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ORIGINAL ARTICLE

Tracking CTX-M gene *in Escherichia coli* isolates from urinary tract infection in over fifty years women

Mohsen Zargar[1*], Ali Javadi[1], ZohrehsadatHosseini[1], TalayehSeyed Shakeri[1]
[1]Department of Microbiology, Qom branch, Islamic Azad University, Qom, Iran
Corresponding author's Email: zmohsen2002@gmail.com

ABSTRACT
Urinary Tract Infection (UTI) is among the most common type of infections in women. Studies have indicated that E. coli is the major cause for this type of infection. The reason for the large involvement of E. coli is the ability of the bacterium in the production of a broad range of beta-lactamases that are involved in the inactivation of the beta-lactam antibiotics; the antibiotics which are widely applied in the treatment of the UTIs.In order to identify the role of E. coli in UTIs in the present study we have focused on the CTX-M gene of the E. coli and the role it may play in conferring the bacterium with the extended spectrum beta-lactamases (ESBL) activity in over fifty years women with the UTI.300 women with over fifty years of age have participated in the present cross sectional study. E. coli was isolated from the urine culture, the identity of which was verified by applying biochemical analysis, and the presence of CTX-M gene was confirmed using PCR.E. coli was isolated from82 out of 300 urine samples' culture. 37.5% of the isolates were found to be cefotaxime resistant and 23.8% were resistant to ceftazidime. As well, 51.21% of the bacteria were shown to be ESBL phenotype and 85.7% of the E. coli isolates were CTX-M gene positive for their genotype.E. coli is the common cause of infection in women over fifty years with the urinary tract infection. As well the prevalent genotype in the E. coli derived urinary tract infection is CTX-M.
Key words: PCR, Urinary Tract Infection, *E.coli*, *CTX-M*, Antibiotic susceptibility.

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INTRODUCTION

Urinarytract infections (UTIs) are among the most common types of infections which were the subject of many investigations during the past twenty years. As well, UTIs and problems associated with this type of infection has made many clinical sectors to be engaged with the disease and created too much burden and cost to the health services. *E. coli*, and to a lesser extent other *Enterobacteriaceae* such as *Klebsiella* and *Proteus* were identified to be the cause of acquired UTI from the community without clinical symptoms, however, as far as studies have indicated *E. coli* plays the major role in UTIs [1].

In order to treat a specific pathogen, it is important that the pattern of the antibiotic resistance of the pathogens related to the disease to be well known, especially the common types of pathogens. *Enterobacteriaceae* family of bacteria has well shown to have a wide range of resistance against antibiotics either acquired through exposure to the antibiotics or innate. This family of bacteria is among the most important common type of pathogens which is resistant to the antibiotics [2].

In order to prevent infections and proper treatment, as a requirement, it is necessary that information regarding both the common infective bacteria and the pattern of their resistance to the antibiotics to be permanently made up to date [3]. *E. coli* is able to produce extended spectrum beta-lactamases (EBSLs), among which, the CTX group of the enzymes which confer the bacteria resistance against beta-lactam group of antibiotics. Since *E. coli* constitutes a large proportion of the both clinical and non-clinical infections, thus understanding the pattern of the resistance to the beta-lactam antibiotics or sensitivity of these bacteria toward beta-lactams would substantially help the successful treatment and control of this type of bacteria [4].

UTIs are common type of infection in the middle age population which necessitates long term care and associates with a high mortality rate. 40% of the hospitalized elder patients show UTI and the common cause was found to be *E. coli*[5]. Due to the importance of the sanitation in over 50 year women with UTI, in addition to the better understanding of the pattern of the resistance to beta-lactams, as well as the

presence of the CTX-M gene (the gene that codes for beta-lactamase) we carried out the present study. Our results indicate the important role of *E. coli* in over fifty years women's UTI and CTX-M gene involvement in the resistance against beta-lactam antibiotic.

