Types and Prevalence of Carbapenem-Resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* Complex in Northern Taiwan

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The frequency of the carbapenem-resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* (CRACB) complex increases annually in our hospitals. However, the types and prevalence of carbapenemases among isolates still remain unclear. In this study, we identified and collected 672 carbapenem-resistant isolates from a medical center in Northern Taiwan between April and December of 2010. There were 577 genospecies 2 (*Acinetobacter baumannii*), 79 genospecies 13TU, and 16 genospecies 3 isolates. The isolates had an acquired *bla*_{OXA-24}-like gene, which was confirmed by sequencing for the encoded OXA-72 carbapenemase, and were often associated with high-level carbapenem resistance. These CRACB complex isolates remained susceptible to colistin (100%). The genotyping of isolates was conducted using pulsed-field gel electrophoresis with *Apa*I digestion. In most clonally related groups, patients were from both branch hospitals. The results indicate that interhospital dissemination of clones occurred. This study provides updated data on the types and prevalence of the CRACB complex. In addition, it presents a warning on the emergence and spread of CRACB complex harboring *bla*_{OXA-24}-like genes in northern Taiwan.

Acinetobacter is an important nosocomial pathogen. Within the genus, the vast majority of species of clinical origin belong to the genetically closely related genospecies 2 (*Acinetobacter baumannii*), genospecies 13TU (AG13TU), and genospecies 3 (AG3). These genomic species are phenotypically similar and are collectively referred to as the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex with genospecies 1 (*Acinetobacter calcoaceticus*). Carbapenems have been widely used for treating the multi-drug-resistant *A. calcoaceticus*-*A. baumannii* complex. However, the carbapenem-resistant *A. calcoaceticus*-*A. baumannii* (CRACB) complex is increasingly reported as being caused by carbapenem-hydrolyzing class D β -lactamases (CHDLs), including OXA-51-like, OXA-23-like, OXA-24-like, and OXA-58-like genes, or metallo- β -lactamases (MBLs), including VIM, IMP, and SIM (1). In addition to β -lactamases, carbapenem resistance in the *A. calcoaceticus*-*A. baumannii* complex might also result from porin or penicillin-binding protein modifications (2, 3). According to an epidemiological investigation, the prevalence of the CRACB complex among nosocomial bacteria has risen in rank from the eighth (2006) to the first (2012) in our hospitals. This study analyzes the molecular characterization of carbapenemases in CRACB complex isolated in Taiwanese hospitals.

MATERIALS AND METHODS

Isolates and data collection. All clinical isolates of the CRACB complex with imipenem or meropenem MICs of ≥ 16 μ g/ml were collected from a medical center (comprising 2 branches) in northern Taiwan between April and December of 2010. The identification was performed using the VITEK2 system (bioMérieux Vitek Systems Inc., Hazelwood, MO). MICs were determined using the agar dilution method.

Pulsed-field gel electrophoresis (PFGE). Isolates were typed using PFGE (4) following the digestion of intact genomic DNA with *Apa*I (Bio-labs). DNA fragments were separated using 1% (wt/vol) agarose gels in a 0.5% Tris-borate-EDTA (TBE) buffer by using a CHEF DRII apparatus (Bio-Rad, Hercules, CA) at 6 V/cm, pulsed from 5 to 20 s, for 19 h at 14°C (5). A computer-assisted analysis was also performed using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was per-

