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Molecular characteristics and antibiotic resistance profiles of *Escherichia coli* strains isolated from urinary tract infections in children admitted to children's referral hospital of Qom, Iran

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Parole chiave: Escherichia coli, Infezioni del tratto urinario, pediatria, β*-lattamasi a spettro esteso, ERIC-PCR*

Abstract

Background. *Urinary tract infections (UTIs) are a highly prevalent infection among children and Escherichia coli is one of the most important pathogens causing pediatric UTIs. Production of extended spectrum* β*-lactamase (ESBL) enzymes is an important factor in the emergence of antibiotic resistance among these bacteria. This study aimed to determine the resistance patterns, the frequency of ESBL-encoding genes and the genetic diversity of E. coli strains isolated from children with UTIs who were admitted to children's referral hospital of Hazrat Masoumeh, Qom, Iran.*

Methods. *A total of 102 consecutive non-duplicative strains of E.coli that were isolated from children with UTIs were included into the study. Antibiotic susceptibility of the isolates was determined by disk diffusion method according to the CLSI guidelines. The ability of the isolates to produce ESBLs was phenotypically determined by both combined disk test and double disk synergy test. The ESBL encoding genes (bla $_{CTX-M}$, bla $_{SHV}$, and bla $_{TEM}$) in phenotypically confirmed ESBL-positive isolates was detected by PCR method. The genetic relatedness of the isolates was designated by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR).*

Results. *Eighty-three percent (n=85) of the children were female. Most of the infected boys (88%, n=15) were less than 1 year of age and most of the infected girls (48%, n=41) aged 1 to 6 years old. The highest sensitivity was observed to nitrofurantoin (8%, n=8), followed by amikacin (12%, n=12) and piperacillin-tazobactam (17%, n=17). In contrast, the highest resistance rate was seen to ampicillin (94%, n=96) and cefazolin (93%, n=95). Eighty-eight percent (90 out of 102) of the strains were multidrug-resistant (MDR). Fifty-eight percent (n=59) of the strains were ESBL-positive and results of the combined disk test was in concordance with PCR. The bla$_{CTX-M}$ was the most frequent ESBL encoding gene (88%, n=52), followed by bla$_{TEM}$ (54%, n=32), and bla$_{SHV}$ (15%, n=9). Based on the ERIC-PCR technique, isolates were clustered in*

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13 different types. There was no relationship between different ERIC types and origin of the isolates (i.e. hospitalized or outpatients), ESBL-producing ability, and antibiotic resistance patterns.

Conclusion. *High prevalence of ESBL-positive isolates of E. coli (58%) was found in our study and all of them were MDR. In addition, there were statistically significant differences in the resistance rates of ESBL-producers, and non-producers to some antibiotics, which result in limiting their therapeutic options. Continuous surveillance of the emergence of ESBL-producing isolates and their antibiotic resistance profiles as well as using appropriate typing methods is needed for reducing their spread, selecting appropriate treatment regimens and finding hospital outbreaks.*

Introduction

Urinary tract infections (UTIs) are the most frequent bacterial infections in children (1, 2). Without early diagnosis and adequate treatment of UTI, it may cause some sequelae such as progressive destruction of renal structure, renal scarring, chronic renal failure, urinary stones, failure to thrive, enuresis, hypertension, and septicemia (2-4). *Escherichia coli* is the most common pathogen responsible for childhood UTIs (5). In recent years, the antibiotic resistance of *E. coli* had an increasing trend and, consequently, a limitation of the options of empirical therapy (3, 5). This is mostly due to the production of the extended spectrum β-lactamase (ESBL) enzymes (3) that can hydrolyze the β-lactam ring of antibiotics (6) and confer resistance to penicillins, third-generation cephalosporins, and monobactams (1). Moreover, most of these ESBL- producing strains are resistant to non-β-lactam antibiotics, because the plasmids that carry the ESBL-encoding genes may also carry the resistance encoding genes of other antibiotic classes (7, 8). Therefore, the frequency of infections caused by ESBL- producing bacteria and their resistance patterns should be reported by clinical laboratories to diminish their impact on patient's treatment (7). On the other hand, identification of the genetic relationship between isolates can be useful to control the infection source and to take preventive measures (9). The aim of this study was to determine the resistance patterns, the frequency of ESBL-encoding genes and the genetic diversity of *E. coli* strains isolated from children with UTI who were admitted to children's hospital of Hazrat Masoumeh, Qom, Iran.

