CORRESPONDENCE

Comparison of the carbapenem inactivation method (CIM) and modified carbapenem inactivation method (mCIM) for the detection of carbapenemase-producing organisms

Sir,

The phenotypic detection of carbapenemase-producing organisms (CPO) is an important adjunct to genotypic detection methods.¹ Phenotypic methods such as the well described Carba-NP test are utilised in diagnostic laboratories that do not have access to more expensive gene detection platforms, and for the detection of CPO harbouring novel resistance genes.² The Carbapenem Inactivation Method (CIM) was first described by Van der Zwaluw *et al.* in 2015 and detects the presence of carbapenemase activity in bacteria via the hydrolysis of meropenem.³ However when the Clinical and Laboratory Standards Institute (CLSI) updated its performance standard in 2017, a modified version of this test was recommended: the modified CIM (mCIM).⁴ The CIM and mCIM offer several advantages over other phenotypic detection methods (e.g., Carba-NP): they are simple to perform, use readily available, inexpensive consumables and do not have a subjective colorimetric endpoint.^{4,5} To date, there has been extensive evaluation of the CIM,^{6–10} limited published studies evaluating the mCIM test,¹¹ and only one head-to-head comparison of the two methods.¹² The aim of our study was to directly compare the performance of the mCIM and CIM in the setting of a local *bla*_{KPC} outbreak, previously described by Chang *et al.*,¹³ and then by using a broader range of reference laboratory acquired isolates.

During the previously described *bla*_{KPC} outbreak,¹³ 67 isolates with an elevated minimum inhibitory concentration (MIC) to meropenem or resistance to multiple antibiotic classes were referred from St Vincent's Pathology to the Microbiological Diagnostic Unit (MDU) for molecular characterisation. Forty-seven of these isolates were found to harbour a carbapenemase gene. Table 1 provides details of these isolates and an additional 28 isolates obtained from reference laboratories in Victoria and South Australia. The additional 28 (reference laboratory) isolates included 20 CPOs (predominantly *bla*_{OXA} and *bla*_{VIM}) which were selected to compliment the range of carbapenemase producing isolates in the St Vincent's Pathology set and a further eight isolates with multiple antibiotic class resistance but no carbapenemase gene. The combined isolate sets included 67 CPOs and 29 non-CPOs. The non-CPO isolates were selected for inclusion as they had been referred to a reference laboratory after displaying resistance to multiple classes of antibiotics or a meropenem MIC ≥ 0.5 $\mu\text{g/mL}$ on initial Vitek (bioMérieux, France) antimicrobial susceptibility testing. All 29 non-CPO isolates therefore had been subjected to an in-house broad range polymerase chain reaction (PCR) for carbapenemase genes and had no carbapenemase gene detected. All non-CPOs had subsequent antimicrobial susceptibility performed by E-test (bioMérieux); 11 isolates had a meropenem MIC ≤ 2 $\mu\text{g/mL}$ and 18 isolates had a meropenem

MIC > 2 $\mu\text{g/mL}$ (range 0.125 $\mu\text{g/mL}$ to > 32 $\mu\text{g/mL}$). A head-to-head comparison of the CIM and mCIM was performed using all 96 isolates (67 carbapenemase-producing isolates and 29 non-carbapenemase producing isolates). The 67 St Vincent's Pathology isolates were subcultured from original isolates stored in glycerol at -70°C . Isolates were subcultured onto MacConkey agar for determination of purity and then a repeat subculture was made on Columbia agar with 5% sheep blood (SBA) for testing. Isolate identification was confirmed by MALDI-TOF MS (Bruker Daltonics, Germany) and repetitive subculturing of isolates was avoided to prevent resistance gene dropout. The provenance and number of passages of the reference laboratory isolates was unknown. The CIM was performed according to the original method as described by Van der Zwaluw *et al.*³ In brief, using a 10 μL loop the test isolate was suspended in 400 μL of sterile water in an Eppendorf tube. The suspension was vortexed for 10–15 seconds, after which a 10 μg meropenem antibiotic disk (Oxoid, UK) was added to the tube. The Eppendorf tube was then incubated in ambient air at 35 – 37°C . After 2 h incubation the meropenem disk was carefully removed from the Eppendorf tube with sterile forceps, squeezed gently to remove excess moisture and placed on Mueller–Hinton agar (Oxoid) pre-inoculated with a lawn of susceptible *Escherichia coli* (ATCC strain 25922). After 12–18 h incubation in ambient air at 35 – 37°C the zone of inhibition around the meropenem disk was measured using Vernier callipers. Results were confirmed by a second reader. Both readers were blinded to the

