IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION, IDENTIFICATION, AND MULTI-DRUG RESISTANCE PATTERN OF *ESCHERICHIA COLI* ISOLATED FROM SAMPLES OF URINARY TRACT INFECTED PATIENTS

Dr. Prasad Madhukarrao Deshmukh
Assistant Professor,
Department of Microbiology, Shri Dnyaneshwar Maskuji Burungale Science & Arts College, Shegaon, Dist. Buldana, Maharashtra.

ABSTRACT: -

The most prevalent medical condition among hospitalized and outpatient patients is a urinary tract infection. Multi-drug resistance to antibacterial medications is a further issue that raises concerns among health professionals. The most frequent causative and etiologic agent for urinary tract infections is *Escherichia coli* (*E. coli*). *E. coli* was the most common bacteria, accounting for 27.38% (43/157) of the 157 isolated uropathogens. A large percentage was also seen in females, 62.42% (98/157).

These isolates were all further checked against 12 different antibiotics. *E. coli* showed tremendous resistance towards Amoxyclav 88.42% followed by Ampicillin, 81.79%, Ciprofloxacin, 79.32%, Nalidixic acid, 77.42%, Cefodoxime, 71.47%, Cephalothin, 69.62%, Tetracycline, 67.81%, Ceftazidime/clavulanic acid, 59.28%, Ceftriaxone, 53.80%, Cefepime, 32.75% and Cefoperazone/sulbactam, 23.70%. While Imipenem is the prominent drug of choice for urinary tract infections.

Key Words: *E. coli*, urinary tract infection, multi-drug resistance

INTRODUCTION: -

Due to anatomical differences, urinary tract infections (UTIs) are the most prevalent infection in humans, particularly in women. Due to the evolution of resistance to several antimicrobial drugs, managing urinary tract infections has recently become increasingly difficult (Steadman and Topley, 1998). The most frequent pathogen that can cause urinary tract infections (UTI) in both developed and developing nations is *Escherichia coli* (*E. coli*), more specifically known as Uropathogenic *Escherichia coli* (UPEC) (Samra et al., 2005). Bacterial resistance to various antimicrobial medications is a severe health risk for people all over the world. One of the main reasons for bacterial resistance to antimicrobial drugs is the unwarranted or constant use of certain antimicrobials.

According to Shames *et al*., (2009) and Ogura *et al*., (2009), *E. coli* is a common, diverse, and harmless commensal organism that only needs to acquire virulence factors to become a highly pathogenic organism with the ability to cause diseases like gastroenteritis, extra-intestinal infections, urinary tract infections, and bloodstream infections. In addition to these, it can survive in various environments, which makes it an excellent indicator organism to assess environmental samples and fecal contamination (Feng *et al*., 2002). It belongs to the gamma probacteria's *Enterobacteriaceae* family. According to Wagenlehner *et al*., (2008) and Kashef *et al*., (2010), it is the main infectious agent responsible for urinary tract infections. Additionally, the majority of diarrhea-related deaths in children under the age of five among people around the world are caused by *E. coli*

(Turner *et al.*, 2006).

Antimicrobial resistance in *E. coli* has been noted globally, and the rate of resistance has risen among *E. coli*, which is a serious issue (Kholy *et al*., 2003; Bell *et al*., 2002). Treatment of urinary tract infections becomes more challenging as antimicrobial medication resistance continues to rise. According to Dromigny *et al*., (2005), the majority of people with severe urinary tract infection symptoms were likely treated without doing a bacteriological study in 95% of cases. According to Coque *et al*., (2008), the first drug resistance was described in relation to ampicillin, amoxyclav, trimethoprim, erythromycin, third-generation cephalosporin antibiotics, and tetracycline.

The incidence and susceptibility profiles of *E. coli*, according to Erb *et al*., (2007), show substantial topographical alterations as well as significant variations in different contexts and populations. Antibiotics should be used correctly to reduce the rate of antibiotic resistance (Islam *et al*., 2010). This study's goal was to identify the prevalence and multidrug resistance profile of *E. coli* isolates from individuals with urinary tract infections in hospital settings.

MATERIALS & METHODS: -

In the current investigation, a total of 63 urine samples from individuals with infections of the urinary tract were obtained. Aseptically, in a sterile, clean catch container, fresh midstream urine samples were obtained. Within two hours of collection, urine samples were brought to the lab in sterilized Luria Broth (LB) broth. At 37°C, Luria broth was incubated for an entire night.