Materials and methods

Study population and design

This cross-sectional study was performed on 102 consecutive non-duplicative isolates of *E. coli* collected from children with UTI who were admitted to children's referral hospital of Hazrat Masoumeh, Qom, Iran from March 2015 to March 2016. Urine samples were taken with 4 methods. In the case of toilet-trained children, midstream urine collection method was used and for infants and non-toilet-trained children the urine bag, catheter or suprapubic aspiration methods were applied. The study population included children whose urine culture was positive. Positivity was determined by (a) a bacterial growth of $\geq 10^5$ colony forming units (CFU)/ml for midstream and urine bag samples, (b) a bacterial growth of $\geq 10^4$ for samples collected by catheter and (c) a growth of any count of bacteria for samples acquired by suprapubic aspiration (10, 11). The identity of the isolates was determined by standard biochemical tests such as Gram stain, oxidase, indole, methyl red/ Voges-Proskauer (MR-VP), citrate, fermentation of glucose, lactose, motility, and gas production (12).

Table 1 - General and clinical characteristics of the patients

Characteristics	N (%)
Gender	
Male	17 (17)
Female	85 (83)
Age	
< 1 years	46 (45)
1-6 years	39 (38)
>6 years	17 (17)
Clinical symptoms	
Fever	45 (44)
Fidgetiness	38 (37)
Dysuria	21 (20.5)
Nausea/vomiting	18 (18)
Diarrhea	12 (12)
weight loss	12 (12)
Malodorous urine	10 (10)
Abdominal pain	6 (6)
Poor of appetite	2 (2)
Admission type	
outpatient	55 (54)
inpatient	47 (46)
Hospital wards	
Nephrology	26 (55)
Infectious unit	10 (21)
Gastroenterology	6 (13)
Surgical	3 (6)
Neonatal	1 (2)
Oncology	1 (2)
Previous antibiotic consumption	
Yes	9 (9)
No	58 (57)
ND	35 (34)

ND: Not determined

Ethical considerations

The central microbiology laboratory of the Children's Hospital of Hazrat Masoumeh, Qom, Iran provided the *E. coli* isolates for this study. The clinical and demographic information about the patients (i.e. sex, age, clinical symptoms, admission type, hospital wards and history of antibiotic treatment) were obtained from medical records. The study protocol was approved by the Ethics Committee of Qom University of Medical Sciences, Qom, Iran (IR.MUQ.REC.1393.123).

Antimicrobial susceptibility testing

Antibiotic susceptibility of the isolates was determined using the disk diffusion agar method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (13). The following antibiotic disks were used: amikacin (10μg), gentamicin (10μg), ceftriaxone (30μg), nalidixic acid (30μg), cefixime (5μg), nitrofurantoin (300μg), cefuroxime (5μg), cefazolin (30μg), ampicillin (10μg), piperacillin-tazobactam (100/10μg), trimethoprim-sulfamethoxazole (30μg), and amoxicillin-clavulanic acid (25/10μg). All disks were purchased from Mast Co., UK. *E. coli* ATCC 25922 was used as the quality control of the test.

Identification of multi-drug resistant (MDR) isolates of *E. coli* was performed according to the guidelines proposed by the European Center for Disease Prevention and Control (ECDC, Stockholm S) and the Centers for Disease Control and Prevention (CDC, Atlanta GA) and MDR was defined as being resistant to at least one agent in three or more antimicrobial categories (14).

Determination of ESBL production by phenotypic confirmatory tests

The ability of isolates to produce ESBLs was phenotypically evaluated by both combined disk test and double disk synergy test based on the CLSI guidelines (13).

Combined Disk test

The sensitivity of the isolates to ceftazidime (30 μg), ceftazidime plus clavulanic acid (30/10 μg), cefotaxime (30 μg), and cefotaxime plus clavulanic acid (30/10 μg) disks (Mast Co., UK) were determined on Muller-Hinton agar

(MHA) (Merck Co, Germany). The plates were incubated for 18-24 h at 37°C. If the diameter of the inhibition zone around the cefotaxime / clavulanic acid and ceftazidime / clavulanic acid disks was at least 5 mm greater, compared to disks without clavulanic acid, the isolate was considered as ESBL-producer.

