Acinetobacter baylyi as a Pathogen for Opportunistic Infection[▽]

Te-Li Chen,^{1,2} Leung-Kei Siu,³ Yi-Tzu Lee,¹ Chien-Pei Chen,¹ Li-Yueh Huang,³
Roy Chen-Chih Wu,² Wen-Long Cho,² and Chang-Phone Fung^{1,2,4*}

Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital,¹ Institute of Tropical Medicine, School of Medicine, National Yang-Ming University,² and Division of Clinical Research, National Health Research Institutes,³ Taipei, and Chutung Veterans Hospital, Chutung,⁴ Taiwan

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There are no previous reports of human infection due to *Acinetobacter baylyi*. In this study, we report on six patients with bacteremia due to *A. baylyi*, based on analysis of the 16S-23S rRNA intergenic spacer and the 16S rRNA gene. All six patients had multiple underlying diseases. The infection was nosocomially acquired in five patients. The six clinical isolates had similar ribopatterns, suggesting a clonal relationship. Compared to the reference strain, the clinical isolates were more resistant to antimicrobial agents, especially beta-lactam antibiotics. In three of the isolates, they may have undetermined plasmid mediated class C type beta-lactamases because of the positive results in a double-disk synergy test using 3-aminophenylboronic acid. Two of the clinical isolates retained a level of natural transformability similar to that of the reference strain. None of the patients died, although only three of them received appropriate antimicrobial therapy. This study demonstrates that *A. baylyi* is a potential human pathogen that can cause nosocomial infection in immunocompromised patients.

Classification of the *Acinetobacter* spp., important soil organisms and sources of infection in immunocompromised patients, is difficult because of limited knowledge of their basic biology and ecology (3). *Acinetobacter* species are now defined as strictly aerobic gram-negative coccobacilli that are nonmotile, catalase positive, oxidase negative, and have a DNA G+C content of 39 to 47% (3). They can survive at a wide range of temperatures on animate and inanimate surfaces and are commonly isolated in hospitals, where these organisms are emerging nosocomial pathogens.

At present, the *Acinetobacter* genus has at least 32 genomic species (10). *Acinetobacter baumannii* is the most clinically important species (22), presumably because it can rapidly develop resistance to antibiotics. *Acinetobacter* genomic species 13TU and 3, which are phenotypically similar to *A. baumannii*, are also commonly encountered in nosocomial infections. Along with an environmental species (*Acinetobacter calcoaceticus*), these species are classified as the *A. calcoaceticus*-*A. baumannii* complex (3). This complex is usually identified by commercially available methods based on phenotypic characteristics (4), although more reliable identification requires molecular methods.

The identification of non-*A. calcoaceticus*-*A. baumannii* complex species by phenotypic methods is more difficult (11). Therefore, the clinical significance of many non-*A. calcoaceticus*-*A. baumannii* complex species remains to be determined. However, precise identification is necessary in order to evaluate the clinical significance of a species. For example, the use of a genotypic method (16S rRNA gene analysis) enabled identification of 103 *Acinetobacter* isolates and showed that two

species, *Acinetobacter schindleri* and *Acinetobacter ursingii*, were underreported by previous studies (11).

DNA-DNA hybridization and sequence analysis are the most widely accepted methods for identifying species, including *Acinetobacter* (3). DNA-DNA hybridization is considered the gold standard but is labor-intensive and impractical in most clinical laboratories (15). Sequence analysis of housekeeping genes, including the 16S rRNA gene (13), 16S-23S rRNA intergenic spacer (ITS) (6), *recA* gene (15), *rpoB* and flanking spacers (16), and *gyrB* gene (27), also allows identification of *Acinetobacter* species. Among these genes, the 16S rRNA gene is most commonly used (14). It has been suggested that an unknown isolate can be assigned to a species when its 16S rRNA sequence has a similarity of $\geq 99\%$ with the reference sequence of a well-defined species and when it has at least 0.5% sequence difference to the second nearest species (4, 14).

