



Evaluation of the VITEK2 AST-XN17 card for the detection of carbapenemase-producing *Enterobacterales* in isolates primarily producing metallo β -lactamase

Tomokazu Kuchibiro¹ · Masaru Komatsu² · Katsutoshi Yamasaki³ · Tatsuya Nakamura⁴ · Makoto Niki⁵ · Hisaaki Nishio⁶ · Kaneyuki Kida⁷ · Masanobu Ohama⁷ · Akihiro Nakamura² · Isao Nishi⁸

Received: 19 December 2021 / Accepted: 14 February 2022 / Published online: 24 February 2022
© The Author(s) 2022

Abstract

Carbapenemase-producing *Enterobacterales* (CPE) are not always resistant to carbapenem antimicrobial susceptibility testing (AST) and can be difficult to detect. With the newly created VITEK2 AST-XN17 card, the types of antibiotics measured in AST can be increased. In this study, we evaluated the detectability of CPE using the results of AST with multiple antimicrobial agents with additional measurements of the AST-XN17 card. In addition, we evaluated the CPE detectability of comments on CPE using the VITEK2 Advance Expert System (AES). In total, 169 *Enterobacterales* samples, including 76 non-CPE and 93 CPE, collected from multiple medical institutions in the Kinki region of Japan, were used in this investigation. AST with VITEK2 was performed by adding the AST-XN17 card in addition to the AST-N268 or AST-N404 card. Measurement results were identified using cutoff values, primarily Clinical and Laboratory Standards Institute breakpoints, and the CPE detection capability of each antibiotic was evaluated in several terms, including sensitivity and specificity. The drugs highly sensitive to CPE detection were faropenem (FRPM) > 2 $\mu\text{g}/\text{mL}$ at 100% and meropenem > 0.25 $\mu\text{g}/\text{mL}$ at 98.9%; the highest specificity to CPE detection was for avibactam/ceftazidime (AVI/CAZ) > 8 $\mu\text{g}/\text{mL}$ at 100%. The sensitivity and specificity of each card in the AES output were 86.2% and 94.7% for AST-N404 and AST-XN17 and 91.5% and 90.8% for AST-N268 and AST-XN17, respectively. AST using the VITEK2 AST-XN17 card is a useful test method of screening for CPE.

Keywords Carbapenemase-producing *Enterobacterales* · Antimicrobial susceptibility testing · VITEK2 · Advance Expert System

Introduction

Carbapenems are a commonly used primary therapeutic option for serious infections caused by gram-negative bacilli. They are often considered agents of last resort. In recent years, an increase in carbapenemase-producing *Enterobacterales* (CPE) has been reported, and this has become a global problem [1, 2]. Resistance to carbapenem among *Enterobacterales* is mediated by various mechanisms, including production of carbapenem-hydrolyzing enzymes (so-called carbapenemases), alteration in outer membrane permeability, and in certain circumstances, overproduction of an

AmpC- or extended-spectrum β -lactamase (ESBL)-type enzyme combined with porin loss/modification [1, 3–5]. Carbapenemase is classified as metallo- β -lactamase (MBL) and serine carbapenemase, and several types of carbapenemase-producing genes have been reported in *Enterobacterales* [6]. As there are various types of carbapenemase-producing genes and *Enterobacterales* species, they show different patterns of antibiotic resistance [6]. Among CPE, there are so-called stealth-type strains in which the minimum inhibitory concentrations (MIC) of carbapenem are not determined to be resistant beyond the breakpoint upon antimicrobial susceptibility testing (AST) [7]. Therefore, it may be difficult to detect CPE based only on the results of routine AST such as with imipenem (IPM) and meropenem (MEPM) in case with low MIC values cases. In this study, a newly developed VITEK2 AST-XN17 card (bioMérieux, Marcy-l'Étoile, France) was used. This card allows users to

✉ Tomokazu Kuchibiro
tk_kensa@nagahp.jp

Extended author information available on the last page of the article

perform AST for the measurement of various antibiotics in addition to the standard antibiotics tested by the existing VITEK2 AST card; thus, susceptibility measurement of a wider variety of antibiotics in addition to the AST card used for routine measurement becomes possible. The present study aimed to evaluate the detectability of CPE based on the results of AST with multiple antimicrobial agents in conjunction with additional measurements of the AST-XN17 card.

Materials and methods

The strains evaluated were stock and clinical isolates of β -lactamase-producing *Enterobacteriales* strains collected from 2010 to 2020 by the Naga Municipal Hospital and the Study of Bacterial Resistance in the Kinki Region of Japan. Isolates were obtained from various clinical sources (e.g., blood cultures, urine, and sputum), and there was no duplication of isolates from the same patient. A total of 169 *Enterobacteriales* isolates, including 76 non-CPE (including extended-spectrum β -lactamases and/or AmpC producers) and 93 CPE (79 IMP, 2 VIM, 1 KPC, 2 NDM, 2 OXA, 6 GES, and 1 IMP/GES-producing *Enterobacteriales*) isolates collected from multiple medical institutions in the Kinki region of Japan, were used in this investigation. Confirmation of resistance mechanisms was determined via PCR and sequence analysis, as previously reported [8–12]. The targeted carbapenemase genes were *bla*GES-like, *bla*IMP-1-like, *bla*IMP-2-like, *bla*KPC-like, *bla*oxa-48-like, *bla*NDM-like, and *bla*VIM-like. The amplified products were sequenced using an automated DNA sequencer (ABI 3100, Applied Biosystems, Foster City, CA). In the group containing non-CPE, the PCR results were negative for carbapenemase, and strains suspected of overexpression of chromosomal AmpC or ESBL and/or plasmid-mediated AmpC-producing strains were used. Non-CPE strains were confirmed to be negative by performing phenotypic tests by PCR of the carbapenemase gene and modified carbapenem inactivation method test (mCIM) according to the Clinical and Laboratory Standards Institute (CLSI) M100-S29 [13].

