J Med Sci 2018;35(5):222-227
DOI: 10.4103/jmedsci.jmedsci_158_17

ORIGINAL ARTICLE

Risk Factors and Molecular Epidemiology of Carbapenem-Resistant *Acinetobacter calcoaceticus-baumannii* Complex at a District Hospital in Taiwan

Ching-Mei Yu[1], Chia-Jung Lin[2], Rui-Xin Wu[3], Ching-Hsun Wang[4]

[1]Department of Clinical Pathology, National Defense Medical Center, Tri-Service General Hospital, [4]Department of Internal Medicine, Division of Infectious Diseases and Tropical Medicine, National Defense Medical Center, Tri-Service General Hospital, Taipei, [2]Division of Clinical Laboratory, Tri-Service General Hospital Penghu Branch, [3]Division of Infectious Diseases, Tri-Service General Hospital Penghu Branch, Penghu, Taiwan

Aims: The aims of this study were to identify the risk factors and describe molecular epidemiology carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex (ACB complex) at a district hospital in Taiwan. **Materials and Methods:** This is a case–control study at a district hospital in the Penghu Islands, Taiwan, from May 2014 to June 2016. Patients with carbapenem-resistant ACB complex and controls with carbapenem-nonresistant ACB complex were identified, and relevant clinical data obtained from them were compared. Risk factors for carbapenem-resistant ACB complex isolation were searched using bivariable and multivariable analysis. The available isolates from patients were genotyped using a pulsed-field gel electrophoresis (PFGE) method. **Results:** A total of 70 patients were included in this study (36 cases and 34 controls). A bivariable analysis showed that patients who had a hospital admission within the past 3 months and had a recent nasogastric tube insertion had a tendency for subsequent carbapenem-resistant ACB complex isolation (P = 0.066 and 0.051, respectively). Previous exposure to fluoroquinolones was significantly associated with the occurrence of carbapenem-resistant ACB complex (P = 0.028). Further multivariable analysis showed that previous exposure to fluoroquinolones (odds ratio, 10.477; 95% confidence interval, 1.117–98.270; P = 0.040) was an independent risk factor associated with the occurrence of carbapenem-resistant ACB complex. According to PFGE for available carbapenem-resistant ACB complex isolates, one major clone was disseminated in the hospital. **Conclusions:** The antibiotic selective pressure of fluoroquinolone and interpatient dissemination contributed to the occurrence of carbapenem-resistant ACB complex.

Key words: *Acinetobacter baumannii,* carbapenems, drug resistance, risk

INTRODUCTION

The increasing emergence of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex (ACB complex) in Taiwan was noted. According to the Taiwan Surveillance of Antimicrobial Resistance, the prevalence of carbapenem-resistant ACB complex increased from 3.4% in 2002 to 58.7% in 2010.[1,2] Patients infected by this organism are difficult to treat because of the limited therapeutic options. Many studies in Taiwan regarding the molecular and clinical epidemiology of carbapenem-resistant ACB complex have been reported before.[1-5] However, such studies were mostly conducted in a single or a multicentered hospital setting. The application of such research results for relatively smaller scale hospitals was questionable since the patient population and hospital environment were different. Rare epidemiological studies of carbapenem-resistant ACB complex conducted in a regional or district hospital have been reported.[6,7] Therefore, we initiated this study to identify risk factors and

Received: November 25, 2017; Revised: February 23, 2018; Accepted: May 07, 2018
Corresponding Author: Dr. Ching-Hsun Wang, Department of Internal Medicine, Division of Infectious Diseases and Tropical Medicine, National Defense Medical Center, Tri-Service General Hospital, No. 325, Section 2, Cheng-Kung Road, Neihu 114, Taipei, Taiwan.
E-mail: sasak0308@gmail.com

How to cite this article: Yu CM, Lin CJ, Wu RX, Wang CH. Risk factors and molecular epidemiology of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex at a district hospital in Taiwan. J Med Sci 0;0:0.

describe molecular epidemiology of carbapenem-resistant ACB complex at a district teaching hospital located in the Penghu islands, Taiwan.

MATERIALS AND METHODS

Study design

This investigation was a retrospective case–control study. The study procedures were reviewed and approved by the institutional. The study period was from May 2014 to June 2016, and the approval for the study was obtained from the Institutional Review Board (TSGHIRB, 1-103-05-100). The sample included all hospitalized patients aged 18 or older with ACB complex isolated from clinical specimen during the study period. Patients with ACB complex isolates who were younger than 18 years of age or nonadmitted were excluded from the study. Patients included for the study were separated into two groups. The case group comprised patients who were infected or colonized with carbapenem-resistant ACB complex confirmed by the results of the laboratory database. The control group was composed of patients who acquired an ACB complex that is not resistant to imipenem or meropenem.

