Modified CIM test as a useful tool to detect carbapenemase activity among extensively drug-resistant *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter baumannii*

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Abstract

**Purpose:** Timely detection of carbapenemases is essential for developing strategies to control the spread of infections by carbapenem-resistant isolates. The purpose of this study was to determine the epidemiology of carbapenemase genes among carbapenem-resistant isolates of *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*. In addition, the efficacy of the modified Hodge test (MHT), Carba NP test, and modified carbapenem inactivation method (mCIM) were compared.

**Methods:** A total of 122 carbapenem-resistant clinical isolates including 77 *K. pneumoniae*, 39 *A. baumannii*, and six *E. coli* were collected from hospitalized patients. Three phenotypic methods, including the MHT, Carba NP test, and mCIM were used for investigation of carbapenemase production. In addition, polymerase chain reaction (PCR) was performed to detect carbapenemase-encoding genes.

**Result:** The sensitivity and specificity of the MHT were 75.0% and 100%, respectively. In addition, Carba NP displayed 80.8% sensitivity and 100% specificity, whereas the sensitivity and specificity were 90.4% and 100% for the mCIM test, respectively. Among carbapenem-resistant isolates, 70, 84, and 87 isolates exhibited positive results according to the MHT, Carba NP test, and mCIM, respectively. PCR indicated the presence of one or more carbapenemase genes in 119 of carbapenem-resistant isolates, with *blaKPC* and *blaVIM* being the most commonly encountered. Co-production of *KPC* and OXA-48, *KPC* and VIM*, and *KPC* and IMP* was detected in three, nine, and seven isolates, respectively.

**Conclusion:** Our results confirm that the mCIM test is a useful tool for the reliable detection of carbapenemase activity in enterobacterial isolates, especially in clinical microbiological laboratories with limited resources.

**Keywords:** *Acinetobacter baumannii*, Carba NP, Carbapenemase, mCIM, Carbapenem resistance
Introduction

The worldwide emergence and spread of carbapenemase producers represent a significant threat to public health (Bialvaei et al. 2017). Particularly, this may pose a major problem as carbapenems are becoming more frequently needed to treat infections caused by Gram-negative bacilli (GBN) that produce extended-spectrum beta-lactamases (ESBL) (Vardakas et al. 2012). Carbapenemase enzymes are clustered in different classes that define their hydrolytic profiles; Verona integron-encoded metallo-β-lactamase (VIM), New Delhi MBL (NDM), and imipenemase (IMP) belong to the Ambler class B metallo-β-lactamases (MBLs), Klebsiella pneumoniae carbapenemase (KPC) and GES belong to class A, and OXA-48 and OXA-48-like belong to class D (Zahedi Bialvaei et al. 2015). These profiles are associated with resistance to carbapenems and most β-lactam antibiotics. In addition, mobile genetic elements (plasmids, transposons, etc.) that harbor carbapenemase genes have been documented to spread among GNB, making it important to rapidly and efficiently detect all carbapenemase producers to prevent their further spread (Gniadek et al. 2016).

The epidemiology of carbapenemase-producing bacteria varies by country. The prevalence of KPC-producing GNB is unevenly distributed among the USA, and there are less frequent reports of other carbapenemases, including IMP, VIM, NDM, and OXA-48-like enzymes (Lutgring and Limbago 2016; Guh et al. 2014). The epidemiology is different in other countries. For example, VIM is endemic in Greece, KPC is the most common carbapenemase in Israel, and IMP is endemic in Japan (Lutgring and Limbago 2016). In addition, the highest KPC-mediated resistance in the middle east area has been observed in Afghanistan, Saudi Arabia, and Jordan followed by NDM in Pakistan and OXA in Turkey and Pakistan (Zahedi Bialvaei et al. 2015). Therefore, antimicrobial resistance surveillance is essential for providing the necessary information for formulating local and international antimicrobial guidelines. Phenotypic methods defined by the Clinical and Laboratory Standards Institute (CLSI) to detect carbapenemase producers include the modified Hodge test (removed from the M100-S28 in 2018; CLSI), the Carba NP test, and most recently the modified carbapenem inactivation method (mCIM) (Cunningham et al. 2017; Pierce et al. 2017). While the Carba NP test is relatively easy to implement in the clinical laboratory, the mCIM has been found to be sensitive and specific in several recent studies and easy to perform and implement. Also, it utilizes low-cost materials that are readily available in clinical laboratories (Pierce et al. 2017; Tamma et al. 2017).

