

Emergence and Distribution of Plasmids Bearing the *bla*_{OXA-51}-Like Gene with an Upstream *ISAbal* in Carbapenem-Resistant *Acinetobacter baumannii* Isolates in Taiwan[▽]

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The *bla*_{OXA-51}-like gene with an upstream *ISAbal* (*ISAbal-bla*_{OXA-51}-like gene) was originally found on the chromosomes of carbapenem-resistant or -susceptible *Acinetobacter baumannii* isolates. However, a plasmid-borne *ISAbal-bla*_{OXA-51}-like gene has recently been identified in *Acinetobacter* genomic species 13TU and several *A. baumannii* isolates in Taiwan, and all of the isolates are carbapenem resistant. This study aimed to characterize the plasmids bearing the *ISAbal-bla*_{OXA-51}-like gene and their significance in *A. baumannii*. Among the 117 *ISAbal-bla*_{OXA-51}-like-harboring isolates collected from 10 hospitals in Taiwan, 58 isolates (49.6%) from 24 clones had the genes located on plasmids that likely originated from a common progenitor. Among the 58 isolates, four had additional copy of the *ISAbal-bla*_{OXA-51}-like gene on their chromosomes. Based on the analysis of these four isolates, the plasmid-located *ISAbal-bla*_{OXA-51}-like gene appeared to be acquired via one-ended transposition (Tn6080). The isolates with a plasmid bearing the *ISAbal-bla*_{OXA-51}-like gene had higher rates of resistance to imipenem (98% versus 46.6%; $P < 0.001$) and meropenem (98% versus 69%; $P = 0.019$) than those with the genes chromosomally encoded, which is most likely due to increased gene dosage provided by the higher copy number of associated plasmids. Transformation with a recombinant plasmid harboring only the *ISAbal-bla*_{OXA-51}-like gene was enough to confer a high level of carbapenem resistance to *A. baumannii*, eliminating the possible contribution of other factors on the original plasmids. This study demonstrated that the carbapenem resistance-associated plasmids carrying the *ISAbal-bla*_{OXA-51}-like gene are widespread in *A. baumannii* strains in Taiwan.

Acinetobacter baumannii has emerged as a major pathogen of nosocomial infections in immunocompromised patients and is associated with a high mortality rate (7). The management of this pathogen has become a significant challenge due to the increased emergence of carbapenem-resistant strains (19, 22). It has been shown that most of the carbapenem resistance in *A. baumannii* is due to the production of carbapenemases, especially those belonging to carbapenem-hydrolyzing class D beta-lactamases (CHDLs), which are encoded by the *bla*_{OXA-51}-like, *bla*_{OXA-23}-like, *bla*_{OXA-24}-like, and *bla*_{OXA-58}-like genes (19). Among these CHDL genes, the *bla*_{OXA-51}-like gene is intrinsic to and was originally located on the chromosome of *A. baumannii* (7, 18, 24).

The CHDLs have only a weak carbapenem-hydrolyzing activity (1, 19). However, overexpression of these CHDL genes, driven mostly by promoters provided by their upstream insertion sequences (ISs), is one of the means by which *A. baumannii* acquires a high level of carbapenem resistance. For example, *ISAbal* upstream of the *bla*_{OXA-51}-like gene is associated

with the overexpression of the *bla*_{OXA-51}-like gene and carbapenem resistance in *A. baumannii* (12, 23). However, some isolates harboring the *bla*_{OXA-51}-like gene with an upstream *ISAbal* (*ISAbal-bla*_{OXA-51}-like gene) are still susceptible to carbapenem (9, 14, 16). The reason for this discrepancy in carbapenem susceptibility levels in isolates harboring the *ISAbal-bla*_{OXA-51}-like gene has not been completely clarified. In some isolates, the high level of resistance might be due to interplay between the overexpression of the *bla*_{OXA-51}-like gene and other mechanisms, such as overexpression of an efflux pump (12, 19). However, the overexpression of the CHDL genes by themselves can occasionally confer a high level of carbapenem resistance, especially when the resistance determinants are located on a plasmid, and this is likely due to the higher gene dosage provided by the higher copy number associated with plasmids (3). Recently, we have identified several *A. baumannii* isolates in Taiwan that harbor plasmid-borne *ISAbal-bla*_{OXA-51}-like genes (*ISAbal-bla*_{OXA-82}) (GenBank accession no. GQ352402). Moreover, a plasmid bearing the *ISAbal-bla*_{OXA-51}-like gene (*ISAbal-bla*_{OXA-138}) has also been identified in an *Acinetobacter* genomic species 13TU isolate (15). The mobilization of the *ISAbal-bla*_{OXA-51}-like gene to plasmids and dissemination of these plasmids will further complicate the management of *A. baumannii* infections. This study aimed to characterize the spread of these

