

“Study of Antibiotic sensitivity pattern and detection of fluoroquinolone resistance of Escherichia isolates of urinary tract infection”

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Abstract: The present study was done to detect antibiotic sensitivity pattern and detection of fluoroquinolone resistance of Escherichia isolates of urinary tract infection.

Background: Urinary tract infection (UTI) is the most common bacterial infection in human produced by uropathogenic species of bacteria. Escherichia coli is the most prevalent in our human¹. Escherichia coli is the most common cause of urinary tract infection. Escherichia coli accounts for 75% to 90% of urinary tract infection. At some point in lives, at least 12% men and 10-20% women have a patient like-old age, pregnant women, immune sup present, previous infection (David rah a 2008). Although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women².

Materials and Methods: During the study period, 300 clinical samples of urine samples were collected and process for isolation and identification of E. coli by standard method out of which 70 positive E. coli were isolated and their antibiotic sensitivity were tested by Kirby Bauer disc diffusion method according to CLSI guidelines (2018). E. coli ATCC 25922 strains were used as control organism.

Results: Conclusion: Out of 300 samples 150 are sterile samples and remaining 150 showed growth, in which E. coli 70 (46.66%) was the most commonest organism isolated followed by Klebsiella spp. 33 (22%) and Citrobacter spp. 17 (11.33%), Pseudomonas spp. 15 (10%), Acinetobacter 5 (3.33%), Proteus spp. 4 (2.66%), Enterobacter spp. 3 (2%), Staphylococcus spp. 2 (1.33%), And Candida albicans 1 (0.66%) were the least common organism isolated. In the study showed an increased fluoroquinolone resistance among uropathogenic E. coli isolates. Increased antibiotic resistance trends in UTI patients indicated that it is imperative to rationalize the use of antimicrobials and to use them conservatively.

Key Word: Escherichia coli, Urinary tract infection, Sensitivity, Resistance

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I. Introduction

UTI is the most common bacterial infection in human produced by uropathogenic species of bacteria. Escherichia coli is the most prevalent in our human¹. Escherichia coli is the most common cause of urinary tract infection. Escherichia coli accounts for 75% to 90% of urinary tract infection. At some point in lives, at least 12% men and 10-20% women have a patient like-old age, pregnant women, immune sup present, previous infection (David rah a 2008). Although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women². Uncomplicated UTIs in healthy women have an incidence of 50/1000/year². In 2017, demonstrated that prevalence for UTI among female is higher than male 75.6%, 24.4% respectively and higher prevalence is seen in 16 to 30 age group³.

An estimated 50% of women experience at least one episode of UTI at some stage in their life, while about 20-40% of women have suffered recurrent episode^{4, 5}.

II. Material And Methods

The prospective cross sectional observational study was carried out in department of Microbiology at National Institute of Medical Science & Research, Jaipur, Rajasthan. A total 70 Escherichia coli strains were isolated from various clinical samples of patient.

Study Design: Cross sectional observational study.

Study Location: This study was conducted in Department of Microbiology, National institute of medical science and research centre, Jaipur, Rajasthan.

Study Duration: The study was carried from July 2018 to December 2018

Sample size: 300 patients.

Sample size calculation During the study period 300 mid-stream urine (MSU) were collected at NIMS hospital. Out of 300 samples 150 are sterile samples and remaining 150 showed growth, in which E.coli 70(46.66%) was the most commonest organism isolated followed by Klebsiella spp.33 (22%) and Citrobacter spp. 17(11.33%) , Pseudomonas spp. 15 (10%) , Acinetobacter 5 (3.33%), Proteus spp. 4 (2.66%), Enterobacter spp. 3(2%), Staphylococcus spp. 2(1.33%), And Candida albicans 1 (0.66%) were the least common organism isolated.

Subjects & selection method: The study was conducted in the department of Microbiology, NIMS Medical College and hospital ,Rajasthan from July 2018 to December 2018, During the study period ,70 E.coli organism isolate from 300 urine

specimen of patient of all ages both sexes attending various patient at NIMS Hospital were processed in which 150 samples were sterile. Remaining 150 samples are E.coli 70 (46.66%) was the most common organism isolated followed by Klebsiella spp.32.(21.33%) and Citrobacter spp.17(11.33%), Pseudomonas spp. 15(10%), Acinetobacter 5(3.33%), Proteus spp.4(2.66%), Enterobacter spp.3(2%), Staphylococci 2(1.33%) and Candida albicans 1(0.66%) was the least common organism isolated.

