Original Article

Prevalence of ESBLs-producing Pseudomonas aeruginosa isolates from different wards in a Chinese teaching hospital

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Abstract: This study was to explore the molecular dissemination of P. aeruginosa producing extended spectrum β-lactamase (ESBLs) recovered from the different wards in a teaching hospital, Jilin. Among 240 isolates, 91 strains were isolated from burn wards and 149 strains from surgical wards. A total of 210 strains (87.5%) produced ESBLs, 30 strains (12.5%) didn’t produce ESBLs. All ESBLs isolates showed identical antimicrobial susceptibility profiles. The genotypic prevalence of ESBLs for blaSHV-12, blaTEM-24, blaCTX-M-1, blaCTX-M-2, blaCTX-M-3, blaPER and blaVEB genes was 17.6%, 20.5%, 14.3%, 9.6%, 12.9%, 12.9%, 13.8% and 11.4% respectively. All P. aeruginosa strains producing ESBL had three to six plasmids and contained class 1 integrons, which transferred resistance to E. coli C 600 by conjugation. The data indicated a high prevalence of ESBL among P. aeruginosa isolates in this region and their enzyme types were diverse.

Keywords: Nosocomial infection, Pseudomonas aeruginosa, expended spectrum-β-lactamases

Introduction

P. aeruginosa is an important conditioned pathogen which is known to cause nosocomial septicemia and burn infections, which are very difficult to treat particularly in the burn wards and intensive care units (ICU) [1, 2]. Extended spectrum beta-lactamas (ESBLs)-producing P. aeruginosa have spread rapidly worldwide and pose a serious threat as a healthcare-associated infections [3]. Since the discovery of ESBLs in 1983, their prevalence has been reported threateningly in many regions of the world and now comprises over three hundred variants [4].

ESBLs are plasmid-mediated enzymes that hydrolyze broad spectrum beta-lactams and monobactama, and are strongly inhibited by clavulanate, which are transmitted by plasmids among bacteria in and between hospitals. When inappropriate antimicrobial therapy is used to treat infections caused by ESBL producing bacteria, failure in the clinical treatment will occur frequently. Therefore, if infections with ESBL-producing bacteria can be predicted by the clinical characteristics of patients, can lead to a better selection of antibiotics and help to improve the outcome of infections [5].

This study was done to investigate on antibiotic resistance and molecular epidemiology of P. aeruginosa strain producing ESBLs in patients with wound bury infections and surgical infections in an affiliated hospital in Jilin region.

Materials and methods

Bacteria

A total of 240 clinical isolates of multidrug-resistant P. aeruginosa were consecutively obtained from clinical specimens at different surgical wards in an affiliated hospital in Jilin, in the years 2011 and 2013.

The hospital is a 450-bed tertiary teaching hospital. All the isolates were identified using the API 20E system. The isolates, which were identified as P. aeruginosa by the conventional bio-
Genotypes of prevalent ESBLs producing *Pseudomonas aeruginosa*

chemical methods, were confirmed by VITEK GNI system (bioMerieux Vitek Inc, USA). All strains were cryopreserved in L-B broth containing 15% glycerol at -80°C for further analysis. Reference strains were included as internal standards in all tests: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

The O serotype of the isolates was determined by slide agglutination using commercially available anti-sera (BIO-RAD, Marnes la Coquette, France) as previously described [6].

**Determination of resistance and ESBL screening**

Routine antibiograms were determined by the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institutes (CLSI) [7]. The antimicrobial agents tested were ampicillin, ampicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, cefotaxime, ceftazidime, cefoxitin, cefepime, aztreonam, imipenem, amikacin, tobramycin, gentamycin, ciprofloxacin and sulfamethoxazole-trimethoprim.

ESBL screening was performed by the use of the double disc synergy test. The combination disk method based on the inhibitory effect of clavulanic acid was also used according to the CLSI criteria (CLSI, 2012).

The following antibiotics were used to indicate ESBL production: cefpodoxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefteriaxone (30 µg) and aztreonam (30 µg). In each plate, four discs were placed at inter-disc distances of 25 or 30 nm away from an amoxicillin/clavulanic acid disc (20/10 µg).

The test was considered positive when the difference of zone diameters between the beta-lactam disc and disc containing antibiotic associated with clavulanic acid was > 5 mm.

