

Evaluation of a commercial multiplex real-time PCR panel for identification environmental water antibiotic-resistant bacteria in hemoculture bottles

Pimwan Thongdee¹, Putt Narongdej¹, Passara Wongthai¹, Sirachat Nitchaphanit¹, Kasidach chuenchom², Kanlaya Krataipech², Jantima Raksatham², Dutsadee Peerapongpaisarn³, Wilawan Oransathit³, Sirigade Ruekit³, Nattaya Ruamsap³, Paphavee Lertsethtakarn³, Piraporn Juntanawiwat⁴, Pongthorn Narongroeknawin¹, Tanit Boonsiri^{1*}

¹Department of Microbiology, Phramongkutklao College of Medicine, Bangkok, Thailand.

²Department of Biology, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

³Department of Bacterial and Parasitic Diseases, Walter Reed Army Institute of Research – Armed Forces Research Institute of Medical Sciences (WRAIR-AFRIMS), Bangkok, Thailand.

⁴Division of Microbiology, Department of Pathology, Phramongkutklao Hospital, Bangkok, Thailand.

ARTICLE INFO

Article history:

Received 5 November 2024

Accepted as revised 13 December 2024

Available online 23 December 2024

Keywords:

Antimicrobial resistance, bacterial resistance genes, bloodstream infections, QIAstat-Dx BCID GN plus AMR panel, multiplex real-time PCR panel.

ABSTRACT

Background: Antimicrobial resistance (AMR) is a major public health problem worldwide, and it is increasing, especially in developed and developing countries, including Thailand. Sepsis is a crisis and emergency causing the death of patients with a high mortality rate of 1 in 5 of the global population. Using inappropriate antimicrobial drugs to treat infections increases the chance of drug resistance in the microorganisms, and precise and rapid treatment is essential for patient management.

Objective: This cooperative study aims to evaluate a commercial multiplex real-time PCR panel, QIAstat-Dx BCID GN Plus AMR Panel, to detect antimicrobial-resistant genes in the bloodstream.

Materials and methods: Thirty bacterial samples were used to simulate bloodstream infections. The comparison between the QIAstat-Dx BCID GN Plus AMR Panel and the conventional PCR method was revealed.

Results: Both methods gave concordant results for 12 samples, while results for 15 were discordant. Three samples could not be detected using the QIAstat-Dx BCID GN Plus AMR Panel because *ebc* gene is not included. The QIAstat-Dx BCID GN Plus AMR Panel revealed a sensitivity, specificity, positive predictive value, and negative predictive value of 91.50%, 93.30%, 74.10%, and 98.50%, respectively.

Conclusion: The study indicated that the QIAstat-Dx BCID GN Plus AMR Panel Cartridge can detect common antimicrobial-resistant genes from the bloodstream. However, additional resistant genes should be included in the panel to cover various highly prevalent antimicrobial-resistant genes based on geographical location.

* Corresponding contributor.

Author's Address: Department of Microbiology,
Phramongkutklao College of Medicine, Bangkok,
Thailand.

E-mail address: boonsiri-t@pcm.ac.th

doi: 10.12982/JAMS.2025.029

E-ISSN: 2539-6056

Introduction

Antimicrobial-resistant bacteria have emerged as a significant global public health concern, with a continuously increasing trend observed in developed and developing countries, including Thailand. The majority of pathogens responsible for infectious diseases in Thai communities,¹ as reported by the Ministry of Public Health, Department of Health, Office of Environmental Health in 2017, were implicated in bloodstream infections. The problem of antimicrobial resistance is multifactorial, with factors such as the excessive use of antibiotics and the discharge of contaminated water or waste materials from

hospitals or factories into the environment contributing to the escalation.¹

In 2019, the global Antimicrobial Resistance (AMR) Situation Report revealed that 4.95 million people died from AMR infection, with one in every five children under 5 years old. In 2019, Thailand reported 10,800 deaths attributed to AMR and an additional 43,900 deaths related to AMR.² Sepsis is a critical emergency leading to a high mortality rate, affecting 1 in 5 individuals globally. In Thailand, sepsis stands as the primary cause of death among hospitalized patients, with a tendency to increase.³ Gram-negative bacteria pose a significant medical challenge due to their resistance to antimicrobial agents, especially *Escherichia coli*, *Klebsiella pneumoniae*, other Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.⁴ Over the past decade, these organisms have developed increased drug resistance, leading to prolonged treatment durations to eliminate them.⁵

Among these Gram-negative bacteria, AmpC beta-lactamase (*ampC*) expression in bacteria is a resistance mechanism producing beta-lactamase, an enzyme with similar activity as extended-spectrum beta-lactamases (ESBLs).⁶ Beta-lactamases can hydrolyze the beta-lactam ring, thereby inactivating the antibiotic and conferring resistance to most beta-lactam antibiotics, including penicillin, narrow-spectrum and extended-spectrum cephalosporin and monobactams.⁷ However, AmpC confers resistance to cephamycin and is not inhibited by beta-lactamase inhibitors. Therefore, beta-lactamase can cause bacteria to become resistant to many drugs.

