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Spread of Enterobacteriaceae carrying the PER-1 extended-spectrum β-lactamase gene as a chromosomal insert: a report from Italy

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Sir,

Nowadays, therapeutic failure and increased hospital costs are associated with serious infections caused by PER-1-positive isolates of Enterobacteriaceae.[1] PER-1 extended-spectrum β-lactamase (ESBL), first detected in a *Pseudomonas aeruginosa* clinical isolate, has also been reported in *Alcaligenes faecalis* and *Proteus mirabilis*.[2,3]

In this study, hospital- and community-acquired ESBL producers were investigated by colony-blot hybridization, using random-primed ^{32}P-labelled DNA probes for bla_{TEM}-, bla_{SHV}-, bla_{PER}- and $bla_{CTX\text{-}M}$-type genes and subjected to exhaustive sequencing of identified allelic variants. Particularly, the primers used to study the nature of bla_{PER} and bla_{TEM} alleles were as follows: PER-1/F (5′-ATGAATGTCATTATA AAAGCT) and PER-1/R (5′-TTAATTTGGGCTTAGGG), and TEM/F (5′-ATGAGTATTCAACATTTCCG) and TEM/R (5′-TTACCAATGCTTAATCAGTGAG), respectively. All PCR reactions were performed in a 100 μL volume using 2 μL of a crude bacterial lysate obtained by boiling bacterial suspension (A_{600} 0.15) in sterile distilled water for 10 min as template. The PCR amplicons were directly sequenced on both strands, on two independent amplification products for each isolate, using an ABI PRISM 310 DNA Sequencer (Applied Biosystems, Applera Italia). PER-1-producing isolates were investigated by PFGE analysis and genomic DNA was prepared in agarose plugs as previously described,[4] with the following modifications: bacterial cells [optical density (OD_{600}) 0.7–1.0] were embedded in 2% low-melting-point agarose (LMPA) in SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.5), followed by double protein digestion at 50°C for 16 h in ES proteolysis buffer (0.5 M EDTA, pH 9.0; 1% N-lauryl-sarcosine; 1 mg/mL proteinase K). The plugs were washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to remove excess proteinase K and stored at 4°C. DNA was digested with 40 U of *Sma*I (BioLabs, New England) at 30°C overnight. PFGE analysis of *Sma*I profiles was carried out in a CHEF-DR® II apparatus (Bio-Rad, CA, USA) at 14°C using a switch time of 0.5–15 s at 6 V/cm for 17 h. Similarities among *Sma*I macrorestriction patterns were identified according to established criteria.[5] Conjugation experiments were carried out in Mueller–Hinton broth (Difco Laboratories, Detroit, MI, USA) using *E. coli* K-12 as a recipient and an initial donor/recipient ratio of 1 to 10. Transconjugants were selected on Mueller–Hinton agar with cefotaxime (16 mg/L) plus streptomycin (1000 mg/L). MICs of various antibiotics were determined by Etest (AB Biodisk, Solna, Sweden) according to CLSI guidelines,[6] with *Escherichia coli* ATCC 25922 as a quality control strain.

Overall, 15/583 isolates were found to be PER-1 positive (2.6% of total strains). Isolates included: *Providencia stuartii* ($n = 8$), *P. mirabilis* ($n = 5$), *Providencia rettgeri* ($n = 1$) and *E. coli* ($n = 1$). PER-1-positive strains were detected in 5/11 hospitals located in different Italian regions. Most isolates were obtained from urinary or lower respiratory tract infections and both community and hospitalized patients were involved. Two isolates of *P. stuartii* and one of *E. coli* harboured the bla_{PER} gene only, whereas the remaining 12/15 isolates also carried bla_{TEM} genes [TEM-1 ($n = 6$), TEM-2 ($n = 4$) and TEM-5 ($n = 2$)]. A large plasmid was extracted from isolates carrying both bla_{TEM} and $bla_{PER\text{-}1}$ genes and transferability was assayed for all strains. In contrast, no plasmids were detected in the two *P. stuartii* isolates carrying the PER-1 determinant only. In a Southern-blot experiment, carried out on nylon filters, the $bla_{PER\text{-}1}$ probe yielded a hybridization signal corresponding to the chromosomal DNA band for *P. mirabilis*, *P. stuartii* and *P. rettgeri*. No hybridization signal was observed with plasmidic DNA bands that, instead, were positive when using the bla_{TEM} probe (data not shown). Plasmids were inserted by transformation experiments into *E. coli* HB101 competent cells to verify that only TEM-type β-lactamases were plasmid-encoded whereas PER-1 was chromosome-encoded.

