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Original Article

Predictive biomarkers for drug-resistant *Acinetobacter baumannii* isolates with $bla_{TEM\text{-}1}$, AmpC-type *bla* and integrase 1 genotypes

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Background and Purpose: We tested whether antibiotic susceptibilities of drug-resistant *Acinetobacter baumannii* isolates could be used to predict the clinically important genotypes $bla_{TEM\text{-}1}$, AmpC-type *bla*, and integrase 1 gene (*IntI*1).
Methods: We analyzed 401 *A. baumannii* isolates obtained at Changhua Christian Hospital between April 2001 and March 2002. The isolates were all from blood cultures, and identification of *A. baumannii* was confirmed by API-20NE. Antibiotic susceptibility testing (phenotype) was performed by disk diffusion method. Polymerase chain reaction was used to detect the genes $bla_{TEM\text{-}1}$, AmpC-type *bla* and *IntI*1.
Results: Of 32 *A. baumannii* isolates, 10 possessed $bla_{TEM\text{-}1}$, 21 AmpC-type *bla*, and 26 *IntI*1. Resistance to ceftazidime (CAZ) predicted $bla_{TEM\text{-}1}$ genotype with 63.6% sensitivity, 100% specificity, 55.6% positive predictive value (PPV) and 0% negative predictive value (NPV). Trimethoprim-sulfamethoxazole (SXT) and gentamicin (GM) resistance predicted *IntI*1 genotype with 83.3% sensitivity, 71.4% specificity, 95.2% PPV and 45.4% NPV. No resistance phenotype could predict the AmpC-type *bla* genotype.
Conclusions: CAZ resistance predicted the $bla_{TEM\text{-}1}$ genotype with 100% specificity, and SXT and GM resistance predicted the *IntI*1 genotype with 92.5% PPV. Therefore, antibiotic susceptibilities to CAZ, SXT, and GM can be utilized clinically to detect critical genotypes in *A. baumannii*.

Key words: *Acinetobacter baumannii,* bacterial drug resistance, beta-lactamases genetics, biological markers, genotype

Introduction

Acinetobacter baumannii infection is a serious problem that yields high rates of mortality and morbidity [1-3]. Numerous studies have demonstrated that *A. baumannii* infections have three characteristic features: antibiotic resistance, cross-infection, and inducible resistance [4-6]. The evaluation of predictors of the antimicrobial effect and clinical outcome has been accepted as a useful tool in the design of optimal dosing regimens [7]. Concerning antibiotic resistance, TEM-1 beta (β)-lactamase is one of the main causes of bacterial resistance to β-lactam antibiotics. Therefore, detecting the $bla_{TEM\text{-}1}$ gene has become important.

A unique class 1 integron (GenBank accession number, AY557339) has been found in *A. baumannii* strain 1-43 isolated from Taiwan. Several studies of antibiotic resistance mechanisms in *Acinetobacter* have demonstrated the presence of specific genes located on integrons, such as the integrase 1 gene (*IntI*1) [8]. The presence of type 1 and type 2 integrons has already been described in *A. baumannii* strains of both clinical and environmental origin [9,10]. It has also been reported that epidemic strains of *A. baumannii* usually contain significantly more integrons than non-epidemic strains [10]. Barbosa and Levy showed the impact of antibiotic use on resistance development and persistence [11].

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Concerning the AmpC-type *bla* gene, Hanson has previously pointed out the characteristics of the inducible AmpC β-lactamases such as physical properties, hydrolytic activity, the molecular mechanisms involved in chromosomal expression, and comparative studies between genera on the induction potential of the β-lactamases [12]. Hanson showed that an *ampR* gene is located upstream of the *AmpC* gene, and that the *AmpR* product acts as a repressor on the basal level of *AmpC* biosynthesis and as an activator upon the addition of a β-lactam inducer, and that transformation of a ceftazidime (CAZ)-resistant mutant with *ampD* from *Escherichia coli* restores an inducible phenotype [12]. Those inducible chromosomal genes were detected on plasmids and were transferred to organisms.