Table 1 Carbapenemase-producing isolates and non-carbapenemase-producing isolates subjected to the CIM and mCIM test

Organism	Resistance gene ^a	No. isolates	No. positive CIM	mCIM
Carbapenemase-producing isolates				
Ambler Class A	<i>KPC-2</i>	25	25	25
	<i>KPC-3</i>	1	1	1
Ambler Class B	<i>NDM-1</i>	2	2	2
	<i>NDM-4</i>	1	1	1
	<i>NDM-5</i>	10	10	9
	<i>NDM-7</i>	2	2	2
	<i>VIM-1</i>	1	0	0
	<i>VIM-2</i>	1	1	0
	<i>VIM-5</i>	1	0	0
	<i>IMP-1</i>	1	1	1
	<i>IMP-4</i>	1	1	1
	<i>IMP-14</i>	2	2	2
Ambler Class D	<i>OXA-48</i>	5	5	4
	<i>OXA-23, OXA-51</i>	1	1	0
	<i>OXA-24, OXA-40</i>	1	0	0
	<i>OXA-181</i>	6	6	5
Mixed Ambler Class	<i>NDM-5, OXA-232</i>	5	5	5
	<i>NDM-5, OXA-232, VIM-2</i>	1	1	1
Total		67	64	59
Non-carbapenemase producing isolates				
Ambler Class A (non-carbapenemase)	<i>blaCIT</i>	2	0	0
	<i>blaDHA</i>	2	0	0
Non-β-lactamase method of resistance	<i>nd</i>	25	1	1
Total		29	1	1

^a As determined by reference laboratory PCR.

carbapenemase status of the isolates. Carbapenemase production was recorded as present when there was no zone of inhibition. A positive control (*Klebsiella pneumoniae* ATCC BAA-1705) and a negative control (*Klebsiella pneumoniae* ATCC BAA-1706) were tested in parallel with the isolates. Isolates subsequently found to have produced false negative results were retested under the same conditions once and then subsequently using a longer vortexing time (2 min) and a disk incubation period of 6 h. The mCIM was performed according to the method described in CLSI M100, Performance Standards for Antimicrobial Susceptibility Testing.⁴ In brief, a 1 µL loop of test isolate was suspended in 2 mL of Trypticase Soy Broth (TSB) in a test tube. The test tube was then vortexed for 10–15 seconds after which time a 10 µg meropenem antibiotic disk (Oxoid) was added to the tube. The test tube was incubated in ambient air for 4 h at 35–37°C. After 4 h incubation, the meropenem disk was carefully removed with sterile forceps, squeezing excess moisture from the disk. The 10 µg meropenem disk was then applied to a Mueller–Hinton agar (Oxoid) plate pre-inoculated with a lawn of susceptible *Escherichia coli* (ATCC 29522). After incubation in ambient air at 35–37°C for 18–24 h the zone of inhibition was read using Vernier callipers. Results were confirmed by a second reader. Both readers were blinded to the carbapenemase status of the isolates. As per the CLSI standard, a carbapenemase positive result was recorded for any isolate exhibiting a zone of inhibition of 6–15 mm or presence of growth within a zone of 16–18 mm. If the zone of inhibition was 19 mm or more the isolate was determined to be carbapenemase negative. For isolates with a zone size of 16–18 mm the carbapenemase status was recorded as indeterminate. A positive control (*Klebsiella pneumoniae* ATCC BAA-1705) and a negative control (*Klebsiella pneumoniae* ATCC BAA-1706) were tested in parallel. Isolates producing false negative results were retested under the same conditions and then subsequently using a longer vortexing time (2 min) and a disk incubation period of 6 h.

