JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2007, p. 2829–2834 Vol. 45, No. 9
0095-1137/07/$08.00+0 doi:10.1128/JCM.00997-07

Outbreak of *Pseudomonas aeruginosa* Infections with PER-1 Extended-Spectrum β-Lactamase in Warsaw, Poland: Further Evidence for an International Clonal Complex[▽]

Joanna Empel,[1]* Krzysztof Filczak,[2] Agnieszka Mrówka,[1] Waleria Hryniewicz,[1] David M. Livermore,[3] and Marek Gniadkowski[1]

National Medicines Institute, 00-725 Warsaw,[1] and Central Hospital of the Ministry of Interior and Administration, 02-507 Warsaw,[2] Poland, and Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, London NW9 5EQ, United Kingdom[3]

Received 14 May 2007/Returned for modification 11 June 2007/Accepted 6 July 2007

Forty-one *Pseudomonas aeruginosa* isolates with extended-spectrum β-lactamases (ESBLs) from a hospital in Warsaw, Poland, were analyzed. Thirty-seven isolates from several wards were collected over 9 months in 2003 and 2004. The isolates were recovered from patients with multiple types of infections, mostly respiratory tract and postoperative wound infections. All 41 isolates produced the PER-1 ESBL, originally observed in Turkey but recently also identified in several countries in Europe and the Far East. The bla_{PER-1} gene resided within the Tn*1213* composite transposon, which was chromosomally located. Pulsed-field gel electrophoresis and multilocus sequence typing (MLST) revealed the presence of three separate clones among the isolates. Two of these, corresponding to sequence types (STs) ST244 and ST235, were responsible for parallel outbreaks. Apart from PER-1, all the isolates produced OXA-2 oxacillinase. ST235 isolates additionally expressed a novel enzyme, OXA-74, differing by one amino acid from the OXA-17 ESBL identified originally in PER-1- and OXA-2-positive *P. aeruginosa* isolates from Ankara, Turkey, in 1992. These earlier Ankara isolates with PER-1, OXA-2, and OXA-17 were also classified into ST235, which is a single-locus variant of two other STs, ST227 and ST230. ST227, ST230, and ST235 all correspond to the recently described clonal complex BG11, which seems to be internationally distributed, having spread in Turkey, Greece, Italy, Hungary, Poland, Sweden, and much of Russia. It is associated with various β-lactamases, including PER-1 and VIM metalloenzymes. This work further demonstrates the value of MLST of *P. aeruginosa*.

Pseudomonas aeruginosa is one of the most important nosocomial pathogens, being responsible for various types of infections with more and more limited therapeutic options. This situation is due in large part to the rapid accumulation of antimicrobial resistance mechanisms in *P. aeruginosa*, including extended-spectrum β-lactamases (ESBLs) that confer resistance to oxyimino-β-lactams. PER (*Pseudomonas e*xtended *r*esistant) β-lactamases are one of the rarer ESBL families; however, their prevalence may be increasing. Two PER types have been described previously. PER-1 was identified first, in a *P. aeruginosa* isolate from 1991 recovered in France from a Turkish patient (36, 37). Later, PER-1 was recognized to be widespread in Turkey among strains of *P. aeruginosa*, *Acinetobacter* spp., and *Enterobacteriaceae* and is the most prevalent ESBL in the first of these species (1, 2, 12, 24, 48, 49, 50, 51). PER-2 production among *Enterobacteriaceae* from South America, mostly Argentina, is known to occur (4, 5, 44, 52).

Since 1995, PER-1-producing organisms have been disseminating in Italy (15, 30, 38, 39, 40) and, more recently, in Belgium (6, 34), France (13, 14, 43), Spain (33), Romania (35), Hungary and Serbia (27, 46), Korea (23, 55), Japan (54), and China (20). In Europe, the bla_{PER-1} gene is found mostly in *P. aeruginosa*, where it resides within a specific transposon, named Tn*1213* by Poirel et al. (42) and Tn*4176* by Mantengoli and Rossolini (31). This element is formed by two different, novel insertion sequences of the IS*4* family with almost identical inverted repeats. The expression of PER-1 (and that of PER-2) usually confers clear resistance to oxyimino-β-lactams, especially ceftazidime, ceftibuten, and aztreonam (4, 12, 37, 38).

Although frequent in *Enterobacteriaceae*, ESBLs in *P. aeruginosa* strains in Poland are very rare, with only a few documented identifications of ESBL-producing *P. aeruginosa* isolates (26, 53). In this paper, the first identification of PER-1-producing *P. aeruginosa* isolates in Poland is described and the relationship of the isolates to earlier producer strains from Turkey is explored.

MATERIALS AND METHODS

Clinical isolates. We examined 41 clinical isolates of *P. aeruginosa* from one hospital in Warsaw, Poland (Table 1), a large (~1,000-bed), tertiary-care medical center treating patients from all over Poland, as well as foreign residents and travelers to Warsaw. Thirty-seven isolates were collected during an apparent outbreak of *P. aeruginosa* infections between September 2003 and May 2004; the remaining four were recovered from patients with sporadic infections between July 2005 and January 2006. Over the study period, all the isolates were identified by the hospital microbiology laboratory as nonreplicate *P. aeruginosa* putative ESBL producers by using the double-disk synergy test (22). The isolates were from patients from several wards, mostly those in the intensive care unit (ICU; 21 isolates) and the surgery ward (10 isolates), and were mainly from tracheos-

* Corresponding author. Mailing address: National Medicines Institute, ul. Chełmska 30/34, 00-725 Warsaw, Poland. Phone: (48) 22-851 43 88. Fax: (48) 22-841 29 49. E-mail: jempel@cls.edu.pl.

