Rapid carbapenem inactivation method (rCIM): A new phenotypic method for rapidly detecting carbapenemase-producing *Enterobacterales*

Yameng Liu[1], Liang Luan[2], Jing Liu[2] and Nan Wan[2*]

Abstract

Background: Rapid and accurate methods for detecting carbapenemase-producing *Enterobacterales* (CPE) are essential for improving patient prognosis and preventing the spread of these microbes. In this study, 103 carbapenem-resistant *Enterobacterales* (CRE) isolates were collected from clinical specimens; of these, 55 CRE isolates were included in the retrospective analysis, and 48 CRE isolates were included in the prospective evaluation. Using sequencing results as the gold standard, we evaluated the performance of the rapid carbapenem inactivation method (rCIM) for detecting carbapenemases in comparison with the modified carbapenem inactivation method (mCIM) and CNPt-Direct test. In rCIM, the test isolate was incubated with meropenem (MEM) disks, and the supernatant obtained via centrifugation was incubated with the indicator strain *Escherichia coli* ATCC 25922. Growth of the indicator strain was monitored using a nephelometer.

*Correspondence: wannan@outlook.com

[1]Post Graduate Training Base of General Hospital of Northern Theater Command, Jinzhou Medical University, Jinzhou, Liaoning Province, China

[2]Department of Clinical Laboratory, General Hospital of Northern Theater Command, Shenyang, Liaoning Province, China

Results: The cut-off value of rCIM was 0.50 McFarland units. In retrospective analysis, the percent positive agreement and percent negative agreement of rCIM for detecting carbapenemase were 97.1% and 100%, respectively, and these values were higher than those for mCIM and the CNPt-Direct test. In the prospective evaluation, rCIM was linked to a sensitivity and specificity of 96.4% and 95%, respectively. **Conclusion:** rCIM may be a rapid (<3 h), economical, simple, and reliable method for screening CPE isolates, and it is expected to be routinely implemented in clinical microbiology laboratories.

Keywords: rCIM, carbapenem-resistant *Enterobacterales*, carbapenemase, mCIM, CNPt-Direct test

Background

Enterobacterales comprises a large group of gram-negative bacilli with similar biological characteristics, and these microbes are common causes of hospital-acquired and community-acquired infections [1]. Carbapenems are the last line of defense against *Enterobacterales* infections. The worldwide emergence and spread of carbapenem-resistant *Enterobacterales* (CRE) represent a public health threat [2-3]. The carbapenem resistance mechanisms include the production of carbapenemase, high efflux pumps expression, and production of extended-spectrum β-lactamase (ESBL) and/or AmpC β-lactamase in combination with mutations that decrease the permeability of the bacterial cell to the entry of carbapenems, but carbapenemase production is the main mechanism [4-5]. Studies have revealed that carbapenemase-producing *Enterobacterales* (CPE) has higher toxicity, easier

transmission, poorer prognosis, and higher mortality than non-carbapenemase-producing *Enterobacterales* (NPE) [6]. Therefore, the early and accurate detection of CPE is extremely important for infection control and for epidemiologic and therapeutic purposes.

Although molecular methods remain the gold standard [7-8], they are complex and costly, their use is limited by the targets used in the test, and they are not accessible to all microbiology laboratories worldwide. Therefore, phenotype-based assays are urgently needed for carbapenemase screening in clinical microbiology laboratories. At present, several phenotypic methods are available for detecting carbapenemase, including MALDI-TOF carbapenem hydrolysis assays [7], biochemical methods (Carba NP) [7], immunological tests (RESIST-3 O.K.N., NG-Test CARBA 5) [9-10], growth-based assays (modified Hodge test), and the modified carbapenem inactivation method (mCIM) [7]. The growth-based assays require at least 18–24 h, which delays detection. The MALDI-TOF carbapenem hydrolysis assays, immunological methods, and Carba NP test require only 15 min to 4 h to obtain results. Because of the high cost of mass spectrometers, MALDI-TOF assays cannot be widely implemented in all microbiology laboratories. The immunological methods only target the 3–5 most common carbapenemases. The Carba NP test interprets the result visually based on color changes, and the result is greatly affected by the personnel who judge the results, which can easily lead to false-negative or false-positive results.