The AST of the test strain was performed using VITEK2 Compact (bioMérieux), and the operation procedure was conducted according to the VITEK2 instruction manual.

The software used was VITEK2 version 9.02, and CLSI M100-S29 [13] was used as the judgment breakpoint. For the test strain, AST by VITEK2 was performed using two types of routine cards, AST-N404 and AST-N268, with AST-XN17 used as an additional card. For MIC values, IPM, MEPM, and TAZ/PIPC were measured with AST-N404, and IPM and MEPM were measured with AST-N268. With the AST-XN17 card, MIC values were measured for faropenem (FRPM), ceftiofloxacin (CFX), ertapenem, latamoxef (LMOX), tazobactam/

ceftolozane, and avibactam/ceftazidime (AVI/CAZ). Because AST-N404 and AST-N268 have significantly different algorithms called drug versions for drug measurement in IPM, measurements were performed using both types of cards in IPM and MEPM. If the measurement result was expressed as terminated results and the MIC value could not be calculated, it was judged to be indeterminate and excluded from the aggregation.

The cutoff value of each antibiotic conformed to CLSIM100-S29, and the MIC value of the breakpoints judged to be susceptibility was set as the cutoff value. The judgment was negative when the value was below the cutoff value and positive when the value was above the cutoff value. As a breakpoint for FRPM has not been set in CLSI, MIC 2 μ g/mL was set independently as a cutoff value based on previously reported values [14, 15]. For MEPM, in addition to the CLSI breakpoint, the cutoff value was set to > 0.25 μ g/mL, which is the closest to the EUCAST epidemiological cutoff value [16] in the VITEK2 measurement range. We defined sensitivity, specificity, positive predictive value, and negative predictive value of individual antibiotics to detect CPE using CLSI breakpoint-based cutoff values. Additionally, we created an algorithm for detecting CPE by combining screening with multiple antibacterial agents based on the results of AST.

For IPM and MEPM, we measured the MIC value by the broth microdilution method using the dry plate Eiken (Eiken Chemical, Tokyo). Subsequently, we assessed the sensitivity and specificity of the recommendations from the Advanced Expert System (AES) to detect CPE. We used AES recommendations expressed by two combinations, one of the AST-N404 and AST-XN17 cards and the other with the AST-N268 and AST-XN17 cards, and we evaluated each combination.

Results

The MIC values of each antibiotic measured by VITEK2 are listed in Tables 1 and 2 for CPE and non-CPE isolates, respectively. Table 3 presents the results of CPE detection performance using the sensitivity, specificity, positive predictive value, and negative predictive value of each antibiotic and the evaluation of AES. The highly sensitive antibiotics were FRPM > 2 μ g/mL at 100%, followed by MEPM > 0.25 μ g/mL and CFX > 8 μ g/mL at 98.9%, and the highest specificity was observed for AVI/CAZ > 8 μ g/mL at 100% and MEPM 1 μ g/mL at 96.2%.

Discussion

The AST-XN17 card can measure additional antibiotics that have been reported to be useful as screening markers for CPE, such as FRPM and LMOX [18–20]. The antibiotic

Table 1 Results of minimum inhibitory concentrations (MICs) by VITEK2 in 93 carbapenemase-producing *Enterobacteriales* isolates

