Evaluation of modified carbapenem inactivation method for suspected carbapenemase among Enterobacteriaceae clinical isolates

Beiwei Yu1,2,*, Dong Dong2,3,*, Mingxia Wang1,2, Yan Guo2,3, Dandan Yin4 and Fupin Hu2,3

1Department of Laboratory Medicine, Jinhua People’s Hospital, Zhejiang Province, China
2Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai, China
3NHC Key Laboratory of Clinical Pharmacology of Antibiotics, Fudan University, Shangai, China
4The Clinical Microbiology Laboratory, Department of Nosocomial Infection Control, Children’s Hospital of Fudan University, Shanghai, China

* These authors contributed equally to this work

Correspondence to: Fupin Hu, email: hufupin@163.com

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ABSTRACT

Modified carbapenem inactivation method (mCIM) testing was currently recommended by Clinical and Laboratory Standards Institute (CLSI) for detection of carbapenemase among Enterobacteriaceae clinical isolates. In this study, a panel of 145 clinical strains were collected for evaluating the mCIM for detection of carbapenemase. Antimicrobial susceptibility testing were performed by microbroth dilution and the results were interpreted according to CLSI guidelines. All strains were resistant to ertapenem with high MIC50 and MIC90 (64 mg/L –>128 mg/L). For blaNDM-1-positive or blaOXA-232-positive strains, the zone of inhibition of meropenem were all 6 mm despite the incubation time of 6 h, 18 h or 24 h. For 6 h, the zone of meropenem inhibition for most of carbapenemase-positive isolates were meet the positive criteria 6–15 mm. However, for carbapenemase-negative isolates, the zone of meropenem inhibition were 16–18 mm after 6 h incubation which should be considered indeterminate for standard incubating time such as 18 h or 24 h. After incubating for 18 h or 24 h, the zone of meropenem inhibition were 22–25 mm for carbapenemase-negative isolates and meet the negative criteria. Our study indicate mCIM is a simple and effective method to identify the carbapenemases producers among Enterobacteriaceae clinical isolates.

INTRODUCTION

Production of carbapenemases are main mechanism underlying resistance to carbapenems which are first-line agents with proven efficacy for treatment of severe infections caused by multi-drugs resistant bacteria [1]. Carbapenemase-producing Enterobacteriaceae isolates are usually extensively drug resistant, and infections caused by these pathogens with significant morbidity and mortality present a serious clinical challenge, especially for pediatric patients [2]. Because genes mediated carbapenemase usually located in transferable plasmids and it can potentially spread rapidly, infections caused by carbapenemase-producing Enterobacteriaceae may prove difficult to control once they emerge [3]. For this purpose, rapid and reliable identification of carbapenemase producers in the clinical microbiology laboratory is urgently needed to affect infection...
treatment. Currently, although several commercial tests are available for detection of carbapenem resistance including phenotypic or genotypic tests, none is ideal for all possible carbapenemase genes [4]. In 2017, modified carbapenem inactivation method (mCIM) testing was recommended by Clinical and Laboratory Standard Institute (CLSI) for detection of carbapenemase among Enterobacteriaceae clinical isolates [5]. This method is simple, low-cost to assess phenotypic carbapenemase activity in Enterobacteriaceae and has been demonstrated a sensitivity >99% and specificity >99% for detection of KPC, NDM, VIM, IMP, SPM, SME and OXA-type carbapenemase [1]. In this study, we described a mCIM method with some modification for the identification of carbapenemase-producing Enterobacteriaceae clinical isolates.

MATERIALS AND METHODS

Strains

A panel of 145 clinical strains including 77 bla<sub>KPC-2</sub>-positive K. pneumoniae, 31 bla<sub>NDM-1</sub>-positive K. pneumoniae, 4 bla<sub>NDM-1</sub>-positive E.coli, 5 bla<sub>OXA-232</sub>-positive K. pneumoniae, and 28 carbapenem-susceptible K. pneumoniae were collected from four hospitals in China for evaluating the mCIM for suspected carbapenemase. All isolates were identified by vitek 2 compact system (BioMerieux, France), and the presence of carbapenemase genes was confirmed by specific PCR and sequence analysis. E. coli ATCC25922 was used as quality control strain in antimicrobial susceptibility testing. E. coli NCTC-13476 (bla<sub>VIM</sub> positive) and K. pneumoniae NCTC-13440 (bla<sub>NDM-1</sub> positive) were also used for positive control for mCIM.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing were performed by microbroth dilution and minimum inhibitory concentrations (MICs) were interpreted according to CLSI guidelines [5].

mCIM for suspected carbapenemase

mCIM for detection of carbapenemases among 145 Enterobacteriaceae clinical isolates were performed as described by CLSI [5]. The zone of inhibition of meropenem was recorded after incubating time for 6 h, 18 h, and 24 h, respectively.