In the present study, we used genotypic methods to identify *Acinetobacter baylyi* in six bacteremic patients. Previously, this species was only known to live in the soil. The identification procedures, antimicrobial susceptibility, natural transformability of the isolates and clinical features of the patients are described.

MATERIALS AND METHODS

Isolates of *Acinetobacter* in the present study. A previous study identified 138 *Acinetobacter* isolates to the species level by ITS sequence analysis and verified a new multiplex PCR method for identification of *A. baumannii* (8). Among the 47 non-*A. calcoaceticus*-*A. baumannii* complex species, six were identified as *A. baylyi*. Sequence analysis of 16S rRNA gene and phylogenetic analysis confirmed this identification. The *A. baylyi* strain ADP1 (ATCC 33305) and *A. baumannii* type strain (ATCC 19606^T) were purchased from the Bioresources Collection and Research Center of Taiwan and used as reference strains. The clonality of the isolates was delineated by using an automated ribotyping system (25).

Genotypic identification and phylogenetic analysis. Amplification of 16S rRNA gene was performed as that described previously (13). The ITS and 16S rRNA gene sequences were compared to sequences in GenBank by using the BLAST program. All available ITS and 16S rRNA sequences of different *Acinetobacter* spp., some of which were not well characterized, were included for phylogenetic analysis. All copies of the 16S rRNA gene and the ITSs of *A. baylyi* strain ADP1 from the genome sequence (CR543861) were also used for com-

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 11217, Taiwan. Phone: 886 3 596 2134, ext. 101. Fax: 886 3 595 7196. E-mail: cpfung@vghtpe.gov.tw.

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TABLE 1. Primers used for detection of genes encoding various beta-lactamases and transposase of IS1236 and ISAbal

Target	Primer	Sequence (5'–3')	Source or reference
<i>bla</i> _{PER}	PER-A PER-B	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAGAA	19
<i>bla</i> _{SHV}	OS5 OS6	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTGCTCGG	5
<i>bla</i> _{TEM}	TEM-A TEM-B	TAAAAATTCCTGAAGACG TTACCAATGCTTAATCA	5
<i>bla</i> _{VEB}	VEB1-A VEB1-B	CGACTTCCATTTCCCGATGC GGACTCTGCAACAAATACGC	19
<i>bla</i> _{CTX-M-1}	CTX-M A CTX-M B	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	5
<i>bla</i> _{CTX-M-2 (TOHO-1)}	TOHO-A TOHO-B	ATGTGCAGTACCAGTAA TAGGTACCAGAACCAG	19
<i>bla</i> _{CTX-M-9 (TOHO-2)}	CTX-M-9A Toho 2 B	CTGATGTAACACGGATTGAC TTACAGCCCTTCGGCGAT	5
<i>bla</i> _{ADC-1} to <i>bla</i> _{ADC-7}	ADC-F ADC-B	TCTCTTTTGACGATACGCCTGG GTGGATTTGACGCCGTATGCT	This study
<i>bla</i> _{ADC-8}	ADC-8F ADC-8B	CTGAGTGGTTTGATGGCCTTTTCG GGTGGTTTTCCGCTACAGTTTCTT	This study This study
<i>bla</i> _{OXA-58}	OXA58-F OXA58-B	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	24
MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11	MOXMF MOXMR	CTGCTCAAGGAGCACAGGAT CACATTGACATAGGTGTGGTGC	18
LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	CITMF CITMR	TGGCCAGAACTGACAGGCCAAA TTTCTCCTGAACGTGGCTGGC	18
DHA-1, DHA-2	DHAMF DHAMR	AACTTTCACAGGTGTGCTGGGT CCGTACGCATACTGGCTTTGC	18
ACC	ACCMF ACCMR	AACAGCCTCAGCAGCCGGTTA TTCGCCGCAATCATCCCTAGC	18
MIR-1T, ACT-1	EBCMF EBCMR	TCGGTAAAGCCGATGTTGCGG CTTCCACTGCGGCTGCCAGTT	18
FOX-1 to FOX-5b	FOXMF FOXMR	AACATGGGGTATCAGGGAGATG CAAAGCGCGTAACCGGATTGG	18
IS1236	IS1236 F IS1236 R	CTGCCTGTAAATGCCTGGGTGTC CGGTCTATTTCGATGGCTGTTTG	This study This study
ISAbal	ISAbalF ISAbalR	CACGAATGCAGAAGTTG CGACGAATACTATGACAC	21