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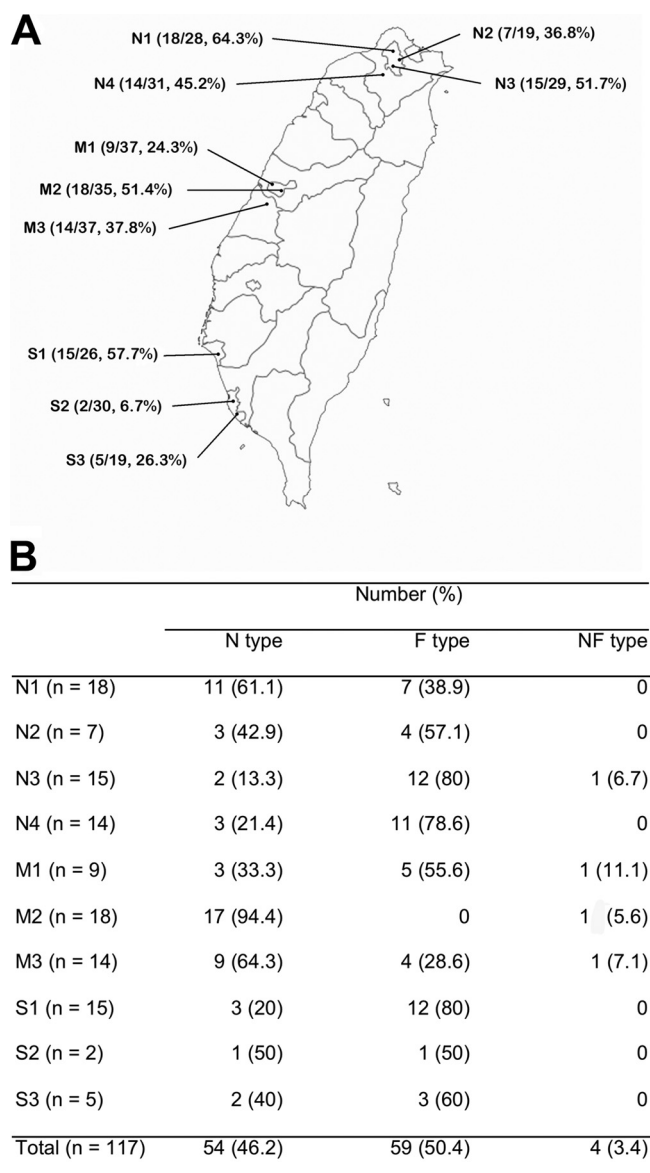


FIG. 1. Isolates harboring the *ISAbal-bla_{OXA-51}*-like gene from 10 hospitals located in different parts of Taiwan. (A) The numerators and denominators in parentheses are the number of isolates harboring the *ISAbal-bla_{OXA-51}*-like gene and the total number of *Acinetobacter baumannii* isolates collected, respectively. (B) Percentage of isolates with either *nuc* (*nuc-ISAbal-bla_{OXA-51}*-like structure, N type) or *fxsA* (*fxsA-ISAbal-bla_{OXA-51}*-like structure, F type) upstream of the *ISAbal-bla_{OXA-51}*-like gene. Four isolates have both genetic structures and were referred to as NF type.

plasmids and their significance in *A. baumannii* isolates in Taiwan.

MATERIALS AND METHODS

Collection of bacteria and genomic species identification of *A. baumannii*. Nonduplicate bacterial isolates that were phenotypically identified as members of the *Acinetobacter calcoaceticus-A. baumannii* complex were collected from 10 medical centers located in different parts of Taiwan (Fig. 1) during the period from July to October 2007. The isolates that were identified to the genomic species level as *A. baumannii* using a multiplex PCR method (2) were included in this study. An *A. baumannii* reference strain, ATCC 15151^T, was used as a

recipient during transformation (3). This strain does not carry carbapenemase genes other than the intrinsic *bla_{OXA-51}*-like gene, which lacks an upstream *ISAbal*.

Detection and sequencing of carbapenemase genes and their upstream ISs. Genes encoding metallo-beta-lactamase (MBL) or CHDLs were detected via PCR as described by Ellington et al. (8) and Higgins et al. (11), respectively. The phenotypic assay for MBL production was carried out using a double-disk synergy test and a combined-disk test with imipenem and EDTA following protocols described by Franklin et al. (10). Detection of *ISAbal*, preceding the *bla_{OXA-51}*-like and *bla_{OXA-23}*-like genes, and *IS1008*, preceding the *bla_{OXA-58}*-like gene, was performed by PCR mapping using a forward primer within the ISs and a reverse primer in the CHDL genes (Table 1). The partial *ISAbal* and full-length *bla_{OXA-51}*-like genes were amplified with LA *Taq* polymerase (Takara Shuzou, Otsu, Japan), TA cloned into the pCR-TOPOII vector (Invitrogen, Carlsbad, CA), and sequenced by Mission Biotech (Taipei, Taiwan).

PCR mapping of the region upstream of the *ISAbal-bla_{OXA-51}*-like gene. Alignment of the sequences surrounding the chromosomally encoded *bla_{OXA-51}*-like gene in *A. baumannii* strains AB307-0294 (accession no. CP001172.1), AB0057 (CP001182.1), ACICU (CP000863.1), SDF (CU468230.2), AYE (CU459141.1), and ATCC 17978 (CP000521.1) identified a gene encoding a sortase (*srt*; open reading frame 13 [ORF13]) (Fig. 2A) and another encoding a transcriptional regulator (ORF14) in the downstream region. A gene encoding a putative suppressor of F exclusion of phage T7 (*fxsA*) was identified immediately upstream of the *bla_{OXA-51}*-like gene (ORF10). Using primers FxsA-F and OXA51-like-R, a fragment (*fxsA-bla_{OXA-51}*-like sequence) of 1,303 bp was generated by PCR. The size of the amplicon increased to 1,920 bp in the presence of an insertion of *ISAbal* between the *fxsA* and *bla_{OXA-51}*-like sequences (*fxsA-ISAbal-bla_{OXA-51}*-like sequence) (Fig. 2A). The isolates having the *fxsA-ISAbal-bla_{OXA-51}*-like genetic structure were referred to as F-type isolates.

