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Original Article

The prediction values of carbapenemase detection methods and carbapenem susceptibility testing for clinical outcomes of patients with *Acinetobacter* bacteremia under carbapenem treatment

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Abstract *Background:* Carbapenem-resistant *Acinetobacter* species have emerged as notorious pathogens causing nosocomial infections. Several phenotypic methods have been developed for detecting carbapenemase production in Enterobacteriaceae. The accuracy of these methods in the prediction of carbapenemase production in *Acinetobacter* species has not been studied well.
Methods: This retrospective study enrolled adult patients with *Acinetobacter* bacteremia from four medical centers in Taiwan between 2012 and 2016. Their demographics and clinical outcomes were recorded. The carbapenem susceptibility of the *Acinetobacter* species was determined using the agar diffusion method. The carbapenemase genes were detected by PCR. Four phenotypic methods, including the modified Hodge test (MHT), modified carbapenem inactivation method (mCIM), Carba NP test, and CarbAcineto NP test were carried out to determine the production of carbapenemase.
Results: We analyzed 257 adults who received initial carbapenem monotherapy for the treatment of *Acinetobacter* bacteremia. Shock within three days of bacteremia and acquisition of carbapenem non-susceptible isolates were independently associated with a higher 14-day and 30-day mortality in patients with *Acinetobacter* bacteremia. Among the four phenotypic tests for carbapenemase detection, MHT using the imipenem disc displayed the greatest sensitivity (94%; 95% confidence interval [CI], 89—97%) and specificity (81%; 95% CI, 73—88%) for predicting imipenem non-susceptibility.
Conclusion: Carbapenem non-susceptibility and shock were independent risk factors for mortality in patients with *Acinetobacter* bacteremia. The MHT could predict the carbapenem susceptibility of *Acinetobacter* isolates. It is a cheap and quick assay, which could be applied in clinical practice.

Introduction

Acinetobacter species have become major nosocomial pathogens and are considered a major cause of high mortality in immunocompromised hosts.[1] The emergence of carbapenem-resistant *Acinetobacter* species threatens the efficacy of carbapenem treatment for infections caused by them.[2,3] The most common mechanism of carbapenem resistance among *Acinetobacter* species is the acquisition of carbapenemase-encoding genes, including genes for carbapenem-hydrolyzing class D β-lactamases (CHDLs) and class B metallo-β-lactamases (MBLs).[4] This may cause the spread of antibiotic resistance and render many β-lactams ineffective, including carbapenem. It is important to early recognize the risk factors for mortality of those infected with these pathogens. Therefore, timely and accurate detection of carbapenem-resistant *Acinetobacter* species could help clinicians adopt preventive measures and use antimicrobial agents appropriately.

There are several molecular and phenotypic methods for detecting carbapenemase production in bacteria. Recognition of the carbapenemase-associated genetic structures such as insertion sequence (IS)*Aba1*-$bla_{\text{OXA-23-like}}$, IS*1008*/IS*1006*-ΔIS*Aba3* -$bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-24}}$-like, $bla_{\text{IMP-like}}$, and $bla_{\text{VIM-like}}$ genes using polymerase chain reaction (PCR) is a well-known molecular method for predicting a higher carbapenem minimum inhibitory concentration (MIC) in *Acinetobacter* isolates.[5] In contrast, there are several phenotypic methods available for detecting carbapenemase production in Enterobacteriaceae.[6—8] However, it is unclear whether these phenotypic methods can also be applied on patients receiving carbapenems for treating *Acinetobacter* infection. This study therefore aimed at evaluating the clinical outcomes of patients receiving carbapenem treatment for *Acinetobacter* bacteremia and assessing the efficacy of the phenotypic methods used to detect carbapenemase production of clinical *Acinetobacter* isolates.