MATERIALS AND METHODS

Sample preparation, cultivation, bacterial isolation, and identification

Urine samples were collected clean catch from 300 over fifty years of age women. Samples were cultivated in Eosin Methylene Blue as well as blood agar applying semi quantitative method and calibrated loop [6]. Bacterial identity was done using specific culture along with observation of the colonies morphology in addition to Gram stain and biochemical analysis. Applying available standard tables, the identity of the bacteria was confirmed at the level of genus and species.

Bacterial evaluation for ESBL production

In order to characterize bacterial potential for the production of the wide spectrum beta-lactamases (ESBL) in the isolated bacteria, Combined Disk method was used according to available standards for the production of ESBL. The method applies inhibitory effect of the clavulanic acid on beta-lactamases according to the Clinical and Laboratory Standards Institute (CLSI) standards [7]. Following to the preparation of the bacterial suspension with a turbidity of 0.5 McFarland they were spread on Mueller Hinton agar plates and then disks containing either cefotaxime or cefatoxime-clavulanic acid were applied onto agar plate at a distance of 20 mm apart from each other. Plates were incubated at 37 $^{\circ}$C for 24 h and the corona which indicates zone of inhibition of the bacterial growth around either types of the antibiotics disks were measured in mm. If zone of inhibition around the disk containing clavulanic acid was 1.5 times more than that of cefotaxime only disks it would indicate ESBL production by that bacterial isolate [8].

Polymerase Chain Reaction (PCR)

Using Gene Runner software the proper thermodynamic features including Tm, GC%, secondary structure formation, and the length of the amplified fragment by applying the following primers for CTX-M gene amplification were obtained. The sequence of the primers were as follow: the forward primer: 5' ATGTGCAGYACCAGTAARGT 3' and the reverse primer: 5'TGGGTRAARTARGTSACCAGA 3'.

DNA extraction was carried out using AccuPrep® Genomic DNA Extraction Kit (Bioneer) and amplification was carried out in 25 µl final volume. The PCR reaction mix was included of 1.5 µl of 50 mM $MgCl_2$, 2.5 µl of the 10X PCR reaction buffer, 1 µl of dNTP mix (10 mM), 1 µl of primers (forward and reverse primers, 50 Pmole) and 1.5 µl of the Taq DNA polymerase in addition to 2 µl of DNA and 14.5 µl of water. PCR was done according to the program shown in table 1. Electrophoresis of the resultant PCR product was run on 1% agarose gel along with molecular size marker as well as positive and negative controls for comparison.*E. coli* ATCC25922 was used as negative control and *Klebsiella pneumonia* ATCC700603 was used as positive control.The amplification was performed according to conditions which are shown in Table 1.

Table 1: PCR program for amplification of the600 bp long CTX-M gene.

PCR program: CTX-M Rep: 35	
Primary Denaturation	**94 ºC 5 min**
Denaturation	**94 ºC 45 S**
Annealing	**55 ºC 45 S**
Extention	**72 ºC 5 min**

RESULTS

Following to the biochemical analysis of the 300 urine samples obtained from over fifty year women, 82 samples (26.7%) were positive for *E. coli* infection. Results obtained from disk diffusion assay shows that 23.8% of the samples were resistant to ceftazidime and 37.5% to the cefatoxim (Figure 1). Applying combined disk assay has indicated that among the 82 *E. coli* resistant isolates 51.21% are ESBL positive and 48.79% were non-ESBL (Figure 2and Figure3).

