Assessment of extended-spectrum β-lactamases and integrons among Enterobacteriaceae in device-associated infections: multicenter study in north of Iran

Masoumeh Bagheri-Nesami, Alireza Rafiei, Gohar Eslami, Fatemeh Ahangarkani, Mohammad Sadegh Rezai, Attieh Nikkhah and Azin Hajalibeig

Abstract

Background: Device-associated nosocomial infections (DA-NIs), due to MDR Enterobacteriaceae, are a major threat to patient safety in ICUs. We investigated on Extended-spectrum β-lactamases (ESBL) producing Enterobacteriaceae and incidence of integrons in these bacteria isolated from ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections (CAUTIs) in 18 governmental hospitals in the north of Iran.

Methods: In this cross-section study, the antibiotic susceptibility test was performed using the MIC method; also, phenotypically detection of ESBL-producing bacteria was carried out by the double-disk synergy (DDS) test. Presence of ESBL-related genes and integron Classes 1 and 2 was evaluated by the PCR method.

Results: Out of a total of 205 patients with DA-NIs, Enterobacteriaceae were responsible for (72.68%) of infections. The most common DA-NIs caused by Enterobacteriaceae were VAP (77.18%), CAUTI (19.46%), and sepsis due to VAP (3.35%). The most frequently Enterobacteriaceae were; Klebsiella pneumoniae 75 (24; 32% ESBL positive), E. coli 69 (6; 8.69% ESBL positive) and Enterobacter spp. 5 (5; 100% ESBL positive). Distribution of ESBL-related genes was as follows: bla-SHV (94.3%), bla-CTX (48.6%), bla-VEB (22.9%) and bla-GES (17.14%). The incidence rate of integron class 1 and class 2 was (82.92%) and (2.9%) respectively. Eight types of ESBL-producing bacteria were observed.

Conclusions: Due to the fact that the emergence rate of ESBL Enterobacteriaceae is increasing in DA-NIs, co-incidence of different types of ESBL genes with integrons in 75–100% of strains in our study is alarming for clinicians and healthcare safety managers. Therefore, regional and local molecular level estimations of ESBLs that are agents of DA-NIs are critical for better management of empiric therapy, especially for patients in ICUs.

Background

Device-associated nosocomial infections (DA-NIs), especially ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections (CAUTIs) pose the greatest threat to patient safety in the ICUs [1–3]. VAP is the most lethal among the two, however, CAUTIs are the most common Enterobacteriaceae that have been indicated as the most common cause of extended-spectrum β-lactamases (ESBL) producing bacteria in ICUs. These bacteria have a plethora of resistance mechanisms and often use multiple mechanisms against the same antibiotic or use a single mechanism to affect multiple antibiotics. Resistance to broad-spectrum cephalosporin is spreading quickly among Enterobacteriaceae and this is mostly related to acquisition of ESBL genes. Isolates that express ESBL phenotypes and hydrolyze the beta lactam antibiotics are often multiple drug resistant (MDR) [4, 5]. The commonly genes related to the ESBL phenotype...
are sulf-hydryl variable (SHV), cefotaxime-beta lactamases (CTX), Vietnam extended-spectrum \( \beta \)-lactamase (VEB) and Guyana Extended-Spectrum \( \beta \)-lactamases (GES) genes. Integrons as mobile DNA elements, are capable of retention and excision of antibiotic-resistant genes. Integrons achieve this by site-specific recombination. The different combinations of gene cassettes can contribute to the diverse genetic organization of integrons. There are five different classes of integrons. Class 1 integrons are the most common type that are present in Enterobacteriaceae.

Class 2 integrons are associated with the Tn7 transposon, whose transposition activity is directed at specific attachment sites on chromosomes or plasmids. Many of the antibiotic-resistant genes found in clinical isolates of Enterobacteriaceae are part of a gene cassette inserted into an integron [6]. Due to the potential of integrons to capture and collect gene cassettes, it is likely that incidence of MDR bacteria such as ESBL-producing Enterobacteriaceae, will become more prevalent in the future and integrons will continue to threaten the usefulness of antibiotics as therapeutic agents [6–8]. ESBL genes can be located on integrons, which may facilitate the spread of such genetic elements. To the best of our knowledge, this study is the first of its kind on ESBL-producing Enterobacteriaceae and incidence of integrons in these bacteria isolated from VAP and CAUTI as a major threat to patient safety in ICU wards, which was conducted in 18 governmental hospitals of Mazandaran province (The largest province in the north of Iran in terms of area and population).

