Journal of Microbiological Methods 163 (2019) 105652

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Development and evaluation of the method for detecting metallo-carbapenemases among carbapenemase-producing *Enterobacteriaceae*

Quanfeng Liao[a,1], Yun Xie[b,1], Chengcheng Wang[c], Zhiyong Zong[c], Siying Wu[a], Ya Liu[a], Weili Zhang[a], Yi Xie[a], Yuling Xiao[a], Mei Kang[a,*]

[a] Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China
[b] Department of Clinical Laboratory, Northwest Women's and Children's Hospital, Xi'an, Shanxi, China
[c] Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China

ARTICLE INFO

Keywords:
Metallo-carbapenemase
Enterobacteriaceae
EDTA synergistic simplified carbapenem inactivation method
EDTA synergistic carbapenem inactivation method

ABSTRACT

A simple EDTA synergistic carbapenem inactivation method (esCIM) based on the simplified carbapenem inactivation method (sCIM) and EDTA synergistic carbapenem inactivation test (eCIM) detected the levels of metallo-β-lactamases (MBLs) carbapenemase. The esCIM method uses EDTA-impregnated antibiotic disk to detect carbapenemase-producing *Enterobacteriaceae* (CPE) directly instead of inculating the disk in the trypticase soy broth (TSB). To determine the sensitivity and specificity of esCIM, 167 carbapenemase-resistant *Enterobacteriaceae* (CRE) isolates were collected, of which, 161 were CPE strains confirmed by PCR. The carbapenemase genes included *blaKPC* (50.9%), *blaNDM* (36.6%), *blaIMP* (6.8%), *blaVIM* (3.1%), and *blaOXA-181* (0.6%). Three isolates carried two different types of genes (*blaKPC* and *blaNDM*), and the remaining six CRE strains lacked the carbapenemase genes. The phenotypic evaluations were performed using both esCIM and eCIM. The esCIM performs better than eCIM in the detection of *blaNDM* and *blaIMP*, and the positive rate of eCIM was 83% and 55% for *blaNDM* and *blaIMP*, respectively. However, in the case of esCIM, the rate increased to 97% and 73%, respectively. For all MBLs, the sensitivity of esCIM and eCIM observed was 91% and 76%, respectively, while the specificity of the two methods was 100%. Taken together, esCIM could be performed easily and interpreted quickly.

1. Introduction

Carbapenem-resistant *Enterobacteriaceae* (CRE) are defined as *Enterobacteriaceae* resistant to any carbapenems such as imipenem, meropenem, or ertapenem. The percentage of CRE has been increased sharply in recent years (Righi et al., 2017; Li et al., 2018). The current reports showed that the primary resistance mechanisms encompass the production of carbapenemases, high discharge pump, hyperproduction of AmpC β-lactamases, or extended-spectrum β-lactamases (ESBLs) combined with altered membrane permeability (Bush and Fisher, 2011; Patel and Bonomo, 2013; Logan and Weinstein, 2017). This phenomenon could be attributed to the localization of carbapenemase genes in a ligated plasmid that can be transmitted horizontally to other bacteria: carbapenemase-producing *Enterobacteriaceae* (CPE) is threatening and associated with significant morbidity and mortality (Tamma et al., 2017; Nordmann et al., 2011).

Carbapenemases can be divided into class A, B, or D based on their molecular characteristics according to the Ambler classification system (Bush and Fisher, 2011; Ambler, 1980). Carbapenemases in class A and D require serine at their active sites and could be inhibited by enzyme inhibitors such as clavulanic acid and tazobactam. Carbapenemases in class B are known as metallo-β-lactamases (MBLs), require zinc ions for β-lactam hydrolysis, and can be inhibited by ethylene diamine tetraacetic acid (EDTA) (Bush and Fisher, 2011; Jean et al., 2015; Bonomo, 2017). The most common carbapenemases in class A are KPC enzymes, while notable transmissible carbapenemases in class B include IMP (imipenem-activated), VIM (Verona integron-encoded MBL), and NDM (New Delhi MBL) enzymes (Bush and Fisher, 2011; Patel and Bonomo, 2013; Walsh, 2010). Common class D carbapenemases include OXA-24-like, OXA-48-like, and OXA-181-like enzymes (Jean et al., 2015).

