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EPIDEMIOLOGY AND SURVEILLANCE

Molecular Epidemiology of Emerging $bla_{OXA-23-Like}$- and $bla_{OXA-24-Like}$-Carrying *Acinetobacter baumannii* in Taiwan

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ABSTRACT The rate of recovery of carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates has increased significantly in recent decades in Taiwan. This study investigated the molecular epidemiology of CRAB with a focus on the mechanisms of resistance and spread in isolates with $bla_{OXA-23-like}$ or $bla_{OXA-24-like}$. All 555 CRAB isolates in our multicenter collection, which were recovered from 2002 to 2010, were tested for the presence of class A, B, and D carbapenemase genes. All isolates with $bla_{OXA-23-like}$ or $bla_{OXA-24-like}$ were subjected to pulsed-field gel electrophoresis, and 82 isolates (60 isolates with $bla_{OXA-23-like}$ and 22 isolates with $bla_{OXA-24-like}$) were selected for multilocus sequence typing to determine the sequence type (ST) and clonal group (CG) and for detection of additional β-lactamase and aminoglycoside resistance genes. The flanking regions of carbapenem and aminoglycoside resistance genes were identified by PCR mapping and sequencing. The localization of bla_{OXA} was determined by S1 nuclease and I-CeuI assays. The numbers of CRAB isolates carrying $bla_{OXA-23-like}$ or $bla_{OXA-24-like}$, especially those carrying $bla_{OXA-23-like}$, increased significantly from 2008 onward. The $bla_{OXA-23-like}$ gene was carried by antibiotic resistance genomic island 1 (AbGRI1)-type structures located on plasmids and/or the chromosome in isolates of different STs (CG92 and novel CG786), whereas $bla_{OXA-24-like}$ was carried on plasmids in CRAB isolates of limited STs (CG92). No class A or B carbapenemase genes were identified. Multiple aminoglycoside resistance genes coexisted in CRAB. Tn*6180*-borne *armA* was found in 74 (90.2%) CRAB isolates, and 58 (70.7%) isolates had Tn*6179* upstream, constituting AbGRI3. bla_{TEM} was present in 38 (46.3%) of the CRAB isolates tested, with 35 (92.1%) isolates containing bla_{TEM} in AbGRI2-type structures, and 61% of *ampC* genes had IS*Aba1* upstream. We conclude that the dissemination and spread of a few dominant lineages of CRAB containing various resistance island structures occurred in Taiwan.

KEYWORDS *Acinetobacter baumannii*, carbapenem resistance, AbGRI, aminoglycoside resistance, transposon

Acinetobacter baumannii has become one of the most important pathogens because of its high prevalence of multidrug resistance, and the rate of recovery of carbapenem-resistant *A. baumannii* (CRAB) isolates has especially increased worldwide during the past few decades (1–3). CRAB isolates are concomitantly resistant to multiple antibiotics, and infections caused by CRAB are associated with higher rates of mortality and prolonged hospital stays because of limited treatment options and the increased chance of inappropriate therapy (2, 4, 5).

The class D carbapenemases are one of the most important mechanisms responsible for carbapenem resistance in *Acinetobacter*, and of these, OXA-23-like and/or OXA-24-like has commonly been reported to be the most prevalent carbapenemase in many

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countries (1, 2, 6). Our prior cross-sectional multicenter studies also showed different distributions of carbapenemase genes among geographic regions in Taiwan (7, 8). The rapid acquisition of carbapenem resistance determinants has been attributed to transposons or plasmids (1, 9). The $bla_{\text{OXA-23-like}}$ gene is almost always found within a transposon, which is commonly associated with an antibiotic resistance genomic island (AbGRI) (9), and $bla_{\text{OXA-24-like}}$ is influenced by clonal spread and sometimes horizontal transfer by plasmids (10). While these cross-sectional studies provided information on and an understanding of the molecular epidemiology and mechanism of carbapenem resistance, little effort was focused on determination of their dynamic changes over time and mechanisms of spread.