**Methods**

**Study population and DA-NIs definitions**

This cross-sectional study was conducted in 18 governmental hospitals that overall contained 1200 ward beds and 100 intensive care unit beds, in Mazandaran province, located in the north of Iran, during 2014 and 2015. This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (Code No: 879 Date: July 9, 2014).

DA-NIs were defined as: Catheter-Associated Urinary Tract Infection; patient with a urinary catheter that had fever, dysuria, frequency, flank pain, suprapubic pain, nausea and vomiting. In addition, the urine culture was positive for \( 10^5 \) colony forming units per mL or more, with no more than two microorganisms isolated or, must have had at least two symptoms such as fever, dysuria, frequency, flank pain, suprapubic pain, nausea and vomiting plus pyuria.

Ventilator-Associated Pneumonia; Ventilator-associated pneumonia was indicated in a mechanically ventilated patient with a chest radiograph that showed new or progressive infiltrates, cavitation, consolidation, or pleural effusion 48 h after hospitalization. The patient must have had at least one of the following criteria: new onset of purulent sputum or change in character of sputum; organism cultured from blood or from a specimen obtained by tracheal aspirate, bronchoalveolar lavage or bronchial brushing, or biopsy.

Sepsis due to VAP; in patients ventilated more than 72 h, and bacteria separated from positive blood culture and tracheal tube aspirate positive culture were similar; while the patients had the symptoms of systemic inflammatory response syndrome (SIRS).

For all the patients whom were subject to ventilator and urinary catheter, certain prevention strategies were used against VAP and CAUTIs.

The prevention strategies for CAUTIs: insert catheters just for appropriate indications; leave catheters only as long as needed; only trained nurses insert and maintain catheters; hand hygiene; Insert catheters using aseptic technique and sterile equipment; aseptic insertion, maintain a closed drainage system; maintain unobstructed urine flow.

The prevention strategies for VAP are: elevation of the head of the bed; oral hygiene care; Prophylaxis interventions for peptic ulcer disease and deep vein thrombosis.

**Sampling and microbiological methods**

For VAP, a deep tracheal aspirate from the endotracheal tube was obtained, and for CAUTI, urine was aseptically aspirated from the sampling port of the urinary catheter for performing gram stain and culture on selective media. Sampling was done by the head nurses and the samples were immediately transported in a transport medium to the microbiology laboratory. All the samples were routinely cultured on MacConkey and blood agar plates. Blood samples were cultured in Blood culture bottles. Isolates were identified at the species level using standard biochemical tests and microbiological methods [9, 10].

**Antibiotic susceptibility test**

Susceptibility of the clinical isolates to routinely used antibiotics was determined by the standard broth dilution (micro dilution) technique. MIC was determined according to the recommendations of the standard protocol of CLSI 2010. The antibiotics were purchased from Sigma chemical company. Antibiotics used in this study were Amikacin, Ciprofloxacin, Imipenem, Gentamicin, Ceftazidime, Tobramycin, Piperacillin-Tazobactam, Cefepime, Colistin and Co-trimoxazole.

**Phenotype detection of extended-spectrum beta-lactamase (ESBL) producing enterobacteriaceae**

ESBL-producing Enterobacteriaceae was detected using the double-disk synergy (DDS) test [11, 12]. ESBL’s presence was assayed using the following antibiotic
disks (MAST, UK): cefotaxime (30 μg), cefotaxime/clavulanic acid (30/10 μg), ceftazidime (30 μg), and ceftazidime/clavulanic acid (30/10 μg). *Escherichia coli* ATCC 25922 strain served as positive controls.

**DNA extraction and detection of ESBL-related genes**

*Enterobacteriaceae* that were phenotypically confirmed as ESBL, were evaluated for ESBL-related genes. DNA of ESBL-positive *Enterobacteriaceae* was extracted using a commercial gene extraction kit (DNA Zist, Iran) according to the company’s instructions. ESBL-positive strains were screened by the PCR method for genes bla CTX, bla VEB, bla GES, bla SHV and also integrons class 1 and class 2. The set of primers and PCR amplification conditions are available in Additional file 1. After performing the PCR reaction, electrophoresis of PCR products was carried out in 2% agarose gel at 70 voltage for 50 min. Then, results were evaluated under UV light on the UV Trans illuminator. In all the experiments, the following reference strains were used as positive controls: *K. pneumoniae* 7881 (CTXM), *K. pneumoniae* 7881 strain (containing SHV), *P. aeruginosa* ATCC 27853 (VEB-1), and *K. pneumoniae* (GES). *E. coli* 96 K062 was used as a positive control for classes 1 and 2 integrons. A non-ESBL-producing strain (*E. coli* ATCC 25922) was used as negative control.

