Evaluation of ESBL Frequency in Enterobacteriaceae Isolated from Clinical Specimens in an AJA Hospital in Tehran

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**ABSTRACT**

**Background:** Antibiotic resistance among bacteria has been one of the health problems in the world since the past. A type of resistance is due to the production of enzymes called beta-lactamases which cause resistance to beta-lactam drugs and led to the emergence of new types of Extended-Spectrum Beta-Lactamases (ESBLs). Due to the importance of ESBL producing strain in uncontrolled hospital infections, the present study was Designed for evaluation of ESBL frequency in isolated Enterobacteriaceae from clinical specimens in an AJA hospital in Tehran.

**Methods:** In this study, 100 clinical specimens collected from AJA. All specimens were identified by standard bacteriological and biochemical methods and antimicrobial susceptibility test was performed using disc diffusion method. Production of broad-spectrum beta-lactamase enzymes was evaluated using a mixed disk method with cefotaxime and ceftazidime antibiotics with clavulanic acid or alone. Results were interpreted according to CLSI guidelines.

**Results:** From 100 specimen, 75 isolates (75%) were confirmed as an Enterobacteriaceae family. Most sensitivities observed in Imipenem (97.33%), Meropenem (94.66%), Piperacillin (68%), and Gentamicin (64%). Overall 42.66% of isolates produced ESBLs.

**Conclusion:** According to the results, broad-spectrum beta-lactamase production in isolated bacteria from a clinical specimen of AJA hospital was high and 42.66% of isolates produced ESBLs. Due to the high resistance of these strains to most common antibiotics, identification of these pathogens, applying control methods in relation to the production and release of this type of bacteria and the correct use of antibiotics should be put on the agenda.


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Introduction

In the last decade, antibiotic resistance in bacteria is considered as a major public health problem. Due to the incorrect use of antibiotics, the number of bacteria resistant to antibiotic agents is increasing rapidly, some of which are of great importance in nosocomial infection. A type of resistance is due to the production of enzymes called beta-lactams, which is a cause of resistance to beta-lactam drugs (1, 2). These enzymes are diverse in bacteria and, in response to selective pressure, antibiotics are constantly being mutated, especially in the active site of the enzyme, which has led to the emergence of new types of Extended-spectrum beta-lactamases (3). The strains produced by these enzymes are resistant to penicillins, broad-spectrum cephalosporins, and Aztreonam (Monobactam), but are inhibited by beta-lactamase inhibitors such as clavulanic acid. The First report of ESBL isolates was reported from Germany in 1983 (4, 5).

The treatment of infections caused by the bacteria that produce these enzymes is complicated and has become a global problem. On the one hand, resistance to a wide range of beta-lactam antibiotics is observed and On the other hand, ESBL genes which transported on a large plasmid is easily transmitted among Enterobacteriaceae members which leads to the accumulation of resistance genes and the production of plasmid strains with multiple resistances which is associated with a failure in the treatment of infections and an increase in the mortality rate of patients and an increase in the financial burden of treatment (6, 7).

In Iran, there is no comprehensive study on this issue. In some studies, drug resistance to third-generation cephalosporins and other broad-spectrum drugs has been reported, which mainly focused on the resistance of commonly used gram-negative bacilli isolated in hospitals against broad-spectrum drugs such as ceftazidime, ciprofloxacin, and Cefitizoxime, third-generation cephalosporins (8).

The beta-lactamases producing bacteria have been considered as a clinical threat and have caused physicians to be concerned about the treatment of infections caused by these organisms (responsible for long-term hospital infections with adverse effects). Our awareness at different times of the frequency of these resistant isolates will increase the effectiveness of various drugs in the treatment of common infections, especially in experimental treatments. Given the increasing and unnecessary consumption of antibiotics and consequently increased antibiotic resistance, increasing medical costs and ultimately increasing mortality and due to the role and importance of the ESBL producing strains in uncontrolled nosocomial infection, the present study was designed In order to evaluate the frequency of ESBL in Enterobacteriaceae isolated from clinical cases in AJA hospital in Tehran.

Material and methods

Isolation and Identification

In this descriptive cross-sectional study, 100 clinical specimens (Includes urine, blood, feces, tissues and them secretions) within 3 months (late July to late October of 2017) collected from AJA hospitals in Tehran and transferred to Faculty of Paramedical Science of AJA University of Medical Sciences. Bacterial and biochemical standard tests include Gram staining, catalase, Sugar fermentation in the TSI medium, indole production and motility in SIM medium - reaction in MR, VP and Simmons’ citrate (IMViC), lysine decarboxylase, H2S production, and urea test were used for identification of isolates (9).