Double-disk synergy test

Briefly, after the bacterial suspension equivalent to 0.5 McFarland standards was inoculated on MHA plates, the amoxicillin-clavulanic acid disk was placed in the center of the plate and ceftriaxone and ceftazidime disks (third-generation cephalosporins) were placed around the amoxicillin-clavulanic acid disk with a distance of 30 mm (center to center). An extension of the edge of the inhibition zone around β-lactam antibiotic (ceftriaxone or ceftazidime) toward the disk containing β-lactam inhibitor (amoxicillin-clavulanic acid) was indicative of ESBL production.

Molecular detection of ESBLs encoding genes

The polymerase chain reaction (PCR) assay was carried out for detection of ESBL encoding genes (bla_{CTX-M}, bla_{SHV}, and bla_{TEM}) in phenotypically confirmed ESBL-positive isolates using specific primers (15). Genomic DNA was extracted by boiling method (16). The assay was fulfilled in a final volume of 25 µl containing 2.5 µl of PCR buffer, 0.2 unit of Taq DNA polymerase, 0.5 µl of dNTP, 0.5 µl of $MgCl_2$, 0.5 µl of each primer, 1 µl of template DNA and 19.8 µl of dH_2O. The thermal cycler conditions included the following steps: a first denaturation cycle at 95°C for 10 min, followed by 30 cycles of 30 s at 95°C, 30 s at 61°C and extension at 72°C for 30 s. The products were analyzed by electrophoresis on 1% agarose gels.

Molecular genotyping

All isolates were typed by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) as described previously (17). Comparison of ERIC-PCR banding patterns was performed using Gelcompar II, version 6.5 (Applied Maths, Sint-Matens-latem, Belgium). Isolates producing fingerprints related by 100% (Dice coefficient/unweighted pair-group method with arithmetic mean [UPGMA]) were allocated to the same ERIC-PCR type. The criterion for related clones was taken as profiles with 80 % or more similar bands.

Data analysis

All statistical studies were accomplished using statistical package SPSS 16.0 (SPSS Inc. Chicago, IL). The differences between resistance rates of ESBL-positive and ESBL-negative isolates, differences in the proportion of ESBL-positive and ESBL-negative strains between female and male patients and between hospitalized and outpatients and differences between the frequencies of ESBL-encoding genes were investigated by chi-square test. The degree of agreement between the combined disk test and double disk synergy test in detection of ESBL-producing isolates was calculated by Cohen's Kappa test. A P value ≤ 0.05 was considered statistically significant.

Results

During the study period, the *E .coli* strains isolated from urine samples of 102 patients less than 14 years of age with a median age of 12 months (interquartile range [IQR], 5-47.25 months) were analyzed. The clinical and demographical characteristics of the patients are listed in Table 1. Eighty-three percent (n=85) of the children were female and 17% (n=17) were male. Most of the studied patients were younger than 1 year of age (45%, n=46). The majority of infected boys (88%, n=15) were < 1 year and most of the infected girls (48%, n=41) were in the age group of 1-6 years. Fever

Table 2 - Antimicrobial resistance rates of ESBL and non-ESBL producing *E. coli* strains

Antibiotics	Resistance rate (n=102) N (%)	ESBL-producing isolates (n=59) N (%)	Non ESBL-producing isolates (n=43) N (%)
Amikacin	12 (12)	7 (12)	5 (12)
Gentamicin	37 (36)	24 (41)	13 (30)
Ampicillin	96 (94)	59 (100)	37 (86)*
Nalidixic acid	86 (84)	54 (91.5)	32 (74)*
Nitrofurantoin	8 (8)	4 (7)	4 (9)
Cefuroxime	92 (90)	59 (100)	33 (77)*
Cefixime	77 (75)	59 (100)	18 (42)*
Cefazolin	95 (93)	59 (100)	36 (84)*
Ceftriaxone	75 (73.5)	59 (100)	16 (37)*
Piperacillin-tazobactam	17 (17)	11 (19)	6 (14)
Trimethoprim-sulfamethoxazole	82 (80)	54 (91.5)	28 (65)*
Amoxicillin-clavulanic acid	57 (56)	38 (64)	19 (44)*

* Significant differences in antibiotic resistance was seen between ESBL-producing isolates and non ESBL-producing isolates

was the most observed clinical symptom (44%, n=45) and poor appetite was the least frequent symptom. Forty-six percent of the cases (n=47) were hospitalized and 54% (n=55) were outpatients. Most of the hospitalized patients (55%, n=26) have bee admitted to the nephrology department. Nine percent (n=9) of the patients had a history of antibiotic consumption.