**Statistical analysis**

Data were analyzed using SPSS software version 16. Descriptive statistics, Chi-square and Fisher’s exact tests were used for statistical analysis.

**Results**

Out of total of 205 hospitalized patients with DA-NIs in ICU wards of the mentioned hospitals during 2014–2015, *Enterobacteriaceae* were responsible of 149 (72.68%) of DA-NIs. The most frequently found *Enterobacteriaceae* were; *Klebsiella pneumoniae* 75 (24; 32% ESBL positive & 51; 68% ESBL negative), *E. coli* 69 (6; 8.69% ESBL positive & 63; 91.30% ESBL negative) and *Enterobacter* spp. 5 (5; 100% ESBL positive). The most common DA-NIs caused by *Enterobacteriaceae* were VAP (77.18%), CAUTI (19.46%) and sepsis due to VAP (3.35%).

The demographic feature of patients with DA-NIs caused by ESBL *Enterobacteriaceae* was as follows; 27 VAP patients (15; 55.5% male and 12; 44.4% female) with average age of 66.5 ± 20.17 years and average duration of hospitalization in the ICU of 28.37 ± 20.03 days; three CAUTI patients (1; 33.3% male and 2; 66.6% female) with average age of 45.66 ± 21.93 years and average duration of hospitalization in the ICU of 19.33 ± 15.63 days; five patients of sepsis due to VAP (2;40% male and 3;60% female) with average age of 60.25 ± 14.79 years and average duration of hospitalization in the ICU of 10.75 ± 1.89 days.

In total, the distribution of ESBL-related genes was 33 (94.3%) bla-SHV, 17 (48.6%) bla-CTX, 8 (22.9%) bla-VEB and 6 (17.14%) bla-GES. Figure 1 that is the illustration of agarose gel, shows the strains containing VEB, SHV, int1 (integron class 1), int2 (integron class 2), GES and CTX genes. The Antibiotic susceptibility pattern of *Enterobacteriaceae* containing ESBL-related genes is shown in detail in Table 1. The rate of antibiotic resistance among strains containing SHV gene was 27.3–78.8% whereas the rate of sensitivity was 6.1–48.5%. On the other hand, Gentamicin and Imipenem had the highest resistance and sensitivity rates respectively. The rate of antibiotic resistance among strains containing CTX gene was 41.2–88.2%, whereas the rate of sensitivity was 5.9–35%. In addition, Gentamicin and Ciprofloxacin had the highest resistance and sensitivity rates respectively. The rate of antibiotic resistance among strains containing VEB gene was 12.5–87%, whereas the rate of sensitivity was 12.5–75% and Ceftazidime and Ciprofloxacin had the highest resistance and sensitivity rates respectively. The rate of antibiotic resistance among strains containing
GES gene was 16.7–83.3% whereas the rate of sensitivity was 16.7–66.7%. On the other hand, Cefepime and Imipenem had the highest resistance and sensitivity rates respectively. The incidence of integrons class 1 and class 2 was 29 (82.92%) and 1 (2.9%) respectively. Antibiotic susceptibility pattern of integron-positive Enterobacteriaceae is shown in Table 2. The rate of antibiotic resistance among integron class 1 positive strain was 35–85%. The only integron class 2 positive strain was *Klebsiella pneumoniae* and this isolate was resistant to all the antibiotics. Eight types of ESBL genes were seen among the isolates. Coincidence of each type of ESBL-producing bacteria and integron class 1 is shown in Table 3. Nine strains contained three ESBL genes (2 strains had GES, VEB, and SHV, 3 strains had GES, CTX, and SHV and 4 strains had VEB, CTX, and SHV). Fourteen strains contained 2 ESBL genes (10 strains had CTX and SHV, one strain had GES and SHV and three strains had VEB and SHV). Twelve strains had only one ESBL gene (11 strains contained

