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RESEARCH NOTE

Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in New Caledonia

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ABSTRACT

Carbapenem-resistant *Acinetobacter baumannii* (CR-*Ab*) ranked third, with a frequency of 24.8%, among 202 strains of multidrug-resistant bacteria isolated from clinical samples in the main hospital of New Caledonia in 2004. All CR-*Ab* isolates were analysed by isoelectric focusing, conjugation, pulsed-field gel electrophoresis and PCR for the presence of carbapenemase genes. Fifty CR-*Ab* isolates produced carbapenemase OXA-23. The isolates belonged to a single clone presenting several subtypes, suggesting an endemic situation. This study further illustrates the widespread prevalence of carbapenemase OXA-23-producing CR-*Ab* isolates in the South Pacific.

Keywords *Acinetobacter baumannii*, carbapenemase, hospital cohort, OXA-23

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Acinetobacter baumannii has emerged worldwide as an important nosocomial pathogen, causing outbreaks particularly in intensive care units, in wards with patients who have serious underlying illness, and in warm countries [1]. Imipenem is among the drugs of choice for treatment of nosocomial infections due to multidrug-resistant (MDR) *A. baumannii* isolates. However, their efficacy is being increasingly compromised by the emergence of carbapenem-hydrolysing β-lactamases of molecular Ambler class B (VIM, IMP) and class D (OXA-23, OXA-58) [1].

The aim of this study was to analyse the molecular mechanisms of carbapenem resistance in *A. baumannii* and to evaluate carbapenem-resistant *A. baumannii* (CR-*Ab*) prevalence among all MDR strains isolated at the central hospital of Noumea (CHT), a 285-bed tertiary-referral centre.

Methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and all Gram-negative bacteria resistant to at least three agents from distinct classes of antibiotics, including extended-spectrum cephalosporin-resistant *Enterobacteriaceae*, ceftazidime-resistant *Pseudomonas aeruginosa*, CR-*Ab*, ceftazidime-resistant *Burkholderia* spp., and clavulanate–ticarcillin-resistant *Stenotrophomonas maltophilia*, were considered to be MDR. The study was conducted during the entire year 2004 in the CHT. During that period, 15 320 patients were admitted. All MDR bacteria included were from clinical samples. Colonization and environmental samples were excluded. Only the first sample was analysed for patients who had more than one MDR bacterial episode involving the same strain during the year of the study. The incidence of MDR bacteria acquisition is expressed as the number of MDR bacteria acquisitions per 1000 patient-days in the hospital.

Standard bacteriological techniques and an automated BacT/Alert system (bioMérieux, Marcy l'Etoile, France) were used to culture bacteria. Bacterial isolates were initially identified by various routine microbiological methods [2]. Identification of *A. baumannii* isolates was confirmed by the detection and sequencing of the intrinsic $bla_{oxa\text{-}51}$-like gene [3]. Susceptibility of the isolates to common antibacterial agents was

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tested using the disk diffusion method, according to CLSI guidelines [4] and the British Society for Antimicrobial Chemotherapy breakpoint for colistin (http://www.bsac.org.uk). MICs of imipenem, meropenem, ceftriaxone, ceftazidime, ampicillin–sulbactam, colistin, rifampicin and tigecycline were determined with the Etest method using Mueller–Hinton agar (Oxoid) according to the manufacturer's recommendations.

Carbapenem-resistant and carbapenem-susceptible *A. baumannii* isolates were typed by macrorestriction analysis of *Apa*I-digested chromosomal DNA separated by pulsed-field gel electrophoresis (PFGE), using a CHEF-DR III apparatus (Biorad) with pulses ranging from 5 to 35 s at a voltage of 6 V/cm at 14°C for 32 h. The criteria used for PFGE pattern interpretation to define epidemiological relatedness were as described by Tenover *et al.* [5]. DNA extraction (genomic and plasmid) and analysis, isoelectric focusing and conjugation assays with rifampicin-resistant *A. baumannii* strain CIP 7020 were performed as described previously [6]. Genes coding for Ambler class B and D carbapenemases were sought by PCR using primers specific for bla_{IMP} [7], bla_{VIM} [8], $bla_{\mathrm{OXA\text{-}58}}$ [9], $bla_{\mathrm{OXA\text{-}23}}$-like [9], $bla_{\mathrm{OXA\text{-}40}}$-like [9] and $bla_{\mathrm{OXA\text{-}51/69}}$-like genes [3]. Similarly, the β-lactamase bla_{TEM} and bla_{AMPC} genes, along with IS*AbaI*, which may be inserted upstream of several β-lactamase genes, were sought by PCR [10,11].