Results of disk diffusion agar method (Table 2) demonstrated that nitrofurantoin was the most effective antibiotic against *E. coli* strains and had the lowest resistance rate (8%, n=8), followed by amikacin (12%, n=12) and piperacillin-tazobactam (17%, n=17). On the other hand, ampicillin (94%, n=96) and cefazolin (93%, n=95) were the least effective antibiotics. According to the guidelines of ECDC and CDC [15], 88% (90 out of 102) of the strains were MDR.

There were differences between the results of the two ESBL phenotypic confirmatory tests. Based on the combined disk test, 59 isolates of *E. coli* (58%) were ESBL-positive. However, the double disk synergy test detected only 49 ESBL-positive isolates and the two methods were in poor agreement (Kappa=0.096). Results of PCR assay was in accordance with the combined disk test and ESBL-encoding genes were detected in all of the ESBL-positive isolates (59/59). Seventy-eight percent (n=46) of ESBL-producing *E. coli* strains were isolated from female patients and 22% (n=13) were isolated from male patients and there was no relationship between ESBL production and patients' gender (P=0.2799). Forty-one percent (n=24) of ESBL-positive isolates were collected from hospitalized patients and 59% (n=35) were isolated from outpatients. Their differences were not statistically significant (P=0.1514).

All of the ESBL-producing isolates (Table 2) were resistant to the tested βlactam antibiotics (i.e. ampicillin, cefuroxime, cefixime, cefazolin, and ceftriaxone) and the most resistance rates in non-ESBL-producing isolates were related to ampicillin (86%, n=37) and cefazolin (84%, n=36). There were statistically significant differences ($P < 0.05$) between the resistance rates of ESBL-positive and ESBL-negative isolates against ampicillin, nalidixic acid, cefuroxime, cefixime, cefazolin, ceftriaxone, trimethoprim-sulfamethoxazole, and

Table 3 - Molecular characteristics of ESBL-encoding genes in *E. coli* isolates (n=59)

Genotype	Isolates N (%)
$bla_{CTX\text{-}M}$. bla_{TEM} . bla_{SHV}	3 (5)
$bla_{CTX\text{-}M}$. bla_{TEM}	23 (39)
$bla_{CTX\text{-}M}$. bla_{SHV}	4 (7)
bla_{TEM} . bla_{SHV}	1 (2)
$bla_{CTX\text{-}M}$	22 (37)
bla_{TEM}	5 (8)
bla_{SHV}	1 (2)

amoxicillin-clavulanic acid. All of the ESBL-producing isolates were MDR.

The frequency of ESBL-encoding genes and their related genotypes are shown in Table 3. The $bla_{CTX\text{-}M}$ was the most frequent gene and was detected in 88% (n=52) of ESBL-positive isolates, followed by bla_{TEM} (54%, n=32), and bla_{SHV} (15%, n=9). There were statistically significant differences between the frequencies of investigated genes ($P < 0.0001$). In addition, $bla_{CTX\text{-}M}$. bla_{TEM} had the highest frequency among ESBL positive strains (44%, n=26).

Genotyping results showed 13 different clusters (A-M) (more than 80% genetic similarity was classified as a cluster) (Figure 1). F and J clusters accounted for 16 and 14 *E. coli* isolates and most of them were isolated from outpatients. Most strains isolated from hospitalized patients were categorized in various clusters and there was little genetic relationship between them. Except for clusters A and M, whose isolates were all ESBL producers, and cluster J, whose 11 out of 14 isolates (78.5%) were ESBL-positive, the other clusters were mixed and included both ESBL-positive and ESBL-negative strains. There was no relationship between bacterial genotypes and antimicrobial resistance profiles of bacteria.

