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Checking the Plentitude of (BLA-SHV) ß-lactamase Gene in Separated E. Colis in Children under 6 Years-Old Infected with Urinary Tract Infection Referring to Tehran Central Pathobiology Laboratory (2014)

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Abstract

Increasing consumption of Antibiotics like β-lactam antibiotics including Penicillins and Cephalosporins has resulted in the creation of refractory E. coli levels to these antibiotics. The aim of this study is investigating the presence of resistant gene to β-lactam antibiotics and third-generation Cephalosporins among separated E. coli levels from urine sample of the referring children under 6 infected with urinary tract infection to Tehran central pathobiology laboratory. 130 s E.coli sample were collected from urine sample of the children. Sensitivity of the collected levels to β-lactam antibiotics including Ampicillin, Amoxicillin and third generation Cephalosporins was measured. Also, PCR was implemented for finding BLA-SHV gen. Among 130 samples under study, 45 samples comported BLA-SHV gen. 100 samples (77%) were resistant toward Amoxicillin and Ampicillin antibiotics and 30 samples (23%) were sensitive to these antibiotics. In this study, resistant percentage rate to non-ß-lactam antibiotics like Amikacin, Gentamicin, Cotrimoxazole, and Ciprofloxacin was respectively 0%, 14%, 66% and 23%. This study has shown the presence of BLA-SHV gen with an abundance of 35% in E. coli bacteria and it also confirms the importance of awareness of growing increase of Antibiotic resistance and reduction and change in antibiotics' consumption strategy.

Keywords: E.coli, Antibiotic resistance, broad ß-lactamases, BLA-SHV gene

Introduction

Urinary tract infection is one of the most common infectious diseases all over the world. Urinary tract infection can only infect lower urinary tract that in this case, it is called bladder infection. It also might involve upper urinary tract that in this case, it is called Pyelonephritis which is the result of microorganisms' presence in urinary tract.

Symptoms related to lower urinary tract include excretion of urine along with burning and pain or urination frequency, whereas, symptoms related to Pyelonephritis include fever and flank pain along with relevant symptoms to lower urinary tract infection. Such symptoms might be ambiguous or unknown in elderly or very young members. Urinary tract infections usually occur more in women than in men. This is because; half of women, during their life are at least infected by an infection. Re-emergence of illness is a common problem. Risk maker factors include: females 'body anatomy, sexual intercourse and family history. Pyelonephritis, in case of occurrence, usually appears after bladder infection. But it might also result from transmitted infection via blood (Nicolle, 2008).

Lower urinary tract infection is also called bladder infection. Its most common symptoms include burning on urination and the need to frequent urination (or urge to urination) and considerable pain. Such symptoms might be sever or medium, and in healthy women, they last on

average 6 days. People who are infected by upper urinary tract infection or Pyelonephritis might show flank pain, fever or nausea and vomiting in spite of common symptoms of lower urinary tract infection. Rarely is the urine bloody or with dirt. Diagnosis of this illness in young and healthy women is only possible through the symptoms. Diagnosis of illness might be problematic in the individuals with ambiguous symptoms; because, bacterias might exist, but no infection is observed. In complicated cases or when treatment fails, urine culture might be important (Woodford and George, 2011). In patients with recurrent infections, we can use low dose of Antibiotics as the preventive measurement. In non-complicated cases, urinary tract infections can easily be treated by consuming Antibiotic in a short-term, though resistance against many used Antibiotics for treating this illness is increasing (Colgan and Williams, 2011). In complicated cases, it may be necessary that treatment courses be extended more or intravenous antibiotics injection might be needed, and in the case that the symptoms didn't improve during two or three days, more diagnostic tests are needed. In women, urinary tract infections are the most common form of bacterial infections, and any year, 10% is added to the rate of urinary tract infections (Lane and Takhar, 2011).

Early diagnosis and rapid antimicrobial treatment are necessary in order for minimizing renal scarring and progressive kidney damages. In patients with suspected UTI, antibiotic treatment usually starts experimentally before culture reply is prepared. Unfortunately, antibiotic resistance is an increasing critical problem in many countries like Iran (Aminzadeh, 2008; Al-Tawfiq and Anani, 2009). In clear cases, we can proceed toward diagnosis and treatment only based on the symptoms, and there is no need for more confirmation through experimental tests. In complex or suspected cases, diagnosis confirmation might be useful through urine analysis and search for the presence of nitrite in urine, white blood cells (leukocytes) or esterase leukocyte (esterase leukocyte test measures the rate of esterase enzyme which is released through white blood cells). One of the other experiments, namely microscopic examination of urine, searches for the presence of red blood cells, white blood cells or bacteria. Urine culture is positive in the case that the number of bacterial colonies is greater or equal to 10^3 colony-forming units (CFU) in common organisms per ml in urinary tract. Antibiotic susceptibility is also testable by this culture, and in this regard, it is useful in selecting antibiotic therapy. However, antibiotic treatment might again result in improvement in women culture result of whom is negative. Because, the symptoms might be ambiguous, and diagnosis in elderly members without trustworthy experiments related to urinary tract infections might be problematic (Rosen et al., 2017).

