Journal of Microbiology, Immunology and Infection 57 (2024) 782—790

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Comparison of ERIC carbapenem-resistant Enterobacteriaceae test, BD Phoenix CPO detect panel, and NG-test CARBA 5 for the detection of main carbapenemase types of carbapenem-resistant Enterobacterales

Yu-Tzu Lin [a], Hsiu-Hsien Lin [b], Kun-Hao Tseng [b], Tai-Fen Lee [c], Yu-Tsung Huang [c], Po-Ren Hsueh [b,c,d,*]

[a] *Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, Taiwan*
[b] *Department of Laboratory Medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan*
[c] *Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan*
[d] *Division of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, China Medical University, Taichung, Taiwan*

Received 8 April 2024; received in revised form 28 May 2024; accepted 30 May 2024
Available online 6 June 2024

KEYWORDS
Carbapenem-resistant Enterobacterales;
Carbapenemases;
ERIC carbapenem-resistant Enterobacteriaceae test;
NG-Test CARBA 5;
BD Phoenix CPO detect panel

Abstract *Background:* This study aimed to assess the performance of three commercial panels, the ERIC Carbapenem-Resistant Enterobacteriaceae Test (ERIC CRE test), the NG-Test CARBA 5 (NG CARBA 5), and the BD Phoenix CPO Detect Panel (CPO panel), for the detection of main types of carbapenemases among carbapenem-resistant Enterobacterales (CRE).
Methods: We collected 502 isolates of carbapenem-resistant Enterobacterales (CRE) demonstrating intermediate or resistant profiles to at least one carbapenem antibiotic (ertapenem, imipenem, meropenem, or doripenem). Carbapenemase genes and their specific types were identified through multiplex PCR and sequencing methods. Subsequently, the ERIC CRE test, CPO panel, and NG CARBA 5 assay were conducted on these isolates, and the results were compared with those obtained from multiplex PCR.
Results: The results indicated that the ERIC CRE test exhibited an overall sensitivity and specificity of 98.1% and 93.6%, respectively, which were comparable to 99.1% and 90.6% for the NG CARBA 5. However, the CPO panel demonstrated a sensitivity of only 56.2% in identifying

* Corresponding author. Departments of Laboratory Medicine and Internal Medicine, China Medical University Hospital, School of Medicine, China Medical University, Taichung, Taiwan.
E-mail address: hsporen@gmail.com (P.-R. Hsueh).

https://doi.org/10.1016/j.jmii.2024.05.013

Ambler classes, exhibiting the poorest sensitivity for class A. Moreover, while the ERIC CRE test outperformed the NG CARBA 5 in identifying multi-gene isolates with multiple carbapenemase-encoding genes, the CPO panel failed to accurately classify these isolates.
Conclusions: Our findings support the utilization of the ERIC CRE test as one of the methods for detecting carbapenemases in clinical laboratories. Nonetheless, further optimization is imperative for the CPO panel to enhance its accuracy in determining carbapenemase classification and address limitations in detecting multi-gene isolates.

Introduction

Carbapenems, a class of broad-spectrum β-lactam antibiotics, are extensively used in hospitals to address instances of treatment failure or severe infections caused by multidrug-resistant (MDR) pathogens in patients.[1] The emergence and widespread prevalence of ESBL-producing Enterobacterales have resulted in increased carbapenem utilization for treating infections caused by these bacteria.[2] The emergence of carbapenem-resistant Enterobacterales (CRE) has presented significant challenges in clinical treatments, representing a critical global public health concern.[3,4] Numerous studies have confirmed that CRE-related infections correlate with higher mortality rates.[5—8]

Currently, there are two known mechanisms that lead to carbapenem resistance. The first mechanism involves bacteria producing β-lactamase along a deficiency or mutation in associated porins, which β-lactamase commonly presents as ESBL or AmpC.[9—12] The second involves bacteria generating carbapenemases, typically carried on plasmids.[13] Among carbapenemase-producing Enterobacterales (CPE), prevalent carbapenemases include *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamases (NDM), Verona integrin-encoded metallo-β-lactamases (VIM), imipenemase (IMP), and oxacillinases (OXA). In the Ambler β-lactamase classification, KPC belongs to class A and is the most prevalent class A β-lactamase among gut bacteria.[14] NDM, VIM, and IMP belong to class B; these β-lactamases can hydrolyze all types of β-lactams and are not inhibited by known inhibitors, requiring metal ions for activity.[14,15] OXA belongs to class D and predominantly comprises OXA-48 and similar enzymes (OXA-48-like). OXA displays lower hydrolytic activity against carbapenems, often accompanying ESBL production in isolates.[16]

Given that carbapenemases typically reside on mobile genetic elements, detecting carbapenemases becomes imperative from an infection control standpoint. Moreover, in clinical settings, different strategies can be employed to treat various carbapenemase types. Currently, there are several new drugs are available, exhibiting varied efficacy against distinct carbapenemase types.[17] For instance, drugs like ceftazidime-avibactam and meropenem-nacubactam lack efficacy against metallo-β-lactamases. Conversely, meropenem-vaborbactam specifically targets class A β-lactamases. Thus, accurate diagnosis of CPE infections is critical for optimizing antimicrobial treatment and establishing effective infection control policies.

Several novel techniques have emerged for detecting CPE such as the modified carbapenem inactivation method (mCIM), Carba NP-direct test, immunochromatographic methods (lateral flow assays), matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), PCR, whole genome sequencing, and others.[18] While some of these methods cannot distinguish between carbapenemase types, others have not gained widespread adoption in clinical laboratories or require additional steps beyond routine procedures. Among these, the immunochromatographic assay, such as the NG-Test CARBA 5 (NG CARBA 5; NG Biotech, Guipry, France) has been validated and offers the advantage of a short turnaround time.[19,20] Conversely, the BD Phoenix CPO Detect Test (CPO penal) has the benefit of simultaneous testing alongside routine antimicrobial susceptibility testing.[21]

This study validated two relatively less documented detection methods: the ERIC™ Carbapenem Resistant Enterobacteriaceae Test (ERIC CRE test; Dynamiker Biotechnology, Tianjin, China), an immunochromatographic assay, and the CPO panel. These methods were compared to the NG CARBA 5 using PCR results as the reference standard. Our study encompassed various species and five common carbapenemases, including variants. Additionally, it focused on multi-carbapenemase genes isolates that currently lack sufficient information.