**Detection of the class A-lactamase genes by PCR**

A series of allele-specific primers were designed with the Primer 5.0 software for detection of the class A-lactamase genes, which were composed of bla<sub>TEM</sub>, bla<sub>SHV</sub>, bla<sub>PER</sub>, bla<sub>VEB</sub>, bla<sub>GES</sub> and bla<sub>CTX</sub>. Genomic DNA from all cases of isolates with ESBLs positive were extracted by boiling method. PCR analysis containing of PCR primers, conditions in addition to subsequent sequencing of PCR product was performed as previously described [8]. The PCR products were electrophoresed on a 2.0% agarose gel.

The DNA band was excised, purified with a TiANGel Minipurification kit, ligated to pGM-T vector (Tiangen Biotech Co. Ltd), transformed into *E. coli* DH-5α competence cells, and the white clones were screened. Nucleotide sequencing was performed directly on cloned fragments using an ABI Prism 377 DNA sequencer. The DNA sequence was carried out with the Blast program available at the website of the National Center for Biotechnology Information.

**Plasmid profiles and transconjugation**

Plasmid DNA was extracted by the alkaline lysis method, and analyzed by electrophoresis on a 0.8% agarose gel containing 0.25 µg of ethidium bromide per ml. *E. coli* V517 was used as a standard size.

To demonstrate that ESBL genes detected by PCR were located on plasmids, resistance transfer experiments using the broth mating method were performed for all isolates. Rifampicin resistant *E. coli* C 600 was used as the recipient. Transconjugants were selected on MacConkey agar (Oxoid, Basingstoke, United Kindom). Antimicrobial susceptibility, a confirmatory test for ESBL phenotype and PCR detection were performed for transconjugants as previously described in the procedures above.

**Statistical analysis**

Statistical analysis was done by Fisher’s exact rest using SPSS version 10.0. A P-value <0.05 was considered statistically significant.

**Results**

**Antibiotic susceptibility testing and ESBL screening**

During the three years period, a total of two hundred and forty of *P. aeruginosa* isolates were recovered from hospitalized patients in the affiliated hospital, Jilin region. Among all isolates, 91 strains were isolated from wounds
Genotypes of prevalent ESBLs producing Pseudomonas aeruginosa

Table 1. Antimicrobial patterns of ESBLs producer and non-ESBLs producer among P. aeruginosa strains

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance rate (%)</th>
<th>ESBLs (n=210)</th>
<th>Non-ESBLs (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>210 100.0</td>
<td>30 100.0</td>
<td></td>
</tr>
<tr>
<td>Ticaricillin</td>
<td>210 100.0</td>
<td>30 100.0</td>
<td></td>
</tr>
<tr>
<td>Ampicillin clavulanic acid</td>
<td>0 0</td>
<td>5 16.7</td>
<td></td>
</tr>
<tr>
<td>Ticaricillin clavulanic acid</td>
<td>0 0</td>
<td>5 16.7</td>
<td></td>
</tr>
<tr>
<td>Cefotixin</td>
<td>170 80.9</td>
<td>10 33.3</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>122 58.1</td>
<td>4 13.3</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>191 90.9</td>
<td>9 30.0</td>
<td></td>
</tr>
<tr>
<td>Ceftipime</td>
<td>110 52.4</td>
<td></td>
<td>13.3</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>110 52.4</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 0</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>123 58.3</td>
<td>8 26.7</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>123 58.3</td>
<td>8 26.7</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole trimethoprim</td>
<td>210 100.0</td>
<td>8 26.7</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>142 67.6</td>
<td>11 36.7</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>189 90.9</td>
<td>11 36.7</td>
<td></td>
</tr>
</tbody>
</table>

ESBL: Extended spectrum beta lactamases.

Table 2. Overview of ESBLs genotypes among P. aeruginosa isolates distributed in different wards

