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Prevalence of Extended-Spectrum- β -Lactamase-producing *Escherichia coli* isolates among uropathogens in a Pediatrics Hospital

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ABSTRACT

Among drug resistance, resistance to β -lactam - antimicrobial drugs is a major concern for the treatment of microbial infections. In recent decades, has been observed a tremendous increase in prevalence of high-level resistance to β -lactam antibiotics in members of Enterobacteriaceae family owing to extended-spectrum β -lactamase (ESBL) enzymes. So, this study decided to characterize the prevalence rate of ESBL- producing *E.coli* isolates in different wards of Pediatrics Ghods Hospital, Qazvin, Iran. Of 380 *E.coli* isolates were achieved from urine clinical samples between March to October 2015 of different wards of Pediatrics Ghods Hospital, Qazvin, the identification process was accomplished using biochemical and microbiological tests. Susceptibility of isolates to 9 different antibiotic disks was characterized by agar disk diffusion method and ESBL- producing *E.coli* isolates were identified and the results were analyzed by spss software. In this study the highest resistance rate in ESBLs producing *E.coli* isolates was to ceftazidime and cefixime (100%). The most effective antibiotic with a sensitivity of about 97% for ESBLs producing *E.coli* isolates was amikacin. Among these 380 isolates 325 from females and 55 of males were isolated. The total numbers of ESBLs producing *E.coli* isolates were 102 cases (28.4%). Of these ESBLs producing *E.coli* isolates 16 cases were belong to men and the remaining were belong to the females. Based on results of this study and other studies from Iran, the prevalence of infection with ESBLs producing Enterobacteriaceae is increasing widely in over the our country and is one of emerging problem in pediatrics population and they act as reservoirs and transmission to community and hospital environment, so the need to improve microbiological diagnostic facilities and antibiotic resistance surveillance in resource-poor settings; to be able to effectively revise antibiotic regimens and avoid emergence of resistance.

Key words: ESBL, Pediatrics, *E.coli*

INTRODUCTION

as *Escherichia coli* are widely used as an expression host for production of recombinant proteins as well as these organisms are the most important opportunistic human pathogens [1, 2]. Different infectious diseases caused by these organisms are; urinary tract infections, pneumonia, septicemia and abdominal infections, they are related with high morbidity and mortality owing to the postponement in proper treatment. The most prevalent species in the Enterobacteriaceae which have been found in clinical samples including *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* Spp [3]. Among drug resistance, resistance to β -lactam - antimicrobial drugs is a major concern for treatment of microbial infections [4]. In recent decades, has been observed a tremendous increase in the

