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AŞIK, GÜLŞAH; ÖZDEMİR, MEHMET; KURTOĞLU, MUHAMMET GÜZEL; YAĞCI, SERVER; ÖKSÜZ, LÜTFİYE; GÜL, MUSTAFA; KOÇOĞLU, MÜCAHİDE ESRA; ÇETİN, EMEL SESLI; SEYREK, ADNAN; BERKTAŞ, MUSTAFA; AYYILDIZ, AHMET; and ÇİFTÇİ, İHSAN HAKKI (2014) "Detection the frequency of PER-1 type extended-spectrum β-lactamase-producing Acinetobacter baumannii clinical isolates in Turkey: a multicenter study," Turkish Journal of Medical Sciences: Vol. 44: No. 6, Article 19. https://doi.org/10.3906/sag-1309-126
Available at: https://journals.tubitak.gov.tr/medical/vol44/iss6/19

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This article is available in Turkish Journal of Medical Sciences: https://journals.tubitak.gov.tr/medical/vol44/iss6/19
Detection of the frequency of PER-1 type extended-spectrum β-lactamase–producing Acinetobacter baumannii clinical isolates in Turkey: a multicenter study

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1. Introduction
Since members of the genus Acinetobacter were realized to be significant nosocomial pathogens, much information has been learned. In the first in vitro studies, most clinical isolates were susceptible to generally used antimicrobial agents so that infections caused by these organisms could be treated relatively easily (1). However, successive surveys have shown increasing resistance among clinical isolates, particularly those belonging to the Acinetobacter baumannii complex, and high proportions of isolates are now resistant to the most commonly used antimicrobial agents.

Over the past decade, nosocomial outbreaks of A. baumannii have been described with increasing frequency, mostly in association with intensive care units, burn units, or surgical wards (2). Resistance to β-lactam antibiotics in Acinetobacter spp. predominantly involves 3 mechanisms: production of β-lactamases, loss of outer
membrane proteins, and upregulation of efflux pumps (2). The most common mechanism of resistance to β-lactam antibiotics is the production of β-lactamases (3). Acquired β-lactamases encoded by mobile genetic elements are an important resistance mechanism in A. baumannii (4).

One of the β-lactamases, Pseudomonas extended resistance (PER-1), was first identified in 1991 in a Pseudomonas aeruginosa strain isolated from a Turkish patient in France (5). The bla\_PER-1 gene is widespread in Turkey, particularly among P. aeruginosa and Acinetobacter spp. strains (6–8). However, PER-1 type β-lactamases among clinical isolates from Turkish hospitals have not been investigated extensively. Furthermore, the current prevalence of PER-1 in the various regions of Turkey remains unknown. Therefore, the aim of this study was to determine the prevalence of PER-1 type β-lactamases in A. baumannii isolates from 11 hospitals in Turkey.

2. Materials and methods

A total of 763 nonrepetitive A. baumannii clinical isolates archived from 9 university hospitals and 2 state hospitals in Turkey from 2008 to 2011 were included in the study. The provinces that participated in the study are shown in the Figure. Antimicrobial susceptibility tests and molecular studies were performed in the microbiology laboratories of Afyon Kocatepe University. The isolates were identified by means of both conventional techniques (including oxidation–fermentation reactions in triple sugar iron agar, oxidase production, and colony appearance) and automated systems, including the VITEK system (bioMérieux VITEK System Inc.; bioMérieux, Marcy l’Etoile, France) and the Phoenix 100 system (Becton Dickinson and Company, Franklin Lakes, NJ, USA). The OXA-51 gene region is species-specific for A. baumannii (4). The identification of species was verified by the presence of the bla\_OXA-51 gene within the A. baumannii genome. The susceptibilities of the isolates to imipenem, meropenem, cefepime, cefoperazone-sulbactam, gentamicin, amikacin, and tobramycin were tested using the standard disk diffusion method on Mueller Hinton (MH) agar plates (Oxoid Ltd., Basingstoke, UK) and using the breakpoints defined by the Clinical and Laboratory Standards Institute (9). Antimicrobials were applied and stored according to the manufacturer’s instructions. A. baumannii ATCC 19606 was used as a reference strain for susceptibility tests.

For DNA extraction, a loopful of bacteria was taken from each fresh overnight culture on MH agar plates. DNA samples were extracted by incubating a pure bacterial suspension at 95 °C for 10 min in a lysis buffer, and debris was removed by centrifugation for 5 min at 12,000 × g. The quantity and quality of the extracted DNA were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA concentration and the ratio of the optical density at 260/280 nm to evaluate the purity of the DNA samples were calculated simultaneously (10).