The resistance phenotype of isolates was evaluated using the following antibiotics: amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin and ciprofloxacin. Isolates were consistently resistant to amoxicillin/clavulanate, ceftazidime and gentamicin, while they were always susceptible to piperacillin/tazobactam and meropenem; some strains were also resistant to cefotaxime (Table 1). Susceptibility to other antibiotics varied depending on the species.

The analysis of PFGE profiles revealed that strains of *P. mirabilis* recovered in the same hospital were closely related, but different from those obtained in other cities. The eight *P. stuartii* isolates collected in the same hospital were clonally related.

Overall, PER-1 ESBL-producing Enterobacteriaceae appear to be emerging in Italy. Adequate surveillance and monitoring

Table 1. Antimicrobial susceptibilities of PER-1-producing Enterobacteriaceae

Strain	β-Lactamase(s)	MIC (mg/L)										
		AMC	TZP	FOX	CTX	CAZ	FEP	IMP	MEM	AMK	GEN	CIP
E. coli PA-126	PER-1	128	8/4	>128	>128	>128	>128	1	0.5	8	16	0.032
P. rettgeri AN-026	TEM-2/PER-1	>128	2/4	4	>32	128	4	2	0.064	2	16	>32
P. stuartii FI-001	PER-1	>128	2/4	8	8	>128	2	4	0.125	8	16	1
P. stuartii FI-070	TEM-1/PER-1	>128	2/4	8	>32	>128	4	4	0.19	4	>128	>32
P. stuartii FI-090	PER-1	>128	2/4	8	16	>128	2	4	0.125	4	32	>32
P. stuartii FI-112	TEM-1/PER-1	>128	2/4	16	16	>128	2	4	0.19	4	16	>32
P. stuartii FI-137	TEM-5/PER-1	>128	2/4	8	>32	>128	2	4	0.125	8	16	1
P. stuartii FI-143	TEM-5/PER-1	>128	2/4	8	16	>128	2	4	0.25	4	16	>32
P. stuartii FI-153	TEM-1/PER-1	>128	2/4	16	16	>128	2	2	0.125	8	16	>32
P. stuartii FI-155	TEM-1/PER-1	>128	2/4	16	>32	>128	2	4	0.19	4	16	>32
P. mirabilis AN-021	TEM-2/PER-1	48	2/4	24	>32	>128	32	8	0.125	32	>128	0.5
P. mirabilis AN-042	TEM-1/PER-1	48	2/4	24	>32	>128	32	8	0.125	32	>128	1
P. mirabilis AN-129	TEM-2/PER-1	48	2/4	24	>32	>128	64	8	0.125	32	>128	1
P. mirabilis NA-15	TEM-2/PER-1	64	2/4	32	>32	>128	32	8	0.125	24	>128	1
P. mirabilis VA-1144	TEM-1/PER-1	64	4/4	24	>32	>128	64	4	0.125	16	>128	>32

AMC, amoxicillin/clavulanate; TZP, piperacillin/tazobactam; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin.
Tazobactam was used at a fixed concentration of 4 mg/L.

are mandatory to avoid further spread of this resistance determinant.

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Transparency declarations

None to declare.

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