The most important foundation of treatment is using antibiotics. Sometimes during the first few days, in spite of antibiotic, phenazopyridine is prescribed in order to help the irritation relief and compulsion removal due to bladder infection. However, due to safety problems associated with its use and especially the increase of the danger of being affected by methemoglobinemia (methemoglobinemia rate higher than normal level of blood), its continuous usage is not recommended (Perrotta et al., 2008; Cubeddu et al., 2009).

The most common factor of urinary tract infection creation is 75-90 % E. coli bacteria, and following that Klebsiella spp. ‹Enterobacter spp ‹Proteus spp and Pseudomonas spp are among gram-negative bacterias of urinary tract infection creation. Among gram-positive bacterias creating urinary tract infections, we can refer to Group B ß-Streptococcus ‹Enterococcus sp. ‹MRSA ‹negative Staphylococcus Coagulase. Also, Bacterias such as Chlamydia and Mycoplasma can be the cause of urinary tract infection that in spite of other microorganisms, infection with this bacterium is considered a part of STD. Infection with the funguses is also observed in some cases (Mabeck et al., 1999).

In this regard, the aim of the present research is evaluating the frequency of ß-lactamase gene (BLA-SHV) in separated E. Colis in referring children under six infected with urinary tract infection to Tehran central pathobiology laboratory (2014) that we studied.

Materials and Methods

Standard method of detector disks of ESBLs

In this method, a ß-lactamase inhibitor is used which is usually Clavulanic acid along with one of Oxy Mino cephalosporins antibiotics such as Cefotaxime or Ceftazidime or Ceftriaxone, in a way that Clavulanic acid results in ESBLs inhibition, so that it reduces the resistance of bacteria toward Cephalosporins, and we can observe growth inhibition region around the disk which includes Clavulanic acid. But, around the disk which lacks Clavulanic acid, growth inhibition region is not observed or it is observed less than semi-sensitive or resistant state. In this case, existence or non-existence of ESBLs in the intended bacteria is discovered. It is necessary to mention that this method is known to Combination disk method test (CDT) due to using two disks in this method.

Needed materials and equipment for recognizing ESBLs

- Mueller-Hinton agar medium
- Used disks in order for recognizing ESBLs which have been provided by MAST company, including:

Cefotaxime disk along with Cefotaxime disk+ Clavulanic acid

- Ceftriaxone disk along with Ceftriaxone disk+ Clavulanic acid
- Sterile physiology serum
- 0.5 standard McFarland tubes
- Bacterial suspension
- Sterile swaps and pence
- Graph rule

Like Antibiotic susceptibility determination methods, medium is prepared and microbial suspension is inoculated to the medium through diffusion from disk method. Using delicate sterile pence, disks are placed at a distance of thirty mm from each other on Mueller-Hinton agar medium. Then, plates are inverted and are placed in incubator at 35 to 37 ° C for 18 to 24 hours. Then, using scaled ruler in indirect light and with naked eye, diameter of growth inhibition zones is measured and the results are evaluated.

The increase of zone diameter to 5 millimeter A≥5mm for any of antibiotics in combination with Clavulanic acid versus their region is lonely considered as a positive result for ESBL production.

Long-term storage and preservation method of bacterias

Phosphate buffered saline (PBS) solution is used for maintaining the intended bacteria. Bacterial samples are cultured in plate densely for a full day and transferred to micro tubes containing PBS in a way that they were taken from grown pure colonies on blood agar medium by means of sterile loop, and a microbial suspension was provided in PBS solution according to standard solution of Makfarland. Micro tubes containing inoculated bacterial suspension in PBS solution can be conserved in freezer for a long time.

Another method of cultivation was new 24 hours samples on blood agar medium and closure of plates' door or parafilm and maintenance in 2-8 c in refrigerator.

In for making sure about sample reservation, all above methods were used.

DNA extraction from separated bacterias for PCR

Separated E.coli bacterias are cultured in LB liquid medium and when the number of bacterias reached to (1×10^9) of bacterial cell, they are divided to two sections and any of them are centrifuged for 3 minutes in dosage (10000 rpm) 9.300 ×g. zincoid solution is carefully vacated.

Add the value of 400 µ L of R renewed solubilization buffer to microbial cell mass and homogenize it by pipetting. Transfer the homogenized sample to L extraction tube and vertex it a bit.

Incubate the sample in 65c for 10 minutes (alternate shaking increases slip process).

Place Extraction L tube in thermo maker and incubate it in 95c for 5 to 10 minutes (alternate shaking increases slip output).