▽ Published ahead of print on 18 July 2007.

TABLE 1. Clinical data, PFGE types, MLST results, and β-lactamases corresponding to the *P. aeruginosa* isolates studied

PFGE type (no. of isolates)	Date(s) (mo/yr) of isolation	Source(s)	Hospital ward(s) or location	MLST[a]		β-Lactamase pIs	β-Lactamase variants[b]
				Allelic profile	ST		
A, subtypes 1 to 5 (37)	9/2003–1/2006	Tracheostomy tube swabs, wound swabs, urine, blood, peritoneal fluid, drainage fluid, ear swabs	ICU, surgery, neurology, cardiology, internal medicine	17-5-12-3-14-4-7	ST244	5.3, 7.7, 8.2	PER-1, OXA-2, OXA-50f
B, subtypes 1 and 2 (3)	2/2004–3/2004	Urine, peritoneal fluid, ear swab	Surgery, neurology, nephrology	38-11-3-13-1-2-4	ST235	5.3, 6.5, 7.7, 8.2	PER-1, OXA-2, OXA-74, OXA-50g
C[c] (1)	7/2005	Tracheostomy tube swab	Neurology	39-6-12-11-3-15-2	ST245	5.3, 7.7, 8.2	PER-1, OXA-2, OXA-50h
D (2)	2/1992–3/1992		Ankara, Turkey	38-11-3-13-1-2-4	ST235	5.3, 6.5, 7.7, 8.2	PER-1,[c] OXA-2,[d] OXA-17,[d] OXA-50g

[a] MLST of eight ST244 isolates, three ST235 isolates, one ST245 isolate, and two Turkish isolates was performed.
[b] PCR analyses of the bla_{PER-1}-, bla_{OXA-2}-, bla_{OXA-10}-, and bla_{OXA-50}-like genes of all isolates were performed; full sets of amplified genes from three representative ST244 isolates and all ST235 and ST245 isolates from Warsaw were sequenced. Only the bla_{OXA-50}-like genes from the Turkish isolates were sequenced in the present study; the other β-lactamase genes of these isolates were characterized previously (11, 12).
[c] Reference 12.
[d] Reference 11.

tomy tube swabs (14 isolates), postoperative wound swabs (12 isolates), and urine (6 isolates). Of all the affected patients, 11, mostly those in the ICU, died; however, all these patients had severe underlying diseases (e.g., cancer, diabetes, circulatory failure, and chronic renal failure), and only in one case could the death be unambiguously attributed to bacterial infection. Other patients were successfully treated according to susceptibility data with either piperacillin-tazobactam or imipenem. Infection control procedures included mostly personnel education and strict contact precautions. The microbiological investigation of the ICU environment did not identify any materials contaminated with infecting organisms, and a personnel carriage investigation was not performed.

The isolates were sent to the National Medicines Institute in Warsaw for further examinations. They were reidentified by the ATB ID32GN test (bioMérieux, Charbonnieres-les-Bains, France) and confirmed as ESBL producers by the double-disk test (22) using ceftazidime (30 μg), cefotaxime (30 μg), and amoxicillin and clavulanate (20 and 10 μg) disks (Oxoid, Basingstoke, United Kingdom).

P. aeruginosa isolates 871 and 873, producing PER-1, OXA-2, and OXA-17 β-lactamases, were identified in 1992 in a hospital in Ankara, Turkey (11, 12), and were included in this work for comparison. One study isolate, 2622/03, was used previously to partially characterize the genetic context of bla_{PER-1} (42).

Antimicrobial susceptibility testing. MICs were evaluated by the Clinical and Laboratory Standards Institute (CLSI) agar dilution method (7) using aztreonam and cefepime (Bristol-Myers Squibb, New Brunswick, NJ); cefotaxime and gentamicin (Polfa Tarchomin, Warsaw, Poland); ceftazidime, ticarcillin, and clavulanate (GlaxoSmithKline, Stevenage, United Kingdom); ciprofloxacin (Bayer AG, Leverkusen, Germany); imipenem (Merck, Rahway, NJ); meropenem (AstraZeneca, Macclesfield, United Kingdom); piperacillin-tazobactam (Wyeth, Pearl River, NY); and tobramycin (ICN Biomedicals, Eschwege, Germany). Clavulanate and tazobactam concentrations in combination tests were fixed at 2 and 4 μg/ml, respectively. *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *E. coli* ATCC 35218 were used as reference strains in susceptibility testing.

PFGE. Pulsed-field gel electrophoresis (PFGE) typing was performed as described by Grundmann et al. (19) or, in the case of isolates with rapidly degrading DNA, as described by Römling and Tümmler (45). DNA was cut using the XbaI restriction enzyme (MBI Fermentas, Vilnius, Lithuania) and electrophoresed in a contour-clamped homogenous electric field DRIII PFGE system (Bio-Rad, Hercules, CA). The results were interpreted according to the criteria of Tenover et al. (47).

MLST. Multilocus sequence typing (MLST) was performed as proposed by Curran et al. (9). Seven internal fragments of the genes *acsA*, *aroE*, *guaA*, *mutL*, *nuoD*, *ppsA*, and *trpE* were amplified, and the resulting PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Sequencing was carried out with primers as corrected at the http://pubmlst.org website by using an ABI 310 automatic sequencer (Applied Biosystems, Foster City, CA). Sequences were edited and assembled with the SEQEDV 10.3 software (Applied Biosystems). The database at http://pubmlst.org was used to assign numbers to particular alleles and to identify sequence types (STs).