Although molecular methods remain the gold standard, they are costly, limited by the targets used specifically in the test, and not accessible to all microbiology laboratories throughout the world (Claridge 3rd 2004). These methods can only detect known carbapenemase-encoding genes, and the number of carbapenemase-encoding genes and allelic variants thereof is expanding rapidly. In contrast, a phenotypic assay may detect carbapenemase activity irrespective of the carbapenemase-encoding gene sequence. In addition, timely detection of carbapenemases is essential for developing strategies to control the spread of infections by carbapenem-resistant isolates and related morbidity and mortality, particularly in specific regions. Therefore, the aims of this study were the characterization of carbapenemase activity in extensively drug-resistant (XDR) A. baumannii, K. pneumoniae, and E. coli clinical isolates and comparing the sensitivity and specificity of the modified Hodge test (MHT), Carba NP test, and mCIM in detecting carbapenem resistance.

Methods

Bacterial isolates

A total of 122 non-duplicate carbapenem-resistant clinical isolates were analyzed. The isolates included 77 K. pneumoniae, 39 A. baumannii, and six E. coli had been collected at various wards between May 2017 and February 2019 in 1000-bed tertiary care of Milad hospital, Tehran, Iran. Bacterial identification was performed by standard biochemical and microbiological tests. All isolates were stored at – 70 °C in trypticase soy broth with 15% glycerol and were subcultured twice on blood agar plates (Merck, Germany) prior to testing. The isolates were obtained from urine specimens (43, 35.2%), followed by tracheal aspirate/fluid (35, 28.7%), wound and soft tissue specimens (34, 27.9%), blood (6, 4.9%), sputum (3, 2.5%), bronchoalveolar lavage (1, 0.8%). Patient’s age ranged from 1 to 93 years old (61.4 ± 20.43), 73 (59.8%) were from female patients, while 49 (40.1%) from males. As a control, 18 carbapenem-resistant, KPC-2 type carbapenem-producing K. pneumoniae clinical isolates were used in this study (Wang et al. 2011).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolated bacteria was determined in vitro, utilizing the disc diffusion method in accordance with the CLSI criteria (Clinical and Laboratory Standards Institute (CLSI) 2018). The tested antibiotics included ceftriaxone, cefotaxime, amoxicillin, cefepime, tazocin, ceftazidime, ciprofloxacin, gentamicin, and colistin (MAST Diagnostics, Merseyside, UK). Susceptibility testing for meropenem and/or imipenem was performed according to the CLSI reference by using E test method (Liofilchem, Italy). Carbapenem-resistant isolates were selected using the CLSI M100-S standard (27th edition) definition, i.e., not susceptible (intermediate or resistant, minimum inhibitory concentrations (MICs) of ≥ 2 μg/ml) to
meropenem and/or imipenem, as defined by the current (Müller et al. 2017).

**The modified Hodge test**

The MHT was carried out on all isolates to detect carbapenemase activity as described by CLSI (Clinical and Laboratory Standards Institute 2017). Briefly, an inoculum of *E. coli* ATCC 25922 was adjusted to 0.5 McFarland turbidity standard, and then a 1/10 dilution was inoculated on the surfaces of Mueller-Hinton agar plates (BD Ltd, USA) by swabbing. Next, after the plates were left undisturbed for 10 min at room temperature, a 10-μg meropenem disk (Oxoid, UK) was placed in the center of each plate. Subsequently, suspected bacteria grown overnight on blood agar plate were inoculated onto the plate in a straight line from the edge of the disk to the periphery of the plate (without touching the disc) by swabbing. The plate was incubated overnight at 37 °C in ambient air for 16–24 h. In negative isolates, the clear zones around the disk remain homogeneous, while carbapenemase-producing isolates cause cloverleaf-like indentation.