The surrounding structures of plasmids bearing the *ISAbal-bla_{OXA-51}*-like gene from an *A. baumannii* strain (pAbSK-OXA-82, accession no. GQ352402) and an *Acinetobacter* genomic species 13 TU strain (accession no. EU670845) were also aligned and compared. A gene encoding a thermonuclease protein (*nuc*; ORF8) (Fig. 2B) was identified in the upstream region of the *ISAbal-bla_{OXA-51}*-like genes in these two plasmids. PCR with primers TMN-F and OXA51-like-R amplified a fragment of 2,388 bp in isolates harboring the *nuc-ISAbal-bla_{OXA-51}*-like structure, and these isolates were referred to N-type isolates. The gene contents of the PCR products of randomly selected isolates were verified by cloning and sequencing.

Identification of the plasmid location of the *nuc-ISAbal-bla_{OXA-51}*-like sequence by Southern blot analysis. Plasmids were purified using a plasmid DNA miniprep kit (Biomax, Taipei, Taiwan) or a plasmid maxikit (Qiagen, Valencia, CA), gel separated, transferred to nylon membranes (Perkin-Elmer, Boston, MA), and hybridized with PCR-generated probes specific for the *bla_{OXA-51}*-like and *nuc* genes. Several representative strains were also selected for mapping the location of the *nuc* gene using an I-CeuI restriction enzyme mapping method (15). After transfer of the I-CeuI-cut fragments to a nylon membrane, Southern blot analysis was performed using PCR-generated probes specific for the 16S rRNA (17) or *nuc* genes. Southern blot analysis was performed using a digoxigenin (DIG) DNA labeling and detection kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions.

Determination of clonal relationships by PFGE. Determination of the clonal relationship of the *A. baumannii* isolates was performed by pulsed-field gel electrophoresis (PFGE) as described previously (13). The restriction enzyme *NotI* and a CHEF-Mapper electrophoresis system (Bio-Rad Laboratories, Richmond, CA) were used. Banding patterns were interpreted according to the criteria recommended by Tenover et al. (21) and cluster analysis using the Molecular Analyst Fingerprinting, Fingerprinting Plus, and Fingerprinting DST software (Bio-Rad Laboratories, Richmond, CA) with the unweighted-pair group method with arithmetic averages (13). Isolates with the same pulsotype were classified as clones.

Antimicrobial susceptibility tests. MICs were determined using the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (4). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control strains during MIC tests. The breakpoint concentrations for all antibiotics were as previously defined (5).

Electrotransformation of recombinant plasmids harboring *ISAbal-bla_{OXA-51}*-like or *bla_{OXA-51}*-like genes. The amplified *ISAbal-bla_{OXA-51}*-like or *bla_{OXA-51}*-like gene was cloned into an *E. coli-A. baumannii* shuttle vector, pYMAb3 (3). pYMAb3 is derived from *E. coli* plasmid pET-28a (Novagen, Madison, WI) and has a plasmid replicon from *A. baumannii* plasmid pMAC (3). *A. bau-*

TABLE 1. Primers used in this study

Primer	Sequence (5' → 3')	Site ^a	Purpose (reference)
IS <i>Aba1</i> -F	CACGAATGCAGAAGTTG	2	PCR mapping (20)
IS1008-F	TCGGCACTTTCAAGGTGAAAT		PCR mapping
OXA-23-like-R	AATAATATTCAGCTGTTTTAATG		PCR mapping
OXA-58-like-R	TTATAAATAATGAAAAACACCCA		PCR mapping
OXA-51-like-R	CTATAAAATACCTAATTKTTCTAA		PCR mapping
OXA-51-F2	ATCTTGCTCGTGCTTCG		Probe
OXA-51-R1	ATATTCCTTGAGGCTGAAC		Probe
FxsA-F	CCACTGATGAACTGTGCTAC	1	PCR mapping
SK17-Ori-F	GCTTAAAAAGACGAAAGACCG	3	PCR mapping
SK17-ORF-2-R	GTAECTCAAATATCTGTCTAG	4	PCR mapping
SK-17-ORF-2-F	CATGCTGGGGATTACCGC	5	PCR mapping
SK-17-ORF-5-R	GCTTCTATTTGGGGAAACGGC	6	PCR mapping
SK-17-ORF-5-F	GATGCCGTTTCCCAAATAG	7	PCR mapping
TMN-R	CTCCATAAGCCTGTGGCTC	8	PCR mapping
TMN-F	GGTATGGCATGGGCCTTC	9	PCR mapping
OXA-51-like-R2	GGATAACTAAAACACCCGTAG	10	PCR mapping
IS <i>Aba1</i> -F2	GGTAAGCACTTGATGGGCAAG	11	PCR mapping
SK-17-ORF-13-R	GGAGTAAAGCAACTCTACCC	12	PCR mapping
16SrRNA-F	AGAGTTTGATCHTGGYTYAGA		Probe (17)
16SrRNA-R	ACGGYTACCTTGTACGACTT		
TMN-F2	GATCCAAAACAGATGAGGGTG		Probe
TMN-R2	TTGAGATTGATTCTTTCGCCAC		Probe

^a As depicted in Fig. 2.

mannii ATCC 15151^T and F-type isolates susceptible to carbapenem were randomly selected as recipient cells. Transformation of the empty shuttle vector was also performed as a control. Electroporation was performed using an ECM 630 electroporator (BTX, San Diego, CA) and 2-mm electrode gap cuvettes as previously described (3). The transformants were selected on agar

plates containing imipenem (8 µg/ml). Transformants carrying an empty shuttle vector or a shuttle vector with the *bla*_{OXA-51}-like gene were also selected on agar plates containing kanamycin (40 µg/ml). Successful transformations were verified by plasmid analysis and PCR mapping of the recombinant plasmid.