In comparison of K. Usha , E kumar ,dvrsaigopal et al 2013 total of 121 GNB were isolated identified by standard biochemical tests E.coli was predominant (37.19%) followed by Pseudomonas spp. (19.83%) ,non fermentive gram negative bacilli (NFGNB) (10.74%), Enterobacter spp. (6.61%) and others(3.30%)⁶

Inclusion criteria:

1. Patients diagnosed with UTI (10-70 Age group) will be seen.
2. Patient not taking prior medications (1-6 month) including antibiotics that may affect the culture results.

Exclusion criteria:

1. Age less than 10 years and more than 70 years of patient.
2. Patient already taking antibiotic/chemotherapy treatment.
3. H/o Pregnancy / lactation.
4. History of Type-2 Diabetes Mellitus.
5. H/o patient with post-surgical UTI.

Procedure methodology

After written informed consent was obtained, a well-designed questionnaire was used to collect the data of the recruited patients respectively

Method of collection of urine specimen

Patient who were clinically suspected of UTI were asked to collect a fresh sample of midstream specimen of urine (MSU) in a wide mouthed universal container with a secure lid. A proper instruction was given to the patient regarding the method of collection of mid-stream urine sample. Male patient was asked to retract the prepuce, cleanse the glans penis with soap and water and then collect the sample from middle of the urine flow. Female patient was instructed to thoroughly clean the an-genital area from front to back, pass urine with labia separated and collect sample from middle portion of stream.

Specimen Transport:

Since urine is an excellent culture medium supporting the rapid growth of many bacteria. It was transported to the laboratory within an hour and processed.

MICROBIOLOGICAL EXAMINATION

A) Culture; Sample was inoculated on media such as Cysteine Lactose Electrolyte Deficient (CLED) Agar incubated at 37⁰ c for overnight.

B) Inoculation of sample: All urine sample were processed immediately and routinely cultured as per standard protocol on CLED agar. These plates were routinely incubated at aerobically and after overnight incubation, they were checked for bacterial growth. The organism was identified by their colony morphology, gram staining characters, motility and other relevant biochemical test as per standard laboratory method of identification.¹⁰

Antimicrobial susceptibility testing: -

Antibiotic susceptibility testing will be performed by Kirby Bauer disk diffusion method by CLSI guidelines 2018. An inoculation with a turbidity equivalent with a 0.5 McFarland standard and Muller Hinton agar is used. The inoculum will be smoothly swabbed over dried surface on Muller Hinton agar plate within 15 minutes and allowed to dry at room temperature. Antibiotic disc will be placed on with pointed forceps and

plates will be incubated at 37⁰ c for 24 hrs. The following antibiotic disc from HI media laboratories will be used for testing. Antibiotic sensitivity testing profile of Kirby Bauer disc diffusion method on Muller Hinton agar media.

The following drugs are used for FLUOROQUINOLONES for Enterobacteriaceae are-Ciprofloxacin , Levofloxacin, Nalidixic acid ,Norfloxacin,Ofloxacin, Gemifloxacin,cinoxacin and for GNB are Ampicilin, Imepenam,Meropanam, Doxycycline, Azethronam ,Cefazolin, Cefoxitin, Amikacin .Result were measured and recorded as compared to that of the according to the Clinical and Laboratory standard institute(CLSI)guideline.

McFarland standard turbidity preparation: To standardize the inoculums density for a susceptibility test, for McFarland turbidity equivalent to a 0.5 McFarland standard was used.

QualityControl; - E. coli ATCCThese Were used as quality control strains for antimicrobial susceptibility testing

III. Result

The present Study of Antibiotic sensitivity pattern and detection of fluoroquinolone resistance of E. coli isolates of urinary tract infection was conducted in the department of Microbiology, National Institute of Medical Sciences (NIMS) Medical College and hospital, from July 2018 to December 2018. During the study period 300 mid-stream urine (MSU) were collected at NIMS hospital. Out of 300 samples 150 are sterile samples and remaining 150 showed growth, in which E.coli 70(46.66%) was the most commonest organism isolated followed by Klebsella spp.33 (22%) and citrobacter spp. 17(11.33%) , Pseudomonas spp. 15 (10%) , Acinetobacter 5 (3.33%), Proteus spp. 4 (2.66%), Enterobacter spp. 3(2%), staphylococcus spp. 2(1.33%), And Candida albicans 1 (0.66%) were the least common organism isolated.