The Taiwan Surveillance of Antimicrobial Resistance (TSAR), a longitudinal multicenter surveillance program, has observed a dramatic increase in the rates of carbapenem resistance among *A. baumannii* isolates over the years (from 3.4% in 2002 to 58.7% in 2010) (11). Using isolates collected in the TSAR program, the aim of this study was to detail the prevalence of and dynamic changes to the carbapenemase gene in CRAB over the 8-year span and the diverse mechanisms that CRAB strains have adopted to become carbapenem resistant. In addition to carbapenems, *A. baumannii* can easily acquire resistance to other antibiotics through a variety of mechanisms, especially lateral gene transfer (1, 12–14). For example, insertion of IS*Aba1* provides promoter activity that drives the overexpression of the downstream AmpC β-lactamases (15). Recent studies also found IS*26*-mediated recombination events in the antibiotic resistance genomic island to be responsible for the different resistance genes and their variants, including those encoding aminoglycoside resistance, observed in *A. baumannii* (16, 17). Therefore, the prevalence of determinants of resistance to β-lactams and aminoglycosides and their mechanisms of spread in representative CRAB isolates were also investigated.

RESULTS AND DISCUSSION

All 555 CRAB isolates were positive for $bla_{\text{OXA-51-like}}$. Among them, 227 (40.9%) had IS*Aba1*-$bla_{\text{OXA-51-like}}$, 292 (52.6%) also had $bla_{\text{OXA-23-like}}$, 59 (10.6%) also had $bla_{\text{OXA-24-like}}$, and 6 (1.1%) also had $bla_{\text{OXA-58-like}}$ (see Table S1). The concomitant existence of the carbapenem-hydrolyzing class D β-lactamases (excluding $bla_{\text{OXA-51}}$ without IS*Aba1* upstream) was found in 40 isolates (see Table S2 in the supplemental material). Twelve isolates were negative for all of the four class D β-lactamase genes as well as the $bla_{\text{OXA-143-like}}$ and $bla_{\text{OXA-235-like}}$ genes. Neither class A (bla_{NMC}, bla_{SME}, bla_{IMI}, bla_{KPC}, and bla_{GES}) nor class B (bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM}, $bla_{\text{SIM-1}}$, and $bla_{\text{NDM-1}}$) β-lactamase genes with carbapenemase activity were detected.

Isolates with IS*Aba1*-$bla_{\text{OXA-51-like}}$ comprised all the CRAB isolates recovered in 2002 and 2004 (6 and 45 isolates, respectively), but this proportion decreased to 15.9% (33/208) in 2010 for the whole island of Taiwan. In contrast, isolates with $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ emerged in 2006, and their prevalence quickly increased, from 4.3% (4/94) and 8.5% (8/94), respectively, in 2006 to 61.4% (124/202) and 8.9% (18/202), respectively, in 2008 and 78.8% (164/208) and 15.9% (33/208), respectively, in 2010 (Table S1) with a regional predominance. By 2010, isolates with $bla_{\text{OXA-23-like}}$ comprised >90% of the CRAB isolates from northern and central Taiwan, while those with $bla_{\text{OXA-24-like}}$ comprised >70% of the CRAB isolates from southern and eastern Taiwan (Fig. 1a and Table S3). Although isolates with $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, or IS*Aba1*-$bla_{\text{OXA-51-like}}$ had similar susceptibility profiles (Fig. 1b), the MICs of several other β-lactams, including cefepime and ampicillin-sulbactam, were higher for $bla_{\text{OXA-23-like}}$ transformants (Table 1). The contribution of OXA-23 to cefepime resistance was shown a decade ago, with the MICs of cefepime for $bla_{\text{OXA-23}}$ transformants being increased by 8-fold (18). A recent kinetic study also showed that OXA-23 is weakly inhibited by sulbactam (19).