IEF of β-lactamases. β-Lactamases were profiled by isoelectric focusing (IEF) as described by Bauernfeind et al. (3) by using a model 111 mini IEF cell (Bio-Rad). Gels were run at pHs over the range of 3 to 10 and were developed with 0.5 mM nitrocefin (Oxoid) or with 0.5 mM nitrocefin plus 0.3 mM cloxacillin (Polfa Tarchomin).

PCR detection and sequencing of β-lactamase (*bla*) genes. Entire protein-encoding regions of all *bla* genes were amplified using the primers shown in Table 2. DNA sequencing was performed as described above, with the appropriate primers presented in Table 2.

Context and location of *bla* genes. The presence of bla_{PER-1} genes within Tn*1213* (42) was checked by separate PCR analyses of two overlapping parts of the element; Table 2 shows the sequences of the primers used. The 5′ part of the element was amplified with Tn*1213*-specific forward primer ISPa12.B or ISPa14.B and the bla_{PER-1}-specific reverse primer PER-1E, whereas the 3′ part was amplified with the forward primer PERC and the reverse primer ISPa13.A or ISPa14.A. The location of bla_{PER-1} genes in chromosomal or plasmid DNA was studied by the I-CeuI technique (28) with probes specific for rRNA genes and bla_{PER-1}. The bla_{PER-1} probe was a ~0.9-kb PCR product obtained with primers PERA and PERD using total DNA of *P. aeruginosa* 2623/03 as a template. The rRNA gene probe was a mixture of PCR amplicons encompassing fragments of 16S and 23S rRNA genes of *E. coli*, obtained as described previously (18). I-CeuI-digested and PFGE-separated DNA of *P. aeruginosa* isolates was blotted onto a Hybond-N+ membrane (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). Probe labeling, hybridization, and signal detection were performed with the ECL random-prime labeling and detection system (Amersham).

Nucleotide sequence accession numbers. The nucleotide sequences analyzed in this work were deposited in the EMBL database under the following accession numbers: AJ854182 for bla_{OXA-74}, AM117126 for $bla_{OXA-50f}$, AM117127 for $bla_{OXA-50g}$, and AM117128 for $bla_{OXA-50h}$.

RESULTS

PFGE and MLST. PFGE revealed three separate clusters of isolates (Table 1). One of these, type A, was predominant, corresponding to 34 isolates from the main collection period and three isolates from patients with sporadic infections; this cluster split into five subtypes. Three other outbreak isolates were classified into PFGE type B, which was differentiated into two subtypes. The remaining sporadic-infection isolate was classified into PFGE type C. No correlation between the PFGE type and the hospital ward or the type of infection was found.

Twelve representative isolates were analyzed by MLST. The results correlated well with the PFGE data (Table 1). Isolates of PFGE type A were classified into ST244, and isolates of PFGE type B corresponded to ST235, whereas the PFGE type C isolate was classified into ST245. All the MLST alleles found

TABLE 2. PCR primers used to analyze β-lactamase genes

Primer designation	Target gene or region	Sequence (5′–3′)	Purpose(s)	Expected size(s) (bp) of amplicon(s) (corresponding primer)[a]	Reference
PERA	bla_{PER-1}	ATGAATGTCATTATAAAAGC	PCR, sequencing	925 (PERD)	12
PERD	bla_{PER-1}	AATTTGGGCTTAGGGCAGAA	PCR, sequencing		12
S	bla_{OXA-50} type	AATCCGGCGCTCATCCATC	PCR, sequencing	867 (AS)	16
AS	bla_{OXA-50} type	GGTCGGCGACTGAGGCGG	PCR, sequencing		16
OXA-2A	bla_{OXA-2}	ATGGCAATCCGAATCTTCG	PCR, sequencing	828 (OXA-2B)	This work
OXA-2B	bla_{OXA-2}	TTATCGCGCAGCGTCCG	PCR, sequencing		This work
OXA-2C	bla_{OXA-2}	CTTGAATGTCGATGCAGGC	Sequencing		This work
OXA-2D	bla_{OXA-2}	GGTCGCAACTGGATACTGC	Sequencing		This work
OXA-10A	bla_{OXA-10} type (bla_{OXA-74})	ATGAAAACATTTGCCGCATATG	PCR, sequencing	801 (OXA-10B)	This work
OXA-10B	bla_{OXA-10} type (bla_{OXA-74})	TTAGCCACCAATGATGCCC	PCR, sequencing		This work
OXA-10C	bla_{OXA-10} type (bla_{OXA-74})	CCTGATGCTCATTCTTTATG	Sequencing		This work
OXA-10D	bla_{OXA-10} type (bla_{OXA-74})	CACCTGAATATCTAGTGCA	Sequencing		This work
ISPa12.B		GATCTCGCTTTACATTTACC	PCR	1,678 (PER-1E)	42
ISPa14.B		GCCTAATTCGATGCCTTAT	PCR	1,993 (PER-1E)	42
PER-1E	bla_{PER-1}	GCACTGGAACACTAAACTCG	PCR		This work
PERC	bla_{PER-1}	ACACAGCTGTCTGAAACCTC	PCR	1,883 (ISPa13.A); 2,528 (ISPa14.A)	12
ISPa13.A		TAACCATATGCACTCAACGG	PCR		42
ISPa14.A		AATCAAATGTCCAACCTGCC	PCR		42

[a] PCR product sizes are shown in conjunction with the forward primer in each primer pair; the corresponding primer in the pair is identified in parentheses.

had known sequences; however, ST244 and ST245 represented novel combinations of alleles.

β-Lactamase content. IEF analyses of crude sonication products from all the study isolates were performed; results are shown in Table 1. All the ESBL-positive isolates expressed β-lactamases with pIs of 8.2, 7.7, and 5.3. Additionally, the three ST235 isolates produced pI 6.5 enzymes. The pI 8.2 β-lactamases ceased to be visualized when the nitrocefin was supplemented with cloxacillin, indicating these to be *P. aeruginosa*-specific AmpC cephalosporinases.