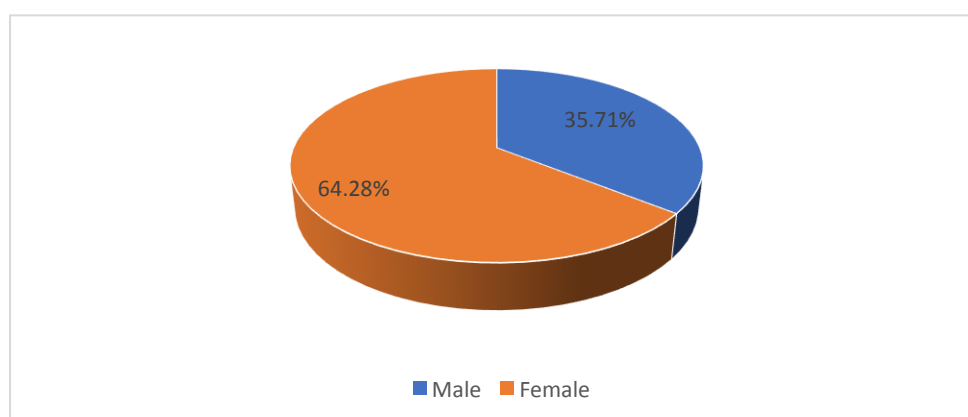
During the study E. coli isolated from urine specimen of patient of all ages were processed further for Study of Antibiotic sensitivity pattern and detection of fluoroquinolone resistance of E. coli isolates of urinary tract infection.

Distribution of E. coli according to gender:

During the study period E. coli isolated from urine specimen of patient of all ages and both sexes were processed.

Table 1: Distribution of E. coli according to gender

S.No.	Sex	Number	Percentage
1.	Male	25	35.71%
2.	Female	45	64.28%
3.	Total	70	100%



As shown in chart 2 Escherichia coli causing urinary tract infections were seen mostly in females 45 (64.28%) than in men 25(35.71%).

Table2. Percentage ratio of GNB drugs

S.NO.	DRUGS	SENSITIVE	Percentage (100%)
1.	Piperacilin-Tezobactum	45	64.28
2.	Ampicilin	39	55.71
3.	Amikacin	58	82.85
4.	Imepenam	59	84.28
1.	Meropenam	45	64.28
2.	Doxtcycline	39	55.71
3.	Gentamicin	53	75.71
4.	Azethronam	30	42.85
5.	Cefepime	39	55.71
6.	Cefazolin	40	57.14
7.	Cefoxitin	43	61.42
8.	Nitrofurantoin	44	62.85