Carbapenem inactivation method (CIM)-based tests have attracted widespread

67 interest in clinical microbiology since 2015, and many studies have described the
68 simplicity, low cost, and high sensitivity and specificity of CIM assays for the
69 detection of CPE [11-14]. CIM assesses the growth of the indicator strain *Escherichia*
70 *coli* ATCC 25922 around meropenem disk that were previously incubated with the
71 test isolate. If meropenem in the disk is hydrolyzed by carbapenemase expressed by
72 the test organism, *E. coli* ATCC 25922 will grow to the edge of the disk or exhibit a
73 diminished zone of growth inhibition. Conversely, a zone of growth inhibition
74 indicates that MEM in the disk is active and that the test isolate does not produce a
75 carbapenemase. mCIM was developed by Dr. Sanchita Das and colleagues
76 (unpublished work) both to overcome the limited sensitivity of CIM for certain
77 enzyme classes and increase the simplicity of the method (purchasing 2 mL of TSB
78 broths versus aliquoting 400 µL of water). In 2017, the Clinical and Laboratory
79 Standards Institute (CLSI) recommended mCIM as the phenotype test for detecting
80 carbapenemase [15-16]. In 2018, CLSI also added mCIM and
81 ethylenediaminetetraacetic acid (EDTA)-CIM into as standard microbial susceptibility
82 tests for screening carbapenemases and differentiating serine enzymes [17].

83 In this study, we introduced the rapid carbapenem inactivation method (rCIM), a
84 new variant of CIM, and judged whether the test isolate produced carbapenemase by
85 measuring the McFarland index of the indicator strain ATCC 25922. The method
86 shortens the detection time to 2–2.5 h. In this study, we compared rCIM with
87 sequencing and other phenotype tests (mCIM and CNPt-Direct) to evaluate its
88 performance and provided evidence for clinical treatment and the prevention of

nosocomial infections.

Results

Establishment of the rCIM positive cut-off value

In the positive control, the MEM disks were inactivated, indicating that the growth of the indicator strain ATCC 25922 was unhindered. The median (min–max) baseline-subtracted values at 1.5 and 2 h were 0.84 (0.79–0.92) and 1.33 (1.31–1.37) McFarland units, respectively. In the negative control, the growth of ATCC 25922 was inhibited by MEM disks, and the median values (min–max) at 1.5 and 2 h were 0.15 (0.13–0.18) and -0.02 (-0.04–0.01) McFarland units, respectively. There was no obvious growth difference between the positive and negative controls before 1.5 h, whereas a significant difference was identified after 1.5 h. According to the calculation rule of cut-off value, we established 0.50 McFarland units as the rCIM positive cut-off value, which was 3-fold higher than the median negative control at 1.5 h, and evaluated the value using a retrospective and prospective collection of 103 CRE isolates.

Retrospective analysis

In the retrospective analysis, the 55 organisms and their sequencing and phenotypic methods results are presented in Table 3. The growth of the indicator strain is presented in Figure 1, and the results indicated an apparent growth difference between the CPE and NPE isolates. At 1.5 h, rCIM accurately detected 31/35 CPE isolates, and the median (min–max) was 1.92 (0.22–2.50) McFarland units. In addition, the value did not reach 0.5 McFarland units (0.44, 0.38, and 0.49, respectively) for three *E.*

cloacae isolates at 1.5 h , but the values all exceeded 0.50 McFarland units (1.10, 0.78, and 0.88, respectively) at 2 h. At 2 h, the majority of isolates (34/35) had reached 0.50 McFarland units with the median (min–max) reaching 2.47 (0.46–3.06). One blaNDM *E. cloacae* isolate grew to 0.46 McFarland units at 2 h. rCIM detected 20/20 NPEs, and the median values (min–max) at 1.5 and 2 h were 0.03 (-0.10–0.17) and 0.05 (-0.12–0.15) McFarland units, respectively. In the retrospective analysis, the percent positive and negative agreement of rCIM for detecting CPE were 97.1% and 100%, respectively, and κ was 0.961.