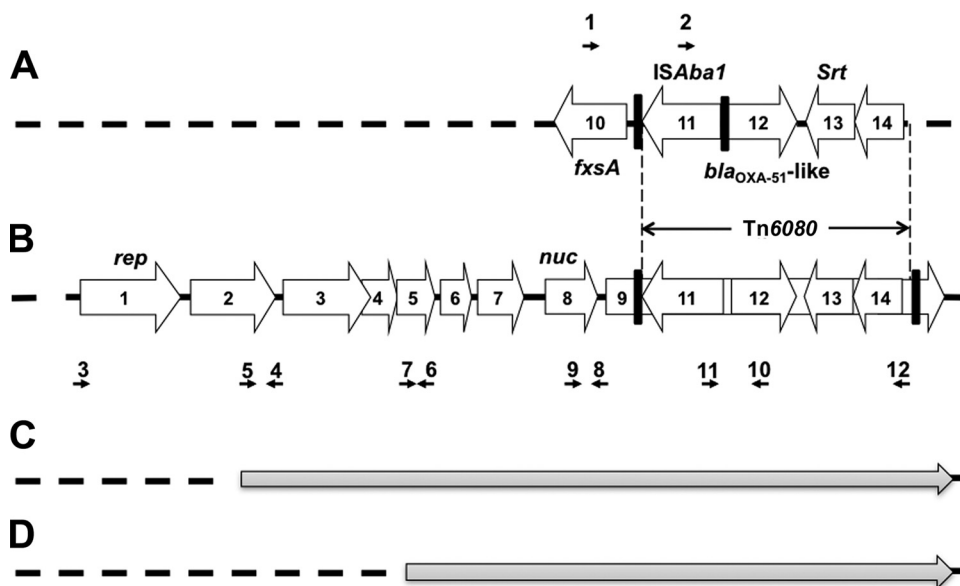


FIG. 2. Schematic map of the partial genetic structure flanking the IS*Aba1*-*bla*_{OXA-51}-like gene. (A) The IS*Aba1*-*bla*_{OXA-51}-like gene on the chromosome of *A. baumannii* with *fxsA* immediately upstream and a gene encoding a sortase (*srt*, ORF13) and another encoding a transcriptional regulator (ORF14) located downstream. The direct repeat (AAGTCTTAT) flanking IS*Aba1* is indicated with a thick vertical line. (B) Plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene and their surrounding sequences. ORF1 encodes a plasmid replicase, ORF2 encodes a protein belonging to the zeta-toxin family, and ORF8 (*nuc*) encodes a thermonuclease. ORF3 to ORF7 and ORF9 encode hypothetical proteins. The detailed sequences and annotation of the ORFs can be found in GenBank (accession no. GQ352402). ORF9 was inserted by a putative transposon, Tn6080, which contained IS*Aba1*-*bla*_{OXA-51}-like-*srt*-ORF14. The direct repeat (AAAAAATA) flanking Tn6080 is indicated with a thick vertical line. Regions with high similarity to that depicted in panel A are indicated by vertical dotted lines. The black horizontal arrows indicate the locations of primers listed in the Table 1. (C and D) Genetic structures of plasmids carrying the IS*Aba1*-*bla*_{OXA-51}-like gene that have partial similarity to (indicated with gray arrows) but lack some of the upstream fragments in (indicated with dotted lines) that depicted in panel B. Figures are not to scale.