Pulsed-field gel electrophoresis (PFGE) was performed on all $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$-positive CRAB isolates to determine whether their increase was due to clonal spread. The results (not shown) indicated that isolates with $bla_{\text{OXA-23-like}}$ be-

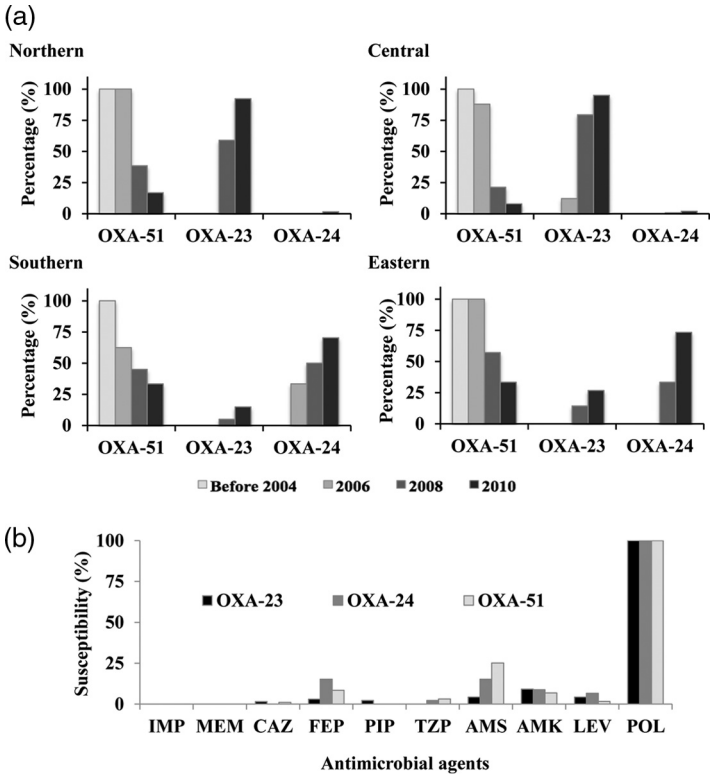

FIG 1 Secular change of carbapenem-resistant *Acinetobacter baumannii* (CRAB) carrying specific carbapenemase genes recovered from different regions of Taiwan from 2002 to 2010 (a) and their susceptibility to antibiotics (b). Isolates carrying multiple carbapenemase genes were counted more than once. OXA-51, CRAB isolates with IS*Aba1*-*bla*$_{OXA-51-like}$; OXA-23, CRAB isolates with *bla*$_{OXA-23-like}$, and OXA-24, CRAB isolates with *bla*$_{OXA-24-like}$. IMP, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; AMS, ampicillin-sulbactam; AMK, amikacin; LEV, levofloxacin; POL, polymyxin B.

longed to diverse clonal groups (pulsotypes 23A to 23E and multiple other distinct pulsotypes), while those with *bla*$_{OXA-24-like}$ (pulsotypes 24A to 24C) were less diverse. A total of 82 representative isolates (60 with *bla*$_{OXA-23-like}$ and 22 with *bla*$_{OXA-24-like}$) from different pulsotypes and collection years (Fig. S1) were selected for multilocus sequence typing (MLST). The sources of these isolates are shown in Table 2. eBURST analysis of the MLST data (Fig. 2) showed that the majority of isolates with *bla*$_{OXA-23-like}$ genes belonged to clonal group 92 (CG92), which is also referred to as international clone II (3, 20, 21). In addition, a new local CG, CG786, was also identified. CG786 includes sequence type 550 (ST550), ST551, ST555, and ST685, as well as three newly assigned STs (ST786, ST789, and ST792). CG92 and CG786 differed in the *gltA*, *cpn60*, *gpi*, and *rpoD* gene loci. In contrast, isolates with *bla*$_{OXA-24-like}$ were highly similar, and all belonged to CG92 (international clone II). CC92 has been prevalent in Asia (22), and