In the retrospective analysis, mCIM accurately detected 32/35 CPEs and 20/20 NPEs, whereas false-negative results were obtained for three isolates (blaKPC *K. pneumoniae*, blaNDM *E. coli*, and blaNDM *E. cloacae*). The percent positive agreement, percent negative agreement, and κ of mCIM were 91.4%, 100%, and 0.886, respectively. CNPt-Direct accurately detected 33/35 CPEs and 20/20 NPEs, including two false-negative results (blaKPC *K. pneumoniae* and blaIMP *K. oxytoca*), and the percent positive agreement, percent negative agreement, and κ were 94.3%, 100%, and 0.923.

Prospective evaluation

The sequencing, rCIM, mCIM, and CNPt-Direct results for 48 organisms are presented in Table 4. The growth of the indicator strain *E. coli* ATCC 25922 is shown in Figure 2. In total, 27/28 CPE isolates were detected by rCIM, and the median values (min–max) at 1.5 and 2 h were 1.32 (-0.07–1.71) and 1.97 (-0.05–2.39) McFarland units, respectively. A false-negative result was obtained for isolate of

blaNDM *P. mirabilis*, reaching -0.05 McFarland units at 2 h. rCIM detected 19/20 NPEs, with the median values (min–max) at 1.5 and 2 h reaching 0.03 (-0.15–0.55) and 0.06 (-0.12–2.16) McFarland units, respectively. A false-positive result was recorded for one PCR-negative *K. pneumoniae* isolate, and the values at 1.5 and 2 h were 0.55 and 2.16 McFarland units, respectively. In the prospective evaluation, the sensitivity, specificity, positive predictive value, negative predictive value, and κ of rCIM were 96.4%, 95%, 96.4%, 95%, and 0.914, respectively.

In the prospective evaluation, mCIM accurately detected 18/28 CPE and 19/20 NPE isolates, and false-negative results were obtained for 10 isolates (three *K. pneumoniae*, two *E. coli*, three *E. cloacae*, one *P. mirabilis*, and one *E. aerogenes* isolate). One PCR-negative *K. pneumoniae* isolate had an inhibition zone diameter of 6 mm, which denoted a false-positive result. The sensitivity, specificity, positive predictive value, negative predictive value, and κ of mCIM were 64.3%, 95%, 94.7%, 65.5%, and 0.557, respectively. CNPt-Direct accurately detected 23/28 CPE and 19/20 NPE isolates, whereas false-negative and false-positive results were recorded for five isolates (two *K. pneumoniae*, one *E. coli*, one *P. mirabilis*, and one *E. aerogenes* isolate) and one isolate (PCR-negative *K. pneumoniae*), respectively. CNPt-Direct had a sensitivity, specificity, positive predictive value, negative predictive value, and κ of 82.1%, 95%, 95.8%, 79.2%, and 0.750, respectively. The sensitivity and consistency of rCIM were better than those of the other phenotypic tests (mCIM and CNPt-Direct), but there was no difference among the groups concerning specificity.

Discussion

Carbapenemase-encoding genes are mostly located in mobile genetic elements such as bacterial plasmids, integrants, and transposons, and they can easily spread among bacteria when the outer membrane is damaged and/or permeability increases, thereby explaining the widespread spread of carbapenem-resistant Enterobacteriaceae isolates globally [18-19]. Therefore, a method for rapidly and effectively detecting carbapenemase is urgently required. In this study, we evaluated a new phenotypic method for the rapid detection of carbapenemases. rCIM correctly detected 61/63 CPE and 39/40 NPE isolates, giving an overall sensitivity of 96.8% and specificity of 97.5%, which were slightly lower than the values reported by Muntean *et al.* [20]. These lower results may be related to the insufficient sample size. In general, rCIM is an excellent method for the rapid screening of CPE.

