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Prevalence and antibiotic susceptibility pattern of extended spectrum beta lactamases producing *Escherichia coli* isolated from outpatients with Urinary Tract Infections in Babol, Northern of Iran

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ABSTRACT

The incidence of community-acquired UTIs due to extended-spectrum beta-lactamase (ESBL) producing *E. coli* isolates, has increased worldwide and is considered a great problem in the treatment of infections. The aim of this study was to determine the prevalence of ESBL producing *E. coli* isolates in urine samples of outpatients in Babol, North of Iran. A total of 3699 urine samples from outpatients referred to Yahyanejad Hospital, Babol, Iran, were collected during 15 months, from March 2016 to June 2017. The samples were processed for bacterial culture using conventional methods, while antimicrobial susceptibility testing for *E. coli* isolates was performed by the disc diffusion method. The prevalence of ESBL producing *E. coli* isolates were assessed by Double-Disc tests. Of 3699 tested sample, 201 samples showed the growth of pathogens and among them, 106 isolates (52/7%) were *E. coli*. The rate of ESBL producing *E. coli* isolates was 25/4%. The majority (81/5%) of the isolates were from females. High percent resistance was found against ceftazidime, cefotaxime, ceftriaxone, cefixime and ciprofloxacin, ranging from 61-100%, while the highest percent susceptibility was seen to meropenem, piperacillin-tazobactam (100%), followed by nitrofurantoin and amikacin (91%). The high prevalence of ESBL producing *E. coli* isolates from outpatients calls for the need to aware of this increasing resistance between uropathogens and update bacterial susceptibility data and to set up our empirical therapy accordingly.

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections that are caused by gram negative bacteria. These can be Community-acquired or nosocomial [1,2]. More than 25% of all women experience some types of UTI at least once during their lifetime [3]. UTI is an absolute risk factor for bladder cancer and renal cell carcinoma. Indeed, clinicians often start empirical antimicrobial therapy before the culture results are available [4].

Escherichia coli (*E. coli*) is the most common cause of UTI, and nowadays we notice an increasing antibiotic resistance between *E. coli* associated infections worldwide. The well-spring of creating this resistance is the overuse of

antibiotics. It is reported that urinary Tract isolates of *E. coli* from outpatient clinics have shown an increased resistance to certain antibiotics, including ampicillin and ciprofloxacin, rendering them near useless [5].

Beta lactams are the most frequent antibiotics that are prescribed by physicians. The main mechanism of resistance to these antibiotics, especially within gram negative bacteria, is the production of extended-spectrum beta-lactamases (ESBLs) [6,7]. These enzymes can completely hydrolyze oximino-beta lactams such as the third generation cephalosporins. The majority of produced ESBL enzymes have been reported in *E. coli* and *klebsiella pneumoniae*, but also are found in other enterobacteriaceae [8,9]. ESBLs have a wide effect on penicillins and cephalosporins and aztreonam, but they do not effect carbapenems. ESBLs are prevented by

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beta-Lactamase inhibitors like clavulanic acid, tazobactam and sulbactam [9]. Treatment of infections caused by ESBL-producing bacteria has become a world problem and has led to an increase in the death of patients, as well as a greater financial burden for treatment [10]. Because of rising antibiotic resistance between organisms and the high prevalence of ESBL producing *E. coli* in the community and its importance in managing UTIs, local hospital-based knowledge of the uropathogens and their antibiotic susceptibility pattern is necessary and helpful for physicians to choose appropriate empirical treatment [11,12]. Therefore, the goal of this study was to evaluate the prevalence of ESBL enzymes in *E. coli* isolates in outpatients with UTI.

MATERIALS AND METHODS

Sample Collection and Bacteriology

The present study is a cross-sectional study and was performed in shahid Yahyanejad Hospital, Babol, Iran from March 2016 to June 2017. Midstream specimens of urine from outpatients were obtained for semi-quantitative urine culture on blood agar and MacConkey agar by way of the standard loop method. After 24 hours incubation at 37°C, colonies were counted. Samples with colonies of more than 10⁵ are considered as significant bacteriuria. *E. coli* isolates were identified with Gram-stains and biochemical tests containing oxidase, indole, methyl red, simmons citrate, lysine decarboxide, urease and glucose fermenting in TSI (Triple Sugar Iron) Agar medium and as described in standard bacteriological methods [13].

Antibiotic susceptibility test

Antibiotic sensitivity pattern to Clinical Laboratory Standard Institute (CLSI) guidelines was determined by the disc diffusion method on Muller-Hinton agar [14]. *E. coli* isolates were tested against common antibiotics used in the hospital: Amikacin (AMK 30 µgr), nitrofurantoin (NIT 30 µgr), ciprofloxacin (CIP 5 µgr), cefotaxime (CTX 30 µgr), ceftazidime (CAZ 30 µgr) meropenem (MEM 10 µgr), ceftriaxone (CRO 30 µgr), piperacillin-tazobactam (PTZ 100 µgr), ampicillin-sulbactam (SAM 20 µgr). All antimicrobial discs were obtained from MAST chemical co, England.