Many clinical microbiologists are unaware of plasmid-encoded AmpC β-lactamases because phenotypic detection is difficult at best and these β-lactamases can be misidentified as extended spectrum β-lactamases. Hanson has described a new inducible AmpC-type β-lactamase, the AmpC-type *bla* gene, from a clinical isolate of a new member of the *Enterobacteriaceae* family [12]. Thus, we chose this AmpC-type *bla* gene as an antibiotic resistance inducible biomarker.

An imipenem-resistant *A. baumannii* isolate has emerged in Changhua Christian Hospital (CCH), Taiwan. The bla_{TEM-1}, AmpC-type *bla*, and *IntI*1 genes are notorious for providing antibiotic resistance to *Enterobacteriaceae*, including *A. baumannii* [13]. In this study, we investigated whether common antibiotic susceptibility tests could predict the presence of some of the important antibiotic resistance genes in clinical isolates of *A. baumannii*. In order to test this hypothesis, we compiled and analyzed a dataset of 401 *A. baumannii* infections and evaluated whether antibiotic susceptibilities correlated with bla_{TEM-1}, AmpC-type *bla*, and *IntI*1 genotypes.

Methods

A cross-sectional, prospective study of patients with *A. baumannii* infection was conducted using documented infections as per the microbiological records from the Microbiology Laboratory of CCH. We analyzed 401 *A. baumannii* infections documented between April 1, 2001, and March 31, 2002. Infectious disease specialists reviewed the medical records, and a microbiologist examined all of the *A. baumannii* strains during this period.

Microbiological investigations

Blood cultures were performed in every patient with suspected sepsis. The BACTEC NR-860 system (Becton Dickinson Diagnostic Instrument Systems, Franklin Lake, NJ, USA) was used to detect pathogens. *A. baumannii* was presumptively identified using colony morphology, Gram staining, oxidase testing (Dry Slide; Difco Laboratories, Detroit, MI, USA), and catalase testing. Identification of *A. baumannii* was confirmed using the API-20NE kit (bioMérieux Vitek, Hazelwood, MO, USA). All *A. baumannii* strains were also tested with the 10% lactose reaction. Routine antibiotic sensitivity testing was performed using the disk diffusion method (BBL, Sensi-Disc; Becton Dickinson, Cockeysville, MD, USA), according to the guidelines of the National Committee for Clinical Laboratory Standards [14].

Antibiotic sensitivity testing was performed with β-lactams (amoxicillin-clavulanate [SAM], piperacillin [PIP], PIP-tazobactam [TZP], flomoxef [FEP], CAZ, and imipenem-cilastatin [IPM]), aminoglycosides (amikacin [AN], gentamicin [GM], and netilmicin [NN]), fluoroquinolones (ciprofloxacin [CIP] and ofloxacin), doxycycline (D), and trimethoprim-sulfamethoxazole (SXT). Several antibiotics, including SXT, CIP, AN, GM, SAM, PIP, and IPM were tested using the Etest (A-B biodisk, Solna, Sweden). The definition of the "mutiresistant phenotype group" was those *A. baumannii* resistant to more than 3 kinds of those aminoglycosides (AN, GM, NN), fluoroquinolones (CIP), tetracycline (D), and SXT, in addition to some of the β-lactam antibiotics (SAM, PIP, TZP, FEP, CAZ, IPM).

Molecular detection of the bla_{TEM-1}, AmpC-type *bla*, and *IntI*1 genes

As previously described, three sets of specific primers were used to amplify the target genes, including the bla_{TEM-1} gene [13], the AmpC-type *bla* gene [15], and the *IntI*1 gene [10]. The bla_{TEM-1} primer C1 (5'-GGGAATTCTCGGGGAAATGTGCGCGGAAC-3') and bla_{TEM-1} primer C2 (5'-GATCCGAGTAAACTTG GTCTGACAG-3') were used to detect the bla_{TEM-1} gene. The AmpC primer P1 (5'-TAAACACCACATATGTT CCG-3') and AmpC primer P2 (5'-CGGAACATAT GTGGTGTTTA-3') were used to detect the AmpC-type *bla* gene. The *IntI*1 primer F (5'-CAGTGGACATA AGCCTGTTC-3') and *IntI*1 primer R (5'-CCCGAGG CATAGACTGTA-3') were used to detect the *IntI*1 gene.