PCR detection and sequencing of *bla* genes. The genomic DNA of each isolate was tested by PCR for the presence of bla_{PER-1}- and bla_{OXA-2}-type genes, which might encode the pI 5.3 and pI 7.7 β-lactamases, respectively. Amplicons of the expected sizes of ~0.9 and ~0.8 kb, respectively, were obtained in all cases. The three ST235 isolates additionally were positive for bla_{OXA-10}-type genes, possibly coding for the pI 6.5 enzymes and yielding amplicons of the expected size of ~0.8 kb. Finally, all the isolates were positive for the bla_{OXA-50}-type (*poxB*) genes, recently found to be characteristic of *P. aeruginosa* (16, 25), giving PCR products of the expected size (~0.85 kb).

Three ST244 isolates and all the ST235 and ST245 isolates were selected for sequencing of the entire coding regions of their amplified *bla* genes. Sequence analysis confirmed the presence of classical bla_{PER-1} and bla_{OXA-2} in all these isolates, with sequences identical to those described originally (10, 37). The bla_{OXA-10}-type genes of the ST235 isolates specified a novel β-lactamase variant, now designated OXA-74 (http://www.lahey.org/studies/webt.htm). bla_{OXA-74} differed by two mutations, C197→T and A218→G (numbered from the first ATG codon of the coding region), from bla_{OXA-10} (21). These mutations cause two amino acid substitutions in OXA-74, Ala69Val and Asn76Ser (numbered according to the class D β-lactamase scheme [8]), respectively, compared to OXA-10. The Ala69Val substitution differentiates OXA-74 from OXA-17, identified in *P. aeruginosa* isolates from Turkey that also produced PER-1 and OXA-2 (11, 12).

Three allelic variants of bla_{OXA-50} specific to the three STs were found (Table 3). A comparison of the sequences of the

TABLE 3. Nucleotide sequences of identified bla_{OXA-50} alleles in the *P. aeruginosa* isolates studied

Strain or ST/allele	Nucleotide at position[a]:														
	9	21	36	46	66	74	96	105	126	145	282	289	327	492	500
PAO1/bla_{OXA-50}	T	T	T	A (Thr)	C	A (Gln)	C	G	C	C (Arg)	G	C (Arg)	C (Asp)	A	G (Arg)
DM1253/*poxB27*[b]	C									T (Cys)			G (Glu)		A (His)
COL-1/$bla_{OXA-50b}$[c]		C	C	G (Ala)		G (Arg)	A	T	T		A			G	
ST244/$bla_{OXA-50f}$	C									T (Cys)		T (Cys)	G (Glu)		A (His)
ST235/$bla_{OXA-50g}$		C	C	G (Ala)		G (Arg)	A	T	T		A				
ST245/$bla_{OXA-50h}$	C				T								G (Glu)		A (His)

[a] Nucleotide positions are numbered consecutively, starting with the first ATG codon of the bla_{OXA-50} coding sequence. Only nucleotide differences with respect to the sequence of the bla_{OXA-50} gene from *P. aeruginosa* PAO1 (GenBank accession no. AE004964) (16) are shown. In cases in which polymorphisms cause amino acid differences, the amino acid symbols are given in parentheses.

[b] GenBank accession no. AY597445; reference 25.

[c] GenBank accession no. AY306131; reference 16.

TABLE 4. Antimicrobial susceptibilities of clinical isolates[a]

Strain or ST (no. of isolates)	MIC (μg/ml) of:													
	TIC	TIM	PIP	TZP	CTX	CAZ	CAZ-CLA	FEP	ATM	IPM	MEM	GEN	TOB	CIP
ST244 (37)	256–>512	32–256	16–>256	8–>256	128–>256	>256	4–128	32–>256	64–>256	0.5–>32	2–>32	8–>64	2–>64	>32
ST235 (3)	512	256	64	64	128	128–>128	16–32	64–128	128–>128	8	16	>64	>64	16–>16
ST245 (1)	512	32	32	16	>256	>256	32	256	>256	4	4	4	2	1
P. aeruginosa ATCC 27853	16	16	2	2	16	1	2	2	2	2	0.5	2	0.5	0.25
E. coli ATCC 25922	4	8	2	2	0.06	0.25	0.125	0.06	0.06	0.06	0.015	0.5	0.25	0.015

[a] Abbreviations: TIC, ticarcillin; TIM, ticarcillin with clavulanate; PIP, piperacillin; TZP, piperacillin-tazobactam; CTX, cefotaxime; CAZ, ceftazidime; CLA, clavulanate; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MEM, meropenem; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin.

bla_{OXA-50}-type alleles with those available in databases (16, 25) revealed that these sequences were unique. $bla_{OXA-50f}$ alleles from the ST244 isolates and $bla_{OXA-50h}$ from the ST245 isolate differed by five and four nucleotides, respectively, from the bla_{OXA-50} gene of *P. aeruginosa* PAO1 (16), and their closest relative was *poxB27*, previously identified in the *P. aeruginosa* strain DSM 1253 of unreported origin (25). The $bla_{OXA-50g}$ allele of the ST235 isolates was much different from the other bla_{OXA-50} alleles, with 8, 13, and 12 polymorphisms compared with bla_{OXA-50}, $bla_{OXA-50f}$, and $bla_{OXA-50h}$, respectively. It differed by only a single silent mutation from $bla_{OXA-50b}$, identified in *P. aeruginosa* COL-1 from France (16).