Based on sequences of \textit{bla\_PER-1} and \textit{bla\_OXA-51} of A. baumannii retrieved from the National Center for Biotechnology Information Entrez database, oligonucleotide primer sets PER-1F (5’-ATG AAT GTC ATT ATA AAA GC-3’), PER-1R (5’-AAT TTG GGC TTA GGG CAG AA -3’), OXA-51F (5’-TCA GCA AGA GGC ACA GTT TG-3’), and OXA-51R (5’-GCT GAA CAA CCC ATC CAG TT-3’) were designed and used to amplify a single polymerase chain reaction (PCR) product of 925 bp and a 188-bp fragment, respectively. The annealing temperatures were 52 °C and 54 °C for the PER-1 and OXA-51 primer pairs, respectively. Conventional assays were used to optimize the PCR reaction using a TECHNE-TC-512 thermal cycler (Barloworld Scientific, Burlington NJ, USA) for detection of \textit{bla\_PER-1} and \textit{bla\_OXA-51} genes.

Conventional PCR reactions with genomic DNA were performed in a 25-µL mixture according to the manufacturer’s protocol for the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific). The PCR reaction steps were as follows: preheating at 95 °C
for 10 min; 35 cycles consisting of 95 °C for 30 s, 52 °C for PER-1 and 54 °C for OXA-51 30 s, and 72 °C for 1 min; and a final extension incubation at 72 °C for 4 min. After optimization, PCR assays were completed using the real-time method (SLAN, Shanghai Odin Science & Technology Co, Shanghai, China). Real-time PCR reactions were performed in a 25-µL mixture according to the manufacturer's protocol for the PCR master mix. The real-time PCR reaction steps were as follows: initial denaturation at 95 °C for 5 min; 30 cycles consisting of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min; and a final extension incubation at 72 °C for 2 min.

To confirm the PCR product, melting curve analysis and 1.0% agarose gel electrophoresis were performed. The chi-square test was used to compare proportions. P < 0.05 was deemed to indicate statistical significance. Statistical analyses were conducted using the SPSS 17 (SPSS Inc., Chicago, IL, USA).

### 3. Results

A total of 763 unique *A. baumannii* strains had been identified previously in clinical microbiology laboratories at study centers between 2008 and 2011. All of the 763 strains tested positive using primers specific for OXA-51. Among the isolates, the rates of resistance were as follows: 100% for the third-generation of cephalosporin (ceftazidime, ceftriaxone, and cefotaxime) 94.8% for cefepime, 73.6% for cefoperazone-sulbactam, 76.3% for meropenem, 74.5% for imipenem, 77.1% for gentamicin, 75.3% for amikacin, and 36.5% for tobramycin. Although the frequency of resistance to all antibiotics was high, especially against carbapenems when grouped according to year, there were no significant differences among the rates of antimicrobial resistance in the various locations in Turkey (chi-square test; P > 0.05). The regions, years, and antimicrobial resistance distributions of *A. baumannii* isolates are summarized in the Table.

### Table. PER-1 frequencies and the antimicrobial resistance profiles of *A. baumannii* isolates from various hospitals in Turkey.