Methods

Bacteria isolates

CRE was defined as intermediate or resistant to at least one carbapenem antibiotic (ertapenem, imipenem, meropenem, and doripenem), in accordance with the CLSI guidelines.[22] This determination was established based on MIC values of ≥1 mg/L for ertapenem, ≥2 mg/L for imipenem, ≥2 mg/L for meropenem, and ≥2 mg/L for doripenem. The CRE isolates utilized in this study were randomly collected from the China Medical University Hospital (CMUH) and National Taiwan University Hospital (NTUH) between 2019 and 2022. A total of 502 isolates were included in present study, comprising 442 *K. pneumoniae*, 43 *Enterobacter cloacae* complex, 12 *Escherichia coli*, 3 *Klebsiella oxytoca*, 1 *Klebsiella aerogenes*, and 1 *Serratia marcescens*. Among the 502 isolates, 30 isolates were resistant only to ertapenem or were intermediate to the

tested carbapenem antibiotics. All the isolates were cultured on sheep blood plates at 37 °C for 18 h before test.

Determination of carbapenemase-encoding genes by multiplex PCR

Isolates were initially cultured on sheep blood plates and subsequently in tryptone soy broth at 37 °C. DNA extraction was carried out using the QIAamp UCP Pathogen Mini Kit (QIAGEN, Hilden, Germany) from overnight cultures. Multiplex PCR was employed to detect eleven common carbapenemase-encoding genes including bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{KPC}, bla_{NDM}, bla_{BIC}, $bla_{OXA\text{-}48\text{-}like}$, bla_{AIM}, bla_{DIM}, bla_{GIM}, and bla_{SIM}, as previously described.[23,24] In evaluating the detection capabilities of NG CARBA-5, ERIC CRE test, and CPO panel in this study, the PCR results were considered the reference standard. Carbapenemase variants were determined by PCR followed by sequencing as previous described[24–27] and the sequences were analyzed by the beta-lactamase database.[28]

ERIC CRE test

A total of 491 isolates were subjected to the ERIC CRE test (Fig. 1), an *in vitro* qualitative assay designed for the detection of the five common carbapenemase families (KPC, NDM, OXA-48, IMP, and VIM). The test utilizes double antibody sandwich and colloidal gold immunochromatography (lateral flow) methods. For each isolate, 300 μl of extraction buffer was combined with a full 1 μl inoculation loop of bacterial samples, and 200 μl of this suspension was then transferred into the test cassette. Results were interpreted after a 15-min incubation at room temperature, with reliability diminishing if read after more than 30 min of incubation.

(https://en.dynamiker.com/index/index/pro_info/aid/666.html).

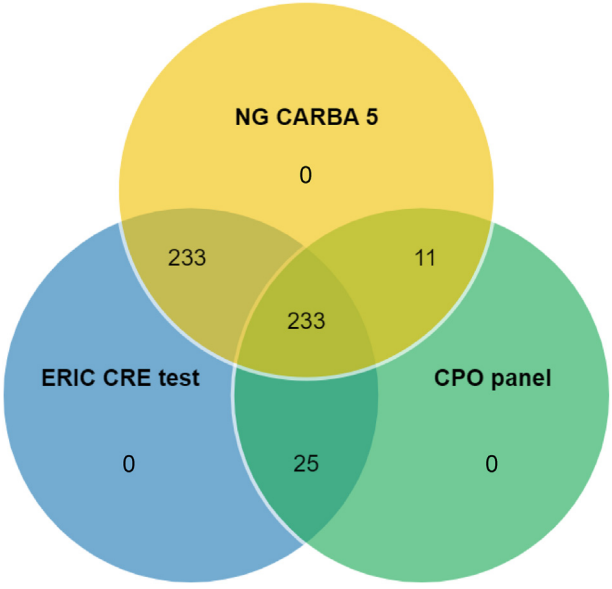


Figure 1. Number of isolates tested with NG CARBA 5, the ERIC CRE test and CPO panel. The yellow circle indicates the number of isolates tested using NG CARBA-5, the blue circle represents the number of isolates tested using the ERIC CRE test, and the green circle indicates the number of isolates tested using the CPO panel.

NG CARBA5

A total of 477 isolates underwent testing using NG-Test® CARBA-5 (Fig. 1), a visual multiplex immunochromatographic (lateral flow) qualitative assay designed to detect and differentiate the five most prevalent carbapenemase families (NDM, IMP, VIM, OXA-48 and KPC) and their most clinically relevant variants in less than 15 min from a bacterial colony. The test was performed following the manufacturer's operating manual (https://www.ngbiotech.com/ng-test-carba-5/). The variants listed in the "variants detected by NG-Test CARBA 5 in publications" section of the NG CARBA 5 instructions are included in Table S1.

BD Phoenix CPO Detect Test (CPO panel)

A total of 269 isolates were included in the assessment of CPO panel (Fig. 1). This automated carbapenemase detection was conducted using the BD Phoenix Automated Microbiology System instrument (BD Diagnostic Systems) with the Phoenix™ NMIC-500 panel which includes the CPO Detect test (CPO panel). The test was executed following the manufacturer's operating manual, and the CPO panel results were interpreted utilizing a BD Phoenix algorithm. Bacterial colonies grown on sheep blood plates were suspended in Phoenix ID broth with McFarland 0.25. These suspensions were then inoculated into the test panel. The CPO panel provides a single result among five categories: carbapenemase producer, Class A carbapenemase producer, Class B carbapenemase producer, Class D carbapenemase producer and carbapenemase negative. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive control of carbapenemase producing strains, respectively.