Hospital setting

The study was conducted at the district teaching hospital in Penghu, Taiwan, with 210 general ward beds and 10 Intensive Care Unit (ICU) beds. It provides acute medical and surgical services to the residents of the Penghu Islands, with an average of 200 admissions per month.

Clinical and microbiologic data collection

The clinical data of identified patients in both groups were recorded through medical chart review: demographic characteristics, comorbidities, severity of the underlying disease according to the McCabe and Jackson classification,[8] exposure to antimicrobial agents 2 weeks before ACB complex isolation, indwelling devices, and clinical outcomes.

ACB complex isolates from the clinical specimen of identified patients were verified with the VITEK 2 automated system (bioMérieux Inc., Marcy-l'Etoile, Rhône, France). ACB complex included *A. calcoaceticus, A. baumannii*, *Acinetobacter* genomic species 3, and 13TU, which were phenotypically indistinguishable using a routine laboratory identification method.[9] Susceptibility of ACB complex isolates was measured by the VITEK 2 automatic system using an automated broth microdilution method. According to the guidelines from the 2016 Clinical and Laboratory Standards Institute, carbapenem-resistant ACB complex is an isolate resistant to imipenem or meropenem defined as the minimal inhibitory concentration ≥8 μg/mL.[10] The susceptibility of identified carbapenem-resistant ACB complex isolates to other antimicrobial agents was also recorded. The British Society for Antimicrobial Chemotherapy tigecycline breakpoints were used as the interpretative criteria.[11]

The clonal relationship of available carbapenem-resistant ACB complex isolates was analyzed by a pulsed-field gel electrophoresis (PFGE) using the restriction enzyme ApaI (New England Biolabs, Ipswich, MA, USA). The banding patterns of PFGE were analyzed with BioNumerics software (Applied Maths, Kortrijk, Belgium) using the Dice coefficient similarity index and unweighted pair group method. PFGE patterns were compared and analyzed according to Tenover *et al*.[12] Strains with PFGE profiles of more than 80% were considered closely related.

Statistical analysis

The results were analyzed using a commercially available software package (SPSS, version 16.0; SPSS Inc., Chicago, IL, USA). The categorical variables were analyzed using Chi-square or Fisher's exact test as appropriate. Continuous variables were compared using Mann–Whitney test. A multivariable analysis was performed using a logistic regression to identify the independent risk factors associated with the isolation of the carbapenem-resistant ACB complex. Variables with $P < 0.1$ on bivariable analysis were considered for inclusion in a multivariable analysis. All P values were two-tailed, and $P < 0.05$ was considered as statistically significant.

RESULTS

From May 2014 to June 2016, the annual incidence of colonization or infection due to carbapenem-resistant ACB complex per 1000 discharges was 2.8, 3.5, and 2.2 in 2014, 2015, and 2016, respectively. During the study period, 36 patients at our institution had carbapenem-resistant ACB complex and 34 had carbapenem-nonresistant ACB complex. Table 1 summarized the clinical characteristics of identified patients and controls with carbapenem-resistant ACB complex and carbapenem-nonresistant ACB complex, respectively. Demographic features, disease severity, comorbidities, and clinical outcomes (14-day and inhospital mortality) were similar in both groups. The bivariate analysis was shown in Table 2. Patients in the carbapenem-resistant ACB complex group had a higher rate of recent admission within 3 months and insertion of indwelling nasogastric tubes as compared with those in the nonresistant complex group, but these differences did not reach significance ($P = 0.066$ and 0.0051, respectively). Of the antimicrobials previously

exposed, fluoroquinolone was the only class that showed a significant difference between groups (22.2% and 2.9%, $P = 0.028$). In the multivariable analysis shown in Table 3, previous fluoroquinolone exposure was independently associated with the presence of ACB complex (odds ratio = 10.477, $P = 0.040$).

The microbiology of carbapenem-resistant ACB complex isolates was shown in Table 4. The most common isolation region was from the respiratory tract. Carbapenem-resistant ACB complex isolates were resistant to multiple antibiotics, and colistin was the only agent with an excellent activity against these isolates. Seven of the 36 carbapenem-resistant ACB complex isolates from respiratory tract were available and used for molecular typing as shown in Figure 1. Of the seven isolates, one isolate belonged to pulsotype A; one isolate belonged to pulsotype B; four isolates belonged to pulsotype C, which was further subdivided into two subtypes (C1 and C2); and one isolate belonged to pulsotype D.