The main causes of hospital-acquired infections are *K. pneumoniae* and *E. coli*, with limited drugs available for treatment. In beta-lactamase-producing *E. coli* strains, AMR can be verified by phenotypic expression characteristics and by detecting the beta-lactamase genes. Furthermore, Amp C origin - *Enterobacter cloacae* (EBC) is included in the beta-lactamase class C group.⁸ Primary genes responsible for beta-lactam resistance include ambler classification class A enzymes, such as cefotaximase (CTX-M), sulfhydryl variable (SHV), temoniera beta-lactamase (TEM), *Pseudomonas* extended resistance beta-lactamase (PER), Vietnamese extended-spectrum beta-lactamase (VEB) and Guiana extended-spectrum beta-lactamase (GES). Recently, beta-lactamases have emerged that are active against carbapenems (carbapenemases), which are found primarily in enterobacterales. The three types of carbapenemases comprise *K. pneumoniae* carbapenemases (KPCs), metallo-beta-lactamases (MBLs), and oxacillinases (OXAs). The KPCs belong to the serine class A (functional group 2f) beta-lactamases and are resistant to all beta-lactam drugs but may still be affected by beta-lactamase inhibitors. MBLs are class B beta-lactamases, functional group 3a, capable of hydrolyzing all beta-lactam drugs but are not inactivated by beta-lactamase inhibitors. The most widely distributed MBLs are the IMP-1 (for imipenem resistance) and VIM-1 (Verona integron encoded MBL) types. MBL has recently been identified, mainly in *E. coli* strains, and has

been designated NDM-1 (New Delhi MBL). The infections caused by carbapenem-resistant enterobacterales (CRE) strains have been associated with up to 71% in-hospital mortality.^{9,10}

The widespread dissemination of colistin resistance has recently garnered significant attention from the scientific community due to its critical role as the last line of defense against carbapenem-resistant enterobacterales infections and its potential for horizontal gene transfer. The mobilized colistin resistance (*mcr*) gene has been identified as the key gene responsible for this unique form of resistance. Colistin, also known as polymyxin E, is an antimicrobial agent effective against various gram-negative bacilli, particularly those in the enterobacteriaceae famil.¹¹

Aminoglycoside-modifying enzymes (AMEs) are another kind that confer antibiotic resistance through enzymatic modifications, changing the structure of aminoglycoside antibiotics and reducing their binding affinity to the 30S ribosomal subunit. Aminoglycosides bind to the aminoacyl-tRNA recognition site (A-site) of the 30S ribosomal subunit, inhibiting polypeptide synthesis and subsequent bacterial cell death. AMEs are commonly expressed by the aminoglycoside resistance methylase (*armA*) and aminoglycoside 3-N-acetyltransferase (*aac*) genes.¹²

Antimicrobial drug use to treat infectious diseases usually increases the chance of drug resistance as a survival mechanism for the pathogen.¹³ Therefore, Accurate diagnosis and early testing for AMR are crucial for selecting the proper treatment quickly, helping to reduce the severity of the disease and lower the risk of death. Therefore, this research focused on evaluating the effectiveness of the multiplex real-time PCR panel in detecting drug-resistant genes in bloodstream infections.

Materials and methods

Bacterial identification, antimicrobial susceptibility testing, and preparation

A total of 30 bacterial strains were used in this research, including 28 strains from Walter Reed Army Institute of Research—Armed Forces Research Institute of Medical Sciences (WRAIR-AFRIMS) and 2 standard strains (*E. coli* ATCC 8739 and *E. coli* ATCC 25922), which were used as negative controls. All 28 bacterial strains were isolated from environmental water.

Each bacterial isolate was initially identified for its species and antimicrobial susceptibility testing (AST) using the BD Phoenix™M50 automated microbiology system (BD Diagnostic Systems, Sparks, MD). Antimicrobial susceptibility results were interpreted according to the CLSI, EUCAST, and La Société française de microbiologie (SFM) guidelines.¹⁴⁻¹⁶ Conventional molecular detection of AMR genes was performed as described previously.¹⁷

Isolate preparation for the QIAstat-Dx BCID GN plus AMR panel

Each strain was cultured in tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD, USA) at 37 °C for 24 hrs, then adjusted to a bacterial concentration of 1.0

McFarland (equivalent to $\sim 3.0 \times 10^8$ CFU/mL), and 1 mL was inoculated in a blood culture bottle (Becton Dickinson, Sparks, MD, USA) with 9 ml of human whole blood donor. The blood culture bottle was incubated in an automated blood culture system following the instruction of BD BACTEC™ (Becton Dickinson, Sparks, MD, USA) until the blood culture bottle showed positive.

At bottle positivity, 100 μ L of blood was withdrawn, placed in a microcentrifuge tube, and then transferred to the QIAstat-Dx BCID GN Plus AMR Panel cartridge (Qiagen, Thailand) included primers for detecting 18 resistance gene; AmpC, CTX-M, IMP, KPC, NDM, OXA-23, OXA-48 like, OXA-24/40, OXA-58, SHV, TEM, VIM, PER, *armA*, *acc (6')-Ib*, VEB, GES and SPM. Next, the QIAstat-Dx BCID GN Plus AMR Panel cartridge was inserted in the QIAstat-Dx Analyzer 1.0 (Qiagen, Thailand) based on real-time automated PCR assay.

Data analysis

The results of the QIAstat-Dx BCID GN Plus AMR Panel were compared to reference results generated by conventional PCR and antimicrobial drug susceptibility testing performed by WRAIR-AFIRMS. Statistical analyses were performed using STATA/BE, 18.0 Software, including sensitivity, specificity, and positive and negative predictive values. The limit of detection was defined as the lowest

concentration at which each target was detected $\geq 95\%$ of the time. At least 20 replicates were performed at the determined limit of detection concentration.

