To assess dissemination of OXA-23–producing strains of *Acinetobacter baumannii*, we obtained 20 carbapenem-resistant, OXA-23–producing isolates from different regions. Their clonal relationship was assessed by pulsed-field gel electrophoresis and multilocus sequence typing. We identified 8 sequence types, including 4 novel types. All except 2 strains belonged to 2 main European clonal lineages. The *bla* \(_{OXA-23}\) gene was either located on the chromosome or on plasmids and associated with 4 genetic structures.

*A. baumannii* is a gram-negative organism that is increasingly recognized as a major pathogen causing nosocomial infections, including bacteremia and ventilator-associated pneumonia, particularly in patients admitted to intensive care units (1). Several studies have shown the geographically widespread occurrence of multidrug-resistant *A. baumannii* strains, which suggested a clonal relatedness of these strains. Three international *A. baumannii* clones associated with multidrug resistance (European clones I, II, and III) have been reported (2).

Increasing resistance to carbapenems has been observed worldwide in the past decade, frequently mediated by production of class D \(\beta\)-lactamases with carbapenemase activity. Three acquired class D \(\beta\)-lactamases with carbapenemase gene clusters have been described in *A. baumannii*, which correspond to *bla* \(_{OXA-23}\)-like, *bla* \(_{OXA-48}\)-like, and *bla* \(_{OXA-58}\)-like genes (3). The *bla* \(_{OXA-23}\) gene, first characterized in Scotland (4), has been increasingly reported worldwide. *A. radioresistens* was recently identified as the progenitor of the *bla* \(_{OXA-23}\)-like genes (5). Clonal outbreaks of carbapenem-resistant and OXA-23–producing *A. baumannii* have been reported in many countries, such as Bulgaria (6), People’s Republic of China (7), Brazil (8), Iraq (9), Afghanistan (9), and French Polynesia (10).

Genetic acquisition of the *bla* \(_{OXA-23}\) gene was investigated and transposons Tn2006, Tn2007, and Tn2008 were identified as genetic structures harboring this gene (10–12). In Tn2006, the *bla* \(_{OXA-23}\) gene is flanked by 2 copies of the insertion sequence IS\(_{Aba1}\), which are located in opposite orientations (Figure 1). The functionality of Tn2006 has been recently demonstrated (13). Tn2008 is similar to Tn2006 but lacks the second copy of IS\(_{Aba1}\) and the *bla* \(_{OXA-23}\) gene is associated with 1 copy of IS\(_{Aba4}\) (which differs from IS\(_{Aba1}\)) in Tn2007 (Figure 1) (14). As reported for strains from United Arab Emirates and Bahrain, the *bla* \(_{OXA-23}\) gene can be associated with only 1 copy of IS\(_{Aba1}\) (14,15). We studied the clonal relationship and genomic environment of sequences surrounding the *bla* \(_{OXA-23}\) gene among a collection of OXA-23–producing isolates from 15 countries.

**Materials and Methods**

**Bacterial Strains and Susceptibility Testing**

Twenty OXA-23–producing *A. baumannii* clinical isolates were obtained from 15 countries. These isolates had been obtained from patients hospitalized in intensive care units from December 2003 through March 2008. Isolates were obtained from tracheal aspirates (n = 3), bile (n = 1), urine (n = 4), wounds (n = 1), respiratory tract (n = 1), blood (n = 4), and sputum (n = 1). The isolates were initially chosen after preliminary pulsed-field gel electrophoresis (PFGE)–based typing had identified 13 pulsotypes. Isolates
were obtained from France (n = 4), Vietnam (n = 1), New Caledonia (n = 1), Thailand (n = 1), Australia (n = 1), Tahiti (n = 1), Reunion (n = 2), South Africa (n = 1), United Arab Emirates (n = 2), Libya, (n = 1), Bahrain (n = 1), Egypt (n = 1), Belgium (n = 1), Algeria (n = 1), and Brazil (n = 1).

