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The Prevalance of Beta *Lactamase* Producing *Escherichia coli* Strains Isolated from the Urine Samples in Valiasr Hospital

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ABSTRACT

Resistant strains of Uropathogenic *Escherichia coli* have been considered as the most prevalent cause of UTIs. Recently, the incidence of extended spectrum beta lactamases producing *E. coli* has been increased and causes severe illnesses. Resistance to β-lactam antimicrobial agents in *E. coli* is primarily mediated by β-lactamases. The classical TEM-1 enzyme is the predominant plasmid-mediated β-lactamases of gram-negative rods. This study aimed to detect E. coli in urine samples from patients at the valiasr hospital, Rasht, Iran to study the antibiotic susceptibility pattern of bacterial isolates as well as analyze the distribution of ESBL strains and TEM-1 enzyme. Seven hundred thirty one urine samples of hospitalized patients and Outpatient of valiasr hospital were collected and immediately cultured. Presence of ESBL strains and TEM-1 enzyme have been diagnosed using the PCR method. Susceptibility of *E. coli* isolates against 9 commonly used antibiotics has been studied usimg the disk diffusion method. Of 731 urine samples, 149 samples (20.38%) were positive for *E. coli*. Women had the higher distribution of *E. coli* than men ($P< 0.05$). We found that 30-40 years old patients had the highest incidence of *E. coli*. E. coli strains had the highest levels of resistance to amoxicillin (97.31%), cephalexin (80.53%), tetracycline (75.16%) and co amoxiclav (69.12%). Of 149 *E. coli* isolates, 58 samples were ESBL (38.92%) and the incidence of TEM-1 enzyme was 21.47%. Special health care should be performed on management of Urinary tract infections in public place. We suggested use of gentamicin and ciprofloxacin antibiotics in order to treatment of UTIs in patients. High presence of ESBL strains suggested the need to orient much attention over antibiotics.

Key words: *Escherichia coli, Beta Lactamase Enzymes,* Urine samples.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most frequent infectious diseases around the world. Urinary tract infections contain a variety of illnesses including cystitis (infection of the bladder) and pyelonephritis (infection of the kidney). Forty to fifty percent of people and especially women have UTIs throughout their lives (1) . The Uropathogenic *Escherichia coli* (*E. coli* (UPEC)) strains are the most mutual cause of the UTIs (2).

Treatment of diseases caused by this bacterium often requires antimicrobial therapy; however, antibiotic-resistant strains of bacteria cause more severe diseases for longer periods of time than their antibiotic-susceptible counterparts. Today, due to the widespread presence of different kinds of Extended Spectrum Beta Lactamase (ESBL), many reports suggestedthat the UPEC strains have been resistant to commonly used antibiotics (3,4). Treatments of the cases of infections associated with ESBL-producing bacteria is very

difficult, because the genes that are responsible for bacterial resistance by plasmids have been performed (5,6).

Enzymes of ESBL have the ability of creating resistance against cephalosporins, penicillins and menobacterums. ESBL were appeared after advent of cephalosporins (7). These enzymes are the modified kinds of primary enzymes, TEM-1, TEM-2 and SHV-1. These changes mainly involve changes in one or more amino acid sequences (8,9).

TEM-1 is the prevalent beta lactamase in gram negative bacteria. This enzyme is considered as the cause of 90 % resistantance to ampicillin (3). TEM-2 is derived from TEM-1; the only difference between them is one amino acid, but they operate identical. SHV-1 is another prevalent beta lactamase. Contrary to TEM having found in different microorganisms, SHV was identified in just a limited numbers (10,11). In recent years, a new family of ESBL has been recognized, named CTX-M. This new enzyme is alike of SHV and TEM in just 14 % of features (4). Isolation of antibiotic-resistant genes and the prevalence of ESBL *E. coli*, through providing information about the epidemiology and prevention of infections caused by *E. coli*.

The purpose of this study was to investigate the epidemiology, medicine sensitivity, and the resistance of bacteria producing beta lactamase, in Iranian military personnel, and people who are related to them, i.e. the soldiers and the families of employees.