Results

Diagnostic performance of QIAstat-Dx BCID GN Plus AMR Panel in comparison with PCR

Eleven genes were detected using the QIAstat-Dx BCID GN Plus AMR Panel: CTX-M, GES, SHV, VEB, TEM, AmpC, NDM, OXA-23, OXA-48 like *armA* and *acc (6')-Ib*. This panel could not detect EBC, *mcr-3*, and OXA-resistant genes, as they are not part of the panel. When comparing the results of this panel to results from conventional PCR, 10 bacterial strains showed concordant results, 15 strains gave discordant results, and 3 strains carried resistant genes that the panel excluded (Table 1). The calculated values for sensitivity (91.50%), specificity (93.30%), positive predictive value (74.10%), negative predictive value (98.50%), and detection limit (3.0×10^8 CFU/mL) of the panel are shown in Table 2. The strength of agreement between QIAstat-Dx BCID GN Plus AMR Panel and PCR method for detecting drug-resistant genes was categorized as a substantial correlation with a kappa value of 0.71 (Table 2). The calculated values for each AMR gene are shown in Table 3.

Table 1. Comparison of bacterial drug resistance gene test results between conventional PCR and QIAstat-Dx BCID GN plus AMR panel.

No.	Pathogens	Beta-lactamases class A group (ESBL)												Beta-lactamases (class C group)				Carbapenem (class B group)				Carbapenem/Oxacillinase (class D group)						Aminoglycoside-modifying resistance gene				Mobilized colistin resistance gene			
		CTX-M		GES		SHV		VEB		TEM		AmpC		EBC		NDM		OXA		OXA-23		OXA-48 like		armA		aac (6')-Ib		mcr-3							
		PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.				
1	<i>Enterobacter cloacae</i> WOH-005-5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	ND	Neg	Neg	Neg	ND	Neg	ND				
2	<i>Enterobacter cloacae</i> WOH-006-3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	ND	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	ND	Neg	Neg	Neg	ND	Neg	ND				
3	<i>Escherichia coli</i> WOH-008-2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	ND	Neg	Neg	Neg	ND	Neg	ND				
4	<i>Escherichia coli</i> WOH-009-6	Pos	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	ND	Neg	Neg	Neg	ND	Neg	ND				
5	<i>Escherichia coli</i> WOH-010-1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	ND	Neg	Neg	Neg	ND	Neg	ND				
6	<i>Klebsiella pneumoniae</i> WOH-010-3	Pos	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Pos	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	Neg	Neg	ND				
7	<i>Acinetobacter baumannii</i> WOH-010-4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Pos	Neg	ND	Neg	Neg	Neg	Pos	ND	Neg	ND	Neg	Neg	Neg	Neg	ND				
8	<i>Klebsiella pneumoniae</i> WOH-010-5	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	Neg	Neg	ND				
9	<i>Enterobacter cloacae</i> WOH-010-6	Neg	Pos	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Pos	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				
10	<i>Escherichia coli</i> WOH-010-9	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				
11	<i>Klebsiella pneumoniae</i> WOH-010-10	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Pos	Neg	ND	Neg	Neg	Neg	Pos	ND	Neg	ND	Neg	Neg	Neg	Neg	ND				
12	<i>Enterobacter cloacae</i> WOH-010-11	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	Neg	Neg	ND				
13	<i>Escherichia coli</i> WOH-010-12	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Pos	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				
14	<i>Escherichia coli</i> WOH-010-13	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Pos	Neg	Neg	ND				
15	<i>Klebsiella pneumoniae</i> WOH-010-14	Pos	Neg	Pos	Neg	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Pos	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	Neg	Neg	ND				
16	<i>Escherichia coli</i> WOH-010-15	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				
17	<i>Escherichia coli</i> WOH-010-16	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Pos	ND	Neg	Neg	ND				
18	<i>Acinetobacter baumannii</i> WOH-010-17	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				
19	<i>Enterobacter cloacae</i> WOH-010-19	Neg	Neg	Pos	Neg	Pos	Neg	Neg	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND	Neg	Neg	Neg	Neg	Neg	ND				

Pos = Positive / Neg = Negative / ND = Not determined

Table 1. Comparison of bacterial drug resistance gene test results between conventional PCR and QIAstat-Dx BCID GN plus AMR panel (continued).

No.	Pathogens	Beta-lactamases class A group (ESBL)												Beta-lactamases (class C group)				Carbapenem (class B group)				Carbapenem/Oxacillinase (class D group)				Aminoglycoside-modifying resistance gene				Mobilized colistin resistance gene	
		CTX-M		GES		SHV		VEB		TEM		AmpC		EBC		NDM		OXA		OXA-23		OXA-48 like		armA		aac (6')-Ib		mcr-3			
		PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.	PCR	Q.st.		PCR	Q.st.	
20	<i>Enterobacter cloacae</i> WOH-010-20	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	ND	Pos	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
21	<i>Enterobacter cloacae</i> WOH-010-21	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	ND	Pos	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
22	<i>Escherichia coli</i> WOH-010-23	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Neg	ND	Neg	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
23	<i>Enterobacter cloacae</i> WOH-010-24	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Neg	ND	Pos	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
24	<i>Escherichia coli</i> WOH-010-26	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
25	<i>Klebsiella pneumoniae</i> WOH-010-27	Pos	Pos	Neg	Neg	Pos	Pos	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	ND	Neg	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
26	<i>Enterobacter cloacae</i> WOH-010-31	Neg	Neg	Neg	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Neg	ND	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
27	<i>Enterobacter cloacae</i> WOH-010-32	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Pos	Pos	Neg	Neg	ND	ND	Pos	Neg	ND	ND	Neg	Neg	ND	ND	Neg	ND	Pos	Neg	Neg	ND	
28	<i>Escherichia coli</i> WOH-010-33	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Neg	ND	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
29	<i>Escherichia coli</i> ATCC 8739	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Neg	ND	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	
30	<i>Escherichia coli</i> ATCC 25922	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	ND	Neg	ND	ND	ND	Neg	Neg	ND	ND	Neg	ND	Neg	Neg	Neg	ND	

Pos = Positive / Neg = Negative / ND = Not determined

Table 2. QIAstat-Dx BCID GN Plus AMR Panel performance to detect AMR genes in comparison with conventional PCR

Performance	QIAstat-Dx BCID GN Plus AMR Panel
Sensitivity	91.50 % (79.60 % – 97.60 %)*
Specificity	93.30% (89.10 % – 96.20 %)*
Positive predictive value (PPV)	74.10 % (61.00 % – 84.70 %)*
Negative predictive value (NPV)	98.50 % (96.20 % – 99.60 %)*
Detection limit	3.0 x 10 ⁸ CFU/ml
Agreement between tests kappa	0.71 (0.66 - 0.76)*

*Data are presented as percentage (95% confidence interval; CI) for all parameters except the detection limit (CFU/ml).