Then, using a calibrated loop that delivered 0.01ml of each sample, each sample was inoculated on plates of MacConkey agar, Cysteine Lactose Electrolyte Deficient (CLED) agar, and Eosin Methylene Blue (EMB) agar. Plates were then incubated for 24 hours at 37°C overnight. In addition, the E. coli isolates in which we are most interested were found through analysis of their morphological, cultural, and biochemical traits.

Using the single disc diffusion method recommended by Bauer-Kirby (1966), the antibiotic resistance pattern of E. coli isolates was examined. For this, 3 ml of sterile nutrient broth tubes were injected with a loop full of bacterial culture and incubated at 37°C for 4-6 hours. The nutritional broth tube was then used to moisten a clean cotton swab. The Mueller-Hinton agar plates' whole surface was inoculated with this swab. Mueller-Hinton agar was now covered with an antibiotic disc using sterile forceps, and the plates were then incubated at 37°C for an overnight period.

The following antibiotics were used in the current study, Amoxyclav (30µg), Ampicillin (20µg), Ciprofloxacin (5µg), Nalidixic acid (10µg), Imipenem (10µg), Tetracycline (10µg) and different generation cephalosporin viz, Cefepime (30µg), Cefodoxime (30µg), Cefperazone/sulbactum (20/10µg), Cephalothin (30µg), Ceftazidime/clavalunic acid (20/10µg) and Ceftriaxone (30µg). Every antibiotic was purchased from Hi Media Laboratory in India. A quality control strain that was obtained from MTCC (443) was used as a control strain in the present investigation.

RESULTS AND DISCUSSION: -

A total of 63 urine samples were obtained for the current study from the district civil hospital in Amravati, Maharashtra. These allowed for the isolation of 157 different varieties of Gram-positive and Gram-negative bacteria. *Escherichia coli* was the most frequent etiologic agent in our investigation, accounting for 68.25% (43/63) of all cases. The prevalence of several uropathogens isolated from urine samples is shown in Fig.1. From the isolates females (68.79%) accounted more as compared to males (31.21%). Gender-wise distribution of several uropathogens was shown in Fig.2. Figure 3 depicts the resistance pattern of *Escherichia coli* against 12 different antibiotics.

Using guidelines from the Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee on Clinical Laboratory Standards (2006), the resistance pattern of *Escherichia coli* isolates was determined in the current investigation.

Table 1: The frequency of *Escherichia coli* and other bacteria that are isolated from urine samples			
Sr. No.	Bacteria	No. of bacterial isolate	Percentage of isolated bacteria
1	*Escherichia coli*	43	68.25%
2	*Klebsiella pneumoniae*	39	61.90%
3	*Staphylococcus aureus*	31	49.20%
4	*Pseudomonas aeruginosa*	23	36.50%
5	*Bacillus subtilis*	21	33.33%
	Total	157	-

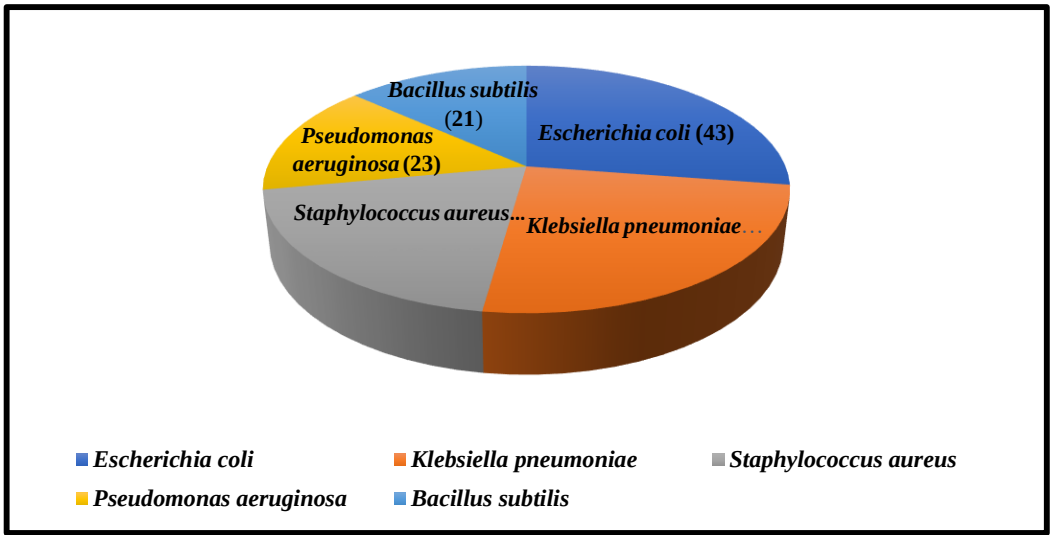

Fig. 1. The frequency of *Escherichia coli* & other bacteria that are isolated from urine samples