Antimicrobial susceptibility assessment

Susceptibility and resistance of isolates evaluated by disc Agar diffusion method (10). For this purpose, 12 antibiotic discs were used include aztreonam (30μg), Piperacillin (100μg), Gentamicin (10μg), Imipenem (10μg), Meropenem (10μg), Cefepime (30μg),
Ceftazidime (30μg), Cefotaxime (30μg), Ceftizoxime (30μg), Ceftriaxone (5μg), Ciprofloxacin (30μg) and Nalidixic acid (30μg) purchased from the company Himedia.

**Phenotypic evaluation for the production of ESBL**

Screening of ESBL producing organisms was performed by combined disk method with Ceftazidime, Cefotaxime, Ceftazidime/Clavulanic Acid (30 / 10 μg) and Cefotaxime /Clavulanic Acid (30 / 10 μg) antibiotic disk (Himedia co.). Briefly, 0.5 McFarland suspension was prepared from pure bacterial culture, and after that cultured in Mueller Hinton Agar. After 15 min, Ceftazidime, Ceftizoxime, Ceftazidime/Clavulanic Acid and Cefotaxime/Clavulanic Acid disks was placed with 20 mm spacing and incubated for 24h at 37°C. ESBL production was determined by increasing the diameter of the inhibition zone of 5 mm or more around the disc of ceftazidime + clavulanic acid or cefotaxime + clavulanic acid (6, 11).

Disc diffusion sensitivity and ESBL evaluations were interpreted based on CLSI protocols (10). The standard strain of Escherichia coli ATCC 25922 was used as a positive control.

**Results**

From 100 specimens, 61 samples (61%) for women and 39 (39%) for men; 64 (64%) of the cases were hospitalized and 36 (36%) cases were related to outpatients. After confirmatory tests, 75 (75%) of 100 samples were confirmed as Enterobacteriaceae family members (Table 1).

The results of assessing the sensitivity of a range of antibiotics used in hospitals using a disk diffusion method are presented in Table 2. The highest susceptibility and resistance of the isolates to the studied antibiotics studied were shown in diagram 1 and 2, respectively.

After antibiogram for selected antibiotics, for confirmation of ESBL producing isolates, the combined discs of cefotaxime/clavulanic acid, ceftazidime/clavulanic acid were used (Fig 1). Accordingly, 42.66 % (32 isolates) confirmed as the ESBL producing organisms. The frequency of positive and negative ESBL strains was shown in Table 3.

**Discussion**

The phenomenon of drug resistance is immediately recognized several years after the mass consumption of antibiotics in human populations and is seen in single or multiple drugs forms. With isolates resistant to antibiotics, antibiotic-resistant Enterobacteriaceae are also significantly removed from clinical specimens (5). Empirical treatment of drug-resistant infections increases costs, complications for the patient and endangers the patient's life. The choice of antibiotics should be based on the type of pathogen, antibiotic susceptibility patterns, antibiotic resistance mechanisms, a pharmacokinetic model of the drug, drug tolerance, and patient safety. For this reason, all treatment centers must have a codified policy and appropriate programs to ensure proper, effective and economical administration of antibiotics to prevent the development of resistant microorganisms and reduce their release (12).

Shahchehraei et al., in a study of 150 isolates of Klebsiella pneumoniae in Tehran, resistance to Piperacillin, cefotaxime, and ceftazidime were reported 55%, 32%, and 31% reported, respectively. Also, none of the isolates showed resistance to imipenem (13). Ghenaat and Sadeghian evaluated the antibiotic resistance of 1261 isolates against ceftazidime, ciprofloxacin and ceftizoxime, and it was found that the isolates had a relatively high sensitivity to all three drugs (14). In a similar study by Eslami and Najari Pirayesh, the resistance to ceftazidime, ciprofloxacin, and ceftizoxime was reported to be about 40% (15). Rastegar Lary et al. reported 14% of clinical isolates to ceftazidime (8). Cheung et al., determined resistance pattern of 36243 isolates. They found that 5.5% of Escherichia coli, 16.6% of Klebsiella pneumoniae and 6.8% of Pseudomonas aeruginosa isolates were resistant to ceftazidime (16). In the study, Mansuri et al.
resistance levels of *Escherichia coli* isolate to ciprofloxacin was 41% (17). In the study of Mohammadi Mehr et al. in 2007, *Escherichia coli* resistance to ciprofloxacin and gentamicin was 58.33% and 27.77% respectively (18). In a study conducted by Mohammadi et al. in Falavarjan, the resistance to Nalidixic Acid was reported to be 20.1% (19). In a study conducted by Madani et al. in 2006 in Kermanshah, resistance to meropenem, cefotaxime, and ceftriaxone was 11.8%, 30.4% and 29.8%, respectively (20).