Table 3. QIAstat-Dx BCID GN Plus AMR Panel performance to detect each AMR genes in comparison with conventional PCR.

Drug-resistant genes	Performance of QIAstat-Dx BCID GN Plus AMR Panel			
	Sensitivity	Specificity	Positive predictive value (PPV)	Negative predictive value (NPV)
CTX-M	100.00% (73.50% - 100.00%)*	94.40% (72.70% - 99.90%)*	92.30% (64.00% - 99.80%)*	100.00% (80.50% - 100.00%)*
GES	83.30% (35.90% - 99.60%)*	100.00% (85.80% - 100.00%)*	100.00% (47.80% - 100.00%)*	96.00% (79.60% - 99.90%)*
SHV	100.00% (47.80% - 100.00%)*	88.00% (68.80% - 97.50%)*	62.50% (24.50% - 91.50%)*	100.00% (84.60% - 100.00%)*
VEB	66.70% (9.40% - 99.20%)*	85.20% (66.30% - 95.80%)*	33.30% (4.30% - 77.70%)*	95.80% (78.90% - 99.90%)*
TEM	92.30% (64.00% - 99.80%)*	100.00% (80.50% - 100.00%)*	100.00% (73.50% - 100.00%)*	94.40% (72.70% - 99.90%)*
AmpC	50.00% (1.30% - 98.70%)*	100.00% (87.70% - 100.00%)*	100.00% (2.50% - 100.00%)*	96.60% (82.20% - 99.90%)*
NDM	100.00% (47.80% - 100.00%)*	84.00% (63.90% - 95.50%)*	55.60% (21.20% - 86.30%)*	100.00% (83.90% - 100.00%)*
OXA-48 like	100.00% (2.50% - 100.00%)*	96.60% (82.20% - 99.90%)*	50.00% (1.30% - 98.70%)*	100.00% (87.70% - 100.00%)*

*Data are presented as percentage (95% confidence interval; CI)

Sensitivity, specificity, PPV, and NPV were not calculated for OXA, OXA-23, EBC, *armA*, *aac(6')*-I, and MCR-3 due to insufficient data.

Discrepancies between QIAstat-Dx BCID GN Plus AMR Panel and antimicrobial susceptibility testing

Thirteen antibiotic drugs were designated for antimicrobial susceptibility testing, and the results are presented in Table 4. The correlation between the antimicrobial susceptibility testing and the panel is presented in Table 5, 6 and 7. All drug-resistant genes of the beta-lactamase group showed positive results with 100.00% concordance with phenotypic resistance to ampicillin. Some of these genes also showed concordance with cefazolin, ampicillin-sulbactam, cefepime, ceftazidime,

ceftazidime-avibactam, and piperacillin-tazobactam resistance. For the carbapenem/oxacillinase group, the NDM and OXA-23 genes showed positive results with 100.00% concordance with resistance to ertapenem, imipenem, and meropenem. In contrast, the OXA-48-like gene showed a positive result with 100.00% concordance with resistance to ertapenem and imipenem. The *armA* gene of the aminoglycoside group showed positive results with 100.00% concordance with resistance to amikacin and gentamicin.