Presence of the bla\textsubscript{OXA-23} gene was screened by PCR by using specific primers (OXA-23-A 5′-GA ATTCCATGAAATAATTTGCTTC-3′ and OXA-23-B 5′-CGGGATCCCCGTTAATAATTCAGGTC-3′) and additional sequencing (ABI 3100 sequencer; Applied Biosystems, Foster City, CA, USA). Susceptibility patterns to β-lactam antimicrobial drugs were determined by using a standard disk diffusion method according to published standards (16) and Etest strips (AB Biodisk, Solna, Sweden). Isolates were identified by using 16S rRNA gene sequencing (17).

Clonal Relationships

Isolates were typed by using ApaI macrorestriction analysis and PFGE according to the manufacturer’s recommendations (Bio-Rad, Marnes-la-Coquette, France). Bacteria were grown in a medium appropriate for the strain until an optical density of 0.8 to 1 at 600 nm was reached. One milliliter of cells was centrifuged, washed, and resuspended in 10 mmol/L Tris, pH 7.2, 20 mmol/L NaCl, 50 mmol/L EDTA. Immediately after resuspension, an equal volume of 2% low melting point InCert agarose (Bio-Rad) was added. Solid agarose plugs were lysed at 37°C for 2 h in 1 mL of lysis buffer (10 mmol/L Tris, pH 7.2, 50 mmol/L NaCl, 0.5% sodium laurylsarcosine, 0.2% sodium deoxycholate) supplemented with 20 mg/L of lysozyme. The plugs were then incubated at 55°C for 16 h with proteinase K buffer (10 mmol/L EDTA, pH 8, 0.2% sodium deoxycholate, 1% sodium laurylsarcosine) supplemented with 20 mg/L of proteinase K. Plugs were washed with Tris-EDTA buffer containing 1 mmol/L phenylmethylsulfonyl fluoride (Sigma, St. Louis, MO, USA) and 3× with Tris-EDTA buffer at room temperature.

Whole-cell DNA of A. baumannii isolates was digested with ApaI overnight at room temperature (New England Biolabs, St. Quentin-en-Yvelines, France). Electrophoresis was performed on a 1% agarose gel with 0.5× Tris-borate-EDTA buffer by using a CHEF DRII apparatus (Bio-Rad). Samples were subjected to electrophoresis at 14°C, 6 volts/cm, and a switch angle with 1 linear switch ramp of 3–8 s for 10.5 h, and then for 12–20 s for 10.5 h.

Identification of PCR-based sequence groups was conducted by using 2 multiplex PCR assays designed to selectively amplify group 1 or group 2 alleles of the gene encoding outer-membrane protein A (ompA), the gene encoding part of a pilus assembly system required for biofilm formation (csuE), and the gene encoding the intrinsic carbapenemase gene of A. baumannii (bla\textsubscript{OXA-23}) (18). Clonal relationships were established by multilocus sequence typing (MLST) by using 7 standard housekeeping loci (citrate synthase [gltA], gyrase B [gvrB], glucose dehydrogenase B [gdhB], recombination A [recA], chaperone 60 [cpn60], glucose-6-phosphate isomerase [gpi], and RNA polymerase [rpoD]) as described (18). Sequencing of internal fragments was performed by using BigDye fluorescent terminators and primers described (19). Sequences were compared with the A. baumannii database at the MLST Website (http://mlst.zoo.ox.ac.uk). To supplement epidemiologic results, we performed a second MLST typing using the scheme developed by Nemec et al. (20). Sequences of the 7 housekeeping genes were analyzed by using an A. baumannii database (www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html).

**Southern Blot Analysis and Location of bla\textsubscript{OXA-23} Gene**

Southern blot analysis was performed by using total genomic DNA digested with EcoRI, separated by electro-
Carbapenemase Gene of A. baumannii

Cloning Experiments
To identify entire transposon structures containing the bla\textsubscript{OXA-23} gene in different isolates and determine their location in the target DNA, a cloning procedure was used. Some data had been reported for 6 of 20 isolates (11). Total DNA was digested with either Sac\textsubscript{i} or Sal\textsubscript{i}, ligated into the Sac\textsubscript{i} or Sal\textsubscript{i} sites of plasmid pBK-CMV (kanamycin-resistant cloning vector), and the recombinant plasmids were transformed into Escherichia coli TOP10, as described (14). Recombinant plasmids were selected on tetracycline soy agar plates containing ticarcillin (50 mg/L) and rifampin (50 mg/L).