Polymerase chain reaction (PCR) amplifications were carried out in 20-μL volumes containing 0.25 μL of template DNA, 0.025 mM deoxynucleoside triphosphate, 1 μL of 10X PCR buffer, 1 U of *Taq* polymerase (Perkin-Elmer Applied Biosystems, Foster City, CA, USA), 1.5 mM magnesium chloride, and 0.5 μM of each primer. PCR amplification was performed with the MJ Research DNA engine (Peltier Thermal Cycle, PTC-200; MJ Research, Watertown, MA, USA). Amplification procedures were resolved by electrophoresis on 1.5% agarose gel with 0.5X Tris-borate-ethylenediamine tetra-acetic acid buffer containing ethidium bromide, and were visualized under ultraviolet light. All PCR amplifications were performed in duplicate. PCR products were purified with the Mini-M™ plasmid DNA extraction system (Viogene, CA, USA). Purified PCR products were sequenced with dye terminators on an ABI 377 automatic sequencer (Perkin Elmer Applied Biosystems). DNA sequences were compared to the National Center for Biotechnology Information database.

Statistical analysis

We used the finite mixture for categorical dataset according to the antibiotic resistance phenotypes. Results were considered significant if the *p* value was less than 0.05. All data were analyzed using a personal computer equipped with SAS software v6.12 (SAS, Cary, NC, USA).

Table 1. Genotypes and antibiotic susceptibility phenotypes in 401 clinical *Acinetobacter baumannii* isolates

Cluster	Grouping	SXT	CIP	D	AN	FEP	TZP	SAM	PIP	CAZ	IPM	GM-10	NN	TEM-1	AmpC	*IntI*1	N
1	1	R	R	I	R	R	R	S	R	R	S	R	R	No	No	No	49
1	2	R	R	R	R	R	R	R	R	R	S	R	R	No	No	No	30
1	3	R	R	R	R	R	R	S	R	R	S	R	R	No	No	No	24
1	4	R	R	R	R	R	R	I	R	R	S	R	R	Yes	No	Yes	20
1	5	R	R	R	R	S	R	R	I	R	R	R	R	Yes	Yes	Yes	16
1	6	R	R	R	R	R	R	S	R	R	R	R	R	Yes	No	Yes	17
1	7	R	R	R	R	S	R	R	R	I	S	R	R	Yes	Yes	Yes	1
1	8	R	R	R	R	R	I	I	R	R	S	R	R	No	Yes	Yes	21
2	9	S	R	S	R	S	S	R	R	R	R	S	S	No	No	No	14
2	10	S	S	S	S	R	S	S	I	S	S	S	S	Yes	Yes	No	12
2	11	R	S	S	S	S	S	S	R	S	S	S	S	No	Yes	Yes	11
2	12	S	S	S	S	S	I	S	R	I	S	S	S	No	No	Yes	13
2	13	S	R	S	S	S	S	S	S	S	S	S	S	No	Yes	Yes	11
2	14	S	S	S	S	S	S	R	S	S	S	S	S	No	Yes	Yes	12
2	15	R	S	S	S	S	S	S	S	S	S	R	R	No	Yes	Yes	10
2	16	S	S	S	S	S	S	S	S	S	S	R	R	No	No	No	10
2	17	S	S	S	S	S	S	S	S	S	S	S	S	Yes	Yes	Yes	8
2	18	R	S	I	I	S	S	S	I	S	S	R	R	No	No	Yes	8
2	19	S	S	S	S	S	S	S	I	S	S	S	S	Yes	Yes	Yes	15
2	20	R	S	S	S	S	S	S	I	I	S	R	R	No	No	Yes	1
2	21	R	S	R	I	S	S	S	I	I	S	R	R	Yes	Yes	Yes	12
2	22	R	R	S	S	I	R	S	R	R	S	S	S	Yes	Yes	Yes	9
2	23	R	R	R	S	R	R	S	R	R	S	S	S	No	Yes	Yes	8
2	24	R	R	R	S	R	I	I	R	R	S	R	R	No	Yes	Yes	7
3	25	R	S	S	R	R	I	S	R	R	R	R	R	No	Yes	Yes	5
3	26	R	R	R	R	S	R	R	R	I	S	R	R	No	Yes	Yes	8
3	27	R	R	R	R	S	I	S	R	R	S	R	R	No	Yes	Yes	9
3	28	R	R	R	S	I	R	S	R	R	S	R	R	Yes	Yes	Yes	12
3	29	R	R	R	R	I	R	I	R	R	S	R	R	No	No	Yes	5
3	30	R	R	R	R	I	R	I	R	R	S	R	S	No	Yes	Yes	6
3	31	R	R	R	R	I	I	S	R	R	S	R	R	No	Yes	Yes	8
3	32	R	R	R	R	R	R	S	R	R	S	R	R	No	Yes	Yes	9