Table 4. Antimicrobial drug susceptibility testing results

No.	Pathogens	Beta-lactamases drugs							Carbapenem drugs			Aminoglycoside drugs		Colistin drug
		Ampicillin	Ampicillin-Sulbactam	Cefazolin	Cefepime	Ceftazidime	Ceftazidime-Avibactam	Piperacillin-Tazobactam	Ertapenem	Imipenem	Meropenem	Amikacin	Gentamicin	Colistin
1	<i>Enterobacter cloacae</i> WOH-005-5	R	R	R	S	S	S	S	S	I	S	S	S	R
2	<i>Enterobacter cloacae</i> WOH-006-3	R	R	R	S	S	S	S	S	I	S	S	S	X
3	<i>Escherichia coli</i> WOH-008-2	R	I	X	S	S	S	S	S	S	S	S	S	X
4	<i>Escherichia coli</i> WOH-009-6	R	S	R	R	I	S	S	S	S	S	S	S	X
5	<i>Escherichia coli</i> WOH-010-1	R	I	X	S	S	S	S	S	S	S	S	R	X
6	<i>Klebsiella pneumoniae</i> WOH-010-3	R	R	R	R	R	S	R	R	R	I	S	R	X
7	<i>Acinetobacter baumannii</i> WOH-010-4	R	R	R	R	R	R	R	R	R	R	R	R	X
8	<i>Klebsiella pneumoniae</i> WOH-010-5	R	R	R	S	R	S	S	R	I	R	S	R	X
9	<i>Enterobacter cloacae</i> WOH-010-6	R	R	R	R	R	R	R	R	R	R	R	R	X
10	<i>Escherichia coli</i> WOH-010-9	R	I	R	S	S	S	S	S	S	S	S	R	X
11	<i>Klebsiella pneumoniae</i> WOH-010-10	R	R	R	R	R	R	R	R	R	R	R	R	X
12	<i>Enterobacter cloacae</i> WOH-010-11	R	R	R	S	S	S	S	R	S	S	S	I	R
13	<i>Escherichia coli</i> WOH-010-12	R	R	R	R	R	R	R	R	R	R	R	R	X
14	<i>Escherichia coli</i> WOH-010-13	R	S	R	R	R	S	S	S	S	S	S	R	R
15	<i>Klebsiella pneumoniae</i> WOH-010-14	R	R	R	R	R	S	R	S	S	S	S	R	R
16	<i>Escherichia coli</i> WOH-010-15	R	I	R	R	R	S	S	S	S	S	S	R	X
17	<i>Escherichia coli</i> WOH-010-16	R	R	R	R	R	S	R	R	R	R	R	R	X
18	<i>Acinetobacter baumannii</i> WOH-010-17	R	I	R	R	R	R	R	R	R	R	S	S	X
19	<i>Enterobacter cloacae</i> WOH-010-19	R	R	R	R	R	R	R	R	R	R	R	R	X
20	<i>Enterobacter cloacae</i> WOH-010-20	R	S	R	I	R	S	S	S	S	S	S	R	R
21	<i>Enterobacter cloacae</i> WOH-010-21	R	R	R	R	R	R	R	R	R	R	S	S	X
22	<i>Escherichia coli</i> WOH-010-23	R	I	R	I	S	S	S	S	S	S	S	R	X
23	<i>Enterobacter cloacae</i> WOH-010-24	R	R	R	R	R	R	R	R	R	R	R	R	X
24	<i>Escherichia coli</i> WOH-010-26	R	I	R	R	R	S	S	S	S	S	S	R	X
25	<i>Klebsiella pneumoniae</i> WOH-010-27	R	I	R	R	R	S	S	S	S	S	S	S	X
26	<i>Enterobacter cloacae</i> WOH-010-31	R	R	R	R	R	S	S	S	S	S	S	S	X
27	<i>Enterobacter cloacae</i> WOH-010-32	R	R	R	R	R	R	R	R	R	R	S	R	X
28	<i>Escherichia coli</i> WOH-010-33	R	S	R	R	I	S	S	S	S	S	S	S	X
29	<i>Escherichia coli</i> ATCC 8739	S	S	S	S	S	S	S	S	S	S	S	S	X
30	<i>Escherichia coli</i> ATCC 25922	S	S	S	S	S	S	S	S	S	S	S	S	X

I = Indeterminate, R = Resistant, S = Susceptible, X = Not detected.

Table 5. Discrepancies between QIAstat-Dx BCID GN Plus AMR Panel and antimicrobial drug susceptibility testing for Beta-lactam antibiotic class.

Antimicrobial drug susceptibility testing	QIAstat-Dx BCID GN Plus AMR Panel											
	Beta-lactamases class A group (ESBL)										Beta-lactamases class C group	
	CTX-M		GES		SHV		VEB		TEM		AmpC	
	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)
Ampicillin												
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	15 (88.2)	13 (100.0)	23 (92.0)	5 (100.0)	20 (90.9)	8 (100.0)	22 (91.7)	6 (100.0)	16 (88.9)	12 (100.0)	27 (93.1)	1 (100.0)
S	2 (11.8)	0 (0.0)	2 (8.0)	0 (0.0)	2 (9.1)	0 (0.0)	2 (8.3)	0 (0.0)	2 (11.1)	0 (0.0)	2 (6.9)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ampicillin_Sulbactam												
I	3 (17.6)	5 (38.5)	8 (32.0)	0 (0.0)	7 (31.8)	1 (12.5)	8 (33.3)	0 (0.0)	2 (11.1)	6 (50.0)	8 (27.6)	0 (0.0)
R	11 (64.7)	5 (38.5)	11 (44.0)	5 (100.0)	9 (40.9)	7 (87.5)	11 (45.8)	5 (83.3)	10 (55.6)	6 (50.0)	15 (51.7)	1 (100.0)
S	3 (17.6)	3 (23.1)	6 (24.0)	0 (0.0)	6 (27.3)	0 (0.0)	5 (20.8)	1 (16.7)	6 (33.3)	0 (0.0)	6 (20.7)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cefazolin												
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	13 (76.5)	13 (100.0)	21 (84.0)	5 (100.0)	18 (81.8)	8 (100.0)	20 (83.3)	6 (100.0)	16 (88.9)	10 (83.3)	25 (86.2)	1 (100.0)
S	2 (11.8)	0 (0.0)	2 (8.0)	0 (0.0)	2 (9.1)	0 (0.0)	2 (8.3)	0 (0.0)	2 (11.1)	0 (0.0)	2 (6.9)	0 (0.0)
X	2 (11.8)	0 (0.0)	2 (8.0)	0 (0.0)	2 (9.1)	0 (0.0)	2 (8.3)	0 (0.0)	0 (0.0)	2 (16.7)	2 (6.9)	0 (0.0)
Cefepime												
I	1 (5.9)	1 (7.7)	2 (8.0)	0 (0.0)	2 (9.1)	0 (0.0)	1 (4.2)	1 (16.7)	1 (5.6)	1 (8.3)	2 (6.9)	0 (0.0)
R	8 (47.1)	11 (84.6)	16 (64.0)	3 (60.0)	11 (50.0)	8 (100.0)	15 (62.5)	4 (66.7)	10 (55.6)	9 (75.0)	18 (62.1)	1 (100.0)
S	8 (47.1)	1 (7.7)	7 (28.0)	2 (40.0)	9 (40.9)	0 (0.0)	8 (33.3)	1 (16.7)	7 (38.9)	2 (16.7)	9 (31.0)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime												
I	0 (0.0)	2 (15.4)	2 (8.0)	0 (0.0)	2 (9.1)	0 (0.0)	2 (8.3)	0 (0.0)	2 (11.1)	0 (0.0)	2 (6.9)	0 (0.0)
R	10 (58.8)	9 (69.2)	15 (60.0)	4 (80.0)	11 (50.0)	8 (100.0)	14 (58.3)	5 (83.3)	10 (55.6)	9 (75.0)	18 (62.1)	1 (100.0)
S	7 (41.2)	2 (15.4)	8 (32.0)	1 (20.0)	9 (40.9)	0 (0.0)	8 (33.3)	1 (16.7)	6 (33.3)	3 (25.0)	9 (31.0)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftazidime_Avibactam												
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	6 (35.3)	3 (23.1)	7 (28.0)	2 (40.0)	5 (22.7)	4 (50.0)	6 (25.0)	3 (50.0)	5 (27.8)	4 (33.3)	8 (27.6)	1 (100.0)
S	11 (64.7)	10 (76.9)	18 (72.0)	3 (60.0)	17 (77.3)	4 (50.0)	18 (75.0)	3 (50.0)	13 (72.2)	8 (66.7)	21 (72.4)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Piperacillin_Tazobactam												
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	7 (41.2)	5 (38.5)	9 (36.0)	3 (60.0)	6 (27.3)	6 (75.0)	8 (33.3)	4 (66.7)	6 (33.3)	6 (50.0)	11 (37.9)	1 (100.0)
S	10 (58.8)	8 (61.5)	16 (64.0)	2 (40.0)	16 (72.7)	2 (25.0)	16 (66.7)	2 (33.3)	12 (66.7)	6 (50.0)	18 (62.1)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 6. Discrepancies between QIAstat-Dx BCID GN Plus AMR Panel and antimicrobial drug susceptibility testing for Carbapenem antibiotic class.

