Introduction. Pseudomonas aeruginosa is an important opportunistic clinical pathogen, which is associated with several clinical infections that are usually difficult to treat because of resistance to multiple antimicrobials. The production of extended-spectrum β-lactamases (ESBLs) is an important mechanism of β-lactam resistance. The aims of this study were to determine the prevalence of ESBLs, antimicrobial susceptibility, and to detect the blaTEM, blaSHV, and blaCTX-M genes.

Methods. In this study, carried out from March 2013 to December 2014, 266 P. aeruginosa isolates were collected from patients admitted to teaching hospitals of Qazvin and Tehran, Iran. All isolates were initially screened for ESBL production by disk diffusion method and were further confirmed using a combined disk method. Antimicrobial susceptibility of ESBL-producing isolates was determined by standard disk diffusion method. Polymerase Chain Reaction (PCR) and sequencing techniques were employed for detection of blaTEM, blaSHV, and blaCTX-M genes.

Results. In total, 262 (98.5%) P. aeruginosa isolates were non-susceptible to the used extended spectrum cephalosporins, and, among these, 75 (28.6%) isolates were ESBL producers. Fifty-nine (78.7%) of ESBL-producing isolates showed multidrug-resistance pattern. Of 75 ESBL-positive isolates, the blaTEM-1 (26.7%) was the most common gene, followed by blaCTX-M-15 (17.3%), blaSHV-1 (6.7%), and blaTEM-12 (4%), either alone or in combination.

Conclusions. The results of this study showed the notable prevalence of ESBLs among the clinical isolates of P. aeruginosa in Iran, indicating the urgency for the implementation of appropriate follow-up measures for infection control and proper administration of antimicrobial agents in our medical settings.
larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V [13, 14]. Detection of ESBLs is important for the surveillance and epidemiological studies of their transmission in medical settings [15]. One major concern regarding the spread of ESBL-producing *P. aeruginosa* within hospital settings is the treatment failure to infections caused by this organism due to the limitations in therapeutic choices [9, 16]. There are few reports describing the prevalence of TEM- or SHV-type ESBLs in *P. aeruginosa* in Iran. The aims of this study were to determine the prevalence of ESBLs and to detect the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>-types ESBL genes among clinical isolates of *P. aeruginosa* collected from hospitals of Tehran and Qazvin, two central provinces of Iran.

**Methods**

**Bacterial isolates**
The clinical isolates of *P. aeruginosa* (one isolate per patient) were collected from hospitalized patients of Tehran and Qazvin provinces from March 2013 to December 2014. The bacterial isolates were collected from different clinical specimens, including urine, wounds, sputum, bronchoalveolar lavage (BAL), trachea, and blood. These isolates were obtained from patients admitted to intensive care units (ICUs), internal medicine, general surgery, neurology, neurosurgery, and infectious disease wards. Specimens of these patients were sent to the microbiology laboratory of the hospitals under study. The study was approved by the ethics committee of Qazvin University of Medical Sciences (code IR.QUMS.REC.1394.147). Written informed consent was obtained from all subjects enrolled in this study. All isolates were identified as *P. aeruginosa* using standard microbiological and biochemical tests [17]. The isolates were stored at -70°C in trypticase soy broth (TSB) containing 20% glycerol and subcultured twice prior to testing.

**ESBL screening**
All isolates were initially screened for ESBLs production using the standard disc diffusion method, using cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefpodoxim (30µg), and aztreonam (30µg). Isolates which were non-susceptible to any of third generation cephalosporins were selected for ESBLs detection phenotypically. The antibiotic disks were purchased from Mast (Mast Diagnostics Group Ltd). *P. aeruginosa* ATCC 27853 was used as a control strain in antimicrobial susceptibility testings.

**Confirmation of ESBL production**
Phenotypic confirmatory tests [18], which were designed for detecting ESBLs in *K. pneumoniae* and *E. coli*, were also performed by comparing the inhibition zone of disks containing ceftaxime or ceftazidime with and without clavulanic acid. *E. coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used as the quality control strains in antimicrobial susceptibility testing.

