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Identification of *Acinetobacter baumannii* by Detection of the $bla_{\text{OXA-51-like}}$ Carbapenemase Gene Intrinsic to This Species

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$bla_{\text{OXA-51-like}}$ was sought in clinical isolates of *Acinetobacter* species in a multiplex PCR, which also detects $bla_{\text{OXA-23-like}}$ and class 1 integrase genes. All isolates that gave a band for $bla_{\text{OXA-51-like}}$ identified as *A. baumannii*. This gene was detected in each of 141 isolates of *A. baumannii* but not in those of 22 other *Acinetobacter* species.

Acinetobacter baumannii is an increasingly important nosocomial pathogen that particularly affects critically ill patients. It is associated with multiple antibiotic resistance, and many widespread strains are resistant to almost all antibiotics currently in use (4, 7, 8, 9, 10). There is mounting evidence that *A. baumannii* has a naturally occurring carbapenemase gene intrinsic to this species (5, 11, 13). The first report of this gene described $bla_{\text{OXA-51}}$ (2), but since then a large number of closely related variants have been found (with OXA numbers 64, 65, 66, 67, 68, 69, 70, 71, 75, 76, 77, 83, 84, 86, 87, 88, 89, 91, 92, 94, and 95) (1, 5, 11), and we have referred to them collectively as "$bla_{\text{OXA-51-like}}$" genes. Carbapenem resistance has only been associated with these genes when the insertion sequence IS*Aba1* is upstream (11) and is not an indicator of whether an isolate has such a gene.

Although it is clear that $bla_{\text{OXA-51-like}}$ genes are present in at least the vast majority of isolates of *A. baumannii*, there has been some debate as to whether they are present in all isolates of this species (3). If they are consistently found and are also unique to this species, then their detection could provide a simple and convenient method of identifying *A. baumannii* which could more easily be carried out than current definitive methods, such as amplified rRNA gene restriction analysis (12) (ARDRA), and which would be more reliable than biochemical identification (e.g., by API), which is most commonly used. Since *A. baumannii* is clinically by far the most significant of the *Acinetobacter* species, the ability to distinguish it rapidly from other members of the genus would be highly valuable.

Here we describe the results of testing large numbers of well-characterized clinical *Acinetobacter* isolates for $bla_{\text{OXA-51-like}}$ genes by PCR with group-specific primers (13) and compare the results with those obtained by ARDRA. Detection of $bla_{\text{OXA-51-like}}$ can be carried out as part of a multiplex, and two such multiplexes (one detects all the known groups of OXA carbapenemase genes in *Acinetobacter* [13], and the second detects $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, and the class 1 integrase gene) are in current use in our laboratories; the class 1 integrase gene is a useful marker for outbreak strains of *A. baumannii* (6, 10).

All isolates of *Acinetobacter* received by the United Kingdom reference laboratories between November 2005 and March 2006 were included (170 isolates). Every isolate was compared with previous isolates by pulsed-field gel electrophoresis (PFGE) of ApaI-digested DNA, using BioNumerics software, as described previously (9, 10). The set included 64 isolates of OXA-23 clone 1 (the most prevalent genotype in the United Kingdom), 22 isolates of the South East clone, 18 isolates of the T strain, and 1 isolate each of the W strain (known to belong to European clone 1) and the "uncertain" strain. These outbreak strains have all been described previously and iden-

TABLE 1. Primers used in the multiplex PCR

Primer	Sequence	Target	Amplicon size (bp)	Reference
OXA-51-likeF	5′-TAA TGC TTT GAT CGG CCT TG-3′	$bla_{\text{OXA-51-like}}$	353	13
OXA-51-likeR	5′-TGG ATT GCA CTT CAT CTT GG-3′			
OXA-23-likeF	5′-GAT CGG ATT GGA GAA CCA GA-3′	$bla_{\text{OXA-23-like}}$	501	13
OXA-23-likeR	5′-ATT TCT GAC CGC ATT TCC AT-3′			
Int1F	5′-CAG TGG ACA TAA GCC TGT TC-3′	Class 1 integrase gene	160	6
Int1R	5′-CCC GAG GCA TAG ACT GTA-3′			

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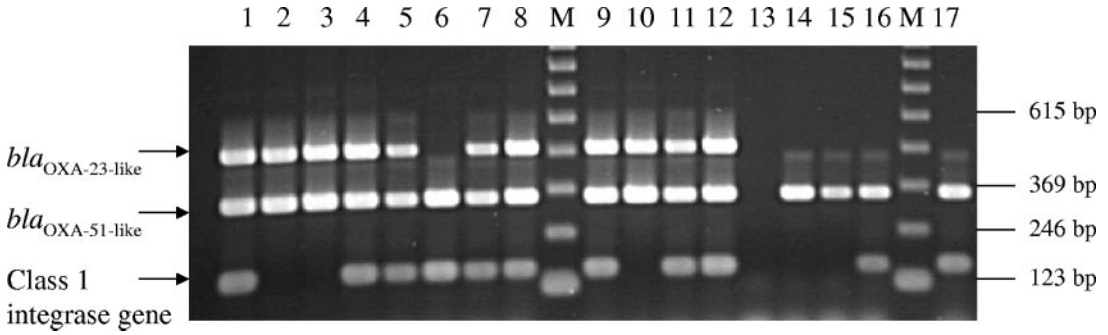