Antimicrobial drug susceptibility testing	QIAstat-Dx BCID GN Plus AMR Panel					
	Carbapenem (class B group)			Carbapenem/Oxacillinase (class D group)		
	NDM		OXA-23		OXA-48 like	
	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)
Ertapenem						
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	4 (19.0)	9 (100.0)	11 (39.3)	2 (100.0)	11 (39.3)	2 (100.0)
S	17 (81.0)	0 (0.0)	17 (60.7)	0 (0.0)	17 (60.7)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Imipenem						
I	3 (14.3)	0 (0.0)	3 (10.7)	0 (0.0)	3 (10.7)	0 (0.0)
R	2 (9.5)	9 (100.0)	9 (32.1)	2 (100.0)	9 (32.1)	2 (100.0)
S	16 (76.2)	0 (0.0)	16 (57.1)	0 (0.0)	16 (57.1)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem						
I	1 (4.8)	0 (0.0)	1 (3.6)	0 (0.0)	0 (0.0)	1 (50.0)
R	2 (9.5)	9 (100.0)	9 (32.1)	2 (100.0)	10 (35.7)	1 (50.0)
S	18 (85.7)	0 (0.0)	18 (64.3)	0 (0.0)	18 (64.3)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 7. Discrepancies between QIAstat-Dx BCID GN Plus AMR Panel and antimicrobial drug susceptibility testing for Aminoglycoside antibiotic class.

Antimicrobial drug susceptibility testing	QIAstat-Dx BCID GN Plus AMR Panel			
	Aminoglycoside-modifying resistance gene			
	<i>armA</i>		<i>aac (6')-Ib</i>	
	Neg N (%)	Pos N (%)	Neg N (%)	Pos N (%)
Amikacin				
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
R	5 (17.9)	2 (100.0)	6 (25.0)	1 (16.7)
S	23 (82.1)	0 (0.0)	18 (75.0)	5 (83.3)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gentamicin				
I	1 (3.6)	0 (0.0)	0 (0.0)	1 (16.7)
R	16 (57.1)	2 (100.0)	13 (54.2)	5 (83.3)
S	11 (39.3)	0 (0.0)	11 (45.8)	0 (0.0)
X	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Discussion

A bloodstream infection occurs when the body responds to bacteria or other diseases, causing widespread inflammation. If severe, it can lead to shock, organ failure, and a life-threatening situation. Early treatment is crucial.¹⁸ Doctors start by evaluating the patient's symptoms and performing blood cultures, which take 3 to 5 days for results. Due to the urgency, they must quickly diagnose and prescribe broad-spectrum antibiotics. Delayed or incorrect treatment increases the risk of death.¹⁹ In recent years, molecular biology methods have been employed to detect pathogens in sepsis. This has significantly reduced the detection time while showcasing excellent diagnostic performance.^{20, 21}

