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Outbreak of Carbapenem-Resistant *Acinetobacter baumannii* Producing the Carbapenemase OXA-23 in a Tertiary Care Hospital of Papeete, French Polynesia

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Carbapenem-resistant *Acinetobacter baumannii* isolates were obtained from 24 patients between March and May 2004 at the Centre Hospitalier de Polynésie Française, Tahiti, French Polynesia. The isolates were multidrug resistant, produced the carbapenemase OXA-23, and belonged to a single clone presenting several subtypes, suggesting an endemic situation. This study further illustrates the global spread of this kind of β-lactamase-mediated resistance.

Imipenem and meropenem are among the drugs of choice used to treat nosocomial infections due to multidrug-resistant *Acinetobacter baumannii* isolates. However, their efficacy is being increasingly compromised by the emergence of carbapenem-hydrolyzing β-lactamases of molecular Ambler class B (metalloenzymes) and D enzymes (oxacillinases) (1, 2, 3, 12, 17). Whereas the metalloenzymes are of IMP and VIM types, the carbapenem-hydrolyzing oxacillinases are members of three subgroups of enzymes: the OXA-23, OXA-24, and OXA-58 enzymes (2, 5, 9, 15, 16, 17). Outbreaks of OXA-type carbapenemase-producing *A. baumannii* strains have been reported worldwide: OXA-24 in Madrid, Spain (4, 5); OXA-23 in Spain and in Curitiba, Brazil (2, 8); OXA-58 in Toulouse, France (11); and OXA-40 in Bilbao, Spain (13). The aim of this study was to analyze the molecular mechanism of carbapenem resistance in *A. baumannii* strains isolated at the Centre Hospitalier de Polynésie Française (CHPF), the main hospital of Papeete, Tahiti, French Polynesia, that is located in the middle of the Pacific Ocean.

From March to May 2004, 24 carbapenem-resistant *A. baumannii* isolates were isolated from 24 patients hospitalized at the CHPF, a 353-bed tertiary care hospital. The patients' ages ranged from 8 to 80 years (mean age, 54 years). Nineteen patients (80%) were colonized and 5 were infected (20%) according to the French national recommendations, which derived from those of the Centers for Disease Control and Prevention (7, 10). The sites of infection were urinary tract (2 patients), intravenous catheters (2 patients), and the respiratory tract (1 patient). The patients were scattered throughout the hospital, being mostly in the intensive care unit (41.7%), and the motives for hospital admission were diverse (Table 1). Seventy-seven percent of the patients had a bacterial infection prior to the isolation of *A. baumannii* isolates and received an appropriate antibiotic treatment. Thirty-three percent of the patients died after *A. baumannii* isolation, but the death rate was 26.3% with colonization and 60% when the patient was infected. Infection with multidrug-resistant *A. baumannii* was considered by the clinicians to have contributed to the death of patient 3, despite amikacin and colistin treatment. The four other infected patients were cured from their infection without treatment. Two of them died from their underlying disease (patients 2 and 10; Table 1).

Isolates were identified using the API 32GN system (BioMérieux, Marcy l'Etoile, France). Pulsed-field gel electrophoresis (PFGE) was performed with ApaI-restricted whole-cell DNAs embedded in 1% agarose plugs and separated in a 1% pulsed field-certified agarose gel using a contour-clamped homogeneous electric field DRII system (Bio-Rad, Marnes-La-Coquette, France), as previously described (11). Routine antibiograms were determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) and interpreted as recommended by the Clinical and Laboratory Standards Institute (6). MICs of carbapenems were determined with E-tests (AB BIODISK, Solna, Sweden) performed on Mueller-Hinton agar plates (Oxoid, Basingstoke, United Kingdom) with incubation at 37°C for 24 h. DNA extractions (genomic and plasmid) and analysis, isoelectric focusing, and conjugation assays with rifampin-resistant *A. baumannii* strain CIP 7020 were performed as described previously (11).