MATERIAL AND METHOD

Samples and *Escherichia coli* identification

From January to April 2013, a total of 731 urine samples were collected from patients who suffered from UTIs. All of these patients were from the army and were referred to the Valiasr Hospital Gilan , Rasht , Iran. Presence of UTIs in pediatrics was confirmed using the ultrasound technique (12). Most of patients had been handling urine catheter for a week before they got UTIs. Strong urge to urine frequently even immediately after the bladder is emptied, painful burning sensation when urinating, cloudy and bloody urine with bad smell and in some cases fever, chills and nausea are the most commonly detected symptoms in pediatric patients. In order to decrease potential bacterial, cellular and artifactual contamination all urine samples were collected from midstream. Urine samples were collected using the Suprapubic Aspiration (SPA) method based on the standard technique of NICE (2007) (13).

Totally, 3 mL of each sample was blended with 225 mL of nutrient broth (Merck, Germany) for 2 min at normal speed, using a Stomacher lab blender and incubated at 37 °C for 24 h. A 1 mL sample of the nutrient broth culture was mixed with 9 mL of MacConkey broth (Merck, Germany) and further incubated at 37 °C for 24 h. One loop of each tube was streaked on MacConkey agar (Merck, Germany). A typical purple colony of *E. coli* was streaked on *Eosin Methylene Blue agar* (EMB agar; Merck, Germany) plate and incubated at 37 °C for 24h. Green colonies with a metallic luster were considered as typical *E. coli* colonies. Such colonies were confirmed as *E. coli* using standard biochemical tests (e.g., Methyl red, Voges-Proskauer, Indole, and Citrate utilization tests). *E. coli* isolates were stored in Tryptic Soy Broth (TSB, Merck, Germany) containing 20% glycerol at "70˚C for further characterization.

Antimicrobial susceptibility pattern

Pattern of antimicrobial resistance was examined using the simple disk diffusion technique. The Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084) medium was used for this purpose. Antibiotic resistance of UPEC strains against 15 commonly used antibiotics was determined using the instruction of Clinical and Laboratory Standards Institute guidelines (CLSI 2012) (14). Susceptibility of *E. coli* isolates were tested against amoxicillin (10 u/disk), tetracycline (30 µg/disk), gentamycin (10 µg/disk), ciprofloxacin (5 µg/disk), cotrimoxazole (30 µg/disk), ceftriaxone (30 µg/disk), ceftizoxime (30 µg/disk), cephalexin (30 µg/disk), and *co-amoxiclav* (3 µg/disk) antibiotic agents (Oxoid). All of the inoculated plates were aerobically incubated at 37 °C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012) (14). In all reactions, the *E. coli* ATCC 25922 was used as quality control organisms.

To selected bacteria producing ESBL, DDST method and Mueller Hinton Agar culture was used (15). Inhibit the growth being seen in each of the disks used in combination with clavulanic acid sign of ESBL production by the bacteria. Results were reported based on clinical and laboratory standards institute

DNA and Plasmid extraction, PCR amplifications and gel electrophoresis

Bacteria were cultured overnight on Luria-Bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies using the DNA extraction kit (DNP™, CinnaGen, Iran) according to manufacturer's instruction. All *E. coli* colonies were also confirmed using the PCR technique (16). A PCR method was done with a total volume of 50 µL including 2 mM $Mgcl_2$, 1 µM of forward primer, 1 µM of reverse primer (specified for the 16S rRNA gene of the *E. coli*), 5 µL PCR buffer 10X, 200 µM dNTP (Fermentas), 1 U Taq DNA polymerase (Fermentas) and 2.5 µL DNA template. The DNA was then amplified by 31 successive cycles of denaturation at 95°C for 45 s, primer annealing at 59°C for 60 s, and DNA chain extension at 72°C for 60 s. The programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg,Germany) PCR device was used in all PCR reaction.

DNA plasmid was extracted using the boiling method with respect to the previously published protocol (17,18). Set of primers used for detection of TEM -1 gene of *E. coli* strains are shown in Table 1. The PCR protocol of previous study was used for TEM-1 amplification (19).PCR products were electrophoresed using 2% agarose gels which was stained with ethidium bromide at 90 V for 6 h using 1× TBE (0.89 M Tris borate, 0.02 M EDTA, pH 8.3) as the running buffer. All products were examined under ultraviolet illumination. A set of molecular weight standards (Fermentas, GmbH, Germany) ranging from 100 bp to 2000 bp was included on each gel.