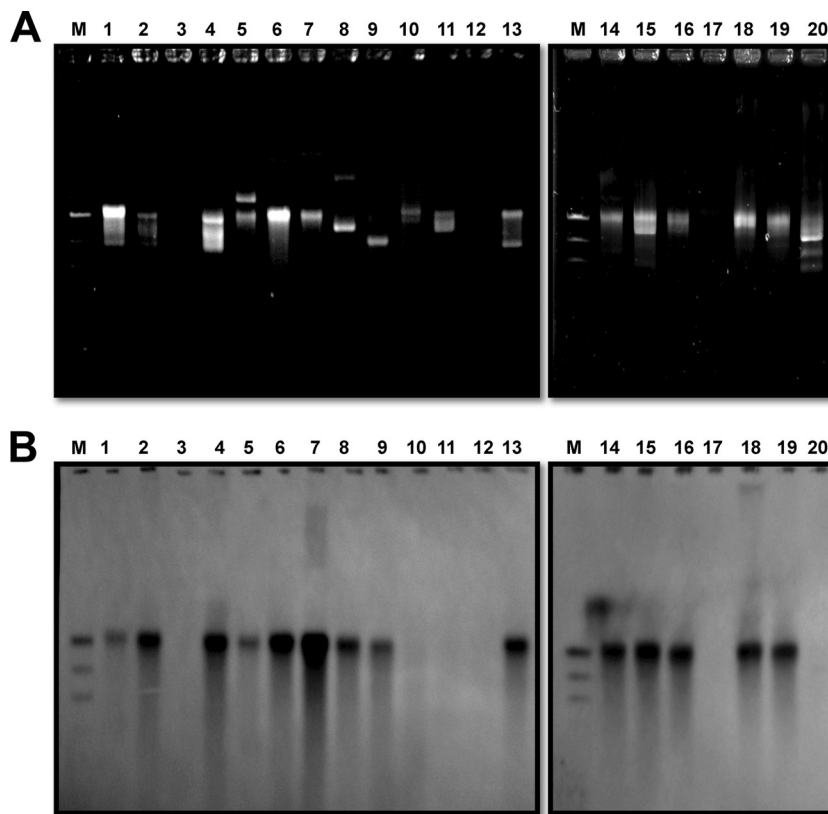


FIG. 3. Southern blot analysis depicting the plasmid location of the *bla*_{OXA-51}-like gene. (A) Gel migration patterns of the isolates. (B) Hybridization with the *bla*_{OXA-51}-like probe. The plasmids in lanes 3, 10, 11, 12, 17, and 20 were from isolates harboring the *fxsA*-*ISAbal*-*bla*_{OXA-51}-like structure (F type), and those in lanes 1, 2, 5, 6, 7, 8, 9, 13, 14, 15, 16, 18, and 19 were from isolates harboring the *nuc*-*ISAbal*-*bla*_{OXA-51}-like structure (N type). The plasmid in lane 4 was from an NF-type isolate.

Statistical analysis. The antimicrobial resistance rates of N-type and F-type isolates were compared using the chi-square test with Yates correction or Fisher's exact test with SPSS version 17 for Windows (SPSS, Chicago, IL). A *P* value of <0.05 was considered significant.

Nucleotide sequence accession numbers. The nucleotide sequences of *bla*_{OXA-172} to *bla*_{OXA-177} were assigned accession no. HM113558 to HM113563 in the GenBank database.

RESULTS

Bacterial isolates harboring the *ISAbal*-*bla*_{OXA-51}-like gene with different upstream genes. A total of 291 *A. baumannii* isolates were collected from 10 hospitals during the study period. One hundred seventeen isolates (40.2%) had *ISAbal* next to the *bla*_{OXA-51}-like gene (*ISAbal*-*bla*_{OXA-51}-like gene). The prevalence of the isolates harboring the *ISAbal*-*bla*_{OXA-51}-like gene ranged from 6.7 to 64.3% among the different hospitals (Fig. 1A). The sequence upstream of the *ISAbal*-*bla*_{OXA-51}-like gene could be PCR amplified with primers for either *nuc* (*nuc*-*ISAbal*-*bla*_{OXA-51}-like sequence, N type) or *fxsA* (*fxsA*-*ISAbal*-*bla*_{OXA-51}-like sequence, F type) in 54 and 59 isolates, respectively. Four isolates had both the *nuc* and *fxsA* upstream sequences, indicating that they had at least two copies of the *ISAbal*-*bla*_{OXA-51}-like gene, and were referred to as NF type. The percentages of N-type and F-type isolates were different among the 10 hospitals (Fig. 1B). Most of the isolates having the *ISAbal*-*bla*_{OXA-51}-like gene did not carry other CHDL genes. Among the N-type isolates, one (from hospital M3) also

had an *IS1008*-*bla*_{OXA-58} gene and three (all from hospital M2) had *ISAbal*-*bla*_{OXA-23}-like genes. Among the F-type isolates, one (from hospital N4) also had a *bla*_{OXA-24}-like gene. No MBL production was detected in the isolates.

Location of the *nuc*-*ISAbal*-*bla*_{OXA-51}-like structure in N- and NF-type isolates. Previous studies have shown that *nuc*-*ISAbal*-*bla*_{OXA-51}-like structures can be found in *Acinetobacter* plasmids (GenBank accession no. GQ352402 and EU670845). Hence, we determined whether the N-type or NF-type isolates also had plasmids harboring the *nuc*-*ISAbal*-*bla*_{OXA-51}-like structure. The results demonstrated that the N-type and NF-type isolates had different plasmid patterns (Fig. 3A), but Southern blot analysis revealed that all of them had a plasmid of similar size that harbored the *bla*_{OXA-51}-like (Fig. 3B) and *nuc* (data not shown) genes. The sizes of the plasmids were comparable to that of pAbSK-OXA-82. In addition, based on the I-CeuI mapping method, *nuc* was localized to a plasmid (Fig. 4B, lanes 3 and 4) that also contained the *bla*_{OXA-51}-like gene (data not shown) in N-type and NF-type isolates.

