Pharmaceutical Sciences Asia

Pharm Sci Asia 2023; 50(4), 331-336

DOI:10.29090/psa.2023.04.23.652

Research Article

Detection of carbapenemase producing *Enterobacterales* by MALDI-TOF mass spectrometry in Samutprakan Hospital, Thailand

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ABSTRACT

A rapid phenotypic carbapenemase-producing *Enterobacterales* (CPE) detection method was established using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Sixty-four carbapenem-resistant *Enterobacterales* (CRE) strains from Samutprakan Hospital were examined using MALDI-TOF MS by the ertapenem hydrolysis method and the susceptibility was compared with a modified carbapenem inactivation method (mCIM). Drug resistance genes were detected by polymerase chain reaction (PCR). The ertapenem drug and bacterial strains were mixed and drug hydrolysis owing to CPE activity was confirmed by specific molecular masses of ertapenem [M+H]+ at 476.5 m/z (±500 ppm), with peak disappearance judged as carbapenemase-positive. The most common CRE species were *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*. From 58 CPE strains, 17 strains of *K. pneumoniae* (29.3%) harbored bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes together, while 33 strains of *K. pneumoniae* (56.9%), 6 strains of *E. coli* (10.3%) and 1 strain of *Ent. cloacae* (1.75%) carried bla_{NDM} or $bla_{OXA\text{-}48\text{-}like}$ genes alone and 1 strain of *K. pneumoniae* (1.75%) contained bla_{KPC}. After 3 h of incubation with ertapenem, all 58 drug-resistant strains revealed disappearance of the ertapenem-specific waveform peak at 476.5 m/z, whereas 6 strains of CRE (non-CPE) revealed the ertapenem-specific waveform peak. The MALDI-TOF MS and mCIM data were 100% consistent. The MALDI-TOF MS based ertapenem hydrolysis assay was demonstrated as a rapid and accurate method to detect carbapenemase activity of *Enterobacterales* strains that can be routinely performed in clinical microbiology laboratories.

Keywords:
CPE, MALDI-TOF MS, Ertapenem hydrolysis, *Enterobacterales*

1. INTRODUCTION

Carbapenem-resistant *Enterobacterales* (CRE), especially carbapenemase-producing *Enterobacterales* (CPE) have recently emerged and spread rapidly all over the world, precipitating the need for the development of rapid and reliable methods for antimicrobial susceptibility testing (AST). Both phenotypic and genotypic methods have been used for carbapenemase detection[1-2], with these molecular methods still the gold standard and more reliable for detecting CPE. These techniques include polymerase chain reaction (PCR), hybridization and whole genome sequencing (WGS)[3-5]. Carbapenemase encoding genes; bla_{NDM}, bla_{VIM} bla_{IPM} and $bla_{OXA\text{-}48\text{-}like}$ are examples of the most common resistant genes widely distributed among *Enterobacterales* clinical isolates[6]. A phenotypic method for screening carbapenemase producers as the modified carbapenem inactivation method (mCIM) is suggested by the Clinical Laboratory Standard Institute (CLSI)[7] as a reference standard but has limitations with results delayed by at least 18 h.

Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) analysis has recently been introduced in clinical microbiology for species identification[8]. This method is based on the microbial identification of characteristic protein fingerprints of

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bacteria to quickly distinguish closely related microbial strains. The analysis of unique protein peaks in the mass spectrum allows discrimination of bacteria at the strain level. This rapid and reliable method for AST and the potential of MALDI-TOF MS to achieve the gold standard have been explored. All published MALDI-TOF MS assays are based on the same principle that aims to detect the disappearance of the native carbapenem and/or production of the carbapenem hydrolysis product after incubation of the tested strain with a carbapenem molecule[9]. This study focused on the detection of carbapenemase activity in *Enterobacterales* using MALDI-TOF MS analysis.

2. MATERIALS AND METHODS

2.1. Strain collection

Sixty-four *Enterobacterales* strains were collected between April and October 2019 at the Clinical Microbiology Laboratory of Samutprakan Hospital, Thailand with species identification and antimicrobial susceptibility testing carried out using MicroScan WalkAway 96 plus (Beckman Coulter, USA). The MIC was interpreted according to the CLSI criteria[7]. All strains were resistant to at least one type in the carbapenem group (ertapenem, meropenem, imipenem and doripenem). These CRE strains were isolated from urine, pus, blood and sputum. All strains were characterized for their carbapenemase producing ability at the phenotypic and molecular level. *K. pneumoniae* ATCC BAA-1706 (carbapenemase negative), *K. pneumoniae* ATCC BAA-1705 (*bla*$_{KPC}$ positive), and *K. pneumoniae* ATCC BAA-2146 (*bla*$_{NDM}$ positive) were used as internal controls for PCR, mCIM and MALDI-TOF MS testing.