FIG. 1. Multiplex PCR results to amplify fragments of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-51-like}}$, and the class 1 integrase gene from *Acinetobacter* spp. Representatives of OXA-23 clone 1 (lanes 1, 4, 5, 7, 8, 9, 11, and 12), the SE clone (lanes 16 and 17), and strains A (lane 6), C (lanes 2 and 3), and D (lane 10) are included. Isolates in lanes 13, 14, and 15 were sporadic. All isolates except that in lane 13 identified as *A. baumannii* and had $bla_{\text{OXA-51-like}}$; the isolate in lane 13 was identified as *A. lwoffii* and lacked this gene. M, 123-bp ladder.

tified as *A. baumannii* (9, 10). The remaining isolates received consisted of sporadic strains (40 isolates) (defined by unique PFGE profiles and the absence of the class 1 integrase gene) and minor strains (24 isolates). Thirty-eight of these were further characterized by ARDRA (12).

All isolates were subjected to the multiplex PCR to detect $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, and class 1 integrase genes (Table 1). PCRs were carried out as previously described (10) in 25-μl reaction volumes with 3 μl of extracted DNA, 12.5 pmol of each primer, and 1.5 U of *Taq* DNA polymerase in 1× PCR buffer containing 1.5 mM $MgCl_2$ (QIAGEN) and 200 μM of each deoxynucleoside triphosphate. Conditions for the multiplex were the following: 94°C for 3 min, and then 35 cycles at 94°C for 45 s, at 57°C for 45 s, and at 72°C for 1 min, followed by a final extension at 72°C for 5 min. A single PCR (using only the OXA-51-like primers) was also used to seek $bla_{\text{OXA-51-like}}$ in representatives of *Acinetobacter* genomic species 1 to 17 (inclusive) as well as other *Acinetobacter* species. Conditions were the same, except that an annealing temperature of 60°C was used.

All 106 isolates of the main outbreak strains of *A. baumannii* were PCR positive for $bla_{\text{OXA-51-like}}$ genes (Fig. 1). However, it was the minor strains, most of which were negative for the class 1 integrase gene, and the sporadic strains, detailed in Table 2, which provided a greater challenge for this method, since these were the most diverse. Thirty-eight of these isolates (which included representatives of minor strains A to F) were investigated further (Table 3). All those that were positive for $bla_{\text{OXA-51-like}}$ (13 sporadic isolates and representatives of strains A to E) identified as *A. baumannii* by ARDRA. All those that were PCR negative for $bla_{\text{OXA-51-like}}$ (18 sporadic isolates and a representative of strain F) identified as other *Acinetobacter* species.

Although integrons are a good marker for outbreak strains of *A. baumannii*, increasingly we have found multiple representatives of $bla_{\text{OXA-23-like}}$-positive genotypes which are PCR negative for the class 1 integrase gene, such as strains B and C (Table 2). In today's climate of carbapenem therapy, the association between outbreak strains and integrons may break down among isolates with carbapenemase genes such as $bla_{\text{OXA-23-like}}$, which confer high level carbapenem resistance, and we will continue to monitor this.

$bla_{\text{OXA-51-like}}$ was also sought in reference isolates of *Acinetobacter* genomic species 1 to 17 and other *Acinetobacter* spe-

TABLE 2. PFGE and PCR results obtained on sporadic and minor outbreak strains

Strain designation by PFGE[a]	No. of isolates	No. of genotypes	No. with $bla_{\text{OXA-51-like}}$	Comment(s)
A	3	1	3	$bla_{\text{OXA-23-like}}$ negative, class 1 integrase positive
B	7	1	7	$bla_{\text{OXA-23-like}}$ positive, class 1 integrase negative
C	7	1	7	$bla_{\text{OXA-23-like}}$ positive, class 1 integrase negative
D	2	1	2	$bla_{\text{OXA-23-like}}$ positive, class 1 integrase negative
E	3	1	3	$bla_{\text{OXA-23-like}}$ and class 1 integrase negative
F	2	1	0	$bla_{\text{OXA-23-like}}$ and class 1 integrase negative
Sporadic	40	40	13[b]	All class 1 integrase negative

[a] Strains A, B, C, D, and E were all identified as *A. baumannii* by ARDRA, and strain F was identified as genomic species 10 (see Table 3).
[b] All identified as *A. baumannii* by ARDRA (see Table 3).