Table 2: Gender-specific distribution of several uropathogens in numbers					
Gender	**Uropathogens**				
	E. coli	***K. pneumoniae***	***S. aureus***	***P. aeruginosa***	***B. subtilis***
Male (%)	20	11	08	07	03
Female (%)	35	26	19	15	13
Total	55	37	27	22	16

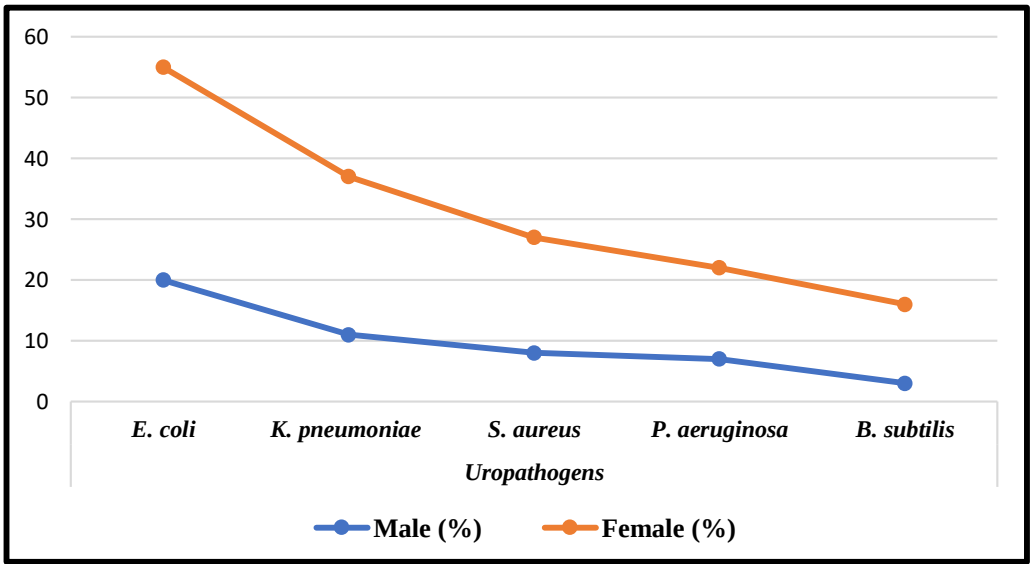

Fig. 2 Gender-specific distribution of several uropathogens in numbers

Table 3: *Escherichia coli's* pattern of resistance towards 12 different antibiotics			
Sr. No	**Antibiotics**	**No. of resistant isolates**	**% of resistant isolates (n=43)**
1	Amoxyclav (30µg)	39	90.70%
2	Ampicillin (20µg)	35	81.40%

3	Ciprofloxacin (5µg)	34	79.07%
4	Nalidixic acid (10µg)	37	86.05%
5	Cefodoxime (30µg)	32	74.42%
6	Cephalothin (30µg)	28	65.12%
7	Tetracycline (10µg)	27	62.79%
8	Ceftazidime/clavalunic acid (20/10µg)	22	51.16%
9	Ceftriaxone (30µg)	21	48.84%
10	Cefepime (30µg)	18	41.86%
11	Cefperazone/sulbactam (20/10µg)	16	37.21%
12	Imipenem (10µg)	00	00%