Genes coding for Ambler class B and D carbapenemases were sought by PCR using primers specific for the bla_{IMP} (19), bla_{VIM} (19), $bla_{\text{OXA-23}}$-like (9), $bla_{\text{OXA-26}}$-like (2), and $bla_{\text{OXA-58}}$ genes (16). Similarly, the class A β-lactamase bla_{TEM} gene and the chromosomal class C β-lactamase bla_{AMPC} gene were sought by PCR (11, 18). The presence of IS*AbaI* inserted upstream of a bla_{AMPC}-β-lactamase gene was sought by PCR as previously described (14, 18). PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Courtaboeuf, France) and sequenced on both strands with an automated sequencer (ABI 3100; Applied Biosystems, Foster City, Calif.). The nucleotide and deduced amino acid sequences were analyzed with software available over the Internet (http://www.ncbi.nlm.nih.gov/).

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TABLE 1. Case history of patients with OXA-23-producing *A. baumannii*[e]

Isolate no.	Date of hospitalization (mo/day/yr)	Age (yr)	Sex	Ward	Date of isolation (mo/day/yr)	Underlying condition	Site of isolation	Diagnosis[a]	Treatment[b]	Outcome[c]	PFGE pulsotype[f]
1	03/03/04	44	F	Gastroenterology	03/22/04	Heart failure	Rectal swab	C			A_1
2	03/05/04	58	M	Cardiology ICU	03/22/04	Heart failure	Central catheter	Infection			A_6
3	02/15/04	45	F	ICU	04/10/04	Asthma	Tracheal aspiration	Infection	AM, CS	Deceased	A_1
4	03/28/04	50	M	ICU	04/13/04	Respiratory failure	Rectal swab	C			A_6
5	02/09/04	62	M	Internal medicine	04/14/04	Paraplegia	Rectal swab	C			A_6
6	04/10/04	22	M	ICU	04/20/04	Polytraumatism	Rectal swab	C			A_6
7	03/31/04	39	M	Surgery	04/19/04	Arthritis	Rectal swab	C			A_6
8	04/08/04	79	M	Internal medicine	04/20/04	Cerebral vascular	Urinary	Infection			A_1
9	04/06/04	35	F	Endocrinology	04/22/04	Diabetes	Urinary	Infection			A_5
10	03/23/04	68	F	ICU	04/22/04	Heart failure	Catheter	Infection			A_4
11	03/28/04	61	M	ICU	04/23/04	Tetraplegia	Catheter	C			A_4
12	08/30/03	58	M	Internal medicine	04/23/04	Tetraplegia	Rectal swab	C			A_3
13	04/03/04	76	M	Internal medicine	04/26/04	Erysipelas	Rectal swab	C			A_3
14	04/20/04	30	M	ICU	04/27/04	Respiratory failure	Rectal swab	C			A_4
15	03/16/04	20	M	Gastroenterology	05/02/04	Leptospirosis	Rectal swab	C			A_1
16	04/16/04	50	F	ICU	05/04/04	Meningitis	Rectal swab	C			A_5
17	04/28/04	43	F	Emergency	05/04/04	Erysipela	Rectal swab	C			A_1
18	05/02/04	8	F	Surgery	05/10/04	Polytraumatism	Rectal swab	C			A_5
19	05/05/04	80	M	ICU	05/11/04	Cellulitis	Rectal swab	C			A_2
20	03/14/04	77	M	Internal medicine	05/12/04	Cellulitis	Rectal swab	C			A_1
21	04/13/04	67	M	Pneumology	05/14/04	Leukemia	BAF[d]	C			A_3
22	05/01/04	66	M	Surgery	05/18/04	Digestive bleeding	Rectal swab	C			A_1
23	04/21/04	76	M	ICU	05/19/04	Respiratory failure	Central catheter	C			A_3
24	05/09/04	59	M	ICU	06/01/04	Epilepsy	Urinary catheter	C			A_3

[a] Infection or colonization (C) status was established based on clinical data.
[b] Only patients that received antibiotic treatment for their *A. baumannii* infections are shown. The treatment consisted of amikacin (AM) and colymicine (CS).
[c] Unless-specified, the outcome was favorable with respect to the *A. baumannii* infection.
[d] BAF, bronchoalveolar fluid.
[e] All isolates were imipenem resistant and positive for bla_{OXA-23} by PCR. ICU, intensive care unit.
[f] Pulsotypes are according to Tenover et al. (20).