**Carba NP and CarbAcineto NP**

All isolates have also been tested for carbapenemase activity with Carba NP and CarbAcineto NP as defined by CLSI (Clinical and Laboratory Standards Institute 2017). Briefly, one third to one half of a 10-μl inoculation loop of the tested isolate, recovered from Mueller-Hinton Agar (BD Ltd, USA), was resuspended in 100 μl of commercially available lysis buffer (B-PERII, Thermo Scientific Pierce, Rockford, USA). Next, 100 μl of diluted phenol red (Merck, Germany) solution containing 0.1 mM ZnSO₄ (Merck, Germany) (pH = 7.8) and 6 mg/ml Imipenem was added. The phenol red solution, with no antibiotic, was used as a control tube for each isolate. Both tubes were vortexed and incubated at 37 °C for a maximum of 2 h. The color of the test tube changed to full yellow or orange, resulting from the hydrolysis of imipenem into a carboxylic derivative, leading to a decrease of the pH value (Dortet et al. 2014). For Acinetobacter, this protocol has two modifications: the use of a full 10-μl loop of culture as an inoculum in order to increase the enzyme quantity and 100 μl NaCl (5 M) solution instead of the lysis buffer, avoiding any buffer effect.

**The modified carbapenem inactivation method**

The mCIM was performed as described earlier by van der Zwaluw et al. (2015), with some modifications. Briefly, a suspension was made with a 10-μl inoculation loop from a 1-day-old culture, taken from a Mueller-Hinton agar plate in 500 μL of sterile tryptic soy broth (TSB; Merck, Germany) and vortexed to obtain a homogenous suspension. Subsequently, a 10-μg meropenem disk (Oxoid, UK) was immersed in the suspension and incubated for 2–3 h at 37 °C. After incubation, the disk was carefully picked from the suspension using a forceps, placed on a Mueller-Hinton Agar plate pre-inoculated with a susceptible *E. coli* ATCC 25922 using a suspension equivalent to 0.5 McFarland and subsequently incubated overnight at 37 °C in ambient air. A zone of ≤ 15 mm was considered positive for carbapenemase production, and a clear zone of ≥ 19 mm was considered negative. Zones of 16 to 18 mm or ≥ 19 mm with colonies present within the zone were considered indeterminate. Quality control was performed by testing a carbapenemase-positive (*K. pneumoniae* ATCC BAA-1705) and a carbapenemase-negative (*K. pneumoniae* ATCC BAA-1706) control strain on each day of testing. For additional controls, meropenem disks were incubated in Trypticase soy broth (TSB) or water alone (no organisms) for 2 h at 37 °C. The dry meropenem disk were also applied to the *E. coli* lawn, and zones were evaluated after overnight incubation to ensure they fell within CLSI quality control (QC) ranges for meropenem and *E. coli* ATCC 25922.

**Detection of carbapenem resistance genes**

Total DNA was extracted from all strains using the High Pure Template Kit (Roche Diagnostics, Germany) following the manufacturer’s instructions. Five carbapenemase genes, including *blaKPC*, *blaNDM*, *blaOXA-48*-like, *blaIMP*, and *blaVIM* genes, were amplified using previously described primers (Schecher et al. 2009; Nordmann et al. 2011; Poirel et al. 2012; Pavelkovich et al. 2014). Amplification was performed in the thermal cycler (Eppendorf, Germany) and the amplified products were analyzed in 2% (w/v) agarose gel (100 V for 45 min). Quality control included testing an isolate positive or negative for each target on each day of testing. Additionally, a 16S rRNA gene internal control was included in each PCR. Of note, multiplex polymerase chain reaction (PCR) was repeated for isolates that showed discrepant results in the form of negative PCR and positive Carba NP test or mCIM.