2.2. Molecular characterization of carbapenemase genes

DNA was extracted from all strains using the modified boiling lysis method[10]. Briefly, each strain was cultured on a blood agar plate and incubated at 35°C for 16-24 h. One to five colonies of bacterial growth were suspended in nuclease-free water and the turbidity was adjusted to 0.5 McFarland standard. Then, 0.5 mL of the suspension was boiled at 100°C for 10 min in a thermal block (Polystat 5, Bioblock Scientific, Illkirch, France), followed by centrifuging at 19,000×g for 5 min. An aliquot of DNA supernatant (1.25 ng/µL) was used directly as a template for PCR assay. The 20 µL PCR master mix consisted of 1x buffer, 2.5 mM $MgCl_2$, 2 mM dNTPs, carbapenemase encoding gene primers: *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-23-like}$, *bla*$_{OXA-48-like}$[11], 5U Taq DNA polymerase, and nuclease-free water with 2.5 ng of DNA template. The amplification conditions were 94°C for a 3 min initial strand separation, 33 cycles at 94°C for 20 sec, 61°C for 15 sec, 72°C for 45 sec, and a 3 min final elongation step at 72°C. The amplification products were separated by electrophoresis on 2% agarose gel stained with nucleic acid staining solution (RedSafe™). The expected product sizes for carbapenemase genes were 310, 640, 470, 246, 390 and 755 bp.

2.3. Phenotypic detection of carbapenemase-producing *Enterobacterales*

The modified carbapenem inactivation method (mCIM) was performed on all strains according to the CLSI guidelines[7]. In brief, a 1 µL loopful of bacteria was resuspended in a 2 mL tube of trypticase soy broth (TSB). A meropenem disk was placed in each tube and incubated at 35°C for 4 h±15 min. The disk was then removed and applied on Mueller-Hinton agar (MHA) plates freshly plated with 0.5 McFarland suspension of *E. coli* ATCC 25922 strain and incubated at 35°C for 16-20 h. The mCIM was considered negative if the zone size was ≥19 mm, positive if the zone size was 6-15 mm, or intermediate (defined as positive) if pinpoint colonies were present within a 16-18 mm zone.

2.4. MALDI-TOF MS based ertapenem hydrolysis assay

The ertapenem hydrolysis assay followed the modified method of Haijun et al.[12]. The strains were grown for 18-24 h on MHA (Francisco Soria Melguizo. S.L. Madrid.). A 4 McFarland concentration suspension of the organism was prepared in microtubes with 50 µL of an ertapenem 0.5 mg/mL solution (Invanz, Merck Sharp & Dohme, NJ) and incubated 2, 3 and 4 h at 35°C. At each incubation period, 100 µL of the bacterial suspension was removed and centrifuged at 14,000 rpm for 2 min. Then, 1.5 µL of supernatant was taken to target plate, dried, and covered with 1.5 µL of substrate. After drying in a block heater at 50°C, the target plate was examined using Autof ms 1,000 (Autof ms 1,000, Zhengzhou Autobio). The blank control was the same level of antimicrobial solution. Finally, the spectra were analyzed by Autof Analyzer software with parameter settings: 60 Hz laser 40 shots, Detector 2.57 kV, Repulsion 20.00 kV, Elicitation 2.00 kV, Focus 7.00 kV; Relative molecular mass tested: 0-1,000. To interpret the MALDI-TOF MS results, peaks were automatically selected with the following parameter settings: peak detection algorithm: centroid; signal-to-noise threshold, 2; relative intensity threshold, 0%; minimum intensity threshold, 100; peak width, 02 m/z; height, 80% and compared with the molecular masses of ertapenem [M+H]+at 476.5 m/z (±500 ppm). The disappearance of the waveform peak was judged as carbapenemase-positive while showing the waveform peak was interpreted as carbapenemase-negative[12-13].