prevalence of high-level resistance to β -lactam antibiotics in members of *Enterobacteriaceae* as same as the other important healthcare-associated pathogen "*Acinetobacter baumannii*" that can cause of life threatening infections [5-15]. ESBLs are bacterial enzymes that cause resistance to broad-spectrum antibiotics [16]. These enzymes hydrolysis and inactivate beta-lactam antibiotics before reaching to the Penicillin binding proteins (PBP) in cytoplasmic membrane [17]. Encoding these enzymes occurs by plasmids or chromosomes and are related with mobile genetic elements such as integrons and transposons and can be carrying genes which encode resistance to other antibiotics classes such as sulfonamides, aminoglycosides, trimethoprim/ sulfamethoxazole, and quinolones [18]. It is estimated that ESBLs around 50% affect non-hospitalized patients [19]. The main concern is associated coresistances to other classes of antibiotics which aid the dissemination of multi-resistant isolates [20]. Timely identification of ESBL producing bacteria is becoming increasingly important from aspect of suitable treatment and effective infection control in hospitals. Patients with infections caused by ESBL producers have delay in start of proper treatment compared with patients with non-ESBL infections [21]. It is be noted that delay in treatment will lead to undesirable results and increased mortality [22]. CTX-M β -lactamases producing *Enterobacteriaceae*, that are generally found in outpatients and UTIs, are naturally as well as resistant to several antibiotics such as quinolones, aminoglycosides and sulfonamides including ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole, respectively [23]. Various bacteria are ESBLs producing, but in meantime *E.coli* and *Klebsiella* have more contribution [17]. Urinary tract infections caused by ESBLs producing *E.coli* are increasing around the world and reducing the drug choice to a restricted number of antibiotics in treatment [24]. The second cause of community-acquired infection and nosocomial infection are belong to the Urinary tract infections (UTI) and most prevalent gram negative bacterium with related to this infection is *E. coli* with prevalence rate 90% [25]. The most common ESBLs from Western and Asian countries are Extended- Spectrum β -Lactamases which derived from SHV, TEM and CTX which are located on the large plasmids and create strains that are resistant to treatment [16, 26]. The most important risk factors for susceptibility to infection with ESBL- producing bacteria are; gastrointestinal tract colonization, continued length of stay in ICU, arterial and venous catheterization, infants with low birth weight, prior antibiotic use, and mechanical ventilation [27]. Pediatrics wards and hospitals are good settings for the transmission of infection and young children are susceptible to many infections because of insufficient immunity [28]. The neonates at highest risk for colonization and infection with ESBL- producing organisms [29]. Among these factors, several studies have revealed that renal abnormalities, septicemia, systemic disease, hospitalization within the previous 3 months predisposing to the UTI [30]. So, this study decided to characterize the prevalence rate of ESBL- producing *E.coli* isolates of urine samples in different wards of *Pediatrics Ghods Hospital, Qazvin*.

MATERIALS AND METHODS

Urine Samples Collection / Isolation of Bacteria and Antimicrobial Susceptibility Testing

551 urine samples were collected from pediatrics patients during a 6-month period (between March to August, 2015) at *Pediatrics Ghods Hospital, Qazvin* in the Center of Iran. Suprapubic bladder aspiration, midstream clean catches and transurethral bladder catheterization was used. The samples were cultured and inoculated aerobically at 37°C for 24 hours on appropriate media, the identification process of acquired isolates was accomplished using biochemical and microbiological tests, finally lactose fermenting colonies were recognized as *E.coli* [31]. Antimicrobial susceptibility testing was done using standard Kirby-Bauer disk diffusion test on Mueller Hinton agar plate regarding to the Clinical and Laboratory Standards Institute (CLSI) strategies, briefly, by use of an aseptic technique, placed a sterile swab into the broth culture *E.coli* isolates and then softly with streak culturing on Mueller-Hinton agar plate a bacterial lawn was formed, then the plate dried for approximately 5-10 minutes, the antibiotics placed onto the plate. Plates incubated overnight at temperature of 37 °C [32]. The Antibiotics included in present study were: amikacin (30 μ g), ampicillin (10 μ g), trimethoprim-sulfamethoxazole (1.25 μ g), gentamicin (10 μ g), ceftriaxone (30 μ g), cefixime (30 μ g), ciprofloxacin (5 μ g), ceftazidime (30 μ g), imipenem (10 μ g). Cephalosporins-resistant isolates for studding of ESBLs using combined disks with cefotaxime, *ceftazidime*, *ceftazidime*(30 μ g) + *clavulanic acid* (10-30 μ g) were applied, ESBL production was determined by increasing the inhibition zone diameter by 5 mm or more around the combination disk (*ceftazidime*+ *clavulanic acid* disk) in compared *ceftazidime* alone [33]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls respectively for quality control in production of ESBL, and afterwards the results were analyzed by spss software (ver. 19.0.0; SPSS Inc., Chicago, IL) where significant differences in variables were analyzed by χ^2 test. P value < .05 was considered indicative of a statistically significant.