Abbreviations: SXT = trimethoprim-sulfamethoxazole; CIP = ciprofloxacin; D = doxycycline; AN = amikacin; FEP = flomoxef; TZP = piperacillin-tazobactam; SAM = amoxicillin-clavulanate; PIP = piperacillin; CAZ = ceftazidime; IPM = imipenem-cilastatin; GM = gentamicin; NN = netilmicin; TEM-1 = $bla_{\text{TEM-1}}$ gene; AmpC = AmpC-type *bla* gene; *IntI*1 = integrase 1 gene; N = total number; R = resistant; S = sensitive; I = intermediate

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Results

A total of 401 strains of *A. baumannii* were analyzed for their antibiotic resistance phenotype (the dataset of the microbiological results is summarized in Table 1). The results of antimicrobial susceptibility testing showed that nearly 90% of the *A. baumannii* strains were resistant to first-, second-, and third-generation cephalosporins (except CAZ). Furthermore, those strains were usually resistant to penicillins (except ampicillin-sulbactam) as well.

These isolates can be divided into 3 clusters according to their antibiotic resistance phenotypic profile. Cluster 1 included 70.0% of the isolates and comprised the "mutiresistant phenotype", which can be divided into 8 subgroups. Cluster 2 included 20.3% of the isolates and comprised the "sensitive phenotype", which can be divided into 16 subgroups. Cluster 3 included 9.0% of the total isolates and comprised the "variant phenotype", which can be divided into 8 subgroups.

To confirm the resistant phenotypes of the 32 *A. baumannii* subgroups, we compared the results of susceptibility tests from the disk diffusion method and the Etest method. There was no significant difference between those two methods (unpublished data). *A. baumannii* type CH 1 was the most common in cluster 1 and was multidrug-resistant except for SAM, IMP and D; it represented the typical multidrug-resistant *A. baumannii*. *A. baumannii* type CH 7 was an imipenem-resistant strain (minimal inhibitory concentration, 128 μg/dL).

We investigated whether there was a relationship between antibiotic susceptibilities and the presence of the bla_{TEM-1} gene in clinical *A. baumannii* strains (Table 2). Six bla_{TEM-1} (+) and 15 bla_{TEM-1} (–) strains were sensitive to FEP, while 4 bla_{TEM-1} (+) and 7 bla_{TEM-1} (–) strains were resistant to FEP (p=0.703); none of the bla_{TEM-1} (+) and 14 bla_{TEM-1} (–) strains were sensitive to CAZ, while 10 bla_{TEM-1} (+) and 8 bla_{TEM-1} (–) strains were resistant to CAZ (p=0.001). Thus, the CAZ-resistant phenotype predicted the bla_{TEM-1} genotype with a sensitivity of 63.6% (14/22), a specificity of 100% (10/10), a positive predictive value (PPV) of 55.6% (10/18), and a negative predictive value (NPV) of 0% (0/14).

Data on the relationship between antibiotic susceptibilities and the *IntI*1 gene in clinical *A. baumannii* strains are shown in Table 3. Four *IntI*1 (+) strains and 4 *IntI*1 (–) strains were sensitive to SXT, while 22 *IntI*1 (+) and 2 *IntI*1 (–) strains were resistant to SXT (p=0.023). Five *IntI*1 (+) and 5 *IntI*1 (–) strains were sensitive to GM, while 21 *IntI*1 (+) and 1 *IntI*1 (–) strains were resistant to GM (p=0.006). Thus, SXT- and GM-resistant phenotypes predicted *IntI*1 genotype with a sensitivity of 83.3% (5/6), a specificity of 71.4% (20/28), a PPV of 95.2% (20/21), and an NPV of 45.4% (5/11).

