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Antimicrobial resistance surveillance among hospitalized and non-hospitalized extend-spectrum beta-lactamase producing Escherichia coli from four tertiary-care hospitals in Isfahan, Iran; 2008-2011

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For the first time we launched a prospective laboratory-based Antimicrobial Resistance Surveillance System in Isfahan province, Iran. In a cross-sectional study prevalence of ESBL (extend-spectrum beta-lactamase) producing Escherichia coli isolates and their antimicrobial susceptibility patterns according to CLSI (Clinical and Laboratory Standards Institute) guideline were studied in four tertiary-care hospitals from January 2008 to February 2011. Data were extracted from the participating hospital and converted centrally into a standard format using WHONET 5.6 software (WHO, Geneva, Switzerland). Among a total of 2035 consecutive clinical isolates identified as E. coli, 898 (44.1%) and 432 (21.2%) were ESBL producers for hospitalized and non-hospitalized patients respectively (95% confidence intervals: 1.3771 to 2.013, Odds ratio: 1.6649, P<0.0001). Two studied hospitals presented 64 and 56% ESBL producing E. coli. The both non-hospitalized and hospitalized isolates were more resistant to ampicillin (84.4 and 94.9% respectively), trimethoprim/sulfamethoxazole (60.1 and 84.8% respectively) and cefazolin (42 and 72.2% respectively). In non-hospitalized isolates, nitrofurantoin (with only 10.2% resistant) and ciprofloxacin (31%) were the effective antibiotics. Imipenem (2.5%), amikacin (17%) and nitrofurantoin (23.1%) were the effective antibiotics against hospitalized E. coli isolates recommended these agents as the first choices for patients’ treatment. Our results showed importance of continued antibiotic surveillance that will provide succession in the efforts of infection control programs for the future.

Key words: Antibiotic sensitivity pattern, Antimicrobial resistance surveillance, ESBL producing Escherichia coli, WHONET 5.6 software.

INTRODUCTION

Antimicrobial resistance is one of the significant problems worldwide leads to curative failure of therapy and it has been a threat to the patient’s safety in both developing and developed countries (Blomberg et al., 2007; Mylette et al., 2001). Therefore, awareness of the local prevalence of pathogens and their antimicrobial sensitivity patterns is essential for clinicians. Establishing surveillance systems integrate clinical and laboratory data and using that the necessary data can be captured and strengths of both data sets can be combined. There is evidence that the wiser use of antimicrobials may
reduce the rate of resistance emerges (Coque et al., 2008; Spanu et al., 2002; Winokur et al., 2001). Thus information from surveillance of antimicrobial resistance provides a powerful tool for the control of resistance. The aim of antimicrobial resistance surveillance is providing the information that is necessary to obtain an approach to the management of communicable diseases that diminishes morbidity and mortality.

The main applications of surveillance information are to optimize the use of antimicrobials and assist in the prevention and control of antimicrobial resistance at the local, regional and national levels (Coque et al., 2008; Spanu et al., 2002; Winokur et al., 2001; Yagi et al., 2000). WHONET is a computer program designed to meet the needs of surveillance that was mentioned earlier. It helps in forming hospital drug policy, identification of hospital outbreaks and recognition of quality control problems in the laboratory (Coque et al., 2008; Spanu et al., 2002; Sahm et al., 2001).

Extended spectrum beta lactamase (ESBL) production is one of the different mechanisms of drug resistance in Gram-negative bacilli predominantly present in Escherichia coli and Klebsiella pneumoniae (Bisson et al., 2002; Einhorn et al., 2002; Emery and Weymouth 1997; Harris et al., 2007; Lautenbach et al., 2001; Minarini et al., 2007). Infectious Diseases Society of America listed ESBL-producing Klebsiella sp. and E. coli as one of the six drug-resistant microbes to which new therapies are urgently needed (Johann and Laupland 2008; Kim et al., 2002; Warren et al., 2008). ESBLs are a group of active-site serine enzymes that are able to hydrolyse a wide range of β-lactams, including the most recently developed cephalosporins and monobactam (Johann and Laupland 2008; Moyo et al., 2010; Nazik et al., 2011).

To date, official information has not been available on the overall occurrence of ESBL-producing isolates in most Iran hospitals.

Thus, the authors aimed to determine the prevalence of ESBL-producing E. coli by launching of an “Antimicrobial Resistance Surveillance” program from 2008 in Isfahan province, Iran. The Objective of this study was to evaluate the prevalence and susceptibility pattern of Escherichia coli isolates producing (ESBLs) in Isfahan, Iran from 2008 to 2011.