Add binding buffer B6 ۴۰۰ Lµ to the sample and vertex it a bit.

Load the sample in RTA spin filter set and place it in room temperature for 1 minute. Then centrifuge it in standard centrifuge (12000 rpm) in 4oo.13 × g. Shed the liquid and turn over RTA spin filter in RTA receiver tube.

Add the Wash buffer I 500 µl and centrifuge it in (10000 rpm) and dosage 9.300 × g for 1 minute. Shed the liquid and RTA receiver. Place RTA spin filter in a new RTA receiver tube. Add the wash buffer II 600 µl and centrifuge it for one minute in dosage (10000 rpm) 9.300 × g. Shed the liquid and return RTA spin filter to RTA spin filter and finally centrifuge it for 3 minutes in maximum speed in order for the ethanol to be quietly detoxified.

RTP Bacteria DNA kit contents:

30 ml Extraction Tube

30 ml Resuspension Buffer R

30 ml Binding Buffer B6

30 ml Elution Buffer D

Wash Buffer I 30ml (final volume ml 60)

Wash Buffer II 18ml (final volume ml 60)

RTA spin filter set 50

RTA Reciever Tube 50

1.5 ml Reciever Tubes 50

In order for extracting blaSHV gen, RTP Bacteria DNA Mini commercial kit made in Germany was used the procedures of which are as the following:

The first stage:

Add 30 ml of 96-100% ethanol to wash Buffer I bottle and blend it a bit and always close the bottles' door firmly.

Add 42 ml of 96-100% ethanol to wash Buffer II bottle and blend it a bit and always close the bottle's door tightly.

Selecting (Forward) and (Reverse) primers in order for PCR of blaSHV gen

A) In order for searching for finding blaSHV gen in DNA of separated E. coli bacterias and based on the information of nucleotide sequence of this gen in papers 17 and then adjustment of this data with nucleotide sequence information of this gen in NCBI genbank site, forward and reverse primers were selected with the following sequences:

Forward: 5′-TCATCGAAAAACACCTTG-3′

Reverse: 5′-TCCCGCAGATAAATCACC-3′

Sharma (2013) in a conducted study in 2013 regarding the spread of blaSHV gen in Klebsiella spp and and E. coli, designed the following primers:

F. P: 5`-GTCAGCGAAAAACACCTTGCC-3`

R. P: 5`- GTCTTATCGGCGATAAACCAG – 3

Kariuki et al in the year 2012 in a conducted study on ß-lactamase gene in E.coli designed the following primers:

F: 5' – TTCGCCTGTGTATTATCTCCCTG-3'
R: 5' –TTAGCGTTGCCAGTGYTCG-3'
In the year 2013, Krishnamurthy et al in a conducted study
regarding genotypic and phenotypic methods for recognizing generating Klebsiella pneumonia of ESBLs, designed the following primers:
F: 5'- GGGTAATTCTTATTTGTCGC- 3'
R: 5'- TTAGCGTTGCCAGTGCTC -3'
Regarding selecting the primer, we should consider some points. For example, the intended primer should have 18-25 nucleotides and G=C sequences should exist more at both ends. Tm of the used primers shouldn't differ from each other more than 5 c. Some parts of the gene can be used that don't change. We also should consider the number of nucleotides and lack of complementary sequence in designed primers and lack of palindromic sequence.
Procurement of PCR reaction
PCR reaction was conducted in final volume of 50 microliter. PCR materials were supplied from Cinnagen Company.
The following materials were respectively added to
eppendorf tubes specified to PCR:
PCR water: 18 microliter
PCR buffer: 2.5 microliter
dNTP: 1 microliter (equal proportions of dATP,dCTP,dGTP,dTTP was used).
Forward primer: 0.5 microliter
Reverse primer: 0.5 microliter
Then the materials were vertexed a stage and after that 2 microliter of genomic DNA and finally 0.5 microliter of Taq DNA Polymerase enzyme was added to them and they immediately were transferred to thermo cycler system (eppendorf-model Company).
PCR reaction was implemented in 35 cycles according to table 1.

Table 1: execution stages of PCR reaction

PCR reaction stages		Temperature (c)	Time(second)
Denaturation	The first stage	95	300
	The next stages	95	30
Annealing		60	45
Extention		72	30
Final Extention		72	600

Electrophoresis
In order for constructing electrophoresis gel, initially, 2 grams of Agarose is blended with 100 milliliter TBE (%2). The obtained mixture is heated in microwave for several times so that it boils but not overflows until a lucid solution is obtained. After complete coolness, gel was poured in special format of electrophoresis gel in the liquid state and the intended specific comb was placed in

gel and gel was allowed to chill and be solidified. Then, TRAY format was placed in electrophoresis tank containing electrophoresis buffer and comb was expelled from that quietly.