3. RESULTS AND DISCUSSION

CRE strains have recently emerged and become a

public health threat worldwide, including in Thailand. In this study, most sample types were urine (48.4%) and sputum (42.2%), with other samples pus (6.2%) and blood (3.2%). The most common CRE species were *K. pneumoniae* (55/64 (85.9%)), *E. coli* (6/64 (9.4%)) and *Ent. cloacae* (3/64 (4.7%)) concurring with previous reports[14-15]. Carbapenemase genes in 64 CRE strains were characterized by the PCR method. Results revealed that 58 strains were carbapenemase producers and 6 strains were non-carbapenemase producers following non-enzymatic mechanisms and were also related to carbapenem resistance by other mechanisms such as porin alteration[9]. Among the 58 CPE strains, 17 strains of *K. pneumoniae* (29.3%) harbored bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes together, while 33 strains of *K. pneumoniae* (56.9%), 6 strains of *E. coli* (10.3%), and 1 strain of *Ent. cloacae* (1.75%) carried bla_{NDM} or $bla_{OXA\text{-}48\text{-}like}$ genes alone and 1 strain of *K. pneumoniae* (1.75%) contained bla_{KPC} (Table 1).

The prevalence of carbapenemase species such as KPC, NDM, IMP, and OXA-48 family enzymes is dependent on geographic region. NDM is mostly found in Southeast Asian countries, while OXA-48-like enzymes are usually found in European countries, in the Middle East (Turkey) and in Mediterranean countries including North Africa, with KPC commonly prevalent in the Americas and European countries[16-18]. In Thailand, NDM, OXA-48-like and IMP enzymes are frequently detected

Table 1. PCR, mCIM and MALDI-TOF MS test results for all 64 isolates.

CRE (n)	PCR method					mCIM method		MALDI-TOF MS (peak at 476.5 m/z)	
	NDM	OXA-48 like	KPC	NDM + OXA-48 like	ND*	Pos	Neg	Pos	Neg
E. cloacae (3)	0	1	0	0	2	1	2	1	2
E. coli (6)	6	0	0	0	0	6	0	6	0
K. pneumoniae (55)	9	17	1	24	4	51	4	51	4
Total (64)	15	18	1	24	6	58	6	58	6