parison. Analysis of 16S rRNA gene was performed by comparison of a continuous stretch of ca. of 1,330 bp, which corresponded to nucleotide positions 102 to 1435 of the homologous *Escherichia coli* gene (EU014689). The phylogenetic analysis was performed with the neighbor-joining algorithm in the CLUSTAL W program (version 1.83), and the resulting phylogenetic tree was displayed with TreeView software (version 1.6.6).

Phenotypic identification. For phenotypic identification, the ID 32 GN and Vitek 2 systems (both from bioMérieux, Marcy l'Etoile, France) were used according to the manufacturer's protocols. The results from the 32 GN system were interpreted with the database version 3.1. The identification with Vitek 2 system was performed by using the colorimetric-based GN card, and the results were interpreted by the Advanced Expert system (version VT2-R04.03).

Antimicrobial susceptibility testing. The MIC of isolates was determined by the broth dilution method with the use of an automated Sensititre susceptibility plate (TREK Diagnostic Systems, Ltd., United Kingdom) according to the recommendation of the Clinical Laboratory Standard Institute (9) or as recommended by the manufacturer. *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 reference strains were included for quality control.

Detection of ESBLs, AmpC type beta-lactamases, and insertion sequences (ISs). Beta-lactamase production was detected by the colorimetric nitrocefin disk assay (Remel, Lenexa, KS) according to the manufacturer's instructions. Phenotypic assay for extended-spectrum beta-lactamase (ESBL) production was carried out by using double-disk synergy test on the plates with or without cloxacillin (200 µg/ml) according to the method of Poiriel et al. (20). Detection of the plasmid-mediated class C enzymes was performed by disk potentiation and double-disk synergy test with the use of 3-aminophenylboronic acid (APB) as previously described (26). Genes encoding ESBLs, AmpC type beta-lactamases (including *Acinetobacter*-derived cephalosporinase [ADC]), *tnpA* of IS1236, and ISAbal were detected by PCR method using the primers listed in Table 1. Templates of *Enterobacteriaceae* harboring different ESBL genes, AmpC genes,

and *A. baumannii* harboring ADC gene were included in the PCR as a positive control.

Natural transformation assay. An *Acinetobacter-E. coli* shuttle vector, pOXA58-2, was used as donor DNA in the transformation assay. This shuttle vector has a pET backbone (kanamycin resistance) and contains fragments of *A. baumannii* ATCC 19606^T plasmid origin and *bla*_{OXA58} gene. The electrotransformation of the pOXA58-2 to *A. baylyi* ATCC 33305 could confer a kanamycin and imipenem resistance to the recipient cells (unpublished data). To perform the natural transformation assay, 40 µl of overnight culture was diluted 1:10 in fresh Luria-Bertani broth medium and incubated at 30°C for 2 h with shaking (180 rpm). One microgram of pOXA58-2 was then added. After incubation for another 4 h, 50 µg of DNase I was added to the culture. Aliquots of 0.1 ml were removed and plated on Mueller-Hinton plate with or without 1 µg of imipenem/ml to examine the number of transformants and viable cells, respectively. The transformation of pOXA58-2 was verified by PCR method using the primers OXA-58-F and OXA-58-B. *A. baumannii* ATCC 19606^T was included in the assay for comparison.

Clinical information. All patient data were retrospectively retrieved from medical records. A patient's antimicrobial therapy was considered to be appropriate if the isolate of *A. baylyi* demonstrated in vitro susceptibility to at least one of the antimicrobial agents used within 3 days. The present study was conducted under the approved protocol of Joint Institutional Review Board of Taipei Veterans General Hospital. Since the data were obtained from retrospective chart review, informed consent was not required.