Table 2. Antibiotic susceptibility phenotypes of the bla_{TEM-1} gene of clinical *Acinetobacter baumannii* strains

Phenotype	bla_{TEM-1}		Total (n = 32) No. (%)	p
	Yes (n = 10) No. (%)	No (n = 22) No. (%)		
Flomoxef				
Sensitive	6 (60.0)	15 (68.2)	21 (65.6)	0.703
Resistant	4 (40.0)	7 (31.8)	11 (34.4)	
Piperacillin-tazobactam				
Sensitive	5 (50.0)	13 (59.1)	18 (56.3)	0.631
Resistant	5 (50.0)	9 (40.9)	14 (43.8)	
Amoxicillin-clavulanate				
Sensitive	7 (70.0)	19 (86.4)	26 (81.3)	0.346
Resistant	3 (30.0)	3 (13.6)	6 (18.8)	
Piperacillin				
Sensitive	1 (10.0)	10 (45.5)	11 (34.4)	0.106
Resistant	9 (90.0)	12 (54.5)	21 (65.6)	
Ceftazidime				
Sensitive	0 (0.0)	14 (63.6)	14 (43.8)	0.001
Resistant	10 (100.0)	8 (36.4)	18 (56.3)	
Imipenem-cilastatin				
Sensitive	7 (70.0)	21 (95.5)	28 (87.5)	0.079
Resistant	3 (30.0)	1 (4.5)	4 (12.5)	

Table 3. Antibiotic susceptibility phenotypes of the integrase 1 gene (*IntI*1) of clinical *Acinetobacter baumannii* strains

Phenotype	*IntI*1		Total (n = 32) No. (%)	*p*
	Yes (n = 26) No. (%)	No (n = 6) No. (%)		
Trimethoprim-sulfamethoxazole				
Sensitive	4 (15.4)	4 (66.7)	8 (25.0)	0.023
Resistant	22 (84.6)	2 (33.3)	24 (75.0)	
Ciprofloxacin				
Sensitive	7 (26.9)	5 (83.3)	12 (37.5)	0.018
Resistant	19 (73.1)	1 (16.7)	20 (62.5)	
Doxycycline				
Sensitive	9 (34.6)	6 (100.0)	15 (46.9)	0.006
Resistant	17 (65.4)	0 (0.0)	17 (53.1)	
Amikacin				
Sensitive	11 (42.3)	5 (83.3)	16 (50.0)	0.172
Resistant	15 (57.7)	1 (16.7)	16 (50.0)	
Flomoxef				
Sensitive	16 (61.5)	5 (83.3)	21 (65.6)	0.637
Resistant	10 (38.5)	1 (16.7)	11 (34.4)	
Piperacillin-tazobactam				
Sensitive	12 (46.2)	6 (100.0)	18 (56.3)	0.024
Resistant	14 (53.8)	0 (0.0)	14 (43.8)	
Amoxicillin-clavulanate				
Sensitive	22 (84.6)	4 (66.7)	26 (81.3)	0.310
Resistant	4 (15.4)	2 (33.3)	6 (18.8)	
Piperacillin				
Sensitive	7 (26.9)	4 (66.7)	11 (34.4)	0.148
Resistant	19 (73.1)	2 (33.3)	21 (65.6)	
Ceftazidime				
Sensitive	9 (34.6)	5 (83.3)	14 (43.8)	0.064
Resistant	17 (65.4)	1 (16.7)	18 (56.3)	
Imipenem-cilastatin				
Sensitive	23 (88.5)	5 (83.3)	28 (87.5)	1.000
Resistant	3 (11.5)	1 (16.7)	4 (12.5)	
Gentamicin				
Sensitive	5 (19.2)	5 (83.3)	10 (31.3)	0.006
Resistant	21 (80.8)	1 (16.7)	22 (68.8)	
Netilmicin				
Sensitive	6 (23.1)	5 (83.3)	11 (34.4)	0.011
Resistant	20 (76.9)	1 (16.7)	21 (65.6)	