Detection of CPE is challenging in clinical laboratories. Although genotypic assays (such as PCR and DNA microarray tests) are the "gold standard" for detecting the drug-resistant genes rapidly and specifically, some laboratories are unable to carry out the testing due to the

* Corresponding author at: Department of Laboratory Medicine, West China Hospital of Sichuan University, No. 37 Guoxue Lane, Chengdu 610041, China.
E-mail address: kangmei@sina.com (M. Kang).
[1] These authors contributed equally to this work and should be considered co-first anthors

https://doi.org/10.1016/j.mimet.2019.105652
Received 20 January 2019; Received in revised form 6 June 2019
Available online 13 June 2019

lack of equipment, especially in developing countries. However, phenotypic detections are convenient and manageable methods and play a vital role in screening carbapenemases. Over the last decade, a number of phenotype-based assays have been developed, including rapid colorimetric-based assays (manual and commercial versions of the Carba NP) (Nordmann et al., 2012), the modified carbapenem inactivation method (mCIM), EDTA synergistic carbapenem inactivation test (eCIM) for detecting MBLs with mCIM (CLSI, 2017, 2018), and the simplified carbapenem inactivation method (sCIM) (Jing et al., 2018). The Carba NP test requires the acquisition of dedicated reagents (with associated costs and training needs). It is interpreted subjectively and is poorly sensitive to the detection of the OXA-48-type. mCIM and sCIM are employed to detect carbapenemases. Although sCIM is based on the mCIM that saves 4 h incubation time, it cannot distinguish MBLs from carbapenemases. In the present study, based on the mCIM and eCIM, we designed a simple EDTA synergistic carbapenem inactivation method (esCIM) for detecting carbapenemases in *Enterobacteriaceae* and compared it with polymerase chain reaction (PCR) and mCIM methods.

2. Materials and methods

2.1. Bacteria

All CRE strains (167) were isolated from two centers: West China Hospital Sichuan University ($n = 156$) and Wuhan Fourth Hospital ($n = 11$) and were found to be resistant to any carbapenem. The strains were identified using the Vitek2 compact automatic microbial analysis system (BioMerieux, France). All isolates utilized in this study belonged to *Klebsiella* spp. (126), *Escherichia coli* (22), *Enterobacter* spp. (10), *Citrobacter* spp. (one), *Raoultella ornithinolytica* (one), and *Enterobacter cloacae* (seven). Each phenotypic test was performed using a stock strain stored at −80 °C. The isolate was cultured at 35 ± 2 °C for 18–24 h on blood agar (Autobio Diagnostics Co., Ltd., China) before testing.

2.2. PCR detection of carbapenemase genes

The bacterial chromosomal DNA was extracted by boiling a volume of 10 μL loop germs suspended in 800 mL distilled water for 10–15 min at 100 °C. Subsequently, the suspension was harvested by centrifugation at 12,000 rpm for 5 min at 4 °C. The resulting supernatant extracted from *Enterobacteriaceae* was applied as a template for the PCR assay. Primers for *blaKPC*, *blaIMP*, *blaVIM*, *blaNDM*, and *blaOXA* are listed in Table 1. PCR was performed as described previously (Doyle et al., 2012): A volume of 14.5 μL of PCR Master Mix (AOKE, Chengdu, China) was mixed with 2 μL of forward and reverse primers in a 25-μL reaction. Then, 1 μL of sample from each test isolate was added to the mixture. The PCR program consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were separated by agarose gel electrophoresis.

