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Genes Encoding OXA-134-Like Enzymes Are Found in *Acinetobacter lwoffii* and *A. schindleri* and Can Be Used for Identification

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***bla*$_{OXA-134}$ genes and variants were sought in 21 species of *Acinetobacter* and found in *A. lwoffii*, genomic species 9 (regarded as synonyms), and *A. schindleri*. Sequencing revealed a 9-bp deletion in the gene in the type strain of genomic species 9 (ATCC 9957) relative to the gene in the type strain of *A. lwoffii* (ATCC 15309). Primers based on the gene without the deletion gave specific amplification of 29 of 30 clinical isolates of *A. lwoffii*/genomic species 9.**

Species within the *Acinetobacter* genus are difficult to distinguish except by molecular methods. Hospital isolates are dominated by *A. baumannii*, which is usually resistant to most antibiotics and can relatively readily be identified by detection of the *bla*$_{OXA-51-like}$ intrinsic carbapenemase gene or by other PCR methods (3, 5, 14). However, it is clear that other species are also clinically important, with *rpoB* sequencing having been instrumental in providing a means of species identification facilitating assessment of the prevalence and clinical relevance of some of the less common species (6, 8, 15, 16). Among these, *A. lwoffii*/genomic species 9 (now considered synonyms for the same species) (10) is relatively commonly found from blood, sometimes as a result of contamination from the skin but also associated with bacteremia (12, 13), and a rapid method of detection would be useful. Recently, a carbapenem-hydrolyzing class D β-lactamase, OXA-134, has been described in *A. lwoffii*, providing a potential target for species-specific identification (4). In the original study, the gene and its variants (referred to as *bla*$_{OXA-134-like}$) were sought using flanking primers, but we chose to amplify a smaller, internal fragment so that detection could be combined with amplification of variable regions of the *rpoB* gene. This provides an internal control for *Acinetobacter* species in the PCR and affords the possibility of identifying the non-*lwoffii* isolates by sequencing the *rpoB* amplicon, if required.

We have used a similar approach for detecting *A. baumannii* for some time, with primers for amplification of the intrinsic *bla*$_{OXA-51-like}$ being combined with those for amplification of *rpoB*; primers for detection of acquired OXA and class 1 integrase genes are also included (Table 1) (14, 17). The PCR is carried out using the Qiagen multiplex PCR kit as recommended by the manufacturers, with an annealing temperature of 57°C, in a reaction mixture volume of 25 μl. However, a substantial proportion of blood isolates of this genus identify as *A. lwoffii*, and for these, screening for this species is often more relevant.

Primers OXA-134_29F2 and OXA-134_309R (Table 1) were designed from consensus regions of the *bla*$_{OXA-134a}$ sequence of isolate AL3 (GenBank accession number HQ122933) and the corresponding sequence of the type strain of *A. lwoffii* (ATCC 15309), kindly provided by Jacqueline Chan following whole-genome sequencing. PCRs were carried out in 25-μl volumes containing 10 pmol of each primer (OXA-134_29F2, OXA-134_309R, and *rpoB* primers Ac696F and Ac1598R), 1× Qiagen CoralLoad buffer, deoxynucleoside triphosphates (200 μM each), 2 μl cell lysate, and *Taq* DNA polymerase (1.5 U). Reaction conditions were 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 45 s, and 72°C for 1 min, followed by a 5-min final extension at 72°C.

This first version of the PCR was tested on type strains of the former genomic species 1 to 17, most of which are now named species (http://www.bacterio.cict.fr/a/acinetobacter.html) (Table 2) and 80 clinical isolates received from United Kingdom hospitals, previously subjected to the *bla*$_{OXA-58-like, -23-like, -51-like, -40-like, -143}$/class 1 integrase gene PCR and *rpoB* sequencing. These included 30 isolates that had been identified as *A. lwoffii*/genomic species 9 and representatives of other clinically relevant species, as follows: *A. ursingii* ($n = 4$), *A. parvus* ($n = 5$), *A. schindleri* ($n = 4$), *A. gyllenbergii* ($n = 1$), *A. soli* ($n = 1$) (species not otherwise represented), *A. calcoaceticus* ($n = 2$), *A. baumannii* ($n = 10$), *A. pittii* ($n = 3$), *A. nosocomialis* ($n = 2$), *A. junii* ($n = 1$), *A. haemolyticus* ($n = 1$), *A. radioresistens* ($n = 4$), *A. johnsonii* ($n = 3$), genomic species 13BJ ($n = 1$), genomic species 16 ($n = 1$), *A. bereziniae* ($n = 3$), and *A. baylyi* or *A. guillouiae* ($n = 3$). Reaction products were separated by agarose gel electrophoresis followed by staining with GelRed (3× in 0.1 M NaCl) and photography under UV illumination or on a QIAxcel instrument (Qiagen) using the high-resolution cartridge and 3-kb/15-bp alignment marker.