| Antibiotic susceptibility pattern of *Enterobacteriaceae* containing ESBL related genes | *K. pneumoniae* | *Enterobacter* spp | *E. coli* |
|-----------------------------------------------|-----------------|-------------------|-----------|
| SHV, CTX, VEB, GES | N = 24 | N = 5 | N = 6 |
| SHV | CTX | VEB | GES |
| SHV | CTX | VEB | GES |
| SHV | CTX | VEB | GES |
| R | 50 | 50 | 25 | 25 |
| I | 27.2 | 28.5 | 50 | 25 |
| S | 22.7 | 21.5 | 50 | 25 |
| R | 40.9 | 50 | 0 | 25 |
| I | 31.8 | 28.5 | 50 | 25 |
| S | 27.2 | 21.5 | 50 | 25 |
| R | 36.3 | 50 | 25 | 50 |
| I | 27.2 | 28.5 | 25 | 25 |
| S | 36.3 | 21.5 | 50 | 25 |
| R | 81.8 | 85.7 | 50 | 100 |
| I | 45.4 | 0 | 0 | 0 |
| S | 13.6 | 14.2 | 50 | 25 |
| R | 72.7 | 57.1 | 75 | 50 |
| I | 27.2 | 35.7 | 25 | 25 |
| S | 13.6 | 7.1 | 0 | 25 |
| R | 59 | 64.2 | 50 | 25 |
| I | 31.8 | 28.5 | 50 | 50 |
| S | 9.1 | 7.1 | 0 | 25 |
| R | 63.6 | 64.2 | 50 | 75 |
| I | 31.8 | 28.5 | 75 | 25 |
| S | 4.5 | 7.1 | 25 | 0 |
| R | 72.7 | 85.7 | 25 | 100 |
| I | 9.1 | 7.1 | 25 | 0 |
| S | 18.1 | 7.1 | 50 | 0 |
| R | 72.7 | 71.4 | 50 | 25 |
| I | 27.2 | 14.2 | 50 | 25 |
| S | 13.6 | 14.2 | 50 | 25 |
| R | 81.8 | 85.7 | 75 | 75 |
| I | 18.2 | 14.2 | 25 | 25 |

Table 1

Resistant (R), Intermediate (I), Sensitive (S)

---

GES gene was 16.7–83.3% whereas the rate of sensitivity was 16.7–66.7%. On the other hand, Cefepime and Imipenem had the highest resistance and sensitivity rates respectively. The incidence of integrons class 1 and class 2 was 29 (82.92%) and 1 (2.9%) respectively. Antibiotic susceptibility pattern of integron-positive *Enterobacteriaceae* is shown in Table 2. The rate of antibiotic resistance among integron class 1 positive strain was 35–85%. The only integron class 2 positive strain was *Klebsiella pneumoniae* and this isolate was resistant to all the antibiotics. Eight types of ESBL genes were seen among the isolates. Coincidence of each type of ESBL-producing bacteria and integron class 1 is shown in Table 3. Nine strains contained three ESBL genes (2 strains had GES, VEB, and SHV, 3 strains had GES, CTX, and SHV and 4 strains had VEB, CTX, and SHV). Fourteen strains contained 2 ESBL genes (10 strains had CTX and SHV, one strain had GES and SHV and three strains had VEB and SHV). Twelve strains had only one ESBL gene (11 strains contained
SHV and one strain had CTX). Coincidence of isolates that contained different types of ESBL genes and integron class 1 was 75–100%, which was statistically significant ($P > .05$).

**Discussions**

DA-NIs due to MDR bacteria are a serious threat to patient safety, being among of the most serious causes of morbidity, mortality and economic burden in developing countries such as Iran. Various studies have shown that the DA-NIs are a serious issue in Iran [13–16] But no studies had specifically addressed and evaluated the ESBL genes and mobile genetic elements such as integrons in *Enterobacteriaceae* as common agents of DA-NIs patients in Iran. It was found in this study that ESBL-producing *Enterobacteriaceae* were causative agents of 23% of DA-NIs in the region. Rosenthal et al. surveyed DA-NIs in 55 ICUs of eight developing countries and found that VAP posed the greatest risk (41%), followed by CVC-related bloodstream infections (30%) and CAUTI (29%). On the other hand, they reported *Enterobacteriaceae* were agents of about 27% of VAPs and 42% of CAUTIs [16]. In our study, overall 56% of *Enterobacteriaceae* were resistant to