**Statistical analysis**

Data were analyzed with SPSS 23.0 (SPSS Inc., Chicago, IL). Diagnostic sensitivity and specificity calculations with 95% confidence intervals were done as described by Pasteran et al. (2009), using the results of carbapenem resistance gene PCR as the gold standard. Indeterminate or invalid results were classified as false-negative results when they occurred in carbapenemase-producing isolates and as false-positive results when they occurred in non-carbapenemase-producing isolates. The kappa coefficient was calculated to assess agreement between testing sites. Comparison between groups was performed by
positivity of the Carba NP test is shown in Fig. 1. There were false-negative results in 16 isolates in Carba NP test. The false-negative results missed by the Carba NP test were linked to \( \text{bla}_{\text{VIM}} \) (8/16), \( \text{bla}_{\text{IMP}} \) (5/16), and \( \text{bla}_{\text{KPC}} \) (3/16) carbapenemases. The test was found negative in all those isolates displaying no reduced sensitivity against any of the carbapenems. The sensitivity of the Carba NP test was determined as 80.8% and a specificity of 100% in the isolates studied (Table 2).

**mCIM test results**

All 122 isolates of carbapenem-resistant bacteria were tested, and among them 87 (71.3%) isolates showed positive results by the mCIM (Fig. 1). It was observed that 12 isolates tested by the mCIM were determined as false negative. All the 12 isolates of positive carbapenemase gene, which were negative by the mCIM test, included 11 and one isolate of \( A.\ baumannii \) and \( K.\ pneumoniae \), respectively. The false-negative results missed by the mCIM test were linked to \( \text{bla}_{\text{VIM}} \) (6/12), \( \text{bla}_{\text{IMP}} \) (3/12), \( \text{bla}_{\text{KPC}} \) (2/12), and \( \text{bla}_{\text{OXA-48}} \) (1/12) carbapenemases.

All the isolates were detected to be positive by the end of 6 h. The negative isolates were incubated overnight, but the result did not change. All the isolates displaying no reduced sensitivity against any of the carbapenems were found to be negative by the mCIM test. The sensitivity and specificity of the mCIM test in the isolates studied was determined to be 90.4% and 100%, respectively. We observed that the mCIM test has a slightly higher sensitivity than the Carba NP test (90.4% vs. 80.8%, respectively; \( p = 0.018 \)).

**Detection of carbapenemase genes**

All isolates were tested for five carbapenemase genes by PCR. Of all carbapenem-resistant isolates, PCR results demonstrated that 119 (97.5%) isolates harbored at least one carbapenemase gene; none of the five carbapenemase genes were detected in the isolates by the PCR method (Fig. 2). One isolate yielding carbapenemase production by the mCIM test was negative for the evaluated genes. The number of isolates that carried these genes is shown in Table 3. The most frequently identified carbapenemase genes were \( \text{bla}_{\text{KPC}} \) identified in 59 isolates (48.3%), \( \text{bla}_{\text{VIM}} \) identified in 49 isolates (40.1%), followed by

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**Table 1** Antimicrobial susceptibility testing for 122 nonduplicated isolates of \( K.\ pneumoniae \), \( A.\ baumannii \), and \( E.\ coli \) strains isolated over study period.

| Bacteria                  | CIP | GM | CAZ | CRO | CTX | AMS | CPM | TZ | CS | IPM | MEM |
|---------------------------|-----|----|-----|-----|-----|-----|-----|----|----|-----|-----|
| \( K.\ pneumoniae \) \((n = 77)\) | 76  | 1  | 76  | 1   | 76  | 1   | 76  | 0  | 76 | 0   | 16  | 75  |
| \( A.\ baumannii \) \((n = 39)\)    | 39  | 0  | 39  | 0   | 39  | 0   | 39  | 0  | 39 | 0   | 38  | 39  |
| \( E.\ coli \) \((n = 6)\)            | 6   | 0  | 6   | 0   | 6   | 0   | 6   | 0  | 6  | 0   | 6   |

