PER-1 is still widespread in Turkish hospitals among *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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Abstract

PER-1 type β-lactamases were screened among ceftazidime-resistant clinical isolates of *Acinetobacter* spp. and *Pseudomonas aeruginosa*. A total of 176 non-repetitive isolates (84 *Acinetobacter* spp. and 92 *P. aeruginosa*) were collected during a three month surveillance period. Isolates were obtained from seven intensive care units of seven university hospitals. All strains were screened for *bla* _PER-1_ alleles by PCR. Of the strains, 31% and 55.4% of *Acinetobacter* spp. and *P. aeruginosa* were positive for *bla*_ _PER-1_ type genes, respectively.

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1. Introduction

*Pseudomonas aeruginosa* and *Acinetobacter* spp. are significant pathogens, particularly in intensive care units, in Turkey [1,2]. These microorganisms exhibit resistance to a wide range of antibiotics by adjusting the expressions or functionality of some inherited structures, such as porins, pumps and PBPs [3–6]. However, acquired β-lactamases have been reported very fre-

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this epidemiological study, we surveyed PER-1 type \(\beta\)-lactamases among ceftazidime-resistant clinical isolates from seven intensive care units of seven tertiary care hospitals located in specific parts of Turkey.

2. Materials and methods

2.1. Bacterial strains and microbiological studies

Seven university hospitals from specific different parts of Turkey were asked to collect consecutive, non-replicate clinical isolates of \textit{P. aeruginosa} and \textit{Acinetobacter} spp. during the three months, April to June, in 2003.

The strains were re-identified in our laboratory by methods described elsewhere [9] and stored at \(-20^\circ\text{C}\) until the study date.

MICs were determined by an agar dilution technique on Mueller-Hinton agar (Oxoid, Basingstoke, UK) with an inoculum of \(10^6\) CFU per spot according to Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines. Endpoints were read after 18 h of incubation at \(37^\circ\text{C}\).

Antimicrobial agents, their sources and ranges tested were: amikacin (Eczacibasi; 0.125–64 mg L\(^{-1}\)); aztreonam (Bristol-Myers S; 0.25–1024 mg L\(^{-1}\)); cefepime (Bristol-Myers S; 0.251024 mg L\(^{-1}\)); ceftazidime (Glaxo-SmithKline; 0.25–1024 mg L\(^{-1}\)); ciprofloxacin (Bayer, 0.064–128 mg L\(^{-1}\)); gentamicin (IEU; 0.25–32 mg L\(^{-1}\)); imipenem (Merck, 0.064–128 mg L\(^{-1}\)); meropenem (Astra-Zeneca; 0.064128 mg L\(^{-1}\)); piperacillin/tazobactam (Wyeth; 0.251024 mg L\(^{-1}\)); cefoperazone-sulbactam (Pfizer; 4256 mg L\(^{-1}\)).

\textit{P. aeruginosa} ATCC 27853 was the control strain in the agar dilution test.

2.2. PCR, RAPD and sequence analysis

Genomic DNA for \(\beta\)-lactamase screening by PCR tests were extracted by simply by incubating dense bacterial suspensions at 95 \(^\circ\text{C}\) for 10 min and sedimenting the debris for 10 min at 12,000 g. For random amplified polymorphic DNA (RAPD) analysis, however, a more concentrated and purified DNA was required. For this purpose, DNA was isolated by the lytic aid of guanidine thiocyanate lysis. The method is described elsewhere [9] and stored at \(-20^\circ\text{C}\) until the study date.

The sequences and annealing temperature of primers are as follows: PER-1, PERA 5'-GTA GTT ACT GCC TCG ACG CT-3' and PERB 5'-TCA AAT TGA TAC GCA GTC TGA-3' \((T_m = 57\, ^\circ\text{C})\). PCR cycles consisted of 5 min denaturation at 95 \(^\circ\text{C}\) followed by 35 cycles of 1 min at the indicated annealing temperature, 2 min at 72 \(^\circ\text{C}\) and 30 s at 94 \(^\circ\text{C}\), followed by 10 min of final incubation at 72 \(^\circ\text{C}\). Products were separated on a 1.5 \% agarose gel at a constant 120 V, stained with ethidium bromide and visualized on a UV lamp.

Genomic relatedness of strains was evaluated by random amplified polymorphic DNA analysis (RAPD) with ERIC primers, ERIC-1 R, 5'-AAC CTC GTG GGG ATT CA-3' and ERIC-2, 5'-AAG CTC ATG ACT GGT GTG AGC G-3' [14]. For RAPD analysis, the master mixture composition was the same as in the screening tests indicated above. But, the amplification cycles differed with as the initial denaturation followed by 25 cycles of 3 min at 39 \(^\circ\text{C}\) with an increase of 0.3 \(^\circ\text{C}\) every cycle, 2 min at 72 \(^\circ\text{C}\) and 1 min at 94 \(^\circ\text{C}\) and 30 cycles of 2 min 44 \(^\circ\text{C}\), 3 min 72 \(^\circ\text{C}\), and 1 min at 94 \(^\circ\text{C}\). A final extension for 1 h at 72 \(^\circ\text{C}\) completed the procedure [14,15].

Both strands of PCR products were sequenced. After gel purification of the products, the sequencing was performed with the same primers used for amplification. The sequence analysis method was dye terminator cycle sequencing with the ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, Calif.). The assay was carried out according to the standard protocol. Data were collected on an ABI 377 automated fluorescence sequencer.