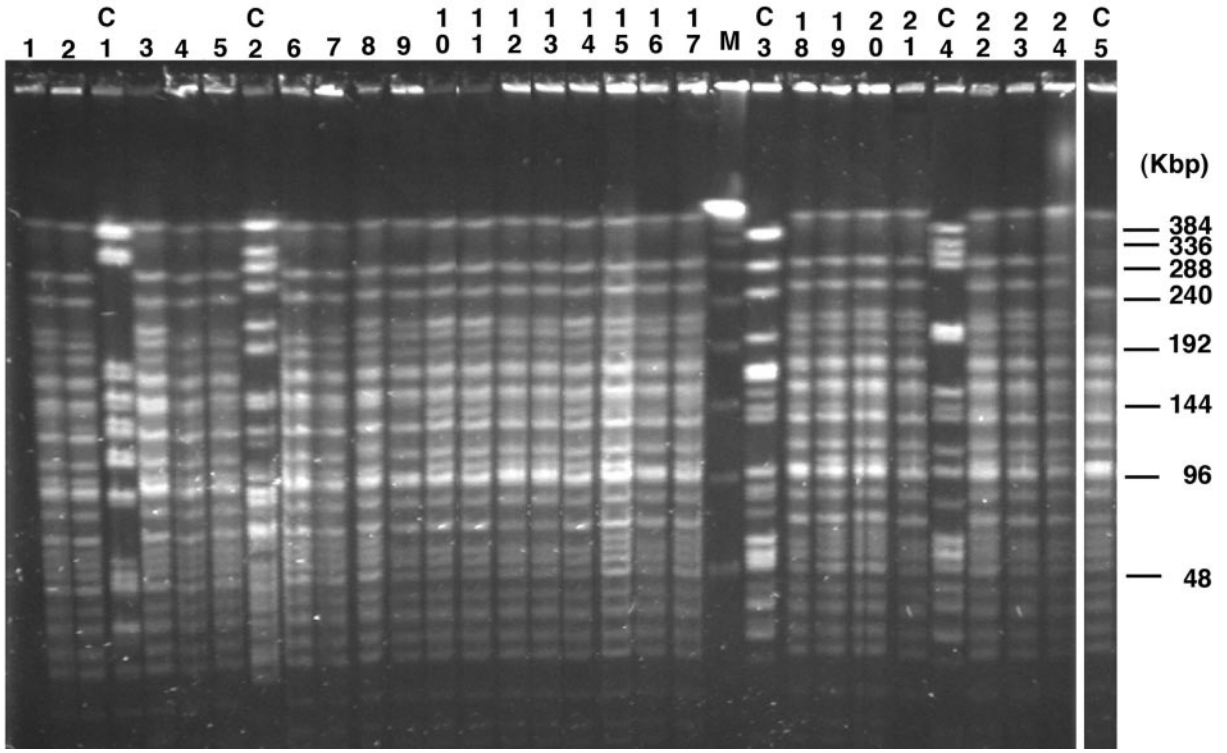

FIG. 1. PFGE patterns of *A. baumannii* isolates. The assigned numbers of *A. baumannii* isolates are shown over the lanes of the gel and correspond to those of Table 1. The positions of molecular size markers in kilobases (M) are shown on the right side of the gel. Lanes C1, C2, C3, and C4 correspond to carbapenem-susceptible strains isolated during the study period. Lane C5 corresponds to *A. baumanii* strain C5, which was isolated in Montpellier in 1999 from a patient directly transferred from Papeete.