MATERIALS AND METHODS

Study design and selection of participating hospitals

We initiated a prospective laboratory based surveillance study of antimicrobial resistance (Antimicrobial Resistance Surveillance System) in Isfahan province, Iran from September 1, 2008. This was a cross-sectional study and to obtain representative data regarding to infections caused by ESBL producing E. coli, four tertiary-care participating hospitals were chosen based on most receiving patients number, containing 470-bed Gharazi hospital, 950-bed Alzahra hospital, 134-bed Fatemeh-Zahra and 493-bed Shariati hospital. These hospitals act as referral hospitals for a broad area of Isfahan province and cover a population of about 1,500,000.

Sample collection

This study included all clinical specimens submitted for bacterial culture at the microbiology laboratories of mentioned hospitals. Both hospitalized and non-hospitalized infections have been conducted. Hospitalized infections were defined as patients confined to bed in hospital and non-hospitalized infections were defined as infections in patients that had no previous contacts with hospitals or long-term care facilities in the last 2 weeks. Clinical samples included: urine, blood stream, sputum, wound and abscess and others. Urine sample collection was case dependent and included urine from midstream urine, catheter, or suprapubic aspiration. A wound infection was identified by the presence of purulent discharge from the incision with erythematous cellulitis, inflammatory acid, pain, and demonstrable fluid collection noted on ultrasound after surgery. Aspirates were obtained by preparing the wound and abscess area with alcohol, inserting a sterile needle through the healing incision and aspirating fluid into a sterile syringe. Drawn blood samples from the patients were cultured on blood agar media and incubated at 35°C for 18–24 h.

Characterization and Identification of Bacteria from Clinical Specimens

Bacteria recovered from clinical specimens were identified by standard biochemical methods (Forbes et al., 1998). The samples were cultured on nutrient agar, Mac Conkey agar, Blood agar and Eosin Methylene Blue (EMB) agar (purchased from Himedia Company). The plates were incubated at 35°C for 24 h and the pure isolates characterized and identified according to Gram stains and biochemical tests such as catalase, oxidative, indole production, citrate utilization, triple iron sugar utilization, urea test, Oxidative-fermentative test with glucose, ONPG test, and methyl red-Yoses Proskauer as described in standard bacteriological methods (Forbes et al., 1998).

ESBL production assay

All E. coli isolates from clinical specimens were tested for ESBLs production. The ESBL-producing were determined by the phenotypic method using screening Kirby-Bauer disk diffusion method according to the (CLSI) (2006). A confirmatory test of ESBL-production was performed with the double-disk (combined-disk) method. The zones of inhibition of each isolate were tested on Mueller-Hinton agar plates with the disks containing 30 μg of cefazidime and cefotaxime alone and in combination with 10 μg of clavulanic acid, respectively (all above disk purchased from Mast Company). An organism was classified as having an ESBL producing phenotype if the zone of inhibition produced by at least one combination disk was more than 5 mm larger than that produced by the corresponding antimicrobial disk without clavulanic acid. Consecutive nonduplicate clinical isolates of ESBL-producing E. coli were collected for further investigation.

Antibiotic susceptibility test

Antibiotic sensitivity pattern of isolates to common antibiotics used in the hospital was determined by the Kirby Bauer’s disc diffusion method on Mueller-Hinton agar. Choice of antibiotic disks was determined by (CLSI) guidelines (Wayne, 2006). All isolates were tested against beta-lactam agents, including ampicillin, ceftazidime,
Table 1. The patient’s demographics and overall prevalence of *E. coli* isolates in the clinical samples.

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>Number</th>
<th>Sex</th>
<th>Patient</th>
<th>Specimen type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-hospitalized</td>
<td>Hospitalized</td>
</tr>
<tr>
<td>Gharazi</td>
<td>626</td>
<td>547</td>
<td>79</td>
<td>386</td>
</tr>
<tr>
<td>Alzahra</td>
<td>659</td>
<td>435</td>
<td>224</td>
<td>250</td>
</tr>
<tr>
<td>Fatemeh-Zahra</td>
<td>343</td>
<td>277</td>
<td>66</td>
<td>217</td>
</tr>
<tr>
<td>Shariati</td>
<td>407</td>
<td>314</td>
<td>93</td>
<td>287</td>
</tr>
<tr>
<td>total</td>
<td>2035</td>
<td>1573</td>
<td>462</td>
<td>1140</td>
</tr>
</tbody>
</table>

**Data collection**

Data were extracted from the participating hospital and converted centrally into a standard format using WHONET 5.6 software (WHO, Geneva, Switzerland), with duplicates eliminated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2006 and CLSI 2010).