Species	β-lactamase content	No. of strains	MICs(μg/mL) of BMD			AST-N404			AST-N268			XN17			
			IPM	MEPM	IPM	MEPM	IPM	MEPM	IPM	MEPM	FRPM	ETPM	CFX	LMOX	TAZ/PIPC
<i>C. freundii</i> [8]	IMP-1	2	4	16	4 to ≥16	≥16	8 to ≥16	≥16	≥8	≥8	≥64	≥64	≥128	16	≥16
	IMP-6	4	≤0.5 to 2	4 to >16	1	8 to ≥16	8 to ≥16	0.5 to 4	8 to ≥16	≥8	1 to ≥8	≥64	≤4	2 to 8	2 to ≥16
	IMP-19	1	>16	>16	8	≥16	8	≥16	≥8	1	≥64	≥64	≥128	16	≤0.12
	VIM-2	1	2	≤0.5	1	4	4	2	≥8	≥8	≥64	≥64	≥128	16	≥16
<i>E. cloacae</i> [7]	IMP-1	4	1 to 2	2 to 8	1 to 8	≥16	8 to ≥16	≥16	≥8	1 to ≥8	≥64	≥64	64 to ≥128	16 to ≥32	≥16
	IMP-1, GES-4	1	8	16	8	≥16	≥16	≥16	≥8	≥8	≥64	≥64	≥128	16	≥16
	IMP-6	1	1	>16	0.5	≥16	≥16	≤0.25	≥8	≥8	≥64	≥64	16	≥32	≥16
<i>E. coli</i> [17]	VIM-2	1	1	1	≥16	≥16	≥16	≥16	≥8	≥8	≥64	≥64	8	≥32	≥16
	IMP-6	1	2	>16	1	≥16	≥16	≤0.25	≥8	≥8	≥64	≥64	≤4	≥32	≥16
	IMP-6	12	≤0.5	≤0.5 to >16	≤0.25 to 4	≥16	≤0.25 to 4	≥16	4 to ≥8 to 8	0.25 to ≥8	≥64	≥64	≤4 to 32	4 to ≥32	2 to ≥16
	IMP-6	2	1	>16	≤0.25	≥16	≤0.25	≥16	≥8	2 to ≥8	≥64	≥64	≤4	8	≥16
<i>K. aerogenes</i> [1]	IMP-6	1	1	16	1	≥16	2	≥16	≥8	≥8	≥64	≥64	≤4	≥32	≥16
	NDM-5	1	8	>16	≥16	≥16	≥16	≥16	≥8	≥8	≥64	≥64	≥128	≥32	≥16
	IMP-6	1	≤0.5	>16	4	≥16	4	≥16	≥8	≥8	≥64	≥64	≥128	≥32	≥16
<i>K. oxytoca</i> [4]	IMP-6	1	≤0.5	>16	≤0.25	≥16	≤0.25	≥16	≥8	≥8	≥64	≥64	16	≥32	≥16
	IMP-34	3	≤0.5	4 to >16	≤0.25 to 2	≥16	≤0.25 to 2	≥16	≥8	≥8	≥64	≥64	≤4 to 16	8 to ≥32	8 to ≥16
<i>K. pneumoniae</i> (50)	GES-4	4	≤0.5 to 1	≤0.5 to 1	2 to 4	1	2 to ≥16	1 to 4	≥8	0.25 to 2	≥64	≤4 to 8	64 to ≥128	8 to ≥32	0.25 to 1
	GES-4	1	1	1	4	1	4	4	≥8	2	≥64	8	≥128	≥32	1

Table 1 (continued)

Species	β-lactamase content	No. of strains	MICs (μg/mL) of BMD		AST-N404		AST-N268		XN17		TAZ/CTTZ	TAZ/PIPC	LMOX	CFX	ETPM	FRPM	CFX	LMOX	TAZ/PIPC	TAZ/CTTZ	AVI/CAZ
			IPM	MEPM	IPM	MEPM	IPM	MEPM	FRPM	ETPM											
IMP-1	ESBLs/PABL	2	≤0.5 to 4	4 to 8	8 to ≥16	≥16	8 to ≥16	≥16	≥8	≥8	≥64	8 to ≥128	≥64	≥64	≥8	≥8	≥64	≥64	8 to ≥128	≥32	≥16
IMP-6		1	2	8	≥16	≥16	≥16	≥16	≥8	≥8	≥64	64	≥64	≥64	≥8	≥8	≥64	≥64	64	≥32	≥16
IMP-6	CTX-M-2	34	≤0.5 to 2	≤0.5 to >16	≤0.25 to 4	≤0.25 to ≥16	≤0.25 to 2	≤0.25 to ≥16	4 to ≥8	0.25 to ≥8	32 to ≥64	≤4 to ≥128	16 to ≥64	16 to ≥64	0.25 to ≥8	0.25 to ≥8	0.25 to ≥8	0.25 to ≥8	≤4 to ≥128	2 to ≥32	1 to ≥16
IMP-6	CTX-M-2, DHA	1	1	>16	2	≥16	2	≥16	≥8	≥8	≥64	32	≥64	≥64	≥8	≥8	≥64	≥64	32	8	≥16
IMP-8		1	8	8	≥16	4	≥16	4	≥8	≥8	≥64	≥128	≥64	≥64	≥8	≥8	≥64	≥64	≥128	≥32	≥16
IMP-19	CTX-M-1	1	0.5	0.25	4	4	≥16	4	≥8	0.25	≥64	8	≥64	≥64	≥8	≥8	≥64	≥64	8	≥32	≥16
IMP-19	CTX-M-3	1	1	0.25	4	1	≥16	1	≥8	1	≥64	16	≥64	≥64	1	≥8	≥64	≥64	16	≥32	≥16
IMP-34		1	1	2	8	≥16	≥16	≥16	≥8	2	≥64	32	≥64	≥64	≥8	≥8	≥64	≥64	32	≥32	≥16
KPC-2	CTX-M-14	1	>16	>16	≥16	≥16	≥16	≥16	≥8	≥8	≥64	≥128	≥64	≥64	≥8	≥8	≥64	≥64	≥128	≥32	1
OXA-48		1	1	0.5	4	4	4	4	≥8	≥8	≤4	≥128	16	≥64	≥8	≥8	≥64	≥64	≥128	1	0.25
OXA-181		1	2	1	2	1	2	2	≥8	≥8	16	≥128	16	≥64	≥8	≥8	≥64	≥64	≥128	≥32	0.5
IMP-1		1	8	16	≥16	4	1	4	≥8	0.5	≥64	≤4	≥64	≥64	0.5	≥8	≥64	≥64	≤4	8	≥16
NDM-1	CTX-M-65	1	8	4	≥16	≥16	≥16	≥16	≥8	≥8	32	8	16	≥64	≥8	≥8	≥64	≥64	8	≥32	≥16
IMP-1		1	16	16	≥16	≥16	≥16	≥16	≥8	≥8	≥64	8	≥64	≥64	≥8	≥8	≥64	≥64	8	16	2
GES-5		1	8	>16	8	≥16	8	≥16	≥8	≥8	≥64	≥128	≥64	≥64	≥8	≥8	≥64	≥64	≥128	8	2
IMP-1		2	16 to >16	16 to >16	8 to ≥16	≥16	≥16	≥16	≥8	≥8	≥64	64 to ≥128	≥64	≥64	≥8	≥8	≥64	≥64	64 to ≥128	16 to ≥32	≥16