Methods

Hospital setting and study population

This retrospective study was conducted from January 2012 to December 2016 at 4 medical centers in Taiwan: Changhua Christian Hospital (CCH, 1676 beds) in Central Taiwan and Mackay Memorial Hospital (MMH, 2055 beds), Taipei Veterans General Hospital (TVGH, 2900 beds), and Tri-Service General Hospital (TSGH, 1712 beds) of National Defense Medical Center in Northern Taiwan. We recruited patients aged ≥20 years with at least one blood culture positive for *Acinetobacter* species and symptoms and signs of infection for the study. Case patients were defined as individuals whose blood cultures grew *Acinetobacter* species without other concomitant microorganism(s) and who had received a type II carbapenem (imipenem and meropenem) as initial monotherapy within 24 h of onset of bacteremia, lasting for a minimum of 24 h. Patients receiving inappropriate dosages of carbapenem for end

organ(s) function and those having incomplete medical records were excluded. The protocol was approved by the hospitals' institutional review boards (CCH: IRB No. 140514, MMH: IRB No. 14MMHIS125, TVGH: IRB No. 2014-07-006CC, TSGH: IRB No. 1-103-05-100).

Data collection

We reviewed medical records to extract patient information, including demographic characteristics, comorbidities, duration of intensive care unit (ICU) and hospital stay, receipt of invasive procedures at the time of bacteraemia onset, infectious foci as well as the schedule, regimens, and doses of antimicrobials administered. The onset of bacteremia was defined as the day when the blood culture yielding *Acinetobacter* species was obtained. The all-cause 14-day and 30-day mortality rates were used as endpoints and defined as death occurring within 14 and 30 days of onset of bacteremia, respectively. The status of patients, discharged before the 30-day limit, was determined by reviewing their outpatient records or contacting them. No patient was lost to follow-up.

Bacterial identification and chemical compounds

We used the initial isolate for the microbiological studies. The bacteria were phenotypically identified as *Acinetobacter* species using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). *Acinetobacter baumannii* was identified using multiplex PCR.[9] Isolates identified as non-*baumannii Acinetobacter* species were further analyzed at the genomic level by 16S—23S ribosomal DNA intergenic spacer sequence analysis.[10] All antimicrobials were purchased from Sigma—Aldrich (St. Louis, MO, USA).

Antimicrobial susceptibility testing

The carbapenem MICs were determined by agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI).[11] Antimicrobial susceptibility was interpreted according to CLSI standards.[12] Specifically, the CLSI susceptible, intermediate, and resistant breakpoints for the imipenem and meropenem MIC against *Acinetobacter* spp. are $\leq$2, 4, and $\geq$8 mg/L, respectively.

Molecular method for confirmation of carbapenem resistance-associated genes

We performed multiplex PCR assays to detect the CHDL genes ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, and $bla_{\text{OXA-143-like}}$).[13] MBLs were identified by PCR assays, including a multiplex PCR with primers specific for the bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{SPM}, and $bla_{\text{GIM-1}}$ genes,[14] and a PCR assay for detecting the presence of $bla_{\text{NDM-1}}$.[15] We analyzed upstream locations of insertion sequences IS*Aba1* of $bla_{\text{OXA-51-like}}$ or $bla_{\text{OXA-23-like}}$, and IS*1008* or IS*1006* of $bla_{\text{OXA-58-like}}$ by PCR mapping.[14,16—18]

Phenotypic methods for carbapenemase production detection

1. The modified Hodge test

The MHT was performed according to the CLSI guidelines.[12] A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of saline was prepared. A 10 μg meropenem or imipenem susceptibility disc was centrally placed in the Mueller-Hinton agar (MHA) plate. A clover leaf-like indentation of the carbapenem-susceptible reference strain *E. coli* 25922 growing along the test *Acinetobacter* strain growth streak within the disk diffusion zone after 16—24 h of incubation indicated a positive MHT result.[12]