**Statistical analyses**

Differences between proportions were analyzed using the Chi-square test. Association was determined by calculation of the odds ratio (OR) with 95% confidence intervals (CI). The null hypothesis was rejected for values of *p*<0.001.

Statistical analyses were performed with SPSS version 17 software (SPSS Inc., USA) and Microsoft Office Excel 2007.

**RESULTS**

**Patients’ data, characterization and identification of bacteria from clinical specimens**

Bacteria recovered from clinical specimens were identified by standard biochemical methods. During the 3-years study period (From January 2008 to February 2011), a total of 2035 consecutive clinical isolates were identified as *E. coli* at four hospitals under study. Table 1 shows the patients demographics in each hospital and the overall prevalence of these organisms in the clinical samples (collected using WHONET 5.6 software).

The sources of these 2035 isolates were as the following: 1804 (88.6%) from urine cultures, 73 (3.65%) from blood cultures, 64 (3.15%) from wound cultures, 19 (0.92%) from sputum or bronchoscopy specimens, 20 (0.98%) from abscess cultures and 55 (2.7%) from other clinical samples. Females had higher overall prevalence (77.3%) than the males (22.7%) as shown in Table 1. A total of 895 (44%) isolates were implicated in hospitalized infections and 1140 (56%) isolates were implicated in nonhospitalized infections.

**Prevalence of ESBL-producing *E. coli***

All 2035 *E. coli* isolates were examined for ESBL production. Among these isolates, in case of nonhospitalized infections, 19.7 and 19.8% were simultaneously resistant to ceftazidime and cefotaxime respectively and these data for hospitalized infections were 59.4 and 64.2% to ceftazidime and cefotaxime respectively. Confirmatory test of ESBL-production with the double-disc (combined-disc) method showed 21.2 and 44% ESBL production for nonhospitalized infections and hospitalized infections respectively (*P*<0.001). Frequency of ESBL-producing *E. coli* in hospitalized and nonhospitalized patients in 4 selected hospitals globally and in each hospital separately is showed in Table 2A and 2B. Globally, among the 2035 *E. coli* isolates in all selected hospitals for hospitalized and nonhospitalized patients, 898 (44.1%) and 432 (21.2%) were ESBL producers respectively (*P*<0.001) (Table 2A). In case of nonhospitalized infections, ESBL-producing *E. coli* percentages for selected hospitals were as following: 21% Gharazi hospital, 27% Alzahra hospital, 27% Fatemeh-Zahra...
Table 2. Frequency of ESBL-producing *E. coli* in hospitalized and nonhospitalized patients in 4 selected hospitals globally and in each hospital separately with *P* value <0.001.

<table>
<thead>
<tr>
<th>ESBL-producing <em>E. coli</em></th>
<th>Frequency (%)</th>
<th>Significance level</th>
<th>Odds ratio</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalized</td>
<td>44.1</td>
<td><em>P</em> = 0.0057</td>
<td>2.762</td>
<td>1.6786</td>
</tr>
<tr>
<td>Non-hospitalized</td>
<td>21.2</td>
<td><em>P</em> &lt; 0.0001</td>
<td>8.988</td>
<td>4.8681</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P</em> = 0.0645</td>
<td>1.849</td>
<td>1.6561</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P</em> &lt; 0.0001</td>
<td>6.905</td>
<td>5.101</td>
</tr>
</tbody>
</table>

95% confidence intervals (CI): 1.3771 to 2.013, Odds ratio: 1.6649, z statistic: 5.263, Significance level (*P* value): *P* < 0.0001.

Table 3. Frequency of ESBL producing *E. coli* in male and female patients in four hospitals globally.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency (%)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>43.2</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Female</td>
<td>33.9</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

hospita and 19.8% Shariati hospital (Table 2B). These data in hospitalized infections were as following: 31% Gharazi hospital, 64% Alzahra hospital, 25.4% Fatemeh-Zahra hospital and 56% Shariati hospital (table 2B). A number of 533 female patients among 1573 with *E. coli* infection were ESBL positive (33.9%) and this result for male was 200 patients among 462 (43.2%) (Table 3).