5 microliter of any PCR product was blended with 1 microliter of Erythrogel and they were poured in any sinks of Agarose gel. 3 microliter of DNA Ladder (300-1000 bp) was poured in the first sink as the marker. Electrophoresis conditions were adjusted on 130 volt for half an hour.

After necessary time elapse, gel was placed in Ethidium bromide for 15 minutes and after that bleaching in distilled water was accomplished. Gel was observed and photographed following proximity to ultraviolet in trans-illuminator system. Extracted DNA from Klebsiella pneumoniae ATCC 700603 standard stem was used as positive control of SHV gen and extracted DNA from E. coli ATCC 25922 was used for negative control.

Results

The results of separation and recognition of bacterias causing urinary tract infection

In this study, 130 E. coli strains were under microbiologic evaluation during 3 months from October to December of 2014 from 3500 urine samples related to referring under 6 children to central pathobiology laboratory.

230 samples were recognized as positive regarding bacterial infection. 85 % of the infection agent was E. coli bacteria and the remained 15% included other gram-negative bacterias such as Klebsiella. Proteus sp. Enterobacter sp.sp, Pseudomonas aeroginosa and gram-positive bacterias such as Coag. Positive, Enterococcus sp., Group A Streptococci, Group B Streptococci Staphylococci and candidiasis infection. Regarding gender, 104 cases 80% were girl and 26 cases 20% were son. Following cultivating the samples on blood agar medium and EMB and maintenance in 37c for 24 hours, purple and often with metallic luster colonies through gram staining and biochemical differential tests and Antibiogram tests and samples PCR were confirmed. In anti-bio gram studies, 77% of E. coli isolates were resistant to ampicillin and amoxicillin antibiotics. BLA-SHV ß-lactamase resistance gene was searched among E. coli isolates through PCR method and this gen was specified in 35% of E. coli strains.

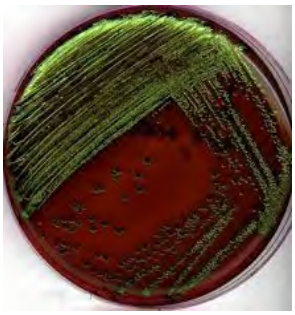

Figure 1: shows E. coli 24 hours medium in EMB agar medium.

Figure 2: shows microscopic view of E. coli which is in form of gram-negative bacillus

Figure 3: shows differential test of IMViC related to E. coli

Sensitivity evaluation results of E. coli strains to ß-lactam group antibiotics through disk-inserting method

Sensitivity of bacterial strains toward ampicillin antibiotics, amikacin, amoxicillin, carbenicillin, cephalexin, cephalothin, cefotaxime, cefixime, ceftriaxone, ceftizoxime, ciprofloxacin, ofloxacin, cotrimoxazole, gentamicin, nitrofurantoin and nalidixic acid was measured through disk-inserting method. Figure 4 shows sensitivity evaluation through diffusion from resistant one strain disk.

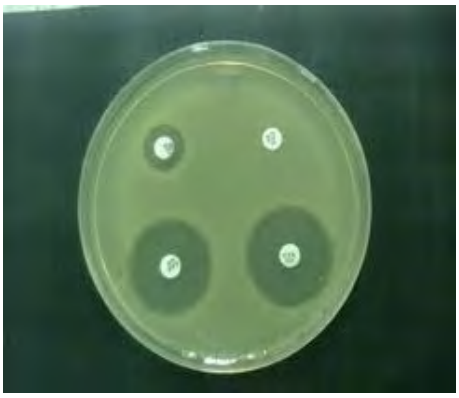

Figure 4: shows sensitivity evaluation to the studied antibiotics through diffusion from resistant one strain disk.

Diagram 1 and 2 show the percentage of sensitive, semi-sensitive, and resistant strains to ciprofloxacin and ampicillin antibiotics through disk-inserting method in this study.

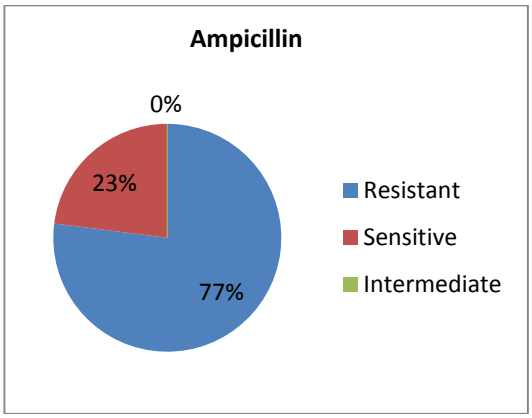

Diagram 1: frequency ratio of the number of sensitive, semi-sensitive and resistant E. coli strains to ampicillin antibiotic through disk-inserting