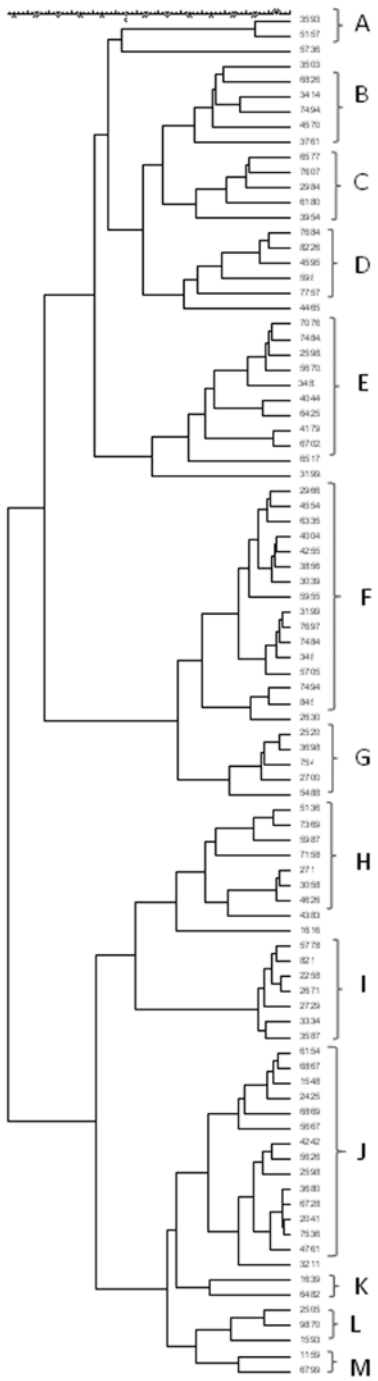

Fig. 1 - Dendrogram of *E. coli* isolates analyzed by ERICD-PCR method. The scale at the top represents the genetic distance between the isolates. Similarity analysis of results was obtained using the Dice coefficient/unweighted pair-group method with arithmetic mean (UPGMA)

Discussion

In this study, it was found that the incidence of UTI differs by sex and age.

The majority of patients were female (83%, n=85) and 48% of them (n=41) were in the age group of 1-6 years; however, most of the infected boys (88%, n=15) were younger than 1 year of age. In the study conducted by Parajuli *et al.,* (5), similar results were observed and UTI was more prevalent in female patients of the age groups 1-4 and 5-9 years. Other studies also emphasized that UTI is more prevalent in boys < 6 months of age and in school-girls (18, 19). Some studies concluded that UTI was more common in male patients (3, 20, 21), contrary to our results. The higher rates of UTI in the infant boys might be due to the more common occurrences of obstructive congenital urogenital abnormalities in boys (19). Being uncircumcised is another factor that may be associated with high odds of UTI in infant boys (22, 23). The high prevalence of UTI in girls is probably because of different immune status, anatomical differences in urinary tract, low-level hygiene, moist environment of vagina and periurethral tracts that increases the risk of pathogen growth and infection caused by fecal flora (18, 24).

In this study, a high prevalence of ESBL-positive *E. coli* (58%) was reported and, as it was noted, there was a poor agreement between the results of the two ESBL phenotypic confirmatory tests (i.e. combined disk test and double disk synergy test). The differences observed between the results of the two tests might be due to the production of β-lactamases other than ESBLs, such as AmpC β-lactamases and TEM β-lactamases, which are resistant to β-lactamase inhibitors such as clavulanic acid, so preventing the detection of ESBLs and resulting in false negatives. On the other hand, the overproduction of TEM and SHV β-lactamases by bacteria can affect the results of ESBL phenotypic confirmatory tests (25). The application of tazobactam or sulbactam, that are weak inducers of AmpC β-lactamases and of cefepime, a fourth generation cephalosporin, which is stable to AmpC β-lactamase, can be a solutions for this problem (26). Benedic *et al*. (27) showed, for the double disk synergy test, that the sensitivity of the test is affected by the distance between the disks and the type of applied disks; actually, using smaller distance between the disks (e.g. 2.5 cm or 2 cm) and all four disks (i.e. ceftazidime, cefotaxime, ceftriaxone and aztreonam) can improve the sensitivity of the test. So, it can be stated that the double disk synergy test has low sensitivity for detection of ESBL positive organisms and needs some modifications (26).