Data analysis

The 95% confidence intervals (CI) for sensitivity and specificity were calculated by using the Newcombe-Wilson method[29] with VassarStats: Statistical computation website (http://vassarstats.net). To address the CPO panel's limitation in providing a single outcome, isolates with multiple genes were excluded from sensitivity and specificity calculations. Moreover, considering the potential for unclassified outcomes from the CPO panel, two scenarios were contemplated. The first scenario was named as "unclassified as consistent," meaning that regardless of the Ambler class determined by PCR for the isolate, when the CPO panel did not classify the carbapenemase and only provided a result of "carbapenemase producer," it was considered a "true positive." The second scenario was termed "unclassified as inconsistent," where the result was considered a false negative when the CPO panel did not classify the carbapenemase.

Given the misclassifications observed in the CPO panel, these results were simultaneously factored in as both false negatives and false positives when computing the overall sensitivity and specificity. For instance, if PCR results reported Ambler class B but the CPO panel indicated Ambler class D, one false negative is counted for class B and one false positive for class D.

Results

Carbapenemase gene profile among CRE isolates

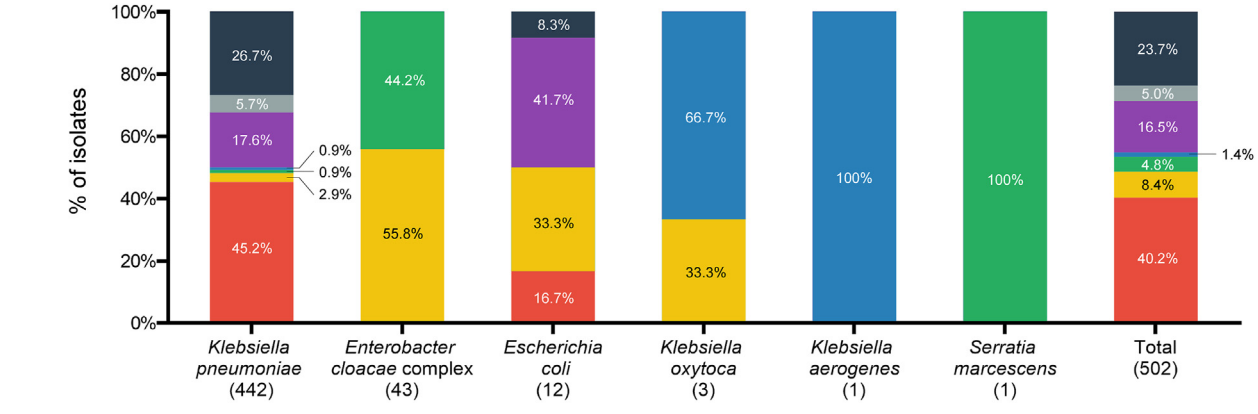
Out of the 502 CRE isolates, 76.3% (383/502) were found to carry carbapenemase genes (CPE) (Fig. 2). Among these CPE isolates, the majority (93.5%, 358/383) contained a single carbapenemase gene, whereas 6.5% (25/383) carried multiple genes. The predominant carbapenemase gene was bla_{KPC}, detected in 52.7% (202/383) of the isolates followed by $bla_{OXA\text{-}48\text{-}like}$ (21.7%, 83/383), bla_{NDM} (11.0%, 42/383), bla_{IMP} (6.3%, 24/383), and bla_{VIM} (1.8%, 7/383). The other six carbapenemase genes were not detected in any isolates. Isolates carrying bla_{NDM} and bla_{IMP} were mainly from the *E. cloacae* complex, while the rest were from *K. pneumoniae*.

Among the collected isolates, five variants of bla_{KPC} variants were identified, including $bla_{KPC\text{-}2}$ (n = 209), $bla_{KPC\text{-}17}$ (n = 2), $bla_{KPC\text{-}33}$ (n = 1), $bla_{KPC\text{-}91}$ (n = 3), and $bla_{KPC\text{-}144}$ (n = 1). The prevalent bla_{NDM} variant was $bla_{NDM\text{-}1}$, observed in 45 isolates, accompanied by four isolates carrying $bla_{NDM\text{-}4}$ and two isolates with $bla_{NDM\text{-}5}$. Three different variants of bla_{IMP} were detected: $bla_{IMP\text{-}8}$ (n = 23), $bla_{IMP\text{-}20}$ (n = 1), and $bla_{IMP\text{-}23}$ (n = 4). For bla_{VIM}, all five isolates carried $bla_{VIM\text{-}1}$. Additionally, 85 isolates harbored $bla_{OXA\text{-}48}$ and $bla_{OXA\text{-}181}$ was detected in 7 isolates. Fifteen carbapenemase genes were not identified with specific variant types.

Among the 119 isolates without detected carbapenemase genes, nine isolates were intermediate to all the tested carbapenem antibiotics. Additionally, 38 isolates were resistant to only one type of carbapenem, with 21 isolates were resistant only to ertapenem.

Sensitivity and specificity of NG CARBA-5, ERIC CRE test, and CPO panel

The selective results obtained from the NG CARBA-5 and the ERIC CRE test among seven CRE isolates are depicted in Fig. 3. The NG CARBA 5 exhibited an overall sensitivity and specificity (with 95% CI) of 99.1% (97.3—99.8%) and a specificity of 90.6% (83.9—94.9%), while the ERIC CRE test showed an overall sensitivity (with 95% CI) of 98.1% (95.9—99.2%) and a specificity (with 95% CI) of 93.6% (87.4—97%) (Table 1). The NG-CARBA 5 displayed sensitivity and specificity exceeding 98.8% for various carbapenemases, except for a sensitivity of 87.5% in VIM. Similarly, the ERIC CRE test showed a lower sensitivity in VIM (60.0%), while other sensitivities were above 97.8%. The CPO panel results indicated that under the "unclassified as consistent" scenario, all three Ambler classes exhibited over 90.0% sensitivity and specificity, with class A performing the best (Table 2). However, under the "unclassified as inconsistent" scenario, the sensitivity of class A dropped to only 4.4%, with successful classification limited to only 4 KPC-carrying isolates (Tables 2 and 4). These findings indicate the notably poor classification ability of the CPO panel for class A carbapenemases.