Comparative typing of ST235 isolates and *P. aeruginosa* isolates from Turkey. The similar complexities of the β-lactamase contents of the ST235 (PFGE type B) isolates, producing PER-1, OXA-2, and OXA-74, and two *P. aeruginosa* isolates identified in 1992 in Ankara, Turkey, producing PER-1, OXA-2, and OXA-17 (11, 12), suggested possible relatedness. The two Ankara isolates, therefore, were compared with isolates from Warsaw by PFGE, MLST, and sequencing of bla_{OXA-50}-type genes. In the PFGE analysis, the two Turkish isolates were classified into a separate type, type D, but their DNA banding patterns showed clear similarity to those of PFGE type B (data not shown). Moreover, the isolates from Ankara corresponded to ST235 by MLST and carried the $bla_{OXA-50g}$ gene variant, which revealed a fundamental relationship.

Location of *bla* genes. A previous study of a PFGE type A isolate showed that its bla_{PER-1} gene was chromosomally located, inside a Tn*1213* composite transposon (42). PCRs demonstrated that this genetic context of bla_{PER-1} was conserved in all of the Warsaw clones, irrespective of ST and PFGE type, and in the isolates from Turkey (Table 1). I-CeuI analysis, carried out with several isolates representing Warsaw ST244 and ST235 isolates and with the two Turkish isolates, confirmed the chromosomal location of bla_{PER-1} (data not shown).

Susceptibility testing. Most of the study isolates were resistant to multiple drugs, including β-lactams, aminoglycosides, and ciprofloxacin (Table 4). All but seven isolates were, however, susceptible to piperacillin by CLSI (though not European) criteria, and all but one were susceptible to piperacillin-tazobactam on the same basis. Seventeen isolates were intermediately susceptible or resistant to imipenem, and 27 isolates were intermediately susceptible or resistant to meropenem.

DISCUSSION

This study is the first report of PER-1-producing microorganisms in Poland. For years, PER β-lactamases were thought to be significant only in Turkey (PER-1) and Argentina (PER-2) (4, 12, 32, 41, 50, 51). However, the recent identification of PER-1 producers in several European countries and in the Far East suggests their proceeding dissemination (20, 27, 33, 35, 46, 54). In Korea, as in Turkey, PER-1 production by *Acinetobacter* spp. has been reported often, but in Europe, it has been identified mostly in *P. aeruginosa*.

With 37 patients in several wards affected between September 2003 and May 2004, the outbreak described in the present study is one of the largest caused by *P. aeruginosa* with PER-1 to be described so far. Molecular typing revealed two clones among the outbreak isolates, with one of these, ST244 (PFGE type A), being greatly predominant. The clonal dissemination of *Acinetobacter baumannii*, *P. aeruginosa*, or *Proteus mirabilis* strains with PER-1 in hospitals in Korea, Turkey, Italy, and Belgium has been reported previously, but the corresponding outbreaks were limited and restricted to ICUs (6, 23, 30, 39, 51). The concurrent presence of two or more unrelated clones of *A. baumannii* or *P. aeruginosa* with the PER-1 enzyme in some Korean, Turkish, and Italian centers has been reported previously (1, 38, 55).

In all of the study isolates, bla_{PER-1} resided in the same transposon, Tn*1213* (Tn*4176*), as in many producers from Turkey, Italy, France, and Belgium (31, 42). The constant genetic environment of bla_{PER-1} in organisms from multiple countries implies a common origin and horizontal transmission of the gene, even though it often occurs in chromosomal DNA (42). PER-1 expression was inferred to be the major source of oxyimino-β-lactam resistance in the study isolates, although they also produced other β-lactamases. The ST235 isolates expressed a novel oxacillinase, OXA-74, which differed by one amino acid only from the OXA-17 β-lactamase from *P. aeruginosa* isolates with PER-1 obtained in Ankara in 1992 (12). OXA-17 has ESBL activity conferring cefotaxime resistance (11), and it is possible that OXA-74 does as well. The ST235 isolates did not differ significantly from ST244 isolates (without OXA-74) in resistance levels; however, the β-lactam MICs for the ST235 isolates were more often at the upper end of the MIC ranges.

It is difficult to identify the origin and molecular epidemiology of PER-1-producing *P. aeruginosa* strains in Warsaw. Genetic similarity in terms of β-lactamase content, ST, and the

bla$_{OXA-50}$ allele demonstrated the relatedness of the minor outbreak clone, ST235, to two Ankara isolates from 1992 (11, 12). One may therefore propose the import of ST235 from Turkey and the transfer of *bla*$_{PER-1}$ to ST244, responsible for the main outbreak, and later to ST245. However, the first of the Warsaw ST235 isolates was recovered in February 2004, 6 months after the first ST244 isolate. It is entirely possible that the ST235 strain had been present but undetected before the main ST244 infection outbreak; nevertheless, the origins of *P. aeruginosa* strains with the PER-1 enzyme in the hospital remain unclear.

The recently introduced *P. aeruginosa* MLST scheme (9) has permitted interesting epidemiological analyses. Apart from the Warsaw (2004) and Ankara (1992) isolates examined here, ST235 isolates have been identified as producers of VIM-2 or VIM-4 metallo-β-lactamases circulating since 2001 in northern Poland (J. Empel and M. Gniadkowski, unpublished results). ST235 also has been found in three distant Russian cities (Moscow, Omsk, and Krasnodar; 2002 to 2004), though the β-lactamase content of these isolates was not reported (http://pubmlst.org). Moreover, two single-locus variants (SLVs) of ST235, namely, ST227 (with VIM-1) and ST230 (with VIM-4), have recently been described in Italy (where such isolates were obtained between 1997 and 2003) and in Greece and Sweden (where such isolates were obtained between 2001 and 2002), respectively. ST227 and ST230 have been classified as clonal complex BG11 (17), and being an SLV of these lineages, ST235 provides a link between them and also belongs to the BG11 complex. Most probably, BG11 is a truly international complex that has been disseminating over a long period and a wide area, acquiring various resistance determinants in particular locales. By contrast, of the two other STs identified here, ST245 is a singleton among the STs so far reported, whereas ST244 has an SLV (ST336) but one on which no clinical data have yet been presented (http://pubmlst.org). ST244 and ST245 have not yet been reported elsewhere. It is noteworthy, finally, that the distribution of STs perfectly matched that of the *bla*$_{OXA-50}$ alleles seen, confirming the validity of the MLST scheme and suggesting that *bla*$_{OXA-50}$ may be another potential clonality marker for *P. aeruginosa*.

