

# Genetic Structures at the Origin of Acquisition and Expression of the Carbapenem-Hydrolyzing Oxacillinase Gene *bla*<sub>OXA-58</sub> in *Acinetobacter baumannii*

Laurent Poirel and Patrice Nordmann\*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris,  
Faculté de Médecine Paris-Sud, Université Paris XI, K-Bicêtre, France

Received 17 October 2005/Returned for modification 3 January 2006/Accepted 3 February 2006

**Genetic structures surrounding the carbapenem-hydrolyzing oxacillinase gene *bla*<sub>OXA-58</sub> were characterized in a series of OXA-58-positive *Acinetobacter baumannii* strains isolated from different countries. We showed that in most of the cases, acquisitions of the *bla*<sub>OXA-58</sub>-containing overall structure, including insertion sequence elements, may be likely the results of recombination events. In type strain *A. baumannii* MAD, the genetic structure surrounding the *bla*<sub>OXA-58</sub> gene was bracketed by two 27-bp repeated sequences. The isolation of a clonally related OXA-58-negative *A. baumannii* isolate that possessed the same plasmid backbone as *A. baumannii* MAD but lacked this genetic structure indicated that the mechanism of acquisition could be reversible. Parts of the structure identified in *A. baumannii* MAD were conserved in other *bla*<sub>OXA-58</sub>-positive isolates from various European countries. Primer extension experiments showed that *bla*<sub>OXA-58</sub> expression was related to promoter sequences brought by different insertion sequence elements, such as an IS*Aba3*-like element, IS*Aba1*, IS*Aba2*, and IS*I8*. This work identified novel structures at the origin of acquisition and expression of a carbapenem-hydrolyzing  $\beta$ -lactamase identified in non-clonally related *A. baumannii* isolates.**

The increasing number of worldwide reports of  $\beta$ -lactamase-mediated carbapenem resistance in *Acinetobacter baumannii* raises the question of the genetic structures that may be at the origin of their acquisition (18, 29). Those carbapenem-hydrolyzing  $\beta$ -lactamases are metallo- $\beta$ -lactamases (Ambler class B  $\beta$ -lactamases) and oxacillinases (Ambler class D  $\beta$ -lactamases). Integrons are common vehicles for acquisition of metallo- $\beta$ -lactamase genes. However, carbapenem-hydrolyzing oxacillinase (CH $\beta$ L) genes have not been reported in class 1 integrons. The *bla*<sub>OXA-48</sub> gene identified in a carbapenem-resistant *Klebsiella pneumoniae* isolate was associated with an IS*I999* insertion sequence element (19) and *bla*<sub>OXA-23</sub> with an IS*Aba1* element (12, 13, 17). In addition, genetic structures for acquisition of the *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-25</sub>, *bla*<sub>OXA-26</sub>, *bla*<sub>OXA-27</sub>, and *bla*<sub>OXA-40</sub> genes of *A. baumannii* isolates are unknown (1, 4, 10).  $\beta$ -Lactamases OXA-51 and OXA-69, which are very similar and share less than 63% amino acid identity with the two latter groups and which may be considered, in view of recent findings (11), as naturally produced enzymes, would define a third group of those oxacillinases (5, 6). In addition, we have recently characterized  $\beta$ -lactamase OXA-58, which belongs to a fourth group of CH $\beta$ Ls (20). The *bla*<sub>OXA-58</sub> gene was found to be plasmid located in most cases. This oxacillinase gene has been identified in a carbapenem-resistant *A. baumannii* isolate that was at the origin of a nosocomial outbreak in Toulouse, France (9), and further studies showed that carbapenemase OXA-58 was very widespread, since it has been detected in *A. baumannii* isolates recovered from different countries from

southern Europe, the Balkans, and central Turkey (16) and also from Argentina, Kuwait, and the United Kingdom (J. Coelho, N. Woodford, M. Afzal-Shah, and D. Livermore, 6th Int. Symp. Biol. Acinetobacter, abstr. pC7, 2004). The aim of the present study was to investigate the genetic structures associated with the *bla*<sub>OXA-58</sub> genes, since they have been reported worldwide and may be the main cause for carbapenem resistance in *A. baumannii*.

This work has been in part previously presented (L. Poirel, S. Marqué, C. Héritier, and P. Nordmann, 15th Eur. Cong. Clin. Microbiol. Infect. Dis, abstr. P425, 2005).

## MATERIALS AND METHODS

**Bacterial isolates.** Fourteen *bla*<sub>OXA-58</sub>-positive *A. baumannii* isolates were included in this study (Table 1). The *bla*<sub>OXA-58</sub> gene was searched for by using a PCR technique with primers OXA-58A and OXA-58B as described previously (16). In addition, two *bla*<sub>OXA-58</sub>-negative *A. baumannii* isolates not listed in Table 1 were included. They had been recovered in Toulouse, France, where an outbreak due to *bla*<sub>OXA-58</sub>-positive *A. baumannii* had occurred (9) from a patient and from a hospital environment. They have been included here, since they were of the same pulsotype as the epidemic strain (pulsotype E) (Table 1). Isolates had been identified by using the API32GN systems (bioMérieux, Marcy-l'Étoile, France). In this study, we have selected a single representant of each pulsotype as previously determined (16). In addition, strains belonging to the identical pulsotype but possessing different plasmids were also studied (Table 1). Electrocompetent *Escherichia coli* DH10B (GIBCO BRL, Life Technologies, Cergy Pontoise, France) was used as the recipient strain in transformation experiments.