2. The modified carbapenem inactivation method

The mCIM was performed by inoculating a loopful (10 μl) of organism from the culture grown on solid media into 2 ml tryptic soy broth (TSB) along with a 10 μg meropenem disc for 4 h.[7] A zone diameter of 6—15 mm was classified as positive (positive for carbapenemase production), 16—18 mm as indeterminate (requiring further testing to confirm the presence or absence of carbapenemase production), and $\geq$19 mm as negative (negative for carbapenemase production).[19] Moreover, when multiple small bacterial colonies were observed growing within a zone of inhibition of $\leq$18 mm, the result was interpreted as positive, while it was interpreted as indeterminate for a zone diameter of $\geq$19 mm. A narrow ring of growth around the meropenem disk, representing carryover of the test organism from the TSB, was ignored.[7,19]

3. The Carba NP test

The Carba NP test was performed and interpreted according to the simplified CNPt-direct protocol.[8] Bacterial colonies grown overnight on MHA were scraped and suspended in two Eppendorf tubes containing 100 μl of 20 mM Tris—HCl lysis buffer and vigorously mixed. This lysate was mixed with 100 μl of an aqueous indicator solution consisting of 0.1% (vol/vol) of Triton X-100, 0.05% phenol red, and 0.1 mmol/L ZnSO4, previously adjusted to pH 7.8. A total of 6 mg/ml imipenem was added in one of the tubes (reaction tube) while no antibiotic was added in the other (control) tube. Tubes were incubated at 35 °C and observed for 2 h. The color change from red to orange/yellow in the reaction tube indicated a positive result.

4. The CarbAcineto NP test

The CarbAcineto NP test, a modified test to detect carbapenemase-producing *Acinetobacter* spp., was performed and interpreted as previous described.[20] A loopful (10 μl) of the tested bacteria was scraped and suspended in two Eppendorf tubes (A and B) containing 100 μl of 5 M NaCl. 100 μl of indicator solution containing phenol red was prepared and added in tubes A (control) and B (reaction).[21] In tube B (reaction), 6 mg/ml imipenem was added. The

Table 1 Demographic and clinical characteristics of patients with *Acinetobacter* bacteremia stratified by 14-day mortality.

Characteristic	All (n = 257)	14-day survivor (n = 174)	14-day non-survivor (n = 83)	P
Demographic characteristics				
Age, median (IQR), years	74 (59–81)	75 (59–82)	71 (58–80)	0.202
Male sex	178 (69.3)	117 (67.2)	61 (73.5)	0.386
Acquired in intensive care unit	138 (53.7)	89 (51.1)	49 (59.0)	0.285
Length of hospitalization before bacteremia, median (IQR), days	20 (10–37)	20 (10–39)	20 (10–34)	0.435
Comorbid condition, No. (%)				
Alcoholism	18 (7.0)	11 (6.3)	7 (8.4)	0.603
Liver cirrhosis	23 (8.9)	12 (6.9)	11 (13.3)	1.000
Chronic kidney disease	93 (36.2)	63 (36.2)	30 (36.1)	1.000
Type 2 diabetes mellitus	92 (35.8)	64 (36.8)	28 (33.7)	0.678
Chronic obstructive pulmonary disease	59 (23.0)	38 (21.8)	21 (25.3)	0.531
Hypertension	99 (38.5)	73 (42.0)	26 (31.3)	0.131
Coronary artery disease	43 (16.7)	33 (19.0)	10 (12.0)	0.211
Congestive heart failure	45 (17.5)	32 (18.4)	13 (15.7)	0.726
Collagen vascular disease	12 (4.7)	10 (5.7)	2 (2.4)	0.348
Malignancy	62 (24.1)	45 (25.9)	17 (20.5)	0.436
Chemotherapy	23 (8.9)	17 (9.8)	6 (7.2)	0.642
Immunosuppressive therapy	21 (8.2)	15 (8.6)	6 (7.2)	1.000
Trauma	7 (2.7)	6 (3.4)	1 (1.2)	0.434
Shock within 3 days	96 (37.4)	51 (29.3)	45 (54.2)	<0.001
APACHE II score, median (IQR)	26 (18–30)	24 (17–29.3)	28 (21–33)	0.001
Invasive procedure use				
Abdominal drainage	38 (14.8)	24 (13.8)	14 (16.9)	0.574
Arterial catheter	121 (47.1)	84 (48.3)	37 (44.6)	0.596
Central venous catheter	178 (69.3)	113 (64.9)	65 (78.3)	0.031
Hemodialysis	44 (17.1)	30 (17.2)	14 (16.9)	1.000
Ventilator	190 (73.9)	126 (72.4)	64 (77.1)	0.451
Infection source				
Respiratory tract	132 (51.4)	89 (51.1)	43 (51.8)	1.000
Urinary tract	10 (3.9)	8 (4.6)	2 (2.4)	0.508
Catheter-related	34 (13.2)	20 (11.5)	14 (16.9)	0.243
Intra-abdomen	13 (5.1)	8 (4.6)	5 (6.0)	0.762
Soft tissue or wound	9 (3.5)	8 (4.6)	1 (1.2)	0.279
Primary bacteremia	59 (23.0)	41 (23.6)	18 (21.7)	0.874
Resistance profiles of bloodstream isolate				
Carbapenem non-susceptible	161 (62.6)	97 (55.7)	64 (77.1)	0.001
Therapy[a]				
Appropriate antimicrobial therapy	138 (53.7)	105 (60.3)	33 (39.8)	0.002
Monotherapy with carbapenem	137 (53.3)	97 (55.7)	40 (48.2)	0.256
Combination therapy	120 (46.7)	77 (44.3)	43 (51.8)	0.256
Tigecycline-based therapy	12 (4.7)	5 (2.9)	7 (8.4)	0.060
Colistin-based therapy	25 (9.7)	14 (8.0)	11 (13.3)	0.188
Sulbactam-based therapy	9 (3.5)	7 (4.0)	2 (2.4)	0.723