<table>
<thead>
<tr>
<th>City</th>
<th>Year</th>
<th>Location in Turkey</th>
<th>n</th>
<th>PER-1 (+) n/%</th>
<th>PER-1 (-) n/%</th>
<th>Antimicrobial resistance rate. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afyonkarahisar</td>
<td>2008</td>
<td>West</td>
<td>42</td>
<td>10/23.8</td>
<td>32/76.2</td>
<td>47.6 54.8 100 52.3 83.3 71.4 64.2</td>
</tr>
<tr>
<td>Kahramanmaraş</td>
<td>2008</td>
<td>Southeast</td>
<td>32</td>
<td>15/46.9</td>
<td>17/53.1</td>
<td>65.6 65.6 87.5 50.0 71.8 68.7 12.5</td>
</tr>
<tr>
<td>Van</td>
<td>2008</td>
<td>East</td>
<td>62</td>
<td>46/74.2</td>
<td>16/25.8</td>
<td>45.2 50.1 95.1 73.5 90.3 70.9 9.7</td>
</tr>
<tr>
<td>Afyonkarahisar</td>
<td>2009</td>
<td>West</td>
<td>79</td>
<td>21/26.6</td>
<td>58/73.4</td>
<td>60.8 60.8 97.5 67.1 48.1 48.1 32.9</td>
</tr>
<tr>
<td>İstanbul</td>
<td>2009</td>
<td>Marmara</td>
<td>41</td>
<td>9/22.0</td>
<td>32/78.0</td>
<td>92.6 92.6 100 73.8 85.3 80.5 51.2</td>
</tr>
<tr>
<td>Konya</td>
<td>2009</td>
<td>Middle</td>
<td>65</td>
<td>21/32.3</td>
<td>44/67.7</td>
<td>93.8 92.3 100 92.3 72.3 72.3 53.8</td>
</tr>
<tr>
<td>Bolu</td>
<td>2009</td>
<td>North</td>
<td>43</td>
<td>31/72.1</td>
<td>12/27.9</td>
<td>81.4 81.4 100 88.3 100 88.3 32.5</td>
</tr>
<tr>
<td>Afyonkarahisar</td>
<td>2010</td>
<td>West</td>
<td>105</td>
<td>1/0.9</td>
<td>104/99.1</td>
<td>83.8 82.9 97.1 61.9 80.0 66.6 39.0</td>
</tr>
<tr>
<td>Ankara</td>
<td>2010</td>
<td>Middle</td>
<td>50</td>
<td>1/2.0</td>
<td>49/98.0</td>
<td>60.0 60.0 88.0 70.0 58.0 76.0 52.0</td>
</tr>
<tr>
<td>Erzurum</td>
<td>2010</td>
<td>East</td>
<td>49</td>
<td>7/14.3</td>
<td>42/85.7</td>
<td>69.4 69.4 91.8 77.5 65.3 71.4 40.8</td>
</tr>
<tr>
<td>Isparta</td>
<td>2010</td>
<td>South</td>
<td>94</td>
<td>11/11.7</td>
<td>83/88.3</td>
<td>72.3 76.6 82.9 60.6 76.6 88.3 20.2</td>
</tr>
<tr>
<td>Konya</td>
<td>2010</td>
<td>Middle</td>
<td>74</td>
<td>11/14.9</td>
<td>63/85.1</td>
<td>98.7 98.7 98.7 97.3 93.2 79.7 52.7</td>
</tr>
<tr>
<td>Van</td>
<td>2011</td>
<td>East</td>
<td>18</td>
<td>4/22.2</td>
<td>14/77.8</td>
<td>94.4 94.4 100 88.8 88.8 83.3 27.7</td>
</tr>
<tr>
<td>Elazığ</td>
<td>2011</td>
<td>East</td>
<td>9</td>
<td>0/0</td>
<td>9/100</td>
<td>77.7 88.8 88.8 77.7 66.6 88.8 22.2</td>
</tr>
</tbody>
</table>

TOTAL 763 188/24.6 575/75.4 74.5 76.3 94.8 73.6 77.1 75.3 36.5

The concentration and the purity of the extracted DNA from a representative study group of 25 randomly selected test samples were assessed using the NanoDrop ND-1000 spectrophotometer. The mean ± standard deviation (SD) concentration of extracted DNA was 22.36 ± 7.11 ng/µL (minimum–maximum values, 8.97–49.37 ng/µL). The mean ± SD purity of extracted DNA samples (A260/A280) was 1.69 ± 0.09 (minimum–maximum values, 1.60–1.85).

PER-1 was detected by PCR using specific primers in 188 (24.6%) of 763 isolates. The annual PER-1 detection frequencies were 52.2%, 35.9%, and 8.3% in 2008, 2009, and 2010, respectively. The rates of PER-1–producing isolates were 47.6%, 42.4%, 17.5%, 13.6%, and 11.7% in the northern, eastern, middle, western, and southern regions of Turkey, respectively. The rates of PER-1–producing isolates differed significantly among the regions of Turkey (chi-square test; P < 0.001). Furthermore, the rates of PER-1–producing isolates in Afyonkarahisar Province were 23.8%, 26.1%, and 1% in 2008, 2009, and 2010, respectively (chi-square test; P < 0.001); in Van Province they were 74.2% and 22.2% in 2008 and 2011, respectively (chi-square test; P < 0.001). There were statistically significant differences between the rates of isolation of PER-1–producing strains from different hospitals in the same city, and in different cities in the same region of Anatolia (chi-square test, P = 0.017). Other results are summarized in the Table.

4. Discussion

A. baumannii has become a major cause of hospital-acquired infections, because this pathogen is difficult to control due to its prolonged environmental survival. Moreover, treatment is complicated by its ability to develop resistance to multiple antibiotic agents. Thus, in the last 10 years, A. baumannii has emerged as a highly problematic pathogen because few antibiotics are effective against this organism (11).

A. baumannii has become resistant to almost all antimicrobial agents currently available, including broad-spectrum β-lactams and quinolones. Most strains are resistant to aminoglycosides and cephalosporins (11). Our results demonstrate that all isolates were resistant to third-generation cephalosporins. In addition, cefoperazone/sulbactam and aminoglycoside resistance rates (except those for tobramycin) were around 70% at the various centers in Turkey. Because of these very high resistance rates, these agent classes are unlikely to play an important role in the treatment of A. baumannii infections.