Results
Clonal Relatedness of the Isolates
Twenty carbapenem-resistant A. baumannii isolates were obtained from 15 countries (Table). All isolates were highly resistant to ticarcillin (MIC >256 mg/L) and showed a high level of resistance to ceftazidime (MIC >256 mg/L), except isolates Ab14 (MIC 4 mg/L) 861 and DOS (MIC 8 mg/L). All isolates were resistant to imipenem and meropenem (MIC ≥16 mg/L) (Table).

Multiplex PCR for identification of sequence groups showed 10 isolates that belonged to group 1 according to Turton et al. (18), eight that belonged to group 2, and 2 isolates that did not belong to groups 1 or 2. The 10 isolates that belonged to group 1 and corresponded to European clone II (18) were classified into 2 sequence types (STs), ST22 and ST53, according to MLST analysis (18). ST22 (1–3–3–2–2–7–3) was the most frequent type identified. Nine isolates were identified: 2 from France and 1 each from Vietnam, New Caledonia, Thailand, Australia, Ta-hiti, Reunion, and South Africa. A single European clone II isolate was classified as ST53 (1–3–3–2–2–3), a single-locus variant of ST22. Among 10 other isolates, 8 belonged to group 2 (corresponding to European clone I). Four STs were identified: ST25 (10–12–4–11–1–9–5) (Libya, United Arab Emirates, and Bahrain), ST44 (10–12–4–11–4–9–5) (United Arab Emirates and Algeria), and 2 new STs, 1 for isolates from Reunion and Egypt (10–12–4–11–4–16–5) and another related ST identified in the single isolate from Belgium (10–12–4–11–4–4–5). These 4 STs differ by 1 locus. The 2 most recent isolates from France and Brazil did not belong to European clones I or II and corresponded to 2 STs (1–22–3–11–1–9–7 and 12–18–12–1–15–9–19, respectively) (Table). Although 8 STs were identified in this collection, 9 pulsotypes were characterized by PFGE according to the criteria of Tenover et al. (23) (Figure 2).

According to MLST analysis developed by Nemec et al. (20), all isolates that belonged to European clone II had the same sequence type (ST2) (2,2-2,2,2-2,2), including isolate 585, which had a distinct but related ST in the first analysis. Among isolates that belonged to European clone I, two sequence types were determined: ST20 (3–1–1,1–5–1–1) (Libya, United Arab Emirates, Bahrain) and ST1 (1,1,1–5–1–1) (United Arab Emirates, Reunion, Egypt, Belgium, Algeria). Isolates 910 (Reunion), 861 (Egypt), and BEL (Belgium) were included in ST1. These isolates had a distinct ST according to methods of Bartual et al. (19). The 2 most recent isolates were classified into 2 STs, a new ST (3–2,2,2,5–4–8) for isolate DOS (France) and ST15 (6,6–8–2–3–5–4) for isolate 877 (Brazil) (Table).

Location and Transferability of the bla\textsubscript{OXA-23} Gene
Location of the bla\textsubscript{OXA-23} gene was evaluated by using the I-CeuI method. Eleven isolates had the bla\textsubscript{OXA-23} gene on the chromosome, with a hybridization signal for an ≈40-kb band for isolate AS1 and an ≈200-kb band for 10 isolates (Table). Nine isolates carried the bla\textsubscript{OXA-23} gene on a plasmid and 1 isolate had 2 copies of the bla\textsubscript{OXA-23} gene, 1 on the chromosome and 1 on a 7-kb plasmid (Table).

To examine the copy number of the bla\textsubscript{OXA-23} gene in different A. baumannii genomes, we performed Southern blot hybridization on EcoRI-digested DNA fragments using a 589-bp DNA probe specific for the bla\textsubscript{OXA-23} gene. Sixteen isolates showed only 1 copy of the bla\textsubscript{OXA-23} gene. Isolates BEL, Ab14, and DOS had 2 copies of the bla\textsubscript{OXA-23} gene on different plasmids, and Ab13 had 1 copy on the chromosome and 1 copy on a plasmid according to results of the I-CeuI technique.