**Molecular detection of ESBL-encoding genes**
ESBL-producing isolates were subjected to Polymerase Chain Reaction (PCR) targeting *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-8</sub>, *bla*<sub>CTX-M-9</sub>, and *bla*<sub>CTX-M-25</sub> genes using the specific primers listed in Table I. Plasmid DNA was extracted using plasmid mini extraction kit (Bioneer Company, Korea). The PCR amplifications were performed in a thermocycler (Applied Biosystems, USA) as follows: 96°C for 5 min and 35 cycles of 1 min at 96°C, 1 min at a specific temperature for each primer and 1 min at 72°C and a final extension step of 10 min at 72°C. Amplification reactions were prepared in a total volume of 25 μl (24 μl of PCR master mix plus 1 μl of

| Targets | Primer Sequences (5’-3’) | Annealing Temperature (°C) | References |
|---------|-------------------------|---------------------------|------------|
| TEM     | ATGACTATTTCAACATTCGG    | 50                        | 19         |
|         | GACATCTACAATGTAAATC     |                           |            |
| TEM (Sequencing) | TAACCATGATGATAACCTA    | 50                        | 19         |
|         | CCGATCATCGTCAAGTAAGAAA |                           |            |
| SHV     | CTAAACTCGGCTTATCGGGG    | 50                        | 19         |
|         | ATGCCGCTTGTCGCGGAGG    |                           |            |
| SHV (Sequencing) | ACTGCTTTTGCCGCCGAG    | 56                        | 19         |
|         | CAGTCCGTTCCCCGCAAG    |                           |            |
| CTX-M-1 group | ATGCAGTACGAGAGTTCG    | 55                        | 20         |
|         | TGGGTAGCAGTATGGCC      |                           |            |
| CTX-M-2 group | ATGCAGTACGAGAGTTCG    | 55                        | 20         |
|         | TGGGTAGCAGTATGGCC      |                           |            |
| CTX-M-8 group | ACTTCAGCCACACAAGTCATTA | 55                        | 20         |
|         | CGAACGACAGGACATCCAG    |                           |            |
| CTX-M-9 group | ATGCAGTACGAGAGTTCG    | 55                        | 20         |
|         | CCTTGCCGCGAGATTCC      |                           |            |
| CTX-M-25 | CACAGAATGGAATGCAGC    | 50                        | 21         |
|         | TCACACAGGATGATGAG      |                           |            |
template DNA) including 5 ng of genomic DNA, 2.0 U of Taq DNA polymerase, 10 mM dNTP mix at a final concentration of 0.2 mM, 50 mM MgCl₂ at a final concentration of 1.5 mM, 1 μM of each primer, and 1X PCR buffer (final concentration). PCR products were electrophoresed on a 1% agarose gel at 100 volts and then were stained with ethidium bromide solution and finally visualized in gel documentation system (UVtec, UK). The purified PCR products were sequenced by the Macrogen Company (Seoul, Korea). The sequence alignment and bioinformatics analyses were performed using the Basic Local Alignment Search Tool (BLAST) online program of the National Center for Biotechnology Information (freely available at http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis was performed for descriptive statistics, including frequencies, cross-tabulation of microbiological, clinical, and demographic characteristics, using the commercial software Statistical analysis software package (version 16, IBM SPSS Corporation, Armonk, NY, USA).

Results

During the study period from March 2013 to December 2014, a total of 266 isolates of P. aeruginosa were collected from different clinical specimens including blood (94 isolates; 35.3%), urine (80 isolates; 30.1%), wounds (29 isolates; 10.9%), trachea (26 isolates; 9.8%), sputum (19 isolates; 7.1%), and BAL (18 isolates; 6.8%). Isolates were obtained from patients admitted to intensive care units (99-37.2%), internal medicine (64-24.1%), infectious diseases (61-22.9%), general surgery (26-9.8%), neurosurgery (12-4.5%), and neurology (4-1.5%) wards. One hundred and twenty-five (47%) were male and 141 (53%) were female. Out of the 266 P. aeruginosa isolates, 262 (98.5%) isolates were non-susceptible to at least one of the antibiotics used in the screening test and, among these, 75 (28.6%) isolates were identified as potential ESBL producers using combined disk method. Fifty-nine (78.7%) of ESBL-producing isolates were found to be multidrug-resistant (MDR), i.e. showed intermediate or fully resistance to at least three different classes of antimicrobial agents including β-lactams, aminoglycosides, and fluoroquinolones. Amikacin (58.7%) and piperacillin-tazobactam (53.3%) showed the highest rates of susceptibility among antimicrobials tested in this study, respectively (Table II). Further, 41 (54.7%) and 40 (53.5%) ESBL-producing isolates were fully or intermediate resistant to meropenem and imipenem, respectively. ESBL-producing isolates were mainly recovered from urine (30.2%), followed by wound (28.3%) samples. The patients affected were mainly those admitted in ICU (54.7%) and the internal wards (45.1%), respectively (Table III). Of the 75 P. aeruginosa isolates with ESBL phenotype, blaTEM-1 (20-26.7%) was the most common gene, followed by blaCTX-M-15 (13-17.3%), blaSHV-1 (5-6.7%), and blaSHV-12 (3-4%), either alone or in combination (Table IV). In this study, isolates were, instead, negative for blaCTX-M-2, blaCTX-M-3, and blaCTX-M-9 group genes, as well as for blaCTX-M-25.