**Cloning of the *bla*<sub>OXA-58</sub>-surrounding sequences and sequencing.** Total DNAs of the *A. baumannii* isolates were extracted as described previously (10). Those DNAs were used as templates in standard PCR conditions for detection of *bla*<sub>OXA-58</sub>-surrounding sequences. Total DNA of *A. baumannii* MAD was digested partially by Sau3AI restriction enzyme, ligated into the BamHI site of plasmid pBK-CMV, and transformed in the *E. coli* DH10B reference strain, as described previously (22). Recombinant plasmids were selected on Trypticase soy (TS) agar plates containing amoxicillin (50  $\mu$ g/ml) and kanamycin (30  $\mu$ g/ml). The cloned DNA fragments of several recombinant plasmids were sequenced on both strands with an ABI 3100 sequencer (Applied Biosystems, Foster City,

\* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.ap-hop-paris.fr.

TABLE 1. Features of the OXA-58-positive *A. baumannii* isolates used in this study

| Strain                    | Pulsotype <sup>a</sup> | Geographical origin | Plasmid size (kb) or location | Structure as referred to in Fig. 1 | Additional IS  | Source or reference |
|---------------------------|------------------------|---------------------|-------------------------------|------------------------------------|----------------|---------------------|
| <i>A. baumannii</i> MAD   | E                      | Toulouse, France    | 30                            | 2                                  | IS <i>Aba2</i> | 20                  |
| <i>A. baumannii</i> 328   | I                      | Lille, France       | 30                            | 6                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> 203   | J                      | Mâcon, France       | 40                            | 3                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> 253   | K                      | K.-Bicêtre, France  | 40                            | 3                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> CH22  | E                      | Seville, Spain      | 27                            | 5                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> CH17  | F                      | Seville, Spain      | 40                            | 4                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> CH28  | G                      | Seville, Spain      | 25                            | 7                                  | IS <i>Aba2</i> | 16                  |
| <i>A. baumannii</i> 18    | B                      | Iasi, Romania       | C <sup>b</sup>                | 8                                  | None           | 16                  |
| <i>A. baumannii</i> 73    | C                      | Iasi, Romania       | 15                            | 8                                  | None           | 16                  |
| <i>A. baumannii</i> 24    | D                      | Iasi, Romania       | 20                            | 10                                 | None           | 16                  |
| <i>A. baumannii</i> CH29  | H                      | Ankara, Turkey      | 21                            | 9                                  | IS <i>I8</i>   | 16                  |
| <i>A. baumannii</i> 10    | N                      | Ankara, Turkey      | 20                            | 10                                 | None           | This study          |
| <i>A. baumannii</i> 3601  | L                      | Istanbul, Turkey    | 20                            | 10                                 | None           | This study          |
| <i>A. baumannii</i> 13015 | M                      | Istanbul, Turkey    | 22                            | 11                                 | IS <i>Aba1</i> | This study          |

<sup>a</sup> Pulsotypes are referring to reference 16, except for the two additional lanes, L and M, obtained in this present study.

<sup>b</sup> C, chromosomal location.

Calif.). The entire sequence provided in this study was made of sequences of several plasmids that contained overlapping cloned fragments. The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available on the Internet at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>).

**PCR mapping of the *bla*<sub>OXA-58</sub>-surrounding sequences and identification of insertion sequence (IS) elements.** Precise determination of the genetic structures surrounding the *bla*<sub>OXA-58</sub> gene in *A. baumannii* MAD allowed us to design a series of primers for PCR amplifications from the other *A. baumannii* isolates tested. The primers used in this study are listed in Table 2, and their locations are indicated in Fig. 1. The primers specific for the different IS elements identified are also listed in Table 2. Identification of the IS elements located in the 5' end of the *bla*<sub>OXA-58</sub> gene was performed by sequencing PCR products generated with primer SM2 in combination with OXA-58B.

**Genetic support.** Plasmid DNA extraction was attempted by the Kieser method (15). Plasmid extracts were subsequently analyzed by electrophoresis on a 0.7% agarose gel.

**PFGE.** Pulsed-field gel electrophoresis (PFGE) analysis was done according to the manufacturer's instructions (Bio-Rad, Ivry-sur-Seine, France). *Apa*I restriction enzyme (Amersham Pharmacia Biotech, Orsay, France) was used for genotyping of the *A. baumannii* isolates, as described previously (21, 28).

**Determination of the transcription initiation sites by 5'-RACE.** Total RNA was isolated from different *A. baumannii* isolates by using the RNeasy Midi kit (QIAGEN, Courtaboeuf, France). 5'-rapid amplification of cDNA ends (5'-RACE) reactions were performed using 5 µg of total RNA with the 5'-RACE system kit (version 2.0; Invitrogen, Life Technologies, Cergy Pontoise, France). The first-strand synthesis was primed with specific primer OXA-58-5'ext, and amplification of the target cDNA was performed with the dC-tailed cDNAs as templates by using the newly described primers AAP and AUAP (Table 2). The 5'-RACE PCR products were cloned into pCRBluntII-Topo (Invitrogen), and each clone with a distinct insert size was sequenced. Analysis of the cloned sequence allowed the determination of the transcription initiation site and subsequently the promoter sequences. For each transcription assay, at least 10 clones were analyzed, and the entire experiment was repeated twice for all strains studied.

**Nucleotide sequence accession numbers.** The nucleotide sequences of the insertion sequences reported in this paper have been submitted to the IS Finder website (<http://www-is.biotoul.fr>). The entire sequence identified for the *A. baumannii* MAD isolate and described in that study has been deposited in the EMBL/GenBank nucleotide sequence database and has been assigned accession no. AY665723.