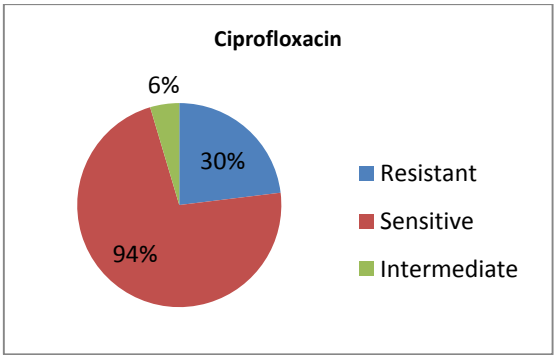

Diagram 2: frequency ratio of the number of sensitive, semi-sensitive and resistant E. coli strains to Ciprofloxacin antibiotic through disk-inserting method

From the summation of 130 studied samples, 100 samples namely 77% were resistant to ampicillin antibiotic and 30 samples namely 23% were sensitive to ampicillin antibiotic and in opposite, 30 samples namely 23% were resistant to ciprofloxacin antibiotic and 94 samples namely 72% were sensitive to ciprofloxacin antibiotic and 6 samples namely 5% were semi-sensitive to ciprofloxacin antibiotic.

PCR results of BLA-SHV gen in E. coli strains

After separation and microscopic and biochemical recognition of E. coli strains and implementing antibiotic sensitivity determination experiments on them, PCR experiments were implemented on strains using related primers to BLA-SHV gen and PCR product was electrophoresed. PCR products of the strains with BLA-SHV gen showed a band with molecular weight of 210bp (7.77 kDa) in agarose gel (figure 5).

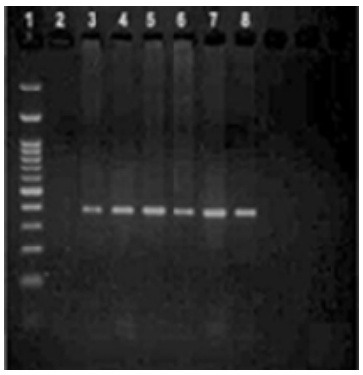

Figure 5: electrophoresis of PCR product of BLA-SHV gen in E. coli strains, DNA Ladder sink 1, negative control sink 2 (E. coli ATCC 25922), positive control sink 3 (ATCC 700603 Klebsiella pneumonia), 4 to 8 sinks of E. coli strains with BLA-SHV gen

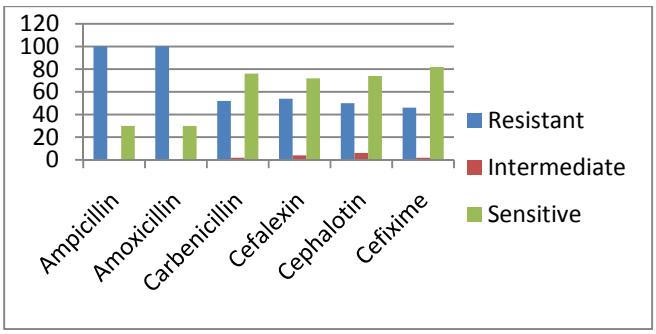

Diagram 3: frequency percentage of sensitivity and resistance to ß-lactam group antibiotics in separated E. coli strains in under 6 children infected with urinary tract infection

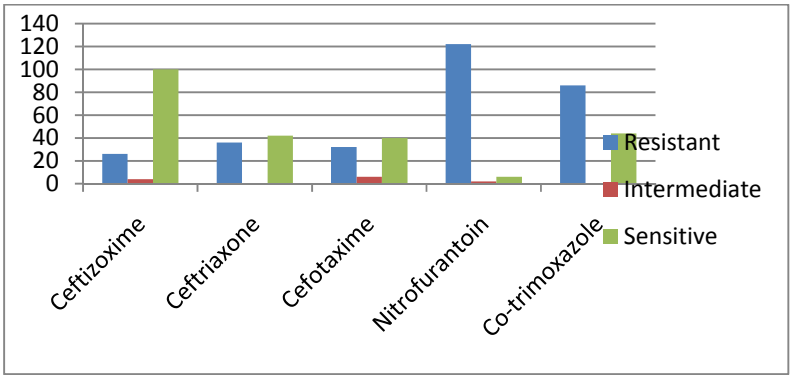

Diagram 4: frequency percentage of sensitivity and resistance to the antibiotics of cephalosporins, nitrofurantoin, cotrimoxazole group in separated strains of E. coli in under 6 children infected with urinary tract infection

