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Clinical Strain of *Pseudomonas aeruginosa* Carrying a bla_{TEM-21} Gene Located on a Chromosomal Interrupted Tn*A* Type Transposon

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A clinical isolate of *Pseudomonas aeruginosa* was found to produce a clavulanic acid-inhibited extended-spectrum β-lactamase with a pI of 6.4. PCR, cloning, and sequencing experiments showed that the corresponding bla_{TEM-21} gene was part of a chromosomally located Tn*801* transposon disrupted by an IS*6100* element and adjacent to an *aac(3)-II* gene.

Most extended-spectrum β-lactamases (ESBLs) produced by enterobacteria derive from the common plasmid-mediated penicillinases TEM-1, TEM-2, and SHV-1 (14). ESBLs have recently emerged in *Pseudomonas aeruginosa*, where they still remain infrequent. In this bacterial species, ESBLs are mainly OXA derivatives belonging to Ambler class D or newly emerging class A β-lactamases such as PER-1 (23) and VEB-1 (9). However, occasionally some enzymes of the TEM or SHV families, previously (TEM-4, TEM-24, and SHV-2a) or newly (TEM-42) described, have been also found (11–13, 19). These observations suggest that ESBLs widespread in the *Enterobacteriaceae* family may be increasingly found in *P. aeruginosa* (13), which could also be a reservoir for the dissemination of this kind of enzyme. We report here the production of an unusual TEM derivative, TEM-21, in a clinical strain of *P. aeruginosa*.

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P. aeruginosa Pa141 was isolated in 1997 in a private laboratory in Bordeaux, France, from the urinary tract of a 68-year-old woman living in a nursing home, with Alzheimer's disease and carrying an indwelling urinary tract catheter. This strain belonged to serogroup P2, and by the disk diffusion method it was resistant to all antipseudomonal β-lactams except for imipenem. A marked synergistic effect between clavulanic acid and cefotaxime, ceftazidime, or aztreonam was observed, suggesting the presence of an ESBL. Indeed, the isolate remained susceptible to the combinations of ticarcillin and clavulanic acid and of piperacillin and tazobactam. In addition, Pa141 was resistant to gentamicin, tobramycin, netilmicin, ciprofloxacin, and fosfomycin. These data were confirmed by MIC determination (Table 1) by an agar dilution method in Mueller-Hinton medium according to official guidelines (http://www.sfm.asso.fr). Isoelectric focusing of crude β-lactamase extracts was performed on a pH 3.5 to 10 Ampholine polyacrylamide gel and revealed by the iodine procedure, with benzylpenicillin (75 μg/ml) as substrate. By this method the Pa141 strain produced a single β-lactamase of pI 6.4 cofocusing with the TEM-21 reference enzyme (22).

Total DNA of *P. aeruginosa* Pa141 was extracted as previously described (18) and subjected to PCR amplification with primers specific for the bla_{TEM} genes (22), and the PCR product was sequenced on both strands with the D Rhodamine dye terminator kit (Perkin-Elmer, Courtaboeuf, France) and an automatic sequencer (ABI 377; Perkin-Elmer). The nucleotide sequence of the ESBL-encoding gene differed from that of the bla_{TEM-21} gene previously described (22) by two silent mutations at positions 369 (C→T) and 624 (G→A) according to Sutcliffe's numbering system (21). The TEM-21 protein derives from the parental TEM-2 enzyme, like TEM-24 (12) and TEM-42 (11), two other ESBLs found in *P. aeruginosa*. TEM-21 differs from TEM-2 by three amino acid substitutions, Glu104→Lys, His153→Arg, and Gly238→Ser, according to the numbering system of Ambler et al. (2). First described for a *Klebsiella pneumoniae* isolate in Tunisia (3), TEM-21 is rarely encountered and is much less frequent than TEM-24 and TEM-3 in France (7). However, the bla_{TEM-21} gene has been already detected and sequenced in a *Morganella morganii* strain (22) from the same region where Pa141 was recovered.

