Molecular Characterization of β -Lactamase Genes and Their Genetic Structures in *Acinetobacter* Genospecies 3 Isolates in Taiwan[†]

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The genetic structure of β -lactamases in *Acinetobacter* genospecies 3 (AG3) isolates in Taiwan was studied to analyze their high rates of resistance to β -lactams, including carbapenems (57.9%). *bla*_{IMP-1} and *bla*_{IMP-8} were located in a class 1 integron. *bla*_{OXA-58} was bracketed by IS*Aba3*. A novel TnpF-like integrase gene was identified upstream of *bla*_{VEB-3}. Adjacent to the 5' sequence of the *bla*_{ADC} gene, *folE* was identified. Four new *Acinetobacter*-derived cephalosporinase (ADC) enzymes were found, which clustered phylogenetically with published AG3 ADC proteins.

Acinetobacter genospecies 3 (AG3), belonging to the *A. calcoaceticus*-*A. baumannii* complex, exists in the natural environment and the clinical setting. It was reported to be the most frequently isolated *Acinetobacter* species in Hong Kong, Sweden, and Ireland (3, 4, 32). Carbapenem-resistant or multi-drug-resistant (MDR) AG3 has been found globally in the past decade (3, 12, 18, 20, 36).

In *Acinetobacter* spp., β -lactamases are the most common contributors to resistance to extended-spectrum cephalosporins and carbapenems, including extended-spectrum β -lactamases (ESBL), metallo- β -lactamases (MBL), carbapenem-hydrolyzing class D β -lactamases (CHDL), and *Acinetobacter*-derived cephalosporinases (ADC) (24, 27). Mobile genetic elements, including insertion sequences (IS) and integrons, may contribute to β -lactamase overproduction and dissemination (26, 31). Several carbapenemases (IMP-1, IMP-4, VIM-2, OXA-23, and OXA-58) and versatile AmpC cephalosporinases (ADC-12 to ADC-23) have been found in AG3 (2, 3, 12, 20, 22, 36) but with few studies on their genetic support to elucidate possible transmission mechanisms.

Nineteen AG3 clinical isolates were collected from three hospitals in Taiwan from 1999 to 2007 and were identified using the API 32GN kit (bioMérieux, Marcy-l'Etoile, France) and *recA* and *rpoB* sequencing (15, 16). Sixteen distinct pulsotypes were identified among the isolates, and each blood isolate displayed a different pulsotype, indicating nonclonal relatedness.

The MICs of antimicrobials were determined using the broth microdilution method, except the MIC of sulbactam, which was determined with agar dilution method (5). More than half of AG3 isolates were resistant to the β -lactams tested (57.9 to 94.7%), including carbapenems (57.9%), and all were susceptible to sulbactam, tigecycline, and colistin (Table 1). Fifteen isolates (78.9%) were resistance to ≥ 3 classes of anti-

TABLE 1. Antimicrobial susceptibility testing results for *Acinetobacter* genospecies 3 isolates from three medical centers in Taiwan^d

Antimicrobial agent ^a	MIC (μ g/ml)			Resistance (%)
	Range	50%	90%	
Piperacillin	8– ≥ 128	≥ 128	≥ 128	63.2
Piperacillin-tazobactam	8– ≥ 128	≥ 128	≥ 128	57.9
Ampicillin-sulbactam	4– ≥ 32	4	≥ 32	15.8
Ceftriaxone	8– ≥ 64	≥ 64	≥ 64	63.2
Ceftazidime	4– ≥ 64	≥ 64	≥ 64	68.4
Cefepime	4– ≥ 32	≥ 32	≥ 32	63.2
Aztreonam	16– ≥ 32	≥ 32	≥ 32	94.7
Imipenem	0.25–64	16	32	57.9
Meropenem	0.5–64	16	32	57.9
Tobramycin	≤ 0.5 – ≥ 16	8	≥ 16	42.1
Gentamicin	1– ≥ 16	8	≥ 16	52.6
Amikacin	16– ≥ 64	32	≥ 64	52.6
Ciprofloxacin	0.12–16	0.25	2	5.3
Sulbactam ^b	0.5–4	2	4	0
Tigecycline ^c	≤ 0.12 –2	0.12	0.5	0
Colistin	1–2	1	2	0

^a The breakpoints used were according to CLSI guidelines (6).