In total, 202 single clinical isolates of MDR bacteria were collected during the study period, representing a rate of 1.9 episodes per 1000 patient-days. The distribution of MDR bacteria isolated is shown in Table 1. CR-*Ab* (24.8%) ranked third behind expanded-spectrum cephalosporin-resistant *Enterobacteriaceae* (36.2%) and methicillin-resistant *S. aureus* (34%). The incidence rate of CR-*Ab* was 0.48 per 1000 patient-days in hospital, with higher rates in the intensive care unit (1.7) and in the respiratory ward (1.2).

Table 1. Multidrug-resistant (MDR) bacteria isolated in 2004

	No. of isolates (%)[a]	Incidence per 1000 patient-days
Expanded-spectrum cephalosporin-resistant *Enterobacteriaceae*	73 (36.2)	0.7
Methicillin-resistant *Staphylococcus aureus*	68 (33.5)	0.6
Imipenem-resistant *Acinetobacter baumannii*	50 (24.8)	0.48
Ceftazidime-resistant *Pseudomonas aeruginosa*	7 (3.5)	0.07
Ticarcillin–clavulanate-resistant *Stenotrophomonas maltophilia*	4 (2)	0.04
Vancomycin-resistant *Enterococcus* spp.	0	0
All MDR bacteria	202	1.9

[a]Percentage of total isolates.

All CR-*Ab* isolates were resistant to most agents tested: all β-lactams (including imipenem and meropenem), quinolones and aminoglycosides (except for amikacin and tobramycin, which remained active in 90% and 88%, respectively, of the cases). All CR-*Ab* isolates were susceptible to colistin. The MIC range in mg/L was as follows: 64 to >256 for ceftazidime, >256 for ceftriaxone, >32 for imipenem and meropenem, 0.5–1 for colistin and rifampicin, 12–24 for ampicillin–sulbactam, and 4 to >256 for tigecycline. CR-*Ab* represented 64% of all *A. baumannii* isolates obtained during the entire year 2004 (50/78 strains, data not shown).

Carbapenem-resistant and carbapenem-susceptible *A. baumannii* isolates were subjected to PFGE (Fig. 1). Imipenem-susceptible isolates displayed significant variability, and none appeared to be genetically related to carbapenem-resistant isolates, which clustered into a single PFGE type represented by two subtypes, A_1 and A_2. CR-*Ab* isolates were found in different wards, across all ethnic and age groups (data not shown), and were seen throughout 2004 at the CHT; moreover, the number of isolates did not follow a clear epidemic curve. The PFGE profile of CR-*Ab* strains differed from those of the reference strains expressing extended-spectrum β-lactamases, e.g. VEB-1 and PER-1, and from those of strains expressing carbapenem-hydrolysing oxacillinases, e.g. OXA-40 and OXA-58; however, the profiles were very similar to those of the French Polynesian *A. baumannii* OXA-23 Tah-1 clone [12].