	Klebsiella pneumoniae (442)	*Enterobacter cloacae* complex (43)	*Escherichia coli* (12)	*Klebsiella oxytoca* (3)	*Klebsiella aerogenes* (1)	*Serratia marcescens* (1)	Total (502)
ND	118	0	1	0	0	0	119
Multi-gene	25	0	0	0	0	0	25
$bla_{OXA\text{-}48\text{-}like}$	78	0	5	0	0	0	87
bla_{VIM}	4	0	0	2	1	0	7
bla_{IMP}	4	19	0	0	0	1	24
bla_{NDM}	13	24	4	1	0	0	42
bla_{KPC}	200	0	2	0	0	0	202

Figure 2. Types of cabapenemases genes determined by multiplex PCR among all the CRE isolates. "Multi-gene" indicates the presence of more than one carbapenemase genes. Among *K. pneumoniae*, 11 bla_{KPC} + bla_{NDM}, three bla_{KPC} + bla_{IMP}, one bla_{KPC} + bla_{VIM}, five bla_{KPC} + $bla_{OXA\text{-}48\text{-}like}$, two bla_{NDM} + $bla_{OXA\text{-}48\text{-}like}$, one bla_{IMP} + bla_{VIM} and two bla_{IMP} + $bla_{OXA\text{-}48\text{-}like}$.

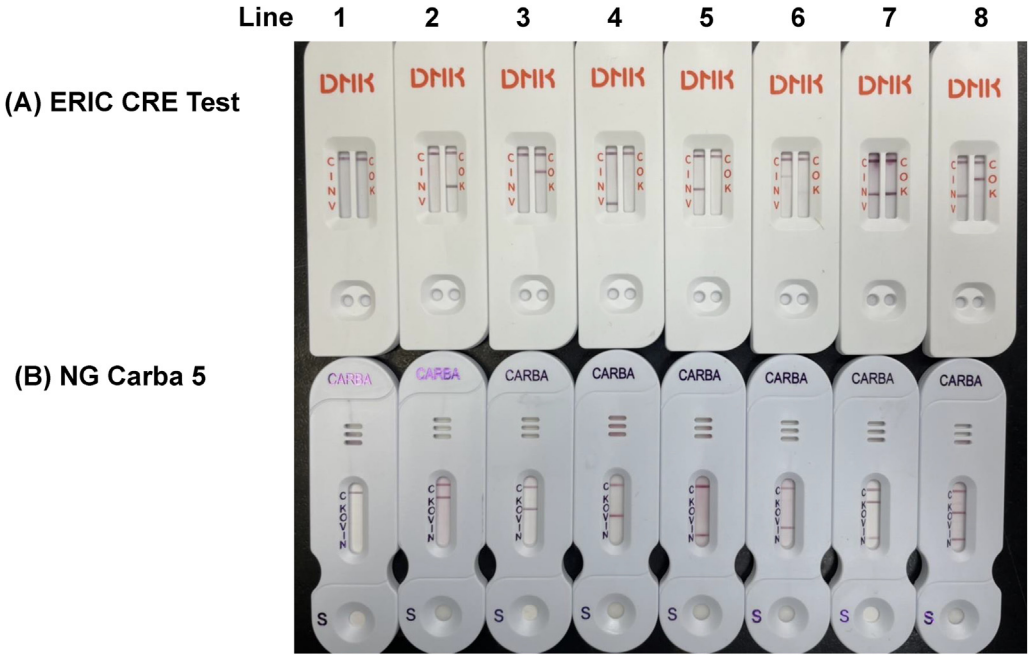


Figure 3. Demonstration of the performance of the two lateral flow assays: (A) the ERIC CRE tes) and (B) the NG CARBA 5 for the detection of five main carbapenemase families (KPC, NDM, OXA-48, IMP and VIM). Line 1, control (C); line 2, positive results for KPC (K); line 3, positive results for OXA-48-like (O); line 4, positive results for VIM (V); line 5, positive results for NDM (N); line 6, positive results for IMP (I); line 7, positive results for both KPC (K) and NDM (D); line 8, positive results for OXA-48 (O) and NDM (N).

Table 1 Comparison of the performance of the NG CARBA 5 and the ERIC CRE test with multiplex PCR.[a]

Results by multiplex PCR	NG CARBA-5						ERIC CRE test					
	TP	FP	FN	TN	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	TP	FP	FN	TN	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
bla_{KPC}	220	1	2	254	99.1 (96.4—99.8)	99.6 (97.5—100.0)	215	1	3	272	98.8 (96.3—99.7)	99.6 (97.7—100.0)
bla_{NDM}	44	5	0	428	100.0 (90.0—100.0)	98.8 (97.2—99.6)	53	0	0	438	100.0 (91.6—100.0)	100.0 (98.9—100.0)
bla_{IMP}	23	3	0	451	100.0 (82.2—100.0)	99.3 (97.9—99.8)	28	1	0	462	100.0 (85.0—100.0)	99.8 (98.6—100.0)
bla_{VIM}	7	0	1	469	87.5 (46.7—99.3)	100.0 (99.0—100.0)	3	4	2	482	60.0 (17.0—92.7)	99.2 (97.8—99.7)
$bla_{OXA\text{-}48\text{-}like}$	85	3	0	389	100.0 (94.6—100.0)	99.2 (97.6—99.8)	90	2	2	397	97.8 (91.6—99.6)	99.5 (98.0—100.0)
Overall	346	12	3	116	99.1 (97.3—99.8)	90.6 (83.9—94.9)	359	8	7	117	98.1 (95.9—99.2)	93.6 (87.4—97.0)

[a] TP, true positive; FP, false positive; FN, false negative; TN, true negative.