Antimicrobial susceptibility pattern

Table 4 shows the susceptibility and resistance percentages of the non-hospitalized and hospitalized isolates and Figure 1 compared antibiotics resistant percentages for each antibiotic in nonhospitalized and hospitalized isolates. In nonhospitalized isolates, the rates of resistance to non-β-lactam agents, including ampicillin, amikacin, gentamicin, trimethoprim/ sulfamethoxazole, nitrofurantoin, Nalidixic acid and ciprofloxacin were 84.4, 8.2, 18.6, 60.1, 10.2, 40.8, and 31.4%, respectively (P<0.001). In nonhospitalized isolates, the rates of resistance to non-β-lactam agents, including, amikacin, gentamicin, trimethoprim/sulfamethoxazole, nitrofurantoin, Nalidixic acid and ciprofloxacin were 8.2%, 18.6, 60.1, 10.2, 40.8, and 31.4%, respectively (P<0.001). In hospitalized isolates, the rates of non-β-lactam antibiotic resistance were as following: 17% for amikacin, 60% for gentamicin, 84.4% for trimethoprim/ sulfamethoxazole, 23.1% for nitrofurantoin and 47.5% for ciprofloxacin (P<0.001). Resistance percentages to β-lactam antibiotic antimicrobial agents in non-hospitalized isolates were as follows: ampicillin 84.4%, ceftazidime 19.7%, cefotaxime 19.8%, cefazolin 42% and cefotizoxime 12.2%. In addition, Resistance percentages to β-lactam antibiotic antimicrobial agents in hospitalized isolates were as follows: ampicillin 94.9%, ceftazidime 59.4%, cefotaxime 64.2%, cefazolin 72.2%, cefepime 62.5%, and imipenem 2.5%. Comparing of antibiotics resistant percentage in nonhospitalized and hospitalized isolates (Figure 1) showed that both nonhospitalized and hospitalized isolates were more resistant to ampicillin, trimethoprim/sulfamethoxazole and cefazolin. In nonhospitalized isolates, amikacin (with only 8.2% resistant), nitrofurantoin (10.2%) and ciprofloxacin (31%) were the effective antibiotics. Imipenem (2.5%), amikacin (17%) and nitrofurantoin (23.1%) were the effective antibiotics against hospitalized *E. coli* isolates.

**DISCUSSION**

The rapid spread of ESBL-producing bacteria worldwide signified needing of a continuous monitoring systems and effective infection control measures (Bradford 2001; Mylette et al., 2001; Spanu et al., 2002). Comprehensive epidemiologic data and characterization of ESBL-producing isolates among hospitalized and nonhospitalized in Iran are still rarely documented and previous studies failed in extend number of patients, long-term study or comprehensive epidemiologic data, despite of their useful content leading to a global view on ESBL-producing bacteria in Iran. For example, Bazzaz et
Table 4. Antimicrobial pattern of ESBL producing *E. coli* isolates from different samples to studied antibiotics in hospitalized and non-hospitalized infections.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Non-hospitalized</th>
<th>Hospitalized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>I (%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>14.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>54</td>
<td>4</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>79.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>75.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>39.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>67.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Amikacin</td>
<td>81.6</td>
<td>10.2</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>85.9</td>
<td>4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>78.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>85.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>54.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Cefepime</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Imipenem</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