In this study, the CRE isolates were mainly *K. pneumoniae* (52.4%), followed by *E. coli* (23.3%) and *E. cloacae* (15.5%). The carbapenem resistance mechanisms of *K. pneumoniae* in our hospital were mainly the production of *Klebsiella pneumoniae* carbapenemase (KPC, 48.1%), which is consistent with relevant domestic reports [21]. There were two false-negative results and one false-positive result for rCIM in this study. In the retrospective analysis, one isolate of blaNDM *E. cloacae* had a growth value of 0.46 McFarland units at 2 h, approaching the positive cut-off value of 0.50, which may be related to the weaker activity of the carbapenemase. Another isolate of blaNDM *P. mirabilis* reached a value of -0.05 McFarland units at 2 h, and the CNPt-Direct result was also negative, which may be related to the non-expression or

low expression of carbapenemase. In addition, a false-positive result was obtained for one PCR-negative *K. pneumoniae* isolate, with the growth of the indicator strain reaching 2.16 McFarland units at 2 h, and the CNPt-Direct result was also positive, probably linked to the limited number of primers used in our study.

In this study, only blaKPC, blaNDM, and blaIMP were detected, and blaVIM- and blaOXA-positive isolates were not found, which was in accordance with the epidemiology of carbapenemase in China [7,22-23]. In the retrospective analysis, three *E. cloacae* isolates exhibited increased growth to 0.44, 0.38, and 0.49 McFarland units at 1.5 h, respectively, but the growth of the indicator strain further increased to 1.10, 0.78, and 0.88 McFarland units at 2 h, respectively, indicating the importance of the additional 30 min of incubation. Another isolate of blaNDM *E. cloacae* had an increase in growth to 0.46 McFarland units at 2 h, approaching the cut-off value of 0.50 McFarland units. To reduce the occurrence of false-negative and false-positive results, the test isolate with growth near the positive cut-off value of 0.50 McFarland units at 2 h needs to be re-tested or further tested using other tests (molecular or immunological methods). In addition, the per-test cost of rCIM is extremely low, requiring only 2.5 mL of TSB broth and two MEM disks, comparable to the requirements for other phenotypic experiments. All isolates used in our study were obtained from blood plates, and the performance of rCIM when applied to other culture media requires further research.

rCIM, a variant of CIM, is a test for the rapid and accurate detection of carbapenemase. CIM and its variants have higher sensitivity than Carba NP when

detecting low-activity carbapenemases [20,24-25]. As reported by Caméléna *et al.* (2018), the CIMPlus test used EDTA as an inhibitor for detecting MBL and phenylboronic acid (PBA) as a class A enzyme inhibitor, and this test can detect carbapenemases at 8 and 20 h and identify the type of carbapenemase at 20 h. CIMplus had sensitivities of 95.7 and 97.8% at 8 and 20 h, respectively, and a specificity of 94.4%, and the characterization was correct for 100, 96.9, and 100% of class A, B, and D enzymes, respectively [26]. According to Bianco *et al.* (2019), the direct β-lactamase inactivation method rapidly detects β-lactamase and carbapenemase from positive blood culture bottles within several hours, and it had a sensitivity of 99% and specificity of 100% when detecting KPC, NDM, and oxacillinase-48 enzymes [27].

The performance of rCIM should be further evaluated using more types of carbapenemase and more diverse bacteria species, such as *Acinetobacter* and *Pseudomonas.* Clavulanic acid and tazobactam can effectively inhibit class A carbapenemases, aztreonam has activity against MBL, and cephalosporins and aztreonam can treat infections caused by class D carbapenemase-expressing microbes. Therefore, we can attempt to add EDTA and PBA to identify the type of carbapenemase in the first incubation of rCIM. We can also attempt to directly detect the production of carbapenemase from positive blood culture bottles and provide timely feedback the results to the clinician.

Conclusion

Our research revealed that rCIM is a rapid (2–2.5 h), inexpensive, and reliable

phenotype test that can quantitatively detect all carbapenemases, and its sensitivity and consistency were higher than those of mCIM and CNPt-Direct, making it routinely useful for small microbiology laboratories.