Table 2 Comparison of the CPO Panel with multiplex PCR.[a]

Results by multiplex PCR	Unclassified as consistent						Unclassified as false					
	TP	FP	FN	TN	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	TP	FP	FN	TN	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
Class A	86	0	4	154	95.6 (88.4—98.6)	100.0 (97.0—100.0)	4	0	86	154	4.4 (1.4—11.6)	100.0 (97.0—100.0)
Class B	65	8	7	164	90.3 (80.4—95.7)	95.3 (90.7—97.8)	64	8	8	164	88.9 (78-7-94.7)	95.3 (90.7—97.8)
Class D	72	3	8	161	90.0 (80.7—95.2)	98.2 (94.3—99.5)	68	3	12	161	85.0 (74.9—91.7)	98.2 (94.3—99.5)
Overall	223	11	19	1	92.1 (87.9—95.1)	8.3 (0.4—40.2)	136	11	106	1	56.2 (49.7—62.5)	8.3 (0.4—40.2)

[a] TP, true positive; FP, false positive; FN, false negative; TN, true negative.

Table 3 The results of the CPO panel among isolates carrying different types of carbapenemases.

Results by multiplex PCR (no. of isolates)	CPO panel results				
	Positive	Class A	Class B	Class D	ND[a]
bla_{KPC} (90)	82	4	0	1	3
bla_{NDM} (42)	0	0	41	1	0
bla_{IMP} (23)	1	0	17	1	4
bla_{VIM} (7)	0	0	6	0	1
$bla_{OXA-48-like}$ (80)	4	0	7	68	1
bla_{KPC} + bla_{NDM} (11)	11	0	0	0	0
bla_{KPC} + bla_{IMP} (3)	2	0	0	0	1
bla_{KPC} + bla_{VIM} (1)	0	1	0	0	0
bla_{KPC} + $bla_{OXA-48-like}$ (5)	2	1	0	2	0
bla_{NDM} + $bla_{OXA-48-like}$ (2)	0	0	0	2	0
bla_{IMP} + bla_{VIM} (1)	0	0	1	0	0
bla_{IMP} + $bla_{OXA-48-like}$ (2)	0	0	1	1	0
ND[a] (2)	0	0	1	0	1
Total (269)	102	6	74	76	11

[a] ND, not detectable.

Concordance of NG CARBA-5, ERIC CRE test, and CPO panel with PCR for detecting CPE isolates

We further assessed the detection results of the NG CARBA-5, the ERIC CRE test, and the CPO panel for CPE isolates, focusing on discrepancies with PCR results. Table 4 and Table S2 listed the incorrect results from the NG CARBA-5 and the ERIC CRE test. The NG CARBA-5 inconsistencies primarily stemmed from detecting additional carbapenemases, mainly NDM (35.7%, 5/14), followed by OXA-48-like with 3 isolates. Conversely, the ERIC CRE test yielded an approximately equal number of additional and missing results. Additional detections predominantly involved VIM (26.7%, 4/15), followed by OXA-48-like, KPC, and IMP. Missing results mainly comprised three isolates of bla_{KPC} and two isolates each of bla_{VIM} and $bla_{OXA-48-like}$. Importantly, these discrepancies did not occur simultaneously within the same isolates, i.e., a specific type of carbapenemases was not mistakenly identified as another.

Regarding the CPO panel, excluding multi-gene isolates and unclassified results, 20 isolates did not align with PCR outcomes (Tables 3 and S2). Among these, nine isolates did not detect carbapenemases, one displayed an additional result, and ten were incorrectly classified. Notably, the most frequent misclassification was identifying OXA-48-like as class B.

Detection among isolation carrying multiple carbapenemases

We also noticed that there were 25 isolates harboring more than one carbapenemase gene. To evaluate the performance of three methods in detecting multiple carbapenemases, 23, 24, and 25 isolates were tested using the NG CARBA-5, the ERIC CRE test and the CPO panel, respectively. In multi-gene isolates, except for both bla_{IMP} and bla_{VIM} being categorized as class B carbapenemases, the CPO panel mostly displayed "carbapenemase producer" results. Among the 10 discrepancies observed compared to PCR results, eight isolates were identified as producing only one class, while the remaining two isolates were not detected. The NG CARBA-5 showed discrepancies with PCR in four out of 23 isolates, and all of which exhibited additional detection of NDM. In contrast, among multi-gene isolates, the ERIC CRE test displayed the best performance among the three methods, with only one isolate showing discordance with PCR results (Table 4).

The carbapenemase variant types of undetected and misidentified results

When the results of NG CARBA-5 differ from those of PCR, it was primarily due to the detection of additional carbapenemases. Only two isolates were not detected with KPC, and they were both KPC-2 variant. Similarly, for the ERIC CRE test, the undetected KPC variants were also KPC-2. The undetected VIM and OXA-48-like variants were VIM-1 and OXA-48, respectively, representing major types prevalent among the collected isolates. Similar situations were observed in the results obtained from the CPO panel.

Table 4 Discrepant results of the NG CARBA 5 and the ERIC CRE test using multiplex PCR as reference.

Results by multiplex PCR	Incorrect pattern (n)	
	NG CARBA-5	ERIC CRE test
bla_{KPC}	KPC + **IMP** (1), KPC + **OXA-48-like** (1), **ND** (2)	KPC + **VIM** (4), KPC + **OXA-48-like** (1), **ND** (3)
bla_{NDM}	NDM + **OXA-48-like** (1)	**KPC** + NDM (1)
bla_{IMP}	**NDM** + IMP (1)	—
bla_{VIM}	—	**ND** (1)
$bla_{OXA-48-like}$	**IMP** + OXA-48-like (2)	**ND** (2)
bla_{KPC} + bla_{IMP}	KPC + **NDM** + IMP (3)	—
bla_{KPC} + bla_{VIM}	—	KPC (1)
bla_{IMP} + bla_{VIM}	**NDM** + IMP + VIM (1)	—
ND[a]	**KPC** (1), **OXA-48-like** (1)	**IMP** (1), **OXA-48-like** (1)
Total	14/477 (2.9%)	15/491 (3.1%)

ND, not detectable.
The bolded portions represent results that are inconsistent with the PCR findings.