While the type strains of *A. lwoffii* (ATCC 15309) and genomic species 9 (ATCC 9957) and field isolates of *A. lwoffii*/genomic species 9 gave the *bla*$_{OXA-134-like}$ band in this PCR, so too did our isolates of *A. schindleri* (4/4). Two isolates (ATCC 9957 and a clinical isolate of *A. lwoffii*/genomic species 9) gave slightly smaller amplicons than the other isolates. The *bla*$_{OXA-134-like}$ products from representatives of each of these groups were sequenced and compared in a BioNumerics (Applied Maths) database (Fig. 1). The gene in the type strain of genomic species 9 (ATCC 9957) has a deletion of 9 bp relative to the gene in the type strain of *A. lwoffii*

Received 2 November 2011 Returned for modification 4 December 2011
Accepted 16 December 2011
Published ahead of print 28 December 2011
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doi:10.1128/JCM.06173-11

TABLE 1 Primers for characterization of isolates of *Acinetobacter* spp.

Target	Primer	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
bla$_{OXA-134-like}$/*rpoB* PCR[a]				
rpoB	Ac696F	TAYCGYAAAGAYTTGAAAGAAG	858	8
	Ac1598R	CGBGCRTGCATYTTGTCRT		
A. lwoffii/g sp. 9 *bla*$_{OXA-134-like}$	**OXA-134F3**	**ACTCAATCSACYCAAGCCA**	**223**	This study
	OXA-134_307R2	**GTTTCTTGCCATCCCATTTA**		
Alternative *bla*$_{OXA-134-like}$ primer pairs[a]				
bla$_{OXA-134-like}$	OXA-134_29F2	TGAGTTGCTTGGGCCTGA	281/290	This study
	OXA-134_309R	GCGTTTCTTGCCATCCC		
bla$_{OXA-134a}$	OXA-134_74F	TCCCATCTCAAAGCATTTC	234	This study
	OXA-134_307R2	GTTTCTTGCCATCCCATTTA		
bla$_{OXA-58-like, 23-like, 51-like, 40-like, -143}$/class 1 integrase gene/*rpoB* multiplex PCR				
rpoB	Ac696F	TAYCGYAAAGAYTTGAAAGAAG	858	8
	Ac1598R	CGBGCRTGCATYTTGTCRT		
bla$_{OXA-58-like}$	mpOXA-58-likeF	AAGTATTGGGGCTTGTGCTG	599	17
	mpOXA-58-likeR	CCCCTCTGCGCTCTACATAC		
bla$_{OXA-23-like}$	mpOXA-23-likeF	GATCGGATTGGAGAACCAGA	501	17
	mpOXA-23-likeR	ATTTCTGACCGCATTTCCAT		
bla$_{OXA-51-like}$	mpOXA-51-likeF	TAATGCTTTGATCGGCCTTG	353	17
	mpOXA-51-likeR	TGGATTGCACTTCATCTTGG		
bla$_{OXA-40-like}$	mpOXA-24-likeF	GGTTAGTTGGCCCCCTTAAA	246	17
	mpOXA-24-likeR	AGTTGAGCGAAAAGGGGATT		
bla$_{OXA-143}$	OXA-143 303F2	GATTTTCAAATGGGACGGT	90	This study
	OXA-143 392R2	GGAACTGCTGAAAGTGCC		
Class 1 integrase gene	Int1F	CAGTGGACATAAGCCTGTTC	160	7
	Int1R	CCCGAGGCATAGACTGTA		

[a] The OXA-134F3/OXA-134_307R2 primer pair (in bold) specifically detected most clinical isolates (29/30) of *A. lwoffii*/genomic species 9; those with the *bla*$_{OXA-134a}$ allele (which are missed) were detected using the OXA-134_74F/OXA-134_307R2 primer pair. The OXA-134_29F2/OXA-134_309R primers amplified *bla*$_{OXA-134-like}$ in *A. lwoffii*, genomic species 9 and *A. schindleri*.

and in most of the field isolates of *A. lwoffii*/genomic species 9 that we sequenced (21/22); one field isolate (isolate AL/9_9) also had this deletion, as does the AL3 isolate in the original description of *bla*$_{OXA-134a}$ (HQ122933). The *A. schindleri* sequences showed less than 90% (~85%) homology to those of the other species and clearly clustered apart from them.