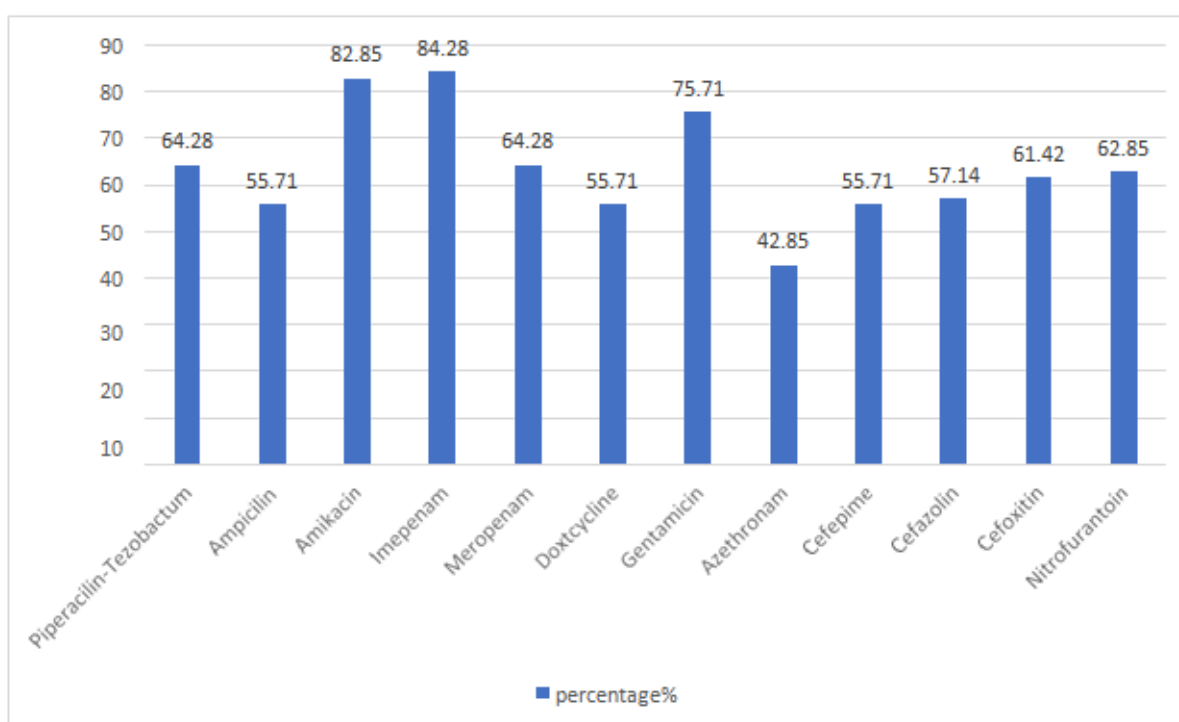


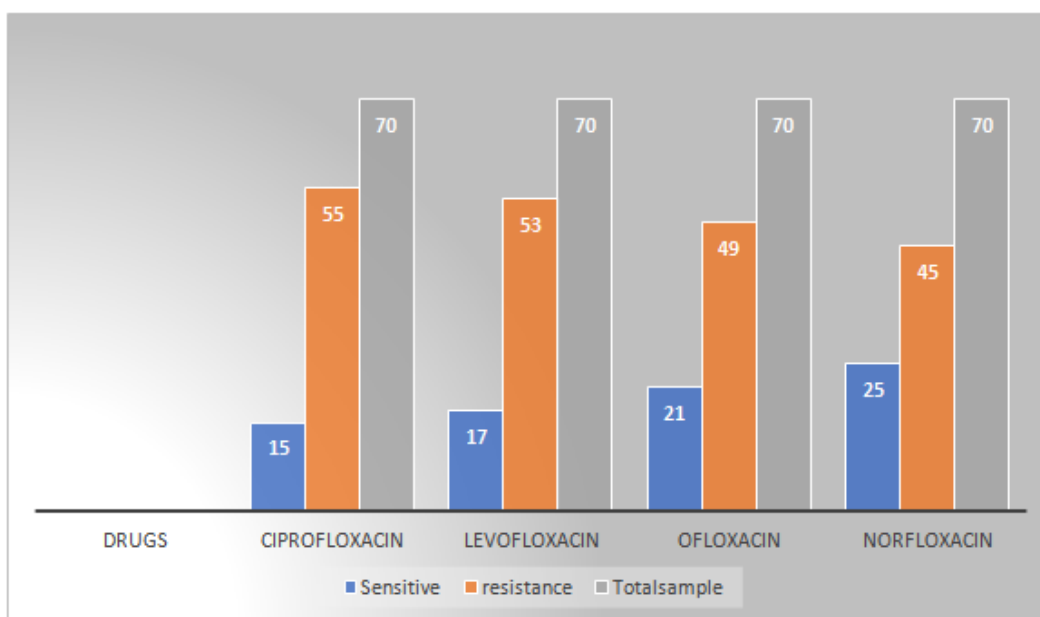
Chart 2. Percentage ratio of GNB drug

As shown in the **table 2**, and **Chart 2** the maximum percentage in IMEPENAM 59(84.28%) & AMIKACIN 58(82.85%), minimum in AZETHRONAM 30(42.85%)

Fluoroquinolone Sensitive/resistant drugs ratio

Table 3. Fluoroquinolone Sensitive/resistant drugs ratio

S.no.	Drugs	Sensitive	Resistance	Total sample
1.	Ciprofloxacin	15	55	70
2.	Levofloxacin	17	53	70
3.	Ofloxacin	21	49	70
4.	Norfloxacin	25	45	70