E. coli ATCC 25922 was used for quality control as CLSI recommendation.

Detection of ESBLs production

The ESBL-producing was determined by application of the Double-Disc (combined-disc) method. The bacterial suspension was prepared with 0/5 Mcfarland dilution and was cultured on Muller-Hinton agar plates. Then, four discs containing ceftazidime (30 µgr) and cefotaxime (30 µgr) alone and in combination with 10 µgr clavulanic acid (CAZ:30 µgr/CA10 µgr) and CTX:30 µgr/CA10 µgr were placed on the agar surface. After incubation at 35±2°C for 24 h, ESBL production was evaluated. A ≥ 5 mm increase in the diameter of inhibition zone produced by one combination disc versus its zone when tested alone, was considered as indicative of ESBL production. *Klebsiella* ATCC 700603 was used for quality control as recommended by CLSI.

Data analysis

All data were analyzed using SPSS software, version 16. The findings were presented as descriptive statistics regarding relative frequency.

RESULTS

During 15 months, a total of 3699 urine samples were obtained. Herein, 201 samples showed growth of pathogens; among them, *E. coli* was the most prevalent isolate (52/7%). The majority (81/5%) of the isolates were from females, while the remaining were from males (18/5%). Figure 1 shows the age distribution of the patients with ESBL producing *E. coli*. The mean age of patients was 46/6 years with a range of 5-81 years. More cases were recorded among the 40-60 year-olds (40/74%).

ESBL positivity was detected in 27 of 106 (25/4%) *E. coli* isolates. Figure 2 shows the susceptibility percentages of ESBL producing *E. coli* isolates to ten selected Antibiotics. Meropenem and piperacillin-tazobactam indicated highest percent susceptibility (100%) to *E. coli* isolates, followed by amikacin (91%), nitrofurantoin (91%) and ampicillin-sulbactam (75%). The rates of resistance in ESBL producing *E. coli* isolates are shown in figure 3. The maximum resistance was seen against ceftazidime (100%), cefotaxime (89%), ceftriaxone (78%), cefixime (67%) and ciprofloxacin (61%). ESBL producing *E. coli* isolates were divided into 6 groups according to their resistance patterns (Table 1).

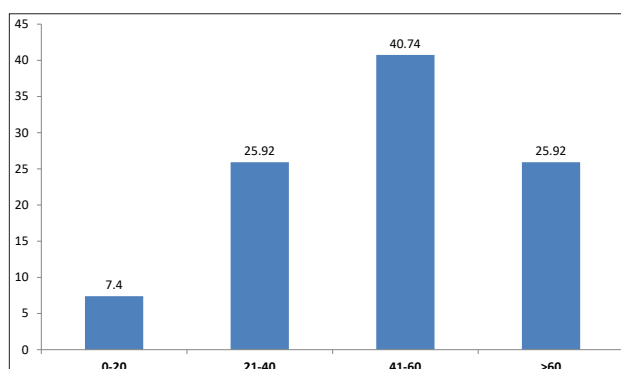


Figure 1. Age distribution of the patients with ESBL producing *E. coli* isolates

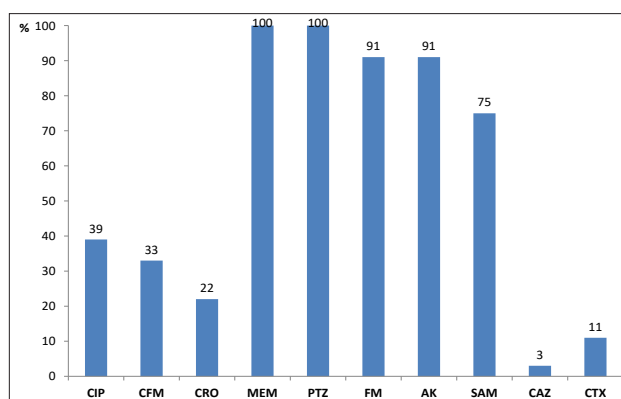
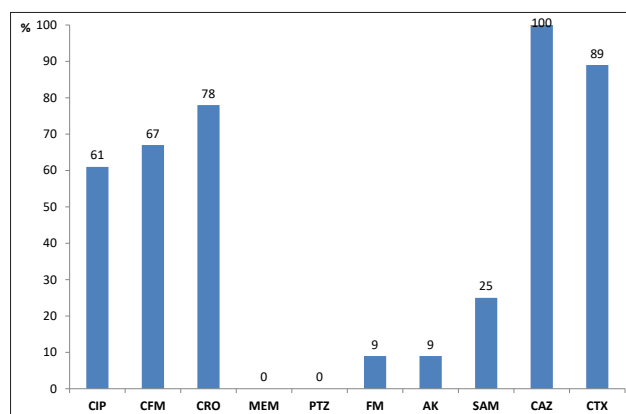


Figure 2. Percentages of antimicrobial susceptibility of ESBL producing *E. coli* isolates

Cyprofloracin (CIP), Cefixime (CFM), Ceftriaxon (CRO), Meropenem (MEM), Piperacillin-tazobactam (PTZ), Nitrofurantoin (FM), Amikacin (AK), Ampicillin-sulbactam (SAM), Ceftazidime (CAZ), Cefotaxime (CTX)