TABLE 1 Susceptibility of transformants with different carbapenemases

Strain	MIC (mg/liter)[a]								
	IMP	MEM	CAZ	FEP	TZP	AMS	AMK	LEV	POL
Wild-type *A. baumannii* ATCC 15151	≤0.25	≤0.25	≤1	≤1	≤4	≤2	≤2	≤0.12	≤0.5
Wild type with shuttle vector pYMAb2	≤0.25	≤0.25	≤1	≤1	≤4	≤2	≤2	≤0.12	≤0.5
Transformant with IS*Aba1*-*bla*$_{OXA-23}$	4	≥16	4	32	≥128	16	≤2	≤0.12	≤0.5
Transformant with *bla*$_{OXA-72}$[b]	4	≥16	4	2	≥128	4	≤2	≤0.12	≤0.5
Transformant with IS*Aba1*-*bla*$_{OXA-82}$[b]	4	≥16	4	2	≥128	≤2	≤2	≤0.12	≤0.5
Transformant with IS*Aba1*-*bla*$_{OXA-66}$[b]	1	2	4	2	≥128	4	≤2	≤0.12	≤0.5

[a]IMP, imipenem; MEM, meropenem; CAZ, ceftazidime; FEP, cefepime; TZP, piperacillin-tazobactam; AMS, ampicillin-sulbactam; AMK, amikacin; LEV, levofloxacin; POL, polymyxin B.

[b]*bla*$_{OXA-72}$ is a variant of *bla*$_{OXA-24-like}$ genes, whereas *bla*$_{OXA-66}$ and *bla*$_{OXA-82}$ are variants of *bla*$_{OXA-51-like}$ genes.

TABLE 2 Clinical information and molecular characteristics of selected CRAB isolates with $bla_{OXA\text{-}23\text{-}like}$ or $bla_{OXA\text{-}24\text{-}like}$

Characteristic	No. (%) of CRAB isolates with:		
	$bla_{OXA\text{-}23\text{-}like}$	$bla_{OXA\text{-}24\text{-}like}$	Overall
Total	60	22	82
Period of isolation			
Before 2006	2 (3.3)	5 (22.7)	7 (8.5)
2008	27 (45)	7 (31.8)	34 (41.5)
2010	31 (51.7)	10 (45.5)	41 (50)
Hospital level			
Medical center	19 (31.7)	9 (40.9)	28 (34.1)
Regional hospital	41 (68.3)	13 (59.1)	54 (65.9)
Region of Taiwan			
Eastern	0 (0)	4 (18.2)	4 (4.9)
Central	44 (73.3)	0 (0)	44 (53.7)
Northern	16 (26.7)	1 (4.5)	17 (20.7)
Southern	0 (0)	17 (77.3)	17 (20.7)
Specimen source			
Respiratory	41 (68.3)	12 (54.5)	53 (64.6)
Pus/discharge	6 (10)	5 (22.7)	11 (13.4)
Blood	4 (6.7)	1 (4.5)	5 (6.1)
Urine	7 (11.7)	2 (9.1)	9 (11)
Others	2 (3.3)	2 (9.1)	4 (4.9)
Patient age group			
Adult	12 (20)	7 (31.8)	19 (23.2)
Elderly	42 (70)	13 (59.1)	55 (67.1)
Pediatric	1 (1.7)	2 (9.1)	3 (3.7)
Unknown	5 (8.3)	0 (0)	5 (6.1)
Presence of other resistance genes			
armA	55 (91.7)	21 (95.5)	76 (92.7)
aphA1	52 (86.7)	20 (90.9)	72 (87.8)
aphA6	3 (5)	1 (4.5)	4 (4.9)
aacC1	48 (80)	20 (90.9)	68 (82.9)
aacA4	54 (90)	20 (90.9)	74 (90.2)
aadB	0 (0)	3 (13.6)	3 (3.7)
aadA1	58 (96.7)	21 (95.5)	79 (96.3)
bla_{TEM}	28 (46.7)	10 (45.5)	38 (46.3)
IS*Aba1-ampC*	34 (56.7)	16 (72.7)	50 (61)
bla_{SHV}	0 (0)	0 (0)	0 (0)
bla_{PER}	0 (0)	0 (0)	0 (0)

our study showed that this clone, carrying either $bla_{OXA\text{-}23\text{-}like}$ or $bla_{OXA\text{-}24\text{-}like}$, is also widespread in Taiwan. In contrast, CG786, which appeared in 2006, has limited its spread locally to northern and central Taiwan (data not shown).