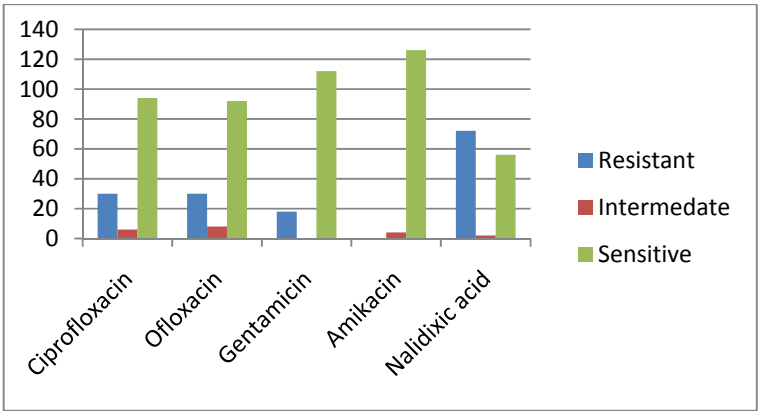

Diagram 5: frequency percentage of sensitivity and resistance to the antibiotics of amino glycosides, quinolones and fluoroquinolones group in the strains of separated E. coli in under 6 children infected with urinary tract infection

Table 2: the results of frequency checking of resistance percentage and sensitivity and semi-sensitivity of E.coli strains to the antibiotics with ß-lactam and also presence and lack of BLA-SHV gen in PCR method

Antibiotic name	Disk-inserting method			Bacteria with BLA-SHV gen		Bacteria without BLA-SHV gen	
	Growth deterrent halation mm (Sensitive, resistant, semi-sensitive)	Bacteria number	Bacteria percentage	No.	Percentage	No.	Percentage
Ampicillin	Sensitive ≥ 17	30	23%	0	0%	30	23%
	Resistant ≤ 13	100	77%	46	35%	54	42%
	Semi-sensitive 14-16	0	0%	0	0%	0	0%
Amoxicillin	Sensitive ≥ 18	30	23%	0	0%	30	23%
	Resistant ≤ 13	100	77%	46	35%	54	42%
	Semi-sensitive 14-17	0	0%	0	0%	0	0%
Carbenicillin	Sensitive ≥ 23	76	59%	0	0%	76	59%
	Resistant ≤ 19	52	40%	46	35%	6	5%
	Semi-sensitive 20-22	2	1%	0	0%	2	1%
Cefalexin	Sensitive ≥ 18	72	55%	0	0%	72	55%
	Resistant ≤ 14	54	42%	46	35%	8	7%
	Semi-sensitive 15-17	4	3%	0	0%	4	3%
Cephalotin	Sensitive ≥ 18	74	55%	0	0%	72	55%
	Resistant ≤ 14	50	42%	46	36%	8	6%
	Semi-sensitive 15-17	6	3%	0	0%	4	3%

Table 3: the results of frequency evaluation of resistance percentage and sensitivity and semi-sensitivity of E.coli strains to the antibiotics with ß-lactam and co-trimoxazole and also presence and lack of BLA-SHV gen in PCR method

Antibiotic name	Disk- inserting method			Bacteria with BLA-SHV gen		Bacteria without BLA-SHV gene	
	Growth deterrent halation mm (Sensitive/resistant/semi-sensitive)(	Bacteria number	Bacteria percent	Number	Percentage	Number	Percentage
Cefixime	Sensitive ≥ 19	82	63%	0	0%	82	63%
	Resistant ≤ 15	46	36%	46	36%	0	0%
	Semi-sensitive 16-18	2	1%	0	0%	2	1%
Ceftizoxime	sensitive ≥ 20	100	77%	16	12%	84	65%
	Resistant ≤ 14	26	20%	26	20%	0	0%
	Semi-sensitive 15-19	4	3%	4	3%	0	0%
Ceftriaxone	Sensitive ≥ 21	42	57%	0	0%	74	57%
	Resistant ≤ 13	36	38%	42	32%	8	6%
	Semi-sensitive 14-20	0	5%	4	4%	1	1%
Cefotaxime	Sensitive ≥ 23	40	60%	0	0%	78	60%
	Resistant ≤ 14	32	35%	42	32%	14	3%
	Semi-sensitive 15-22	6	5%	4	4%	2	1%
Co-trimoxazole	Sensitive ≥ 16	44	34%	6	5%	38	29%
	Resistant ≤ 10	86	66%	40	31%	46	35%
	Semi-sensitive 11-15	0	0%	0	0%	0	0%

Table 4: the results of frequency evaluation of resistance percentage and sensitivity and semi-sensitivity of E.coli strains to aminoglycoside antibiotics and quinolines and fluoroquinolones and nitrofurantoin, and also the presence and non-presence of BLA-SHV gen in PCR method