We also evaluated whether there was a relationship between antibiotic susceptibility phenotypes and the AmpC-type *bla* gene in clinical *A. baumannii* strains (Table 4). One AmpC-type *bla* (+) and 7 AmpC-type *bla* (–) strains were sensitive to SXT, while 4 AmpC-type *bla* (+) and 20 AmpC-type *bla* (–) strains were resistant to SXT (p=0.633). Two AmpC-type *bla* (+) and 19 AmpC-type *bla* (–) strains were sensitive to SAM, while 3 AmpC-type *bla* (+) and 8 AmpC-type *bla* (–) strains were resistant to SAM (p=0.131). Nine AmpC-type *bla* (+) and 5 AmpC-type *bla* (–) strains were sensitive to CAZ, while 17 AmpC-type *bla* (+) and 1 AmpC-type *bla* (–) strains were resistant to CAZ (p=0.627). Thus, no resistant phenotype could accurately predict the AmpC-type *bla* genotype.

Discussion

This is the first study of clinical *A. baumannii* to find the bio-resistant markers and investigate the relationship between three important genotypes ($bla_{\text{TEM-1}}$, AmpC-type *bla*, and *IntI*1) and antibiotic resistance phenotypes in Taiwan. All of the three important genotypes ($bla_{\text{TEM-1}}$, AmpC-type *bla*, and *IntI*1) have clinical consequences:

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Table 4. Antibiotic susceptibility phenotypes of the AmpC-type *bla* gene of clinical *Acinetobacter baumannii* strains

Phenotype	AmpC-type *bla*		Total (n = 32) No. (%)	*p*
	Yes (n = 5) No. (%)	No (n = 27) No. (%)		
Trimethoprim-sulfamethoxazole				
Sensitive	1 (15.4)	7 (66.7)	8 (25)	0.633
Resistant	4 (84.6)	20 (33.3)	24 (75)	
Ciprofloxacin				
Sensitive	1 (26.9)	11 (83.3)	12 (37.5)	0.366
Resistant	4 (73.1)	16 (16.7)	20 (62.5)	
Doxycycline				
Sensitive	1 (34.6)	12 (100)	15 (46.9)	0.284
Resistant	4 (65.4)	15 (0)	17 (53.1)	
Amikacin				
Sensitive	1 (42.3)	13 (83.3)	16 (50)	0.53
Resistant	4 (57.7)	14 (16.7)	16 (50)	
Flomoxef				
Sensitive	1 (61.5)	15 (83.3)	21 (65.6)	0.332
Resistant	4 (38.5)	12 (16.7)	11 (34.4)	
Piperacillin-tazobactam				
Sensitive	1 (46.2)	11 (100)	18 (56.3)	0.821
Resistant	4 (53.8)	16 (0)	14 (43.8)	
Amoxicillin-clavulanate				
Sensitive	2 (84.6)	19 (66.7)	26 (81.3)	0.131
Resistant	3 (15.4)	8 (33.3)	6 (18.8)	
Piperacillin				
Sensitive	1 (26.9)	4 (66.7)	11 (34.4)	0.624
Resistant	4 (73.1)	23 (33.3)	21 (65.6)	
Ceftazidime				
Sensitive	1 (34.6)	8 (83.3)	14 (43.8)	0.627
Resistant	4 (65.4)	19 (16.7)	18 (56.3)	
Imipenem-cilastatin				
Sensitive	5 (88.5)	23 (83.3)	28 (87.5)	0.488
Resistant	5 (11.5)	4 (16.7)	4 (12.5)	
Gentamicin				
Sensitive	1 (19.2)	9 (83.3)	10 (31.3)	0.494
Resistant	4 (80.8)	18 (16.7)	22 (68.8)	
Netilmicin				
Sensitive	1 (23.1)	10 (83.3)	11 (34.4)	0.428
Resistant	4 (76.9)	17 (16.7)	21 (65.6)	

the bla_{TEM-1} gene is associated with antibiotic resistance, the AmpC-type *bla* gene is associated with antibiotic inducibility, and the *IntI*1 is associated with cross-infection [16-19]. At present, selection of appropriate antibiotic therapy can only be done once antibiotic susceptibility results are available.