Sequencing of the $bla_{OXA\text{-}23\text{-}like}$ genes from 60 selected isolates showed that they were all $bla_{OXA\text{-}23}$. PCR mapping of Tn*2006* was carried out, as previous studies suggested (23). Tn*2006* could be embedded in different backbones of Tn*6022*, which belongs to AbGRI1 (9), and our study showed the presence of at least 2 different Tn*6022* constructs, Tn*6166* (*tniD*) and Tn*6166* (Δ*tniD*) (Fig. S2c), which has been found to be widespread in Asia (22). Tn*6166* (*tniD*) was present in 53 isolates, Tn*6166* (Δ*tniD*) was present in 16 isolates, and 11 isolates had both types of Tn*6022*. The remaining 2 isolates had an unidentified genetic environment flanking Tn*2006*. The direct repeat flanking Tn*2006* was sequenced in 10 isolates with different AbGRI1 types, and in all isolates the sequence was ATTCGCGGG (9), indicative of an AbaR4 structure. S1 nuclease and I-CeuI assays were performed on 22 $bla_{OXA\text{-}23}$ isolates, and $bla_{OXA\text{-}23}$ was located in the chromosomes of 16 isolates and on plasmids in 14 isolates; that is, in 8 isolates, $bla_{OXA\text{-}23}$ could be detected both in the chromosome and on plasmids (Fig. S2a

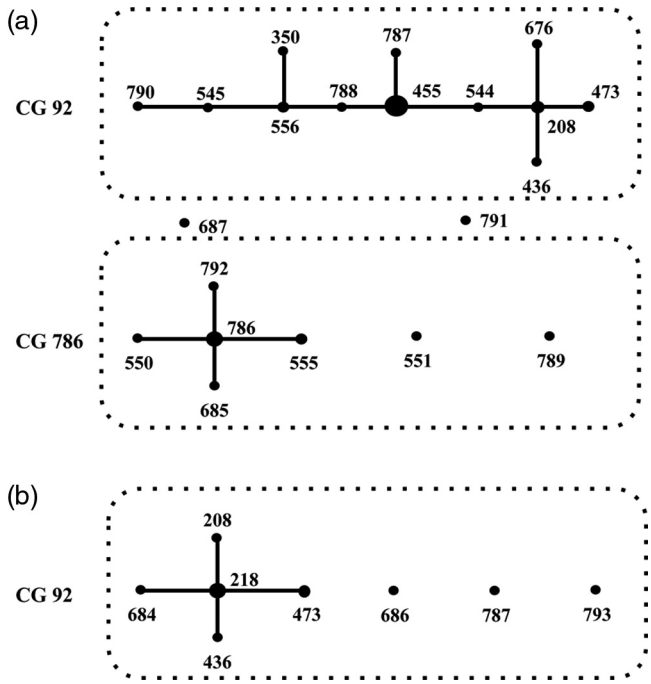

FIG 2 Clonal groups (CGs) of carbapenem-resistant *Acinetobacter baumannii* isolates with *bla*$_{OXA-23-like}$ (a) or *bla*$_{OXA-24-like}$ (b), determined using eBURST analysis. A CG was defined as a group of STs sharing at least 5 identical loci among the 7 housekeeping genes tested.

and b). Among the *bla*$_{OXA-23}$-carrying plasmids with six different sizes identified, a 90-kbp plasmid was the most prevalent (12 of 22 isolates). Although *bla*$_{OXA-23-like}$ has been reported to be located either on plasmids or in the chromosome (23), few studies have reported their presence in both. In contrast to the single copy of *bla*$_{OXA-23-like}$ observed in other studies (22, 24), we detected the carriage of multiple copies of *bla*$_{OXA-23}$ by different AbGRI1 types in some of our isolates (Fig. S2a and b). Further investigation of the presence of AbaR4 within AbGRI1 elsewhere in the chromosome or on plasmids is warranted.