2.3. Developing a new method (esCIM) to detect MBLs

esCIM is a new method to detect metallo-carbapenemase and was based on sCIM and eCIM methods. According to eCIM, 5 mM EDTA was used to detect the Ambler class B enzymes; however, the same EDTA concentration was used to perform esCIM in the present study. Firstly, the imipenem disk was soaked in 5 mM EDTA for 1 min. Subsequently, the disk was be coated with 1–3 colonies of test strains before placing on the Mueller–Hinton agar (MHA) inoculated with 0.5 McFarland standard suspension of *E. coli* ATCC (American Type Culture Collection) 25922 (using the colony suspension method). Simultaneously, another imipenem disk coated only with test strains (sCIM) was also placed on the same MHA plate, followed by incubation at 35 ± 2 °C for 18 h. When the sCIM test is positive, the results of esCIM are interpreted as follows: when the diameter of the inhibitory zone of esCIM is 5 mm larger than that of sCIM, MBL is positive. Any pinpoint colonies should not be considered if existed in the zone. When the diameter of the inhibitory zone of esCIM is < 4 mm of that of sCIM, it can be considered as MBL-negative, indicating that the test strains produce a serine carbapenemase, and the activity of the carbapenemase is not affected by the presence of EDTA; thus, the difference in the diameter between sCIM and eCIM is < 4 mm (CLSI, 2017, 2018). Herein, we utilized 10, 20, 30, and 40 mM EDTA to carry out the steps described above. The positive and negative quality control (QC) strains used in this study were *Klebsiella pneumoniae* ATCC BAA-1705 (blaKPC-positive by PCR), *Klebsiella pneumoniae* ATCC BAA-1706, and *Klebsiella pneumoniae* ATCC BAA-2146 (blaNDM-positive by PCR).

2.4. Evaluation of esCIM and eCIM for detecting CPE

In order to evaluate the sensitivity and specificity of esCIM and eCIM, 167 CRE strains were tested. The eCIM was used together with mCIM. However, in the case of mCIM, an inhibition zone diameter of 6–15 mm or the number of colonies within a 16–18 mm zone was considered to be positive, while a zone of inhibition ≥19 mm was considered to be negative. The interpretation of eCIM is identical to that of esCIM. Also, the esCIM was performed together with sCIM. Interestingly, sCIM is based on mCIM with improved experimental procedures. The testing organisms were smeared onto an imipenem disk, indicating that the strains were inoculated onto the MHA plate following the routine disk diffusion procedure. The plates were allowed to dry for 3–10 min, and then, the side of the disk having bacteria was placed on the MHA plate previously inoculated with *E. coli* ATCC 25922. All the plates were incubated at 35 ± 2 °C for 18 h. The zone of inhibition has a diameter of 6–20 mm; the satellite growth of colonies of *E. coli* ATCC 25922 have a zone diameter ≤ 22 mm, indicating that the isolate was capable of producing carbapenemase. A zone of inhibition ≥26 mm was considered to be negative, while a zone of inhibition of 23–25 mm was considered to be a carbapenemase-indeterminate result (Jing et al., 2018).

Table 1
Primers for the detection of carbapenemase-producing *Enterobacteriaceae*.

Carbapenemase gene	Primer sequences[a]	Amplicon size (bp)	Reference
blaKPC	5′-TGTCACTGTATCGCCGTC-3′ 5′-CTCAGTGCTCTACAGAAAACC-3′	1050	Karuniawati et al., 2013
blaNDM	5′-ATGGAATTGCCCAATATTATGCACCCG-3′ 5′-TCAGCGCAGCTTGTCGGCCATGCG-3′	813	Karuniawati et al., 2013
blaIMP	5′-CTACCGCAGCAGAGTCTTTG-3′ 5′-AACCAGTTTTGCCTTACCAT-3′	587	Jean et al., 2015
blaVIM	5′-GATGGTGTTTGGTCGCATA-3′ 5′-CGAATGCGCAGCACCAG-3′	390	Mushi et al., 2014
blaOXA	5′-GCGTGGTTAAGGATGAACAC-3′ 5′-CATCAAGTTCAACCCAACCG-3′	438	Jean et al., 2015

[a] The first and second primers for each gene are forward and reverse primers, respectively.