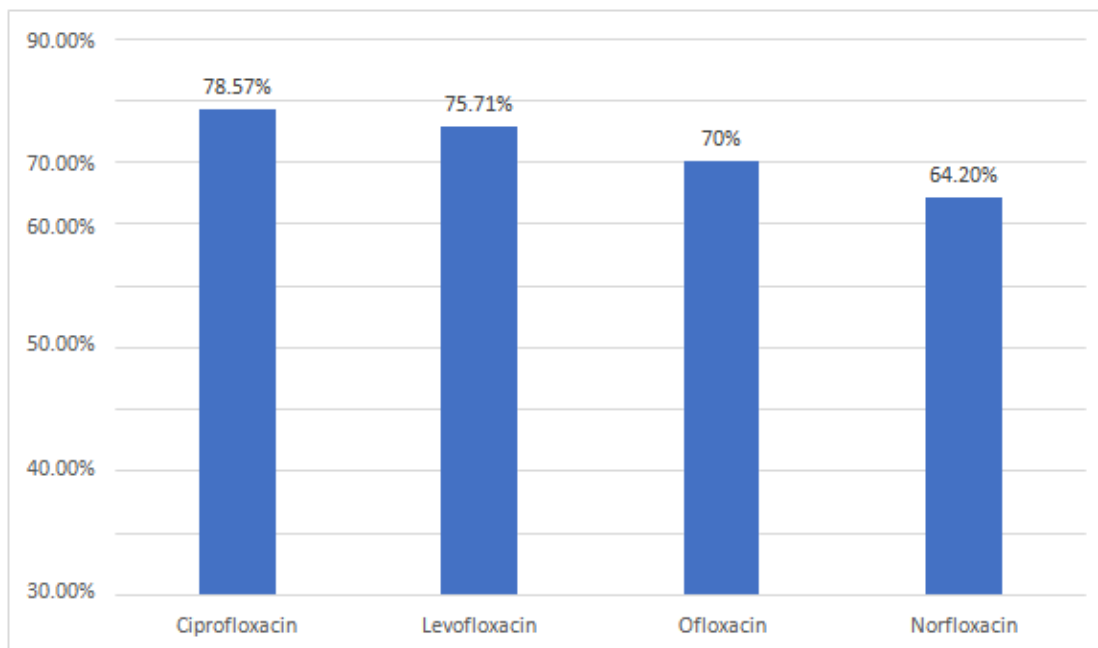


As shown in **chart 3** maximum resistant fluoroquinolone drug is ciprofloxacin (55)

Percentage of Resistant Fluoroquinolone drug

S.No	Drugs	Resistant Percentage
1.	Ciprofloxacin	78.57
2.	Levofloxacin	75.71
3.	Ofloxacin	70
4.	Norfloxacin	64.20

Table 4. Percentage of Resistant Fluoroquinolone drugs



As shown in chart 4 Maximum percentage in fluoroquinone drugs are ciprofloxcon(78.57%) than levofloxacin (.71%), ofloxacin (70%), Norfloxacin (64.20%)

IV. Discussion

Urinary tract infections are the most common bacterial infections in women and accounts for significant morbidity and increases health care costs.⁷ Factors such as the changing in patient population and extensive use and abuse of antimicrobial agents could contribute to changes in the microbial profile of urinary tract isolates. *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infection UTI.⁸ *Escherichia coli* used for study were isolated from urine samples.

The present study was conducted in the department of Microbiology, NIMS Medical College and hospital, Rajasthan from July 2018 to December 2018. During the study period, 70 *E. coli* organism isolate from 300 urine specimen of patient of all ages both sexes attending various patient at NIMS Hospital were processed in which 150 samples were sterile. Remaining 150 samples are *E. coli* 70 (46.66%) was the most common organism isolated followed by *Klebsiella* spp. 32 (21.33%) and *Citrobacter* spp. 17 (11.33%), *Pseudomonas* spp. 15 (10%), *Acinetobacter* 5 (3.33%), *Proteus* spp. 4 (2.66%), *Enterobacter* spp. 3 (2%), *Staphylococci* 2 (1.33%) and *Candida albicans* 1 (0.66%) was the least common organism isolated. In comparison of K. Usha, E. Kumar, D. V. Saigopal et al 2013 total of 121 GNB were isolated identified by standard biochemical tests *E. coli* was predominant (37.19%) followed by *Pseudomonas* spp. (19.83%)

, nonfermentative gram negative bacilli (NFGNB) (10.74%), *Enterobacter* spp. (6.61%) and others (3.30%).⁶

Antimicrobial susceptibility testing to *Escherichia coli* strains isolated from urine samples showed highest resistance Ciprofloxacin (78.57%), Levofloxacin (75.71%) and highest sensitive shown to

Imepanam (84.28%), Amikacin (82.85%) and gentamycin (75.71%). With the use of higher generations of fluoroquinolones for treating *Escherichia coli* causing urinary tract infections, my study showed slight decrease in resistance rates of both decreased sensitive s and resistance rates combined as 79% for ciprofloxacin-first generation drugs, 79% for second generation drugs-levofloxacin 76%.