*ND = Not Detected

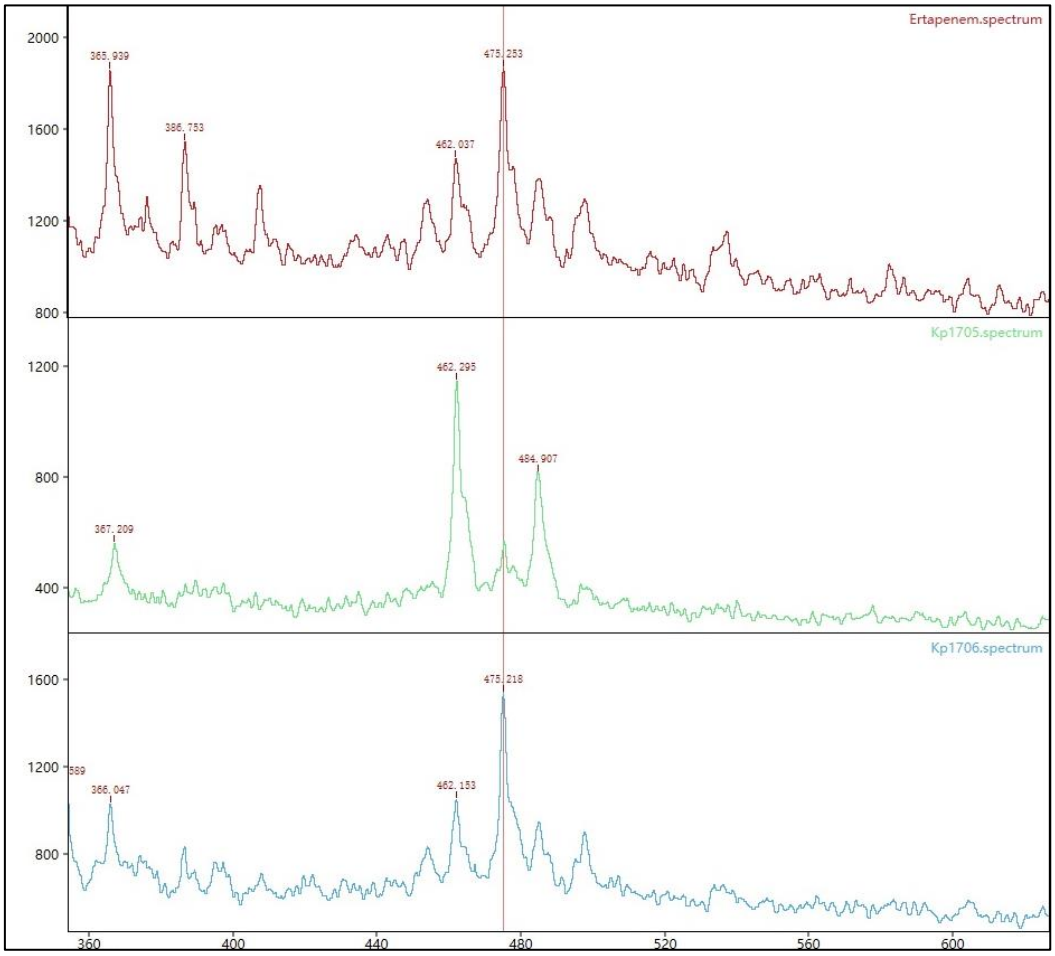

Figure 1. Mass spectrum showing ertapenem-specific waveform peaked around 476.5 m/z (top), disappearance of ertapenem-specific waveform peaked of *K. pneumoniae* ATCC 1705 (positive control) (middle top) and appearance of ertapenem-specific waveform peaked of *K. pneumoniae* ATCC 1706 (negative control) (bottom) at 3 hr-incubation time.

in clinical *Enterobacterales* isolates[10]. Our results supported previous studies showing that NDM and OXA-48-like encoding genes were the most common in Thailand. The *bla*$_{KPC}$ gene was first reported in Thailand in 2014, with dissemination at a very low prevalence[19-20]. This research revealed evidence of *bla*$_{KPC}$ detection in Thailand.

Multiple mechanisms contribute to carbapenem resistance among CRE. It is well known that the detection of genes does not reflect the expression of enzymes. Therefore, phenotypic testing offers a good method to check for carbapenemase production. In this study, the mCIM was selected as the standard method accepted by CLSI for testing with the MALDI-TOF MS method. All 58 strains were mCIM positive, whereas 6 strains of CRE (non-CPE) were mCIM negative. The genetic and mCIM results were 100% consistent. Both PCR and mCIM are widely used in carbapenemase detection. Molecular methods are now available for the verification of the genes responsible for carbapenemase production but these do not detect new mechanisms, while also not always correlating with the phenotype. The mCIM uses inexpensive materials that are readily available in routine microbiological laboratories but requires 18-24 h to report the resistance information.

MALDI-TOF MS is widely used in routine laboratory practice as a rapid and accurate identification technique for bacteria and fungi[9]. Many studies have demonstrated the applicability of this methodology to detect CPE organisms[9,21-23], while numerous sample preparation procedures, incubation times and conditions, and different detection methods have been utilized for different bacterial strains. This experiment followed the method of Haijin et al.[12]; however, an incubation time of 60 min could not detect all the carbapenemase activity, concurring with Carvalhaes et al. who used the VITEK MS platform and reported detection rates as 95% of class A and 87% of class B carbapenemase-producing isolates after 60 min of incubation with ertapenem as the indicator[24]. Therefore, we increased the incubation time to 2, 3 and 4 h. Optimal incubation time was determined as 3 h for microdroplets of the positive control with ertapenem, showing disappearance of the ertapenem-specific waveform peak at 476.5 m/z (±500 ppm) (Figure 1). In all 58 strains, the KPC, NDM, and OXA-48 family enzymes revealed disappearance of the ertapenem-specific waveform peak at 476.5 m/z (±500 ppm), whereas 6 strains of CRE (non-CPE) revealed the ertapenem-specific waveform peak (Figure 2). The ertapenem-specific waveform peak in this study