Cyprofloxacin (CIP), Cefixime (CFM), Ceftriaxon (CRO), Meropenem (MEM), Piperacillin-tazobactam (PTZ), Nitrofurantoin (FM), Amikacin (AK), Ampicillin-sulbactam (SAM), Ceftazidime (CAZ), Cefotaxime (CTX)

Figure 3. Antibiotic resistance pattern in ESBL producing *E. coli* isolates

Table 1. Resistance pattern of ESBL producing *E. coli* isolates

Pattern	Resistance Pattern	Percentage and number of isolates
Pattern 1	Resistant to one drug	18/5 (5)
Pattern 2	Resistant to two drug	29/6 (8)
Pattern 3	Resistant to three drug	22/2 (6)
Pattern 4	Resistant to four drug	22/2 (6)
Pattern 5	Resistant to five drug	3/7 (1)
Pattern 6	Sensitive to all	3/7 (1)



Figure 4. Plate showing ESBL production by Double-Disc method

DISCUSSION

UTIs caused by antibiotic-resistant bacteria are a major problem due to limited choices of treatment. Today, ESBL-producing *E. coli* isolates are the most common pathogens in community-acquired UTIs [15,16]. According to the WHO reports, Iran is one of the countries with the highest antibiotic consumption in the world, but in the global statistics, there is no report of antibiotic use in Iran [17]. In the current study, we evaluated the prevalence of ESBL producing *E. coli* isolates and their antibiotic susceptibility pattern in patients with community acquired UTI.

According to a report by Shakya *et al.*, in Nepal, of 2014, similar to our study, the majority of ESBL producing *E. coli* isolates was from females (81/8%), as was an Indian study in 2006 (66/7%) and Paris in 2015 (81/4%). Hence, women

have a higher prevalence of UTI than men, mainly due to anatomic factors [18-21].

In a large number of studies from different parts of Iran, the prevalence of ESBL positivity UTIs were similar to our study (25/4%), including that from Isfahan in 2012, Kermanshah in 2011, children in North of Iran in 2014, Iran as a whole in 2010 [10, 20,22,23] and also from some countries. In the last, the results were consistent to our study [24-26]. The rate of ESBL producing *E. coli* isolates in Nepal, Paris, Karachi, central India and Shiraz were 91/7%, 69/4%, 54%, 41/6% and 69/2%, respectively, which are high as compared to our results. In contrast, according to a Brazilian report of 2013, the figure was 7/1% which is lower than the results of our study [18,21,27-30]. Contrary to our study, in Isfahan in 2012, *E. coli* isolates showed lower resistance against ceftazidime (32%), cefotaxime (38%) and ciprofloxacin (33/4%), while in Shiraz, resistant to ciprofloxacin and ceftazidim was 38/5% and 63/5%, respectively [20,29]. In Nepal in 2014, Turkey in 2016 and Korea in 2017, resistant to ciprofloxacin, cefotaxime and ceftazidim were also reported lower than our study [18,25,33]. However, ciprofloxacin resistance in Turkish isolates in 2010, in Paris, Saudi Arabia and Jaipur were reported as 84%, 80%, 88/9% and 74/75% respectively, which are high as compared to our study [16,21,24,26].

In a large number of studies, consistent with the results of recent study, high percent susceptibility was found to carbapenems, aminoglycosides and nitrofurantoin [18,20,22,25,29,33] and also in agreement to our results, in Isfahan, Nepal and Korea sensitivity to meropenem was reported 100% [18,20,33]. In Jaipur and Korea, susceptibility to piperacillin-tazobactam was high and similar to our study [26,33]. In India, isolates in 2006 showed highest resistance against nitrofurantoin (80%) [19]. This effect is probability due to widely prescriptions of nitrofurantoin by physicians in the treatment of community-acquired UTI in this region.

Compared to advanced countries, in practice, the use of antibiotic guidelines is not common in Iran and, in fact, there are no local guidelines. The most inconsistent pattern of application has been seen in the prescription of ciprofloxacin and ceftazidim and cefotaxime [17,31]. High percent resistance exists against these antibiotics, probability due to excessive use in recent years. In some studies, ciprofloxacin is the most prescribed antibiotic for treatment of UTI in Iran [17,32]. Thus, knowledge of the local susceptibility pattern of the more frequent UTI pathogens is necessary for choosing appropriate empirical therapy [15], and the results of our study can help us to choose more appropriate antibiotics in UTI treatment.

CONCLUSION

The results of our study showed a high prevalence of ESBL-producing *E. coli* isolates in outpatients. Because of too low resistance to nitrofurantoin, this antibiotic be prescribed in uncomplicated UTIs, while among the parenteral drugs, carbapenems, aminoglycosides and piperacillin-tazobactam can be the choice for complicated UTIs.

In the end, it seems it is necessary to detect and prevent the emergence of resistance and update bacterial susceptibility data for setting up our empirical therapy regime accordingly.

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CONFLICT OF INTEREST:

There is no conflict of interest.

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