TABLE 1 Oligonucleotide primers used in this study

Primer	Sequence (5'–3')	Reference
OXA-51-like forward	TAA TGC TTT GAT CGG CCT TG	6
OXA-51-like reverse	TGG ATT GCA CTT CAT CTT GG	6
OXA-23-like forward	GAT CGG ATT GGA GAA CCA GA	6
OXA-23-like reverse	ATT TCT GAC CGC ATT TCC AT	6
OXA-24-like forward	GGT TAG TTG GCC CCC TTA AA	6
OXA-24-like reverse	AGT TGA GCG AAA AGG GGA TT	6
OXA-58-like forward	AAG TAT TGG GGC TTG TGC TG	6
OXA-58-like reverse	CCC CTCTGCGCTCTACATAC	6
Imp-F	GGA ATA GAG TGG CTT AAY TCT C	7
Imp-R	CCA AAC YAC TAS GTT ATC T	7
Vim-F	GAT GGT GTT TGG TCG CAT A	7
Vim-R	CGA ATG CGC AGC ACC AG	7
Sim-F	TAC AAG GGA TTC GGC ATC G	7
Sim-R	TAA TGG CCT GTT CCC ATG TG	7
ISAba1B	CAT GTA AAC CAA TGC TCA CC	8
ISAba2A	AAT CCG AGA TAG AGC GGT TC	8
ISAba3C	AGC AAT ATC TCG TAT ACC GC	8
IS18B	ACC AGC CAT AAC TTC ACT CG	8
ISAba4A	ATT TGA ACC CAT CTA TTG GC	9

formed using the unweighted pair group method with mathematical averaging (UPGMA), and DNA relatedness was calculated using the band-based Dice coefficient for the whole profile. Isolates were considered to be of the same PFGE type if they were $>87\%$ similar.

PCR detection of carbapenemase genes and insertion sequences. The CHDL- and MBL-encoding genes were detected using PCR methods suggested previously by Woodford et al. (6) and Ellington et al. (7), respectively. PCR was used to detect the insertion sequence (IS) preceding the *bla* gene by using a forward primer of IS and a reverse primer of the

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TABLE 2 Distribution of OXA carbapenemase genes in carbapenem-resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex isolates in this study

Organism	Target allele(s)	No. (%) of isolates
<i>A. baumannii</i>		577
	OXA-51-like	577 (100)
	OXA-51-like only	173 (30.0)
	OXA-51-like + OXA-23-like	391 (67.8)
	OXA-51-like + OXA-24-like	13 (2.2)
AG13TU		79
	OXA-23-like	51 (64.6)
	OXA-24-like	6 (7.6)
	OXA-58-like	2 (2.5)
	IMP-1	1 (1.3)
	OXA-58-like + OXA-24-like	8 (10.1)
	OXA-58-like + IMP-1	11 (13.9)
AG3		16
	OXA-23-like	9 (56.1)
	OXA-24-like	1 (6.3)
	OXA-58-like	1 (6.3)
	IMP-1	1 (6.3)
	OXA-58-like + IMP-1	4 (25.0)

OXA gene. All primers used in this study are listed in Table 1. Sequence similarity searches were conducted using the BLAST program (<http://www.ncbi.nlm.nih.gov/blast>).

RESULTS

Among the 672 nonrepetitive isolates of the CRACB complex, there are 577 *Acinetobacter baumannii* isolates, 79 genospecies 13TU (AG13TU) isolates, and 16 genospecies 3 (AG3) isolates. These isolates originated mainly from intensive care unit patients (62%). All the carbapenem-resistant *A. baumannii* (CRAB) isolates harbored the *bla*_{OXA-51}-like gene (Table 2). This gene is well known to be intrinsically located in *A. baumannii* isolates (8). Other CHDL-encoding genes were detected. The *bla*_{OXA-23}-like gene was the most common (67.8%; 391/577) (Table 2), followed by a lower occurrence of the *bla*_{OXA-24}-like gene (2.2%; 13/577). None of the CRAB isolates had a *bla*_{OXA-58}-like gene or any of the MBL-encoding genes. In total, 173 CRAB isolates (30.0%; 173/577) contained the *bla*_{OXA-51}-like gene only, of which all harbored *ISAbal* upstream. In these cases, there are 7 bases between the IS element and the *bla*_{OXA-51}-like gene. In the isolates possessing both the *bla*_{OXA-51}-like gene and the *bla*_{OXA-23}-like gene (67.8%), *ISAbal* was mostly associated with the *bla*_{OXA-23}-like gene. Thirty-three bases separated the IS element and the *bla*_{OXA-23}-like gene. However, in 16.0% of these isolates, *ISAbal* was associated with both the *bla*_{OXA-23}-like gene and the *bla*_{OXA-51}-like gene. The characterization of the sequences surrounding the *bla*_{OXA-24}-like genes did not reveal any IS elements. In 15.4% of isolates possessing both the *bla*_{OXA-51}-like gene and the *bla*_{OXA-24}-like gene, *ISAbal* was associated with the *bla*_{OXA-51}-like gene.