3. Results

3.1. Carbapenemase genes

The PCR results are shown in Table 3. 161/167 CRE isolates were genetically characterized to be carrying carbapenemase genes: *blaKPC-2* was discovered in 82 (50.9%) isolates, *blaNDM* in 59 (36.6%) isolates (*blaNDM-7* in one, *blaNDM-1* in 28, and *blaNDM-5* in 30 isolates), *blaIMP* in 11 (6.8%) isolates (*blaIMP-4* in nine and *blaIMP-1* in two strains), *blaVIM-1* in five (3.1%) isolates, and *blaOXA-181* in one (0.6%) isolate. Three isolates carried two types of genes (*blaKPC-2* and *blaNDM-1* in two strains, and the another carried *blaKPC-2* and *blaNDM-5*), and the remaining six CRE isolates with mucous characteristic did not carry the carbapenemase genes. These MICs of carbapenem on the six strains were low (low-level expression) with imipenem as well as with meropenem; however, the MIC of ertapenem exceeded 8 μg/mL (Table 3).

3.2. Optimum concentration of EDTA for esCIM

We utilized five concentrations of EDTA (5, 10, 20, 30, and 40 mM) to perform esCIM. The results showed that using 30 mM EDTA had the highest positive rate, and EDTA concentration of negative control inhibited the growth of testing strains with 40 mM EDTA (Table 2).

3.3. Sensitivity and specificity of esCIM

A total of 161 CPEs were detected in the esCIM study. 68 MBLs showed positive results, and the remaining 83 isolates (82 *blaKPC* and one *blaOXA*) were characterized to carrying the serine carbapenemase genes, which showed negative results by esCIM. A total of seven isolates escaped the detection of MBLs including two isolates with *blaNDM* (one *blaNDM-1* in *Klebsiella pneumoniae* and one *blaNDM-5* in *Enterobacter hormaechei*), three *Klebsiella pneumoniae* with *blaIMP-4* (one has mucoid characteristic), and two *Klebsiella pneumoniae* are with *blaVIM-1* (one has mucoid feature). The sensitivity of the esCIM observed in this study was 91% (68/75) and the specificity was 100% (83/83) as compared to the genotype (Table 3**).**

A total of 167 isolates were screened by sCIM, mCIM, esCIM, and eCIM. In order to evaluate the carbapenemases, the sensitivity of sCIM and mCIM was 100%. For 75 MBLs, the sensitivity of the eCIM was 76%; however, with esCIM, it increased to 91%. 57/59 *blaNDMs* were detected definitely by esCIM (97%), while 49 isolates were positive by eCIM (83%). The detection rates were 73% for *blaIMPs* and 60% for *blaVIMs* by esCIM; when tested using eCIM, these rates decreased to 55% and 40%, respectively (Table 3).

Table 4 summarizes the relative pros and cons for eCIM and esCIM, including the following: the sensitivity and specificity, potential false negatives, an approximate cost analysis per test, an interpretation of results, procedural time, the turnaround time, and test reagents, and materials. For MBLs testing, the advantages of esCIM include the readily available reagents and supplies; the outcomes were detected within 18 h. In addition, esCIM does not require the additional 4 h of incubation as compared to eCIM (TABLE 4).

4. Discussion

CPE is a major concern to humans. A recent study showed that CPE is mainly characterized by *blaKPC* and *blaNDM* carbapenemase genes in China; the common carbapenemase genes are *blaKPC-2* and *blaNDM-1* in carbapenem-resistant *Klebsiella pneumoniae* strains and blaNDM-5 in carbapenem-resistant *Escherichia coli* (Wang et al., 2018). Especially, therapeutic regimens are varied based on enzymes: some new drugs such as ceftazidime-avibactam are optimal for Ambler class A carbapenemase as well as some Ambler class C and Ambler class D enzymes (OXA-48). However, these medicines are unavailable for class B carbapenemase. Hence, rapid and accurate phenotypic detection to identify MBLs from carbapenemases is essential.