Antibiotic name	Disk-inserting method			Bacteria with BLA-SHV gen		Bacteria without BLA-SHV gen	
	Growth deterrent halation mm (Sensitive, resistant/semi-sensitive)	Bacteria number	Bacteria percentage	Number	Percentage	Number	Percentage
Ciproflox acin	Sensitive ≥ 21	94	72%	30	23%	64	49%
	Resistant ≤ 15	30	23%	16	12%	14	11%
	Semi-sensitive 16-20	6	5%	0	0%	6	5%
Ofloxacin	Sensitive ≥ 16	92	71%	24	19%	68	52%
	Resistant ≤ 12	30	23%	22	17%	8	6%
	Semi-sensitive 13-15	8	6%	0	0%	8	6%
Gentamici n	Sensitive ≥ 15	112	86%	34	26%	78	60%
	Resistant ≤ 12	18	14%	12	9%	6	5%
	Semi-sensitive 13-14	0	0%	0	0%	0	0%
Amikacin	Sensitive ≥ 17	126	97%	46	35%	80	62%
	Resistant ≤ 14	0	0%	0	0%	0	0%
	Semi-sensitive 15-16	4	3%	0	0%	4	3%
Nitrofuran toin	Sensitive ≥ 17	122	94%	40	31%	82	63%
	Resistant ≤ 14	6	5%	4	4%	2	1%
	Semi-sensitive 15-16	2	1%	2	1%	0	0%
Nalidixic acid	Sensitive ≥ 19	56	64%	10	8%	46	35%
	Resistant ≤ 13	72	31%	36	28%	36	28%
	Semi-sensitive14-18	2	5%	0	0%	2	1%

Table 5: presence percentage of BLA-SHV gen and non-presence of BLA-SHV gen in the studied sensitive and resistant E. coli strains

Antibiotic name	Resistant		Sensitive	
	Presence of BLA-SHV gen	Non-presence of BLA-SHV gen	Presence of BLA-SHV gen	Non-presence of BLA-SHV gen
Ampicillin	35%	42%	0%	23%
Amoxicillin	35%	42%	0%	23%
Carbenicillin	35%	5%	0%	59%
Cefalexin	35%	7%	0%	55%
Cephalotin	36%	6%	0%	55%
Cefixime	36%	0%	0%	63%
Ceftizoxime	20%	0%	12%	65%
Ceftriaxone	32%	6%	0%	57%
Cefotaxime	32%	3%	0%	60%
Co-trimoxazole	31%	35%	5%	29%

Table 6: presence percentage of BLA-SHV gen and non-presence of BLA-SHV gen in the studied resistant and sensitive E. coli strains

Antibiotic name	Resistant		Sensitive	
	Presence of BLA-SHV gen	Non-presence of BLA-SHV gen	Presence of BLA-SHV gen	Non-presence of BLA-SHV gen
Ciprofloxacin	12%	11%	23%	49%
Ofloxacin	17%	6%	19%	52%
Gentamicin	9%	5%	26%	60%
Amikacin	0%	0%	35%	62%
Nitofurantoin	4%	1%	31%	63%
Nalidixic acid	28%	28%	8%	35%

From 130 separated E.coli bacterias based on the results of PCR experiments of BLA-SHV gen, 46 bacterias with this gen were recognized. The most resistance with 100 isolated bactrerias

(77%) was observed about ampicillin and amoxicillin antibiotics that all 35% bacterias with BLA-SHV gen were in this group. 54 resistant bacterias to ampicillin and amoxicillin antibiotics lacked BLA-SHV gen.

Respectively, resistant strains to carbenicillin with 40% (52 strains), ceftriaxone with 38% (36 strains), cefxime with 36% (46 strains) and cefotaxime with 35% (32 strains) and resistant strains to cephalexin and cephalotin antibiotics with 42% (54 strains) showed the most antibiotic resistance after resistant strains to ampicillin and amoxicillin. Separated Klebsiella strains showed the most sensitivity, respectively Amikacin with 97% (126 strains), Gentamicin with 86% (112 strains), ciprofloxacin with 72% (94 strains) and ofloxacin with 71% (92 strains).

Table 7: Separated E.coli frequency based on age and gender of infected children to urinary tract infection

Microorganism		Girl			Boy			The number of tested samples
		0-1 months	1 months-3 years	3-6 years	0-1 months	1 months -3 years	3-6 years	
E. coli	No.	8	75	21	10	11	5	130
	Percentage	6.1%	57.7%	16.2%	7.7%	8.5%	3.8 %	100%

Table 8: frequency of resistant E.coli strains to antibiotics with ß-lactam based on age and gender of children

ß-lactam antibiotics		Girl			Boy			The number of all resistant samples to antibiotic
		0-1months	1 months -3 years	3-6 years	0-1 months	1months -3 years	3-6 years	
Ampicillin	Number	5	65	15	6	6	3	100
	Percentage	5%	65%	15%	6%	6%	3%	100%
Ceftriaxone	Number	2	30	9	3	4	2	50
	Percentage	4%	60%	18%	6%	8%	4%	100%

Table 9: frequency of E.coli strains with BLA-SHV gen based on age and gender of infected children to urinary tract infection

E. coli Strains		Girl			Boy			The number of under study samples
		0-1 months	1 months -3 years	3-6 years	0-1 months	1 months-3 years	3-6 years	
The presence of SHV gen	No.	3	28	8	2	4	1	46
	Percentage	6.5%	61%	17%	4.5%	9%	2%	35%

Statistical analysis of the data

Finally, the obtained results were evaluated using version 21 SPSS statistical program in order for determining P value indicator.