|               | *Klebsiella pneumoniae* | *Enterobacter* spp | *E.coli* |
|---------------|-------------------------|-------------------|---------|
|               | Integron class 1 | Integron class 2 | Integron class 1 | Integron class 2 | Integron class 1 | Integron class 2 |
| **N**        | 24                     |                  | 5               |                  | 6               |                  |
| **Integron class 1** |            |                  | **Integron class 2** |            |                  | **Integron class 2** |
| **Amikacin** | R | 55 100 | 75 - | 66.6 - |                  |                  |
|              | I | 25 0 | 25 | 16.6 |                  |                  |
|              | S | 20 0 | 0 | 16.6 |                  |                  |
| **Ciprofloxacin** | R | 45 100 | 0 - | 33.3 - |                  |                  |
|              | I | 35 0 | 0 | - |                  |                  |
|              | S | 20 0 | 100 | 66.6 |                  |                  |
| **Imipenem** | R | 35 0 | 0 | 16.6 |                  |                  |
|              | I | 30 0 | 0 | 16.6 |                  |                  |
|              | S | 35 100 | 75 | 66.6 |                  |                  |
| **Gentamicin** | R | 80 100 | 75 - | 83.3 - |                  |                  |
|              | I | 5 0 | 0 | 16.6 |                  |                  |
|              | S | 15 0 | 25 | - |                  |                  |
| **Ceftazidime** | R | 55 100 | 50 - | 50 - |                  |                  |
|              | I | 30 0 | 50 | 33.3 |                  |                  |
|              | S | 15 0 | 0 | 16.6 |                  |                  |
| **Tobramycin** | R | 60 0 | 75 - | 33.3 - |                  |                  |
|              | I | 30 100 | 0 | 16.6 |                  |                  |
|              | S | 10 0 | 25 | 50 |                  |                  |
| **Piperacillin-Tazobactam** | R | 70 100 | 75 - | 16.6 - |                  |                  |
|              | I | 30 0 | 25 | 66.6 |                  |                  |
|              | S | 0 0 | 0 | 16.6 |                  |                  |
| **Cefepime** | R | 80 100 | 75 - | 33.3 - |                  |                  |
|              | I | 10 0 | 25 | 16.6 |                  |                  |
|              | S | 10 0 | 0 | 50 |                  |                  |
| **Colistin** | R | 60 0 | 50 - | 33.3 - |                  |                  |
|              | I | 25 100 | 25 | 16.6 |                  |                  |
|              | S | 15 0 | 25 | 50 |                  |                  |
| **Co-trimoxazole** | R | 85 100 | 75 - | 33.3 - |                  |                  |
|              | I | - - | 0 | - |                  |                  |
|              | S | 15 0 | 25 | 66.6 |                  |                  |

* R resistant, I intermediate, S sensitive

Bagheri-Nesami *et al.* Antimicrobial Resistance and Infection Control (2016) 5:52
natural text
Additional file

Additional file 1: Table S1. The set of primers and PCR amplification conditions. (DOCX 12 kb)

Abbreviations
CAUTI: Catheter-associated urinary tract infections; CTX: Cefotaxime-beta-lactamases; OVC: Central venous catheter; DA-Nls: Device associated nosocomial infections; ESBL: Extended-spectrum beta-lactamases; GES: Guyana Extended-Spectrum ß-lactamases; ICU: Intensive care unit; MIC: Minimum inhibitory concentration; Nls: Nosocomial infection; PCR: Polymerase chain reaction; SHV: Sul-f-hydryl variable; VAP: Ventilator-associated pneumonia; VEB: Vietnam extended-spectrum ß-lactamase

Acknowledgments
This article was a part of a specialty’s thesis of Pediatric of Dr Azin Hajalibeig and was supported by the Vice-Chancellor for Research at Mazandaran University of Medical Sciences (Grant Number: 879).

Funding
Mohammad Sadegh Rezai received Research grants of Vice-Chancellor for Research at Mazandaran University of Medical Sciences with grant number 879.

Availability of data and materials
All data analysed during this study are included in this published article.

Authors’ contributions
Mohammad Sadegh Rezai and Masome Bagheri Nesami designed the project, collected data and wrote the manuscript. Gohar Eslami, Azin Hajalibeig, Fatemeh Ahangarkani and Attieh NIKkhah collected data. Alireza Rafiei carried out laboratory examinations. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences. (DOX 12 kb)
IncHI2, IncP1-α, and IncFI groups. Antimicrob Agents Chemother. 2006;50(8):2741–50.

28. Sidjabat HE, et al. Identification of blaCMY-7 and associated plasmid-mediated resistance genes in multidrug-resistant Escherichia coli isolated from dogs at a veterinary teaching hospital in Australia. J Antimicrob Chemother. 2006;57(5):840–8.