Chromatographs were analyzed and compared to the reference sequence with the aid of the freely distributed software ChromasPro (http://www.techneIySium.com.au/ChromasPro.html).

2.3. Isoelectric focusing

\(\beta\)-Lactamases were extracted by freezing and thawing a dense suspension of bacteria in 0.1 \(N\)-\((2\text{-acetamido})-2\text{-aminoethanesulfonic acid (ACES)}-\text{NaOH buffer (pH 7.0)}\) as indicated elsewhere [9]. Crude \(\beta\)-lactamase extracts were analyzed with acrylamide gels with a pH range of 3–10 (Ampholyte sol. from Fluka), run at a constant power and visualized by overlaying a 1 mM...
suspension of nitrocefin (Oxoid). The details of this experiment are described elsewhere [9]. Results were interpreted relative to control enzymes, PER-1 (5.3), TEM-1 (pI 5.4), OXA-14 (pI 6.2) and SHV-1 (pI 7.6). SHV-1, TEM-1 and OXA-14 were gifts from David Lиверморе and PER-1 was from a previous study [16].

3. Results

Table 1 shows MICs and the resistance percentages to the nine antimicrobial agents tested. Resistance to this panel of antibiotics in this collection was overall very high. Of note, however, was the difference in the resistance rates of imipenem and meropenem. Among this collection, imipenem resistance rates were higher. The distribution of \( \text{bla} \text{PER-1} \) is shown in Table 2. PER-1 type enzymes are found to be widespread and prevalent and almost evenly distributed among the contributing centers (data not shown).

Table 2
Multiple antibiotic resistance phenotypes of \( \text{bla} \text{PER-1} \) negative and positive isolates

<table>
<thead>
<tr>
<th>MR</th>
<th>( \text{bla} \text{PER-1} ) n (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9 (15.5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>31 (53.4)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18 (31)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0</td>
<td>1 (2.4)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10 (24.4)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11 (26.8)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>19 (46.3)</td>
</tr>
</tbody>
</table>

\( a \) %, percentages of columns.

\( b \) MR, multiple resistance phenotypes. 0, resistant to only ceftazi-dime; 1, resistant to ceftazidime plus one other antibiotic from any of (a) Carbapenems (imipenem \( \geq 4 \mu \text{g mL}^{-1} \) or meropenem \( \geq 4 \mu \text{g mL}^{-1} \)), (b) Aminoglycosides (gentamicin \( \geq 4 \mu \text{g mL}^{-1} \) or amikacin \( \geq 16 \mu \text{g mL}^{-1} \)) or (c) Ciprofloxacin \( \geq 1 \mu \text{g mL}^{-1} \); 2–3, resistant to ceftazidime plus two or three of the above mentioned antibiotic classes.

Multiple antibiotic resistance phenotypes according to \( \text{bla} \text{PER-1} \) occurrences are shown in Table 3. This comparison demonstrated no significant differences in multiple antibiotic resistance phenotypes between \( \text{bla} \text{PER-1} \) positive and negative strains.

Seven \( P. \) aeruginosa and seven \( \text{Acinetobacter} \) spp., one from each center, were randomly selected. RAPD analysis of seven \( \text{Acinetobacter} \) spp. and seven \( P. \) aeruginosa selected from different centers confirmed the multi-clonal spread of \( \text{bla} \text{PER1} \) (Fig. 1). PER genes from RAPD-typed strains were sequence-analyzed and, except for a few silent mutations, all were confirmed to be PER1.

4. Discussion

This study demonstrated that PER-1 is still a major resistance determinant of \( \text{Acinetobacter} \) spp. and \( P. \) aeruginosa in Turkish hospitals. In a similar survey in 1996, PER-1 type enzymes had been detected in 60% (33 of 55) and 38.4% (40 of 104) of ceftazidime-resistant \( \text{Acinetobacter} \) spp. and \( P. \) aeruginosa, respectively [9]. In this study, the rate among \( \text{Acinetobacter} \) spp. was found to be significantly lower, at 31% (26 of 84), \( p < 0.05 \), compared to the survey of 1996; whereas the rate among \( P. \) aeruginosa is not significant. The figures in this study are somewhat different. Nevertheless, PER-1 is still widespread and highly prevalent.

Resistance to major antibiotics was found to be extremely high. The collection in this study represents only ceftazidime-resistant isolates. Since, ceftazidime...
resistance is extremely high in Turkish hospitals [17,18], the high resistance rates to major antibiotics found in this study is significant. On the other hand, PER1 was not significantly more related to multiple antibiotic resistance phenotypes. Moreover, among Acinetobacter spp. of this study, only one of 26 bla<sub>PER-1</sub> positive isolates was resistant to imipenem; whereas, of PER-1 negative isolates, 21 of 58 were resistant to imipenem (data not shown). PER-1 type enzyme positive strains, in other words, are not more resistant than others. However, independent of antibiotic resistance, PER-1 was found to be significantly related to adverse clinical outcomes in Turkish hospitals [19].

This study documented that PER-1 is still a significant health problem in Turkey as it is in some other countries, and deserves merits more attention.

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References


Fig. 1. RAPD patterns of selected strains. A, Acinetobacter and B, P. aeruginosa; lanes M, DNA marker (Gene Ruler, Fermentas); Lanes, 1–7 represent strains from different centers.