^b The breakpoints used were defined based on the guidelines of a Spanish antibiogram committee (the MENSURA group) for nonfermenting Gram-negative bacteria (susceptible, ≤ 8 μ g/ml; intermediate, 16 μ g/ml; resistant, ≥ 32 μ g/ml) (23).

^c The breakpoints used were defined based on the U.S. Food and Drug Administration (FDA) susceptibility breakpoints for the *Enterobacteriaceae* (susceptible, ≤ 2 μ g/ml; intermediate, 4 μ g/ml; resistant, ≥ 8 μ g/ml) (25).

^d Nineteen isolates were tested.

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TABLE 2. Characteristics of IMP/OXA-58-coproducing *Acinetobacter* genospecies 3 isolates examined in the present study^a

Isolate no.	MIC (μg/ml) of antimicrobial agent										Presence of gene, genetic element, or gene group (product)										Integron cassette gene array	Pulsotype			
	PIP	CRO	CAZ	FEP	IPM	MEM	ATM	SAM	TZP	SUL	ESBL	MBL	CHDL ^b	ADC	IS <i>Abal</i>	IS <i>Abal2</i>	IS <i>Abal3</i>	IS <i>78</i>	IS <i>1008</i>	rep <i>Ad1</i>			int1		
1	>64	>32	>32	>16	32	16	>16	16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	C	
2	>64	>32	>32	>16	32	16	>16	≤8	>64	4	–	+	(IMP-8) + (OXA-58)	+	(ADC-43)	+	–	–	+	–	–	+	(int11)	bla _{IMP-8-aac6-II-aadA4}	L
4	>64	>32	>32	>16	64	64	>16	≤8	64	2	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	+	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4^b}	F
5	>64	>32	>32	>16	16	16	>16	≤8	64	2	–	+	(IMP-1) + (OXA-58)	+	(ADC-43)	–	+	–	–	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	M
6	>64	>32	>32	>16	16	32	>16	16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	K
7	>64	>32	>32	>16	32	16	>16	16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	L
8	>64	>32	>32	>16	32	16	>16	<8	64	2	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4}	B
10	>64	>32	>32	>16	32	32	>16	16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	I
11	>64	>32	>32	>16	32	16	>16	<8	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	I
15	>64	>32	>32	>16	16	32	>16	>16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	–	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	J
18	32	>32	>32	>16	32	32	>16	>16	>64	4	–	+	(IMP-1) + (OXA-58)	+	(ADC-18)	+	–	–	+	–	+	+	(int11)	bla _{IMP-1-aac6-II-aadA4} , dfrA1- <i>orfC</i>	D

^a Abbreviations: PIP, piperacillin; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; MEM, meropenem; ATM, ampicillin-sulbactam; SUL, sulbactam; ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase; CHDL, carbapenem-hydrolyzing class D β-lactamase.
^b The detected CHDL genes, including *bla*_{OXA-23}-like, *bla*_{OXA-24}-like, *bla*_{OXA-51}-like, and *bla*_{OXA-58}-like.

FIG. 1. Schematic representation of the genetic structure of β -lactamases identified in *Acinetobacter* genospecies 3 isolates. The open reading frames of identified gene are shown as arrows, with the direction of transcription indicated by arrowheads and filled diamonds indicating the *attC* sites of the cassettes. β -Lactamases genes are shown as hatched arrows, transposase genes as filled arrows, and other identified genes as gray arrows. Filled arrowheads indicate the locations of the primers for PCR mapping used. 5' and 3' conserved segments are shown as bold lines, the inserts of recombinant plasmid as thin lines, and the identical genetic structure as dash lines. The *Sau3A1* restriction sites used for subcloning are indicated. Terminal repeat invert repeats (IRt), insertion sequence common region (ISCR1), and integron recombination core site (RCS) are represented by white circles. IRt, 25-bp right-hand inverted repeat is identical to In4-type integron. ISCR1, 34-bp fragment matches the right end of ISCR1; RCS, 7-bp sequence is identical to the integron consensus GTTRRRY recombination core site.

biotics that were potentially effective against the *Acinetobacter* spp. and were classified as MDR AG3 (9). In this study, sulbactam, tigecycline, and colistin reveal their roles as effective agents for AG3, which is similar to the situation for carbapenem-resistant *A. baumannii* (19).