The *A. baumannii* isolates tested positive by PCR for the acquired $bla_{\mathrm{OXA\text{-}23}}$-like and bla_{TEM}-like genes, and for the naturally occurring $bla_{\mathrm{OXA\text{-}51}}$-like and bla_{AMPC}-like genes. Sequencing of the amplified fragments confirmed the presence of $bla_{\mathrm{OXA\text{-}23}}$, $bla_{\mathrm{OXA\text{-}51}}$, $bla_{\mathrm{TEM\text{-}1}}$ and bla_{AMPC} genes, identical to those identified in *A. baumannii* OXA-23 Tah-1 [12]. PCR analysis revealed that $bla_{\mathrm{OXA\text{-}23}}$ was not embedded in a class 1 integron, but was surrounded by IS*AbaI* sequences in a manner similar to that of the prototype $bla_{\mathrm{OXA\text{-}23}}$ gene, as described by Corvec *et al.* [10]. Isoelectric focusing analysis confirmed that, in addition to OXA-23 (pI 6.9), the chromosomal class C β-lactamase (pI >9.0) and TEM-1 penicillinase (pI 5.4) were also expressed (data not shown). Plasmid analysis

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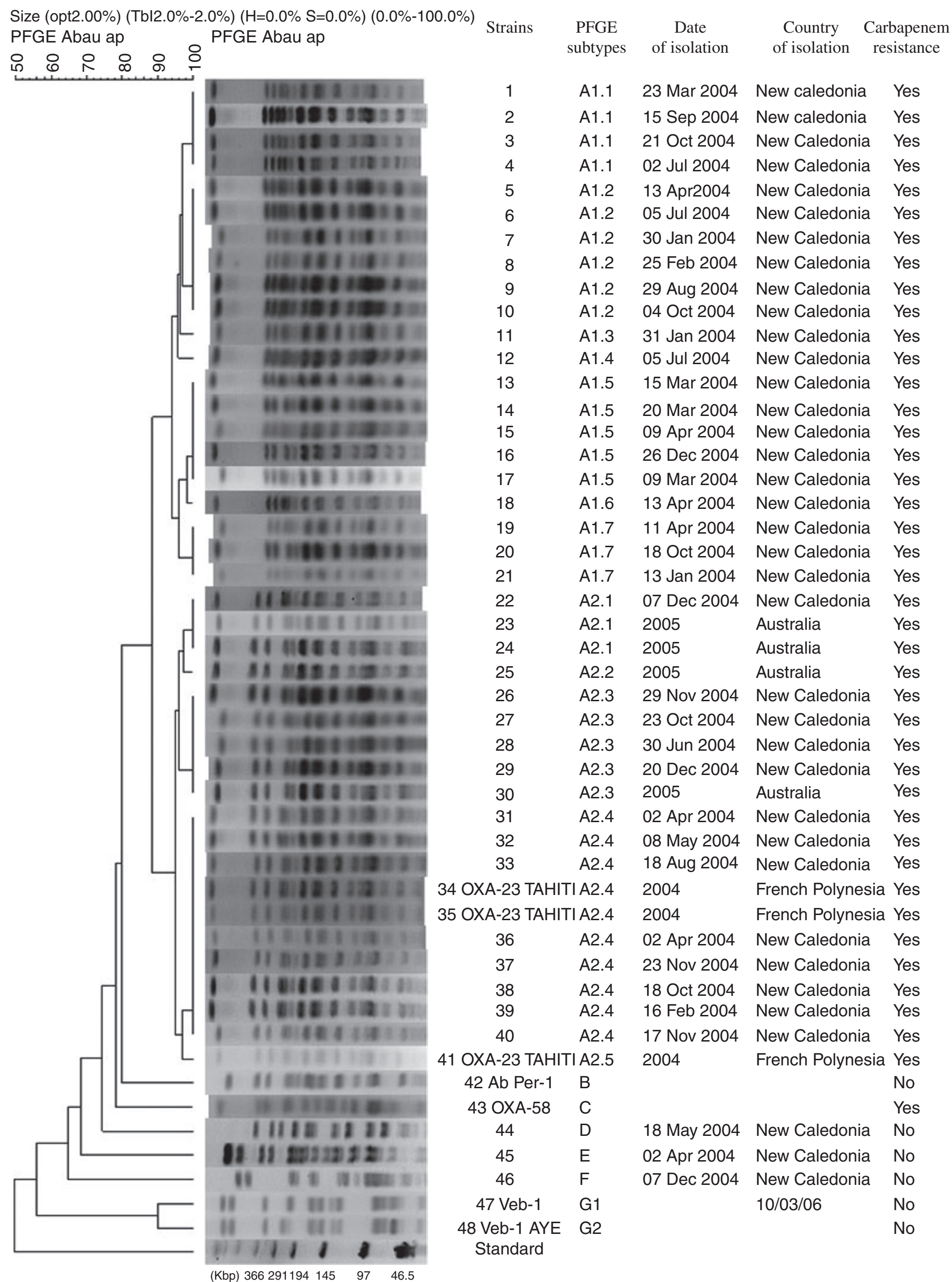