The similar detection principles of esCIM and eCIM are based on the fact that carbapenemases can hydrolyze carbapenem and that MBLs could be inhibited by EDTA (Bush and Fisher, 2011; Bonomo, 2017). Unlike eCIM, disks used for esCIM method were coated with 30 mM EDTA for 1 min instead of immersing into TSB with 20 μL 0.5 M EDTA. In the current study, the additional 4 h to incubate the strains and the materials used in the eCIM method are not required: 5 mL glass test tube, 1 μL incubating loop, or TSB culture media. Thus, the operation in the esCIM experiment has fewer steps and is more convenient than the eCIM. When carbapenemase-producing bacteria are coated on imipenem disks, the enzyme hydrolyzes the antibiotics on the paper, leading to a reduction in the size of the inhibition zone.

Additionally, six carbapenem-resistant *Klebsiella pneumoniae* strains without carbapenemase genes were tested negative by sCIM and mCIM. Due to various mechanisms in CRE, abnormal porin expression might confer resistance to ertapenem (Doumith et al., 2009). The MICs of imipenem and meropenem on these strains were low, while the MIC of ertapenem often exceeded 8 μg/mL; all presented a mucoid characteristic. We speculated that the genes of porin *OMPK35* and *OMPK36* express abnormally, and further studies would be essential to substantiate the findings.

PCR results showed that VIM, NDM, and IMP enzymes were produced by *Enterobacteriaceae* in this study. Compared to eCIM, esCIM was more sensitive (91% vs. 76%) and equally specific (100%), while esCIM performed better than eCIM with respect to the detection of *blaNDM* (97% vs. 83%) and *blaIMP* (73% vs. 55%). However, both methods were unsatisfactory in detecting *blaVIM* (60% vs. 40%). Interestingly, one mucoid *Klebsiella pneumoniae* with *blaIMP-4* showed false-negative result through eCIM and esCIM, and the other two non-mucoid *Klebsiella pneumoniae* strains that expressed *blaIMP* also showed false-negative result by esCIM; however, eCIM increased the number to four. For *blaVIMs*, two mucous isolates showed false-negative results for eCIM detection and one for esCIM. Though all MBLs can be inhibited by EDTA, the test results of *blaVIMs* were dissatisfactory, which might be related to the different inhibitory effects of EDTA on different enzymes.

Using PCR, three carbapenem-resistant *Klebsiella pneumoniae* isolates were confirmed to produce multiple enzymes, including both *blaKPCs* and *blaNDMs*. The eCIM and esCIM failed to detect *blaNDM* for multi-carbapenemase. With respect to the strains with multi-carbapenemases, especially for isolates producing *blaKPC* and *blaNDM*, we designed methods based on different mechanisms to evaluate the phenotype. Boric acid inhibits the serine enzyme (Bush and Fisher, 2011).

Table 2
Study on optimum concentration of EDTA for esCIM.

PCR (n)	esCIM (5 mM EDTA)		esCIM (10 mM EDTA)		esCIM (20 mM EDTA)		esCIM (30 mM EDTA)	
	MBLs (+)	MBLs* (−)	MBLs (+)	MBLs* (−)	MBLs (+)	MBLs* (−)	MBLs (+)	MBLs* (−)
NDM(59)	2	57	54	5	55	4	57	2
IMP(11)	0	11	2	9	6	5	8	3
VIM(5)	0	5	1	4	3	2	3	2

+ Positive; − negative; MBLs* (−) the testing isolate produces a serine carbapenemase; esCIM EDTA synergistic simplified carbapenem inactivation method.

Table 3
Laboratory detection of 167 CRE isolates.