Methods

Retrospective isolates

Two separate cohorts were included, including a retrospective cohort of 55 carbapenem-resistant *Enterobacterales* isolates and a prospective cohort of 48 clinical isolates. The retrospective isolates were obtained from our hospital between January 2016 and September 2018 and previously were molecularly characterized (PCR and gene sequencing) to identify carbapenemase genes. The retrospective collection included 35 CPE isolates carrying the following carbapenemase genes: *blaKlebsiella pneumoniae* carbapenemase (KPC), nine isolates; *bla*New Delhi metallo-β-lactamase (NDM), 23 isolates; *bla*imipenemase (IMP), two isolates; *bla*KPC plus NDM, one isolate; and NPE, 20 isolates. The 55 CRE isolates consisted of *E. coli* (n = 16), *Enterobacter cloacae* (n = 11), *Klebsiella pneumoniae* (n = 22), and *Klebsiella oxytoca* (n = 6).

Prospective isolates

From October 2018 to May 2019, 48 clinical CRE isolates were collected in our hospital for prospective research, including 28 CPE and 20 NPE isolates. The CPE isolates carried the following carbapenemase genotypes: *bla*KPC, 17 isolates; *bla*NDM, eight isolates; *bla*KPC plus NDM, one isolate; and *bla*NDM plus IMP, two isolates. This collection of CRE isolates included the following organisms: *K.*

pneumoniae (n = 32), *E. cloacae* (n = 5), *E. coli* (n = 8), *E. aerogenes* (n = 1), *E. asburiae* (n = 1), and *Proteus mirabilis* (n = 1). All isolates were stored at −80°C, and after thawing, the isolates were subcultured on a blood agar plate before testing. Each isolate was identified via MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany). Carbapenem (imipenem, MEM, and ertapenem) susceptibility testing was performed using the Vitek 2 Compact gram-negative panel (bioMérieux, Marcy l'Étoile, France) and K-B disk diffusion method and interpreted according to the 2018 CLSI guidelines.

rCIM

rCIM was derived from mCIM [16]. Briefly, two 10-µg MEM disks (Oxoid Ltd., Hampshire, United Kingdom) were added to 1 mL of distilled water containing two 10-µL loopfuls of an overnight isolate, incubated for 30 min at 35 ± 2°C, and centrifuged at 10,000 rpm for 5 min (high-mucus isolate with a positive mucus filament test needs to be centrifuged twice). The prepared 2.5 mL of tryptic soy broth (TSB) (Oxoid Ltd.) were taken from a 4°C refrigerator and left at room temperature for 30 min, after which a 1.0 McFarland unit suspension of *E. coli* ATCC 25922 was created. Then, 500 µL of the centrifugal supernatant were added, and the mixture was incubated at 35 ± 2°C for 2 h. The growth of the indicator strain *E. coli* was assessed using a nephelometer (bioMérieux) every 30 min, and the McFarland unit results were recorded after subtracting the baseline. For every experiment, the positive and negative controls were *K. pneumoniae* ATCC BAA-1705 and ATCC BAA-1706, respectively.

Other phenotypic methods

mCIM was performed following the 2018 CLSI guideline [16-17]. A 1-μL loopful of the CRE isolate was suspended in 2 mL of TSB containing a 10-μg MEM disk and incubated for 4 ± 0.25 h at 35 ± 2°C. Subsequently, the MEM disk was removed from the TSB broth at the end of the 4-h incubation using a 10-μL inoculation loop and placed on a Mueller–Hinton agar plate freshly plated with a 0.50 McFarland unit suspension of *E. coli* ATCC 25922. Then, the plate was incubated at 35 ± 2°C for 18–24 h. The mCIM result was interpreted according to the inhibition zone, as shown in Table 1.