Table 1: Clinical characteristics of patients with carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex

Variable	Case group (*n*=36)*	Control group (*n*=34)*	*P*
Demographic characteristics			
Age (years)†	80.5 (68.5-85.0)	79.0 (55.0-86.0)	0.902
Male, *n* (%)	27 (75)	27 (79.4)	0.778
Comorbidity, *n* (%)			
Cerebrovascular disease	12 (33.3)	8 (23.5)	0.364
Congestive heart failure	5 (13.9)	4 (11.7)	1.000
Chronic obstructive pulmonary disease	4 (11.1)	5 (14.7)	0.731
Diabetes mellitus	13 (36.1)	7 (20.5)	0.151
Liver cirrhosis	3 (8.3)	2 (5.8)	1.000
Malignancy	8 (22.2)	9 (26.4)	0.679
Renal insufficiency	10 (27.8)	8 (23.5)	0.684
McCabe classification, *n* (%)			
Nonfatal	13 (36.1)	13 (36.1)	
Ultimately fatal	8 (22.2)	6 (16.6)	
Rapidly fatal	15 (41.6)	15 (41.6)	
Clinical outcomes, *n* (%)			
14-day mortality	11 (30.6)	5 (14.7)	0.114
Inhospital mortality	12 (33.3)	10 (29.4)	0.724

*The case group comprised patients with carbapenem-resistant ACB complex, and the control group was composed of patients who acquired a carbapenem-nonresistant ACB complex. †Data are presented as median (interquartile range). ACB complex=*Acinetobacter calcoaceticus-baumannii* complex

DISCUSSION

In this study, we described the risk factor and molecular epidemiology of carbapenem-resistant ACB complex in our hospital using a case–control study and a PFGE method. According to this study, previous fluoroquinolone use is a risk factor associated with the isolation of carbapenem-resistant ACB complex. Interpatient transmission further contributed to the dissemination of carbapenem-resistant ACB complex.

Previous studies on carbapenem-resistant ACB complex in Taiwan reported that the risk factors associated with the isolation of carbapenem-resistant ACB complex were carbapenem use, duration of hospital stay, and admission in ICU.[1,6,13] The current study revealed that previous fluoroquinolone use is the only factor associated with the isolation of carbapenem-resistant ACB complex. This difference in the results is attributed to the different clinical settings in the district hospital as compared with the medical centers or regional hospitals in Taiwan. In the district hospital, the decreased severity of disease in inpatients resulted in a short hospital stay, lower rates of ICU admissions, and decreased carbapenem use. As described earlier, previous exposure to fluoroquinolone was a risk factor associated with the isolation of carbapenem-resistant ACB complex.[14,15] This result indicated the selective pressure of antibiotic remained responsible for the occurrence of carbapenem-resistant ACB complex in district hospitals. According to this study, epidemiological data of multiple drug-resistant organisms among different hospital levels may be varied, and measures to control multiple drug-resistant organisms spreading in Taiwan should be individualized according to local epidemiologic data to become cost-effective.

The PFGE findings in this study revealed the evidence of clonal dissemination between patients in the hospital. Specifically, an outbreak occurred from May 2014 to October 2014 in the medical wards caused by carbapenem-resistant ACB complex. After the investigation, results revealed

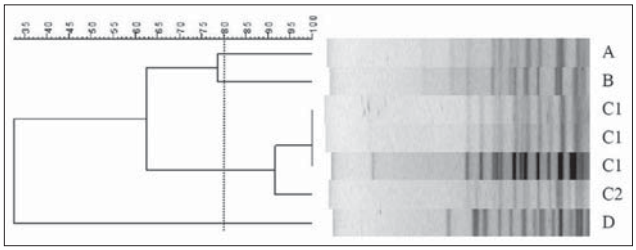

Figure 1: Pulsed-field gel electrophoresis profiles of seven *Acinetobacter calcoaceticus-baumannii* complex isolates from patients. The scale indicates percent similarity. The dotted line indicates the 80% similarity. Four pulsotypes (A–D) and five pulsosubtypes (A–B, C1–C2, and D) were identified

Table 2: Bivariable analysis of patients with carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex

Variable	Case group (*n*=36)*	Control group (*n*=34)*	*P*
Factors related to hospitalization, *n* (%)			
ICU stay before isolation	9 (25.0)	8 23.5)	0.886
Length of hospital stay[†,‡]	1.5 (0–76)	2 (0–7)	0.367
Recent admission within 3 months	31 (86.1)	23 (67.6)	0.066
Comorbidity, *n* (%)			
Cerebrovascular disease	12 (33.3)	8 (23.5)	0.364
Congestive heart failure	5 (13.9)	4 (11.7)	1.000
Chronic obstructive pulmonary disease	4 (11.1)	5 (14.7)	0.731
Diabetes mellitus	13 (36.1)	7 (20.5)	0.151
Liver cirrhosis	3 (8.3)	2 (5.8)	1.000
Malignancy	8 (22.2)	9 (26.4)	0.679
Renal insufficiency	10 (27.8)	8 (23.5)	0.684
Previous antibiotic exposure, *n* (%)			
Third-generation cephalosporin	1 (2.7)	5 (14.7)	0.102
Fourth-generation cephalosporin	2 (5.5)	0	0.493
Carbapenem	4 (11.1)	1 (2.9)	0.358
Fluoroquinolone	8 (22.2)	1 (2.9)	0.028
Glycopeptide	2 (5.5)	0	0.493
Penicillins/beta-lactamase inhibitor	11 (30.5)	9 (26.4)	0.705
Indwelling medical devices, *n* (%)			
Mechanical ventilation	13 (36.1)	11 (32.3)	0.741
Central venous catheter[§]	6 (16.6)	2 (5.8)	0.261
Nasogastric tube	28 (77.8)	19 (55.9)	0.051
Surgical drain	5 (13.9)	7 (20.5)	0.457
Urinary catheter	17 (47.2)	17 (50.0)	0.816

*The case group comprised patients with carbapenem-resistant ACB complex, and the control group was composed of patients who acquired carbapenem-nonresistant ACB complex, [†]Data are presented as median (interquartile range), [‡]Days of stay before isolation of ACB complex, [§]Including double-lumen catheter for hemodialysis, central venous catheter and peripherally inserted central catheter. ACB complex=*Acinetobacter calcoaceticus-baumannii* complex; ICU=Intensive Care Unit

Table 3: A multivariate analysis of the risk factors associated with the occurrence of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex

Variable	OR (95% CI)	*P*
Recent admission within 3 months	2.336 (0.770–7.088)	0.134
Nasogastric tube	2.903 (0.785–10.736)	2.903
Previous fluoroquinolone exposure	10.477 (1.117–98.270)	0.040

CI=Confidence interval; OR=Odds ratio

that the outbreak was related to poor adherence to hand hygiene among bedside caregivers, which has been reported earlier.[16] In addition to our results, intrahospital and interhospital clonal dissemination among different levels of hospitals in Taiwan have been reported.[4,6,7,17] This worrisome phenomenon was attributed to the frequent patient transfers to the different levels of hospitals in Taiwan and poor adherence to hand hygiene among health-care workers. To control the clonal dissemination of carbapenem-resistant ACB complex in Taiwan, patients with carbapenem-resistant ACB complex were informed before transferring them to other hospitals, and strict adherence to hand hygiene among health-care workers was emphasized.

This study had several limitations. First, the relatively small sample size and retrospective case–control design may possibly subject to selection bias and recall bias. In addition, causal relation also could not verified. Therefore, the results must be carefully interpreted. Second, the identification system used in our hospital was unable to identify the difference among ACB complex isolates, and the difference between species may bias our analysis.[18] Third, this study was conducted in a district hospital in the outlying islands

Table 4: Source of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex isolates and related antimicrobial susceptibility

	Case group (*n*=36)*	Control group (*n*=34)*
Source of isolation, *n* (%)		
Respiratory tract	21 (58.3)	16 (47.1)
Urinary tract	13 (36.1)	7 (20.6)
Cerebrospinal fluid	1 (2.8)	0
Catheter	1 (2.8)	1 (2.9)
Blood	0	5 (14.7)
Wound	0	5 (14.7)
Antimicrobial susceptibility, *n* (%)		
Ceftriaxone	0	2 (5.8)
Ceftazidime	0	18 (52.9)
Cefepime	2 (5.5)	22 (64.7)
Gentamicin	4 (11.1)	22 (64.7)
Piperacillin/tazobactam	0	15 (44.1)
Ciprofloxacin	1 (2.8)	17 (50.0)
Trimethoprim-sulfamethoxazole	6 (16.6)	19 (55.9)
Tigecycline†	21 (63.6)	30 (90.1)
Colistin	36 (100)	33 (97.1)

*The case group comprised patients with carbapenem-resistant ACB complex and the control group, †Only 33 isolates were tested for tigecycline antimicrobial susceptibility composed of patients who acquired carbapenem-nonresistant ACB complex. ACB complex=*Acinetobacter calcoaceticus-baumannii* complex

of Taiwan; thus, our findings were not generalizable to other hospitals of the same level in Taiwan. Therefore, future prospective study of multihospital of district level may be helpful for validating our results.