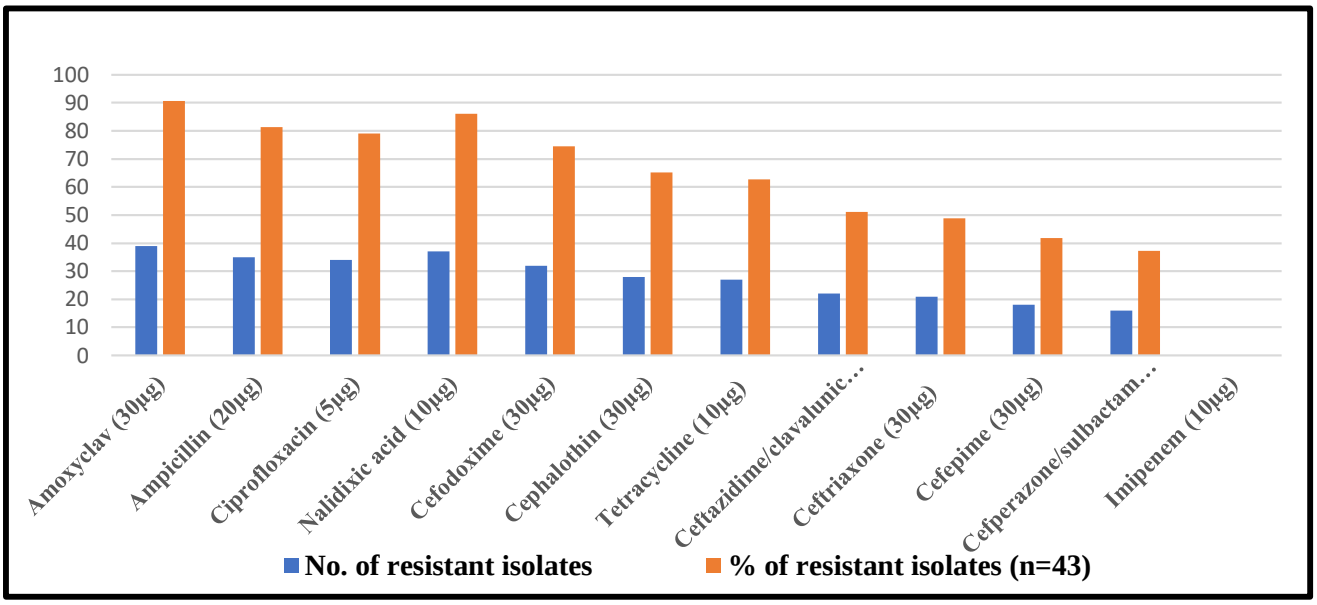

Fig. 3 *Escherichia coli* pattern of resistance towards 12 different antibiotics

The findings of the present study showed that a high proportion of resistance to eleven distinct antibiotics was seen. Amoxyclav (30µg) (90.70%), Ampicillin (20µg) (81.40%), Ciprofloxacin (5µg) (79.07%), Nalidixic acid (10µg) (86.05%), Cefodoxime (30µg) (74.42%), Cephalothin (30µg) (65.12%), Tetracycline (10µg) (62.79%), Ceftazidime/clavalunic acid (20/10µg) (51.16%), Ceftriaxone (30µg) (48.84%), Cefepime (30µg) (41.86%), Cefperazone/sulbactam (20/10µg) (37.21%), and Imipenem (10µg) (00%) are the antibiotics.

Since imipenem was extremely susceptible to all *Escherichia coli* isolates and no one strain was discovered to be resistant, imipenem was the most effective antibiotic and is now the medicine of choice for community-acquired urinary tract infections.

DISCUSSION: -

According to the research that is currently available, the most frequent pathogen found in urine tract infections recovered from patients was *Escherichia coli*. The majority of uropathogens were found in female samples. Akram *et al*. in 2007, conducted a similar investigation. According to Manges *et al*. (2006), the most common pathogen detected in patients with community-acquired urinary tract infections was *Escherichia coli*. Additionally, Zahera *et al*. (2011) demonstrated that the most frequent bacterial isolate discovered in urine samples was *Escherichia coli*.

The current investigation found that 81.40% of *Escherichia coli* strains were resistant to ampicillin, which is concerning. A near about similar set of results, showing 94.29% ampicillin resistance, was discovered by Rawat *et al*. (2010).

The level of fluoroquinolone resistance among the E. Coli isolates in this investigation, including ciprofloxacin, was likewise high. The percentage of E. Coli isolates resistant to ciprofloxacin was 79.07%. The findings of this study are consistent with earlier research, including 84% by Ahmad et al. (2009) and 90.28% by Rawat et al. (2010). The current results of our investigation, however, differ slightly. According to Kiffer et al. (2007), there was a lower-than-expected rate of ciprofloxacin resistance.

In our investigation, 62.79% of the *Escherichia coli* isolates showed high resistance to tetracycline. Yengkokpam *et al*. (2007) discovered a comparable kind of outcome, noting that 73.30% of isolates were found to be tetracycline resistant.