Partial genetic structures around the *ISAbal*-*bla*_{OXA-51}-like gene. PCR mapping with different primer sets (locations are depicted in Fig. 2A and B) showed that all the F-type and NF-type isolates had a genetic structure as depicted in Fig. 2A, and a direct repeat (AAGTCTTAT) was generated upon the insertion of *ISAbal* between the *fxsA* and *bla*_{OXA-51}-like genes. For the N-type and NF-type isolates, most (89.7%) had a

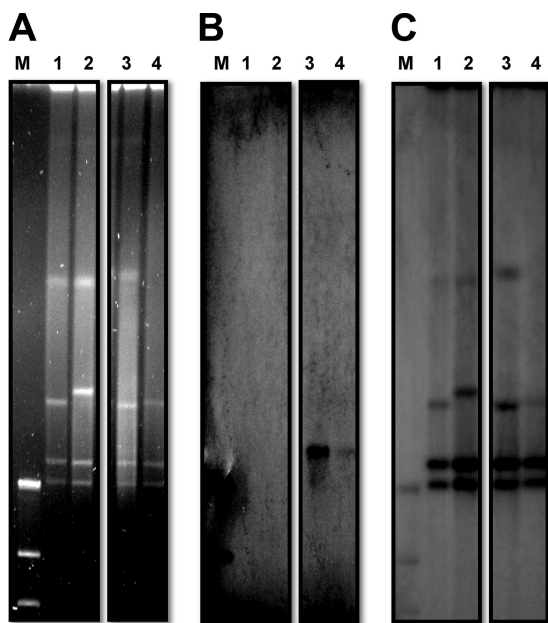


FIG. 4. Identification of the plasmid location of *nuc* by I-CeuI mapping. (A) Pulsed-field gel electrophoresis of I-CeuI-restricted genomic fragments. (B and C) Hybridization of the fragments with *nuc* (B) or 16S rRNA (C) probes. Lanes M, DNA molecular weight marker (DIG labeled); lanes 1, I-CeuI-restricted fragments from an isolate harboring only the *bla*_{OXA-51}-like gene without an upstream IS*Aba1* sequence; lanes 2, I-CeuI-restricted fragments from an isolate harboring the *fxsA*-IS*Aba1*-*bla*_{OXA-51}-like structure (F type); lanes 3, I-CeuI-restricted fragments from an isolate harboring the *nuc*-IS*Aba1*-*bla*_{OXA-51}-like structure (N type); lanes 4, I-CeuI-restricted fragments from an isolate harboring both genetic structures (NF type).

genetic arrangement around the *nuc*-IS*Aba1*-*bla*_{OXA-51}-like structure that was similar to that found in pAbSK-OXA-82 (Fig. 2B). The *srt* gene (Fig. 2B, ORF13) and a gene encoding a transcriptional regulator (Fig. 2B, ORF14) were also located downstream of a plasmid bearing IS*Aba1*-*bla*_{OXA-51}-like genes. The IS*Aba1*-*bla*_{OXA-51}-like-*srt*-ORF14 genetic structure was found inserted into ORF9 on the plasmid and flanked by a direct repeat (AAAAAATA), indicating the mobilization of these structures from the chromosome by one-ended transposition. The putative transposon carrying IS*Aba1*-*bla*_{OXA-51}-like-*srt*-ORF14 was designated Tn6080. Six isolates lacked some of the upstream structure, as depicted in Fig. 2C (five isolates) and D (one isolate).

Clonal relationship of N-type and NF-type isolates. The N-type and NF-type isolates belonged to 24 clones (Table 2). Nine of the clones were found in at least two hospitals. This indicated clonal dissemination between hospitals, with clone VII being the most widely disseminated.

It was found that most hospitals frequently contained isolates belonging to multiple clones, except hospital S1, in which all three isolates belonged to clone VII. The four NF-type isolates each belonged to different clones. F-type isolates that had the same pulsotype as the NF-type isolate were found in hospitals N3 and M1 (data not shown).

Antimicrobial MICs for the N-type and F-type isolates. After excluding the NF-type isolates and those that encoded other CHDL genes, the antimicrobial MICs for N-type (50 isolates) and F-type isolates (58 isolates) were compared. The carbapenem MICs for the N-type isolates were higher than those for the F-type isolates (Fig. 5). The N-type isolates had

TABLE 2. Characteristics of the *Acinetobacter baumannii* isolates harboring plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene^a

Isolate group and hospital	Pulsotype (no. of isolates and plasmid type ^b)	CHDL gene	Amino acid at position ^c :						
			42 (K)	106 (G)	129 (I)	167 (L)	199 (E)	222 (W)	226 (P)
N type									
N1	IV (2B), VI (2B), VII (2B, 2C), XII (1B), XIII (1B), XXIV(1B)	<i>bla</i> _{OXA-82}				V			
N2	IX (1B), XIV (1B), XXII (1D)	<i>bla</i> _{OXA-176}		S		V			
N3	IV (1B)	<i>bla</i> _{OXA-174}				V			K
N3	X (1B)	<i>bla</i> _{OXA-172}			V		L		
N4	I (1B), IV (1B)	<i>bla</i> _{OXA-82}				V			
N4	II (1B)	<i>bla</i> _{OXA-177}				V	G		
M1	III (1B), XV (1B), XVI (1C)	<i>bla</i> _{OXA-172}			V		L		
M2	V (2B), VII (3B), IX (8B), XIV (1B), XV (1B), XVII (1B)	<i>bla</i> _{OXA-172}			V		L		
M2	IX (1B)	<i>bla</i> _{OXA-173}			V		L	L	
M3	XVIII (1B), XIX (2B), XX (1B), XXI (3B), XXIII (1B)	<i>bla</i> _{OXA-82}				V			
M3	XI (1B)	<i>bla</i> _{OXA-175}	R			V			
S1	VII (3B)	<i>bla</i> _{OXA-82}				V			
S2	XIII (1B)	<i>bla</i> _{OXA-82}				V			
S3	VII (1B), VIII(1B)	<i>bla</i> _{OXA-82}				V			
NF type									
N3	I (1C)	<i>bla</i> _{OXA-82}				V			
M1	XI (1B)	<i>bla</i> _{OXA-82}				V			
M2	IX (1C)	<i>bla</i> _{OXA-82}				V			
M3	XXII (1B)	<i>bla</i> _{OXA-82}				V			

^a Abbreviations: CHDL, carbapenem-hydrolyzing class D beta-lactamase; N type, isolates harboring the *nuc*-IS*Aba1*-*bla*_{OXA-51}-like structure; NF type, isolates having both the *nuc*-IS*Aba1*-*bla*_{OXA-51}-like and *fxsA*-IS*Aba1*-*bla*_{OXA-51}-like structures (both *bla*_{OXA-51}-like genes in NF-type isolates are *bla*_{OXA-82}).