Mating-out assays were performed by using the 10 plasmid-positive strains as donor strains and rifampin-resistant A. baumannii BM4547 as the recipient strain. Five transconjugants were obtained; all had a 130-kb plasmid that did not provide additional antimicrobial drug resistance.
to the *A. baumannii* recipient strain, except in 1 case (co-resistance to kanamycin and amikacin on a *bla*<sub>OXA-23</sub>-carrying plasmid that originated from isolate 1190). Plasmids carrying the *bla*<sub>OXA-23</sub> gene in isolates Ab14, DOS, BEL, and 877 were not self-transferable (Table) (24).

### Variability of Genetic Structures Flanking the *bla*<sub>OXA-23</sub> Gene

The 10 isolates that belonged to European clone II had a *bla*<sub>OXA-23</sub> gene that was part of *Tn2006*. The 9-bp direct repeat (DR) that corresponded to duplication of the *Tn2006* target site, which was consistent with a transposition event, was identified in the 9 ST22/ST2 isolates. *Tn2006* was inserted in different locations on the chromosomes of those isolates (Table). For isolates 240, 512, 810, 859, 883, and Aus, the insertion occurred between 2 genes encoding hypothetical proteins (DR: GTCATTAAA) (Figure 1). In isolate 761, transposition *Tn2006* was located between a gene encoding a hypothetical protein and a gene encoding an isoleucyl tRNA synthase (DR: ATTCGCGGG). The plasmid-borne *OXA-23* gene that was part of *Tn2006* was inserted between 2 genes encoding hypothetical proteins (DR: ATTCGCGGG). The plasmid-borne *OXA-23*–carrying transposon *Tn2006* was located between a gene encoding a cytochrome D terminale oxidase and a putative transposase (*Tn2006*). In isolate 585, *Tn2006* was inserted into the reading frame in 2 isolates (BEL and Ab14) (Figure 2).