The observed rate of ESBL-positive *E.coli* in this study (58%) was high compared to the result of our previous study (37%) (11) and rates reported by Sedighi *et al*., (27.3%) (2) and Rezai *et al*., (30.5%) (28) from Iran, Moore *et al*., (44%) from Cambodia (29), and Shettigar *et al*., (37.7%) (30) from India. However, higher rates of ESBL-positive *E. coli* were also reported, including the rate reported by Chinnasami *et al*, from India (83%) (31). UTI prophylaxis, long-term hospitalization, the wide application of second and third-generation cephalosporins, underlying diseases and recurrent UTIs could be some contributing factors in the variations observed in the prevalence rates of ESBL-producing *E. coli* isolates. Identification of these predisposing factors of ESBL production can play an important role in adopting effective preventive policies (2, 5, 6, 11).

Results of antibiogram demonstrated that the lowest resistance rate belonged to the nitrofurantoin (8%, n=8) and the highest resistance rate was seen against ampicillin (94%, n=96) and cefazolin (93%, n=95) and 88% of the isolates were found to be MDR. On the other hand, 100% of the ESBL-positive isolates were resistant to the tested β-lactam antibiotics and all of them were MDR. Comparison of the antibiotic resistance patterns of *E. coli* strains in

this study with our previous research (11), revealed the increasing trends in resistance against aminoglycosides, cefuroxime, cefixime, and ceftriaxone. In the study performed by Sedighi *et al*., from Iran (2), no resistance to nitrofurantoin was observed (0%) and resistance rates to cefixime (34.2%), ceftriaxone (32.5%), gentamicin (17.5%), amikacin (5.8%), trimethoprim/ sulfamethoxazole (70.8%), and nalidixic acid (40.9%) were much lower than our results. In addition, 34.2 % of their isolates were known as MDR, that was lower than our study. Sharma *et al.,* from India (3) reported resistance rates similar to our study; however, the rates of resistance to amoxicillin-clavulanate (96%) and nalidixic acid (98.7%) were higher than in our study. In their survey, a high percentage of the isolates (90%) were MDR, and this was close to our result. One of the reasons for the high level of antibiotic resistance and MDR rates in this study could be due to the high rate of ESBL production by *E. coli* strains. Because ESBL-producing isolates are not only resistant to β-lactam antibiotics and can display co-resistance to other antibiotic classes, including quinolones, aminoglycosides, tetracyclines and sulfonamides (8, 10, 32).

In the current study, the $bla_{\text{CTX-M}}$ gene was more frequent (88%) than the two other ESBL-encoding genes (i.e. bla_{TEM} (54%) and bla_{SHV} (15%)). In the study done by Ramadan *et al.,* from Egypt (24), the $bla_{\text{CTX-M}}$ gene was more prevalent (43.24%) than bla_{SHV} gene (8.1%), and that was similar to our results. However, in the study performed by Rezaei *et al.,* from Iran (28), the bla_{TEM} gene was the most prevalent gene (49%), followed by bla_{SHV} (44%), $bla_{\text{CTX-M}}$ (28%), and bla_{VEB} (8%) genes.

In the present study, the genetic relatedness of the isolates was delineated by the ERIC-PCR method and 102 isolates were categorized into 13 distinct types. In the study performed by Gündogdu *et al.,* (32), 296 isolates of *E. coli* recovered from patients (both adults and children) with UTIs were typed using RAPD-PCR technique and were arranged in 32 common and 87 single types, suggesting a high diversity among the isolates. In other study conducted by Rejiba *et al*. from Tunisia (33), 47 strains of *E. coli* that were isolated from different specimens of children, were analyzed by the ERIC-PCR method and were clustered in 32 different genotypes that represent an extensive diversity. A possible reason for the lower diversity of our studied isolates could be that we analyzed the strains that were isolated from urine samples of children with UTIs, while - in the two mentioned studies - isolates originated from different clinical specimens (e.g. urine, fecal swab, pus, blood, intra-abdominal peritonitis, wound, and trachea) and from both children and adults, that may result in high genetic variety.

Our results demonstrated that there was no correlation between different genotypes and origin of the isolates (i.e. hospitalized or outpatients), ESBL-producing ability and antibiotic resistance patterns. Similar to our study, Marialouis *et al*. (34) reported that MDR and ESBL-positive isolates were distributed in all clusters. In contrast to this study, Kazemian and his colleagues (9) showed that there was a relationship between RAPD types and antibiotic susceptibility patterns of the isolates.