^b The plasmid type refers to the genetic structures depicted in Fig. 2, with type B having all the known partial sequences as depicted in Fig. 2B, type C as depicted in Fig. 2C, and type D as depicted in Fig. 2D.

^c The reference amino acids (in parentheses) and their positions are from OXA-66.

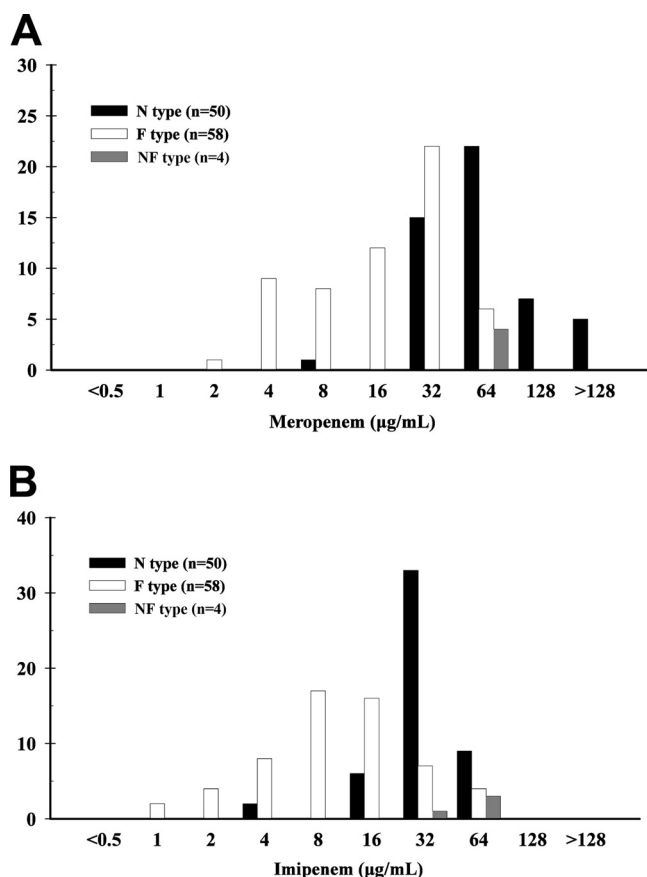


FIG. 5. Meropenem and imipenem MICs for isolates harboring plasmids bearing the *nuc-ISAbal-bla*_{OXA-51}-like structure (N type), the chromosomally encoded *fxsA-ISAbal-bla*_{OXA-51}-like structure (F type), or both genetic structures (NF type).

a higher imipenem resistance rate (98% versus 46.6%, $P < 0.001$) and a higher meropenem resistance rate (98% versus 69%, $P = 0.019$) than the F-type isolates. Unexpectedly, the N-type isolates also had a higher rate of resistance to sulbactam (80% versus 58.6%, $P = 0.029$). The N-type and F-type isolates had similar rates of resistance to cefepime (90% versus 98.3%, $P = 0.094$), ceftazidime (96 versus 98.3%, $P = 0.595$), piperacillin-tazobactam (90% versus 84.5%, $P = 0.573$), amikacin (98% versus 98.3%, $P = 1.000$), and ciprofloxacin (98% versus 98.3%, $P = 1.000$). All the isolates were susceptible to colistin.

Sequences of ISAbal-*bla*_{OXA-51}-like genes. Among the 54 N-type isolates, most of the *bla*_{OXA-51}-like genes were *bla*_{OXA-82} (27 isolates [50%]), followed by *bla*_{OXA-172} (20 isolates [37%]). Other *bla*_{OXA-51}-like genes included *bla*_{OXA-176} (three isolates) and *bla*_{OXA-173}, *bla*_{OXA-174}, *bla*_{OXA-175}, and *bla*_{OXA-177} (one isolate each). OXA-172 was different from OXA-82 at three amino acids (Table 2). *bla*_{OXA-82} could be found in isolates from different hospitals, but *bla*_{OXA-172} was found mostly in isolates from two hospitals in central Taiwan. Among 23 randomly selected F-type isolates, their *bla*_{OXA-51}-like genes were *bla*_{OXA-82} (39.1%), *bla*_{OXA-66} (34.8%), *bla*_{OXA-80} (21.7%), and *bla*_{OXA-83} (4.3%). Interestingly, the two copies of the *bla*_{OXA-51}-like gene in the four NF-type

isolates were all *bla*_{OXA-82}. The putative promoter sequences and their distances from *bla*_{OXA-51}-like were identical in the N, F, and NF-type isolates (data not shown).