The β -lactamases and resistance determinants were detected by PCR (8, 13, 14, 20, 28, 30, 31, 34, 35), including Ambler class A ESBL genes (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{VEB}, and *bla*_{PER}), Ambler class B MBL genes (*bla*_{IMP}, *bla*_{VIM}, *bla*_{GIM}, *bla*_{SPM}, and *bla*_{SIM}), an Ambler class C AmpC β -lactamase genes (*bla*_{ADC}), Ambler class D carbapenemase genes (*bla*_{OXA-23-like}, *bla*_{OXA-24-like}, *bla*_{OXA-51-like}, and *bla*_{OXA-58-like}), integrase genes (*int11*, *int12*, and *int13*), IS elements (IS*Aba1*, IS*Aba2*, IS*Aba3*, and IS*I8*), transposase genes, and a putative replicase gene, *repAci1*. IS1008 was studied using the primers ATGGCATCA GCTATCGTG and CCTGTGCTCAATATCTACTGGA, and the putative TnpF-like integrase gene, identified in this study, was investigated with the primers GCAGACAGCTCTATCG TTAAAG and TTTGTATCACCTTCCTGCTG. The PCR amplification results showed that one isolate contained *bla*_{VEB} (isolate 3), 11 harbored both *bla*_{IMP} (10 had IMP-1 and 1 had IMP-8) and *bla*_{OXA-58-like}, and all had *bla*_{ADC}. Twelve isolates (63.2%) harbored the class 1 integron but none had the class

2 or 3 integron. Eleven isolates (57.9%) had insertion sequence IS*Aba1*, 3 (15.8%) had IS*Aba2*, 18 (94.7%) had IS*Aba3*, 2 (10.5%) had IS*I8*, and 14 (73.7%) had IS1008. *repAci1* was detected in one *bla*_{OXA-58-like}-positive isolate and one *bla*_{OXA-58-like}-negative isolate. The TnpF-like integrase gene was identified only in the *bla*_{VEB}-positive isolate. The concomitant presence of MBL and CHDL was detected in 11 AG3 isolates (Table 2) that had been found in 1 *A. baumannii* strain in Taiwan and 2 in Greece (13, 33). Having both MBL and CHDL may not be rare in *Acinetobacter*, and its clinical impact is yet to be studied.

Sequence analysis of integron PCR products revealed that 11 isolates had *bla*_{IMP}-*aac6-II*-*aadA4* and/or *dfrA1*-*orfC* cassettes (Table 2), and 1 carried the *aphA15*-*aadA1* cassette (isolate 3). The *bla*_{ADC} genes were PCR amplified with the primers FolE-F (CGTGCGGCTAAAGCTTT) and ADC-AG3-R (TTATTTCTTTATTGCATTYARYAC), designed from the *A. baumannii* strain AB0057 *folE* (1) and AG3 *bla*_{ADC} sequences (2). Four novel ADC enzymes (ADC-41 to -44) were identified and clustered (17) with 13 other AG3 ADC proteins (except ADC-5). Thirteen isolates (68.4%) had ADC-18, two had ADC-43, and the other four each had ADC-22, ADC-41, ADC-42, or ADC-44.