_CRO_ ceftriaxone, _CTX_ cefotaxime, _AMS_ amoxicillin, _CPM_ cefepime, _TZ_ tazocin, _CAZ_ ceftazidime, _CIP_ ciprofloxacin, _GM_ gentamicin, _CS_ colistin, _IPM_ imipenem, _MEM_ meropenem
Table 2 Performance of three phenotypic methods compared with the molecular detection of carbapenemase-producing isolates in the study

| Bacteria            | mCIM* (%) (95% CI), n = 122 | CarbaNP (%) (95% CI), n = 122 | MHT† (%) (95% CI), n = 122 |
|---------------------|----------------------------|-------------------------------|---------------------------|
|                     | Sensitivity                 | Specificity                   | Sensitivity               | Specificity |
| *K. pneumoniae (n=77)* | 96.25                      | 100                           | 91.66                     | 100         |
| *A. baumannii (n=39)* | 79.71                      | 100                           | 56.52                     | 100         |
| *E. coli (n=6)*      | 85.71                      | 100                           | 100                       | 100         |
| **Total**            | 90.4                       | 100                           | 80.8                      | 100         |

*Modified carbapenem inactivation method; †Modified Hodge test

The results of this study showed that the antibiotic resistance rates are high among studied clinical strains. In line with other studies conducted in Iran, almost all isolates were resistant to three or more antibiotics tested except colistin, indicating the highest antibacterial activity (Ranjbar and Farahani 2019). In the present study, high MIC values for carbapenems were detected and indicated a markedly reduced efficacy of these agents that could be due to their uncontrolled availability and/or overuse. In addition, we have shown recently that the prevalence of MDR *A. baumannii* in Iran increased from 50% in 2001–2007 to 74% in 2010–2015, with a mean prevalence of 71% (Bialvaei et al. 2017). The trade among nations, for instance, Iran, Iraq, and Turkey, which reports the highest number of MDR cases is another possible reason behind such increase in the prevalence of resistance.

The PCR results, as the gold standard technique, confirmed that 119 isolates harbored one or more carbapenemase genes, *bla*KPC and *bla*VIM, being the most common, in agreement with some studies reporting the high prevalence of *bla*KPC and *bla*VIM carbapenemases (Papadimitriou-Olivgeris et al. 2019). However, *bla*NDM was not detected in any of the investigated strains, while in a study by (Hosseinzadeh et al. 2018), more than 10% of the isolates carried the *bla*NDM gene. Co-production of different carbapenemases in a single isolate being reported increasingly for several Enterobacteriaceae species in various studies (Poirel et al. 2004; Bakthavatchalam et al. 2016; Cizmeci et al. 2017). In our collection, we have identified nine *K. pneumoniae* isolates co-producing KPC and VIM, five *K. pneumoniae* and two *E. coli* isolates co-producing KPC and IMP, and three *K. pneumoniae* isolate co-producing KPC and OXA-48 carbapenemases.

Recently, some phenotypic tests were designated for laboratory detection of carbapenemase activity. Although some studies have emphasized the 100% sensitivity and specificity, some other studies have not confirmed these findings in comparison with molecular methods (Hammoudi et al. 2014; Akhi et al. 2017). Therefore, proper performance of these methods is necessary under different conditions with various isolates obtained.
worldwide. MHT was recommended by the Clinical and Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing (2009) as a screening method for carbapenemase. However, it has been removed from the recent guideline (Clinical and Laboratory Standards Institute (CLSI) 2018). The advantages of the method include it being easy to perform and not needing special reagents or media (Akhi et al. 2017). The sensitivity and specificity of the MHT in our study was lower than in the results obtained by two other methods (Table 2). The Carba NP test was developed by Nordmann & Poirel (Nordmann et al. 2012) in 2012; however, this method was subsequently modified. Carba NP, with high sensitivity and rapid detection (≤ 2 h), can detect not only all the known carbapenemases, but it identifies also newly emerging carbapenemases, compared with molecular methods (Nordmann et al. 2012). In this study, the Carba NP test had a sensitivity of 80.8% and a specificity of 100%. This agrees with several studies evaluating the Carba NP test versus gold-standard PCR, which reported variable sensitivities of 72–93% and specificity of 100% (Tijet et al. 2013; Österblad et al. 2014; Hombach et al. 2015; Bayramoğlu et al. 2016a). Many studies have attributed the variable sensitivities and false-negative results encountered with the Carba NP test to many factors, including enzymes with weak carbapenemase activity (e.g., blaOXA-48-like enzymes and blaGES-3), isolates with mucoid property, size of the bacterial inoculum, early reading before completion of 2 h of incubation, and present but unexpressed carbapenemase genes (Österblad et al. 2014). In agreement with the current study, several studies reported false-negative Carba NP tests with the same dominating enzymes (NDM, KPC, and VIM) and related to mucoid Klebsiella strains, besides other factors linked to agar type and ion content for cultivation of the isolate (Hombach et al. 2015). In this study, the false-negative results missed by the Carba NP test were linked to blaVIM, blaIMP, and blaKPC carbapenemases.