[a] All patients received carbapenem treatment in this study. The definition of combination therapy indicates treatment with carbapenems and at least one antibiotic other than carbapenems.
Data are median value (IQR, interquartile range) for continuous variables and number of cases (%) for categorical variables.
Abbreviations: APACHE II, Acute Physiologic and Chronic Health Evaluation II.

tubes were incubated at 37 °C for a maximum of 2 h and observed. The carbapenemase produced by the bacterial strains could hydrolyze the imipenem into a carboxylic derivative and result in a decrease in pH. This would induce a detectable color change of the indicator (phenol red) solution (from red to yellow/orange). In the presence of non-carbapenemase-producing strains, both tube A and tube B showed red color. In terms of the presence of carbapenemase-producing strains, the tube A showed red and tube B displayed yellow/orange. However, when both tubes A and B showed yellow/orange color, it indicated a non-interpretable result.

Table 2 Logistic regression analysis of predictors for 14-day mortality among patients with *Acinetobacter* bacteremia.

Demographic or characteristic	Univariable analysis		Multivariable analysis	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Shock within 3 days	2.856 (1.662–4.907)	<0.001	2.558 (1.438–4.549)	0.001
APACHE II score	1.053 (1.021–1.086)	0.001		
Central venous catheter	1.949 (1.061–3.580)	0.031		
Carbapenem non-susceptible	2.674 (1.478–4.838)	0.001	2.125 (1.014–4.451)	0.046
Appropriate antimicrobial therapy	0.434 (0.254–0.740)	0.002		

Abbreviations: CI, confidence interval; APACHE II, Acute Physiologic and Chronic Health Evaluation II.

Statistical analysis

PASW for Windows version 26 (SPSS, Chicago, IL, USA) was used for all data analyses. The $\chi 2$ test with Yates correction or Fisher's exact test was used to compare categorical data; the Student's t test or Mann–Whitney rank sum test was used to analyze continuous variables as appropriate. Logistic regression models were used to explore independent risk factors for 14-day and 30-day mortality. We performed univariate analyses separately for each of the risk factor to ascertain the odds ratio (OR) and 95% confidence interval (CI). All biologically plausible variables with a *p* value of <0.20 in the univariate analysis were considered for inclusion in the logistic regression model in the multivariable analysis. A backward selection process was utilized. While the carbapenem susceptibility and the results of carbapenemase phenotypic methods may influence each other, these factors were included for multivariable analysis separately. A *p* value of <0.05 was considered statistically significant.