The genetic environment of the β -lactamase genes was sought by PCR mapping and plasmid random cloning (Fig. 1). The *bla*_{IMP-1} and *bla*_{IMP-8} genes are located in a class 1 integron and followed by the aminoglycoside-resistant genes *aac6-II* and *aadA4* (Fig. 1A), which appears to be a common genetic structure in *Acinetobacter* species (13, 36). Comobilization of the two classes of antimicrobial resistance genes by the same integron suggests the possibility of selecting carbapenem resistance by aminoglycoside use. The *bla*_{OXA-58} gene in AG3 is bracketed by *ISAb3* (Fig. 1B), which is structurally similar to previous reports for several different *Acinetobacter* genospecies (22) and *A. baumannii* on the plasmid pABIR (37). The prokaryotic type 1 GTP cyclohydrolase *folE* gene, associated with sulfonamide resistance in bacteria (7), was identified in the 5'-adjacent sequence of the ADC gene (Fig. 1C).

In *A. baumannii*, *bla*_{VEB-1} has been identified in cassette arrays of a class 1 integron with 3'-end conserved-segment (3'-CS) duplications in the French AYE strain (10), and *bla*_{VEB-1a} is linked to *ISCR2* in Argentinian strains (29). In a difference from the chromosome location reported previously (38), the plasmid location of *bla*_{VEB-3} in our isolate was identified using the I-CeuI mapping technique (28) and Southern hybridization of the plasmid. The upstream region of *bla*_{VEB-3} was cloned (21) and selected with 5 μ g/ml ceftazidime (Sigma, St. Louis, MO). *bla*_{VEB-3} is located in a complex class 1 integron of 3'-CS duplications containing insertion sequence *IS6100*, the *ISCR1* IS common region elements, and a novel integrase TnpF-like protein (Fig. 1D). Furthermore, the TnpF-like integrase gene reveals as low a % G+C content (34.3%) as *Acinetobacter* (11), and its encoded protein exhibits high amino acid sequence identity to the TnpF protein from aquatic and soil bacteria. The TnpF-like integrase carried by the AG3 plasmid may provide a route for horizontal gene transfer between clinical and environmental strains.

Our results suggest that AG3 may obtain the antibiotic resistance genes, especially β -lactamase genes, from clinical or environmental *Acinetobacter* spp. through different gene acquisition mechanisms that include integrons, IS elements, and a novel integrase.

Nucleotide sequence accession numbers. The nucleotide sequences of our reported ADC-type enzymes were deposited in GenBank under the accession numbers FJ744160 (ADC-41), FJ744161 (ADC-42), FJ744162 (ADC-43), and FJ744163 (ADC-44). The genetic surrounding sequences of β -lactamase genes identified were assigned the accession numbers GQ864268 (*bla*_{IMP}), FJ744165 (*bla*_{OXA-58}), FJ744164 (*bla*_{ADC}), and GQ926879 (*bla*_{VEB-3}).

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REFERENCES

- Adams, M. D., K. Goglin, N. Molyneux, K. M. Hujer, H. Lavender, J. J. Jamison, I. J. MacDonald, K. M. Martin, T. Russo, A. A. Campagnari, A. M. Hujer, R. A. Bonomo, and S. R. Gill. 2008. Comparative genome sequence