al. (2009) determine the prevalence of (ESBL) producing *Escherichia coli* in one 900-bed general teaching hospital (59.2%) during 4 months and Mirzaee et al. (2009) showed 56% (n=140) of *E. coli* isolates produced ESBLs from 3 university hospitals in Tehran during 6 months. Therefore, for first time we launched a prospective surveillance study of laboratory based antimicrobial resistance (Antimicrobial Resistance Surveillance
System) in Isfahan province, Iran. In this surveillance study, the prevalence of ESBL-producing E. coli in hospitalized and non-hospitalized infections has been studied during three years and WHONET software as an analysis program helping in forming hospital drug policy was used. The prevalence of ESBL-producing E. coli under antimicrobial resistance surveillance system has been studied in several countries (Coque et al., 2008; Minarini et al., 2007; Pattarachai et al., 2008; Sahm et al., 2001; Sherley et al., 2004; Spanu et al., 2002; Sturm et al., 2010; Winokur et al., 2001; Yagi et al., 2000). The overall prevalence of ESBL-producing E. coli obtained in this study in both hospitalized and non-hospitalized patients, exceptionally in two studied hospitals, was higher than the others studies containing different developed and developing countries. For example, our results in compare with published data from all Europeans countries such as France (Coque et al., 2008), Italy (Coque et al., 2008; Spanu et al., 2002), Netherlands (Sturm et al., 2010), Germany (Coque et al., 2008), and Spain (Coque et al., 2008; Winokur et al., 2001) also in United States (Sahm et al., 2001), Australia (Sherley et al., 2004), Japan (Yagi et al., 2000), Tanzania (Blomberg et al., 2007), Thailand (Pattarachai et al., 2008) and Pakistan (Riaz et al., 2011), showed the high prevalence of ESBL-producing isolates in present study. Two hospitals centers studied in present study (Alzahra and Shariati) that had very high prevalence in hospitalized patients and high odds ratio, are teaching-treatment hospitals that the large amount of third-generation cephalosporins are consumed as prophylaxis and actually third-generation cephalosporins are the first treatment option because of high risks that are presented in these hospitals (for example nosocomial infections risk). Organisms producing ESBL are typically multi drug resistant and previous exposure to an antibiotic, especially to extended-spectrum cephalosporins is a risk factor for infection with ESBL-producing bacteria (Bradford 2001; Einhorn et al., 2002; Mylette et al., 2001; Smet et al., 2010). Therefore, the very high prevalence of ESBL-producing isolates described in this study was probably due to the large amount consumption of third-generation cephalosporins. Although the high prevalence of ESBL producers isolated from those hospitals suggests that ESBL-producing strains are already endemic in these hospitals. In addition, the high prevalence of ESBL producing E. coli not only in nonhospitalized patients, but also in global nonhospitalized patients in compared to other studies revealed a concerning about spread of these agents in Isfahan province of Iran. Males had a higher prevalence of infection due to ESBL producers E. coli than females despite low number of infection. This result was despite the results reported earlier by Pattarachai et al. (2008).

Comparing of antibiotics resistant percentage in nonhospitalized and hospitalized infections showed that for all used antibiotics, hospitalized isolates were more resistant than non-hospitalized ones, similar to findings from other studies (Coque et al., 2008; Smet et al., 2010; Warren et al., 2008). In addition, both nonhospitalized and hospitalized isolates were more resistant to first line drugs including ampicillin, trimethoprim/sulfamethoxazole and cefazolin. This result that is similar to other studies in developing countries (Blomberg et al., 2007; Pattarachai et al., 2008; Riaz et al., 2011) perhaps due to wide use of these drugs because of their relatively cheap cost and easily administration. In nonhospitalized isolates, amikacin (not used for nonhospitalized patients routinely), nitrofurantoin and ciprofloxacin were the effective antibiotics. Imipenem, amikacin and nitrofurantoin were the effective antibiotics against hospitalized ESBL-producing E. coli isolates. The progressive increase of ESBL producing E. coli revealed the need to re-evaluate current antibiotic therapy for these infections especially considering this fact that antibiotic susceptibility pattern for different local is variable (Garau, 2008). Even though not recommended, presented study showed nitro-furantoin had considerable effect on serious infections caused by ESBL-producing E. coli in hospitalized patients and therefore considered to be an alternative rather than a first-line therapeutic agent for this clinical syndrome. The most ESBL-positive isolates were susceptible to imipenem, indicating that this agent is the best option drug for treating serious infections caused by ESBL-producing E. coli. The carbapenems antibiotics including ertapenem, imipenem, and meropenem have widely recognized as the first choice for the treatment of serious infections caused by ESBL-producing Enterobacteriaceae (Bradford 2001; Harris et al., 2007). However, high cost, the necessity for the parenteral route of administration, and broad spectrum of activity that may help infections with yeasts and bacteria with the potential selection of carbapenem-resistant variants are the potential drawbacks of their use (Johann and Laupland 2008). We showed that amikacin and nitrofurantoin are also the effective antibiotics against serious infections caused by ESBL-producing E. coli in studied local.

Further studies aimed at finding the molecular mechanisms of resistance will provide a better understanding of the epidemiology associated with ESBL-producing E. coli in Iran.

Conclusion

Establish systems for monitoring antimicrobial resistance in hospitals and the community and link these findings to resistance and disease surveillance data are fundamental to developing treatment guidelines accurately and to assessing the effectiveness of interventions appropriately. For first time we launched a prospective Antimicrobial Resistance Surveillance System in Isfahan province, Iran. Our result showed very high ESBL producing E. coli prevalence in Isfahan province that alarm an emerging public-health concern, showed emergence
need for developing a treatment guideline for antibiotic consumption. Continued surveillance will provide an important function for success in the efforts of infection control programs for the future and it is a critical step for controlling the growing worldwide threat of antimicrobial drug resistance.

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