RESULTS

Genotypic identification and ribotyping of the *A. baylyi* isolates. The ITS sequences of the six clinical isolates showed 98

FIG. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of 37 *Acinetobacter* reference (including 16 type) strains. Note the cluster of six clinical isolates with the *A. baylyi* strains. Selected bootstrap analysis (100×) values are shown at the branching points. Scale bar = 0.01 estimated base change per nucleotide. The accession number of the sequence is in parentheses.

to 99% identity with that of several *A. baylyi* reference strains deposited in GenBank. In the genome of *A. baylyi* strain ADP1, there were seven copies of ITSs. They can be divided into two groups based on length and sequence (86.59% sequence similarity between each group). In the phylogenetic analysis, the ITSs from both groups were grouped together with those from the six clinical isolates and other reference strains of *A. baylyi* (data not shown).

In the comparison of the 16S rRNA gene sequences, the six clinical isolates also had the highest similarity (99% for four isolates; 100% for two isolates, accession no. EU604244 and EU604245) with several reference strains of *A. baylyi*. The seven copies of the 16S rRNA gene from strain ADP1 is nearly identical to one another, except in one copy the nucleotide changed from A to G at nucleotide position 949. For phylogenetic study, the clinical isolates and the *A. baylyi* strains formed

a separate cluster within the *Acinetobacter* genus (Fig. 1). The 16S rRNA sequences of these six clinical isolates had 97.31 to 97.87% similarity with that of *Acinetobacter* genomic 13TU (ATCC 17922) and 97.15 to 97.71% similarity with that of *A. baumannii* (ATCC 19606^T). These were the second and third nearest species. The ribopatterns of the six clinical isolates were similar to one another but different from the reference strain (Fig. 2).

Phenotypic identification. The 32 GN system identified five of the clinical isolates as *A. baumannii* (actually equivalent to *A. calcoaceticus*-*A. baumannii* complex) but had a low discriminatory level (<80% probability). The major difference in the assimilation test between *A. baylyi* and *A. calcoaceticus*-*A. baumannii* complex was L-arabinose and D-ribose, which were negative for *A. baylyi* but positive in 93 and 77%, respectively, of the *A. calcoaceticus*-*A. baumannii* complex isolates (provided

FIG. 2. Dendrogram of ribopatterns generated from clinical isolates and reference strain of *A. baylyi*. The dendrogram was constructed by unweighted pair-group method for arithmetic averages clustering analysis.

by the database). One clinical isolate was identified as *A. johnsonii* (91.8% probability). Unexpectedly, the reference strain ATCC 33305 was repeatedly identified as *Ralstonia pickettii*. Indeed, *R. pickettii* was the second nearest species identified for two of the clinical isolates but had a low probability (28.5%). The identification obtained by Vitek 2 assigned three isolates as *A. baumannii* with an “excellent” or “very good” confidence level (>90% probability). Three of the clinical isolates were identified as *Pseudomonas* spp., and the reference strain was identified as *A. lwoffii* with a “good” confidence level.

Antimicrobial susceptibility testing. The reference strain was susceptible to all of the tested antibiotics except aztreonam, to which it showed intermediate resistance (Table 2). Compared to the reference strain, the clinical isolates were more resistant to antimicrobial agents, especially to many classes of beta-lactams. All clinical isolates were susceptible to imipenem, aminoglycosides, levofloxacin, trimethoprim-sulfamethoxazole, and chloramphenicol.

Detection of ESBL, AmpC type beta-lactamase, ISs, and transformation assay. All clinical isolates produced beta-lactamase based on nitrocefin test, but the phenotypic assays failed to demonstrate the production of ESBL, and the inhibitory zone around the disk containing extended-spectrum

cephalosporin did not increase in size in the presence of cloxacillin. Three of the clinical isolates (isolates 133, 9255, and 1477) showed positive results in the double-disk synergy test with the use of APB. With the PCR methods, only the reference strain but none of the clinical isolates gave a band corresponding to *bla*_{ADC-8} gene. In addition, no known ESBL and other AmpC beta-lactamase genes could be detected in the reference or clinical isolates. *IS1236* was detected in the reference strain and all but one of the clinical isolate (723), but *ISAbal* was found only in isolate 9255. Among the six clinical isolates, two retained a similar level of natural competence as the reference strain (Table 3). Three clinical isolates showed a decrease in competence but were still more transformable than the *A. baumannii* type strain (Table 3).