Fig. 1. Pulsed-field gel electrophoresis (PFGE) patterns of *Acinetobacter baumannii* isolates. The assigned numbers of *A. baumannii* isolates are shown on the left of the figure. The positions of molecular size markers in kilobases (standard) are shown under the last gel. Associated data with pulsotypes are shown on the right side of the gel. Lanes 41, 42, 46 and 47 correspond to reference strains supplied by Hôpital de Bicêtre, Paris, France: Ab Per-1 [17], Ab Oxa-58 [18], Ab Veb-1 and Ab Veb-1 AYE [2,19], respectively. Lane 28 corresponds to the *A. baumannii* strain carrying OXA-23 carbapenemase, which was isolated in Tahiti, French Polynesia, in 2004 [12]. Cluster analysis was performed by the unweighted pair group method with arithmetic averages (UPGMA), and the percentage relatedness was calculated using the band-based Dice coefficient with a tolerance setting of 1.5% band tolerance and 1.5% optimization. Only bands above 48 kb were considered for analysis. Isolates corresponding to an 87% clustering threshold were considered to belong to the PFGE pattern [20]. Gel images were analysed with BioNumerics version 4.5 software (Applied Maths, Kortrijk, Belgium).

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revealed a 60-kb plasmid, albeit inconsistently. If detected, it was transferred to rifampicin-resistant *A. baumannii* CIP 7020 (at a low frequency of transfer, 10^{-7}). This plasmid carries only the bla_{OXA-23} gene, *OXA-23*, without additional antibiotic resistance markers.

Outbreaks of OXA-23-producing *A. baumannii* isolates have been reported repeatedly in Europe, South America and Asia [12–16]. This study identified an OXA-23-positive *A. baumannii* clone with an alarmingly high frequency among all MDR bacteria in 2004. The frequency of isolation of CR-*Ab* in this study was as high as that of methicillin-resistant *S. aureus* and expanded-spectrum cephalosporin-resistant *Enterobacteriaceae*; this is unusual and worrying, as a prevalence of <1% has been described by the national French network (the Nosocomial Infection Alert, Investigation and Surveillance Network, http://www.invs.sante.fr/surveillance/raisin). CR-*Ab* isolation varies widely in different countries [13] and has been mostly sporadic. Interestingly, in New Caledonia, a retrospective analysis of our bacteriology database of antibiotic resistance susceptibility patterns revealed that this CR-*Ab* strain might have been present in the hospital since at least December 2001, and has now spread to all wards of the hospital. The recently described *Ab*OXA-23 strain Tah-1, responsible for an outbreak in French Polynesia [12], showed 100% identity in PFGE patterns and resistance gene characterization with some New Caledonian isolates. Tahiti, an island also located in the Pacific Ocean, but separated from New Caledonia by about 6000 km, illustrates the extent of the geographical distribution of this carbapenemase gene in the South Pacific region. Moreover, clinical strains isolated in 2005 in Westmead Hospital, Sydney (Australia), with an identical antibiotic susceptibility profile, displayed a similar PFGE pattern and expressed OXA-23 in a manner similar to the endemic CR-*Ab* strain (S. Le Hello, personal communication).

In conclusion, in New Caledonia, OXA-23-producing *A. baumannii* isolates represent an endemic lineage, which is present in neighbouring countries in the Pacific. These findings highlight the importance of being aware of local specificities in MDR bacterial ecology and resistance levels for initiating proper antimicrobial empirical therapy and for implementing effective infection control policies in a general hospital.

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TRANSPARENCY DECLARATION

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The authors declare that they have no conflict of interest in relation to this work.

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