Statistical Analysis

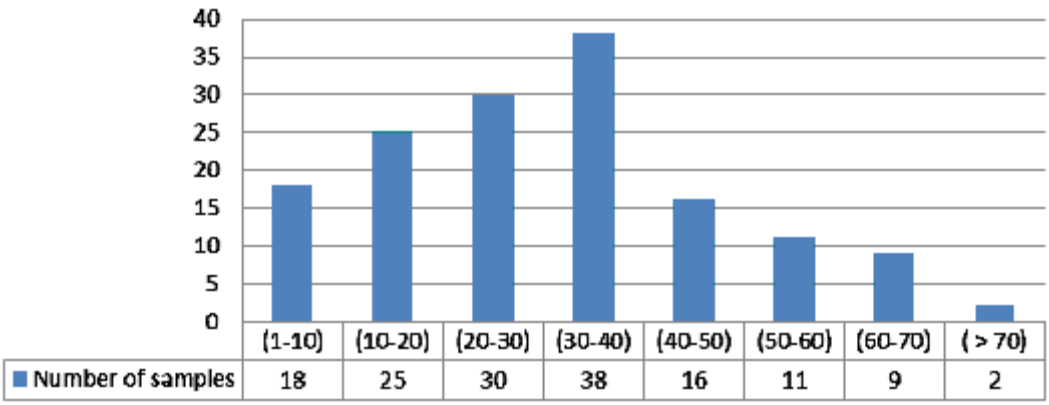
The data were analyzed using SPSS (Statistical Package for the Social Sciences) software and *P* values were calculated using Chi-square and Fisher's exact tests to identify statistically significant relationships between the distribution of *E. coli* and antibiotic resistance properties of the UPEC strains. A *P* value < 0.05 was considered statistically significant.

Ethical issues

All samples were in compliance with ethical issues and patients satisfaction.

RESULTS

Of 731 urine samples studied, 149 samples (20.38%) were positive for *E. coli*. Distribution of *E. coli* in men and women patients were 69.12% and 30.87%, respectively. There were significant differences in the incidence of *E. coli* between men and women (*P*< 0.05). Senile distribution of *E. coli* in the urine samples of army force is shown in figure 1. Results showed that 30-40 years old patients had the highest incidence of *E. coli*. We found statistically significant differences in the incidence of *E. coli* between 30-40 years old



patients and over 70 years old patients and 60-70 years old patients.

Table 2 shows the susceptibility of *E. coli* strains to commonly used antibiotics. We found that bacterial strains of our study had the highest levels of resistance against amoxicillin (97.31%), cefalexin (80.53%), tetracycline (75.16%), co amoxiclav (69.12%) and cotrimoxazole (68.45%). The most effective drugs were gentamicin and ciprofloxacin. There were significant differences in the incidence of antibiotic resistance between amoxicillin and gentamicin and tetracycline and ciprofloxacin ($P< 0.05$). The results of our investigation showed that from 149 positive samples for *E. coli* strains, 58 samples were ESBL positive (38.92%), while 91 samples were ESBL negative (61.07%). The results of PCR technique showed that 32 out of 149 *E. coli* strains (21.47%) were positive for TEM-1 gene (E+ and T+), while 26 strains had no TEM-1 gene (E+, T-). We found statistically significant differences between the incidence of ESBL positive and negative strains and also between the incidence of TEM-1 positive and negative strains ($P< 0.05$).