Conclusions

High prevalence of ESBL-positive isolates of *E. coli* (58%) was found in our study and all of them were MDR. In addition, there were statistically significant differences in the resistance rates to some antibiotics between ESBL-producers and non-producers, which result in limiting their therapeutic options. Continuous surveillance of the emergence of ESBL-producing isolates and their antibiotic resistance profiles, as well as using appropriate typing

methods, is needed for reducing their spread, selecting appropriate treatment regimens and finding hospital outbreaks.

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Riassunto

Caratteristiche molecolari e profili di resistenza agli antibiotici di ceppi di Escherichia coli isolati da infezioni del tratto urinario in bambini ricoverati nell'ospedale di riferimento di Qom, Iran

Contesto. Le infezioni del tratto urinario (ITU) sono un'infezione molto diffusa tra i bambini e l'*Escherichia coli* è uno dei più importanti patogeni che causano ITU pediatriche. La produzione di enzimi β-lattamasi a spettro esteso (BLSE) a spettro esteso è un fattore importante nell'emergere della resistenza agli antibiotici tra questi batteri. Questo studio mirava a determinare i modelli di resistenza, la frequenza dei geni codificanti BLSE e la diversità genetica dei ceppi di *E. coli* isolati da bambini con ITU che sono stati ammessi all'ospedale di riferimento di Hazrat Masoumeh, Qom, Iran.

Metodi. Nello studio sono stati inclusi in totale 102 ceppi consecutivi non duplicati di *E. coli* isolati da bambini con ITU. La suscettibilità agli antibiotici degli isolati è stata determinata mediante metodo di diffusione del disco secondo le linee guida CLSI. La capacità degli isolati di produrre BLSE è stata determinata fenotipicamente sia dal test combinato del disco sia dal test di sinergia del doppio disco. I geni codificanti BLSE (bla CTX-M, bla SHV e bla TEM) in fenotipi confermati isolati BLSE -positivi sono stati rilevati mediante metodo PCR. La parentela genetica degli isolati è stata designata mediante PCR intergenico ripetitivo enterobatterico (ERIC-PCR).

Risultati. L'ottantatre percento (n = 85) dei bambini era di sesso femminile. La maggior parte dei ragazzi infetti (88%, n = 15) aveva meno di 1 anno di età e la maggior parte delle ragazze infette (48%, n = 41) di età compresa tra 1 e 6 anni. La massima sensibilità è stata osservata con nitrofurantoina (8%, n = 8), seguita da amikacina (12%, n = 12) e piperacillina-tazobactam (17%, n = 17). Al contrario, il più alto tasso di resistenza è stato osservato con ampicillina (94%, n = 96) e cefazolina (93%, n = 95). L'ottantotto percento (90 su 102) dei ceppi erano multiresistenti (MR). Il 58% (n = 59) dei ceppi era BLSE -positivo e i risultati del test combinato del disco erano in accordo con la PCR. Il blaCTX-M era il gene di codifica BLSE più frequente (88%, n = 52), seguito da blaTEM (54%, n = 32) e blaSHV (15%, n = 9). Sulla base della tecnica ERIC-PCR, gli isolati sono stati raggruppati in 13 diversi tipi. Non c'era alcuna relazione tra i diversi tipi di ERIC e l'origine degli isolati (cioè ospedalizzati o ambulatoriali), la capacità di produrre BLSE e gli schemi di resistenza agli antibiotici.

Conclusioni. Nel nostro studio è stata riscontrata un'elevata prevalenza di isolati di *E. coli* (58%) positivi per BLSE e tutti erano MR. Inoltre, ci sono state differenze statisticamente significative nei tassi di resistenza dei produttori di BLSE e dei non produttori in alcuni antibiotici, il che ha portato a limitare le loro opzioni terapeutiche. È necessaria una sorveglianza continua dell'emergenza degli isolati che producono BLSE e dei loro profili di resistenza agli antibiotici, nonché l'uso di metodi di tipizzazione appropriati per ridurne la diffusione, selezionare i regimi terapeutici appropriati e individuare epidemie ospedaliere.

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