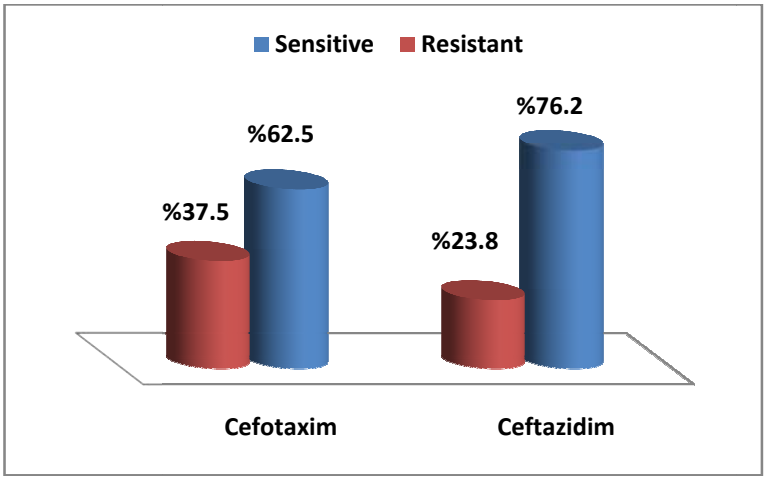

Figure 1. The anti-biogram pattern of the cefotaxime and ceftazidime.

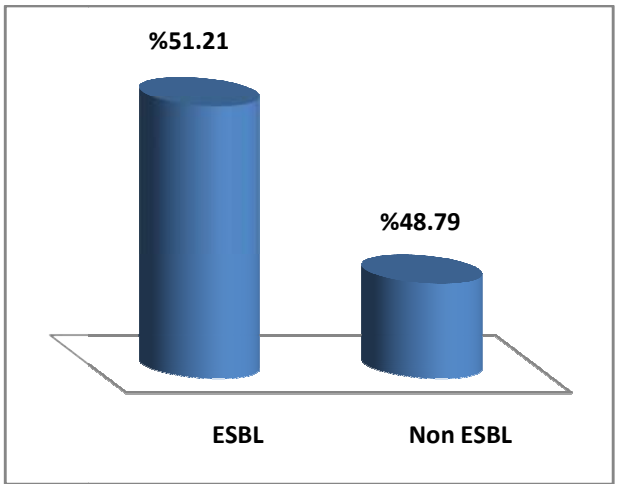

Figure 2. The phenotypic abundance of the broad spectrum beta-lactamases.

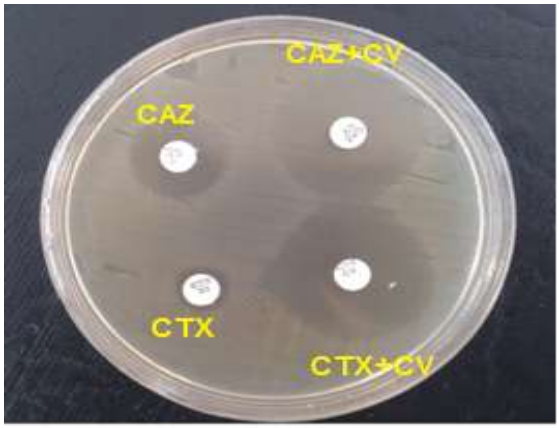

Figure 3. Combination test in order to determine the ESBL phenotype.

Further experiment was carried out by using PCR on CTX-M gene, the product of which is a 600 bp amplification product. We found that 85.71% of the *E. coli* isolates are positive for CTX-M and 14.29% are negative for this gene (Figure 4).