Discussion

P. aeruginosa has recently emerged as a major cause of healthcare-associated infections, especially in immunocompromised people and burn patients [22, 23]. The treatment of P. aeruginosa infections is increasingly

| Antibiotics         | S (%) | I (%) | R (%) |
|---------------------|-------|-------|-------|
| Amikacin            | 44 (58.7) | 10 (13.3) | 21 (28) |
| Piperacillin-tazobactam | 40 (53.3) | 9 (12) | 26 (34.7) |
| Imipenem            | 35 (46.7) | 9 (12) | 31 (41.3) |
| Meropenem           | 34 (45.3) | 7 (9.3) | 34 (45.3) |
| Cefepime            | 30 (40) | 1 (1.3) | 44 (58.7) |
| Piperacillin         | 26 (54.7) | 8 (10.7) | 41 (54.7) |
| Ciprofloxacin       | 24 (52) | 1 (1.3) | 50 (66.7) |
| Gentamicin          | 22 (29.3) | -     | 53 (70.7) |
| Ceftazidime         | 22 (29.3) | 4 (5.3) | 49 (65.3) |
| Tobramycin          | 22 (29.3) | 3 (4) | 50 (66.7) |
| Cefotaxime          | 21 (28) | 2 (2.7) | 52 (69.3) |
| Levofloxacin        | 18 (24) | 2 (2.7) | 55 (73.3) |
| Ticarcillin         | 17 (22.7) | 1 (1.3) | 57 (76) |
| Aztreonam           | 14 (18.7) | 10 (13.3) | 51 (68) |
| Carbenicillin       | 14 (18.7) | 3 (4) | 58 (77.5) |
| Ceftriaxone         | 10 (13.3) | 7 (9.3) | 58 (77.5) |
| Cefotaxime          | 5 (6.7) | 8 (10.7) | 62 (82.7) |

S: Sensitive, I: Intermediate, R: Resistant.
complicated due to both intrinsic and acquired resistance to the most commonly prescribed antibiotics in hospital settings [2, 24]. The emergence of ESBL has become a matter of serious concern for the treatment of patients in Iran. ESBL detection is not routinely tested in most laboratories in Iran. There are only few reports on prevalence of ESBL among P. aeruginosa isolates in our country. In the present study, 26.7%, 17.3%, 6.7%, and 4% of ESBL-producing P. aeruginosa isolates carried bla<sub>TEM</sub>-1, bla<sub>SHV</sub>-12, bla<sub>CTX-M</sub>-15, and bla<sub>PER</sub>, respectively [36]. In Japan, Uemura et al. showed the presence of bla<sub>SHV</sub>-12 among P. aeruginosa isolates collected from burn patients [37]. Polotto et al. reported that the bla<sub>CTX-M-2</sub> (19.6%) gene was the most prevalent ESBL gene in Brazil [38]. Together, these data indicate successful spread of the ESBL-encoding genes around the world.
Conclusions

Findings of the our study show a high prevalence of ESBL-producing *P. aeruginosa* isolates carrying *bla*<sub>CTX-M-15</sub> and *bla*<sub>SHV-12</sub> genes in Iran. The presence of ESBL-producing bacteria within the healthcare setting in Iran should be considered a public health concern both therapeutically and epidemiologically. As such, the identification, treatment, and infection control and management of patients infected with these organisms is of prime necessity.

Acknowledgments

This study was financially supported by Research Deputy of Qazvin University of Medical Sciences, Qazvin, Iran. The authors declare no conflict of interest

Authors' contributions

AP initiated the study design, project and protocol development, and data analysis; TNF performed the data quality control and manuscript writing; EZ collected data and performed microbiological and molecular experiments; KHA was involved in the editing of the manuscript. All authors read and approved the final draft of the manuscript.

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Received on January 7, 2016. Accepted on February 20, 2017.

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