Transfer resistance by conjugation to an azide-resistant strain of *Escherichia coli* HB101 or a rifampin-resistant mutant of *P. aeruginosa* ATCC 27853, by the most efficient filter mating technique (6), did not yield any transconjugants ($<10^{-8}$). Despite repeated attempts with an alkaline-lysis method (4) and the Qiagen (Courtaboeuf, France) plasmid DNA Midi kit, plasmid DNA analysis of *P. aeruginosa* Pa141 did not show any plasmid, and transformation by electroporation of plasmid DNA extract into *E. coli* HB101 was unsuccessful. A Southern blot hybridization with total DNA of Pa141 and a bla_{TEM} probe argued for the chromosomal location of the bla_{TEM-21} gene in this strain (data not shown). Then, the whole-cell DNA of Pa141 was totally restricted by *Hin*dIII and ligated into the *Hin*dIII site of pBK-CMV cloning vector. *E. coli* XL1Blue strains harboring the recombinant plasmids were selected on Mueller-Hinton agar plates containing 100 μg of ampicillin/ml and 50 μg of kanamycin/ml and exhibited the β-lactam and aminoglycoside resistance pattern of Pa141, by MIC determination (Table 1) and isoelectric focusing. After plasmid DNA extraction, a double-restriction digestion with *Hin*dIII and *Pst*I

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TABLE 1. Antimicrobial susceptibilities of the clinical strain Pa141, *E. coli* XL1Blue harboring the recombinant plasmid pMF6, and the reference strain

Antimicrobial agent(s)[a]	MIC (μg/ml) for:		
	P. aeruginosa Pa141	*E. coli* XL1Blue (pMF6)	*E. coli* XL1Blue
Ticarcillin	>512	>512	4
Ticarcillin + CLA	32	128	4
Ceftazidime	32	64	0.2
Ceftazidime + CLA	4	0.2	0.2
Cefotaxime	256	64	<0.1
Cefotaxime + CLA	256	<0.1	<0.1
Cefsulodin	512	>512	32
Cefepime	64	8	<0.1
Cefpirome	128	256	<0.1
Piperacillin	128	256	2
Piperacillin + TZB	8	1	1
Imipenem	2	0.2	0.2
Aztreonam	32	32	<0.1
Aztreonam + CLA	8	<0.1	<0.1
Gentamicin	512	>512	0.5
Tobramycin	128	32	0.5
Netilmicin	>512	32	0.5
Amikacin	16	2	2

[a] CLA, clavulanic acid, 2 μg/ml; TZB, tazobactam, 4 μg/ml.

enzymes followed by electrophoresis on an 0.8% agarose gel allowed us to evaluate the size of the large fragment (ca 6.7 kb) inserted in the recombinant plasmid pMF6.

Genes encoding the TEM type ESBLs are supposed to be located on transposons of the Tn*A* family as are those for the TEM-1 and TEM-2 parental enzymes. However, while the plasmid or chromosomal origin of these genes is usually determined, their precise genetic location is rarely specified (10). In order to analyze the genetic environment of the bla_{TEM-21} gene in Pa141, a part of the recombinant plasmid pMF6 was sequenced with laboratory-designed primers. The sequences immediately upstream and downstream from the bla_{TEM-21} gene exhibited 100% DNA identity with a part of transposon Tn*801* from *P. aeruginosa* (5) (Fig. 1). Indeed, the right side included the IR_R (inverted repeat) of the transposon and the left one contained part of the resolvase gene (*tnpR*, 305 pb). Tn*801* is closely related to Tn*3* and comprises the bla_{TEM-2} gene. The resolvase gene of Tn*801* was truncated or simply interrupted by the insertion sequence (IS) IS*6100* (858 bp sequenced of 880 bp, up to the *Hin*dIII site present in IS*6100*) (Fig. 1). When TEM type ESBL-encoding genes have been situated on transposons, the Tn*A* elements have been generally no longer mobile, due to their disruption and subsequent loss of transposition functions (10). Likewise, IS*6100* has been already found in plasmids, transposons, and integrons, where it frequently truncates genes (15–17, 20). At the 3′ end of Tn*801* the expected IR_R was found adjacent to an *aac(3)-II* (also called *aacC2*) gene which encodes a 3-*N*-aminoglycoside acetyltransferase conferring gentamicin, tobramycin, and netilmicin resistance. The gene and the 34 nucleotides between the gene and the recombination point were identical to those previously described for the *Enterobacteriaceae* (1, 24) (Fig. 1). The −35 sequence of some *aac(3)-II* genes was found to be complemented by an IS*140* element inserted 43 bp upstream from the gene, creating an improved promoter structure (1). With the software available at the Baylor College of Medicine Search Launcher website (http://searchlauncher.bcm.tmc.edu), the analysis of the promoter sequences of the present *aac(3)-II*