Fluoroquinolones resistance increased significantly with patient age because of decreased immune function and overall more frequent fluoroquinolones exposure than that for younger patients (Lauron Becnel Boyd; et al; 2008). Female: Male *Escherichia coli* urinary tract infections is 19%: 28.9% in 2002.

according to Spanish national surveillance study and my study shows 64.28%:35.71%. Less in males is due to prostatic secretion that contains zinc which acts as bactericidal substance and in females reduces the ability of microorganisms to attach to uroepithelial cells and do not penetrate

In china ciprofloxacin resistance is increasing 46.6 to 59.4% in 1998- 2002 studies while it is 62% and 18% with decreased sensitivity in my study 78.57%.

Fluoroquinolones resistance is higher in developing countries than in developed countries. My study shows higher fluoroquinolones resistance rates due to increasing prescriptions without noticing resistant pattern of fluoroquinolones towards urinary *Escherichia coli* as ours is an under developed country (Hafizah Y. Chenia; 2006). Fluoroquinolones resistance is an Independent risk factor for mortality of patients due to delay in the initiation of appropriate antimicrobial therapy for patients with fluoroquinolone resistance infection and now it is no larger significant, after adjusting for inadequate empirical therapy (Ebbing Lautenbach; et al; 2005)⁹

Fluoroquinolones are the drug of choice for urinary tract infections for their high bacteriological and clinical cure rates as well as low rates of resistance among uropathogens. But due to increased prescriptions more frequently in past few years, increase in fluoroquinolone resistant *Escherichia coli* has occurred which is accounting for significant morbidity and health care cost.

V. Conclusion

In the study showed an increased fluoroquinolone resistance among uropathogenic *E. coli* isolates. Increased antibiotic resistance trends in UTI patients indicated that it is imperative to rationalize the use of antimicrobials and to use them conservatively

References

- [1]. Grueling TL. Urinary Tract Infection in Women. In: Litwin MS, Saiga CS, editors. *Urological Diseases in America*. Washington DC: US Government Publishing Office; 2004. 153-83
- [2]. Anas Asif, timelier Shahensharia et al. Increasing incidence of ESBL type Resistance among Urinary Tract Infecting *Escherichia coli* in New Delhi, India. *ijopaar*; 2017. 1(1); pp.48-61
- [3]. De Backer D, Christians T, Heightens S, de Sutter A, Slobbering EE, Verhaegen G. Evolution of bacterial susceptibility pattern of *Escherichia coli* in uncomplicated urinary tract infections in a country with high antibiotic consumption: A comparison of two surveys with a 10-year interval. *J Antimicrobe Chemother*. 2008;62:364-8.
- [4]. Kramer S, Home J, Demidov D. Treatment of lower urinary tract infection in pregnancy. *International Journal of Antimicrobial Agents*. 2001;17(4):279-82
- [5]. Alemu A, Mages F, Shivered Y, Tares K, Kasur A, Anagen B. Bacterial profile and

- drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar teaching Hospital, Northwest Ethiopia. 2012;5:197
- [6]. K. Usha , E kumar ,dvrsaigopal Strategies for the production recombinant protein in E.coli(2013);43:456-458
- [7]. Iravani A. Advanced in the understanding of urinary tract infections in young women. Urology 1991; 37: 503-511.
- [8]. Warren JV, Abrutyn E, Hebel R, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for the treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Clin Infect Dis 1999; 29:745-58.
- [9]. AJ.LUNG,C.-M.LIN,J.M.KIM,M.R.MARSHALL,R.NORDSTEDT,N.P. THOMPSON, and C. Destruction of Escherichia coli O157:H7 and Salmonella enteritidis ISSN:3202
- [10]. Farshad S, Ranjbar R, Anvarinejad M, Shahidi MA, Hosseini M. Emergence of Multi Strains of Escherichia coli Isolated from Urinary Tract Infection The Open Conference Proceedings Journal, 2010: 1:192-96

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