Results

We analyzed 257 patients from four medical centers (CCH: 24, MMH: 45, TVGH: 142, and TSGH: 46) with documented *Acinetobacter* species mono-microbial bacteremia. The overall 14-day mortality rate of patients with *Acinetobacter* bacteremia was 32.3% (83 of 257). Table 1 compares the demographic and clinical characteristics of the survivors and nonsurvors at 14 days after *Acinetobacter* bacteremia. Nonsurvivors were more likely to have shock within three days of bacteremia (54.2% vs. 29.3%, $p < 0.001$), a higher APACHE II score (28 vs. 24, $p = 0.001$), receipt of central venous catheter implantation (78.3% vs. 64.9%, $p = 0.031$), but were less likely to have receipt of appropriate antimicrobial therapy (39.8% vs. 60.3%, $p = 0.002$). The bloodstream isolates obtained from nonsurvivors had a significantly greater rate of non-susceptibility to carbapenems than those from survivors (77.1% vs. 55.7%, $p = 0.001$). There were no significant differences in the infection foci and the therapeutic regimens between survivors and nonsurvivors. Factors that significantly predicted 14-day mortality in logistic regression analysis are shown in Table 2. Multivariate analysis revealed that shock within three days of bacteremia (OR 2.558; 95% CI 1.438–4.549; $p = 0.001$) and acquisition of carbapenem non-susceptible isolates (OR 2.125; 95% CI 1.014–4.451; $p = 0.046$) were the risk factors independently associated with 14-day mortality. The risk factors for 30-day mortality were analyzed in the same fashion as the above analysis and the results were shown as Supplementary Table S1 and Table S2. In addition to shock within three days of bacteremia (OR 2.603; 95% CI 1.486–4.563; $p = 0.001$) and acquisition with carbapenem non-susceptible isolates (OR 2.396; 95% CI 1.197–4.794; $p = 0.014$), a higher APACHE II score (OR 1.035; 95% CI 1.001–1.070; $p = 0.042$) were independently associated with 30-day mortality (Supplementary Table S2).

A. baumannii (141, 54.9%) was most frequently detected species, followed by *Acinetobacter nosocomialis* (82, 31.9%), *Acinetobacter pittii* (23, 8.9%), and *Acinetobacter soli* (5, 1.9%). Of these 257 isolates, 145 (56.4%) and 144 (56.0%) were non-susceptible to imipenem and meropenem, respectively. There were 133 (51.8%) isolates had at least one carbapenemase gene. MHT analysis using the imipenem and meropenem discs showed 157 (61.1%) and 92 (35.8%) isolates positive for carbapenemase production, respectively.

Table 3 shows the sensitivity and specificity of each method for predicting the carbapenem non-susceptibility and presence of carbapenemase-associated genes in all the isolates. Overall, the phenotypic methods had specificities greater than 81% and sensitivities ranging from 16 to 94% for predicting carbapenem non-susceptibility The MHT using the imipenem disc had the best sensitivity (94%) and a specificity of 81% for predicting imipenem non-susceptibility while that using the meropenem disc had a specificity of 100% and sensitivity of 64% for predicting meropenem non-susceptibility. Among the phenotypic tests, the MHT using the imipenem disc also had the best sensitivity (86%) for predicting the presence of carbapenemase-associated genes; however, the Carba NP test had the highest specificity (90%). On the other hand, the carbapenem non-susceptibility had a sensitivity of 90% for predicting the presence of carbapenemase-associated genes. The presence of carbapenemase-associated genes had a sensitivity of 78% and 80% for predicting imipenem and meropenem non-susceptibility, respectively. The imipenem and meropenem MIC distribution of the study isolates and the corresponding values for different methods of detecting carbapenemase production are shown in Supplementary Table S3 and Supplementary Table S4,

Table 3 Accuracy of different assays for carbapenem non-susceptibilities and carbapenemase detection in 257 clinical *Acinetobacter* isolates.