ESBLs extended-spectrum β-lactamases, PABL plasmid mediated AmpC β-lactamase, BMD broth microdilution method, IMP imipenem, MEPM meropenem, FRPM faropenem, ETPM entapenem, LMOX latamoxef, TAZ/PIPC tazobactam/piperacillin, TAZ/CTTZ tazobactam/ceftiozane, AVI/CAZ avibactam/ceftazidime

Table 2 Results of minimum inhibitory concentrations (MICs) by VITEK2 in 76 non-carbapenemase-producing *Enterobacteriales* isolates

Species	β-lactamase content		No. of strains	MICs (µg/mL) of BMD		AST-N404		AST-N268		XN-17						
	PABL	ESBLs		IPM	MEPM	IPM	MEPM	FRPM	ETPM	CFX	LMOX	TAZ/PIPC	CTLZ/TAZ	AVI/CAZ		
<i>E. cloacae</i> [2]	C-AmpC	2	2–4	≤0.5 to 2	1	≤0.25	2	≤0.25	2 to 4	≤0.12	≥64	≤4	≤4	≤0.25	≤0.12 to 0.5	
	CTX-M-1	4	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5 to 1	≤0.12	≤4	≤4	≤4	≤0.25 to 16	≤0.12	
<i>E. coli</i> (38)	CTX-M-2	4	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5 to 2	≤0.12 to 0.25	≤4 to 16	≤4	≤4	2 to 16	≤0.12 to 0.5	
	CTX-M-9	4	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.12	≤4	≤4	≤4 to 16	≤0.25	≤0.12	
SHV-12	5	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5 to 1	≤0.12	≤4 to 16	≤4	≤4	≤0.25	≤0.12		
		TEM-20	1	≤0.5	≤0.5	0.5	≤0.25	≤0.25	2	0.5	8	≥128	4	0.25		
TEM-52	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.12	≤4	≤4	≤4	≤0.25	≤0.12		
CIT	8	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	1 to ≥8	≤0.12 to 0.25	≥64	≤4 to 16	≤4	≤4 to 64	≤0.25 to 2	≤0.12 to 0.25	
		DHA	3	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	2 to 4	≤0.12	≥64	≤4	≤4 to 32	≤0.25	≤0.12	
MOX	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≥8	≤0.12	≥64	≤4	16	0.5	≤0.12	≤0.12		
CTX-M-1	5	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.12 to 0.25	≤4	≤4 to 16	≤4	≤4 to 16	≤0.25	≤0.12 to 0.25	
CTX-M-1 ₁ -M-9	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.12	16	8	≤4	8	1	0.25	
CTX-M-2	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	2	0.25	≥64	≤4	8	2	0.5	0.5	
CTX-M-9	2	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	2 to 4	0.25	≥64	≤4 to 16	32	4 to ≥32	≤0.12 to 0.5	≤0.12 to 0.5	
CTX-M-9	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	≤0.25	4	≤0.12	≥64	≤4	≤4	4	0.25	0.25	
		SHV	1	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	1	≤0.12	≥64	≤4	16	1	0.25	0.25
<i>K. aerogenes</i> [3]	C-AmpC	3	2 to 8	1 to 4	4	1	2	≤0.25 to 4	≥8	≤0.12 to ≥8	≥64	≤4 to 32	≤4 to 32	≤0.25 to 8	≤0.12 to 2	
			CIT	2	≤0.5	≤0.5	≤0.25 to 0.5	≤0.25	≤0.5 to 1	≤0.12	≤4 to 16	≤4	≤4	≤0.25	≤0.12	
<i>K. oxytoca</i> [4]	DHA	1	≤0.5	≤0.5	2	≤0.25	≤0.25	≥8	1	≥64	≥64	≤4	≥128	16	0.5	0.5
			MOX	1	≤0.5	≤0.5	1	≤0.25	≤0.25	≥8	0.25	≥64	≥64	16	0.5	0.5
<i>K. pneumoniae</i> [19]	CTX-M-1	2	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	1 to 4	≤0.12 to 0.25	≤4	≤4	≤4	32	16	0.5	0.5
			CIT	3	≤0.5	≤0.5	0.5 to 1	≤0.25	≥8	0.25	≤0.12 to 0.25	≥64	8 to ≥128	8 to ≥128	to ≥32	to ≥32
CTX-M-2	1	≤0.5	≤0.5	0.5	≤0.25	≤0.25	2	≤0.12	8	≤4	≤4	≤4	≤0.25	≤0.12	≤0.12	
CTX-M-9	2	≤0.5	≤0.5	≤0.25	≤0.25	≤0.25	1 to 4	≤0.12	≤4	≤4	8 to ≥128	8 to ≥128	≤0.25	≤0.12	≤0.12	
CIT	3	≤0.5	≤0.5	2	≤0.25	≤0.25	0.5 to 1	≥8	0.25	≥64	8 to ≥128	8 to ≥128	≤0.25	0.25 to 0.5	0.25 to 0.5	

Table 2 (continued)