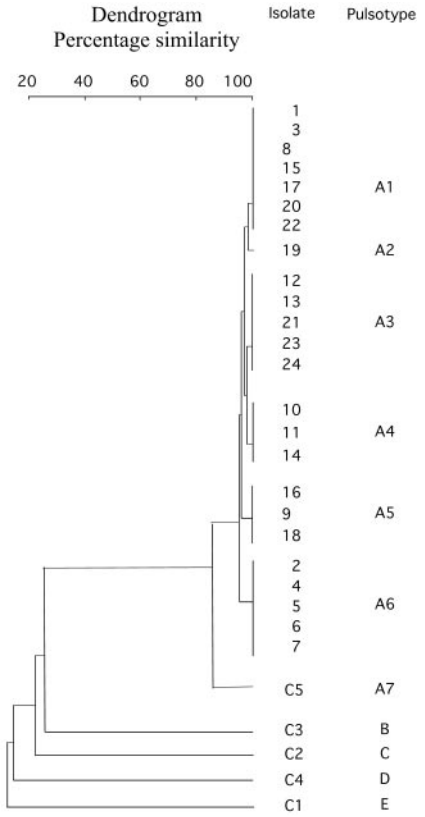

FIG. 2. Dendrogram obtained after digitization of the PFGE gel. ApaI macrorestriction patterns were digitized and analyzed using Taxotron software (Institut Pasteur, Paris, France) to calculate Dice coefficients of correlation and to generate a dendrogram by the unweighted pair group method using arithmetic averages clustering. The scale indicates the level of pattern similarity. Capital letters on the right side indicate macrorestriction types based on visual interpretation of PFGE results according to the criteria of Tenover et al. (20).

The 24 *A. baumannii* isolates were resistant to all β-lactams, including carbapenems (with imipenem and meropenem MICs of >32 μg/ml), ciprofloxacin, fosfomycin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole. The isolates remained susceptible to colistin (MIC, 0.5 μg/ml), rifampin (MIC, 1 μg/ml), and to aminoglycosides, except isolates 3, 4, 6, and 7, which were resistant to gentamicin and netilmicin.

The *A. baumannii* isolates were positive for $bla_{\text{OXA-23}}$-like, bla_{TEM}-like, and the natural and chromosomally located bla_{AMPC} genes. Sequencing of the amplified fragments confirmed the presence of $bla_{\text{OXA-23}}$, $bla_{\text{TEM-1}}$, and bla_{AMPC} genes. As previously described, the $bla_{\text{OXA-23}}$ gene was not embedded in a class 1 integron (9, 17, and data not shown). The genetic environment was similar to that of the prototype $bla_{\text{OXA-23}}$ gene, with insertion sequence IS*abaI* inserted upstream of this gene, as revealed by PCR analysis (9, 14, 18).

Isoelectric focusing confirmed that in addition to OXA-23 (pI 6.9), the chromosomal class C β-lactamase (pI >9.0) and TEM-1 (pI 5.4) were also expressed (data not shown).

Plasmid analysis revealed a 60-kb plasmid in all strains that was mobilizable to rifampin-resistant *A. baumannii* CIP 7020 (frequency of transfer, 10^{-6}). This plasmid expressed only β-lactamase OXA-23, and no additional resistance marker was identified according to results of routine antibiograms performed with the transconjugants and testing the same antibiotics as for the parental strains. The isolates from the different wards of the hospital gave similar PFGE patterns (Fig. 1), differing by a maximum of three bands. Six different subtypes were observed, suggesting clonal dissemination of the isolates (Fig. 2). The four carbapenem-susceptible control isolates from the same hospital differed from the carbapenem-resistant strain according to their PFGE patterns (Fig. 1 and 2).