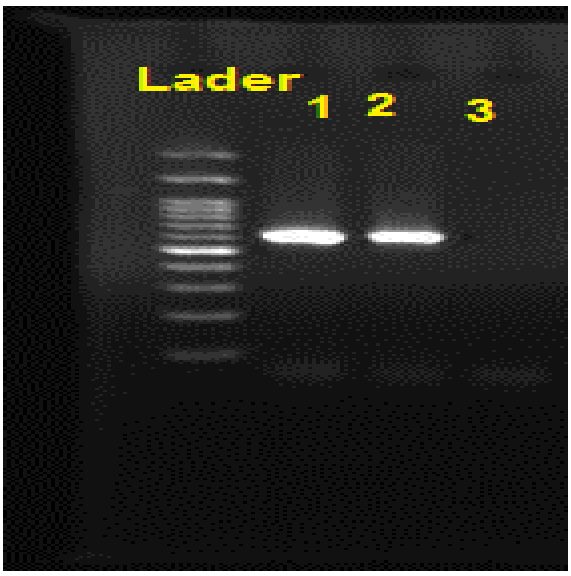

Figure 4. Agarose gel electrophoresis of the amplified CTX-M gene. The presence of the 600 bp amplification product in the positive control and the sample (lanes 1 and 2) is an indication of the presence of the CTX-M. Lane 3 indicates the negative control.

DISCUSSION

UTIs are among the most prevalent infections which were the subject of intensive investigations during the past twenty years. As a clinical issue, UTI has engaged a large portion of the facilities to be allocated forthe disease management and has made the involvement of a wide spectrum of the clinical facilities, sectors, and departments inevitable. Either acquired from the community or not, in most instances the source of UTIs was found to be associated with *E. coli*. However in the other cases the origin of infection could be other species such as *Enterobacteriaceae* for example *Klebsiella* and *Proteus*. In the hospitalized patients with UTI infection, or in the individuals that have had antibiotic treatment, as well as in patients with urinary catheter, *E. coli* was found to the uppermost and the most common cause of infection (40% of cases) [9,10]. *E. coli* is able to produce ESBL beta lactamases such as CTX and PER enzymes which confer the bacterium a strong resistance against beta-lactam antibiotics.

Since *E. coli* plays a major role in the infections; both in the hospitalized and non-hospitalized patients, a better understanding of the mechanisms involved in the resistance against beta-lactam antibiotics in this bacterium or sensitivity toward antibiotics has attracted much of interests and research toward identification of the factors involved in the process of resistance [11]. Following to the identification of beta-lactams by Abraham and Chain in 1940, there was a huge research activity in order to identify and classify of the beta-lactamases. Recent studies have well documented a fast and increased resistance of the bacteria against beta-lactam antibiotics [12]. In the present study, through working on urine samples of the 300 middle-aged women with clinical signs of infection, we found that 82 samples were infected with the *E. coli*. Among these infected samples 51.21% were ESBL positive while the rest (48.71%) were negative for ESBL. Our further analysis through application of the PCR has revealed that CTX-M gene could be a candidate in the process of beta-lactam resistance in the *E. coli*. A high proportion of ESBL isolate were CTX-M positive as well, which indicates, relatively high prevalence of this gene in middle-aged women.

There are many studies regarding induction of the CTX-M gene in UTIs. As a result sufficient information is now availbale supporting this notion and have proven the involvement of this gene in the middle-aged women resistance against antibiotic due to *E. coli* infection. In 2007, Hohas studied the CTX-M gene in *E. coli* isolates from UTIs of women with different age range. And found that among the 63 *E. coli* isolate from women with age range 41 – 64 years only 4 isolates had CTX-M gene activated [13]. Hesieh (2010) hasfound that among 321 over 50 years of age patients with UTI 18 *E. coli* isolates were ESBL positive [14]. The lower ESBL found in Hesieh's study compared to what we have obtained (higher percentage of ESBL) could be expressed in part to be due to the higher sanitation both in the personal life style and in the population on which Hesiehhas carried out study. Mendonca and co-workers (Portugal, 2007) have isolated 181 *E. coli* from patients referred to the hospitals among which 119 (65%) were ESBL phenotype, a percentage more than what we have obtained in our study. Furthermore they found that 76% of the patients with over 60 years of age were CTX-M [15], a percentage that shows conformity with our results. It should be mentioned that other genes rather than CTX-M are also involved in the expression of ESBL

family of enzymes as well. Considering these studies and what was obtained in our investigation it could be proposed that the prevalence of CTX-M gene in the over 50 years women from different ethnicities and societies depends on and health care and sanitary status of that the societies added to geographical and environmental factors.