CONCLUSIONS

We first described the epidemiology of carbapenem-resistant ACB complex in the district hospitals in Taiwan. Results show that cautious fluoroquinolone use and strict adherence to hand hygiene among health-care workers are critical to halt the emergence of carbapenem-resistant ACB complex.

Financial support and sponsorship

Supported by grants from the Tri-Service General Hospital, National Defense Medical Center (TSGH-C105-182), Taiwan.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Su CH, Wang JT, Hsiung CA, Chien LJ, Chi CL, Yu HT, *et al.* Increase of carbapenem-resistant *Acinetobacter baumannii* infection in acute care hospitals in Taiwan: Association with hospital antimicrobial usage. PLoS One 2012;7:e37788.
2. Kuo SC, Chang SC, Wang HY, Lai JF, Chen PC, Shiau YR, *et al.* Emergence of extensively drug-resistant *Acinetobacter baumannii* complex over 10 years: Nationwide data from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC Infect Dis 2012;12:200.
3. Hsieh WS, Wang NY, Feng JA, Weng LC, Wu HH. Types and prevalence of carbapenem-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex in Northern Taiwan. Antimicrob Agents Chemother 2014;58:201-4.
4. Kuo SC, Yang SP, Lee YT, Chuang HC, Chen CP, Chang CL, *et al*. Dissemination of imipenem-resistant *Acinetobacter baumannii* with new plasmid-borne bla(OXA-72) in Taiwan. BMC Infect Dis 2013;13:319.
5. Siegel JD, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in health care settings, 2006. Am J Infect Control 2007;35:S165-93.
6. Lin MF, Yang CM, Lin CH, Huang ML, Tu CC, Liou ML, *et al.* Clinical features and molecular epidemiology of multidrug-resistant *Acinetobacter calcoaceticus*-A *Baumannii* complex in a regional teaching hospital in Taiwan. Am J Infect Control 2009;37:e1-3.
7. Chen CM, Liu PY, Ke SC, Wu HJ, Wu LT. Investigation of carbapenem-resistant *Acinetobacter baumannii* isolates in a district hospital in Taiwan. Diagn Microbiol Infect Dis 2009;63:394-7.
8. McCabe WR, Jackson GG. Gram-negative bacteremia I. Etiology and ecology. Arch Intern Med 1962;110:847-55.
9. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: Emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-82.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing Tisew. PA: Clinical and Laboratory Standards Institute; 2016.
11. Hope R, Parsons T, Mushtaq S, James D, Livermore DM. Determination of disc breakpoints and evaluation of etests for tigecycline susceptibility testing by the BSAC method. J Antimicrob Chemother 2007;60:770-4.
12. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA,

Murray BE, Persing DH, *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233-9.
13. Sheng WH, Liao CH, Lauderdale TL, Ko WC, Chen YS, Liu JW, *et al.* A multicenter study of risk factors and outcome of hospitalized patients with infections due to carbapenem-resistant *Acinetobacter baumannii*. Int J Infect Dis 2010;14:e764-9.
14. Cheng VC, Chen JH, So SY, Wong SC, Yan MK, Chau PH, *et al.* Use of fluoroquinolones is the single most important risk factor for the high bacterial load in patients with nasal and gastrointestinal colonization by multidrug-resistant *Acinetobacter baumannii*. Eur J Clin Microbiol Infect Dis 2015;34:2359-66.
15. Tan CK, Tang HJ, Lai CC, Chen YY, Chang PC, Liu WL, *et al*. Correlation between antibiotic consumption and carbapenem-resistant *Acinetobacter baumannii* causing health care-associated infections at a hospital from 2005 to 2010. J Microbiol Immunol Infect 2015;48:540-4.
16. Wang CH, Li JF, Huang LY, Lin FM, Yang YS, Siu LK, *et al.* Outbreak of imipenem-resistant *Acinetobacter baumannii* in different wards at a regional hospital related to untrained bedside caregivers. Am J Infect Control 2017;45:1086-90.
17. Chen TL, Lee YT, Kuo SC, Hsueh PR, Chang FY, Siu LK, *et al.* Emergence and distribution of plasmids bearing the blaOXA-51-like gene with an upstream ISAba1 in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. Antimicrob Agents Chemother 2010;54:4575-81.
18. Zhang HZ, Zhang JS, Qiao L. The *Acinetobacter baumannii* group: A systemic review. World J Emerg Med 2013;4:169-74.