Species	β-lactamase content	No. of strains	MICs (μg/mL) of BMD		AST-N404	AST-N268	XN-17	FRPM	ETPM	CFX	LMOX	TAZ/PIPC	CTLZI/TAZ	AVI/CAZ
			IPM	MEPM										
ESBLs	DHA	8	≤0.5 to 4	≤0.5 to 4	≤0.25 to 2	≤0.25 to 1	≥8	0.25 to ≥8	32 to ≥64	≤4 to ≥64	≤4 to ≥64	32 to ≥128	4 to ≥32	0.25 to 8
	MOX	1	≤0.5	≤0.5	≤0.25	1	≥8	0.5	≥64	≥64	≥64	16	2	1
	CTX-M-9	2	≤0.5	≤0.5	≤0.25 to 0.5	≤0.25	≥8	0.25	≥64	≤4	≤4	8	≤0.25 to 16	0.25 to 0.5
<i>P. mirabilis</i> [5]	CTX-M-2	3	≤0.5 to 1	≤0.5	2 to ≥16	2 to ≥16	≥8	≤0.12	8 to 32	≤4	≤4	≤4	≤0.25 to 8	≤0.12 to 0.25
	CIT	1	≤0.5	≤0.5	8	8	≥8	≤0.12	≥64	≤4	≤4	≤4	8	≤0.12
<i>S. marcescens</i> [1]	CTX-M-2	1	≤0.5	≤0.5	4	2	≤0.5	≤0.12	32	8	8	≤4	0.5	≤0.12
	C-AmpC	1	4	2	1	4	≥8	≥8	≥64	≥64	≥64	≥128	8	2

ESBLs extended-spectrum β-lactamases, PABL plasmid mediated AmpC β-lactamase, BMD broth microdilution method, IPM imipenem, MEPM meropenem, FRPM faropenem, ETPM ertapenem, LMOX latamoxef, TAZ/PIPC tazobactam/piperacillin, TAZ/CTLZI tazobactam/ceftiozane, AVI/CAZ avibactam/ceftazidime

with the highest sensitivity was FRPM > 2 μg/mL, showing 100% sensitivity, as previously reported [19], and was considered to be very useful as a primary screening. However, because the specificity was as low as 56.6%, distinguishing CPE alone was challenging, and judging in combination with other agents seems highly useful. Screening for a combination of the most specific AVI/CAZ > 8 μg/mL and the most sensitive FRPM > 2 μg/mL yielded very useful results. If these results are obtained, the strain is likely an MBL-producing strain (65/65). Avibactam is a non-β-lactam-β-lactamase inhibitor that inhibits the activities of Ambler class A β-lactamases, including ESBLs and KPC, class C β-lactamases, and some class D β-lactamases [20]. The in vitro antibacterial activity of AVI/CAZ is likely to be effective against class A (including KPC) and class D (including OXA-48) CPE and AmpC and ESBL-producing *Enterobacteriales* with porin loss/modification [20, 21].

However, AVI/CAZ is unlikely to show antibacterial activity in vitro because avibactam has no inhibitory effect on MBL. These characteristics of in vitro antibacterial activity of AVI/CAZ are similar to the results of this study and appear to be useful as specific markers for the differentiation between MBL and serine-carbapenemase.

Next to FRPM, MEPM > 0.25 μg/mL showed the highest sensitivity. In this study, we measured the MIC of MEPM using both the AST-N404 and AST-N268 cards; however, no difference in MIC values was observed between the two cards. The MIC values of IPM and MEPM obtained with VITEK2 tended to be higher in CPE than those obtained using the broth microdilution method. Therefore, it seems that the sensitivity of CPE screening at MEPM > 0.25 μg/mL was increased. When FRPM > 2 μg/mL and MEPM > 0.25 μg/mL were positive, the possibility of metallo-carbapenemase production was very high, similar to when AVI/CAZ > 2 μg/mL was positive, whereas the possibility of serine-carbapenemase production was suggested when AVI/CAZ > 2 μg/mL was negative. These results suggest that AVI/CAZ > 2 μg/mL may be a cutoff value that can classify MBL and serine-carbapenemase. Based on these results, it is possible to conduct CPE confirmation and genotyping tests. LMOX > 8 μg/mL showed a high positive/negative concordance rate and was considered useful for screening. Owing to the high specificity of LMOX, it may be useful to combine LMOX with FRPM. However, caution is required because the sensitivity to GES-type carbapenemase is low (1/6) and there is a tendency to show false positives for MOX-type plasmid-mediated AmpC β-lactamase.

The algorithm for CPE screening using the AST results of VITEK2 is shown in Fig. 1. The results of this study may provide useful information for examining the necessity of conducting carbapenemase-detection tests. In addition, the recommendations of the VITEK2 AES for inferring carbapenemase production showed high detection performance for

Table 3 Evaluation of carbapenemase-producing *Enterobacteriales* detection sensitivity and specificity for each drug and VITEK2 Advance Expert System