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REFERENCES

1. Zhanel, G.G., Laing, N.M., Nichol, K.A., Palatnick, L.P., Noreddin, A., Hisanaga, T., Johnson, J.L., the NAVRESS Group & Daryl J. Hoban, D.J., (2003) Antibiotic activity against urinary tract infection (UTI) isolates of vancomycin-resistant enterococci (VRE): results from the 2002 North American Vancomycin Resistant *Enterococci* Susceptibility Study (NAVRESS). J AntimicrobChemother, 52(3):382-8.
2. Paterson, D.L., (2006) Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. MED., 119(6):S20-S28.
3. Bang, C.s.,Kinnunen, A.,Karlsson, M.,Önnberg, A., Söderquist, B. &Persson, K. (2014). The antibacterial effect of nitric oxide against ESBL-producing uropathogenic*E. coli* is improved by combination with miconazole and polymyxin B nonapeptide. BMC microbial., 14(1): 65.
4. Rodriguez-Bano, J., Alcalá, J.C., Cisneros, J.M., Grill, F., Oliver, A., Horcajada, J.P., Tórtola, T., Mirelis, B., Navarro, G., Cuenca, M., Esteve, M., Peña, C., Llanos, A.C., Cantón, R.&Pascual, A. (2008). Community infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. Arch. Intern. Med., 168(17):1897-902.
5. Beveridge, L.A., Davey. P.G., Philips & G. &McMurdo, M.E. (2011). Optimal management of urinary tract infections in older people. Clin. Interv. Aging.,6:173-180.
6. Pallett, A. & Hand, K. (2010) Complicated urinary tract infections: practical solutions for the treatment of multi resistant Gram-negative bacteria. J. antimicrob. Chemother. 65(3):25-33.
7. Cockerill, F. (2011), Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement. Clinical and Laboratory Standards Institute. 31(1):42-46.
8. Naiemi, N., Duim, B. & Bart, A. (2006). A CTX-M extended-spectrum {beta}-lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonasmaltophilia*. J. Med. Microbiol. 55:1607-1618.
9. Al-Badr, A. &Ghadeer Al-Shaikh, G. (2013). Recurrent Urinary Tract Infections Management in Women. Sultan QaboosUniv Med J. 13(3):359-367.
10. Mody, L. &Juthani-Mehta, M. (2014). Uninary tract infections in older women. JAMA., 311(8):874
11. Hooton, T.M., Bradley, S.F.,Cardenas, D.D., Colgan, R., Geerlings, S.E.,Rice, J.C., Saint, S., Schaeffer, A.J., Tambayh, P.A., Tenke, P. & Nicolle, L.E.Infectious Diseases Society of America.(2010), Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin. Infect. Dis., 50(5):625-663.
12. Lakshmi, R., Nusrin, K.S., Sharon Ann, Georgy, &Sreelakshmi, K.S. (2014), Role of beta lactamase in antibiotic resistnce: A review. Int. Res. J. Pharm. 5(2):37-40.
13. Ho, P.L., Poon, W.W.N., Loke, S.L., Leung, M.S.T., Chow, K. H., Wong, R.C.W., Yip, K.S., Lai E.L. & Kenneth W.T. (2007), Community emergence of CTX-M type extended-spectrum β-lactamases among urinary *Escherichia coli* from women. J. Antimicrob. Chemother., 60(1):140-144.
14. Hsieh, C.J., Shen, Y.H. & Hwang, K.P. (2010) Clinical implications, risk factors and mortality following community-onset bacteremia caused by extended-spectrum β-lactamase (ESBL) and non-ESBL producing *Escherichia coli.* J.Microbiol.Immunol.Infect.,43(3):240-248.
15. Mendonça, N.,Leitao, J., Manageiro, V., Ferreira, E. &Canica, M. (2007) Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob. Agents. Chemother., 51(6):1946-1955.

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