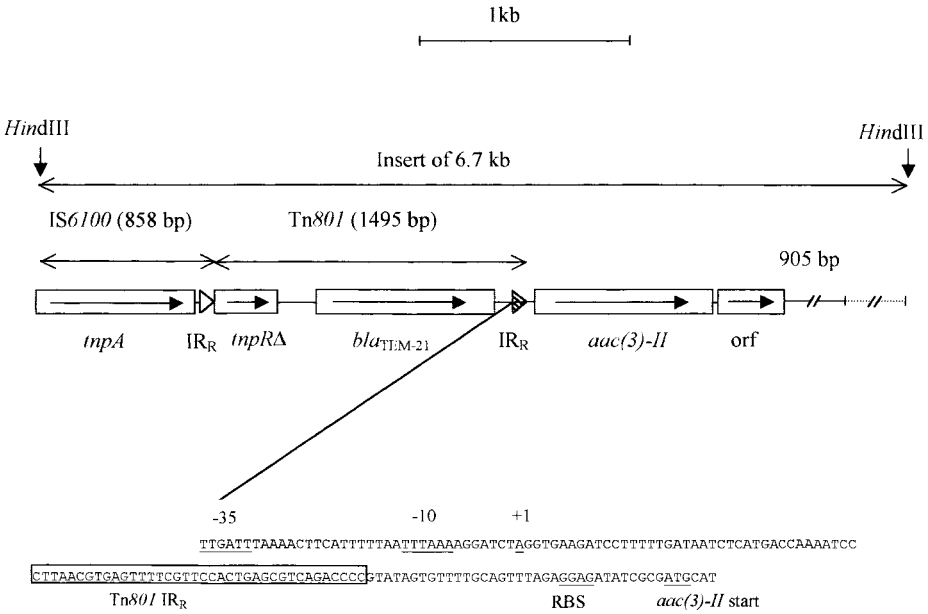

FIG. 1. Schematic representation of the 6.7-kb insert of the recombinant plasmid pMF6. The solid lines represent the sequenced region with the various genes boxed. The horizontal arrows indicate the translation orientation. The open arrowhead represents the terminal inverted repeat of IS*6100*, and the striped arrowhead represents the inverted repeat of Tn*801*. Details on the nucleotide sequence of the *aac(3)-II* promoter region and the recombination point are shown below. The double arrow indicates the size of the IS and transposon homologous regions. The dotted lines indicate the unsequenced fragment, and dashes are used to indicate that the corresponding sequences are not to scale. RBS, ribosomal binding site.

gene indicated that several sequences within the 5′ end of Tn*801* may correspond to putative promoters, including one highly homologous (94%) to *E. coli* promoter sequences for σ^{70} factor (regions −35[TTGATT] and −10[TTTAAA]) (Fig. 1). ISs, but much less commonly transposons, are known to provide mobile promoters for prokaryotic gene expression. Another open reading frame of 321 nucleotides was found 12 bp downstream from the 3′ end of the *aac(3)-II* gene. Except for a Tyr→His substitution at position 84, this open reading frame encoded a protein of 106 amino acids identical to that described in part for plasmids pWP14A and pWP113A, also separated by 12 bp from the same *aac(3)-II* gene (1). A 905-bp sequence at the 3′ end of this gene was determined but did not show any significant homology (<39%) with sequences compiled in the GenBank database. The $bla_{\text{TEM-21}}$ and *aac(3)-II* genes are mostly encountered in the *Enterobacteriaceae*, and they seemed chromosomally located in Pa141, thus supporting the hypothesis that a plasmid derived from enterobacteria became integrated into Pa141 chromosomal DNA either by homologous recombination or by IS- or transposon-mediated specific cointegration (8).

This study is the first description of the $bla_{\text{TEM-21}}$ genetic environment and the presence of this gene in a clinical isolate of *P. aeruginosa*, highlighting once more the broad exchange of resistance genes from *Enterobacteriaceae* to *P. aeruginosa* strains.

Nucleotide sequence accession number. The nucleotide sequences reported in this work are available in the GenBank nucleotide database under the accession no. AF466526.

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