TABLE 2. Oligonucleotide primers used in this study

| Name            | Nucleotide sequence (5'-3')          | Location/use  | No. as referred to in Fig. 1 <sup>a</sup> |
|-----------------|--------------------------------------|---|---|
| OXA-58A         | CGATCAGAATGTTC AAGCGC                | <i>bla</i> <sub>OXA-58</sub>                                  | 1   |
| OXA-58B         | ACGATTTCTCCCCTCTGCGC                 | <i>bla</i> <sub>OXA-58</sub>                                  | 2   |
| IS <i>Aba1A</i> | GTGCTTTGCGCTCATCATGC                 | <i>tnpA</i> of IS <i>Aba1</i>                                 | 16  |
| IS <i>Aba1B</i> | CATGTAAACCAATGCTCACC                 | <i>tnpA</i> of IS <i>Aba1</i>                                 | 17  |
| IS <i>Aba2A</i> | AATCCGAGATAGAGCGGTTTC                | <i>tnpA</i> of IS <i>Aba2</i>                                 | 12  |
| IS <i>Aba2B</i> | TGACACATAACCTAGTGCAC                 | <i>tnpA</i> of IS <i>Aba2</i>                                 | 13  |
| IS <i>Aba3A</i> | CAATCAAATGTCCAACCTGC                 | <i>tnpA</i> of IS <i>Aba3</i>                                 | 3   |
| IS <i>Aba3B</i> | CGTTTACCCCAAACATAAGC                 | <i>tnpA</i> of IS <i>Aba3</i> but not in IS <i>Aba3</i> -like | 4   |
| IS <i>Aba3C</i> | AGCAAATATCTCGTATACCGC                | <i>tnpA</i> of IS <i>Aba3</i> -like and IS <i>Aba3</i>        | 11  |
| IS <i>I8A</i>   | CACCCAACCTTTCTCAAGATG                | <i>tnpA</i> of IS <i>I8</i>                                   | 14  |
| IS <i>I8B</i>   | ACCAGCCATAACTTCACTCG                 | <i>tnpA</i> of IS <i>I8</i>                                   | 15  |
| PreTh           | ATCCAACCATTCATCAAACCTCTGGC           | Left-hand extremity of Re27-1                                 | 6   |
| PreEt           | CTATTTGGTTTTAAGGGGC                  | Right-hand extremity of Re27-2                                | 5   |
| Re27-1          | TTCGTATAACCGCCATTATG                 | Inside the Re27-1 sequence                                    | 7   |
| Re27-2          | AACATAATGGCTGTTATACG                 | Inside the Re27-2 sequence                                    | 8   |
| MAD-Th          | AACAGCAATAGCCATCAAC                  | <i>Thr-Eff</i> gene   | 9   |
| SM2             | AAGTGTCTATATCTCACC                   | Between Re27-1 and IS <i>Aba3</i> -like                       | 10  |
| AAP             | GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG | Used for 5'-RACE  | -   |
| AUAP            | GGCCACGCGTCGACTAGTAC                 | Used for 5'-RACE  | -   |
| OXA-58-5'ext    | CGACTCATACTATGCTCAGC                 | Used for 5'-RACE  | -   |

<sup>a</sup> -, primer not indicated in Fig. 1.

## RESULTS

**Analysis of the plasmid-borne *bla*<sub>OXA-58</sub>-surrounding sequences in reference OXA-58-positive *A. baumannii* MAD.** Previous analysis of the *bla*<sub>OXA-58</sub> downstream-located sequences in *A. baumannii* MAD identified an IS*Aba3* element that was present 28 bp downstream of the stop codon of *bla*<sub>OXA-58</sub> (Fig. 1) (20). IS*Aba3* is a member of the IS1 family and possesses a single open reading frame (ORF) corresponding to a 145-amino-acid transposase. We had showed that a 3-bp duplicated sequence bracketed this IS*Aba3* element, thus suggesting an independent insertion of that mobile structure.

Further sequencing of the right-hand extremity of IS*Aba3* by using several recombinant plasmids obtained in the present study revealed several genes. These genes were (i) *araC1*, encoding a putative 275-amino-acid-long protein sharing 42% amino acid identity with a transcription regulator of *Pseudomonas aeruginosa* belonging to the XylS family; (ii) *lysE*, encoding a putative 201-amino-acid-long protein exhibiting significant similarity with threonine efflux proteins of the LysE family (39% and 33% amino acid identity with those of *Ralstonia solanacearum* and *P. aeruginosa*, respectively); and (iii) *araC2*, encoding a 106-amino-acid-long putative transcription regulator exhibiting 61% amino acid identity with that of *Xyella fastidiosa* (Fig. 1).

Partial analysis revealed that at least part of IS*Aba3* was present 20 bp upstream of the start codon of *bla*<sub>OXA-58</sub>. Further sequencing of recombinant plasmids revealed that, in *A. baumannii* MAD, this IS*Aba3* element was truncated by the insertion of IS*Aba2*, a novel insertion sequence belonging to the IS3 family (IS51 group). IS*Aba2* is 1,306 bp long and possesses two ORFs 74% and 66% identical to those encoding the transposase of IS51. The perfect inverted repeats of IS*Aba2* are 25 bp in length, and its insertion had generated a 5-bp duplication inside the transposase gene of IS*Aba3* (Fig. 1). At the left-hand extremity of IS*Aba2*, the C-terminal extremity of the TnpA transposase of IS*Aba3* element was identified (Fig. 1). However, this transposase sequence was interrupted, since the corresponding 20 last C-terminal amino acids were absent, as well as the right inverted repeat of IS*Aba3*. Thus, this incomplete insertion sequence will be named IS*Aba3*-like element in this work. Precise analysis of the site where the IS*Aba3*-like sequence differed from IS*Aba3* did not give a clue for an insertion mechanism of a foreign DNA. Consequently, the *bla*<sub>OXA-58</sub> gene was not part of a composite transposon made of two IS*Aba3* elements in *A. baumannii* MAD.