Apart from the previously mentioned variants, NDM-1, IMP-8 and IMP-23 were also among the predominant variant types.

Discussion

In this study, we compared the performance of the NG CARBA 5, the ERIC CRE test and the CPO panel. The results indicated that both the NG CARBA 5 and the ERIC CRE test demonstrated high sensitivity and specificity. However, the CPO panel exhibited good sensitivity in detecting carbapenemases but demonstrated relatively poorer performance in carbapenemase classification, particularly in class A. There was no clear evidence of significant impact from variants on the detection in the present study. For multi-gene isolates, the ERIC CRE test exhibited superior performance compared to the NG CARBA 5, while the CPO panel typically failed to provide accurate classification.

In the scenario of "unclassified as consistent," the sensitivity of the CPO panel was 92.1%. Previous reports have indicated sensitivities ranging from 90% to 100%. A large-scale European study reported a sensitivity of 98.8%.[21] Our findings fall within this range, albeit slightly lower. However, considering the classification results, the sensitivity for class A was only 4.4%. Previous studies have also noted low sensitivity for class A (27.0—85.0%).[21,30—32] Since previous studies were conducted in Europe and Singapore and molecular typing was not performed on our isolates, we couldn't determine the reason for the significantly lower sensitivity of class A compared to other reported studies. The large-scale European study reported a high sensitivity for class D (91.8%).[21] However, in our research, the sensitivity for class D was 85.0%, primarily due to misidentifying OXA-48-like as class B. Conversely, previous studies indicated lower sensitivity for class B (69.0—86.7%).[21,30] However, in our case, the sensitivity for class B was higher than that of class D, at 88.9%.

Currently, there are relatively few reports available on the performance of the NG CARBA 5 in detecting multi-gene isolates, often with a limited number of bacterial isolates studied.[33—35] Overall, while immunochromatographic assays require additional handling in clinical laboratories, they generally exhibit better sensitivity and specificity. On the other hand, although the CPO panel allows simultaneous testing with AST, its capability in carbapenemase classification is less effective.

In a validation study comparing the NG CARBA 5 and the Xpert Carba-R, which included 30 multi-gene isolates, the NG CARBA 5 successfully detected 20 isolates.[36] In contrast, in our study, 82.6% (19/23) of multi-gene isolates were successfully identified by the NG CARBA 5. The ERIC CRE test achieved a detection rate of 95.8%. However, due to limitations in reporting methods, the CPO panel is notably less suitable for detecting multi-gene isolates. When genes belong to different classes, they often cannot be identified into a specific category, or only one category can be determined.

This study identified five types of KPC variants: KPC-2, KPC-17, KPC-33, KPC-91, and KPC-144. In Taiwan, KPC, particularly the KPC-2 variant, is predominant among carbapenem-resistant *K. pneumoniae* isolates.[37—39] KPC-17 is also a commonly found variant among *K. pneumoniae* isolates in Taiwan.[39,40]

In the "variants detected by NG-Test CARBA 5 in publications" section of the NG CARBA 5 instructions, KPC-17, KPC-19, KPC-144, IMP-20, and IMP-23 are not listed but they were successfully detected in this study. Additionally, KPC-33, mentioned as potentially undetectable in the NG CARBA 5 instructions, was successfully detected in this study as well.

It's noteworthy that this is the first report of KPC-33, KPC-91 and KPC-144 being reported in Taiwan. This requires close attention as it has been confirmed that KPC-33 and KPC-144 exhibit resistance to ceftazidime—avibactam.[41,42] Two types of IMP variants, IMP-8 and IMP-23, were found in *K. pneumoniae* in this study. The IMP-8 was reported in Taiwan earlier than KPC-2.[43] The first reported carbapenemase-producing *K. pneumoniae* outbreak in Taiwan was caused by IMP-8.[44] As of now, IMP-8 remains the predominant variant of IMP in Taiwan, with sporadic reports of IMP-23.[40,45,46] There are fewer reports regarding carbapenemase-producing *E. cloacae* complex variants in Taiwan. IMP-8 has been previously reported in this context.[8] In this study, an IMP-20 carrying-*E. cloacae* complex isolate was identified. Limited information is available about IMP-20, with current understanding indicating its lower affinity for meropenem.[47] Remarkably, during the testing of this isolate, the NG CARBA 5 additionally reported NDM. The major carbapenemase type identified in *E. cloacae* complex in the present study was NDM, which is very surprising given that, in the past, there has only been one documented case of NDM found in *E. cloacae* in Taiwan, considered to be an imported case from overseas.[48]

Our study has several limitations. Firstly, not all isolates underwent all three tests. Due to challenges in obtaining supplies, we were unable to conduct all tests for every isolate, particularly the CPO panel. Our focus was primarily on CPE, leading to only two carbapenemase-nonproducing isolates being tested using the CPO panel. This limitation hindered our ability to obtain an accurate overall specificity for the CPO panel combination. Secondly, there were fewer isolates of VIM, which might have contributed to the lower sensitivity of the NG CARBA 5 and the ERIC CRE tests towards VIM. Thirdly, the molecular typing of isolates was not included in our study, so it was difficult to assess if some isolates belong to an outbreak. Furthermore, the presence of multi-carbapenemase genes might affect the accuracy of experimental interpretation, as we didn't analyze the expression of each gene, which renders it impossible for us to discern the reasons for the discrepancies in the test results. Additionally, the focus of the tested isolates primarily centered on *K. pneumoniae* and *E. cloacae* complex and carbapenem-susceptible isolates were not included in the present study.