Patients' clinical features. All patients were adults, and four were males (Table 4). The patients had multiple underlying diseases and/or invasive devices. All of the patients, except case 3, acquired the infection nosocomially. Three patients suffered from intracranial hemorrhage and developed bacteremia in the neurological intensive care unit. Case 3, who complained local tenderness over the port-A insertion side, had a purulent discharge, and the pus culture also tested positive for *A. baylyi*. The bacteremia in case 2 was most probably associated with a biliary tract infection, in which the bacteria may have entered

TABLE 2. Antimicrobial susceptibility of a reference strain and 6 clinical isolates of *A. baylyi*

Antimicrobial agent ^a	MIC (μg/ml)						
	ATCC 33305	133	948	4457	9255	723	1477
Gentamicin	0.5	1	0.5	1	1	1	1
Tobramycin	<0.5	1	<0.5	<0.5	<0.5	1	1
Amikacin	<1	2	<1	1	4	2	4
Piperacillin	8	>64	>64	>64	>64	>64	>64
Ampicillin-sulbactam	≤8/4	>16/8	>16/8	≤8/4	>16/8	>16/8	16/8
Piperacillin-tazobactam ^b	8/4	16/4	>64/4	8/4	>64/4	>64/4	>64/4
Ticarcillin-clavulanate ^c	<4/2	>128/2	>128/2	>128/2	>128/2	>128/2	>128/2
Ceftriaxone	8	16	16	4	16	>32	>32
Ceftazidime	4	4	8	4	16	>32	>32
Cefepime	1	2	16	8	>16	16	>16
Aztreonam*	16	16	>16	>16	>16	>16	>16
Imipenem	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Ciprofloxacin	0.06	0.06	0.12	0.12	2	0.06	0.12
Levofloxacin	<0.06	0.12	0.12	0.12	1	0.12	0.12
Chloramphenicol*	8	<2	8	4	4	4	4
TMP/SMX	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

^a *, Criteria used for non-*Enterobacteriaceae* (9). TMP/SMX, trimethoprim-sulfamethoxazole.

^b Fixed concentration of tazobactam at 4 μg/ml.

^c Fixed concentration of clavulanate at 2 μg/ml.

TABLE 3. Results of natural transformation assay

Isolate	Transformation frequency ^a
<i>A. baylyi</i>	
ATCC 33305	$(2.63 \pm 2.36) \times 10^{-5}$
133	$(1.98 \pm 0.82) \times 10^{-5}$
948	$(<1.55 \pm 0.99) \times 10^{-8}$
4457	$(6.43 \pm 0.67) \times 10^{-5}$
9255	$(2.23 \pm 1.32) \times 10^{-7}$
723	$(3.63 \pm 1.88) \times 10^{-7}$
1477	$(5.80 \pm 4.11) \times 10^{-7}$
<i>A. baumannii</i> ATCC 19606 ^T	$(<2.8 \pm 0.6) \times 10^{-8}$

^a Expressed as the number of transformants/number of viable cells (data from triplicate experiments).

via the percutaneous transhepatic cholangial drainage tube. We were unable to determine the routes of infection in the remaining four patients.

All patients presented with fever during bacteremia, with body temperature from 38.2 to 39.7°C (median, 38.8°C). Cases 2 and 4 developed septic shock. Leukocytosis (white blood cell count of $>10^{10}$ cells/liter) was found in cases 1, 2, and 6, and leukopenia (white blood cell count of $<4 \times 10^9$ cells/liter) was found in cases 3 and 5. The value of C-reactive protein, which was available in four patients, ranged from 28.1 to 99.2 mg/liter. Bacterial cultures were monomicrobial in cases 3, 4, 5, and 6. In cases 1 and 2, *Acinetobacter* genomic species 13TU was concomitantly isolated from blood samples. All patients

received antimicrobial therapy with a broad-spectrum beta-lactam antibiotic, with or without an aminoglycoside. Based on our retrospective analysis (Table 4), only three patients received appropriate antimicrobial therapy. None of the patients died from the bacteremia.