Table. 1: Oligonucleotide primers for detection of TEM-1 genes of Uropathogenic *Escherichia coli*

Gene target	Primer Sequence (5'-3')	Size (bp)
TEM-1	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATCA	1080

DISCUSSION

Our work has recognized the high attendance of UPEC strains in the urine samples of Patients to hospital. One possible explanation for the high prevalence of UPEC strains in women is that they have comparatively wide and short urethra. Also, host factors such as changes in normal vaginal flora may put women at higher risk for UTIs. The effects of genetic factors including expression of Lewis blood group Le (a+b-) and Le (a-b-) and HLA-A3 should not be ignored. Our results are in agree with the results of Vollmerhausen *et al*. (2011) and Jadhav *et al*. (2011) (20,21). One possible explanation for the lower prevalence of UPEC strains in men of our study is that they have narrow and tall urethra. Also, all of them were circumcised. Higher incidence of UTIs in uncircumcised boys has been reported previously (22).

Another significant outcome of our enquiry relates to the distributions of antibiotic resistance pattern in UPEC strains. Totally, bacterial strains of our study had the lowest resistance against gentamicin (15.43%) and ciprofloxacin (27.51%), while resistance to amoxicillin (97.31%), cefalexin (80.53%), tetracycline (75.16%), co amoxiclav (69.12%) and cotrimoxazole (68.45%) were high. Of the studies that have been accompanied in this field (23,24,25), all have shown a high distribution of antibiotic resistance against beta lactams

Table 2: Antibiotic susceptibility patterns of Uropathogenic *Escherichia coli* isolated from the urine samples of army force

Antibiotic reaction	Resistant numberpercent		Semi-sensitive numberpercent		sensitive numberpercent	
Gentamicin (GM)	15.43	23	42.95	64	41.61	62
Ciprofloxacin (CP)	27.51	41	35.57	53	36.91	55
Cotrimoxazole (SXT)	68.45	102	23.48	35	8.06	12
Ceftriaxone (SRO)	28.18	42	50.33	75	21.48	32
Ceftizoxime (ZOX)	30.20	45	45.63	68	24.16	36
Cefalexin (CN)	80.53	120	11.40	17	8.06	12
Amoxicillin (AMX)	97.31	145	2.68	4	0	0
Co amoxiclav (AC)	69.12	103	29.53	44	1.34	2
Tetracycline (TE)	75.16	112	48/23	35	34/1	2

antibiotics. High efficiency of beta lactams antibiotics for treatment of the cases of UTIs due to UPEC strains has been reported previously (23,26,27). The results of our study showed that substantial numbers of isolates were resistant to more than one antibiotic agents. Similar investigations have been reported previously (23,25,26,27,2).

In recent years, due to vast usage of antibiotics around the world, many epidemics concerning infections produced by beta lactamase have been observed. ESBL is a specific kind of medicine resistance being reported in 1983 (28). ESBL is usually plasmid oriented, and because it can easily transfer to bacteria, the collection of resistive genes leads to multiple medicine resistance.

Totally, 149 (20.38%) *E. coli* positive samples were isolated among which 58 strains were ESBL positive. After implementing PCR test, 55.17% of isolates had TEM-1 gene. The incidence of antibiotic resistance of ESBL was 7.93% in our investigation. The incidence of ESBL in the studies which was conducted on Japan, USA, Korea, Taiwan, and Hong Kong were 0.1%, 3%, 4.8%, 8.5%, and 12%, respectively. The incidence of TEM-1 positive strains in the studies conducted on USA and Turkey were 4.7% and 25.7%, respectively (18,29).

One possible explanation for the high differences in the levels of antibiotic resistance in various investigation is due to the differences in the idea of doctors in antibiotic prescription. Also,the results of our study and other investigations showed that there is a great supervision over using antibiotics and spreading antibiotic resistance (7,30). In the other hand, various researches regarding the frequency of samples containing ESBL clarified that many differences exist between the results. This is because of varied geographical regions, the time of year the study implemented, and the amount of using antibiotics. It can be concluded that frequency of samples with ESBL is of significant difference.

With increase in resistance of bacteria, finding a treatment would become a harsh challenge, so, the possibility of injuriescan boost. This requires more attention of officials concerning using antibiotics throughout the country. It also needs periodical study of bacteria‘s level of resistance in various regions. It is recommended that to prevent failure, alongside routine experiments, diagnostic tests will also be implemented. Regarding the statistical society of this study, it should be mentioned that much attention to the health of staff working in valiasr hospital (Army hospital), and public places such as barracks, in the country is essential.

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