Of the CRAG13TU isolates, the *bla*_{OXA-23}-like gene accounted for 64.6% (51/79) (Table 2), followed by 7.6% (6/79) possessing the *bla*_{OXA-24}-like gene, 2.5% (2/79) possessing the *bla*_{OXA-58}-like gene, 1.3% (1/79) possessing the MBL-encoding gene IMP-1, 10.1% (8/79) possessing both the *bla*_{OXA-24}-like gene and the *bla*_{OXA-58}-like gene, and 13.9% (11/79) harboring both the

*bla*_{OXA-58}-like gene and the IMP-1 gene. Of the CRAG3 isolates, the *bla*_{OXA-23}-like gene accounted for 56.1% (9/16) (Table 2), followed by 6.3% (1/16) possessing the *bla*_{OXA-24}-like gene, 6.3% (1/16) possessing the *bla*_{OXA-58}-like gene, 6.3% (1/16) harboring the IMP-1 gene, and 25.0% (4/16) harboring both the *bla*_{OXA-58}-like gene and the IMP-1 gene. In all CRAG13TU and CRAG3 isolates, the IMP-1 gene is located in a class 1 integron, and the *bla*_{OXA-58}-like gene is bracketed by *ISAbal*.

FIG 1 Distribution of imipenem MICs in carbapenem-resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex isolates in this study. (A) *Acinetobacter baumannii*; (B) genospecies 13TU; (C) genospecies 3.

TABLE 3 Antibiotic susceptibilities of 677 carbapenem-resistant *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex isolates

Antibiotic	% nonsusceptible (no. of isolates)		
	<i>A. baumannii</i> (<i>n</i> = 577)	AG13TU (<i>n</i> = 79)	AG3 (<i>n</i> = 16)
Ampicillin-sulbactam	96.2 (555)	51.9 (41)	31.3 (5)
Cefpirome	100.0 (577)	100.0 (79)	100.0 (16)
Ceftazidime	100.0 (577)	46.8 (37)	87.5 (14)
Ciprofloxacin	100.0 (577)	24.1 (19)	56.3 (9)
Gentamicin	100.0 (577)	29.1 (23)	43.8 (7)
Tigecycline	63.1 (364)	21.5 (17)	0.0 (0)
Colistin	0.0 (0)	0.0 (0)	0.0 (0)

The CRACB complex isolates harbored the *bla*_{OXA-24}-like gene, the *bla*_{OXA-58}-like gene, and/or the IMP-1 gene, which were more frequently associated with a high level of carbapenem resistance (Fig. 1). These acquired *bla*_{OXA-24}-like genes were confirmed by sequencing for encoding the OXA-72 carbapenemase. All CRACB isolates were also coresistant to ceftazidime and remained susceptible to colistin (Table 3). All CRAB isolates were also coresistant to ceftazidime, ciprofloxacin, and gentamicin. The susceptibility rate was 3.8% for ampicillin-sulbactam and 36.9% for tigecycline. The CRAG13TU isolates still had a 48.1%, 53.2%, 75.9%, 70.9%, and 78.5% susceptibility rate to ampicillin-sulbactam, ceftazidime, ciprofloxacin, gentamicin, and tigecycline, respectively. The CRAG3 isolates had a 68.7%, 12.5%, 43.7%, and 56.2% susceptibility rate for ampicillin-sulbactam, ceftazidime, ciprofloxacin, and gentamicin, respectively. However, CRAG3 isolates were all still susceptible to tigecycline (100%).

Genotyping of isolates was conducted using PFGE with ApaI digestion. The results showed that CRAB isolates that harbored only the *bla*_{OXA-51}-like gene, both the *bla*_{OXA-51}-like gene and the *bla*_{OXA-23}-like gene, and both the *bla*_{OXA-51}-like gene and the *bla*_{OXA-24}-like gene (Fig. 2) exhibited 102, 227, and 7 distinct genotypes, respectively, which were classified into 12, 22, and 1 clonally related groups, respectively. CRAG13TU isolates exhibited 35 distinct genotypes, which were classified into 7 clonally related groups. CRAG3 isolates exhibited 12 distinct genotypes, which

were classified into 5 clonally related groups. In most clonally related groups, patients were from 2 branch hospitals. Thus, the results indicate that the interhospital dissemination of clones had occurred.