Interestingly, 89 bp downstream of the interrupted *tnpA* gene of the IS*Aba3*-like element, a 27-bp sequence termed Re27-1 (5'-TTTCGTATAACCGCCATTATGTTAAAT-3') was identified that was identical to (except a single C→A mismatch at bp position 12) and in the same orientation as another 27-bp sequence (named Re27-2) identified 2,479 bp downstream of *bla*<sub>OXA-58</sub>, located between the *lysE* and *araC2* genes (Fig. 1). This observation strongly suggested that a recombination process was at the origin of the acquisition of the *bla*<sub>OXA-58</sub> locus. This integration of foreign DNA could be the result of a recombination between a Re27 element initially present in the target sequence and another Re27 element

carried by the donor molecule. On the left-hand extremity of Re27-1, a gene (*Est*) (Fig. 1) that encodes a putative 277-amino-acid esterase sharing 61% and 66% amino acid identities with esterases of *E. coli* and *Xanthomonas campestris*, respectively, was identified.

**The *bla*<sub>OXA-58</sub> gene was embedded in various genetic structures.** Analysis of the sequences bracketing the *bla*<sub>OXA-58</sub> gene was evaluated by PCR mapping in different *A. baumannii* isolates. Results indicated that some variability might be observed as indicated in Table 1 and drawn in Fig. 1. A constant feature was the plasmid location of the *bla*<sub>OXA-58</sub> gene (except for *A. baumannii* 18) even though a large variety of different plasmid sizes was noticed (Table 1). Another identified feature was the presence of IS*Aba3* downstream of *bla*<sub>OXA-58</sub> (Fig. 1). However, for several isolates, sequences located on both external extremities of the Re27 repeats lacked, but the overall *bla*<sub>OXA-58</sub> locus including only one Re27 (either Re27-1 or Re27-2) or the two Re27s were identified. This suggested that a recombination process had likely occurred in another target site location (Fig. 1). For some other isolates, both Re27 repeats lacked and did not give any PCR products using all the primers combinations.

Interestingly, for a single isolate (strain CH29) (Fig. 1, lane 9), PCR results indicated that an intact IS*Aba3* element was present upstream of the *bla*<sub>OXA-58</sub> gene. It was truncated by IS18 (see below), but its right-end extremity was present by contrast to what is observed in other strains possessing the IS*Aba3*-like element. Since DNA sequences located downstream of the *bla*<sub>OXA-58</sub> gene were different just after the second copy of the IS*Aba3* element (Fig. 1), it is likely that this structure may correspond in that isolate to a composite transposon made of two IS*Aba3* elements flanking the *bla*<sub>OXA-58</sub> gene.

**Variability of the 5'-end extremity of the *bla*<sub>OXA-58</sub> gene.** Analysis of the region flanking the 5' end of the *bla*<sub>OXA-58</sub> gene indicated that the IS*Aba2* element was not always present. IS*Aba2* was identified at the same position as in *A. baumannii* MAD in six other isolates belonging to different pulsotypes and from several cities in France and Spain (Table 1; Fig. 1). In the other isolates, IS*Aba2* was not identified at that location but was also absent of their whole genome (data not shown). In addition, an IS18 isoform was identified upstream of the *bla*<sub>OXA-58</sub> gene in *A. baumannii* CH29 (Fig. 1, lane 9), and the IS*Aba1* element (13, 26) was identified upstream of the *bla*<sub>OXA-58</sub> gene in *A. baumannii* 13015 (Fig. 1, lane 11). Insertion sequence IS18 belongs to the IS30 family, is 1,074 bp long, possesses a 320-amino-acid transposase and inverted repeats of 20 bp, and generates a 3-bp duplication upon transposition (24). The IS18 isoform we have identified possesses a transposase exhibiting five amino acid substitutions compared to that originally found in IS18. IS*Aba1* belongs to the IS4 family, is 1,180 bp long, possesses two ORFs corresponding to 189 and 178 amino acids, respectively, and 16-bp inverted repeats, and generates a 9-bp duplication upon transposition (13).

**Excision of the *bla*<sub>OXA-58</sub>-borne locus.** Shortly after the outbreak period that had occurred in Toulouse, France, in 2003–2004 (9), hygiene measures, including a systematic search for *A. baumannii* strains in the hospital environment and systematic rectal screening of patients, was implemented.