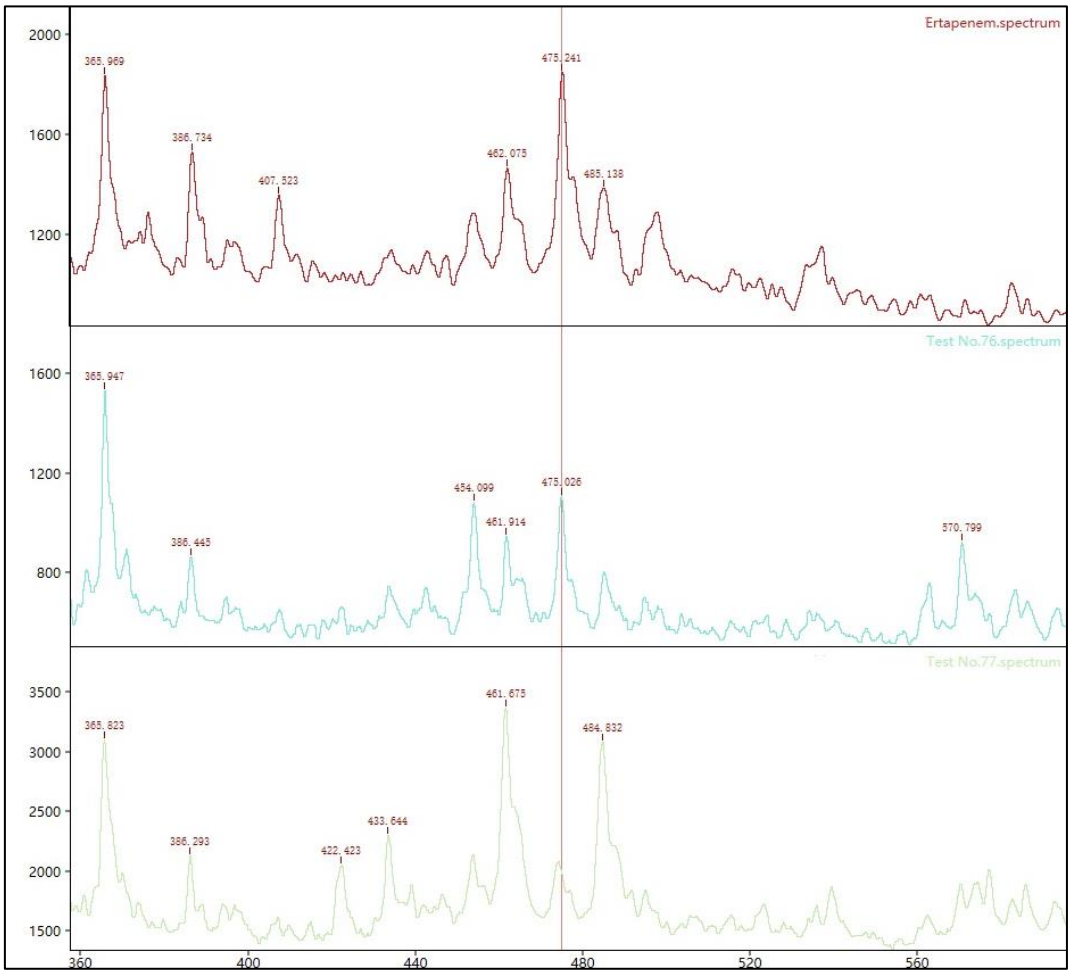

Figure 2. Mass spectrum showing ertapenem-specific waveform peaked around 476.5 m/z (top), appearance of ertapenem-specific waveform peaked of *E. cloacae* (test no. 76) which is CRE (non-CPE) (middle top) and disappearance of ertapenem-specific waveform peaked *of K. pneumoniae* (test no. 77) which is CPE (bottom).

was consistent with results recorded by Johansson et al. and Yu et al. who detected ertapenem by MALDI-TOF MS at 476.5 m/z[25-26], repeating the test three times with the same results. In our study, the detection sensitivity and specificity for CPE using the mCIM and MALDI-TOF MS were both 100%, while positive predictive value and negative predictive value were also 100%. The experimental results indicated agreement between genetic testing and MALDI-TOF MS at 100%. This finding was also supported by Burckardt et al. who revealed that ertapenem was hydrolyzed within 3 h by all carbapenemase activity[27].