TABLE 3. Results of identification tests on isolates of sporadic and minor strains

ARDRA profile[a]					$bla_{\text{OXA-51-like}}$	Identification[c]	No. of isolates
HhaI[b]	AluI	MboI	RsaI	MspI			
1	1	1	2	3	Positive	*A. baumannii*	14[d]
1	1	1	2	1	Positive	*A. baumannii*	4[e]
1	1	1	2	1 + 3	Positive	*A. baumannii*	1[f]
2	1	NT[g]	1	3	Negative	3 or 13TU	9
2	1	NT	1	1	Negative	3 or 13TU	1
2	1	1	1	3	Negative	13TU	1
1	1	2	2	3	Negative	*A. junii*	1
1	4	1	2	2	Negative	*A. haemolyticus* or *A. johnsonii*	3
3	3	2	1	2	Negative	*A. lwoffii*	2
4	2	1	2	3	Negative	10	1[h]
1	2	1	2	2	Negative	15BJ	1

[a] Restriction profiles as defined by Vaneechoutte et al. (12).
[b] Isoschizomer of CfoI.
[c] Species name or genomic species number.
[d] Includes two representatives of strain B and a representative of strain E.
[e] Includes a representative of strain A and of strain C.
[f] Representative of strain D.
[g] NT, not tested.
[h] Representative of strain F.

TABLE 4. Results of the $bla_{OXA-51-like}$ PCR carried out on reference isolates of *Acinetobacter* species

Species and isolate	$bla_{OXA-51-like}$ PCR[a]
A. calcoaceticus, genomic species 1 (ATCC 23055)	Negative
A. baumannii, genomic species 2 (ATCC 19606)	Positive
Acinetobacter genomic species 3 (ATCC 19004)	Negative
A. haemolyticus, genomic species 4 (ATCC 17906)	Negative
A. junii, genomic species 5 (ATCC 17908)	Negative
Acinetobacter genomic species 6 (ATCC 17979)	Negative
A. johnsonii, genomic species 7 (ATCC 17909)	Negative
A. lwoffii, genomic species 8 (ATCC 15309)	Negative
Acinetobacter genomic species 9 (ATCC 9957)	Negative
Acinetobacter genomic species 10 (ATCC 17924)	Negative
Acinetobacter genomic species 11 (ATCC 11171)	Negative
A. radioresistens genomic species 12 (ATCC 43998)	Negative
Acinetobacter genomic species 13 (ATCC 17905)	Negative
Acinetobacter genomic species 13TU (ATCC 17903)	Negative
Acinetobacter genomic species 14 (Bouvet 382)	Negative
Acinetobacter genomic species 15 (Bouvet 79)	Negative
Acinetobacter genomic species 16 (ATCC 17988)	Negative
Acinetobacter genomic species 17 (Bouvet 942)	Negative
A. ursingii (LUH 3292 and LUH 3324)	Negative
A. schindleri (LUH 4595 and LUH 4765)	Negative
A. parvus (LUH 7036)	Negative
A. equinus (LUH 6214)	Negative
"*A. venetianus*" (LUH 7437)	Negative

[a] Identical results were obtained in both a single PCR and in the multiplex for this gene. All isolates were PCR negative for the other target genes in the multiplex (class 1 integrase gene and $bla_{OXA-23-like}$).

cies (Table 4). These included all those likely to be encountered clinically. Only the isolate of genomic species 2 (*A. baumannii*) (type strain ATCC 19606) gave a band. Clinical isolates of other gram-negative organisms tested (*Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Moraxella nonliquefaciens*) failed to give a band.

Many variants of bla_{OXA-51} have been described, and a possible limitation of this PCR is whether it can detect every variant. By design, it should detect all the variants in GenBank to date, with the possible exception of bla_{OXA-75} (there is a single base mismatch with the forward primer in bla_{OXA-75}). In the present study, all 141 isolates of *A. baumannii* found, representing 23 genotypes, gave a band in the $bla_{OXA-51-like}$ PCR, clearly suggesting that this PCR does detect these genes in all the isolates of *A. baumannii* we currently encounter, but we remain alert to the possibility of nondetection of some variants. A further potential problem is that these genes are sometimes associated with IS*Aba1* (11), which may render them mobile.

These results provide evidence that detection of $bla_{OXA-51-like}$ can be used as a simple and reliable way of identifying *A. baumannii*. We have found $bla_{OXA-51-like}$ in every isolate of *A. baumannii* we have investigated in both this and a previous study (11). Furthermore, it is present in the type strain, isolated decades ago. GenBank submissions describing variants are from isolates of *A. baumannii* from many different countries (France, Greece, Turkey, Spain, United Kingdom, South Africa, Hong Kong, Singapore, and Argentina) (e.g., DQ335566 and DQ445683) distributed over four continents (1), clearly suggesting $bla_{OXA-51-like}$ is ubiquitous in *A. baumannii*.

The combination of markers detected in the multiplex described provides powerful information not only on identification as *A. baumannii* but also on probable outbreak potential and allows, to some extent, prediction of likely genotype. For example, the vast majority of isolates of OXA-23 clone 1, the most prevalent genotype in the United Kingdom, give all three bands in this multiplex, while sporadic and more minor strains with $bla_{OXA-23-like}$ lack the class 1 integrase gene. Other common outbreak genotypes of *A. baumannii* are positive for the class 1 integrase gene but lack $bla_{OXA-23-like}$ (Fig. 1). Results from the multiplex can be obtained rapidly and should prove highly useful to clinicians and infection control staff.

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