The amino acid sequences of the OXA-24-like enzymes in all 22 selected isolates showed that they belonged to OXA-72. An outbreak of CRAB has commonly been attributed to the transmission of various *bla*$_{OXA-24-like}$-carrying plasmids (10). Among the *bla*$_{OXA-72}$-positive CRAB isolates that we studied, the S1 nuclease assay showed that the *bla*$_{OXA-72}$ gene was located in six different patterns on 8 plasmids ranging from 9 to 90 kb in size (Fig. S3). PCR mapping indicated that the sequence of a 9-kb *bla*$_{OXA-72}$-carrying plasmid with pattern I was similar to that of a previously reported plasmid carrying *bla*$_{OXA-24-like}$ (pAB-NCGM253; GenBank accession no. NC_021489.1) (14).

The high prevalence of CRAB with *bla*$_{OXA-23-like}$ had been repeatedly reported in cross-sectional surveillance studies (1). The present nationwide longitudinal surveillance revealed dynamic changes to isolates with carbapenemase genes over the 8 study years in Taiwan. The exact reason that isolates with *bla*$_{OXA-23-like}$ replaced those with IS*Aba1*-*bla*$_{OXA-51-like}$ is unknown. Our findings of the diverse clonality of CRAB carrying *bla*$_{OXA-23-like}$, the colocalization of *bla*$_{OXA-23-like}$ in the chromosome and/or on different plasmids, and the presence of different genetic backgrounds flanking *bla*$_{OXA-23-like}$ imply the long and successful evolution of these strains in Taiwan. The same direct repeats flanking Tn*2006* indicated the importance of clonal expansion in isolates with *bla*$_{OXA-23-like}$, with plasmid-borne *bla*$_{OXA-23-like}$ providing other means of transmission (9). The contribution of OXA-23-like to additional resistance to other β-lactams (Table 1) may also provide some advantages over the overproduction of OXA-51-like. The presence of *bla*$_{OXA-24-like}$ in plasmids of similar patterns carried by CRAB isolates with the same CG content indicated that their spread was most likely due

to clonal dissemination and therefore was limited to certain regions of Taiwan (southern and eastern Taiwan).

We also tested for other β-lactamase genes that have been commonly reported in *A. baumannii* (Table 2) (1, 2). The bla_{TEM} gene was present in 38 of 82 isolates (46.3%), but no bla_{SHV} or bla_{PER} gene was identified (Table 2). Since the bla_{TEM} gene is usually seen within AbGRI2 or AbGRI3 (25) and since whole-genome sequencing of one of the isolates found its bla_{TEM} to be located within AbGRI2 (data not shown), a PCR targeting this structure was employed, and 35 (92.1%) of the 38 isolates with bla_{TEM} were positive (where bla_{TEM} was found within ABA1_01191Δ-bla_{TEM}-Δ$tnpA_{1000}$). All 82 isolates also had *Acinetobacter* AmpC genes (also called *Acinetobacter*-derived cephalosporinase [bla_{ADC}]) since its chromosomal location is inherent to *A. baumannii* (1, 26). Overproduction of these AmpCs provides *A. baumannii* intrinsic resistance to penicillins, cephamycins, and even oxyiminocephalosporins (26, 27). Various insertion sequence elements, the most common of which is IS*Aba1*, have been shown to provide strong promoter activity to facilitate overexpression of downstream genes (28). We also found that 50 (61.0%) of our isolates had IS*Aba1* preceding *amp*C.