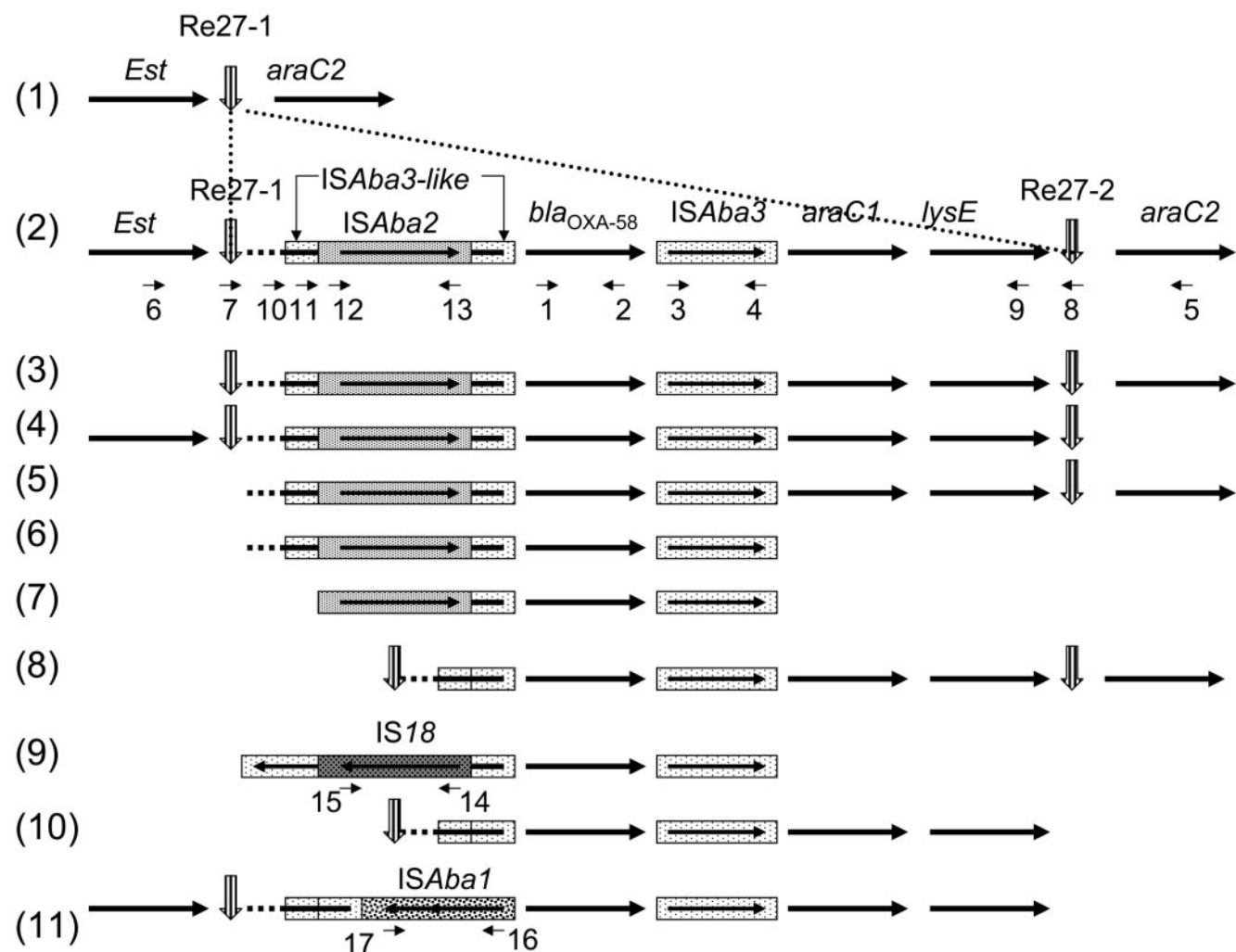


FIG. 1. Schematic map of *bla*<sub>OXA-58</sub>-positive structures identified in *A. baumannii* isolates. (1), the structure identified in two *bla*<sub>OXA-58</sub>-negative isolates; (2), the structure from *A. baumannii* MAD; (3) to (11), structures mapped in other isolates, as indicated in Fig. 1. Genes and their corresponding transcription orientations are indicated by horizontal arrows. The horizontal dotted line indicates the sequence separating the truncated *ISAbA3* element from the Re27-1 repeat sequence. The two different transcription regulator genes (*araC1* and *araC2*), the threonine efflux protein gene (*lysE*) and the esterase gene (*Est*) are indicated. Vertical arrows are for the two Re27 sequences. Positions of the primers are indicated as referred to Table 2. The whole genetic structure which has been acquired or lost by recombination is indicated by the dashed lines. The figure is not to scale.

As a result of this systematic screening, two carbapenem-susceptible *A. baumannii* isolates (MIC of imipenem, 0.5 µg/ml) were recovered from the urine of a patient who did not receive any antibiotic treatment (isolate 9) and from an environmental strain (alimentary peristaltic pump) (isolate 10). PFGE analysis showed that isolates 9 and 10 possessed the same genotype as opposed to the epidemic strain (data not shown). Plasmid analysis showed that they harbored the same ca. 25-kb plasmid, therefore suggesting that it could be related to the plasmid originally found in the epidemic strain (which was 30 kb [20]) without a *bla*<sub>OXA-58</sub> locus. Thus, PCR experiments were performed in order to verify this hypothesis by using primers located on both ends of the repeated sequences (Table 2). Whereas a 5,547 bp-long PCR product was obtained with the *bla*<sub>OXA-58</sub>-positive *A. baumannii* MAD, a 380-bp product was obtained by using DNAs of *A. baumannii* 9 and 10 as tem-

plates. Sequencing of the latter PCR product revealed that the plasmid had indeed lost the *bla*<sub>OXA-58</sub> locus (Fig. 1, lane 1). Only the Re27-1 sequence was identified, indicating that this motif likely corresponded to the target sequence.