Assay	Carbapenems susceptibilities						Carbapenemase production		
	Imipenem non-susceptibility (N = 145)			Meropenem non-susceptibility (N = 144)			Presence of carbapenemase-associated genes[a] (N = 133)		
	No.[b]	%Sensitivity (95% CI)	%Specificity (95% CI)	No.[b]	%Sensitivity (95% CI)	%Specificity (95% CI)	No.[b]	%Sensitivity (95% CI)	%Specificity (95% CI)
MHT using the imipenem disc	136	94 (89–97)	81 (73–88)	134	–	–	115	86 (79–92)	66 (57–74)
MHT using the meropenem disc	–	–	–	–	64 (55–72)	100 (96–100)	75	56 (48–65)	86 (79–92)
mCIM	89	47 (38–55)	89 (81–94)	89	47 (39–56)	93 (81–94)	80	47 (39–56)	83 (75–89)
Carba NP test	23	16 (10–23)	89 (81–94)	23	16 (10–23)	89 (81–94)	24	18 (12–26)	90 (84–95)
CarbAcineto NP test	90	63 (54–70)	95 (89–98)	90	63 (54–70)	95 (89–98)	72	54 (45–63)	81 (73–87)
Carbapenemase-associated genes[a]	113	78 (71–85)	82 (74–89)	115	80 (72–86)	84 (76–90)	–	–	–
Imipenem non-susceptibility		–	–		–	–		85 (77–90)	75 (66–82)
Meropenem non-susceptibility		–	–		–	–		86 (79–92)	77 (68–84)
Carbapenem non-susceptibility		–	–		–	–		90 (84–95)	67 (58–75)

[a] Include IS*Aba1*-bla_{OXA-51}-like, IS*Aba1*-bla_{OXA-23}-like, IS*1008*/IS*1006*-ΔIS*Aba3*-bla_{OXA-58}-like, bla_{OXA-24}-like, bla_{IMP}-like, and bla_{VIM}-like.
[b] Indicates the number of isolates with positive results of the carbapenemase phenotypic methods or carbapenemase-associated genes.
CI, confidence interval; MHT, modified Hodge test; mCIM, modified carbapenem inactivation method.

respectively. Most of the isolates with an imipenem or meropenem MIC ≥16 exhibited positive results of MHT.

The carbapenemase gene associated genetic structures of the isolates were also analyzed. Table 4 shows their carbapenem susceptibility and the results of phenotypic methods for detecting carbapenemase production. Among all the isolates tested, 124 (48.2%) did not have any carbapenem resistance determinants. Among them, there were 31 (25.0%) and 29 (23.4%) isolates non-susceptible to imipenem and meropenem, respectively. There were 109 (42.4%) and 5 (1.9%) isolates harboring only one specific gene encoding CHDLs and MBLs, respectively. Moreover, 19 isolates (7.4%) harbored more than one carbapenemase genes. Among those carrying only one gene encoding CHDLs or MBLs, IS*Aba1*-bla_{OXA-23}-like gene was most frequently detected (48 isolates [18.7%]) followed by IS*Aba1*-bla_{OXA-51}-like gene (34 isolates [13.2%]), and IS*1008*/IS*1006*-ΔIS*Aba3*-bla_{OXA-58}-like (14 isolates [5.4%]). Among those harboring IS*Aba1*-bla_{OXA-51}-like gene (34), 19 (55.9%) and 21 (61.8%) isolates were non-susceptible to imipenem and meropenem, respectively. For the isolates carrying genes encoding CHDLs other than OXA-51-like, the imipenem or meropenem non-susceptibility rate was higher than 85%.

Discussion

This retrospective study was conducted to evaluate the prognostic factors for patients with *Acinetobacter* bacteremia receiving carbapenem therapy. We found that shock within three days of bacteremia and acquisition with carbapenem non-susceptible isolates were risk factors for mortality in patients with *Acinetobacter* bacteremia. We also evaluated the accuracy of four phenotypic tests for detecting carbapenemase production in *Acinetobacter* isolates. We found that carbapenem susceptibility testing and MHT using the imipenem disc exhibited comparable sensitivities (86% *vs.* 85%) for detecting carbapenemase production in *Acinetobacter* isolates and were higher than other phenotypic tests.