The concomitant existence of several aminoglycoside resistance genes (*aphA1*, *aacA4*, *aadA1*, and *armA*) was found in our CRAB isolates of different clonalities, indicating the presence of a common genetic structure (Table 2). In *A. baumannii*, aminoglycoside resistance genes are known to cluster in two separate chromosomal resistance islands, AbGRI2 and AbGRI3 (25, 29). AbGRI2 is an IS*26*-bound structure containing different resistance mechanisms and has been present in international clone 2 isolates since the 1980s. AbGRI3 was recently discovered in *A. baumannii* isolates recovered in the early 2000s, and after that genomic island is another IS*26*-bound structure that contains *armA* (25, 29). The accumulation of different resistance mechanisms in AbGRI2 and AbGRI3 is attributed to the action of IS*26*, thus creating the multiple variants reported in the literature (16, 29). AbGRI2 has been well studied and shown to be conserved in all international clone II isolates (25). In contrast, less is known about the recently discovered AbGRI3 (29). Therefore, the present study focused on aminoglycoside resistance genes in AbGRI3. PCR mapping results showed that 74 of 82 tested isolates harbored the Tn*6180* segment (29), which contained multiple antimicrobial resistance genes (*aacA4*, *catB8*, *aadA1*, and *armA*). Among them, 58 isolates had the upstream Tn*6179* (IS*26*, ΔIS*26*, *aphA1b*, IS*26*), which constituted AbGRI3 (24, 29). The wide dissemination of the Tn*6180*-borne *armA* or AbGRI3 has also been observed worldwide, especially in Japan and East Asia (14, 24, 29).

In conclusion, this nationwide surveillance study showed the simultaneous acquisition of multiple resistance genes via a variety of mechanisms by CRAB to successfully render the majority of antibiotics ineffective. For carbapenem resistance, the nationwide dissemination of isolates with plasmid-borne and/or chromosomal $bla_{OXA\text{-}23\text{-}like}$ carbapenemase genes evolved quickly, resulting in different Tn*6022* clones of diverse genetic backgrounds. In contrast, isolates with $bla_{OXA\text{-}24\text{-}like}$ carbapenemase genes carried by plasmids were relatively conserved and confined to some geographic regions. Other resistance determinants (AbGRI3, IS*Aba1-ampC*, or bla_{TEM}) also accumulated in CRAB isolates of different STs to further render CRAB multidrug resistant.

MATERIALS AND METHODS

Isolate collection for species identification and antimicrobial testing. The CRAB isolates were identified from the collection of *Acinetobacter* isolates recovered as part of the biennial Taiwan Surveillance of Antimicrobial Resistance (TSAR) program from 2002 to 2010 (corresponding to TSAR III to VII). The isolate collection protocol, the participating hospitals, and antimicrobial susceptibility testing have been described previously (11). The bacterial isolates were recovered from clinical samples taken as part of standard care. The study was approved by the Research Ethics Committee of the National Health Research Institutes (EC960205). MICs were determined by a reference broth microdilution method using Sensititre custom-designed plates. Data on the CRAB isolate source are presented in Table S1 in the supplemental material. All CRAB isolates initially identified to be part of the *A. calcoaceticus-A. baumannii* complex by the Vitek I system or Vitek II GN card (bioMérieux, Marcy l'Etoile, France) were further tested to differentiate *A. baumannii* from *A. pittii*, *A. nosocomialis*, and *A. calcoaceticus* by the *gyrB* genospecies PCR typing method (30). All isolates were stored at −80C for subsequent testing.

Detection of carbapenemase genes. PCR was used to detect genes belonging to class A (*bla*$_{NMC}$, *bla*$_{SME}$, *bla*$_{IMI}$, *bla*$_{KPC}$, and *bla*$_{GES}$), class B (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{GIM}$, *bla*$_{SPM}$, *bla*$_{SIM-1}$, and *bla*$_{NDM-1}$), and class D (*bla*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$, *bla*$_{OXA-58-like}$, and *bla*$_{OXA-51-like}$) β-lactamases with carbapenemase activity in all CRAB isolates (31–33). Since IS*Aba1* facilitates the expression of class D carbapenemase genes, such as *bla*$_{OXA-23-like}$ or *bla*$_{OXA-51-like}$, in addition to the mobilization of resistance determinants, the presence of IS*Aba1* upstream of the class D carbapenemase gene described above was determined using a reverse primer specific for IS*Aba1* and a reverse primer specific for the target gene.