DISCUSSION

An annual increase in the proportion of the CRACB complex in Taiwan from 17% in 2003 to 70% in 2013 was documented by the Taiwan Nosocomial Infection Surveillance (TNIS) System. According to the national surveillance data, the proportions of the CRACB complex in Taiwan were higher than those in the United States and Europe. In our study, the CRAB isolates that carry the *bla*_{OXA-23}-like gene were the most common (67.8%). Kuo et al. reported that the occurrence of the *bla*_{OXA-23}-like gene was 58% in hospital C in northern Taiwan (9). A report from South Korea revealed that the occurrence of the *bla*_{OXA-23}-like gene was 77% (10). These results are similar to those in our report. However, a report from another medical center in northern Taiwan revealed that only 3.4% of CRAB isolates harbored the *bla*_{OXA-23}-like gene (11). Reports from 2 hospitals in central Taiwan showed a higher occurrence of the *bla*_{OXA-23}-like gene (93% and 96%, respectively) (12, 13). Meanwhile, the *bla*_{OXA-23}-like gene was less common (0% and 1.1%, respectively) in 2 hospitals in southern Taiwan (14, 15). The prevalence of CRAB isolates carrying the *bla*_{OXA-24}-like gene in our study was 2.2%, a result that is similar to those reported by 2 other hospitals in northern Taiwan (3% and 3.4%, respectively) (9, 11) but differs from those reported by the hospitals in central Taiwan (0% and 0.5%, respectively) (12, 13) and southern Taiwan (14% and 64%, respectively) (14, 15). We propose that the types and prevalence of CHDLs among CRAB isolates might display great geographical differences.

The presence of IS elements upstream of the *bla*_{OXA-51}-like genes, *bla*_{OXA-23}-like genes, and *bla*_{OXA-58}-like genes indicates that they might play a role in their expression by providing promoter sequences. Although the CHDLs encoded by these genes have a relatively weak ability to hydrolyze carbapenems, these enzymes might be overexpressed, resulting in resistance to carbapenems. In isolates containing the *bla*_{OXA-51}-like gene but not containing IS*Aba1* upstream, their resistance to carbapenems might be ex-

FIG 2 Pulsed-field gel electrophoresis patterns of *A. baumannii* isolates harboring both the *bla*_{OXA-24}-like gene and the *bla*_{OXA-51}-like gene, revealed in 7 distinct genotypes that were classified into 3 clusters (A to C). The scale indicates the percentage of genetic similarity. Underlined isolate numbers correspond to patients from different hospitals.

plained by other mechanisms, such as the modification of porins and penicillin-binding proteins or the upregulation of the AdeABC efflux system (16). In isolates possessing both the *bla*_{OXA-51}-like gene and the *bla*_{OXA-23}-like gene, 16.0% had *ISAbal* associated with both the *bla*_{OXA-23}-like gene and the *bla*_{OXA-51}-like gene, indicating that coexpression of these 2 genes occurred. These results differ from those obtained by Turton et al. (17). They reported that *ISAbal* was consistently associated with the *bla*_{OXA-23}-like gene and not with the *bla*_{OXA-51}-like gene. Isolates with the *bla*_{OXA-24}-like gene were more often associated with a high level of carbapenem resistance. Our study did not find any IS elements surrounding the *bla*_{OXA-24}-like genes. Merino et al. demonstrated that all the plasmids isolated from the isolates they collected carried the *bla*_{OXA-24}-like gene flanked by XerC/XerD-like binding sites (18). Their findings show that Xer recombination might be responsible for mobilizing the *bla*_{OXA-24}-like gene. Further experiments will be conducted to confirm this hypothesis.

Large-scale surveys on the prevalence of these carbapenemase genes throughout Taiwan remain scarce. The present study not only demonstrates updated data regarding the molecular epidemiology of carbapenemases in the CRACB complex but also warns of the emergence and spread of the CRACB complex harboring the *bla*_{OXA-24}-like gene in northern Taiwan.

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