In 2009, the CLSI recommended the use of MHT to detect the presence of carbapenemases in carbapenem-susceptible Enterobacteriaceae, claiming that its sensitivity and specificity exceeded 90%.[22] However, our data for *Acinetobacter* species are inconclusive. One study demonstrated that only 2.2% of the meropenem-resistant *A. baumannii* isolates were MHT positive.[23] In contrast, another study found MHT being positive for 95% *Acinetobacter* isolates with imipenem hydrolyzing ability.[24] A recent study also shows that MHT could be used to detect OXA-48 and New Delhi metallo-β-lactamase (NDM) in clinical gram-negative bacilli, including *Klebsiella pneumoniae*, *A. baumannii*, and *Pseudomonas aeruginosa*.[25] Nevertheless, the relatively small sample size of these studies is insufficient to prove the clinical usefulness of MHT to detect carbapenemase production in *Acinetobacter* species. Regardless of the bacterial species, MHT has been found to show good sensitivity for detecting *K. pneumoniae* carbapenemase (KPC),[26,27] VIM,[27,28] IMP,[28] NDM[25] and OXA-48-like enzymes.[25,27] In this study, excluding OXA-51-like, MHT using the imipenem disc and carbapenem

Table 4 Results of carbapenem non-susceptibilities and phenotypic detection methods for *Acinetobacter* isolates with different carbapenemase genes and associated insertion sequences.

Genetic structures harbored by isolates (No.)	Carbapenem susceptibility determined by agar dilution method, No. (%)		Positive results of phenotypic detection methods of carbapenemase production, No. (%)				
	Imipenem NS	Meropenem NS	MHT (imipenem)	MHT (meropenem)	mCIM	Carba NP	CarbAcineto NP
IS*Aba1*-bla_{OXA-51}-like only (34)	19 (55.9)	21 (61.8)	22 (64.7)	7 (20.6)	14 (41.2)	4 (11.8)	4 (11.8)
IS*Aba1*-bla_{OXA-23}-like only (48)	47 (97.9)	47 (97.9)	46 (95.8)	33 (68.8)	20 (41.7)	6 (25.0)	38 (79.2)
IS*1008*/*1006*-ΔIS*Aba3*-bla_{OXA-58} only (14)	13 (92.9)	12 (85.7)	13 (92.9)	6 (42.9)	5 (35.7)	3 (21.4)	3 (21.4)
bla_{OXA-24}-like only (13)	13 (100.0)	13 (100.0)	13 (100.0)	13 (100.0)	7 (53.8)	2 (15.4)	13 (100.0)
bla_{IMP}-like only (3)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	3 (100.0)
bla_{VIM}-like only (2)	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)
bla_{IMP}-like + bla_{VIM}-like (1)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
IS*Aba1*-bla_{OXA-51}-like + IS*Aba1*-bla_{OXA-23}-like (9)	9 (100.0)	9 (100.0)	9 (100.0)	8 (88.9)	6 (66.7)	2 (22.2)	8 (88.9)
IS*Aba1*-bla_{OXA-51}-like + bla_{OXA-24}-like (1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
IS*Aba1*-bla_{OXA-51}-like + bla_{IMP}-like (1)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
IS*Aba1*-bla_{OXA-51}-like + bla_{VIM}-like (5)	4 (80.0)	4 (80.0)	3 (60.0)	0 (0.0)	3 (60.0)	2 (40.0)	1 (20.0)
IS*Aba1*-bla_{OXA-51}-like + bla_{IMP}-like + bla_{VIM}-like (1)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)
IS*Aba1*-bla_{OXA-23}-like + bla_{OXA-24}-like (1)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)
None of the above genetic structure (124)	31 (25.0)	29 (23.4)	42 (33.9)	17 (13.7)	13 (10.5)	12 (9.7)	23 (18.5)
Total isolates (257)	145 (56.4)	144 (56.0)	157 (61.1)	92 (35.8)	76 (29.6)	36 (14.0)	96 (27.4)