***ISAbA1*, *ISAbA2*, and *ISAbA3-like* as enhancers for *bla*<sub>OXA-58</sub> expression.** Using the 5'-RACE PCR technique, the sites of initiation of transcription of the *bla*<sub>OXA-58</sub> gene were mapped in four *A. baumannii* isolates, each having different sequences located upstream of *bla*<sub>OXA-58</sub>, i.e., the presence of *ISAbA1*, of *ISAbA2*, of *IS18*, or of *ISAbA3-like* element only. In *A. baumannii* MAD that possessed an *ISAbA2* element, the +1 transcription start was located 120 bp upstream of the start codon of the *bla*<sub>OXA-58</sub> gene, just inside the *ISAbA3-like* element (Fig. 2A). Interestingly, this promoter, named *P*<sub>Aba2</sub>, was a hybrid, made of a -35 region located inside the *ISAbA2* element and a -10 region located inside the *ISAbA3* element (Fig. 2A). In

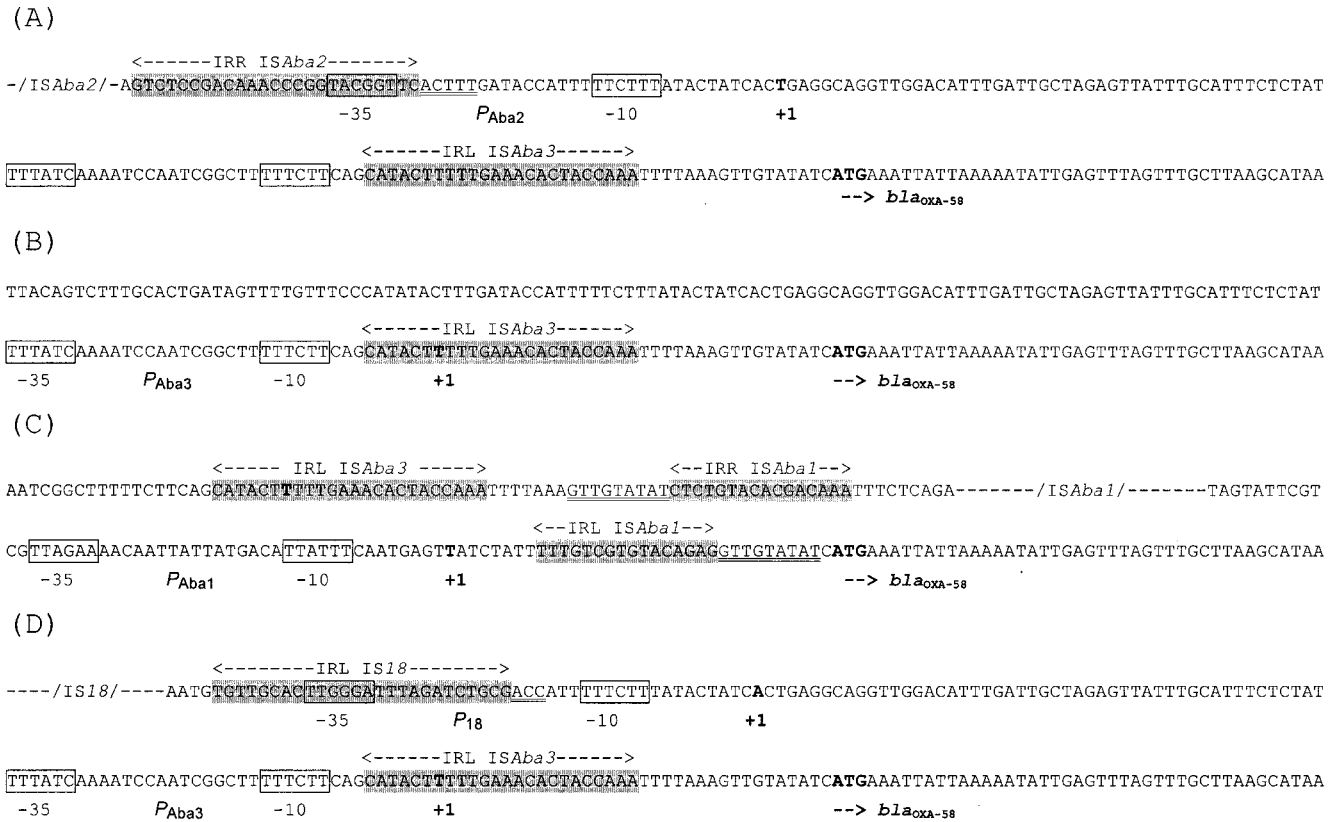


FIG. 2. Promoter structures for *bla*<sub>OXA-58</sub> expression as determined by 5'-RACE experiment. The +1 initiation sites of transcription and the different start ATG codons are in bold. The inverted repeats of the IS elements are shaded in gray. The -35 and -10 motifs of the promoters are boxed. The target site duplications generated by IS transposition, if any, are double underlined. (A) Genetic structure identified from an ISAb2-positive *A. baumannii* CLA isolate; (B) genetic structure identified from an ISAb1-, ISAb2-, and IS18-negative *A. baumannii* 10 isolate; (C) genetic structure identified from an ISAb1-positive *A. baumannii* 13015 isolate; (D) genetic structure identified from an IS18-positive *A. baumannii* CH29 isolate.