Table 10: comparing P Value with the presence of BLA-SHV gen in resistant strains and non-presence of BLA-SHV gen in the studied sensitive strains

Antibiotic		P Value
ßlactams	Penicillins	0.000445
	Cephalosporins	0.000000
Fluoroquinolones		0.008
Quinolones		0.34
Aminoglycosides		0.005
Co-trimoxazole		0.001
Nitrofurantoin		0.213

The results of calculating P value from comparing the presence of BLA-SHV gen in resistant strains and non-presence of BLA-SHV gen in sensitive studied strains for ß-lactam group antibiotics (penicillins, cephalosporins), quinolones, aminoglycosides and cotrimoxazole antibiotic is <0.05 that this problem represents that the presence of BLA-SHV gen in resistant strains and non-presence of BLA-SHV gen in the sensitive studied strains is not accidental and the presence of this gen has been in relation with the induction of resistance to the above antibiotics.

More increase of P Value from the optimum limit for nitrofurantoin and nalidixic acid antibiotics in this paper can result from the association of several gens in resistance affair that in this paper, only BLA-SHV gen has been studied.

Discussion and conclusion

In recent years, prevalence of the infections due to resistant organisms to ß-lactam antibiotics is increasing because of the production of these enzymes. Therefore, recognizing producing strains of broad ß-lactamase has a very important role both in the society and hospital. Because, firstly these strains exist more than whatever they are recognized. Second, ESBLs are a serious threat for nowadays used antibiotics (Krishnamurthy, 1975). Thirdly, the outbreak of infections in health centers is increasing due to selective pressure caused by overdose of broad cephalosporins and lack of effective controlling systems in consuming these drugs. Therefore, only responding to the bacterias with multiple pharmaceutical resistances and rapid and exact recognition of the infections caused by these resistant bacterias and antibiotic treatment are suitable.

Almost 3-5 percent of girls and 1% of boys suffer from urinary tract infection. The average age of catching in girls in the first recognition turn is 2 years, namely in the same age they have urinary continence. In boys, most urinary tract infections occur in the first year of life and they are more common in non- circumcised boys. The amount of prevalence is different depending on age, so that in the first year of life, the ratio of urinary tract infection is 2.8/5.4=boy/son and after 1-2 years, this ratio achieves 1.10. Urinary tract infection is usually caused by colon bacterias. In girls, 75-90% is E. coli infection and after that Klebsiella sp and Proteus sp are created. Urinary tract

infection has usually been mentioned as an important factor in creating insufficiency of kidney final stage. Risk factors in creating urinary tract infection include: gender (girl), non- circumcised boys, urine reflux, urinary obstruction, manipulation of the urinary tract, washing of perineum from back to front, constipation, anatomic abnormalities like labia adherence and neurogenic bladder.

In Iran, different studies have been implemented in this regard that we can refer to the following cases. In accomplished studies by Mobin et al. (2010) on clinical samples which took place in ICU departments of three training hospital of Shariati, Sina, children's medical center; from among 150 isole of enterobacteriaceae family, 89 isole were the producer of ESBL. The most common enterobacteriaceae species producing ESBL included: klebsiella pneumonia 76%, E. coli 60%, enterobacter kolvase 47%. This study showed that producer enterobacteriaceae bacterias of ESBL in Patients in ICU section were prevalent.

In another conducted study by Reza Torshizi et al. (2008) on separated enterobacteriaceae isolates in training hospitals of Shahr-e Kord, the spread of broad ß-lactamase was 28% and frequency of CTX-M ß-lactamase gen in all positive phenotype enterobacteriaceaes was reported 50.5%.

Nowadays, the problem of resistance to ß-lactam drugs and broad cephalosporins is a serious growing problem and due to the limitation of these antimicrobial materials, we need new antibiotics for their replacement. The findings of this research show that in the studied society, the amount of resistance to third generation cephalosporins and also the spread of generating ESBLs strains are rather high. Therefore, in order for controlling the infections caused by such microorganisms, we should consider the following points:

- Reduction and change in consumption strategy of antibiotics
- Increased activity of control committees of hospital infections
- Patients' hygiene observance and control principles of hospital infections
- Exact and rapid recognition of the infections caused by resistant bacterias and suitable antibiotic treatment
- Awareness of the prevalence of ESBLs producing strains in society and hospitals
- Increasing awareness of the society from drug resistances and its dangers for the people and the role of all society members regarding reducing such resistances.

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