TABLE 2 Reference strains used in this study

Genomic species[c]	Species name	Isolate
1	*A. calcoaceticus*	ATCC 23055
2	*A. baumannii*	ATCC 19606
3	*A. pittii*	ATCC 19004
4	*A. haemolyticus*	ATCC 17906
5	*A. junii*	ATCC 17908
6	*Acinetobacter* genomic species 6	ATCC 17979
7	*A. johnsonii*	ATCC 17909
8[a]	*A. lwoffii*	ATCC 15309
9[a]	*Acinetobacter* genomic species 9	ATCC 9957
10	*A. bereziniae*	ATCC 17924
11	*A. guillouiae*	ATCC 11171
12	*A. radioresistens*	ATCC 43998
13BJ	*Acinetobacter* genomic species 13	ATCC 17905
13TU[b]	*A. nosocomialis*	ATCC 17903
14BJ[b]	*Acinetobacter* genomic species 14	Bouvet 382
15	*Acinetobacter* genomic species 15	Bouvet 79
16	*Acinetobacter* genomic species 16	ATCC 17988
17	*Acinetobacter* genomic species 17	Bouvet 942

[a] Probably synonyms for the same species (10).
[b] Probably synonyms for the same species (10).
[c] Genomic species numbering is according to Bouvet and Grimont (1) and Bouvet and Jeanjean (BJ) (2), unless indicated otherwise (TU; Tjernberg and Ursing [13a]).

Among the *A. lwoffii*/genomic species 9 isolates, there were four clusters of *bla*$_{OXA-134-like}$ sequences, that with the deletion being one of them (Fig. 1). On the basis of the type strains, one might anticipate that the allele with the deletion may distinguish genomic species 9 from *A. lwoffii*, but that is not supported by *rpoB* sequence cluster analysis, which shows that the clinical isolate with the deletion shared the same *rpoB* sequence as isolates without it and clustered more closely with ATCC 15309 than ATCC 9957, supporting a single species. In view of the extent of sequence diversity in *bla*$_{OXA-134-like}$, two primer pairs were designed to specifically detect *A. lwoffii*/genomic species 9, one with a forward primer (OXA-134F3) to detect alleles without the deletion and the second with a forward primer (OXA-134_74F) to detect the allele with the deletion; a slightly modified reverse primer (OXA-134_307R2) was used, since this was found to give cleaner PCR products. As before, each pair was used in combination with the *rpoB* primers (Ac696F and Ac1598R), and similar conditions were used (94°C for 3 min followed by 32 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 1 min, followed by a 5-min final extension at 72°C); each primer was present at 8 pmol per reaction mixture. The same panel of isolates was used. All the isolates of *A. lwoffii*/genomic species 9 gave a *bla*$_{OXA-134-like}$ band of the expected size (223 bp) with the OXA-134F3/OXA-134_307R2 primer pair, with the exception of that with the deletion, while none of the other species, including *A. schindleri* and the type strain of genomic species 9, gave this band. All isolates gave the *rpoB* band. When the