*A. baumannii* 24 that possessed the ISAb3-like element upstream of *bla*<sub>OXA-58</sub>, the +1 transcription start was located 35 bp upstream of the start codon of the *bla*<sub>OXA-58</sub> gene, just inside the inverted repeat left (IRL) of the ISAb3-like element (Fig. 2B). A -35 sequence and a -10 sequence separated by 16 bp defined a *P*<sub>Aba3</sub> promoter (Fig. 2B). In *A. baumannii* 13015 that possessed an ISAb1 element, the +1 transcription start was located 34 bp upstream of the start codon of the *bla*<sub>OXA-58</sub> gene, just inside the IRL of ISAb1 (Fig. 2C). This *P*<sub>3</sub> promoter was made of a -35 sequence separated by 16 bp from the -10 sequence (Fig. 2C). This promoter was identical to that brought by ISAb1 when located upstream of *bla*<sub>ampC</sub> gene in *A. baumannii* isolates leading to overproduction of chromosome-encoded AmpC (7, 13). In *A. baumannii* CH29 that possessed an IS18 element, two +1 transcription starts were identified. A +1 transcription start and, consequently, the -35 and -10 sequences were identical to those identified in *A. baumannii* 24, thus indicating that the expression of *bla*<sub>OXA-58</sub> might be in part under the control of promoter *P*<sub>Aba3</sub>, whereas the other +1 signal was located 122 bp upstream of *bla*<sub>OXA-58</sub>, indicating that the -35 (TTG GGA) and -10 (TTTCTT) sequences separated by 17 bp were also acting as promoter sequences (Fig. 2D). Interestingly, as observed with ISAb2, these promoter sequences were

hybrids, since the -35 region is part of the IRL of IS18 whereas the -10 region was part of the ISAb3-like element (Fig. 2).

## DISCUSSION

Upstream and downstream of the *bla*<sub>OXA-58</sub> gene, a previous partial analysis had identified two ISAb3-like elements that could have been related to a composite transposon. We identified here an unusual structure indicating that the acquisition of the *bla*<sub>OXA-58</sub> gene was likely not the result of a transposition process. The mechanism at the origin of such acquisition should likely correspond to homologous recombination. However, it is unlikely that this mechanism could correspond to homology-facilitated illegitimate recombination known to be at the origin of integration of foreign DNA in *Acinetobacter* spp. (8), since no duplication of the target sequence results from this process. To the best of our knowledge, this is the first report of a  $\beta$ -lactamase acquisition process not related to integron, transposon, or insertion sequence features but based on a recombination event. Some interesting features designated as Re elements have been recently identified in association with the *bla*<sub>VEB-1a</sub> gene. These 135-bp sequences were identified upstream and downstream of that  $\beta$ -lactamase gene

in a *Pseudomonas aeruginosa* isolate and in a *Providencia stuartii* isolate (2, 3), but it is not clear whether these unusual structures may result from recombination. Similarly, DR2 direct repeats of 145 bp were identified in the close vicinity of the *bla*<sub>TLA-2</sub> expanded-spectrum  $\beta$ -lactamase gene identified on an antibiotic multiresistance plasmid from a wastewater treatment plant that may indicate an unusual integration process (27).

The lack of the *bla*<sub>OXA-58</sub> gene and its close surrounding sequences in two *A. baumannii* isolates with the sequence defined as a target site being, however, still present suggested strongly a reversibility of that recombination process. Since these structures have been recovered from antibiotic-free samples, it is tempting to speculate that the absence of antibiotic selection pressure may enhance this spontaneous deletion. Despite several attempts, we did not reproduce in vitro the loss of the *bla*<sub>OXA-58</sub>-containing genetic structure in *A. baumannii* MAD.

The variability of the promoter sequences identified upstream of the *bla*<sub>OXA-58</sub> gene among the analyzed isolates was noteworthy. IS*Aba2*, a novel insertion sequence, and IS*I8* provided hybrid promoters enhancing *bla*<sub>OXA-58</sub> expression. Such hybrid promoters generated upon transposition have been already observed, for instance, with IS*I* and IS*2* (14, 23) and also with IS*I8*. Indeed, similar to our observations, IS*I8* identified previously also from an *A. baumannii* isolate activated a silent aminoglycoside resistance gene by providing a  $-35$  promoter sequence of an hybrid promoter (24). In the case of IS*Aba1*, this element has provided the entire promoter sequences for *bla*<sub>OXA-58</sub> expression, as demonstrated in different *A. baumannii* isolates in which IS*Aba1* enhanced expression of the *bla*<sub>AmpC</sub> cephalosporinase gene or the *aac(3)-IIa* acetyltransferase gene (7, 13, 26). The exact same motifs belonging to IS*Aba1* were acting as promoter sequences in these latter cases, thus confirming that IS*Aba1* is a useful tool for gene expression in *A. baumannii* and might be customized for that species, as suggested by Segal et al. (25). The frequent association between *bla*<sub>OXA-58</sub> gene and IS elements could be the consequence of low-level expression of this oxacillinase gene in a wild-type position.

Finally, analysis of several *Acinetobacter* sp. isolates from different geographical origins revealed variable genetic structures sustaining a *bla*<sub>OXA-58</sub> acquisition which could likely contribute similarly to the worldwide spread of this CH $\beta$ L gene in *A. baumannii*.

#### ACKNOWLEDGMENTS

This work was funded by a grant from the Ministère de l'Éducation Nationale et de la recherche (UPRES-EA3539), Université Paris XI, France, and mostly by the European Community (6th PCRD, LSHM-CT-2003-503-335 and LSHM-2005-018705). L.P. is a researcher from the INSERM (Paris, France).

We thank C. Héritier and S. Marqué for their excellent technical contribution. We thank C. Segonds, G. Chabanon, N. Marty, S. Brisse, G. Coman, R. Filip, H. Nazik, C. Bal, and T. Naas for providing us *A. baumannii* isolates. We also thank W. Wackernagel for helpful comments.