NS, non-susceptibility; MHT, modified Hodge test; mCIM, modified carbapenem inactivation method; IS, insertion sequence.

susceptibility testing showed good positive prediction values (>90%) for the presence of OXA-23-like, OXA-58-like, OXA-24-like, IMP, and VIM enzymes. OXA-51 carbapenemase has been widely spread among *A. baumannii*.[16] However, isolates harboring IS*Aba1*-bla_{OXA-51}-like gene showed poor correlation with carbapenem susceptibility and positive MHT results. A possible explanation for this phenomenon is that clinical variants of OXA-51 enzymes exhibit different hydrolytic activities against carbapenems.[29,30] Furthermore, the contribution of IS*Aba1*-bla_{OXA-51}-like to carbapenem resistance varies, depending on its plasmid or chromosome location.[16] This further explains why MHT using the imipenem disc showed better accuracy than the molecular method for predicting imipenem susceptibility. Further investigation is needed to elucidate the cause of this phenomenon.

As carbapenem resistance in Enterobacteriaceae and other gram-negative bacteria may result from mechanisms other than carbapenemase production, there may be discrepancies between the carbapenem susceptibility and MHT result. Therefore, MHT was removed from the CLSI 2018 edition.[31] Instead, the mCIM and CarbaNP test are considered as more reliable methods for carbapenemase detection.[31] In this study, we found that mCIM showed a low sensitivity (47%) for predicting carbapenemase susceptibility and the presence of carbapenemase-associated genes. Additionally, the Carba NP test displayed a much lower sensitivity for predicting carbapenemase susceptibility (16%) and the presence of carbapenemase-associated genes (18%) while the CarbAcineto NP test exhibited a higher sensitivity (63% and 54%, respectively). Overall, these three phenotypic methods failed to provide

reliable accuracy for predicting either carbapenemase susceptibility or the presence of carbapenemase-associated genes in *Acinetobacter* species. In this study, we observed that patients infected with carbapenem non-susceptible isolates had higher 14-day and 30-day mortality rates. This is compatible with our previous study, which found that patients with *Acinetobacter* bacteremia treated with a carbapenem had a worse outcome when the carbapenem MICs of their isolates were resistant to carbapenem ($\geq$8 mg/L).[5] In this study cohort, there was no specific host factor attributable for worse clinical outcome except for shock within three days of bacteremia. This finding is reasonable since septic shock itself is an entity contributing to a higher mortality.[32] While appropriate antimicrobial therapy was associated with better clinical outcome of the patients in this study, there was no statistical significance. This finding reflects the high mortality of the study cohort and highlights the importance of early identification of these pathogens and the need of development of novel antimicrobial agents against these pathogens.

This is the first large scale study for estimating the utilization of various carbapenemase phenotypic methods for predicting the carbapenem susceptibility and existence of carbapenemase genes in *Acinetobacter* species. We also analyzed the predicting factors for mortality in patients with *Acinetobacter* bacteremia. The major limitation of this study is the variation in patient backgrounds and patient care. These factors may influence the outcomes of patients. However, the large sample size strengthened this study. In addition, we performed the multivariate analysis using the clinical characteristics, carbapenem susceptibility, and antimicrobial therapy and determined that acquisition of carbapenem non-susceptibility isolates and shock within three days of bacteremia could predict the 14-day and 30-day mortality of those with *Acinetobacter* bacteremia.

In conclusion, MHT using the imipenem disc could predict the carbapenem susceptibility and outcomes of patients under carbapenem treatment for *Acinetobacter* bacteremia. As MHT is cheap and easy to perform, it could be an important alternative to molecular methods in a resource-limited area.

Declaration of competing interest

All authors declare that they have no relevant conflicts of interest.

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Appendix A. Supplementary data

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