Detection of carbapenem resistance using MALDI-TOF MS has been extensively studied for different Gram-negative bacteria including *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter baumannii* (*A. baumannii*), *Ent. cloacae* and different β-lactam antibiotics[22-23,28]. No false positive or false negative results were recorded from the *Enterobacterales* but two false positive results were reported by Johansson et al.[25] who demonstrated the detection of 9/25 *P. aeruginosa* strains by MALDI-TOF MS. The non-hydrolysis of ertapenem strains was possibly due to additional porin loss, resulting in a very low fraction of ertapenem (if any) reaching the periplasmatic site of action of the VIM-enzyme[29]. In *A. baumannii*, Sharma et al.[28] observed imipenem instead of ertapenem because this organism is intrinsically resistant to ertapenem[7]. They detected complete imipenem hydrolysis as 100% of the metallo-β-lactamases (MBLs) within two hours but an extended incubation of up to 12 h was required for carbapenem-hydrolyzing class D β-lactamases (CHDL) detection because either the oxacillinases from *A. baumannii* had weaker carbapenemase activity or *A. baumannii* grew more slowly than *Enterobacterales* species. This study demonstrated the MALDI-TOF MS based ertapenem hydrolysis assay as a good tool to rapidly detect carbapenemase activity of *Enterobacterales* strains.

4. CONCLUSION

The MALDI-TOF MS technique reliably and quickly detected carbapenemase activity. However, future large-scale testing is required to further evaluate the performance of this method as a suitable tool for rapid detection. This technique showed promise for early detection and control of CPE isolates in a hospital setting, thereby assisting patient therapy.

5. ACKNOWLEDGEMENT

This work was supervised by Assoc. Prof. Dr. Ratana Lawung, lecturer in the Program of Medical Technology Specialty of Clinical Microbiology. Our thanks are also extended to Dr. Namphol Danpipat, a director of Samutprakan Hospital, Mr. Yootapong Meekaewnoi, Head of the Medical Technology and Clinical Pathology Department, and all members of the Clinical Microbiology Laboratory for their support in facilitating this research.

Conflict of Interest
None declared.

Funding
None declared.

Ethics Approval
This experiment was approved by the Research ethical committee of Huachiew Chalermprakiet University, Thailand. (Reference no. OR.1248/2022).

Article info:
Received June 21, 2023
Received in revised form October 4, 2023
Accepted October 9, 2023

Author Contributions
PK - Research design, conducted experiments, performed data analysis, Writing-original draft and contributing to the writing of the manuscript.
WR - Performed data analysis, writing-review and editing of the manuscript.
SJ - Performed data analysis, writing-review and editing of the manuscript.
MS - Conducted experiments, performed data analysis, writing-review and editing of the manuscript.

REFERENCES

1. Zhang Y, Li W, Tian X, Sun R, Zhou S, Jia L, et al. Phenotypic and genotypic characterization of carbapenem-resistant *Enterobacteriaceae* recovered from a single hospital in China, 2013 to 2017. Infect Drug Resist. 2022;15:7679-90.
2. Hu YM, Li J. [Clinical application of mCIM and eCIM combined detection of carbapenemase-producing *Enterobacteriaceae*]. Zhonghua Yu Fang Yi Xue Za Zhi. 2021;55(4):506-11.
3. Kaase M, Szabados F, Wassill L, Gatermann SG. Detection of carbapenemases in *Enterobacteriaceae* by a commercial multiplex PCR. J Clin Microbiol. 2012;50(9):3115-8.
4. Ledeboer NA, Lopansri BK, Dhiman N, Cavagnolo R, Carroll KC, Granato P, et al. Identification of Gram-negative bacteria and genetic resistance determinants from positive blood culture broths by use of the Verigene Gram-negative blood culture multiplex microarray-based molecular assay. J Clin Microbiol. 2015;53(8): 2460-72.
5. Koser CU, Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. Trends Genet. 2014;30(9): 401-7.
6. Temkin E, Adler A, Lerner A, Carmeli Y. Carbapenem-resistant *Enterobacteriaceae*: Biology, epidemiology, and management. Ann N Y Acad Sci. 2014;1323:22-42.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Wayne, PA: CLSI supplement M100; 2020.
8. Murray PR. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology. Clin Microbiol Infect. 2010;16(11):1626-30.
9. Vrioni G, Tsiamis C, Oikonomidis G, Theodoridou K, Kapsimali V, Tsakris A. MALDI-TOF mass spectrometry technology for