Enzyme type	PCR (n)	mCIM		sCIM		eCIM		esCIM	
		+	−	+	−	MBLs(+)	MBLs*(−)	MBLs(+)	MBLs*(−)
Ambler class A	KPC (82)	82 (100%)	0	82 (100%)	0	0	82(100%)	0	82 (100%)
Ambler class B	NDM (59)	59 (100%)	0	59 (100%)	0	49 (83%)	/	57 (97%)	/
	IMP (11)	11 (100%)	0	11 (100%)	0	6 (55%)	/	8 (73%)	/
	VIM (5)	5 (100%)	0	5 (100%)	0	2 (40%)	/	3 (60%)	/
	ALL-MBL (75)	/	/	/	/	57 (76%)	/	68 (91%)	/
Ambler class D	OXA (1)	1	0	1	0	0	1	0	1
Multi-enzyme	KPC and NDM (3)	3	0	3	0	0	3	1	2
	NON-CP CRE (6)	0	6	0	6	/	/	/	/
	All carbapenemases (161)	100%		100%		/		/	

MBLs metallo-β-lactamases; + Positive; MBLs*(−) the testing isolate produces a serine carbapenemase; eCIM EDTA synergistic carbapenem inactivation method; esCIM EDTA synergistic simplified carbapenem inactivation method; CPE carbapenemase-producing *Enterobacteriaceae;* NON-CP CRE non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; Multi-enzyme Isolates harboring more than two types of enzymes (MBL and serine carbapenemase).

Table 4
Relative pros and cons of eCIM and esCIM.

Parameter	eCIM	esCIM
(%; sensitivity, specificity)	76,100	91,100
Potential false negatives	Isolates harboring more than two types of enzymes (MBL and serine carbapenemase) or isolates have mucoid character	Isolates harboring more than two types of enzymes (MBL and serine carbapenemase) or isolates have mucoid character
Procedural time	5 min for initial setup,4 h ± 15 min for inoculating,3–10 min to inoculate plate, and 2 min to read the result on the next days	2 min for initial setup,3–10 min to inoculate plate, and 2 min to read the results on the next days
Turnaround time	22–28 h	16–18 h
Approximate cost per test (US$)	< $1.00	< $1.00
Test reagents and materials	TSB, meropenem disks (10 μg),1 μL inoculation loop, nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots), MHA plates (100 mm or 150 mm), meropenem-susceptible indicator strain – *E. coli* (ATCC 25922),0.5 M EDTA.	Imipenem disks (10 μg), nutrient broth (eg, Mueller-Hinton, TSB) or normal saline (3.0–5.0 mL aliquots), MHA plates (100 mm or 150 mm), *E. coli* (ATCC 25922), 30 mM EDTA
Interpretation of results	Interpret only when the mCIM test is positive. Metallo-β-lactamase positive, A ≥5 mm increase in zone diameter for eCIM vs zone diameter for mCIM. metallo-β-lactamase negative, A ≤4 mm increase in zone diameter for the eCIM vs zone diameter of mCIM, and the testing isolate produce a serine carbapenemase.	Interpret only when sCIM test is positive. Metallo-β-lactamase positive, A ≥5 mm increase in zone diameter for esCIM vs zone diameter for sCIM. metallo-β-lactamase negative, A ≤4 mm increase in zone diameter for the esCIM vs zone diameter of sCIM, and the testing isolate produce a serine carbapenemase.

Next, we performed a series of concentration-based experiments to affirm KPC carbapenemase based on esCIM method, but failed. Furthermore, clavulanic acid was selected to do a similar test (Bush and Fisher, 2011; Bonomo, 2017), and the positive result did not appear. Furthermore, for isolates with multi-carbapenemase, the standard gold method (PCR) needs to be performed.

5. Conclusions

In conclusion, the esCIM is a simple, accurate, and reproducible method for detecting MBLs among CPE. Compared to PCR, the esCIM does not require specific equipment or reagents, and hence, is cost-effective. Compared to eCIM method recommended by CLSI, esCIM is more convenient and has better sensitivity and specificity. This new method is optimal for routine use in most clinical microbiology laboratories for the detection of MBLs and contributes to understanding the local epidemiology of carbapenem resistance for combating the spread of CPE.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank Ji Zeng for providing *Enterobacteriaceae* strains with *blaVIM* carbapenemase gene and Qingyue Yuan for drafting the manuscript.