We point out the importance of the bla_{TEM-1} genotype because β-lactams are very commonly used antibiotics. CAZ resistance predicted the bla_{TEM-1} genotype with a sensitivity of 63.6%, a specificity of 100%, and a PPV of 55.6%. Thus, the CAZ-resistant phenotype can be used clinically to monitor the bla_{TEM-1} genotype. However, further study is necessary to elucidate the mechanism. As far as we know, the bla_{TEM-1} β-lactamase does not hydrolyze cephalosporins; therefore, prediction of bla_{TEM-1} according to CAZ resistance is possibly inappropriate.

The *IntI*1gene is an essential component of integron, and it encodes a site-specific recombinase belonging to the integrase family [8]. Because the *IntI*1 gene was present in nearly all of the *A. baumannii* clinical strains, *IntI*1 detection may be a rapid and simple technique for the routine screening and identification of *A. baumannii* isolates with outbreak potential. *A. baumannii* infections lead to nosocomial outbreaks, and transmission of

CAZ-resistant Gram-negative bacilli, including *A. baumannii,* is very common [20]. Thus, we chose to detect the *IntI*1 gene to monitor the transfer of genetic elements. Of the 32 isolates, 81.3% had the *IntI*1 gene. According to Koeleman et al's study, integrons play an important role in antibiotic resistance and in the epidemic behavior of *A. baumannii* [10]. In the present study, the *IntI*1 gene was commonly found in *A. baumannii* isolates (81.3%), similar to the findings of Gallego and Towner [8]. This is in agreement with previous data showing that integron-located antimicrobial resistance genes are frequently found in epidemic strains of *A. baumannii* [5]. Antimicrobial resistance in *A. baumannii* strains might have been acquired either through horizontal gene transfer or through selection of novel resistant clones. Thus, we believed that integrons could be a potential endemic marker, and that *IntI*1 was the essential component of integron. In this study, SXT and GM resistance predicted the *IntI*1 genotype with a sensitivity of 83.3%, a specificity of 71.4%, a PPV of 95.2%, and an NPV of 45.4%. Thus, the SXT and GM resistance phenotype could be used in clinical screening for the *IntI*1 genotype.

Concerning the AmpC β-lactamase genotype, Bou and Martínez-Beltrán previously demonstrated the presence of the AmpC β-lactamase gene in *A. baumannii* [15] and concluded that the gene was related to cephalosporin resistance. The emergence of resistance to expanded-spectrum cephalosporins is now a major concern for most Gram-negative bacteria, while this problem was initially limited to a number of bacterial species (*Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *Pseudomonas aeruginosa*) that could mutate to hyperproduce their chromosomal class C β-lactamase. The finding of no relationship between antibiotic susceptibility phenotypes and AmpC-type *bla* gene is not surprising. Several reports in the literature have shown that the level of expression of class C β-lactamase is important to determine the resistance phenotype in *Pseudomonas* spp. as well as in *Acinetobacter* spp. Moreover, bacterial permeability, associated with outer membrane protein expression profile, might also play a role. In the current study, no significant correlation between the antibiotic susceptibility phenotypes and genotypes was evident for this AmpC β-lactamase gene.

The present study attempted to find biomarkers that might enable use of antibiotic susceptibility phenotypes to predict three important genotypes (bla_{TEM-1}, AmpC-type *bla*, and *IntI*1) in Changhua, Taiwan. It is the first clinical epidemiological study of the bla_{TEM-1}, AmpC-type *bla*, and the *IntI*1 genes in *A. baumannii* in Taiwan. Although the variability in strain genotype is generally difficult to correlate to antibiotic resistance phenotype, we found that CAZ resistance predicted the bla_{TEM-1} genotype with 100% specificity, and that SXT and GM resistance predicted the *IntI*1 genotype with 92.5% PPV. Therefore, antibiotic susceptibilities to CAZ, SXT, and GM can be utilized to detect critical genotypes in *A. baumannii*.

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