Card NO	Antimicrobial	Cut off value	CPE (n=93)						non-CPE (n = 76)						Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
			Positive			Negative			Positive			Negative						
			Indeterminate	Negative	Positive	Indeterminate	Negative	Positive	Indeterminate	Negative	Positive	Indeterminate	Negative	Positive				
AST-N404	IPM	1	0	46	12	3	61	50.5	83.6	79.7	57.0							
	MEPM	1	0	8	3	73	91.4	96.2	96.6	90.1								
	MEPM	0.25	0	1	7	69	98.9	90.8	92.9	98.6								
AST-N268	IPM	1	0	48	11	65	48.4	85.5	80.4	57.5								
	MEPM	1	0	5	3	73	94.6	96.1	96.7	93.6								
	MEPM	0.25	0	1	8	68	98.9	89.5	92.0	98.6								
AST-XN17	FRPM	2	0	0	33	43	100.0	56.6	73.8	100								
	ETPM	0.5	0	9	6	70	90.3	92.1	93.3	88.6								
	CFX	8	0	1	50	26	98.9	34.2	64.8	96.3								
	LMOX	8	0	5	7	69	94.6	90.8	92.6	93.2								
	TAZ/PIPC	16	0	33	20	54	64.5	73.0	75.0	62.1								
	TAZ/CTLZ	2	0	3	21	53	96.8	71.6	81.1	94.6								
	AVI/CAZ	8	0	28	0	74	69.9	100.0	100	72.5								
Comments of AES																		
AST-N404 + XN17				13	4	72	86.2	94.7	95.3	84.7								
AST-N268 + XN17				8	7	69	91.5	90.8	92.5	89.6								

IPM imipenem, MEPM meropenem, FRPM faropenem, ETPM ertapenem, CFX cefoxitin, LMOX latamoxef, TAZ/PIPC tazobactam/piperacillin, TAZ/CTLZ tazobactam/ceftolozane, AVI/CAZ avibactam/ceftazidime, AES VITEK2 Advance Expert System, PPV positive predictive value, NPV negative predictive value

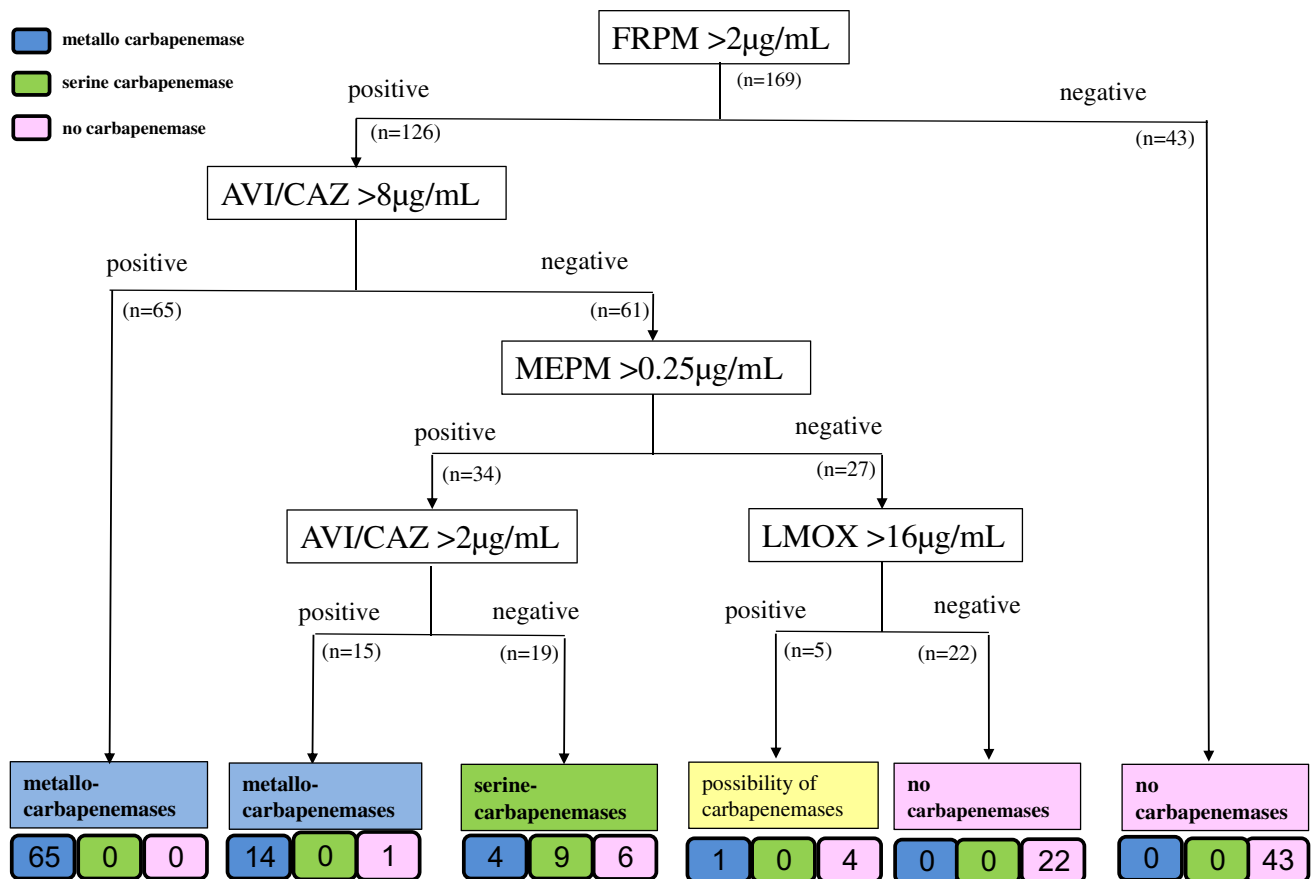


Fig. 1 Algorithm for carbapenemase-producing *Enterobacteriales* (CPE) screening via antimicrobial susceptibility testing using the AST-XN17 card of VITEK2

CPE with either card. The AES has been reported to perform well in the verification of AST results [22]. However, to the best of our knowledge, no studies evaluating the performance of CPE detection using the AES have been reported. This study is the first to evaluate the usefulness of the AES in CPE screening. The AES recommendation is useful for CPE screening, and if CPE are indicated, carbapenemase detection tests should be considered.