RESULTS

Antimicrobial susceptibility testing

The results of antimicrobial agents susceptibility for all of 117 carbapenem-resistant Enterobacteriaceae isolates were detailed in Table 1. All strains were resistant to ertapenem with high MIC<sub>50</sub> and MIC<sub>90</sub> (64 mg/L and >128 mg/L), meropenem with high MIC<sub>50</sub> and MIC<sub>90</sub> (32 mg/L and 128 mg/L), however, 23.1%–49.6% of them were susceptible to ciprofloxacin, gentamicin and amikacin, respectively.

mCIM for suspected carbapenemase production

Results obtained from mCIM indicated (Figures 1 and 2), for bla<sub>NDM-1</sub>-positive and bla<sub>OXA-232</sub>-positive
Figure 1: Zone of Inhibition distribution of meropenem for Enterobacteriaceae clinical isolates at different incubating time.

Figure 2: Modified carbapenem inactivation method for suspected carbapenemase production in Enterobacteriaceae.
1. K6, blakpc-2 positive K. pneumoniae incubating 6 h (zone 6 mm); K24, blakpc-2 positive K. pneumoniae incubating 24 h (zone 6 mm) (arrow indicated); 2. N6, blanDM-1 positive K. pneumoniae incubating 6 h (zone 6 mm); N24, blanDM-1 positive K. pneumoniae incubating 24 h (zone 6 mm) (arrow indicated); 3. O6, blaoxa-232 positive K. pneumoniae incubating 6 h (zone 6 mm); O24, blaoxa-232 positive K. pneumoniae incubating 24 h (zone 6 mm) (arrow indicated); 4. S6, meropenem-susceptible K. pneumoniae incubating 6 h (zone 18 mm); S24, meropenem-susceptible K. pneumoniae incubating 24 h (zone 22 mm) (arrow indicated).
strains, the zone of inhibition of meropenem were all 6 mm despite the incubation time of 6 h, 18 h or 24 h. For 18 h or 24 h, because of presence of colonies within a 16–18 mm meropenem zone for several blaKPC-2 producing K. pneumoniae isolates, mCIM can also differentiate successfully between carbapenemase-positive and carbapenemase-negative Enterobacteriaceae isolates. For 6 h, the zone of meropenem inhibition for all of carbapenemase-positive isolates were meet the positive criteria 6–15 mm. However, for carbapenemase-negative isolates, the zone of meropenem inhibition were 16–18 mm after 6 h incubation which should be considered indeterminate for standard incubating time such as 18 h or 24 h. After incubating for 18 h or 24 h, the zone of meropenem inhibition were 22–25 mm for carbapenemase-negative isolates and meet the negative criteria.

**DISCUSSION**

In the past 10 years, the world-wide increase in carbapenem-resistant organisms has made it even more important to use these “last line” antibiotics which are the most broad-spectrum agents known and are often life-saving therapies for severe infections [6, 7]. According to CHINET surveillance, the resistance rate of K. pneumoniae isolates to carbapenem was increasing rapidly from 2005 to 2015 [1, 8]. Rapid and effective detection of carbapenemases is important for clinicians treating patients with these infections and for infection preventionists to limit the spread of carbapenem-resistant organisms [9]. mCIM recommended by CLSI in 2017 is a simple and inexpensive method to perform and is well established in many clinical microbiology laboratories based on its high sensitivity and specificity to detect carbapenemase-producing Enterobacteriaceae isolates compared with the current published or available phenotype method such as modified hodge testing and Carba-NP method [5]. Modified hodge testing is very simple to perform and no special reagents or media necessary. however, false-positive results can occur in isolates that produce ESBL or AmpC enzymes coupled with porin loss, and false-negative results are occasionally noted for some strains producing NDM carbapenemase. Carba_NP is a rapid method for detection of suspected carbapenemases among Enterobacteriaceae, but special reagents are needed, some of which necessitate inhouse preparation (and have a short shelf life). Given the rapid international spread of carbapenemase-producing isolates and the urgent need of treatment for the infection due to these isolates, a simple and effective mCIM for detection of KPC-2, NDM-1 and OXA-232-type carbapenemases which were the most common carbapenemases among Enterobacteriaceae isolates in China [10, 11] is important.

In CLSI studies [5], one OXA-232-producing K. pneumoniae isolate was negative by mCIM at 4 out of validation sites, however, in this study, all of 5 OXA-232-producing K. pneumoniae isolates were positive by mCIM at different incubating time including 6 h, 18 h and 24 h. In this study, for 6 h, 18 h or 24 h, mCIM demonstrated a sensitivity 100% and specificity 100% for detection of KPC-2, NDM-1, OXA-232-type carbapenemases among Enterobacteriaceae isolates. For 6 h, the indeterminate results occurred for all of carbapenemase-negative Enterobacteriaceae isolates and the results indicated “testing inconclusive for the presence of carbapenemase”. So, for carbapenemase-negative Enterobacteriaceae isolates, it is necessary to extend the incubating time for confirming the production of carbapenemase.

Although carbapenemases involving in this study are only three type of carbapenemase. However, according to the previous studies, blaKPC and blaNDM are the main carbapenemase genes in our country [12]. Other genes including blaOXA-48, blaIPM and blaVIM are rare among Enterobacteriaceae clinical isolates [13]. In some cases, especially for pediatrics infected by CRE, early and rapid detection of carbapenemase among Enterobacteriaceae clinical isolates with 6 h incubation probably is vital the treatment with antimicrobial agents because the infection caused by carbapenem-resistant Enterobacteriaceae clinical isolates are associated with significant morbidity and mortality [14, 15].

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest.

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