References

Ambler, R.P., 1980. The structure of beta-lactamases. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 289, 321–331.
Bonomo, R.A., 2017. Beta-lactamases: a focus on current challenges. Cold Spring Harb. Perspect. Med. 7, a025239.
Bush, K., Fisher, J., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from gram-negative bacteria. Annu. Rev. Microbiol. 65, 455–478.
CLSI, 2017. Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100, 27th edn. Clinical and Laboratory Standards Institute, Wayne, PA.
CLSI, 2018. Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100, 28th edn. Clinical and Laboratory Standards Institute, Wayne, PA.
Doumith, Michel, Ellington, Matthew J., Livermore, David M., Woodford, Neil, 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant Klebsiella and Enterobacter spp. clinical isolates from the UK. J. Antimicrob. Chemother. 63, 659–667.
Doyle, D., Peirano, G., Lascols, C., Lloyd, T., Church, D.L., Pitout, J.D., 2012. Laboratory detection of *Enterobacteriaceae* that produce carbapenemases. J. Clin. Microbiol. 50, 3877–3880.
Jean, S.S., Lee, W.S., Lam, C., Hsu, C.W., Chen, R.J., Hsueh, P.R., 2015. Carbapenemase-producing gram-negative bacteria: current epidemics, antimicrobial susceptibility and treatment options. Future Microbiol 10, 407–425.
Jing, X., Zhou, H., Min, X., Zhang, X., Yang, Q., Du, S., Li, Y., Yu, F., Jia, M., Zhan, Y., Zeng, Y., Yang, B., Pan, Y., Lu, B., Liu, R., Zeng, J., 2018. The simplified carbapenem inactivation method (sCIM) for simple and accurate detection of carbapenemase-producing gram-negative bacilli. Front. Microbiol. 9, 2391.
Karuniawati, A., Saharman, Y.R., Lestari, D.C., 2013. Detection of carbapenemase encoding genes in *Enterobacteriace*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii* isolated from patients at intensive care unit Cipto Mangunkusumo Hospital in 2011. Acta Med. Indones 45, 101–106.
Li, Y., Sun, Q., Shen, Y., Zhang, Y., Yang, J., Shu, L., Zhou, H., Wang, Y., Wang, B., Zhang, R., Wang, S., Shen, Z., 2018. Rapid increase in prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) and emergence of colistin resistance gene mcr-1 in CRE in a hospital in Henan, China. J. Clin. Microbiol. 56 (e01932-17).

Logan, Latania K., Weinstein, Robert A., 2017. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. J. Infect. Dis. 215 (S1), S28–S36.
Mushi, M.F., Mshana, S.E., Imirzalioglu, C., Bwanga, F., 2014. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed. Res. Int. 2014, 303104.
Nordmann, P., Naas, T., Poirel, L., 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. 17, 1791–1798.
Nordmann, P., Poirel, L., Dortet, L., 2012. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. 18, 1503–1507.
Patel, G., Bonomo, R.A., 2013. "Stormy waters ahead": global emergence of carbapenemases. Front. Microbiol. 4, 1–17.
Righi, Elda, Peri, Anna Maria, Harris, Patrick N.A., Wailan, Alexander M., Liborio, Mariana, Lane, Steven W., Paterson, David L., 2017. Global prevalence of carbapenem resistance in neutropenic patients and association with mortality and carbapenem use: systematic review and meta-analysis. Antimicrob. Chemother. 72, 668–677.
Tamma, P.D., Goodman, K.E., Harris, A.D., Tekle, T., Robert, A., Taiwo, A., Simner, P.J., 2017. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae bacteremia*. Clin. Infect. Dis. 64, 257–264.
Walsh, T.R., 2010. Emerging carbapenemases: a global perspective. Int. J. Antimicrob. Agents 36, S8–S14.
Wang, Qi, Wang, Xiaojuan, Wang, Juan, et al., 2018. Phenotypic and genotypic characterization of carbapenem-resistant *Enterobacteriaceae*: data from a longitudinal large-scale CRE Study in China (2012–2016). Clin. Infect. Dis. 67 (S2), S196–S205.