#### REFERENCES

1. Afzal-Shah, M., N. Woodford, and D. M. Livermore. 2001. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D  $\beta$ -lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **45**:583–588.
2. Aubert, D., D. Girlich, T. Naas, S. Nagarajan, and P. Nordmann. 2004. Functional and structural characterization of the genetic environment of an extended spectrum  $\beta$ -lactamase *bla*<sub>VEB</sub> gene from a *Pseudomonas aeruginosa* isolate obtained in India. *Antimicrob. Agents Chemother.* **48**:3284–3290.
3. Aubert, D., T. Naas, M. F. Lartigue, and P. Nordmann. 2005. Novel genetic structure associated with an extended-spectrum  $\beta$ -lactamase *bla*<sub>VEB</sub> gene in a *Providencia stuartii* clinical isolate from Algeria. *Antimicrob. Agents Chemother.* **49**:3590–3592.
4. Bou, G., A. Oliver, and J. Martínez-Beltrán. 2000. OXA-24, a novel class D  $\beta$ -lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrob. Agents Chemother.* **44**:1556–1561.
5. Brown, S., and S. G. Amyes. 2005. The sequences of seven class D  $\beta$ -lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. *Clin. Microbiol. Infect.* **11**:326–329.
6. Brown, S., H. K. Young, and S. G. Amyes. 2005. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. *Clin. Microbiol. Infect.* **11**:15–23.
7. Corvec, S., N. Caroff, E. Espaze, C. Giraudeau, H. Drugeon, and A. Reynaud. 2003. AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. *J. Antimicrob. Chemother.* **52**:629–635.
8. De Vries, J., and W. Wackernagel. 2002. Integration of foreign DNA during natural transformation of *Acinetobacter* sp. by homology-facilitated illegitimate recombination. *Proc. Natl. Acad. Sci. USA* **99**:2094–2099.
9. Héritier, C., A. Dubouix, L. Poirel, N. Marty, and P. Nordmann. 2005. A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem-hydrolyzing oxacillinase OXA-58. *J. Antimicrob. Chemother.* **55**:115–118.
10. Héritier, C., L. Poirel, D. Aubert, and P. Nordmann. 2003. Genetic and functional analysis of the chromosome-encoded carbapenem-hydrolyzing oxacillinase OXA-40 of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **47**:268–273.
11. Héritier, C., L. Poirel, P. E. Fournier, J. M. Claverie, D. Raoult, and P. Nordmann. 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:4174–4179.
12. Héritier, C., L. Poirel, T. Lambert, and P. Nordmann. 2005. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:3198–3202.
13. Héritier, C., L. Poirel, and P. Nordmann. 2006. Cephalosporinase overexpression as a result of insertion of IS*Aba1* in *Acinetobacter baumannii*. *Clin. Microbiol. Infect.* **12**:123–130.
14. Jaurin, B., and S. Normark. 1983. Insertion of IS2 creates a novel *ampC* promoter in *Escherichia coli*. *Cell* **32**:809–816.
15. Kieser, T. 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* **12**:19–36.
16. Marqué, S., L. Poirel, C. Héritier, S. Brisse, M. D. Blasco, R. Filip, G. Coman, T. Naas, and P. Nordmann. 2005. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. *J. Clin. Microbiol.* **43**:4885–4888.
17. Naas, T., M. Levy, C. Hirschauer, H. Marchandin, and P. Nordmann. 2005. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. *J. Clin. Microbiol.* **43**:4826–4829.
18. Nordmann, P., and L. Poirel. 2002. Emerging carbapenemases in gram-negative aerobes. *Clin. Microbiol. Infect.* **8**:321–331.
19. Poirel, L., C. Héritier, V. Tolün, and P. Nordmann. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* **48**:15–22.
20. Poirel, L., S. Marqué, C. Héritier, C. Segonds, G. Chabanon, and P. Nordmann. 2005. OXA-58, a novel class D  $\beta$ -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:202–208.
21. Poirel, L., O. Menuteau, N. Agoli, C. Cattoen, and P. Nordmann. 2003. Outbreak of extended-spectrum  $\beta$ -lactamase VEB-1-producing isolates of *Acinetobacter baumannii* in a French hospital. *J. Clin. Microbiol.* **41**:3542–3547.
22. Poirel, L., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and P. Nordmann. 1999. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum  $\beta$ -lactamase encoded by an *Escherichia coli* integron gene. *Antimicrob. Agents Chemother.* **43**:573–581.
23. Prentki, P., B. Teter, M. Chandler, and D. J. Galas. 1986. Functional promoters created by the insertion of transposable element IS1. *J. Mol. Biol.* **191**:383–393.
24. Rudant, E., Courvalin, P., and T. Lambert. 1998. Characterization of IS*I8*, an element capable of activating the silent *aac(6')-Ij* gene of *Acinetobacter* sp. 13 strain BM2716 by transposition. *Antimicrob. Agents Chemother.* **42**:2759–2761.
25. Segal, H., S. Garry, and B. G. Elisha. 2005. Is IS*Aba1* customized for *Acinetobacter*? *FEMS Microbiol. Lett.* **243**:425–429.
26. Segal, H., R. Thomas, and B. Gay Elisha. 2003. Characterization of class 1 integron resistance gene cassettes and the identification of a novel IS-like element in *Acinetobacter baumannii*. *Plasmid* **49**:169–178.

27. Szczepanowski, R., I. Krahn, B. Linke, A. Goesmann, A. Puehler, and A. Schlueter. 2004. Antibiotic multiresistance plasmid pRSB101 isolated from a wastewater treatment plant is related to plasmids residing in phytopathogenic bacteria and carries eight different resistance determinants including a multidrug transport system. *Microbiology* **150**:3613–3630.
28. 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.
29. Van Looveren, M., H. Goossens, and the ARPAC Steering Group. 2004. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin. Microbiol. Infect.* **10**:684–704.