Carbapenem-resistant *A. baumannii* strains have been isolated in Europe, Asia, and North and South America (1, 2, 4, 5, 8, 13, 16). Outbreaks of OXA-23-producing *A. baumannii* isolates have also been reported in Spain and in Brazil (2, 4, 8). Our study identified OXA-23-positive *A. baumannii* in Tahiti, a remotely located island in the Pacific Ocean, further illus-

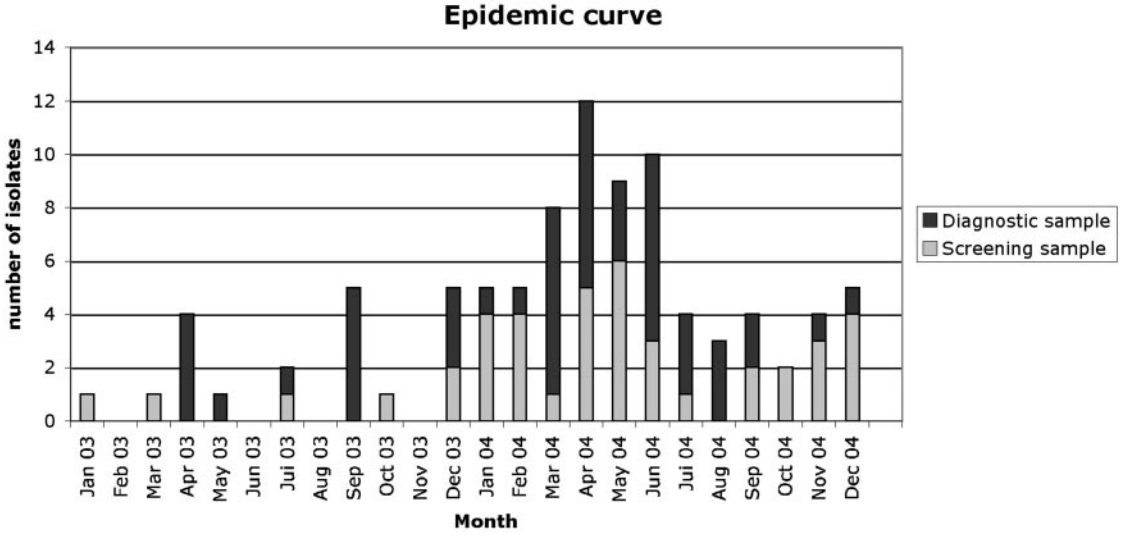

FIG. 3. Epidemic curve of the outbreak.

trating the global spread of this broad-spectrum β-lactamase. The genetic variability observed among the OXA-23-positive *A. baumannii* isolates (six pulsotypes) suggested that these isolates may have been present earlier in the hospital and thus reflect an endemic situation. Indeed, retrospective analysis of a bacteriology database based on antibiotic resistance susceptibility patterns revealed that the index case had been isolated in January 2003, with several sporadic cases until December 2003, when the outbreak started (Fig. 3). Interestingly, in 1999, *A. baumannii* strain C5 was identified at the university hospital of Montpellier (in southern France) from a French tourist previously hospitalized in a private hospital in Papeete. This strain had an identical antibiotic susceptibility profile and a similar PFGE pattern (Fig. 1 and 2) and was OXA-23 positive. These data suggested that this strain could have been present for a much longer period of time on the island.

After May 2004, the epidemic strain was still isolated in screening samples and in clinical specimens (Fig. 3), but implementation of infection control measures (isolation precautions, chlorhexidine hand washing, and barrier protections) and thorough biodecontamination of the rooms of colonized patients led to control of the outbreak but did not eradicate the epidemic strain (Fig. 3). The persistence of this strain is probably a consequence of frequent rehospitalization of colonized patients in the different wards. Indeed, the CHPF is the main hospital of the French Polynesian islands, dealing with acute care patients but also with long-term-care patients. The closest larger hospital is in Auckland, New Zealand, which is 2,600 miles away from Tahiti, thus limiting patient exchange. Overcoming the combination of clonal spread, multidrug resistance, and long-term-care patients that remain carriers is a challenge for the effective control of nosocomial *A. baumannii* infections.

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