DISCUSSION

A. baylyi strain ADP1 is highly competent in natural genetic transformation and metabolic versatility (23) and is the most widely investigated species of *Acinetobacter*. This species is generally considered an environmental bacterium and is frequently isolated from soil and activated sludge (10). There is a recent report of *A. baylyi* being isolated from clinical samples (17). To our knowledge, there have been no reports of *A. baylyi* infection of humans. With the use of genotypic identification method, we were able to identify six clinical isolates of *A. baylyi* from 47 non-*A. calcoaceticus*-*A. baumannii* complex species.

Currently, there is no quantitative definition of *Acinetobacter* species based on ITS sequence analysis. Chang et al. showed that the similarity score between the nearest species of *Acinetobacter* is 92% (6). Although the intraspecies similarities of members of the *A. calcoaceticus*-*A. baumannii* complex are very high (99 to 100%), the intraspecies similarities of species in the non-*A. calcoaceticus*-*A. baumannii* complex are unknown. The ITSs of our clinical isolates best matched those of *A. baylyi*, with a similarity of 98 to 99%. We found two groups of ITSs, with diverse sequence and length, in *A. baylyi* strain

TABLE 4. Clinical characteristics of patients with *A. baylyi* bacteremia^a

Case no.	No. of isolates/yr of isolation	Age (yr)/sex	Underlying diseases	Invasive devices	Ward/day ^b	Treatment	Appropriate antimicrobial therapy	Outcome
1	133/2000	56/M	ICH, postcraniotomy	Arterial line, CVC, foley, ventilator	NICU/6	Ceftazidime plus amikacin for 14 days	Yes	Died of recurrent ICH
2	948/2001	47/M	HBV related cirrhosis, HCC, post-TAE, with tumor recurrent and obstructive jaundice, biliary tract sepsis	PTCD, Foley	Ordinary ward/15	Ampicillin/sulbactam for 9 days	No	Died of malignancy
3	4457/2002	58/M	Plasma cell myeloma, stage III; radiotherapy; chemotherapy	Port-A	Ordinary ward/1	Piperacillin plus amikacin for 10 days; removal of port-A	Yes	Survive
4	9255/2004	69/M	Cerebellar hemorrhage with obstructive hydrocephalus, postcraniotomy; DM, hypertension, renal failure, old pulmonary tuberculosis	Tracheostomy, ventriculoperitoneal shunt	NICU/25	Cefuroxime for 5 days, followed by meropenem for 10 days	No	Died of another episode of pneumonia
5	723/2005	17/F	Acute myelocytic leukemia; chemotherapy	Port-A	Ordinary ward/20	Ceftazidime plus amikacin for 10 days	Yes	Survive
6	1477/2005	83/F	Traumatic ICH, SDH, postcraniotomy, DM, hypertension, old CVA	CVC, Foley, ventilator	NICU/24	Ceftazidime for 10 days	No	Died of recurrent ICH

^a Abbreviations: ICH, intracranial hemorrhage; CVC, central venous catheter; NICU, neurological intensive care unit; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TAE, transarterial embolization; PTCD, percutaneous transhepatic cholangial drainage; DM, diabetes mellitus; SDH, subdural hemorrhage; CVA, central vascular accident.

^b That is, the ward at which bacteremia occurred and that days of bacteremia after hospitalization.

ADP1. *A. haemolyticus* and *A. johnsonii* also have heterogeneity in the number of copies of ITSs (6). However, our phylogenetic analysis clusters the ITSs from both groups in *A. baylyi*. This indicates that analysis of the ITS from either group of *A. baylyi* will not lead to misidentification.