Along with other recent studies, this work illustrates the ongoing dissemination of organisms with PER-type ESBLs. Only time will show whether these ESBLs can mimic the recent and dramatic spread of CTX-M-type enzymes (29). This study is also a good example to demonstrate the value of the wider implementation of MLST in order to identify and track the spread of multidrug-resistant *P. aeruginosa* clones.

ACKNOWLEDGMENTS

We thank Radek Izdebski for his excellent support in PFGE typing and Laurent Poirel and Patrice Nordmann for their very helpful consultations.

This work was financed partially by grant no. 6 PRCD LSHM-CT-2003-503-335 (COBRA) from the European Commission.

REFERENCES

1. **Aktas, Z., L. Poirel, M. Salcioglu, P. E. Ozcan, K. Midilli, C. Bal, O. Ang, and P. Nordmann.** 2005. PER-1- and OXA-10-like β-lactamases in ceftazidime-resistant *Pseudomonas aeruginosa* isolates from intensive care unit patients in Istanbul, Turkey. Clin. Microbiol. Infect. **11:**193–198.
2. **Bahar, G., B. Erac, A. Mert, and Z. Gulay.** 2004. PER-1 production in a urinary isolate of *Providencia rettgeri*. J. Chemother. **16:**343–346.
3. **Bauernfeind, A., H. Grimm, and S. Schweighart.** 1990. A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. Infection **18:**294–298.
4. **Bauernfeind, A., I. Stemplinger, R. Jungwirth, P. Mangold, S. Amann, E. Akalin, O. Ang, C. Bal, and J. M. Casellas.** 1996. Characterization of β-lactamase gene *bla*$_{PER-2}$, which encodes an extended-spectrum class A β-lactamase. Antimicrob. Agents Chemother. **40:**616–620.
5. **Celenza, G., C. Pellegrini, M. Caccamo, B. Segatore, G. Amicosante, and M. Perilli.** 2006. Spread of *bla*$_{CTX-M}$-type and *bla*$_{PER-2}$ β-lactamase genes in clinical isolates from Bolivian hospitals. J. Antimicrob. Chemother. **57:**975–978.
6. **Claeys, G., G. Verschraegen, T. de Baere, and M. Vaneechoutte.** 2000. PER-1 β-lactamase-producing *Pseudomonas aeruginosa* in an intensive care unit. J. Antimicrob. Chemother. **45:**924–925.
7. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement, January 2007. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Couture, F., J. Lachapelle, and R. C. Levesque.** 1992. Phylogeny of LCR-1 and OXA-5 with class A and class D β-lactamases. Mol. Microbiol. **6:**1693–1705.
9. **Curran, B., D. Jonas, H. Grundmann, T. Pitt, and C. G. Dowson.** 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. **42:**5644–5649.
10. **Dale, J. W., D. Godwin, D. Mossakowska, P. Stephenson, and S. Wall.** 1985. Sequence of the OXA-2 β-lactamase: comparison with other penicillin-reactive enzymes. FEBS Lett. **191:**39–44.
11. **Danel, F., L. M., C. Hall, B. Duke, D. Gur, and D. M. Livermore.** 1999. OXA-17, a further extended-spectrum variant of OXA-10 β-lactamase, isolated from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **43:**1362–1366.
12. **Danel, F., L. M. C. Hall, D. Gur, H. E. Akalin, and D. M. Livermore.** 1995. Transferable production of PER-1 β-lactamase in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **35:**281–294.
13. **De Champs, C., C. Chanal, D. Sirot, R. Baraduc, J. P. Romaszko, R. Bonnet, A. Plaidy, M. Boyer, E. Carroy, M. C. Gbadamassi, S. Laluque, O. Oules, M. C. Poupart, M. Villemain, and J. Sirot.** 2004. Frequency and diversity of class A extended-spectrum β-lactamases in hospitals of the Auvergne, France: a 2 year prospective study. J. Antimicrob. Chemother. **54:**634–639.
14. **De Champs, C., L. Poirel, R. Bonnet, D. Sirot, C. Chanal, J. Sirot, and P. Nordmann.** 2002. Prospective survey of β-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolated in a French hospital in 2000. Antimicrob. Agents Chemother. **46:**3031–3034.
15. **Docquier, J. D., F. Luzzaro, G. Amicosante, A. Toniolo, and G. M. Rossolini.** 2001. Multidrug-resistant *Pseudomonas aeruginosa* producing PER-1 extended-spectrum serine-β-lactamase and VIM-2 metallo-β-lactamase. Emerg. Infect. Dis. **7:**910–911.
16. **Girlich, D., T. Naas, and P. Nordmann.** 2004. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **48:**2043–2048.
17. **Giske, C. G., B. Libisch, C. Colinon, E. Scoulica, L. Pagani, M. G. Kronvall, and G. M. Rossolini.** 2006. Establishing clonal relationships between VIM-1-like metallo-β-lactamase-producing *Pseudomonas aeruginosa* strains from four European countries by multilocus sequence typing. J. Clin. Microbiol. **44:**4309–4315.
18. **Gniadkowski, M., I. Schneider, A. Pałucha, R. Jungwirth, B. Mikiewicz, and A. Bauernfeind.** 1998. Cefotaxime-resistant *Enterobacteriaceae* isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. Antimicrob. Agents Chemother. **42:**827–832.
19. **Grundmann, H., C. Schneider, D. Hartung, F. D. Daschner, and T. L. Pitt.** 1995. Discriminatory power of three DNA-based typing techniques for *Pseudomonas aeruginosa*. J. Clin. Microbiol. **33:**528–534.
20. **Hou, T. W., X. L. Yin, C. Y. Jiang, Z. H. Wang, Q. K. Chen, X. Chen, W. Li, and Y. Bai.** 2007. Microbiology and clinical analysis of six cases of hospital-acquired pneumonia caused by *Acinetobacter baumannii*. Zhonghua Jie He He Hu Xi Za Zhi **30:**35–39.
21. **Huovinen, P., S. Huovinen, and G. A. Jacoby.** 1988. Sequence of PSE-2 β-lactamase. Antimicrob. Agents Chemother. **32:**134–136.
22. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
23. **Jeong, S. H., I. K. Bae, S. B. Kwon, K. Lee, D. Yong, G. J. Woo, J. H. Lee, H. I. Jung, S. J. Jang, K. H. Sung, and S. H. Lee.** 2005. Investigation of a nosocomial outbreak of *Acinetobacter baumannii* producing PER-1 extended-spectrum β-lactamase in an intensive care unit. J. Hosp. Infect. **59:**242–248.
24. **Kolayli, F., G. Gacar, A. Karadenizli, A. Sanic, H. Vahaboglu, and the Study Group.** 2005. PER-1 is still widespread in Turkish hospitals among *Pseudomonas aeruginosa* and *Acinetobacter* spp. FEMS Microbiol. Lett. **249:**241–245.
25. **Kong, K. F., S. R. Jayawardena, A. del Puerto, L. Wiehlmann, U. Laabs, B.**