The test strains in this study included many strains collected in the Kinki area of Japan and included many IMP-6 (encoded by the *bla*IMP-6 gene) types. IMP-6, an IMP-type MBL, has been reported to confer a paradoxical IPM-susceptible but MEPM-resistant phenotype to *Enterobacteriales* strains [23, 24]. Therefore, it is considered to be the cause of the bias that the sensitivity of IPM was evaluated as low in this study. In this study, we performed PCR to confirm the carbapenemase gene, which is frequently isolated, and did not perform whole genome sequencing. Therefore, the carbapenemase-negative group may carry a rare carbapenemase gene that was not targeted, which is a limitation of the present study. However, we consider it extremely unlikely, as negative results were obtained in

phenotypic tests using mCIM. Furthermore, as the detection of serine-carbapenemase production such as KPC and OXA-48 is extremely rare in Japan, the small number of these strains examined should be taken into consideration in the interpretation of the results. The lack of detailed studies using serine-type carbapenemase-producing strains is a limitation of the present study and will be a future task.

In conclusion, CPE screening using the results of AST with the AST-XN17 card by VITEK2 appears to be a useful differential method. Establishing a method that does not overlook CPE is also extremely useful in the fields of antimicrobial stewardship and infection control.

Abbreviations AES: Advanced Expert System; AST: Antimicrobial susceptibility testing; CPE: Carbapenemase-producing *Enterobacteriales*; IPM: Imipenem; MEPM: Meropenem; FRPM: Faropenem; AVI/CAZ: Avibactam/ceftazidime; MIC: Minimum inhibitory concentrations; CLSI: Clinical and Laboratory Standards Institute; MBL: Metallo- β -lactamase; ESBL: Extended-spectrum β -lactamases; mCIM: Modified carbapenem inactivation method test; LMOX: Latamoxef

Author contribution Tomokazu Kuchibiro, Masaru Komatsu, and Tatsuya Nakamura conceived the study. Tomokazu Kuchibiro developed the theory and performed the verifications. Tomokazu Kuchibiro, Katsutoshi Yamasaki, Tatsuya Nakamura, Hisaaki Nishio, Makoto Niki, Kaneyuki Kida, Masanobu Ohama, Akihiro Nakamura, and Isao Nishi collected and analyzed the test strains. Tomokazu Kuchibiro, Masaru Komatsu, and Tatsuya Nakamura conceived and planned the experiments. Tomokazu Kuchibiro performed the experiments. Tomokazu Kuchibiro, Masaru Komatsu, Katsutoshi Yamasaki, and Tatsuya Nakamura contributed to the interpretation of the results. Tomokazu Kuchibiro took the lead in writing the manuscript with input from all authors. All authors provided critical feedback and helped shape the research, analysis, and manuscript. Masaru Komatsu and Isao Nishi supervised and managed the entire study process.

Funding This work was supported by the Study of Bacterial Resistance in the Kinki Region of Japan.

Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate All authors have provided consent to participate.

Consent for publication All authors have provided consent for publication.

Conflict of interest All authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.