Evaluated against the 67 St Vincent's Pathology isolates referred to MDU, the CIM test and mCIM both produced a false negative result for one *Klebsiella pneumoniae bla_{VIM-5}*. However, in addition to this isolate the mCIM gave false negative results for a *Acinetobacter baumannii bla_{OXA-23/OXA-51}* and *Escherichia coli bla_{NDM-5}* (Table 1). The CIM test and the mCIM test both produced a false positive result for an *Enterobacter cloacae* that harboured no resistance genes by reference laboratory PCR. The sensitivity of the CIM test was 97.9% and the sensitivity of the mCIM test was 93.6%. The specificity of both tests was 96.5%. Evaluated against the combined sets of isolates (Table 1) the CIM test produced a further two false negative results (*Klebsiella pneumoniae bla_{VIM-1}*, *Acinetobacter baumannii bla_{OXA-24/40}*) and the mCIM produced a further three false negative results (the *Klebsiella pneumoniae bla_{VIM-1}* and *Acinetobacter baumannii bla_{OXA-24/40}* mentioned above and a *Pseudomonas aeruginosa bla_{VIM-2}*) and two indeterminate results (*Klebsiella pneumoniae bla_{OXA-181}*, *Klebsiella pneumoniae bla_{OXA-48}*), reducing the sensitivity to 95.5% and 88.0%, respectively. The specificity for both tests remained unchanged. Modifying the CIM by increasing the vortexing time to 2 min and prolonging the disk incubation time to 6 h resulted in one less false negative result (*Acinetobacter baumannii bla_{OXA-24/40}*). Increasing the vortexing time to 2 min and prolonging the disk incubation time to 6 h for the

mCIM resulted in one less negative result (*Pseudomonas aeruginosa bla_{VIM-2}*) and the two indeterminate results became clearly positive (*Klebsiella pneumoniae bla_{OXA-181}*, *Klebsiella pneumoniae bla_{OXA-48}*).

Tested against isolates obtained during enhanced surveillance for a local *bla_{KPC-2}* outbreak, the CIM offered superior test performance (sensitivity 97.9%, specificity 96.5%). When challenged against a larger set of *bla_{OXA}* producing isolates the sensitivity of the CIM test decreased to 95.5%. Our study has some limitations that warrant discussion. Having been performed in the setting of a local outbreak 38% of the isolates tested were *bla_{KPC}* harbouring Enterobacteriaceae. This is significant as isolates harbouring these resistance genes are very strong carbapenemase producers and are more readily detected than *bla_{IMP}*, *bla_{VIM}* and *bla_{OXA}* harbouring isolates. Having noted this, Miller *et al.*,¹² the only study to demonstrate superiority of the mCIM test, evaluated a set of isolates of which over 50% harboured *bla_{KPC}*. We attempted to correct this bias by obtaining isolates with different Ambler class carbapenemase genes from two reference laboratories, however *bla_{IMP}* and *bla_{VIM}* harbouring isolates and non-Enterobacteriaceae remained underrepresented. Additionally, we did not test any isolates containing the less abundant *bla_{GES}* and *bla_{SME}* carbapenemases. We found that increasing the vortexing and incubation time resulted in detection of weak carbapenemase harbouring isolates that initially tested negative, however as this methodology was not applied to all isolates we could not be certain that this would result in an overall improvement in test performance. Further studies should specifically address this issue.

The CIM and mCIM are effective, simple and cost effective methods for the phenotypic detection of CPO. Both methods are easy to follow and utilise materials readily available in most diagnostic microbiology laboratories. In our hands the CIM test demonstrated superior sensitivity when directly compared to the CLSI recommended mCIM test and took less time to perform. We propose that further head-to-head studies are needed to compare the CIM and mCIM.

