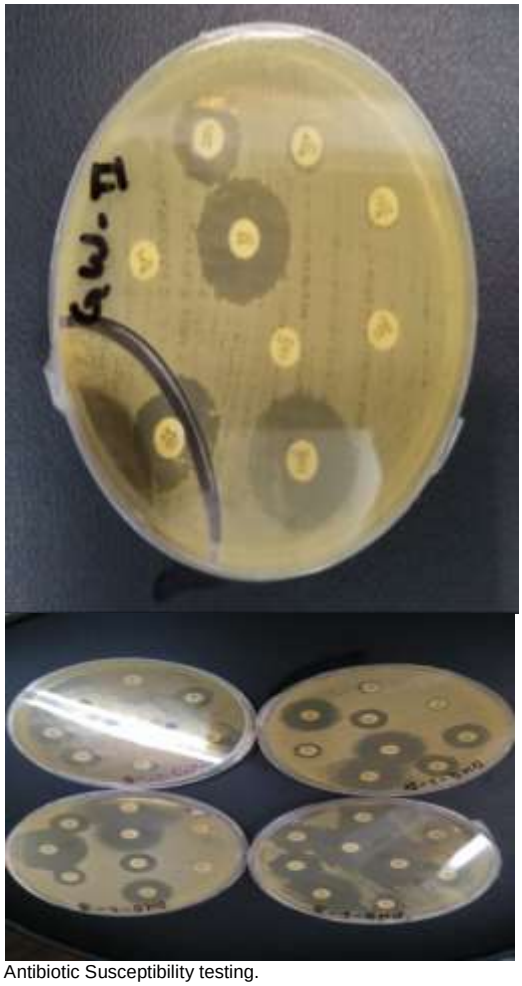

Fig 10: Antibiotic Susceptibility testing.

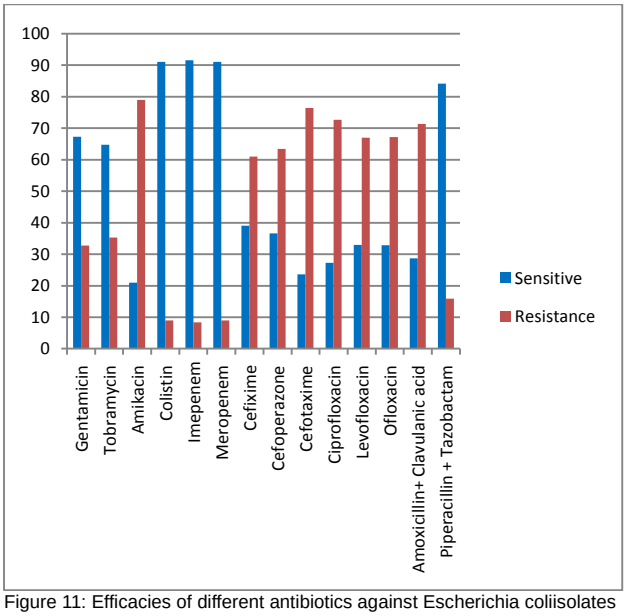

Figure 11: Efficacies of different antibiotics against Escherichia coliisolates

DISCUSSION

Escherichia coli being a well-known nosocomial and the opportunistic pathogen which is responsible to cause infections like

cystitis, pyelonephritis and bacteriuria. Severalmicrobes can cause UTIs though the most common pathogens causing UTI in the community is Escherichia coli, which accounts for approximately 75% of the isolates[2].Gram negative bacteria are considered as the commonest causative urinary pathogens as compared to gram positive bacteria and among the Gram-negative bacteria, Escherichia coli is stated to be the dominant bacterial pathogen of community acquired UTIs and its prevalence rate is highest (43.3 %)[23].

A total number of 180 specimens of urine were collected from patients having symptoms of Urinary tract infections from different private and government hospitals of District Faisalabad.Midstream urine samples were collected in a sterile container by clean catch method from which isolation and antibiogram studies of Escherichia coli were diagnosed through various confirmatory tests including gram staining and biochemical tests[24]. Out of one hundred and eighty samples, 132 (73.3 %) were positive and 48 (26.6%) were negative for the existence of Escherichia coli. The occurrence of Escherichia coli in UTIs according to previous studies was 43% from 89 isolated uropthogens by[25], 76.8% out of 95 urine samples and 66% out of 109 urine samples by[26]. According to these findings, Escherichia coli is rendered as a prominent causative agent of urinary infections particularly in hospitalized patients. The reason for such a high prevalence was long hospital stay and insertion of invasive catheters because this organism has the ability to develop biofilms on their surface.

In current study, occurence of UTI was more in females as compared to male population.Out of 132 confirmed isolates of Escherichia coli, 99 (75%) were isolated from females while 33 (25%) were isolated form males. Similar outcomes have been observed in other studies. According to a study, about78.1 % females were affected from UTI thus showed high prevalence of UTI as compared to males which were affected with 44.5%[27].

Several classes of the antimicrobial drugs have been given in routine prescription for the treatment of UTI whose etiological agent isEscherichia coli [28]. In this current study, CLSI guidelines of 2019 were followed by Kirby-Bauer disc diffusion method which was done to evaluate susceptibility profile by various antibiotics against Escherichia coli. In this study, following antibiotics were used :Amoxicillin-Clavulanic acid (20-10µg), Piperacillin-Tazobactam (100-10 µg), Cefixime (30µg), Cefoperazone, Cefotaxime (30µg), Imipenem (10µg), Meropenem (10µg), Amikacin (30µg), Gentamicin(10µg) , Tobramycin (10µg), Nalidixic acid (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Fosfomycin and Colistin (10µg). The isolates of Escherichia coli were highly susceptible toimepenem, meropenem and then followed by amikacin, gentamicin , Amoxicillin-Clavulanic acid, cefixime, cefotaxime, ciprofloxacin and cefoperazone. Another study revealed that the Escherichia coli was highly susceptible toimipenem (100%), then ampicillin (between 26 and 38%) and piperacillin (between 9 and 24%), whereas an absolute susceptibility was observed with imipenem (100%). The isolates of Escherichia coli were highly resistant to ciprofloxacin (72.7%), Amoxicillin-Clavulanicacid(71.2 %) and then followed by Cefotaxime (56.8%), Cefixime (42.4%), Levofloxacin (32.5%), Gentamicin (28.7%), Ofloxacin (26.5%), Piperacillin-Tazobactam(15.1%), Meropenem (9.0%), Imipenem (8.3%), Tobramycin (8.3%), Amikacin (4.5%) and Colistin (0%).

CONCLUSION

It is concluded that due to this emerging resistance of Escherichia coli towards a variety of antimicrobial drugs, results of antimicrobial susceptibility testing should be followed while prescribing treatment of urinary tract infection to prevent threat of this increasing resistance.

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