References

- Nordmann P, Naas T, Poirel L (2011) Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 17:1791–1798. <https://doi.org/10.3201/eid1710.110655>
- Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P (2012) Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 18:413–431. <https://doi.org/10.1111/j.1469-0691.2012.03821.x>
- Yang D, Guo Y, Zhang Z (2009) Combined porin loss and extended spectrum β -lactamase production is associated with an increasing imipenem minimal inhibitory concentration in clinical *Klebsiella pneumoniae* strains. *Curr Microbiol* 58:366–370. <https://doi.org/10.1007/s00284-009-9364-4>
- Thomson KS (2010) Extended-spectrum- β -lactamase, AmpC, and carbapenemase issues. *J Clin Microbiol* 48:1019–1025. <https://doi.org/10.1128/JCM.00219-10>
- Ean SS, Lee WS, Lam C, Hsu CW, Chen RJ, Hsueh PR (2015) Carbapenemase-producing Gram-negative bacteria: current epidemics antimicrobial susceptibility and treatment options. *Future Microbiol* 10:407–425. <https://doi.org/10.2217/fmb.14.135>
- van Duin D, Doi Y (2017) The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 8:460–469. <https://doi.org/10.1080/21505594.2016.1222343>
- Kayama S, Ohge H, Sugai M (2017) Rapid discrimination of bla IMP-1, bla IMP-6, and bla IMP-34 using a multiplex PCR. *J Microbiol Methods* 135:8–10. <https://doi.org/10.1016/j.mimet.2017.01.013>
- Arlet G, Philippon A (1991) Construction by polymerase chain reaction and use of intragenic DNA probes for three main types of transferable β -lactamases (TEM, SHV, CARB). *FEMS Microbiol Lett* 66:19–25
- Queenan AM, Bush K (2007) Carbapenemases: the versatile β -lactamases. *Clin Microbiol Rev* 20:440–458. <https://doi.org/10.1128/CMR.00001-07>
- Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld KP, Seifert H, Witte W, Higgins PG (2011) Molecular characterization of bla_{NDM-1} in an *Acinetobacter baumannii* strain isolated in Germany in 2007. *J Antimicrob Chemother* 66:1998–2001. <https://doi.org/10.1093/jac/dkr256>
- Dallenne C, Da Costa A, Decré D, Favier C, Arlet G (2010) Development of a set of multi-plex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. *J Antimicrob Chemother* 65:490–495. <https://doi.org/10.1093/jac/dkp498>
- Notake S, Matsuda M, Tamai K, Yanagisawa H, Hiramatsu K, Kikuchi K (2013) Detection of IMP metallo- β -lactamase in carbapenem-nonsusceptible Enterobacteriaceae and non-glucose-fermenting gram-negative rods by immunochromatography assay. *J Clin Microbiol* 51:1762–1768. <https://doi.org/10.1128/JCM.00234-13>
- Clinical and Laboratory Standards Institute (CLSI) (2019). Performance standards for antimicrobial susceptibility testing 29th ed CLSI supplement M100-S29. Wayne PA: CLSI
- Mushtaq S, Hope R, Warner M, Livermore DM (2007) Activity of faropenem against cephalosporin-resistant Enterobacteriaceae. *J Antimicrob Chemother* 59:1025–1030. <https://doi.org/10.1093/jac/dkm063>
- Matsuzaki K, Nishiyama T, Hasegawa M, Kobayashi I, Kaneko A, Sasaki J (1999) In vitro bactericidal activities of new oral penem, faropenem against the various clinical isolates. *Jpn J Antibiot* 52:431–438
- EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (https://www.eucast.org/resistance_mechanisms/)
- Imai W, Sasaki M, Aoki K, Ishii Y, Bonomo RA, Koh TH, Murakami H, Morita T, Tateda K (2017) Simple screening for carbapenemase-Producing Enterobacteriaceae by moxalactam susceptibility testing. *J Clin Microbiol* 55:2276–2279. <https://doi.org/10.1128/JCM.00606-17>
- Hu F, Ahn C, ÓHara JA, Doi Y (2014) Faropenem disks for screening of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol* 52:3501–3502

19. Nakamura T, Kobayashi S, Onuma K, Kusuki M, Hayashi N, Oji G, Tokimatsu I, Saegusa J, Arakawa S (2017) The development of screening methods using the disk diffusion method for carbapenemase-producing Enterobacteriaceae. *Kansenshogaku Zasshi* 91:14–19
20. Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagacé-Wiens PR, Denisuk A, Rubinstein E, Gin AS, Hoban DJ, Lynch JP III, Karlowsky JA (2013) Ceftazidime-avibactam: a novel cephalosporin/β-lactamase inhibitor combination. *Drugs* 73:159–177. <https://doi.org/10.1007/s40265-013-0013-7>
21. de Jonge BL, Karlowsky JA, Kazmierczak KM, Biedenbach DJ, Sahm DF, Nichols WW (2016) In vitro susceptibility to ceftazidime-avibactam of carbapenem-nonsusceptible Enterobacteriaceae isolates collected during the INFORM Global Surveillance Study (2012 to 2014). *Antimicrob Agents Chemother* 60:3163–3169
22. Livermore DM, Struelens M, Amorim J, Baquero F, Bille J, Canton R, Henning S, Gatermann S, Marchese A, Mittermayer H, Nonhoff C, Oakton KJ, Praplan F, Ramos H, Schito GC, Van Eldere J, Verhaegen J, Verhoef J, Visser MR (2002) Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests. *J Antimicrob Chemother* 49:289–300. <https://doi.org/10.1093/jac/49.2.289>
23. Yano H, Kuga A, Okamoto R, Kitasato H, Kobayashi T, Inoue M (2001) Plasmid-encoded metallo-beta-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. *Antimicrob Agents Chemother* 45(5):1343–1348. <https://doi.org/10.1128/AAC.45.5.1343-1348.2001>
24. Kanazawa S, Sato T, Kohira N, Ito-Horiyama T, Tsuji M, Yamano Y (2017) Susceptibility of imipenem-susceptible but meropenem-resistant blaIMP-6-carrying Enterobacteriaceae to various antibacterials, including the siderophore cephalosporin cefiderocol. *Antimicrob Agents Chemother* 61:e00576-e617

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Tomokazu Kuchibiro¹  · Masaru Komatsu² · Katsutoshi Yamasaki³ · Tatsuya Nakamura⁴ · Makoto Niki⁵ · Hisaaki Nishio⁶ · Kaneyuki Kida⁷ · Masanobu Ohama⁷ · Akihiro Nakamura² · Isao Nishi⁸

¹ Department of Clinical Laboratory, Naga Municipal Hospital, 1282 Uchita, Kinokawa, Wakayama 649-6414, Japan

² Department of Clinical Laboratory Science, Tenri Health Care University, Nara, Japan

³ Department of Medical Life Science, Kurashiki University of Science and the Arts, Okayama, Japan

⁴ Department of Medical Technology and Sciences Facility of Health Sciences, Kyoto Tachibana University, Kyoto, Japan

⁵ Department of Infection Control and Prevention, Osaka City University Hospital, Osaka, Japan

⁶ Department of Clinical Laboratory, Shiga Medical Center for Children, Shiga, Japan

⁷ Clinical Laboratory, Japanese Red Cross Otsu Hospital, Shiga, Japan

⁸ Laboratory for Clinical Investigation, Osaka University Hospital, Osaka, Japan