In conclusion, we validated that the ERIC CRE test exhibited comparable sensitivity and specificity to the NG CARBA 5, and even outperforms the NG CARBA 5 in detecting multi-gene isolates. However, the CPO panel demonstrated poor classification capability for class A carbapenemases and tended to have misclassifications, especially with OXA-48-like. Furthermore, the CPO panel exhibited performance limitations in the detection of multi-gene scenarios.

Author contributions

LY, LH, and TK did data analysis and prepared the manuscript. LY and LH conducted the study design, prepared the Figures and reviewed the manuscript. LY, LH, TK, and LT tested the specimens. All authors read and approved the final version of the manuscript.

Funding

This work was supported by grants from the Ministry of Science and Technology (MOST-111-2320-B-039-054 and 111-2622-8-039-001-IE), China Medical University Hospital (DMR-112-031), and China Medical University (CMU110-N-22).

Data availability statement

The data sets used during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare no competing interests.

CRediT authorship contribution statement

Yu-Tzu Lin: Writing — review & editing, Writing — original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hsiu-Hsien Lin:** Writing — review & editing, Writing — original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kun-Hao Tseng:** Writing — review & editing, Writing — original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tai-Fen Lee:** Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Yu-Tsung Huang:** Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Po-Ren Hsueh:** Writing — review & editing, Writing — original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

References

1. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother* 2011;**55**:4943—60.
2. Sheu CC, Lin SY, Chang YT, Lee CY, Chen YH, Hsueh PR. Management of infections caused by extended-spectrum β-lactamase-producing Enterobacteriaceae: current evidence and future prospects. *Expert Rev Anti Infect Ther* 2018;**16**:205—18.
3. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 2017;**8**:460—9.
4. Willyard C. The drug-resistant bacteria that pose the greatest health threats. *Nature* 2017;**543**:15.
5. Liu YC, Lu CY, Yen TY, Chang LY, Chen JM, Lee PI, et al. Clinical characteristics and outcomes of carbapenem-resistant *Enterobacterales* bacteremia in pediatric patients. *J Microbiol Immunol Infect* 2023;**56**:84—92.
6. Chang HJ, Hsu PC, Yang CC, Kuo AJ, Chia JH, Wu TL, et al. Risk factors and outcomes of carbapenem-nonsusceptible *Escherichia coli* bacteremia: a matched case-control study. *J Microbiol Immunol Infect* 2011;**44**:125—30.
7. Chang YY, Chuang YC, Siu LK, Wu TL, Lin JC, Lu PL, et al. Clinical features of patients with carbapenem nonsusceptible *Klebsiella pneumoniae* and *Escherichia coli* in intensive care units: a nationwide multicenter study in Taiwan. *J Microbiol Immunol Infect* 2015;**48**:219—25.
8. Chen CJ, Lu PL, Jian SH, Fu HL, Huang PH, Chang CY. Molecular epidemiology, risk factors and clinical outcomes of carbapenem-nonsusceptible *Enterobacter cloacae* complex infections in a Taiwan university hospital. *Pathogens* 2022;**11**.
9. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev* 2012;**25**:682—707.
10. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 2013;**13**:785—96.
11. Goodman KE, Simner PJ, Tamma PD, Milstone AM. Infection control implications of heterogeneous resistance mechanisms in carbapenem-resistant Enterobacteriaceae (CRE). *Expert Rev Anti Infect Ther* 2016;**14**:95—108.
12. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol* 2018;**56**.
13. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017;**215**:S28—36.
14. Patel G, Bonomo RA. "Stormy waters ahead": global emergence of carbapenemases. *Front Microbiol* 2013;**4**:48.
15. Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new beta-lactamases from gram-negative bacteria. *Annu Rev Microbiol* 2011;**65**:455—78.
16. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* 2012;**67**:1597—606.
17. Jean SS, Gould IM, Lee WS, Hsueh PR. International Society of Antimicrobial Chemotherapy. New drugs for multidrug-resistant gram-negative organisms: time for stewardship. *Drugs* 2019;**79**:705—14.
18. Lee YL, Chen HM, Hii IM, Hsueh PR. Carbapenemase-producing *Enterobacterales* infections: recent advances in diagnosis and treatment. *Int J Antimicrob Agents* 2022;**59**:106528.
19. Baeza LL, Pfennigwerth N, Greissl C, Gottig S, Saleh A, Stelzer Y, et al. Comparison of five methods for detection of carbapenemases in *Enterobacterales* with proposal of a new algorithm. *Clin Microbiol Infect* 2019;**25**:1286 e9—e15.
20. Liao CH, Lee NY, Tang HJ, Lee SS, Lin CF, Lu PL, et al. Antimicrobial activities of ceftazidime-avibactam, ceftolozane-tazobactam, and other agents against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from intensive care units in Taiwan: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan in 2016. *Infect Drug Resist* 2019;**12**:545—52.