Tummler, and K. Mathee. 2005. Characterization of *poxB*, a chromosomal-encoded *Pseudomonas aeruginosa* oxacillinase. Gene **358:**82–92.
26. **Laudy, E. A., A. Cieloch, D. Dzierżanowska, S. Tyski, and J. Patzer.** 2007. GES-1 and TEM-1-like β-lactamases of *Pseudomonas aeruginosa* strains isolated from patients in two Warsaw hospitals. Clin. Microbiol. Infect. **13**(Suppl. s1)**:**S224–S225.
27. **Libisch, B., Z. Lepsanovic, B. Krucso, M. Muzslay, B. Tomanovic, Z. Nonkovic, V. Mirovic, G. Szabo, B. Balogh, and M. Füzi.** 2007. Characterization of PER-1 extended-spectrum β-lactamase producing *Pseudomonas aeruginosa* clinical isolates from Hungary and Serbia. Clin. Microbiol. Infect. **13**(Suppl. s1)**:**S162.
28. **Liu, S.-L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-Ceu-I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
29. **Livermore, D. M., R. Canton, M. Gniadkowski, P. Nordmann, G. M. Rossolini, G. Arlet, J. Ayala, T. M. Coque, I. Kern-Zdanowicz, F. Luzzaro, L. Poirel, and N. Woodford.** 2007. CTX-M: changing the face of ESBLs in Europe. J. Antimicrob. Chemother. **59:**165–174.
30. **Luzzaro, F., E. Mantengoli, M. Perilli, G. Lombardi, V. Orlandi, A. Orsatti, G. Amicosante, G. M. Rossolini, and A. Toniolo.** 2001. Dynamics of a nosocomial outbreak of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β-lactamase. J. Clin. Microbiol. **39:**1865–1870.
31. **Mantengoli, E., and G. M. Rossolini.** 2005. Tn*5393*d, a complex Tn*5393* derivative carrying the PER-1 extended-spectrum β-lactamase gene and other resistance determinants. Antimicrob. Agents Chemother. **49:**3289–3296.
32. **Melano, R. M., A. Corso, A. Petroni, D. Centrón, B. Orman, A. Pereyra, N. Moreno, and M. Galas.** 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum β-lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. J. Antimicrob. Chemother. **52:**36–42.
33. **Miro, E., B. Mirelis, F. Navarro, A. Rivera, R. J. Mesa, M. C. Roig, L. Gomez, and P. Coll.** 2005. Surveillance of extended-spectrum β-lactamases from clinical samples and faecal carriers in Barcelona, Spain. J. Antimicrob. Chemother. **56:**1152–1155.
34. **Naas, T., P. Bogaerts, C. Bauraing, Y. Degheldre, Y. Glupczynski, and P. Nordmann.** 2006. Emergence of PER and VEB extended-spectrum β-lactamases in *Acinetobacter baumannii* in Belgium. J. Antimicrob. Chemother. **58:**178–182.
35. **Naas, T., P. Nordmann, and A. Heidt.** 2007. Intercountry transfer of PER-1 extended-spectrum β-lactamase-producing *Acinetobacter baumannii* from Romania. Int. J. Antimicrob. Agents **29:**226–228.
36. **Nordmann, P., and T. Naas.** 1994. Sequence analysis of PER-1 extended-spectrum β-lactamase from *Pseudomonas aeruginosa* and comparison with class A β-lactamases. Antimicrob. Agents Chemother. **38:**104–114.
37. **Nordmann, P., E. Ronco, T. Naas, C. Duport, Y. Michel-Briand, and R. Labia.** 1993. Characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **37:**962–969.
38. **Pagani, L., E. Mantengoli, R. Migliavacca, E. Nucleo, S. Pollini, M. Spalla, R. Daturi, E. Romero, and G. M. Rossolini.** 2004. Multifocal detection of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β-lactamase in Northern Italy. J. Clin. Microbiol. **42:**2523–2529.
39. **Pagani, L., R. Migliavacca, L. Pallecchi, C. Matti, E. Giacobone, G. Amicosante, E. Romero, and G. M. Rossolini.** 2002. Emerging extended-spectrum β-lactamases in *Proteus mirabilis*. J. Clin. Microbiol. **40:**1549–1552.
40. **Perilli, M., F. De Santis, C. Mugnaioli, G. M. Rossolini, F. Luzzaro, S. Stefani, M. L. Mezzatesta, A. Toniolo, and G. Amicosante.** 2007. Spread of *Enterobacteriaceae* carrying PER-1 extended-spectrum β-lactamase gene as a chromosomal insert: a report from Italy. J. Antimicrob. Chemother. **59:**323–324.
