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Novel PER-variant ß-lactamase Identified in a *Providencia rettgeri* Strain from the United States (USA): Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To characterize the genetic determinant responsible for the ESBL phenotype in a *Providencia rettgeri* bacteremia isolate collected in a hospital in Galveston, Texas, USA.

Methods: During 2007, 283 (10% of all isolates) Enterobacteriaceae isolates from SENTRY Program USA medical sites displayed elevated cephalosporin MIC values (≥2 mg/L) by reference broth microdilution method. ESBL phenotypes were confirmed using Etest strips containing cephalosporins with and without clavulanate. ESBL genes were amplified in a multiplex PCR approach using generic primers for genes encoding PER, VEB, GES, OXA-2 and OXA-10. TEM- and SHV-encoding genes were also amplified and sequenced. Primers comprising the open reading frame of bla_{PER} were used to amplify the entire gene and amplicons were cloned into TOPO and transformed in an *E. coli* host for sequencing. Conjugation experiments were carried out and selected on media containing 500 mg/L of streptomycin and 8 mg/L of ceftazidime.

Results: *P. rettgeri* isolate 25-3141A was recovered in February 2007 from a bloodculture of a 65 years old male patient hospitalized in a Texas hospital. The patient was an inmate of the Texas State Prison for several years prior to admission for evaluation of pneumonia. He was briefly hospitalized a month earlier with diagnosis of HCV cirrhosis and ascites, when he received only one day of cefotaxime. The *P. rettgeri* isolate demonstrated elevated cefepime MIC (4 mg/L) and positive ESBL confirmatory test. Isolate 25-3141A also showed elevated MIC values against fluoroquinolone, trimethoprim/sulfamethoxazole and tetracycline. bla_{PER} amplicons were detected in the multiplex PCR. Recombinant plasmids carrying the entire ß-lactamase encoding gene were sequenced and the analysis of the derived aminoacid sequence showed one aminoacid alteration compared to PER-1 structure. A glycine in position 33 (of bla_{PER-1}) was substituted by a glutamic acid (E33G) that was in this position in all other PER ß-lactamases. This isolate also harboured bla_{TEM-1}. Conjugation failed to yield colonies showing resistance to cephalosporins.

Conclusions: ESBLs, other than SHV and TEM, are becoming more common in the USA with recent reports of CTX-M-producing strains in several medical centers. In this study, we described a novel PER enzyme detected in a *P. rettgeri* that showed a single aminoacid difference compared to PER-1. The diversity in the ß-lactamase types detected among USA isolates is rapidly increasing, changing the ESBL treatment scenario in this country.

INTRODUCTION

PER-type ß-lactamases were initially detected in a *Pseudomonas aeruginosa* strain recovered from a Turkish patient hospitalized in France (1993). Since they were initially described, PER enzymes have been observed among other organisms such as *Acinetobacter* spp. and several Enterobacteriaceae species.

To date, two PER variants, PER-1 and PER-2 have been described and three other types were deposited in the genetic database. PER-1 and PER-2 were both located in similar transposon structure, suggesting a common origin and mobilization mechanism. Furthermore, PER-enzymes have been detected in isolates that also harbor metallo-ß-lactamase encoding genes in geographic regions where both types of resistance determinants are prevalent.

PER-producing isolates have now been reported in several countries, including Argentina, Bolivia, Turkey, Spain, Switzerland, Italy, Belgium, Japan and Korea. However, in Turkey and the Far East this enzyme seems to be more common among *Acinetobacter* spp. isolates, whereas in European countries its occurrence was more often related to *P. aeruginosa*. In the USA, only three PER-producing *Acinetobacter* spp. isolates were detected among soldiers returning from Afghanistan.

In this study, we report the presence of a new PER-enzyme, PER-5, detected in a prison inmate in Texas, USA.

MATERIALS AND METHODS

Bacterial isolates. A total of 2,844 Enterobacteriaceae isolates were collected from 26 USA hospitals during the SENTRY Program (2007). Only one isolate per patient from documented infections were included in the study. Isolates were collected from bloodstream, respiratory tract and skin and skin structures infections according to a common protocol. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing and ESBL confirmation. All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for all antimicrobials were those found in M100-S19 and quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents (M100-S19, 2009).

Isolates displaying the CLSI MIC criteria for ESBL production were confirmed with the clavulanate inhibition Etest (AB BIODISK, Solna, Sweden).

Genotypic detection of ESBL genes, cloning and sequencing of bla_{PER}. A multiplex PCR approach utilizing custom generic primers was used to detect PER, GES, VEB, CTX-M and oxacillinases (OXA-ESBL). PCR amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Primers encoding entire bla_{PER} gene (PER-F 5'-ATG AAT GTC ATT ATA AAA GC-3' and PER-R 5'-AAT TTG GGC TTA GGG CAG AA-3') were used to clone this gene into TOPO vector (Invitrogen, Carlsbad, USA) and transformed in an *E. coli* host. The recombinant plasmid was sequenced.

Plasmid analysis and conjugation. Plasmid extractions were performed using the Plasmid Mini kit (QIAGEN, Hilden, Germany). Plasmid preparations were resolved in 1% agarose gels and the molecular weights were determined by comparison with plasmids harbored by *E. coli* NCTC 50192 and 50193. Plasmid DNA was transferred to nylon membranes by southern blotting and hybridized with a digoxigenin labeled (Roche Diagnostics GmbH, Mannheim, Germany) bla_{PER}-specific probe.