detecting biomarkers of antimicrobial resistance: current achievements and future perspectives. Ann Transl Med. 2018;6(12):240.
10. Ahmed OB, Dablool AS. Quality improvement of the DNA extracted by boiling method in Gram negative bacteria. Int J Bioassays. 2017;6:5347-9.
11. Chuong LV. Molecular identification of broad spectrum β-lactam resistant *Enterobacteriaceae* and *Neisseria gonorrhoeae* [dissertation]. Nakhonpathom: Mahidol University; 2016.
12. Haijun L, Qin B, Xianwei HE, Han Z, Jinfang F. Research and evaluation of rapid detection of β-lactam-resistant enterobacteria based on domestic mass spectrometry Autof msl000. Int J Lab Med. 2014;4:2104-12.
13. Oho M, Funashima Y, Nagasawa Z, Miyamoto H, Sueoka E. Rapid detection method of carbapenemase-producing *Enterobacteriaceae* by MALDI-TOF MS with imipenem/cilastatin (KB) disc and zinc sulfate solution. J Infect Chemother. 2021;27(2): 205-10.
14. Paveenkittiporn W, Lyman M, Biedron C, Chea N, Bunthi C, Kolwaite A, et al. Molecular epidemiology of carbapenem-resistant *Enterobacterales* in Thailand, 2016-2018. Antimicrob Resist Infect Control. 2021;10(1):88.
15. Wangchinda W, Laohasakprasit K, Lerdlamyong K, Thamlikitkul V. Epidemiology of carbapenem-resistant *Enterobacterales* infection and colonization in hospitalized patients at a university hospital in Thailand. Infect Drug Resist. 2022;15:2199-210.
16. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence. 2017;8(4):460-9.
17. Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, et al. Worsening epidemiological situation of carbapenemase-producing *Enterobacteriaceae* in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill. 2019;24(9):1900123.
18. Touati A, Mairi A. Epidemiology of carbapenemase-producing *Enterobacterales* in the Middle East: A systematic review. Expert Rev Anti Infect Ther. 2020;18(3):241-50.
19. Takeuchi D, Kerdsin A, Akeda Y, Sugawara Y, Sakamoto N, Matsumoto Y, et al. Nationwide surveillance in Thailand revealed genotype-dependent dissemination of carbapenem-resistant *Enterobacterales*. Microb Genom. 2022;8(4):000797.
20. Wyres KL, Nguyen TNT, Lam MMC, Judd LM, van Vinh Chau N, Dance DAB, et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. Genome Med. 2020;12(1):11.
21. Dortet L, Bonnin RA, Pennisi I, Gauthier L, Jousset AB, Dabos L, et al. Rapid detection and discrimination of chromosome- and MCR-plasmid-mediated resistance to polymyxins by MALDI-TOF MS in *Escherichia coli*: the MALDIxin test. J Antimicrob Chemother. 2018;73(12):3359-67.
22. Hleba L, Hlebova M, Kovacik A, Cubon J, Medo J. Carbapenemase producing *Klebsiella pneumoniae* (KPC): What is the best MALDI-TOF MS detection method. Antibiotics (Basel). 2021; 10(12):1549.
23. Florio W, Baldeschi L, Rizzato C, Tavanti A, Ghelardi E, Lupetti A. Detection of antibiotic-resistance by MALDI-TOF Mass Spectrometry: An expanding area. Front Cell Infect Microbiol. 2020; 10:572909.
24. Carvalhaes CG, da Silva ACR, Streling AP, Cayo R, Rockstroh AC, Machado AMO, et al. Detection of carbapenemase activity using VITEK MS: Interplay of carbapenemase type and period of incubation. J Med Microbiol. 2015;64(8):946-7.
25. Johansson A, Ekelof J, Giske CG, Sundqvist M. The detection and verification of carbapenemases using ertapenem and matrix assisted laser desorption ionization-time of flight. BMC Microbiol. 2014;14:89.
26. Yu J, Liu J, Li Y, Yu J, Zhu W, Liu Y, et al. Rapid detection of carbapenemase activity of *Enterobacteriaceae* isolated from positive blood cultures by MALDI-TOF MS. Ann Clin Microbiol Antimicrob. 2018;17(1):22.
27. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol. 2011; 49(9):3321-4.
28. Sharma M, Singhal L, Gautam V, Ray P. Distribution of carbapenemase genes in clinical isolates of *Acinetobacter baumannii* & a comparison of MALDI-TOF mass spectrometry-based detection of carbapenemase production with other phenotypic methods. Indian J Med Res. 2020;151(6):585-91.
29. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother. 2009;63(4):659-67.