- analysis of multidrug-resistant *Acinetobacter baumannii*. J. Bacteriol. **190**: 8053–8064.
- Beceiro, A., A. Perez, F. Fernandez-Cuenca, L. Martinez-Martinez, A. Pascual, J. Vila, J. Rodriguez-Bano, J. M. Cisneros, J. Pachon, and G. Bou. 2009. Genetic variability among ampC genes from *Acinetobacter* genomic species 3. Antimicrob. Agents Chemother. **53**:1177–1184.
- Boo, T. W., F. Walsh, and B. Crowley. 2009. Molecular characterization of carbapenem-resistant *Acinetobacter* species in an Irish university hospital: predominance of *Acinetobacter* genomic species 3. J. Med. Microbiol. **58**: 209–216.
- Chu, Y. W., C. M. Leung, E. T. Houang, K. C. Ng, C. B. Leung, H. Y. Leung, and A. F. Cheng. 1999. Skin carriage of acinetobacters in Hong Kong. J. Clin. Microbiol. **37**:2962–2967.
- Clinical and Laboratory Standards Institute. 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, vol. 29, no. 2. Approved standard M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing, vol. 29, no. 3. Approved standard M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
- El Yacoubi, B., S. Bonnet, J. N. Anderson, M. A. Swairjo, D. Iwata-Reuyl, and V. de Crecy-Lagard. 2006. Discovery of a new prokaryotic type I GTP cyclohydrolase family. J. Biol. Chem. **281**:37586–37593.
- Ellington, M. J., J. Kistler, D. M. Livermore, and N. Woodford. 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J. Antimicrob. Chemother. **59**:321–322.
- Falagas, M. E., and D. E. Karageorgopoulos. 2008. Pandrug resistance (PDR), extensive drug resistance (XDR), and multidrug resistance (MDR) among Gram-negative bacilli: need for international harmonization in terminology. Clin. Infect. Dis. **46**:1121–1122.
- Fournier, P. E., D. Vallenet, V. Barbe, S. Audic, H. Ogata, L. Poirel, H. Richet, C. Robert, S. Manganot, C. Abergel, P. Nordmann, J. Weissenbach, D. Raoult, and J. M. Claverie. 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS. Genet. **2**:e7.
- Heuer, H., C. Kopmann, C. T. Binh, E. M. Top, and K. Smalla. 2009. Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low %G+C content. Environ. Microbiol. **11**:937–949.
- Houang, E. T., Y. W. Chu, W. S. Lo, K. Y. Chu, and A. F. Cheng. 2003. Epidemiology of rifampin ADP-ribosyltransferase (*arr-2*) and metallo-beta-lactamase (*bla*_{IMP-4}) gene cassettes in class 1 integrons in *Acinetobacter* strains isolated from blood cultures in 1997 to 2000. Antimicrob. Agents Chemother. **47**:1382–1390.
- Huang, L. Y., T. L. Chen, P. L. Lu, C. A. Tsai, W. L. Cho, F. Y. Chang, C. P. Fung, and L. K. Siu. 2008. Dissemination of multidrug-resistant, class 1 integron-carrying *Acinetobacter baumannii* isolates in Taiwan. Clin. Microbiol. Infect. **14**:1010–1019.
- Koeleman, J. G., J. Stoof, M. W. van der Bijl, C. M. Vandenbroucke-Grauls, and P. H. Savelkoul. 2001. Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. J. Clin. Microbiol. **39**:8–13.
- Krawczyk, B., K. Lewandowski, and J. Kur. 2002. Comparative studies of the *Acinetobacter* genus and the species identification method based on the *recA* sequences. Mol. Cell Probes **16**:1–11.
- La Scola, S. B., V. A. Gundi, A. Khamis, and D. Raoult. 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J. Clin. Microbiol. **44**:827–832.
- Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics **23**:2947–2948.
- Lim, Y. M., K. S. Shin, and J. Kim. 2007. Distinct antimicrobial resistance patterns and antimicrobial resistance-harboring genes according to genomic species of *Acinetobacter* isolates. J. Clin. Microbiol. **45**:902–905.
- Livermore, D. M., and N. Woodford. 2006. The β -lactamase threat in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter*. Trends Microbiol. **14**:413–420.
- Lu, P. L., L. Y. Huang, S. T. Lian, K. Chang, C. L. Lin, I. J. Hwang, W. G. Chiang, Y. H. Chen, S. F. Lin, and L. K. Siu. 2008. How carbapenem-resistant *Acinetobacter* spp. established in a newly constructed hospital. Int. J. Antimicrob. Agents **31**:463–466.
- Ma, L., J. Alba, F.-Y. Chang, M. Ishiguro, K. Yamaguchi, L. K. Siu, and Y. Ishii. 2005. Novel SHV-derived extended-spectrum β -lactamase, SHV-57, that confers resistance to ceftazidime but not ceftazolin. Antimicrob. Agents Chemother. **49**:600–605.
- Marti, S., J. Sanchez-Cespedes, M. D. Blasco, M. Ruiz, P. Espinal, V. Alba, F. Fernandez-Cuenca, A. Pascual, and J. Vila. 2008. Characterization of the carbapenem-hydrolyzing oxacillinase Oxa-58 in an *Acinetobacter* genospecies 3 clinical isolate. Antimicrob. Agents Chemother. **52**:2955–2958.
- Miembros del grupo MENSURA. 2000. Recommendations from MENSURA for selection of antimicrobial agents for susceptibility testing and criteria for the interpretation of antibiograms. Rev. Esp. Quimioter. **13**:73–86.
- Naas, T., P. Bogaerts, C. Bauriaing, Y. Degheldre, Y. Glupczynski, and P. Nordmann. 2006. Emergence of PER and VEB extended-spectrum- β -lacta-