The CNPt-Direct test was derived from the Carba NP test [28]. Solution A of the CNPt-Direct contains 100 μL of 0.05% phenol red solution consisting of 0.1 mmol/L $ZnSO_4 \cdot 7H_2O$ and 0.1% (vol/vol) Triton X-100 (Solarbio, Beijing, China), and the pH of solution was adjusted to 7.8 ± 0.1. Solution B contains 100 μL of solution A supplemented with 12 mg/mL intravenous (i.v.) imipenem-cilastatin sodium (Merck Pharmaceutical Co., Ltd., Hangzhou, China) immediately before use. Solutions A and B were added to a 96-well microtiter plate (Biotech Engineering Co., Ltd., Shanghai, China), and a 1-μL loopful of the test isolate was directly suspended in each well. The 96-well microtiter plate was vortexed for 5–10 s and then incubated at 35 ± 2°C for 2 h. All isolates were tested in triplicate, and the test was repeated in the case of discrepant results. A color change from red to orange/yellow in the solution B reaction well was interpreted as a positive result. Two independent technicians interpreted the results. In each test, *K. pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 were

used as the positive and negative controls, respectively.

PCR detection of carbapenemase genes

In all retrospective and prospective isolates, DNA was extracted using a bacterial DNA extraction kit (Biotech Engineering Co.), and primers were designed to detect the carbapenemase genes (*bla*KPC, *bla*NDM, *bla*IMP, *bla*OXA−48−like, and *bla*VIM), as shown in Table 2. Carbapenemase gene amplification was examined using a PCR kit (Biotech Engineering Co.) with PCR amplification equipment (Hongshi Medical Technology Co., Ltd., Shanghai, China). Briefly, amplification was performed using a protocol consisting of initial denaturation at 94°C for 5 min followed by 36 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, and final elongation at 72°C for 10 min. The PCR products were electrophoresed on a 2% agarose gel at a voltage of 100 for 1 h using an electrophoresis set (Junyi Dongfang Electrophoresis Equipment Co., Ltd., Beijing, China), and the results were read on a gel imager (Junyi Dongfang Electrophoresis Equipment Co., Ltd.). PCR products were selected and sent to an outside company (Biotech Engineering Co., Ltd.) for sequencing, and sequences were aligned using the BLAST software tool.

Statistical analysis

The sensitivity, specificity, and positive and negative predictive values of rCIM and other phenotypic tests were calculated using SPSS 22.0 software, and these results were analyzed using the kappa value (κ) in comparison with the sequencing results. $\kappa > 0.75$ indicated that the two methods are in good agreement, $\kappa = 0.4–0.75$ indicated

moderate agreement, and $\kappa < 0.4$ indicated poor agreement.

Abbreviations

PCR: Polymerase Chain Reaction; CLSI: Clinical and Laboratory Standards Institute; CPE: carbapenemase-producing *Enterobacterales*; CRE: carbapenem-resistant *Enterobacterales*; mCIM: modified carbapenem inactivation method; rCIM: rapid carbapenem inactivation method; ESBL: extended-spectrum β-lactamase; NPE: non-carbapenemase-producing *Enterobacterales*

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Authors' contributions

Conceived and designed the study: NW and YL. Conducted the experiments and analyzed the results: NW, YL, LL, and JL. Drafted the manuscript: YL and NW. All authors contributed to manuscript revision, read and approved the submitted version.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Interpretation of the modified carbapenem inactivation method (mCIM) result

		Zone Size	
mCIM	6 to 15mm or 16 to 18mm with pinpoint colonies in the zone of growth inhibition	16 to18mm or ≥19 mm with pinpoint colonies in the zone of growth inhibition	≥19 mm

Interpretation	Positive (carbapenemase producer)	Indeterminate	Negative (non-carbapenemase producer)

413 **Table 2.** Primers for the detection of carbapenemase-producing *Enterobacterales*

Carbapenemase gene	Primer sequences[a] (5'-3')	Amplicon size (bp)	Reference
blaKPC	CGTCTAGTTCTGCTGTCTTG	798	[29]
	CTTGTCATCCTTGTTAGGCG	232	
blaNDM	GGTTTGGCGATCTGGTTTTC	621	[29]
	CGGAATGGCTCATCACGATC		
blaIMP	GGAATAGAGTGGCTTAAYTCTC	232	[29]
	GGTTTAAYAAAACAACCACC		
blaOXA-48-like	GCGTGGTTAAGGATGAACAC	438	[29]
	CATCAAGTTCAACCCAACCG		
blaVIM	ATCGCAACGCAGTCGTTTGA	318	Corporate
	CCCGCTCGATGAGAGTCCTT		synthesis

414 [a]The first and second primers for each gene are the forward and reverse primers, respectively. KPC, *Klebsiella*
415 *pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; IMP, imipenemase; OXA-48-like,
416 Oxacillinase-48-like; VIM, Verona integron-encoded metallo-β-lactamase.