Electrotransformation of a plasmid carrying the ISAbal-*bla*_{OXA-51}-like or *bla*_{OXA-51}-like gene. To determine whether a plasmid carrying only the ISAbal-*bla*_{OXA-51}-like gene (without the presence of other factors in the original plasmid) can confer a high level of carbapenem resistance, a recombinant plasmid harboring ISAbal-*bla*_{OXA-82} was electrotransformed into ATCC 15151^T and two randomly selected carbapenem-susceptible F-type isolates. The transformants demonstrated an increase in imipenem MIC in recipient *A. baumannii* ATCC 15151^T cells (from 0.5 to 32 µg/ml) and the F-type isolates (from 4 to 64 µg/ml). Transformation of the shuttle vector carrying *bla*_{OXA-82} (without ISAbal) only mildly increased the imipenem MIC for *A. baumannii* ATCC 15151^T (from 0.5 to 2 µg/ml). Transformation of the empty shuttle vector failed to increase carbapenem resistance levels.

DISCUSSION

In addition to the dissemination of extrinsic CHDL genes, including the *bla*_{OXA-23}-like, *bla*_{OXA-24}-like, and *bla*_{OXA-58}-like genes, by plasmids in *A. baumannii* (18), it is now evident that the *bla*_{OXA-51}-like gene and its upstream ISAbal sequence have disseminated in Taiwan via plasmids. The widespread distribution of isolates harboring plasmids bearing the ISAbal-*bla*_{OXA-51}-like gene in Taiwan is mostly due to plasmids spreading among different clones, as the isolated plasmids were all similar in size, contained the same plasmid replicase gene (ORF1) (Fig. 2B), and had similar structures around the ISAbal-*bla*_{OXA-51}-like gene. Clonal spreading of *A. baumannii* also contributed to some extent of the emergence of isolates harboring plasmids bearing ISAbal-*bla*_{OXA-51}-like genes, especially clone VII.

The origin of the ISAbal-*bla*_{OXA-82} plasmid might be deduced by analysis of F-type and NF-type isolates. Presumably, the ISAbal sequence was first transposed at the upstream region of chromosomal *bla*_{OXA-82}, as indicated by the flanking direct repeat (AAGTCTTAT) (Fig. 2A). Later, the genetic structure Tn6080 (ISAbal-*bla*_{OXA-82}-*prt*-ORF14) was copied and integrated into the plasmid in ORF9 via one-ended transposition, as evident by the flanking direct repeat (AAAAA ATA) (Fig. 2B). The one-ended transposition mechanism has also been suggested to be responsible for ISAbal-mediated mobilization of *bla*_{OXA-23} as transposon Tn2007 (6). The origin of the *bla*_{OXA-172} plasmid might have resulted from an independent transposition event, but it could have also originated from mutation of a *bla*_{OXA-82} plasmid, as these plasmids were similar; their CHDL genes differed at only a few nucleotides (data not shown), and the corresponding CHDLs differed at three amino acids (Table 2). Other *bla*_{OXA-51}-like genes were most likely derived from mutation of *bla*_{OXA-82} or *bla*_{OXA-172}.

Compared with the variation of carbapenem MICs in F-type isolates, some of which were below the resistance breakpoint, all but one of the N-type and all NF-type isolates carrying an ISAbal-*bla*_{OXA-51}-like plasmid were not susceptible to carbapenem. The higher carbapenem MIC in N-type relative to F-type isolates did not have any association with their *bla*_{OXA-51}-like promoters, which were identical among the isolates. Increase

gene dosage via high-copy plasmids is the most likely reason for the carbapenem resistance in isolates harboring plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene. Another possibility was that the high level of carbapenem resistance resulted from a synergistic effect from other determinants located on the same plasmid. This was unlikely, as transformation of a plasmid harboring only the IS*Aba1*-*bla*_{OXA-51}-like gene (IS*Aba1*-*bla*_{OXA-82}) was enough to confer a high level of carbapenem resistance. This phenomenon has also been recently demonstrated (16). Interestingly, the N-type isolates also had a higher sulbactam resistance rate, the mechanism of which is undetermined.

The detection of plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene is crucial, as it is central in the treatment of patients and control of plasmid spreading. However, it is not convenient to detect these plasmids by Southern blot analysis. PCR mapping might be a more feasible method for first-step screening. It has been shown that chromosomally carried *bla*_{OXA-51}-like genes are preceded by *fxsA*, based on the analysis of all available *A. baumannii* genomes. In isolates harboring the IS*Aba1*-*bla*_{OXA-51}-like gene, the possibility of mobilization of the IS*Aba1*-*bla*_{OXA-51}-like gene should be noted if PCR mapping fails to identify the upstream *fxsA*. In Taiwan, mapping of *nuc* upstream of the IS*Aba1*-*bla*_{OXA-51}-like gene indicates localization of the IS*Aba1*-*bla*_{OXA-51}-like gene to a plasmid.

In conclusion, plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene are widespread in carbapenem-resistant *A. baumannii* isolates in Taiwan. The origin of the plasmid-borne IS*Aba1*-*bla*_{OXA-51}-like gene is likely a result of transposition. The widespread distribution of these plasmids is due primarily to their spread among different *A. baumannii* clones, although spreading of the clones may also play a role. The plasmids bearing the IS*Aba1*-*bla*_{OXA-51}-like gene are associated with a high level of carbapenem resistance, indicating that detection of these genetic structures and prevention of their further spreading is warranted.

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