<table>
<thead>
<tr>
<th>Genotype</th>
<th>General surgical wards (n=136)</th>
<th>Burn wards (n=74)</th>
<th>Total (n=210)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of isolates (%)</td>
<td>Number of isolates (%)</td>
<td>Number of isolates (%)</td>
</tr>
<tr>
<td>blaTEM</td>
<td>29 (21.3)</td>
<td>14 (18.9)</td>
<td>43 (20.5)</td>
</tr>
<tr>
<td>blaSHV</td>
<td>24 (17.7)</td>
<td>13 (17.6)</td>
<td>37 (17.6)</td>
</tr>
<tr>
<td>blaCTX-M-1</td>
<td>20 (14.7)</td>
<td>10 (13.5)</td>
<td>30 (14.3)</td>
</tr>
<tr>
<td>blaCTX-M-2</td>
<td>10 (7.4)</td>
<td>10 (13.5)</td>
<td>20 (9.6)</td>
</tr>
<tr>
<td>blaCTX-M-3</td>
<td>18 (13.2)</td>
<td>9 (12.1)</td>
<td>27 (12.9)</td>
</tr>
<tr>
<td>blaPER</td>
<td>19 (14.0)</td>
<td>10 (13.5)</td>
<td>29 (13.8)</td>
</tr>
<tr>
<td>blaVEB</td>
<td>16 (11.8)</td>
<td>8 (10.8)</td>
<td>24 (11.4)</td>
</tr>
<tr>
<td>blaCTX-M+SHV</td>
<td>6 (4.4)</td>
<td>3 (4.1)</td>
<td>9 (4.3)</td>
</tr>
<tr>
<td>blaTEM+CTX</td>
<td>7 (5.1)</td>
<td>4 (5.4)</td>
<td>11 (5.2)</td>
</tr>
<tr>
<td>blaSHV+CTX</td>
<td>10 (7.4)</td>
<td>4 (5.4)</td>
<td>14 (6.7)</td>
</tr>
<tr>
<td>blaTEM+SHV+CTX</td>
<td>4 (2.9)</td>
<td>1 (1.4)</td>
<td>5 (2.4)</td>
</tr>
</tbody>
</table>

ESBL: Extended spectrum beta lactamases.

at burn wards and 149 strains from wounds at surgical wards. A total of 210 strains (87.5%, 210/240) produced ESBLs, and 30 strains (12.5%, 30/240) didn’t produce ESBLs.

Generally, all ESBLs producer isolates showed identical antimicrobial susceptibility profiles. They were highly resistant to beta-lactams in addition to aztreonam, with ceftipime (52.4%) showing the lowest resistant among the third generation of cephalosporin antibiotics.

Phenotypic antimicrobial susceptibility to other antibiotics showed resistance to gentamicin, tobramycin, amikacin and sulfamethoxazole-trimethoprim. However, all isolates were sensitive to imipenem, amoxicillin/clavulanic acid and ticarcillin/clavulanic acid.

In non ESBLs producer P. aeruginosa strains, no resistance occurred with imipenem in addition to aztreonam, while the highest resistance observed in cefotaxime and cefazidime (33.3% and 30.0% respectively). Also, some of P. aeruginosa strains were resistant to the whole non-beta lactam antibiotics (lower than 35.0%) and imipenem were found as an effective antibiotic. The resistance patterns of all strains are presented in Table 1.

PCR amplification and sequencing

In this study, all ESBL-producing isolates were subjected to PCR to detect ESBL genes, including blaSHV, blaCTX-M, blaTEM, blaGES, blaPER and blaVEB gene. DNA sequence analysis of PCR products revealed the strains carrying blaSHV were identical to blaSHV-12, blaTEM identical to blaTEM-24. Among P. aeruginosa producing ESBLs, 43 strains (20.5%) contained blaTEM-24 gene, 37 strains (17.6%) were positive for blaCTX-M1 gene, and 30 strains (14.3%) had blaCTX-M2 gene, 27 strains (12.9%) contained blaCTX-M3 gene, 29 strains (13.8%) contained blaPER gene, 24 (11.4%) strains contained blaVEB gene and 20 strains (9.6%) contained blaCTX-M4 gene, respectively. The only gene not detected in all isolates was blaGES.

The blaCTX-M and blaSHV-12 together were present in 14 strains (6.7%), 9 strains (4.3%) carried both blaSHV-12 and blaTEM-24, 11 isolates (5.2%) were positive for blaTEM-24 and blaCTX-M1, 5 isolates (2.4%) carried all three blaSHV-12, blaTEM-24 and blaCTX-M1.

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$bla_{CTX-M}$. Additionally, no significance difference was observed in the distribution of the $bla$ genotypes among the different wards (Table 2). Isolates were all found to be the 0:6 serotype. Furthermore, PCR analysis showed that all ESBL-producing isolates were also carrying *IntI* 1, indicating the presence of a class 1 integron.

**Plasmid profiles and conjugation assay**

All *P. aeruginosa* strains producing ESBLs except the non ESBLs producer strains had three to six plasmids with size ranging from ~10.0 to 45 kb (data not shown).