417 **Table 3.** Results of rCIM, mCIM, and CNPt-Direct for 55 CRE isolates in the
418 retrospective analysis

Organism (n)	Species	No. of	rCIM	mCIM	CNPt-Direct

		solates			
carbapenemase producer (35)					
Class A (9)					
KPC (9)	*K. pneumoniae*	9	+	8/9+	8/9+
Class B (25)					
NDM (23)	*E. coli*	8	+	7/8+	+
	E. cloacae	10	9/10+	9/10+	+
	K. pneumoniae	2	+	+	+
	K. oxytoca	3	+	+	+
IMP (2)	*K. oxytoca*	2	+	+	1/2+
Coproducers (1)					
KPC-NDM (1)	*K. pneumoniae*	1	+	+	+
non-carbapenemase producer (20)					
	E. coli	8	-	-	-
	E. cloacae	1	-	-	-
	K. pneumoniae	10	-	-	-
	K. oxytoca	1	-	-	-

419 rCIM, rapid carbapenem inactivation method; mCIM, modified carbapenem inactivation method; CRE,
420 carbapenem-resistant *Enterobacterales*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi
421 metallo-β-lactamase; IMP, imipenemase; +, positive; −, negative.

422 **Table 4.** Results of rCIM, mCIM, and CNPt-Direct for 48 CRE isolates in the
423 prospective evaluation

Organism (n)	Species	No. of solates	rCIM	mCIM	CNPt-Direct
carbapenemase producer (28)					
Class A (17)					
KPC (17)	*K. pneumoniae*	16	+	14/16+	14/16+
	E. coli	1	+	-	-
Class B (8)					
NDM (8)	*E. coli*	1	+	-	+
	E. cloacae	1	+	-	+
	K. pneumoniae	4	+	3/4+	+
	P. mirabilis	1	-	-	-
	E. aerogenes	1	+	-	-
Coproducers (3)					
KPC-NDM (1)	*E. cloacae*	1	+	+	+
NDM-IMP (2)	*E. cloacae*	2	+	-	+
non-carbapenemase producer (20)					
	E. coli	6	-	-	-
	E. cloacae	1	-	-	-
	K. pneumoniae	12	11/12-	11/12-	11/12-
	E. asburiae	1	-	-	-

424 rCIM, rapid carbapenem inactivation method; mCIM, modified carbapenem inactivation method; CRE,

425 carbapenem-resistant *Enterobacterales*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi

426 metallo-β-lactamase; IMP, imipenemase; +, positive; −, negative

427 **Fig. 1. The growth of the indicator strain *Escherichia coli* ATCC 25922 in the**
428 **retrospective analysis.** The box plot showing the growth of *E. coli* ATCC 25922 at
429 1.5 and 2 h in the retrospective analysis (n = 55). The hollow circles represent the
430 outliers. The horizontal black line at 0.50 McFarland units represents the positive
431 cut-off value. The results are presented as baseline-subtracted McFarland units, and
432 measurements were performed every 30 min.

433 **Fig. 2. The growth of the indicator strain *Escherichia coli* ATCC 25922 in the**
434 **prospective evaluation.** A box plot presenting the growth of *E. coli* ATCC 25922 at
435 1.5 and 2 h in the prospective evaluation (n = 48). The hollow circles represent the
436 outliers, and the asterisks indicate extreme values. The horizontal black line at 0.50
437 McFarland units represents the positive cut-off value. The results are presented as
438 baseline-subtracted McFarland units, and measurements were performed every 30
439 min.