Compared to ITS analysis, analysis of the 16S rRNA gene is a better way to identify *A. baylyi*. The 16S rRNA sequence of the 6 clinical isolates yielded a similarity score of $\geq 99\%$ with sequences of several reference strains of *A. baylyi*. Moreover, there was ≥ 0.5 sequence diversity with the nearest *Acinetobacter* spp. Therefore, based on criteria adopted by most taxonomists (4, 14), the six clinical isolates were confirmed as *A. baylyi*.

The frequency of genetic recombination in *A. baylyi* might cause different ribopatterns between reference strains and clinical isolates, as previously noted in *A. baumannii* (8). Although the ribopatterns of our six clinical isolates were not useful for species identification, they were all very similar, indicating a clonal relationship.

It was well known that phenotypic methods have the limitation for the identification of *Acinetobacter* spp. However, the 32 GN system had a better chance of identifying *A. baylyi* than the Vitek 2 system from clinical samples. We found that the 32 GN system correctly identified all six of the clinical isolates to the genus level, and five were identified as *A. baumannii*, but with low discrimination. The Vitek 2 system identified three isolates as *Acinetobacter* with high probability but misidentified these isolates as belonging to the *A. calcoaceticus*-*A. baumannii* complex. The Vitek 2 misidentified the other three clinical isolates at the genus level. Previous research has also demonstrated that the Vitek 2 fails in the identification of other *Acinetobacter* species, such as *A. ursingii* (11) and *A. lwoffii* (12).

It was previously demonstrated that genome of *A. baylyi* strain ADP1 contains very few traits that are associated with pathogenesis (2). The genome lacks virulence-related genes such as toxins, invasins, and secretory systems, even though it does contain 10 open reading frames encoding hemolysin-like proteins (2). Nevertheless, infection with clinical isolates of *A. baylyi* can result in systemic signs of infection and even septic shock. Notably, four of our bacteremia patients were infected with *A. baylyi* alone, indicating that this species can be pathogenic. All of our affected patients had severe underlying diseases (five with either diabetes mellitus or malignancy), only three patients received appropriate therapy, and yet none of the patients died. Although our sample size is very small, this suggests that *A. baylyi* is an opportunistic pathogen with low virulence.

Interestingly, five of our cases acquired the pathogen nosocomially, in which the reservoir was unknown. Although the clinical isolates of *A. baylyi* were not as resistant to antimicrobial agents as species in the *A. calcoaceticus*-*A. baumannii* complex, they were more antibiotic resistant than the reference strain, especially to beta-lactams, and all of them demonstrated a production of beta-lactamase. Although IS1236 was detected in five isolates and IS*AbaI* was detected in one isolate, none had the known ESBL or AmpC type beta-lactamase gene located downstream of these ISs. Although the inhibitory zones of the extended-spectrum cephalosporins did not increase in size in the presence of cloxacillin, the presence of undetermined plasmid-mediated class C beta-lactamases in some of

the isolates cannot be excluded owing to the positive result in APB test.

Our clinical isolates of *A. baylyi* were susceptible to imipenem, aminoglycosides, levofloxacin, trimethoprim-sulfamethoxazole, and chloramphenicol. The susceptibility to chloramphenicol might differentiate *A. baylyi* from species of the *A. calcoaceticus*-*A. baumannii* complex since 98% (146/149) of the *A. calcoaceticus*-*A. baumannii* complex strains isolated from the same hospital were resistant to chloramphenicol (7). Four of the clinical isolates had diminished transformability. Bacher et al. had also demonstrated the loss of natural competence in *A. baylyi* in an evolutionary experiment (1). Loss of transformability might be due to a corresponding increase in fitness (1).

In conclusion, the present study demonstrated that *A. baylyi* is a potential human pathogen. Based on our study, *A. baylyi* appears to be an opportunistic pathogen that can cause nosocomial infection with a favorable outcome. Clinicians should suspect infection by *A. baylyi* when an isolate from a clinical sample is identified by the 32 GN system as *A. calcoaceticus*-*A. baumannii* complex with low discrimination and the isolate shows susceptibility to chloramphenicol. The isolates can then be subjected to sequence analysis for accurate identification. The high competence of natural transformation and the increasing antibiotic resistance in some of the clinical isolates suggests that this species may become a significant threat in the future.

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