Among the 490 A. baumannii isolates from patients with serious infections in European hospitals participating in the 1997–2000 Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) programs, the 2 carbapenems meropenem and imipenem showed the greatest clinically useful activity (12). Susceptibilities of A. baumannii to meropenem were very high (97%–100%) in all countries except Italy (70%), Turkey (66%), and the United Kingdom (77%). Similar results were observed for imipenem (93%–100%), except for Italy (78%), Turkey (62%), and the United Kingdom (78%) (12). Baran et al. (13) reported that the imipenem resistance rate was lower than indicated in the MYSTIC report for Turkey (53.7%). These reports show that carbapenems (imipenem and meropenem) remain active against many strains; however, increasing numbers of clinical isolates of A. baumannii resistant to carbapenems are now being reported worldwide, reaching levels of ≥90% at some centers (14,15). In the present study, we found that A. baumannii isolates have a high carbapenem resistance rate, which exceeded that in the MYSTIC report. Another important issue is the annual increase in rates of resistance; isolates from Afyonkarahisar, Konya, Istanbul, and Van provinces from the previous years exhibited particularly high rates of resistance. This could be associated with the increased isolation of multidrug-resistant (MDR) species and a change in antibiotic selection protocols. Thus, the use of carbapenem in the treatment of MDR A. baumannii infections has increased in recent years. As a result, a dramatic increase in carbapenem resistance has occurred (Table).

PER-1 β-lactamase has been considered to be significant only in Turkey for a number of years. However, the PER-1 β-lactamase has been detected in P. aeruginosa in many countries, including Turkey, France, Belgium, Spain, Italy, Poland, Romania, Japan, and South Korea (7,16–23). PER-1 production in Acinetobacter spp. has been reported most often in Turkey and Korea (24). PER-1 type β-lactamases in clinical isolates from Turkish hospitals have not been investigated extensively. Only 1 study by Hoşgör et al. (25) in İzmir was published and in that study the bla_{PER-1} gene was detected by PCR in 33 (19.5%) of a total of 169 gram-negative bacteria, including 17 (23.3%) of the 73 P. aeruginosa isolates and 16 (25%) of the 64 A. baumannii complex isolates. The rate of detection of PER-1 was available to date only for 274 A. baumannii isolates from 3 regions of Turkey. Nationwide surveys in Turkey revealed that 46% (1997), 37.8% (2001), 31% (2005), and 35.9% (2007) of A. baumannii isolates produced PER-1, as reported by Vahaboglu et al. (6,26), Kolyai et al. (7), and Erac and Gulay (8), respectively. The current prevalence and distribution of PER-1 in the various regions of Turkey are unknown. In the present study, the rate of detection of PER-1 in 763 A. baumannii clinical isolates collected from different regions, 10 cities, and 11 hospitals was 24.6% (minimum–maximum values, 0%–74.2%). The annual rate of PER-1 detection decreased gradually with time, with the most prominent decrease occurring in 2010. Furthermore, between 2008 and 2011, the resistance rates increased markedly. The data suggest that, in a hospital in which the prevalence of resistant A. baumannii is
increasing and clinical isolates demonstrate new antibiotic resistance patterns, it would be prudent to first consider the likelihood of transmission of these pathogens from exogenous sources. Another important reason for the decreased prevalence of PER-1 is transmission of mobile genes on plasmids among the A. baumannii population.

Differences in PER-1 prevalence have been observed among the regions of Turkey. PER-1 was prominent in isolates from Van Province in the eastern and Bolu Province in the northern region of Turkey. This difference in resistance profiles among regions has been expected by many researchers. We think that the regional differences identified in the present study can be attributed to changes in the patient population and treatment regimen preferences.

In conclusion, A. baumannii as a cause of nosocomial infections has shown a first-order upward trend in recent years. The frequency of detection of PER-1 type extended-spectrum β-lactamases in A. baumannii species has decreased, and PER-1 is no longer a threat in terms of Turkey’s resistance profile. However, the increased carbapenem resistance, together with MDR bacteria, represents a worrisome situation in this species. Thus, the mechanisms responsible for carbapenem resistance in A. baumannii isolates should be investigated. Moreover, strategies to prevent the spread of resistance should be instituted.

Acknowledgments

We thank staff member Şüle Özkan of the Afyon Kocatepe University Microbiology Laboratory for contribution in the performing of the molecular tests. We also thank all the academics who sent A. baumannii strains to our laboratory for this study. There were no financial sources.

References