Recently, the CIM test was introduced and has been shown to be highly sensitive and specific for detection of any type of carbapenemase activity in GNB (van der Zwaluw et al. 2015). We believe that although the CIM test is not as rapid as the Carba NP test (8 h vs. 2 h), it is a highly sensitive, simple, and cost-effective method that can be used in clinical laboratories, particularly those with limited resources, for early detection of carbapenemases. However, the mCIM utilizes readily available reagents not requiring reagent preparation, and results are more objective in nature, as a zone diameter reading is used for interpretation of results (Pierce et al. 2017). In this study, mCIM results gave a sensitivity of 90.4% and a specificity of 100%. These results are in accordance with many other studies evaluating CIM, which showed low false-negative results with a sensitivity of 98.8% (Bayramoğlu et al. 2016a; Bayramoğlu et al. 2016b; Tijet et al. 2016; Laolerd et al. 2018). Like our study, these studies reported no false-positive results, with a specificity of 100%. Jing et al. (2018) introduced the Simplified carbapenem inactivation method (sCIM), which instead of incubating the antibiotic disk in the organism culture media for 4 h as in the mCIM, the organism to be tested was smeared directly onto an antibiotic disk in the sCIM. Based on their results, the sCIM showed high specificity and sensitivity comparable to PCR but has the advantage of being more user-friendly. Yamada et al. (2019) evaluated the detectability of MBL-producing Enterobacterales against three types of MBL inhibitors used in combination with a mCIM. Although inhibitor-combination mCIMs were highly specific (99.0–100%) for the detection of MBL.
Table 3 The results of MHT, Carba NP, and mCIM for isolates tested (n = 122)

| Species (n) | Carbapenemase gene | Meropenem MIC(μg/ml) | Test result for MHT | Carba NP | mCIM |
|-------------|---------------------|----------------------|---------------------|----------|------|
| *Klebsiella pneumoniae* (1) | KPC | 0.094 | − | − | − |
| *K. pneumoniae* (1) | VIM + KPC | 1 | + | − | − |
| *K. pneumoniae* (1) | KPC | 1.5 | + | + | + |
| *K. pneumoniae* (1) | KPC + IMP | 1.5 | + | − | + |
| *K. pneumoniae* (1) | KPC | 2 | + | − | + |
| *K. pneumoniae* (1) | KPC | 3 | + | + | + |
| *K. pneumoniae* (1) | VIM | 3 | + | + | + |
| *K. pneumoniae* (1) | KPC | 6 | + | + | + |
| *K. pneumoniae* (1) | VIM + KPC | 8 | + | + | + |
| *K. pneumoniae* (2) | KPC | 8 | + | − | + |
| *K. pneumoniae* (2) | IMP | 12 | + | + | + |
| *K. pneumoniae* (1) | VIM + KPC | 12 | + | − | + |
| *K. pneumoniae* (1) | VIM + KPC + OXA-48 | 12 | + | + | + |
| *K. pneumoniae* (1) | VIM + IMP | 12 | + | + | + |
| *K. pneumoniae* (3) | KPC | 12 | + | + | + |
| *K. pneumoniae* (2) | KPC | 16 | + | + | + |
| *K. pneumoniae* (1) | KPC | 16 | − | − | + |
| *K. pneumoniae* (3) | IMP | 16 | + | + | + |
| *K. pneumoniae* (1) | VIM + IMP | 16 | − | + | + |
| *K. pneumoniae* (1) | KPC | 24 | + | + | + |
| *K. pneumoniae* (1) | KPC + VIM | 24 | + | + | + |
| *K. pneumoniae* (8) | KPC | > 32 | − | + | + |
| *K. pneumoniae* (14) | KPC | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + KPC | > 32 | + | + | + |
| *K. pneumoniae* (3) | VIM + KPC | > 32 | − | + | + |
| *K. pneumoniae* (1) | - | > 32 | + | + | + |
| *K. pneumoniae* (9) | IMP | > 32 | − | + | + |
| *K. pneumoniae* (5) | VIM | > 32 | + | + | + |
| *K. pneumoniae* (2) | IMP + KPC | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + IMP | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + KPC + IMP | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + OXA-48 + IMP | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + OXA-48 | > 32 | + | + | + |
| *K. pneumoniae* (1) | VIM + OXA-48 | > 32 | + | + | + |
| *Acinetobacter baumannii* (1) | IMP + VIM | 16 | − | − | − |
| *A. baumannii* (1) | VIM | 16 | − | − | − |
| *A. baumannii* (2) | KPC | 16 | + | + | + |
| *A. baumannii* (1) | IMP | 16 | − | − | − |
| *A. baumannii* (12) | VIM | > 32 | − | − | − |
| *A. baumannii* (5) | IMP | > 32 | − | − | − |
| *A. baumannii* (4) | KPC | > 32 | + | + | + |
| *A. baumannii* (5) | VIM | > 32 | + | − | − |
| *A. baumannii* (2) | VIM | > 32 | + | + | + |
authors, they found that sensitivity was dependent on the inhibitor’s concentration.