#### Table. Characteristics of 20 *bla*<sub>OXA-23</sub>–Positive Acinetobacter baumannii Clinical Isolates†

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Date of isolation</th>
<th>Specimen</th>
<th>EC</th>
<th>ST†</th>
<th>Copy no. of <em>bla</em>&lt;sub&gt;OXA-23&lt;/sub&gt;</th>
<th>Genetic location and size, kb</th>
<th>Genetic structure</th>
<th>MIC, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>240</td>
<td>France</td>
<td>2003 Dec</td>
<td>Tracheal</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>512</td>
<td>Tahiti</td>
<td>2004 Mar</td>
<td>Tracheal</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>761</td>
<td>Vietnam</td>
<td>2005 May</td>
<td>Bile</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>810</td>
<td>New Caledonia</td>
<td>2004 Jun</td>
<td>Blood</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>863</td>
<td>Thailand</td>
<td>2006 Jun</td>
<td>Urine</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>883</td>
<td>Reunion</td>
<td>2006 Jun</td>
<td>Unknown</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>Ab13</td>
<td>France</td>
<td>2004 Jun</td>
<td>Urine</td>
<td>II</td>
<td>22/2</td>
<td>2</td>
<td>Chromosome, 200† and plasmid, 70</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>AUS</td>
<td>Australia</td>
<td>2004 Oct</td>
<td>Urine</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>859</td>
<td>South Africa</td>
<td>2006 Jan</td>
<td>Urine</td>
<td>II</td>
<td>22/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>585</td>
<td>France</td>
<td>2004 Jul</td>
<td>Tracheal</td>
<td>II</td>
<td>53/2</td>
<td>1</td>
<td>Chromosome, 200†</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>614</td>
<td>Libya</td>
<td>2004 Oct</td>
<td>Unknown</td>
<td>I</td>
<td>25/20</td>
<td>1</td>
<td>Plasmid, 130</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>AS3</td>
<td>UAE†</td>
<td>2006 Oct</td>
<td>Blood</td>
<td>I</td>
<td>25/20</td>
<td>1</td>
<td>Plasmid, 130</td>
<td>IS<em>Ab</em>1</td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>1190</td>
<td>Bahrain</td>
<td>2008 Mar</td>
<td>Blood</td>
<td>I</td>
<td>41/1</td>
<td>1</td>
<td>Plasmid, 130</td>
<td>IS<em>Ab</em>1</td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>AS1</td>
<td>UAE</td>
<td>2006 Jul</td>
<td>Blood</td>
<td>I</td>
<td>44/1</td>
<td>1</td>
<td>Chromosome, 40‡</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>Ab14</td>
<td>Algeria</td>
<td>2004 Dec</td>
<td>Unknown</td>
<td>I</td>
<td>44/1</td>
<td>2</td>
<td>Plasmid, 25, and plasmid, &gt;150</td>
<td><em>Tn2007</em></td>
<td>4 &gt;16 &gt;32</td>
</tr>
<tr>
<td>910</td>
<td>Reunion</td>
<td>2006 Oct</td>
<td>Unknown</td>
<td>I</td>
<td>New1/1</td>
<td>1</td>
<td>Plasmid, 130</td>
<td><em>Tn2006</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>861</td>
<td>Egypt</td>
<td>2005 Nov</td>
<td>Sputum</td>
<td>I</td>
<td>New1/1</td>
<td>1</td>
<td>Plasmid, 130</td>
<td>IS<em>Ab</em>1</td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
<tr>
<td>BEL</td>
<td>Belgium</td>
<td>2007 Jul</td>
<td>Respiratory tract</td>
<td>New2/1</td>
<td>1</td>
<td>2</td>
<td>Plasmid, 25, and plasmid, &gt;150</td>
<td><em>Tn2007</em></td>
<td>&gt;32 &gt;32 &gt;32</td>
</tr>
</tbody>
</table>

*EC, European clone; ST, sequence type; UAE, United Arab Emirates; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem. The MIC for ticarcillin was >256 μg/mL for all 20 isolates.*

†ST determined by Bartual et al. (19) compared with ST determined by Nemec et al. (20).

‡Size of chromosome band carrying the *bla*<sub>OXA-23</sub> gene, as determined by using the l-Ceut technique.
Only 1 copy of ISAbal was identified upstream of the blaOXA-23 gene in isolates AS3, 1190, 861, and 877. Transposon Tn2008 was identified only in isolate 614 (Figure 1). Sequences of these specific genetic structures have been deposited in Genbank (accession nos. EF127491, EF059914, GQ861438, and GQ861439).

**Discussion**

This study was conducted to define which features may explain the worldwide dissemination of the blaOXA-23 gene in A. baumannii. Isolates were from the Middle East, Europe, and Asia; there were no isolates from North America. Except for 2 isolates, the isolates investigated in this study belonged to European clones I or II. Clustering of A. baumannii isolates was determined by MLST and PFGE; our collection was composed of 13 PFGE types, European clone types, and multilocus sequence typing (MLST) results are shown. *ST, sequence type determined by Bartual et al. (19) compared with ST determined by Nemec et al. (20). Lane M, molecular size markers (48.5 kb).

**References**


**Acknowledgment**

We thank Rémy Bonnin for technical assistance.

This study was supported by a grant from the Ministère de la Recherche, Université Paris XI, Paris, France; grants from the European Community (DRESP2, LSHM-CT-2005-01705, and TROCAR HEALTH-F3-2008-223031); and the Institut National de la Santé et de la Recherche Médicale, France.

Ms Mugnier is a doctoral student at Hôpital de Bicêtre, Institut National et de la Santé et de la Recherche Médicale, Unité, South-Paris Medical School, University Paris-XI in Le Kremlin-Bicêtre, France. Her research interests are genetic plasticity and drug-resistance mechanisms in A. baumannii.


Address for correspondence: Patrice Nordmann, Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 Rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France; email: nordmann.patrice@bct.ap-hop-paris.fr