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Low additional yield from prolonged culture of urine and differential detection of *Actinotignum schaalii* in a private laboratory setting

Sir,

Sterile pyuria in the elderly is regularly seen and may represent many aetiologies, including infection with fastidious organisms, such as *Actinotignum schaalii* (formerly *Actinobaculum schaalii*).

First described in 1997,¹ *A. schaalii* is an increasingly described human pathogen and may easily be overlooked in the high-throughput laboratory due to its slow growth, capnophilic nature and poor growth on MacConkey agar and chromogenic media commonly used for routine urine culture. *Actinotignum schaalii* is also noted to be resistant to agents used for the empiric treatment of urinary tract infections (UTI), namely trimethoprim and fluoroquinolones,² and therefore should be considered when UTI is likely, if routine culture fails to isolate a typical uropathogen, and empiric therapy fails to improve symptoms.

We noted with interest the article by Stevens and Taylor published in the June 2016 issue of *Pathology*,³ in which they

described the isolation and identification of urinary isolates of *A. schaalii* from three Sydney hospitals accounting for approximately 1000 beds. In particular, 20 isolates of *A. schaalii* were detected within the 18-month study period (between May 2012 and November 2013), during which time a total of 59,500 urines were processed (a rate of ~1/3000 compared to a rate of ~1/12 growing *Escherichia coli*). In contrast, our laboratory, which predominantly serves doctors treating patients who are generally well enough to remain in the community and who do not require hospital attendance, reported only 14 isolates of *A. schaalii* in the period from 2012–2016, a rate of 1/195,000 compared to a rate of ~1/9 for *E. coli* (for the year 2015).

Because of this apparent difference in detection of *A. schaalii*, we sought to evaluate the method described for processing urines by Stevens and Taylor on our subset of elderly patients with sterile pyuria with the hope of enhancing detection of fastidious uropathogens, particularly *A. schaalii*.

During the study period in March 2017 we received 16,707 urines which were initially cultured as per laboratory protocol, onto horse-blood agar (HBA) and 'Brilliance UTI' chromogenic agar (Oxoid, UK), and incubated overnight (≥ 16 h) in room air. For the purposes of our evaluation, cultures from patients ≥ 65 years old were selected after meeting the criteria of no growth on primary plates after overnight incubation, associated with a urine white cell count $\geq 50 \times 10^6/L$. Using these criteria, 208 patients (1.2%) were included in our evaluation, comprising 85 (41%) males and 123 females (59%). These specimens were recultured on day 2 onto HBA and chocolate agar (CA) and incubated in 5% CO₂ at 35°C in parallel with the original cultures, for a total of 72 h. Plates were compared daily for growth. Colonies morphologically suspected to be *A. schaalii* were evaluated for concordance with expected phenotypic characteristics (irregular Gram positive bacillus on Gram stain, oxidase/catalase/urease negative and susceptible to penicillin, resistant to trimethoprim and quinolones). Several isolates of *A. schaalii* which had previously been isolated at our laboratory and confirmed via 16S rRNA sequencing were available for morphological and phenotypic comparison throughout the study. Non-*Actinotignum* organisms were identified using a combination of routine phenotypic tests, biochemical testing (Vitek 2; bioMérieux, France), and matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF; Vitek MS; bioMérieux). Isolates were deemed to be 'mixed growth' and received no further work-up if they demonstrated ≥ 3 morphologically distinct organisms. True sterile pyuria was defined as no growth on all plates after 72 h of incubation with enriched atmosphere. Additionally, a screen for urine antimicrobial activity was performed by streaking urine onto plates of a known pan-susceptible *E. coli*; these were examined after overnight incubation for significant growth inhibition.

Of the 208 specimens, the majority (116, 56%) demonstrated mixed growth of ≥ 3 organisms. Only 68 specimens (33%) demonstrated true sterile pyuria, with no growth using either fastidious or typical culture conditions. Twenty-four patients (12%) demonstrated pure growth of a single organism using the fastidious culture method, but of these only seven (3%) were considered possible uropathogens in the absence of obvious specimen contamination (Table 1), with all but one evident at 48 h. Incubation in 5% CO₂ did not