RESULTS

In this study were identified 380 isolates of *E.coli* in cultures from children with ages 0–17 years old. Among these 380 isolates 325 from female and 55 of males were isolated. The total numbers of ESBLs producing *E.coli* isolates were 108 cases (28.4%), (figure 1). Of these ESBLs producing *E.coli* isolates 16 cases were belong to males and the remaining were belong to the females. The highest resistance rate in ESBLs producing *E.coli* isolates was to

ceftazidime and cefixime (100%). The resistance rate for ampicillin and ceftriaxone were 99% and 98% respectively. The resistance rate less than 50% was observed to imipenem, gentamicin and amikacin. The most effective antibiotic with a sensitivity of about 97% for ESBLs producing *E.coli* isolates was amikacin (Table 1).



Figure1 The picture of a ESBL producer *E.coli* isolate

Table 1 The pattern of antibiotics resistance in ESBL producing *E.coli* isolates

CP		AN		IPM		GM		CRO		CFM		CAZ		AM		SXT	
S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
24%	76%	97%	3%	76%	24%	79%	21%	2%	98%	0	100%	0	100%	1%	99%	10%	90%

AN: amikacin, AM: ampicillin, SXT: trimethoprim-sulfamethoxazole, GN: gentamicin, CRO:ceftriaxone, CFM: cefixime, CAZ: ceftazidime, IMP: imipenem

DISCUSSION

As shown in Table 1. The high resistance was seen to cephalosporin antibiotics, of note, this is possibly result from extremely use of antibiotics in our study population, as well as, since the cephalosporins are favorite antibiotics for empirical treatment of urinary tract infections, increased resistance lead to disturbance in process of empirical treatment, but evidences show a relationship between habits of prescribing and antibiotic resistance [34]. Infection with ESBLs producing *Enterobacteriaceae* is increasing widely in over the world and is one of emerging problem that offers an interesting new approach to control infectious agents [35]. Risk factors associated with infection by ESBLs producing *Enterobacteriaceae* in children include prior immunosuppressive therapy, prolonged hospital stays, prior antibiotic use and chronic medical conditions, gastrointestinal comorbidity [46]. ESBL infections also causing negative impact on patients such as increasing hospital costs, length of stay, and mortality rates [47]. The prevalence rate of ESBLs producing *E.coli* isolates in this study was 28.4% which was consistent with other studies conducted in different parts of our country [48-51]. But in some Asia countries such as Lebanon, Korea and turkey the frequency of ESBLs producing *E.coli* isolates has been reported 13.3%, 9.2%, and 17%, respectively [52, 53]. in this study the resistance rate to amikacin was too low (99%), like our results in study carried out by Pourakbari and et al in Tehran, the resistance frequency to mentioned antibiotic was too low, Therefore, this antibiotic can be an effective drug choice for UTI caused by ESBLs producing *E.coli* isolates [54]. Among ESBLs producing *E.coli* isolates from studied children, the resistance to gentamicin was relatively low; the reason for this might be owing to

the less use of this antibiotic in our study population [54]. Fortunately, resistance to imipenem was low too. This finding was in line with other studies conducted in India, Malaysia, Saudi Arabia and Iran [48, 55, 56]. In this study, resistance to trimethoprim-sulfamethoxazole was high and indicates that this drug is not more effective in treatment of UTI and also in consistent to our study one study in America showed that owing to high resistance makes this drug less useful as empiric treatment of UTI in many parts of the world [57]. Pour Akbari and Mansouri and their colleagues also have stated such a high resistance to this antibiotic [58]. But other studies were reported resistance frequency about 50% and 62% to mentioned antibiotic [59, 60]. In total, our study have some limitation, first, we did not perform the MIC test and secondly this results only based phenotypic tests and for further confirmation the molecular tests should be done and for better studying UTI, risk factors associated to its can be reported. So, with regarding the above results, the status of antibiotic resistance in the mentioned study was high and infection control measures should be taken to prevent spreading of more resistance to antibiotics.

CONCLUSION

Based on results of this study and other studies from Iran, the prevalence of infection with ESBLs producing *Enterobacteriaceae* is increasing widely in over the our country and is one of emerging problem in pediatrics population and they act as reservoirs and transmission to community and hospital environment, so the need to develop microbiological diagnostic tests and antibiotic resistance surveillance in hospitals; to be able to avoid coming out of antibiotic resistance.

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