In our investigation, a remarkable 90.70% resistance rate towards amoxyclav was discovered. When this data was compared to findings from another researcher, Mulla *et al*. (2011) demonstrated that amoxyclav was an ineffective medication. The reason behind the resistance of bacteria like *Klebsiella pneumoniae* and *Escherichia coli* to this antibiotic is their synthesis of an enzyme called extended-spectrum β-lactamase, which is mediated by plasmids. In comparison to amoxyclav, 51.16% of the isolates of *Escherichia coli* that we studied showed reduced resistance to ceftazidime/clavulanic acid.

Only 41.86% of the isolates in this experiment were resistant to cefepime, indicating a slightly high susceptibility. Our data revealed almost identical results to those of Khadri *et al*. (2009), who discovered that the proportion of isolates resistant to this antibiotic was lower. As compared with the results of Rawat *et al.*,

According to the results of Rawat *et al.*, (2010) compared with our findings we observed that only 8.75% of *Escherichia coli isolates* were found to be resistant to this antibiotic. In the present investigation, the antibiotic ceftriaxone showed 48.84% resistance. George *et al*. (2012) conducted a similar kind of investigation because only 26.80% of the isolates of *Escherichia coli* were ceftriaxone-resistant.

Nalidixic acid and cephalothin, which account for 86.05% and 65.12% of the resistance rate, respectively, were shown to be highly resistant. Additionally, a high resistance rate of 74.42% against cefodoxime was noted. The current study found that the combination therapy of cefperazone and sulbactum reduced the rate of resistance to this antibiotic, with only 37.21% of *Escherichia coli* isolates resistant to it. Sulbactam is a potent inhibitor of extended-spectrum β-lactamase, so this combination may be used in the future to treat severe nosocomial infections.

Since no one *Escherichia coli* isolate was shown to be resistant in our investigation, imipenem may be the most popular medication of choice. Similarly, imipenem was found to be sensitive to every isolate of *Escherichia coli* by Farshad *et al*. (2010). Adwan *et al*. also noted a significant sensitivity to imipenem (2004).

CONCLUSION: -

The previously mentioned study proved that there is a considerable level of drug resistance to several different kinds of antibacterial drugs. An extremely alarming sign is that every isolation of *Escherichia coli* was resistant to most antibiotics. All of the isolates had extremely high rates of resistance to ciprofloxacin, amoxyclav, ampicillin, nalidixic acid, cefodoxime, cephalothin, and tetracycline. Given that it is effective against every isolate of *Escherichia coli*, imipenem is the drug of choice for treating bacterial infections.

REFERENCES: -

[1] Adwan K, Abu HN, Adwan G, Jarrar N, Abu-Shanab B, Al- Masri M. 2004. Molecular epidemiology of antibiotic-resistant *Escherichia coli* isolated from hospitalized patient with urinary tract infection in Northern Palestine. Polish Journal of Microbiology, 53: 23-26.

[2] Ahmad AS, Syed SA, Mohiruzzaman A, Abu NIS, Mohammad RAM. 2009. Changing trends in uropathogens and their antimicrobial sensitivity pattern. Bangladesh Journal of Medical Microbiology, 3(1): 9-12.

[3] Akram M, Shahid M, Khan AU. 2007. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobial, 6: 274-276.

[4] Bauer AW, Kirby WMM, Sherris JC, Turk M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493- 496.

[5] Bell JM, Turnidge JD, Gales AC, Pfaller M, Jones RN. 2002. Prevalence of extended spectrum beta-lactamase (ESBL)- producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998–99). Diagnostic Microbiolology and Infectious Disease, 42: 193–198.

[6] Clinical and Laboratory Standards Institute. 2006. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Information Supplement. CLSI document M100-S17, Clinical and Laboratory Standards Institute Wayne Pennsylvania.

[7] Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Canton R, Nordmann P. 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerging Infectious Diseases, 14: 195-200.

[8] Dash SK, Chakraborty SP, Mandal D, Roy S. 2012. Isolation and characterization of multi drug resistant uropathogenic *Escherichia coli* from urine samples of urinary tract infected patients. International Journal of Life Science and Pharma Research, 2 (1): 25-39.

[9] Dromigny JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD. 2005. Risk factors for antibiotic resistant *Escherichia coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. Journal of Antimicrobial Chemotherapy, 56: 236–239.

[10] El Kholy A, Baseem H, Hall G, Procop GW, Longworth DL. 2003. Antimicrobial resistance in Cairo, Egypt 1999-2000: a survey of five hospitals. Journal of Antimicrobial Chemotherapy, 51: 625–630.