21. Jonas D, Reuter S, Klassen S, Weber S, Buck M, Giani T, et al. Evaluation of the BD Phoenix CPO detect panel for prediction of Ambler class carbapenemases. *Sci Rep* 2021;**11**:13150.
22. CLSI. *Performance standards for antimicrobial susceptibility testing; 33th informational supplement. CLSI document M100-S33*. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.
23. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;**70**:119—23.
24. Yan JJ, Hsueh PR, Ko WC, Luh KT, Tsai SH, Wu HM, et al. Metallo-β-lactamases in clinical *Pseudomonas* isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. *Antimicrob Agents Chemother* 2001;**45**:2224—8.
25. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2001; **45**:1151—61.
26. Zong Z, Zhang X. blaNDM-1-carrying *Acinetobacter johnsonii* detected in hospital sewage. *J Antimicrob Chemother* 2013; **68**:1007—10.
27. Potron A, Nordmann P, Lafeuille E, Al Maskari Z, Al Rashdi F, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D β-lactamase from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2011;**55**:4896—9.
28. Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, et al. Beta-lactamase database (BLDB) - structure and function. *J Enzym Inhib Med Chem* 2017;**32**:917—9.
29. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med* 1998;**17**:873—90.
30. Thomson G, Turner D, Brasso W, Kircher S, Guillet T, Thomson K. High-stringency evaluation of the automated BD Phoenix CPO detect and Rapidec Carba NP tests for detection and classification of carbapenemases. *J Clin Microbiol* 2017; **55**:3437—43.
31. Simon M, Gatermann S, Pfeifer Y, Reischl U, Gessner A, Jantsch J. Evaluation of the automated BD Phoenix CPO Detect panel in combination with the β-CARBA assay for detection and classification of carbapenemase-producing Enterobacterales. *J Microbiol Methods* 2019;**156**:29—33.
32. Ong CH, Ratnayake L, Ang MLT, Lin RTP, Chan DSG. Diagnostic accuracy of BD Phoenix CPO detect for carbapenemase production in 190 Enterobacteriaceae isolates. *J Clin Microbiol* 2018;**56**.
33. Wang Y, Song H, Xu M, Li D, Ran X, Sun Z, et al. Comparing the broth enrichment-multiplex lateral flow immunochromatographic assay with real time quantitative PCR for the rapid detection of carbapenemase-producing organisms in rectal swabs. *BMC Infect Dis* 2023;**23**:413.
34. Khalifa HO, Okanda T, Abd El-Hafeez AA, Abd El Latif A, Habib AG, Yano H, et al. Comparative evaluation of five assays for detection of carbapenemases with a proposed scheme for their precise application. *J Mol Diagn* 2020;**22**:1129—38.
35. Huang YT, Kuo YW, Lee NY, Tien N, Liao CH, Teng LJ, et al. Evaluating NG-Test CARBA 5 multiplex immunochromatographic and Cepheid Xpert CARBA-R assays among carbapenem-resistant *Enterobacterales* isolates associated with bloodstream infection. *Microbiol Spectr* 2022;**10**: e0172821.
36. Liu Z, Bai L, Liu J, Lei J, Gao X, Tenover FC, et al. Parallel validation of the NG-Test Carba 5 and the Xpert Carba-R for detection and characterization of carbapenem-resistant Enterobacterales causing bloodstream infections. *J Mol Diagn* 2021;**23**:1007—14.
37. Lee YL, Lu MC, Shao PL, Lu PL, Chen YH, Cheng SH, et al. Nationwide surveillance of antimicrobial resistance among clinically important Gram-negative bacteria, with an emphasis on carbapenems and colistin: results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2018. *Int J Antimicrob Agents* 2019;**54**:318—28.
38. Liu PY, Lee YL, Lu MC, Shao PL, Lu PL, Chen YH, et al. National surveillance of antimicrobial susceptibility of bacteremic Gram-negative bacteria with emphasis on community-acquired resistant isolates: report from the 2019 Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). *Antimicrob Agents Chemother* 2020;**64**:e01089. 20.
39. Chen CM, Guo MK, Ke SC, Lin YP, Li CR, Vy Nguyen HT, et al. Emergence and nosocomial spread of ST11 carbapenem-resistant *Klebsiella pneumoniae* co-producing OXA-48 and KPC-2 in a regional hospital in Taiwan. *J Med Microbiol* 2018; **67**:957—64.
40. Tseng IL, Liu YM, Wang SJ, Yeh HY, Hsieh CL, Lu HL, et al. Emergence of carbapenemase producing *Klebsiella pneumonia* and spread of KPC-2 and KPC-17 in Taiwan: a nationwide study from 2011 to 2013. *PLoS One* 2015;**10**:e0138471.
41. Shields RK, Nguyen MH, Press EG, Chen L, Kreiswirth BN, Clancy CJ. Emergence of ceftazidime-avibactam resistance and restoration of carbapenem susceptibility in *Klebsiella pneumoniae* carbapenemase-producing *K pneumoniae*: a case report and review of literature. *Open Forum Infect Dis* 2017;**4**: ofx101.
42. Valencio A, da Silva MA, Santos FF, Polatto JM, Machado MMF, Piazza RMF, et al. Capture ELISA for KPC detection in gram-negative bacilli: development and standardisation. *Microorganisms* 2023;**11**:1052.
43. Ma L, Lu PL, Siu LK, Hsieh MH. Molecular typing and resistance mechanisms of imipenem-non-susceptible *Klebsiella pneumoniae* in Taiwan: results from the Taiwan surveillance of antibiotic resistance (TSAR) study, 2002-2009. *J Med Microbiol* 2013;**62**:101—7.
44. Yan JJ, Ko WC, Tsai SH, Wu HM, Wu JJ. Outbreak of infection with multidrug-resistant *Klebsiella pneumoniae* carrying bla_{IMP-8} in a university medical center in Taiwan. *J Clin Microbiol* 2001;**39**:4433—9.
45. Lai CC, Yu WL. *Klebsiella pneumoniae* harboring carbapenemase genes in Taiwan: its evolution over 20 years, 1998-2019. *Int J Antimicrob Agents* 2021;**58**:106354.
46. Wen LL, Kuo PY, Thuy TTD, Duong TTT, Huang YT, Hsueh PR, et al. Genome-based characterization of conjugative IncHI1B plasmid carrying carbapenemase genes bla_{VIM-1}, bla_{IMP-23}, and truncated $bla_{OXA-256}$in *Klebsiella pneumoniae* NTU107224. *Infect Genet Evol* 2023;**110**:105420.
47. Pal A, Tripathi A. An in silico approach for understanding the molecular evolution of clinically important metallo-β-lactamases. *Infect Genet Evol* 2013;**20**:39—47.
48. Lai CC, Chen CC, Lu YC, Chen HJ, Su BA, Weng TC, et al. Simultaneous three Enterobacteriaceae with different bla_{NDM-1}-encoding plasmids in a patient transferred from mainland China to Taiwan. *Infect Drug Resist* 2018;**11**: 2555—60.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.05.013.