Attempts at isolating plasmids from all the *P. aeruginosa* strains producing ESBL was successful, and repeated attempts to transfer resistance to *E. coli* C600 by conjugation were successful. This indicated that ESBLs containing integrons was probably plasmid-mediated in all ESBLs-producing isolates. Again, phenotypic resistance of ESBLs producers to gentamycin, sulfamethoxazole-trimethoprim, ciprofloxacin and tobramycin was transferred and expressed in *E. coli* C 600 transformants.

**Discussion**

*P. aeruginosa* has now clearly emerged as a leading nosocomial pathogen, because of its ubiquitous nature, ability to survive in moist environments and acquired resistance to the vast majority of antimicrobial drugs [9]. However, the prevalence of ESBL-resistant *P. aeruginosa* has been increasing over recent years.

In this study, ESBLs production was variable from 35.3% in the burn wards to 64.7% in the surgical wards showing a high different in the frequency of ESBLs production.

In the another study in a tertiary care hospital in China 63.5% of *P. aeruginosa* were ESBLs producers while in our study the frequency of ESBLs production by *P. aeruginosa* in the hospital were more than those isolates, nearly to Woodford reporter [10]. In a survey by Yu et al in 2007 in general hospital in China 59.2% of isolates were ESBLs positive and all isolates were susceptible to imipenem [11], our finding also showed all strains had sensitivity toward imipenem.

The prevalence of different ESBLs genotypes varies in different countries and regions. The PER, VEB, GES TEM, SHV and CTX genotypes are prevalent in Asian countries and regions [12]. They have been extensively reported in members of the family Enterobacteriaceae since the early 1980s, whereas they have been described in *P. aeruginosa* only more recently. These enzymes are either of the TEM and SHV types, which are also well known in the Enterobacteriaceae; with the PER type, mostly originating from Turkish isolates; the VEB type from Southeast Asia; and recently, the GES and IBC types, which have been reported in France, Greece and South Africa [13, 14].

When an ESBL is suspected in *P. aeruginosa*, PCR-based molecular techniques may help to identify the gene. The quality of whole-cell DNA used as the template is an important factor for avoiding false-negative results. Primers designed to anneal to the ends of class 1 integrons may also help in the retrieval of PCR products that may contain ESBL genes. Nucleotide sequence analysis of PCR products, whether or not combined with other methods, is still the only acceptable way to accurately discriminate between ESBL genes of the same family.

Based on the conjugation assay of ESBLs producer isolates, all bla genes detected were able to be transconjugants, suggesting that they were plasmid mediated. It is likely that the genes for the TEM- and SHV-type ESBLs in *P. aeruginosa* originated in Enterobacteriaceae, from which the genes were passed by gene transfer. A plasmid location of genes encoding ESBLs of the TEM and SHV series has been reported for $bla_{SHV-12}$, $bla_{TEM-24'}$ and $bla_{TEM-42}$ in *P. aeruginosa*. These data seemed to suggest that plasmid might play an important role in contributing to the horizontal dissemination of antibiotic resistance in the same bacterial species with different serotypes via international travel or trade [15].

The spreading of resistance mediated by TEM, SHV and CTX ESBL in *P. aeruginosa* isolates from surgical and burn wards was largely to a clonal expansion of a TEM, SHV and CTX-carrying strain through the different wards, which was carried on a mobile gene cassette inserted into a class 1 integron located on the plasmid.

Similar studies for published homologous sequences in GenBank revealed that the identi-
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Genetic gene arrays were also found in the same bacterial species with different serotype, in different bacterial species (*E. coli*, *Samonella* sp., and *V. cholerae*), in bacteria from different geographic areas [16].

In this study, we found different ESBLs producers in different part of the hospital. This could be due to population difference in the city and also using of antibiotic is uncontrollable until now. Use of antibiotics and inject-able formulations was high in the region. And different prescriptions may have the result of different resistance to antibiotics [17, 18].

In summary, our data indicated a high prevalence of ESBL among *P. aeruginosa* in this region and their enzyme types were diverse. Spreading dissemination of ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients. The findings of the study added to the increasing recognition of ESBLs and emphasized the need for enhanced surveillance of different ESBLs in this species. The study illustrated the importance of phenotypic and genotypic surveillance to guide control of nosocomial infection in this region.

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**Disclosure of conflict of interest**

None.

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