Transference of ß-lactam resistance determinants was assayed by mating experiments in broth by mixing equal volumes of PER-producing donor and recipient *E. coli* J53 derivatives resistant to azide (Az^R) in the exponential phase of growth. Transconjugants were selected in agar plates containing sodium azide (200 mg/L) and ceftriaxone (1 mg/L) or ceftazidime (8 mg/L).

Nucleotide sequencing accession number. The nucleotide sequence of bla_{PER-5} has been submitted to GenBank nucleotide database and assigned accession number FJ627180.

RESULTS

- Among 283 (9.9% of total) ESBL-producing Enterobacteriaceae isolated in the USA during 2007, only one isolate was positive for bla_{PER} primers.
- This isolate was a *Providencia rettgeri* (25-3141A) recovered from a blood culture (February 2007) of a 65 years old male patient hospitalized in Galveston, Texas (USA).
- The patient was an inmate of the Texas State Prison system for several years prior to admission for evaluation of pneumonia. He was briefly hospitalized a month earlier with diagnosis of HCV cirrhosis and ascites, when he received only one day of cefotaxime.
- Isolate 25-3141A showed a markedly elevated ceftazidime MIC (>16 mg/L) and positive ESBL confirmatory tests. Fluoroquinolone, trimethoprim/sulfamethoxazole and tetracycline MIC values were also at resistant levels (Table 1).
- Sequencing of the entire gene cloned into a recombinant plasmid revealed a gene that was >99% similar to bla_{PER-1} that was subsequently named bla_{PER-5}.

Table 1. Antimicrobial susceptibility pattern of PER-5-producing *P. rettgeri* collected in Galveston, Texas (USA) during the SENTRY Antimicrobial Surveillance Program (2007).

Antimicrobial Agent	MIC (mg/L) bla_{PER-5}-carrying *P. rettgeri* 25-3141A
Aztreonam	8
Ceftazidime	>16
Ceftriaxone	2
Cefepime	2
Piperacillin/Tazobactam	1
Ertapenem	≤0.06
Imipenem	1
Meropenem	≤0.12
Ciprofloxacin	>4
Trimethoprim/Sulfamethoxazole	>2
Amikacin	1
Gentamicin	>16
Tobramycin	2
Tetracycline	>8
Tigecycline	1

Figure 1. Alignment of the amino acid sequences of PER-5 detected during the SENTRY Antimicrobial Surveillance Program 2007 and other PER variants. The aminoacid substitution E33G in PER-5 is underlined.

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PER-1.pro   MNVI I KAVVTASTLLMVSFSSFETSAQSPLLKEQI ESI VI GKKATVGVAVWGPDDLEPLLI NPFEKFPMQSVFKLHLAML  80
PER-2.pro   . . . . T. C. F. . . A. . . LGL. . . VV. . . . . T. . T. . . . . L. . . . . .  80
PER-3.pro   . . . . . .  80
PER-4.pro   . . . . . .  80
PER-5.pro   . . . . . G. . . . . .  80

PER-1.pro   VLHQVDQGKLDLNQTVI VNRAKVLQNTWAPI MKAYQGDEFSVPVQQLLQYSVSHSDNVACDLLFELVGGPAALHDYI QSM  160
PER-2.pro   . . . . . S. T. . . . A. . . . . S. M. . DH. . . . T. A. . . . . Q. . . A. . . L  160
PER-3.pro   . . . . . L. . . . . .  160
PER-4.pro   . . . . . T. . . . . .  160
PER-5.pro   . . . . . .  160

PER-1.pro   GI KETAVVANEAQMHADDQVQYQNWTSMKGAAEI LKKFEQKTQLSETSQALLWKWMVETTTGPERLKGLLPAGTVVAHKT  240
PER-2.pro   . V. . A. . . . . A. . QV. Q. . . . K. . . . . Q. . . . . I . . . .  240
PER-3.pro   . . . . . .  240
PER-4.pro   . . . . . .  240
PER-5.pro   . . . . . .  240

PER-1.pro   GTSGI KAGKTAATNDLGI I LLPDGRPLLVAVFVKDSAESSRTNEAI I AQVAQTAYQFELKKLSALSPN  308
PER-2.pro   . . . . VR. . . . . A. V. M. . . . . E. . . . . A. . . . . V. . D  308
PER-3.pro   . . . . . .  308
PER-4.pro   . . . . . .  308
PER-5.pro   . . . . . .  308
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- Aminoacid sequence analysis showed that PER-5 had one aminoacid alteration compared to PER-1 structure (Figure 1). A glycine in position 33 (of bla_{PER-1}) was substituted by a glutamic acid (E33G). This substitution was not observed in any other PER-variant (Figure 1). Isolate 25-3141A also harboured bla_{TEM-1}.
- Plasmid preparations showed the absence of plasmid bands and hybridization results suggested that bla_{PER-5} was not located on a plasmid. Conjugation attempts failed to yield colonies showing resistance to cephalosporins.

CONCLUSIONS

- Until recently, ESBLs in the USA were considered to be predominantly of TEM- and SHV-types. This scenario quickly changed with the recent emergence and spread of CTX-M-producing strains.
- The finding of a new PER-variant in the USA increases the diversity of ESBL-like enzymes in this country highlighting another potential limitation for the use of broad-spectrum cephalosporins.

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