### Conclusions

High prevalence of extensively drug-resistant *A. baumannii*, *K. pneumoniae*, and *E. coli* with resistance to different classes of antimicrobial agents mediated by carbapenemases can be a main challenge for treatment with serious implications. We found both the mCIM and the CLSI Carba NP test to be accurate for detection of carbapenemases among carbapenem-resistant isolates. However, the mCIM was superior in being of higher sensitivity compared with the Carba NP test. Overall, the mCIM and Carba NP test offer the chance for easy implementation of carbapenemase detection in routine laboratories and can be employed to give timely and actionable clinical results. Moreover, they can contribute to better antimicrobial stewardship of carbapenems through prudent control of carbapenemase producers.

### Code availability

The code is available from the corresponding author upon request.

### Authors’ contributions

Abed Zahedi Bialvaei participated in the methodology, data collection, data analysis and drafted a manuscript. Alireza Dolatyar Dehkharghani and Farhad Asgari participated in design of the study and critical revision of the manuscript. Mohammad Rahbar participated in study supervision and designed the experimental scheme, and carried out the overall planning and improvement of the manuscript. All authors have read and approved the final manuscript.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its tables and figures).

### Declarations

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

The participant has consented to the submission of this article to the journal. We confirm that the manuscript, or part of it, has neither been published nor is currently under consideration for publication. This work and the manuscript were approved by all coauthors.

**Competing interests**

The authors declare that they have no conflicts of interest.

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### Table 3 The results of MHT\(^*\), Carba NP, and mCIM\(^\circ\) for isolates tested (n = 122) (Continued)

| Species (n)          | Carbapenemase gene | Meropenem MIC\(^{\mu g/ml}\) | Test result for MHT | Carba NP | mCIM |
|----------------------|--------------------|----------------------------|----------------------|----------|------|
| *A. baumannii* (2)   | IMP                | > 32                       |                       | +        | –    |
| *A. baumannii* (1)   | VIM                | > 32                       |                       | +        | +    |
| *A. baumannii* (1)   | -                  | > 32                       | –                    | –        | –    |
| *A. baumannii* (2)   | VIM + IMP          | > 32                       | –                    | –        | –    |
| *Escherichia coli* (1) | OXA-48 + KPC    | 16                         | +                    | +        | +    |
| *E. coli* (1)       | OXA-48             | 16                         | +                    | +        | +    |
| *E. coli* (1)       | OXA-48             | > 32                       | –                    | +        | –    |
| *E. coli* (1)       | IMP                | > 32                       | –                    | +        | –    |
| *E. coli* (1)       | VIM                | > 32                       | +                    | +        | –    |

\(^*\)Modified Hodge test; \(^\circ\)Modified carbapenem inactivation method; \(^\ast\)Minimum inhibitory concentration
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