Transformation of resistance determinants to confirm their contribution. The transformation experiment was performed as previously described (15). In brief, the PCR products were amplified with Phusion high-fidelity DNA polymerase (Finnzymes, Espoo, Finland) and confirmed by sequencing. After being cloned into the shuttle vector pYMAb2, electroporation into strain ATCC 15151 was performed with a Gene Pulser electroporator (Bio-Rad, Hercules, CA, USA) and 2-mm-electrode-gap cuvettes. Transformants were selected on the basis of kanamycin resistance, and sequencing was performed to confirm the presence of each resistance gene. The MICs of the transformants were determined by use of a Vitek II system (bioMérieux, Marcy l'Etoile, France).

Selection of emerging isolates on the basis of PFGE. All CRAB isolates carrying either *bla*$_{OXA-23-like}$ or *bla*$_{OXA-24-like}$, or both, were subjected to pulsed-field gel electrophoresis (PFGE) after digestion with ApaI as previously described (34). The stained gels were photographed and analyzed using BioNumerics software (Applied Maths), and dendrograms were generated to determine the relatedness of these isolates. Isolates with ≥80% similarity were designated to be in the same cluster (pulsotype). Isolates were selected for the additional studies described below from each PFGE cluster at a ratio of 1/5, and the isolates with the least similarity to the other isolates within each cluster were chosen. If there were fewer than 5 isolates in a cluster, only one isolate was chosen. However, many isolates with a *bla*$_{OXA-23-like}$ gene were not clustered, and 19 isolates were randomly selected from among these isolates. A total of 82 isolates, including 60 with *bla*$_{OXA-23-like}$ and 22 with *bla*$_{OXA-24-like}$, were selected (Fig. S4).

MLST. The 82 isolates were subjected to multilocus sequence typing (MLST) according to the Oxford scheme using primers specific for seven housekeeping genes listed on the PubMLST website (https://pubmlst.org/abaumannii/), and the sequences were compared to those in the MLST (Oxford) database. New alleles were designated via the PubMed website (http://pubmlst.org/abaumannii/submission.shtml). The eBURST (v3) algorithm (http://eBURST.mlst.net/) was used to assess the evolutionary relationship of STs. A clonal group (CG) was defined as a group of STs sharing at least 5 identical loci among the 7 housekeeping genes tested.

Detection of genes responsible for resistance to other β-lactams and aminoglycosides and their genetic structure. The 82 isolates were also tested for the presence of other prevalent class A and C β-lactamase genes (*bla*$_{TEM}$, *bla*$_{PER}$, *bla*$_{SHV}$, and *ampC*) (35) and genes responsible for aminoglycoside resistance, including the *armA*, *aphA1*, *aphA6*, *aacC1*, *aacA4*, *aadB*, and *aadA1*genes (36). PCR mapping was further performed on all 82 isolates to elucidate the genetic environment of *bla*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$ (7, 8, 22), or other resistance genes, if present (14, 24, 25). The AbGRI2-like structure in which *bla*$_{TEM}$ was embedded was detected using primers published previously (25). Sequencing of the PCR products was performed by the DNA Sequencing Core Lab, National Health Research Institutes.

Localization of *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$. S1 nuclease and I-CeuI assays were used to determine if the *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$.genes were present on a plasmid or in the chromosome (37). Among the 82 isolates mentioned above, 44 (22 with *bla*$_{OXA-23-like}$ and 22 with *bla*$_{OXA-24-like}$) were selected (Fig. S4). Briefly, bacterial cells embedded in agarose gel plugs were first digested with S1 nuclease or I-CeuI, followed by PFGE, and then transferred to a nylon membrane. Both the S1 nuclease and I-CeuI digests were hybridized with labeled target genes, and the I-CeuI digests were further hybridized with labeled 23S rRNA genes amplified from our clinical isolates.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01215-17.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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We have no competing interests to declare.

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