The evaluation of QIAstat-DX in this study revealed that it exhibited high sensitivity (91.5%), specificity (93.3%), and negative predictive value (98.5%). In contrast, the panel indicated a moderate positive predictive value (74.10%). Moreover, false negative results (8.5%) were found because EBC was not included in the panel. Based on our results, the panel performs better to detect resistant genes NDM and SHV in 4 and 3 bacterial strains, respectively (Table 1). Conventional PCR has a higher chance of giving false negatives if there are issues during the DNA extraction step.²² Moreover, a similar study shows that this panel can provide faster results than conventional PCR.²³ However, a limitation of the panel was demonstrated in our study because 2 genes of the beta-lactam groups (EBC and OXA) and 1 gene (*mcr-3*) of colistin resistance were excluded by the panel. EBC is an enzyme belonging to Class C beta-lactamases, also called AmpC beta-lactamases or cephalosporinases, which degrade drugs in the cephalosporin group. The gene responsible for producing EBC is located on the chromosome. However, it has been recently discovered that the gene can also be plasmid-located, the same as for FOX and DHA enzymes.²⁴ OXA is an enzyme belonging to Class D carbapenemases. Currently, more than 45 types of OXA-type carbapenemases have been identified.²⁵ However, the QIAstat-Dx BCID GN Plus AMR Panel can only detect specific variants, including OXA-23, OXA-48 like, OXA-24/40, and OXA-58. *mcr-3* gene, associated with colistin resistance, and is plasmid-located. Colistin is the last option for treating infections

caused by gram-negative bacteria resistant to almost all other drugs.²⁶ Our study suggested that the panel should include EBC, OXA, and *mcr-3* genes because these genes can be found in some strains of *E. cloacae*, *E. coli*, and *K. pneumoniae*.²⁷ Moreover, the genotypic detection of beta-lactamase and carbapenem genes revealed 100% determinant for a phenotypically resistant to ampicillin, ertapenem, and imipenem. In contrast, the *aac6_Ib* gene showed the lowest determinant for a phenotypically resistant to amikacin, with 16.7% (Table 7). Based on our research findings, the presence of antimicrobial resistance genes may not directly cause antibiotic resistance in the bacterial isolates we studied.²⁸

This suggests that other genes might be involved in the mechanism of antimicrobial resistance.²⁹ Although the QIAstat-Dx BCID GN Plus AMR Panel helps detect resistance genes more efficiently, we recommend additional antibiotic susceptibility tests, especially when the panel shows a negative result.³⁰ The test kit has some limitations, such as its inability to detect gram-positive bacteria causing bloodstream infections.^{31,32} Moreover, bloodstream infections can resist multiple drug groups, not just beta-lactams and aminoglycosides, but also other antibiotics like polymyxins (colistin).³³ This interpretation maintains the scientific context while making the language more accessible and straightforward for general understanding.

Conclusion

This study evaluated the QIAstat-Dx BCID GN Plus AMR Panel for its ability to detect Gram-negative bacteria and associated antimicrobial resistance genes, focusing on bloodstream infections potentially caused by environmental antibiotic-resistant bacteria. In contrast, the panel demonstrated high sensitivity and specificity, but its limitations were detecting specific key resistance genes, such as EBC, OXA, and *mcr-3*. Additionally, the bacterial isolates used in this study were not directly derived from bloodstream infections, which may impact their applicability in clinical settings. The study highlights the panel's potential to aid in rapidly diagnosing bloodstream infections caused by environmental antibiotic-resistant bacteria, though further validation with clinical

bloodstream isolates is necessary. To improve its diagnostic utility, future iterations of the panel should include a broader range of resistance genes relevant to both clinical and environmental pathogens. Complementary phenotypic antibiotic susceptibility testing remains essential, particularly in cases where the panel does not detect resistance genes.

Conflict of interest

The authors declare no conflict of interest related to this study.

Funding information

The study was supported by the Department of Microbiology, Phramongkutklao College of Medicine.