- mases in *Acinetobacter baumannii* in Belgium. J. Antimicrob. Chemother. **58**:172–182.
25. Pankey, G. A. 2005. Tigecycline. J. Antimicrob. Chemother. **56**:470–480.
 26. Peleg, A. Y., H. Seifert, and D. L. Paterson. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. **21**:538–582.
 27. Poirel, L., and P. Nordmann. 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. **12**:826–836.
 28. Poirel, L., S. Figueiredo, V. Cattoir, A. Carattoli, and P. Nordmann. 2008. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. Antimicrob. Agents Chemother. **52**:1252–1256.
 29. Poirel, L., P. D. Mugnier, M. A. Toleman, T. R. Walsh, M. J. Rapoport, A. Petroni, and P. Nordmann. 2009. ISCR2, another vehicle for *bla*_{VEB} gene acquisition. Antimicrob. Agents Chemother. **53**:4940–4943.
 30. Poirel, L., and P. Nordmann. 2006. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*_{OXA-58} in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **50**:1442–1448.
 31. Ruiz, M., S. Marti, F. Fernandez-Cuenca, A. Pascual, and J. Vila. 2007. Prevalence of IS*Aba1* in epidemiologically unrelated *Acinetobacter baumannii* clinical isolates. FEMS Microbiol. Lett. **274**:63–66.
 32. Tjernberg, L., and J. Ursing. 1989. Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. APMIS **97**:595–605.
 33. Tsakris, A., A. Ikonomidis, S. Pournaras, L. S. Tzouveleakis, D. Sofianou, N. J. Legakis, and A. N. Maniatis. 2006. VIM-1 metallo-beta-lactamase in *Acinetobacter baumannii*. Emerg. Infect. Dis. **12**:981–983.
 34. Woodford, N., M. J. Ellington, J. M. Coelho, J. F. Turton, M. E. Ward, S. Brown, S. G. Amyes, and D. M. Livermore. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents **27**:351–353.
 35. Yeh, K. M., A. Kurup, L. K. Siu, Y. L. Koh, C. P. Fung, J. C. Lin, T. L. Chen, F. Y. Chang, and T. H. Koh. 2007. Capsular serotype K1 or K2, rather than *magA* and *rmpA*, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in Singapore and Taiwan. J. Clin. Microbiol. **45**:466–471.
 36. Yum, J. H., K. Yi, H. Lee, D. Yong, K. Lee, J. M. Kim, G. M. Rossolini, and Y. Chong. 2002. Molecular characterization of metallo-beta-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new integrons carrying the *bla*_{VIM-2} gene cassettes. J. Antimicrob. Chemother. **49**:837–840.
 37. Zarrilli, R., D. Vitale, A. Di Popolo, M. Bagattini, Z. Daoud, A. U. Khan, C. Afif, and M. Triassi. 2008. A plasmid-borne *bla*_{OXA-58} gene confers imipenem resistance to *Acinetobacter baumannii* isolates from a Lebanese hospital. Antimicrob. Agents Chemother. **52**:4115–4120.
 38. Zong, Z., S. R. Partridge, and J. R. Iredell. 2009. A *bla*_{VEB-1} variant, *bla*_{VEB-6}, associated with repeated elements in a complex genetic structure. Antimicrob. Agents Chemother. **53**:1693–1697.