41. **Petroni, A., A. Corso, R. Melano, M. L. Cacace, A. M. Bru, A. Rossi, and M. Galas.** 2002. Plasmidic extended-spectrum β-lactamases in *Vibrio cholerae* O1 El Tor isolates in Argentina. Antimicrob. Agents Chemother. **46:**1462–1468.
42. **Poirel, L., L. Cabanne, H. Vahaboglu, and P. Nordmann.** 2005. Genetic environment and expression of the extended-spectrum β-lactamase bla_{PER-1} gene in gram-negative bacteria. Antimicrob. Agents Chemother. **49:**1708–1713.
43. **Poirel, L., A. Karim, A. Mercat, I. Le Thomas, H. Vahaboglu, C. Richard, and P. Nordmann.** 1999. Extended-spectrum β-lactamase-producing strain of *Acinetobacter baumannii* isolated from a patient in France. J. Antimicrob. Chemother. **43:**157–158.
44. **Quinteros, M., M. Radice, N. Gardella, M. M. Rodriguez, N. Costa, D. Korbenfeld, E. Couto, and G. Gutkind, and the Microbiology Study Group.** 2003. Extended-spectrum β-lactamases in *Enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. Antimicrob. Agents Chemother. **47:**2864–2867.
45. **Römling, U., and B. Tümmler.** 2000. Achieving 100% typeability of *Pseudomonas aeruginosa* by pulsed-field gel electrophoresis. J. Clin. Microbiol. **38:**464–465.
46. **Szabó, D., L. Rokusz, Z. Juhasz, J. Szentándrassy, K. Katona, and K. Nagy.** 2007. The first isolation of PER-1-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains in Hungary. Clin. Microbiol. Infect. **13**(Suppl. s1)**:**S378.
47. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
48. **Vahaboglu, H., F. Coskunkan, O. Tansel, R. Ozturk, N. Sahin, I. Koksal, B. Kocazeybek, M. Tatman-Otkun, H. Leblebicioglu, M. A. Ozinel, H. Akalin, S. Kocagoz, and V. Korten.** 2001. Clinical importance of extended-spectrum β-lactamase (PER-1-type)-producing *Acinetobacter* spp. and *Pseudomonas aeruginosa* strains. J. Med. Microbiol. **50:**642–645.
49. **Vahaboglu, H., S. Dodanli, C. Eroglu, R. Ozturk, G. Soyletir, I. Yildirim, and V. Avkan.** 1996. Characterization of multiple-antibiotic-resistant *Salmonella typhimurium* stains: molecular epidemiology of PER-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. J. Clin. Microbiol. **34:**2942–2946.
50. **Vahaboglu, H., L. M. C. Hall, L. Mulazimoglu, S. Dodanli, I. Yildirim, and D. M. Livermore.** 1995. Resistance to extended-spectrum cephalosporins, caused by PER-1 β-lactamase, in *Salmonella typhimurium* from Istanbul, Turkey. J. Med. Microbiol. **43:**294–299.
51. **Vahaboglu, H., R. Ozturk, G. Aygun, F. Coskunkan, A. Yaman, A. Kaygusuz, H. Leblebicioglu, I. Balik, K. Aydin, and M. Otkun.** 1997. Widespread detection of PER-1-type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob. Agents Chemother. **41:**2265–2269.
52. **Vignoli, R., G. Ravela, M. I. Mota, N. F. Cordeiro, P. Power, E. Ingold, P. Gadea, A. Sirok, F. Schelotto, J. A. Ayala, and G. Gutkind.** 2005. Enteropathogenic *Escherichia coli* strains carrying genes encoding the PER-2 and TEM-116 extended-spectrum β-lactamases isolated from children with diarrhea in Uruguay. J. Clin. Microbiol. **43:**2940–2943.
53. **Weldhagen, G. F., L. Poirel, and P. Nordmann.** 2003. Ambler class A extended-spectrum β-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. Antimicrob. Agents Chemother. **47:**2385–2392.
54. **Yamano, Y., T. Nishikawa, T. Fujimura, T. Yutsudou, M. Tsuji, and H. Miwa.** 2006. Occurrence of PER-1-producing clinical isolates of *Pseudomonas aeruginosa* in Japan and their susceptibility to doripenem. J. Antibiot. **59:**791–796.
55. **Yong, D., J. H. Shin, S. Kim, Y. Lim, J. H. Yum, K. Lee, Y. Chong, and A. Bauernfeind.** 2003. High prevalence of PER-1 extended-spectrum β-lactamase-producing *Acinetobacter* spp. in Korea. Antimicrob. Agents Chemother. **47:**1749–1751.