Ethical approval

IRBTA948/2566 Code : S063b/66

References

- [1] Jantarabenjakul W, Anugulruengkitt S, Puthanakit T, Pancharoen C. Drug-resistant bacteria: Drug-resistant organisms in pediatrics: Diagnosis and treatment. 1st Bangkok: Active Print; 2019: 288 [in Thai].
- [2] Global Database for Tracking Antimicrobial Resistance (AMR) Country Self-assessment Survey (TrACSS). Available from: <https://amrcountryprogress.org/#/download-responses>.
- [3] Tancharoen L, Pairattanakorn P, Thamlikitkul V, Angkasekwinai N. Epidemiology and burden of sepsis at Thailand's largest university-based National Tertiary Referral Center during 2019. *Antibiotics*. 2022 Jul 5;11(7):899.
- [4] Phumart P, Phodha T, Thamlikitkul V, Riewpaiboon A, Prakongsai P, Limwattananon S. Health and economic impacts of antimicrobial resistant infections in Thailand: A preliminary study. *J Health Syst Res*. 2012; 6(3): 352-60 (in Thai).
- [5] Indrawattana N, Vanaporn M. Nosocomial infection. *J Med Health Sci*. 2015; 22(1): 81-92.
- [6] Thawornwan U, Kongsanan P, Chaisukda S, Eampokalap B. Incidence of AmpC β -lactamase producing bacteria isolated in Bamrasnaradura Infectious Disease Institute during July-September 2007. *J Bamrasnaradura Infect Dis Inst*. 2007; 2(1): 32-9 (in Thai).
- [7] Wongut-sa P. Extended-spectrum β -Lactamases producing in *Escherichia coli* and *Klebsiella pneumoniae* isolated from specimens of patients in Ratchaburi Hospital (2009-2013). *J Med Public Health Region 4*. 2013; 17(3): 222-9 (in Thai).
- [8] Shanthi M, Sekar U, Arunagiri K, Sekar B. Detection of Amp C genes encoding for beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. *Indian J Med Microbiol*. 2012; 30(3): 290-5. doi: 10.4103/0255-0857.99489.
- [9] Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol*. 2018; 4(3): 482-501. doi: 10.3934/microbiol.2018.3.482.
- [10] Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W Sabour AA, et al. An overview on phenotypic and genotypic characterisation of carbapenem-resistant Enterobacterales. *Medicina*. 2022; 58(11): 1675. doi: 10.3390/medicina58111675.
- [11] Gharaibeh MH, Shatnawi SQ. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: A review. *Vet World*. 2019; 12(11): 1735-46. doi: 10.14202/vetworld.2019.1735-1746.
- [12] Doi Y, Wachino JI, Arakawa Y. Aminoglycoside Resistance: The emergence of acquired 16S Ribosomal RNA methyltransferases. *Infect Dis Clin North Am*. 2016; 30(2): 523-37. doi: 10.1016/j.idc.2016.02.011.
- [13] Banerjee R, Patel R. Molecular diagnostics for genotypic detection of antibiotic resistance: current landscape and future directions. *JAC Antimicrob Resist*. 2023; 5(1): dlad018. doi.org/10.1093/jacamr/dlad018.
- [14] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 34th CLSI supplement M100. Wayne: CLSI; 2024.
- [15] European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing. 2024 [cited 2024 Nov 4]. Available from: https://www.eucast.org/ast_of_bacteria.
- [16] European committee on antimicrobial susceptibility testing. La Société française de microbiologie (SFM) guidelines. 2024 [cited 2024 Nov 4]. Available from: <https://www.eucast.org/>.
- [17] Ruekit S, Srijan A, Serichantalergs O, Margulieus KR, Mc Gann P, Mills EG, et al. Molecular characterization of multidrug-resistant ESKAPEE pathogens from clinical samples in Chonburi, Thailand (2017-2018). *BMC Infect Dis*. 2022; 22(1): 695. doi: 10.1186/s12879-022-07678-8.
- [18] Kiattimanaroj A. The development of a model of care for patients with sepsis, Wapipathum Hospital. *Acad J Mahasarakham Provincial Public Health Office*. 2021; 5(9): 27 (in Thai).
- [19] Maenchantrath C. Detection of bacteria in blood using the Bactec™FX: Procedure manual. Sparks: Becton Dickinson and Company; 2020.
- [20] Sun J, Zhao S, Wei C, Liu J, Chen J, Xing L, et al. A diagnostic test of real-time PCR detection in the diagnosis of clinical bloodstream infection. *Ann Palliat Med*. 2022; 11(10): 3224-30. doi: 10.21037/apm-22-1071.
- [21] Trung NT, Thau NZ, Bang MH, Song LH. PCR-based sepsis@Quick test is superior in comparison with blood culture for identification of sepsis-causative pathogens. *Sci Rep*. 2019; 9(1): 13663. doi: 10.1038/s41598-019-50150-y.
- [22] Department of Disease Control. Thailand's integrated antimicrobial resistance surveillance with One Health Approach guideline. Nonthaburi: Ministry of Public Health; 2019: 112.
- [23] Roschanski N, Fischer J, Guerra B, Roesler U. Development of a multiplex real-time PCR for the

- rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-Type AmpCs in Enterobacteriaceae. PLoS ONE. 2014; 9(7): e100956. doi.org/10.1371/journal.pone.0100956.
- [24] Thummeepak R, Kongthai P, Leungtongkam U, Sitthisak S. Distribution of virulence genes involved in biofilm formation in multi-drug resistant *Acinetobacter baumannii* clinical isolates. Int Microbiol. 2016; 19(2): 121-9. doi: 10.2436/20.1501.01.270.
- [25] Juntanawiwat P, Thunyaharn S, Visawapoka U, Samosornsuk W, Samosornsuk S. The prevalence of OXA-type genes in carbapenem-resistant *Acinetobacter baumannii* isolated from critically ill patients in the intensive care unit of Phramongkutklao Hospital. J Med Tech Phys Ther. 2016; 28(2): 120-8.
- [26] Wongut-sa P, Khumdee P, Samosornsuk W, Yansombat J, Samosornsuk S. Prevalence of colistin resistance among third-generation cephalosporins- or carbapenem-resistant enterobacteriaceae Isolated from clinical specimens in Ratchaburi Hospital, Thailand. J Med Tech Assoc Thailand. 2020; 48(1): 60 (in Thai).
- [27] Ku YH, Lee MF, Chuang YC, Yu WL. Detection of plasmid-mediated β -lactamase genes and the emergence of a novel AmpC (CMH-1) in *Enterobacter cloacae* at a Medical Center in Southern Taiwan. J Clin Med. 2019; 8(1): 8. doi: 10.3390/jcm8010008.
- [28] Department of Medical Services, Health Systems Research Institute. EE-AMR Tool Thailand. 2021 [cited 2024 Nov 4]. Available from: <https://kb.hsri.or.th/dspace/handle/11228/5684>.
- [29] Swe-Han K, Pillay M. Amikacin-resistant *Acinetobacter* species mediated by the *aphA6* gene associated with clinical outcome at an academic complex hospital in KwaZulu-Natal Province. S Afr Med J. 2020; 110(1): 49-54. doi.org/10.7196/samj.2020.v110i1.14045.
- [30] Abouelfetouh A, Torky AS, Aboulmagd E. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. Antimicrob Resist Infect Control. 2019 Dec;8(1):185. doi.org/10.1186/s13756-019-0611-6.
- [31] Ramachandran G. Gram-positive and gram-negative bacterial toxins in sepsis. Virulence. 2014; 5(1): 213-8. doi: 10.4161/viru.27024.
- [32] Wang Q, Li X, Tang W, Guan X, Xiong Z, Zhu Y, et al. Differential Gene Sets Profiling in Gram-Negative and Gram-Positive Sepsis. Front Cell Infect Microbiol. 2022 Feb 9;12:1-11. doi.org/10.3389/fcimb.2022.801232.
- [33] Olaitan A, Morand S, Rolain JM. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol. 2014; 5: 643. doi: 10.3389/fmicb.2014.00643.