Prevention of drug resistance is one of the most important issues in the treatment of infections in the community. Due to the increased prevalence of resistance to antibiotics, prompt and timely detection of resistant strains is necessary in order to select appropriate treatment options and prevent the spread of resistance. In comparing the present study with other studies in this field, we have seen similarities and differences in the results. Differences can be found between the source of the infection and the type of sample examined, the infection control system, the different duration of hospitalization, the high and arbitrary use of antibiotics And the difference in patterns of antibiotic use in different regions (12). In the case of high sensitivity to carbapenem antibiotics, the lack of access to these drugs (due to the hospitalization of these drugs) for the community and also high resistance to some of the antibiotics studied, is unusual and incorrect use It is also available without restriction of these drugs.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>15</td>
<td>46.8%</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>8</td>
<td>25%</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td><em>Serratia spp</em></td>
<td>2</td>
<td>6.2%</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>2</td>
<td>6.2%</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>3</td>
<td>9.3%</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100%</td>
</tr>
</tbody>
</table>

ESBL producing bacteria are often resistant to several antibiotic classes, leading to treatment failure and serious problems. The first ESBL isolates were reported in Germany in 1983 and subsequently from various countries around the world which are considered as new health emergencies. Nowadays, ESBL producing pathogens are the leading cause of hospital infections, such as urinary tract infections, catheter-related infections, neonatal meningitis, respiratory infections, and sepsis, which rising rapidly. These strains have created livelong problems for clinical microbiologists, clinicians, professional infection control specialists, and antibacterial drugs manufacturers (5, 6).

In this study, ESBL production rate was 42.66%. In studies conducted in Iran and around the world, in the same way, different results have been reported on the production of ESBL in Enterobacteriaceae family members. In a study by Moyo, in Tanzania, form 270 urinary *E. coli* pathogens and *Klebsiella* species, 122 cases (45.2%) was ESBL producing pathogens (21). Kader et al. In Saudi Arabia, ESBL producing a...
potential of 2,755 of *Escherichia coli* and *Klebsiella pneumoniae* clinical specimens were tested and showed that 268 (11%) isolates were ESBL positive (22). Gulaya and colleagues in İzmir, Turkey examined 44 strains of *Klebsiella pneumoniae*, randomly isolated from nosocomial infection, which 84% of strains were ESBL positive (23). Christine et al. evaluated the 139 strains of *Klebsiella pneumoniae* isolated from 19 laboratories in 11 states of USA with dual disc method and showed that 114 (84%) of isolates were ESBL producing strains (24). In a study in the United States, from 906 isolates of Enterobacteriacea 83 (9%) was ESBL producing strains (25). In the study of Tashakori et al. in Rafsanjan, 19.66% of the isolates were resistant to third-generation cephalosporins, which 10.77% of them were ESBL positive (26). In the study of Nakhkhai Moghadam in Mashhad, 35 (32.11%) ESBL positive isolates from 109 bacteria were positive for extended spectrum beta-lactamase test (27). Behroozi et al. reported that from 620 *Escherichia coli* isolates, 132 (21%) and from 115 *Klebsiella pneumoniae* 18 (15%) isolates produced the β-lactamase enzyme (28). In the study of Babaei et al. in Gorgan, resistance to cefotaxime was observed in 70 isolates of Escherichia coli, of which 62 (88.6%) were identified in the confirmatory test as ESBL positive (29). In a study by Sharifi Yazdi and his colleagues in Khoy, of the total 188 isolates of *Escherichia coli*, 56 (29.8%) isolates produced ESBL (30). In the study of Mirsalahian and his colleagues in Tehran, form 394 strains of *Escherichia coli* 25.25% were ESBL positive (11). Masjedian and colleagues examined 148 strains of *Escherichia coli* with combined and dual disc diffusion method which 51 (76 cases) and 70% (49 cases) were ESBL producing strains, respectively (31).

The prevalence of ESBL-producing strains can be attributed to the inappropriate and incorrect use of antibiotics, the long-term hospitalization of individuals in different parts of hospitals, aging (immunity reduction), and so on.

**Conclusion**

In conclusion, the prevalence of ESBL productive strains is relatively high, and given the importance of this issue in treatment and general health and its costs, it is necessary to select an appropriate antibiotic for the treatment of infections suspected to organisms that produce β-lactamase. On the other hand, due to the high resistance of these strains to most common antibiotics and recently even resistance to carbapenems, the daily identification of these pathogens, the use of control methods related to the production and release of this type of bacteria, as well as appropriate use of antibiotics should be put on the agenda.

Given the increasing trend of ESBL strains in hospitals, it is suggested that phenotypic screening and, if possible, genotypic detection of these strains be made prior to the administration of antibiotics in order to minimize the serious consequences. It is also recommended that accurate statistics of the presence of these strains in the hospital made regularly to control and prevent the dissemination and to be provided to physicians. On the other hand, it is recommended that the administration of broad-spectrum antibiotics and main hospital antibiotics be taken by specialist doctors to minimize the occurrence of such cases.
Figure 1. Highest sensitivity of the isolates to the studied antibiotics.

Figure 2. Highest resistance of the isolates to the studied antibiotics.
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Conflict of interest

None declared.

Financial disclosure

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References

15. Eslami M, Najar Peerayeh. Phenotypic and