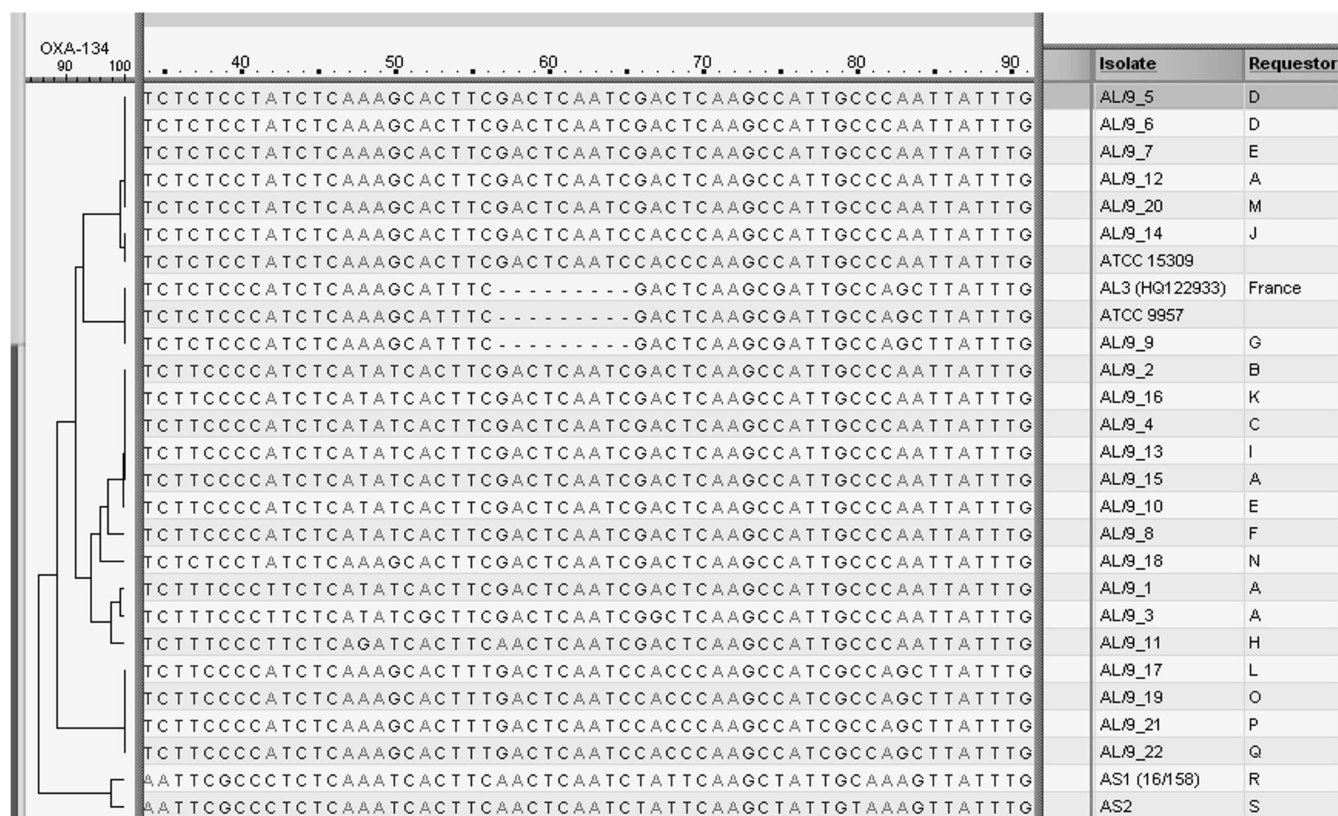

FIG 1 Screenshot of comparison of partial $bla_{OXA-134-like}$ sequences corresponding to nucleotides 37 to 310 of the coding sequence of $bla_{OXA-134a}$ (GenBank accession number HQ122933) showing the region with the 9-bp deletion in ATCC 9957, AL3, and clinical isolate AL/9_9, using BioNumerics (Applied Maths) software. Isolates were identified as *A. lwoffii*/genomic species 9 (AL/9_1 to AL/9_22) or *A. schindleri* (AS1 and AS2) by *rpoB* sequence cluster analysis. Each isolate was from a different patient, from United Kingdom hospitals A to S, collected between 2009 and 2011. Comparison was by the unweighted-pair group method with arithmetic mean (UPGMA).

OXA-134_74F/OXA-134_307R2 primer pair was used, the type strain of genomic species 9 and the isolate with the deletion gave the $bla_{OXA-134-like}$ band; some of the other *A. lwoffii*/genomic species 9 isolates gave slightly larger, fainter bands. We recommend screening with the OXA-134F3/OXA-134_307R2 primer pair to identify *A. lwoffii*/genomic species 9, which will detect most isolates. Those with the deletion, which will be missed, can be identified either by sequencing the *rpoB* amplicon or by screening with the OXA-134_74F/OXA_307R2 primer pair.

The finding of highly similar alleles of $bla_{OXA-134-like}$ in *A. lwoffii* and genomic species 9 supports their being assigned to a single species. Our results on isolates from 30 patients from 22 hospitals indicate that most field isolates of *A. lwoffii*/genomic species 9 do not have the deletion associated with the type strain of genomic species 9. Although there is some potential for intrinsic OXA genes to move between species (e.g., $bla_{OXA-51-like}$ from *A. baumannii* to *A. nosocomialis* [formerly genomic species 13TU] [9] and $bla_{OXA-23-like}$ from *A. radioresistens* to *A. baumannii* [11]), $bla_{OXA-134-like}$ was only found in the species described here among our panel. The PCR using the OXA-134F3/OXA-134_307R2 primer pair provides a rapid method of specific detection of *A. lwoffii*/genomic species 9. Should confirmation of the identification be required, the *rpoB* amplicon can additionally be sequenced.

Nucleotide sequence accession numbers. The partial $bla_{OXA-134-like}$ sequences of ATCC 9957, two clinical isolates of *A. lwoffii*/genomic species 9 (AL/9_7 and AL/9_10), and a clinical isolate of *A. schindleri* (AS1) are deposited in GenBank under accession numbers JN203134, JN804564, JN804565, and JN203135, respectively.

ACKNOWLEDGMENTS

We are grateful to Jacqueline Chan and Mark Pallen at the University of Birmingham for providing the $bla_{OXA-134-like}$ sequence of the type strain of *A. lwoffii*, to the Genomic Services Unit at Centre for Infections, Colindale, for sequencing services, and to colleagues in hospital laboratories for sending these isolates.

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