[11] Erb A, Stürmer T, Marre R, Brenner H. 2007. Prevalence of antibiotic resistance in *Escherichia coli*: overview of geographical, temporal, and methodological variations. European Journal of Clinical Microbiology and Infectious Diseases, 26: 83–90.

[12] Farshad S, Ranjbar R, Anvarinejad M, Shahidi M, Hosseini M. 2010. Emergence of multi-drug resistant strains of *Escherichia coli* isolated from urinary tract infection. The Open Conference Proceedings Journal, 1**:** 192-196.

[13] Feng P, Weagant S, Grant M. 2002. Enumeration of *Escherichia coli* and coliform bacteria. Bacteriology Analysis Manual, (8^{th} edition) FAD/Centre for Food Safety and Applied Nutrition.

[14] George FD, Gbedema YS, Agyare C, Adu F, Boamah EV, Tawiah AA, Saana MS. 2012. Antibiotic resistance pattern of *Escherichia coli* isolates from hospitals in Kumasi, Ghana. International Scholarly Research Network. 2012: 1-5.

[15] Islam MS, Khondkar P, Islam MA. 2010. Isolation and in vitro antibiotic resistance and susceptibility pattern of a strain of *E. coli*. Bangladesh Pharmaceutical Journal. 13(1):34-37.

[16] Kashef N, Djavid GE Shahbazi S. 2010. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran. Iranian Journal of Infection in Developing Countries. 4(4): 202–206.

[17] Khadri H and Alzohairy M. 2009. High prevalence of multi- drug-resistance (MDR) and extended spectrum beta lactamases (ESBL) producing bacteria among community-acquired urinary tract infections (CAUTI). Journal of Bacteriology Research, 1(9): 105-110.

[18] Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW. 2006. The changing prevalence of drug-resistant Enterobacteriaceae groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiology and Infection, 134: 425–431.

[19] Manjunath GN, Praksh R, Vamseedhar A, Shetty K. 2011. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore, International Journal of Biological and Medical Research, 2(2): 504-507.

[20] Ogura Y, Ooka T, Iguchi A, Toh H, Asadulghani M, Oshima K, Kodama T, Abe H, Nakayama K, Kurokawa K, Tobe T, Hattori M, Hayashi T. 2009. Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*, Proceedings of the National Academy of Sciences of the United States of America, 106: 17939–17944.

[21] Rawat V, Umesh, Paul P. 2010. Antibiotic resistance pattern of urinary tract isolates of *Escherichia coli* from Kumaun region. National Journal of Integrated Research in Medicine, 1(4): 43-46.

[22] Samra KSS, Cenk S, Horu G, Mehmet G, Beril O. 2005. Increasing antimicrobial resistance of *Escherichia coli* isolates from community-acquired UTI during 1998-2003 in Manisa Turkey, *Japan* Journal of Infectious Disease. 58: 159-161.

[23] Shames SR, Auweter SD, Finlay BB. 2009. Co-evolution and exploitation of host cell signalling pathways by bacterial pathogens. International Journal of Biochemistry and Cell Biology, 41: 380–389.

[24] Steadman R and Topley N. 1998. The virulence of *Escherichia coli* in Urinary tract. Chapter 3. In: Brumfit W, Jercmy MT, Hamilteon Miller Eds. Urinary tract infections. 1^{st} ed. Chapman and Hall Publication, London. 37-41.

[25] Turner SM, Scott-Tucker A, Cooper LM, Henderson IR. 2006. Weapons of mass destruction: virulence factors of the global killer enterotoxigenic *Escherichia coli*. FEMS Microbiology Letters, 263(1): 10–20.

[26] Wagenlehner FM, Naber KG, Weidner W. 2008. Rational antibiotic therapy of urinary tract infections. Med Monatsschr Pharm, 3(1): 385–390.

[27] Yengkokpam C, Ingudam D, Yengkokpam IS, Jha BK. 2007. Antibiotic susceptibility pattern of urinary isolates in Imphal (Manipur), India, Nepal Medical College Journal, 9(3): 170-172.

[28] Zahera M, Rastogi C, Singh P, Aram S, Khalid S, Kushwaha A. 2011. Isolation, Identification and characterization of *Escherichia coli* from urine samples and their antibiotic sensitivity pattern. European Journal Experimental Biology, 1(2): 118-124.