Laboratory detection of acquired carbapenemases – evaluation of phenotypic methods and a commercial molecular assay.

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Introduction

- The worldwide dissemination of extended-spectrum-ß-lactamase (ESBL) producing Enterobacteriaceae has resulted in greater dependence on carbapenems for the treatment of infections by these organisms.
- The emergence of acquired carbapenemases in such isolates are of major concern as these confer resistance to most β-lactams and are usually associated with other non-β-lactam resistance determinants, leaving limited treatment options.
- Early detection of these isolates is beneficial for appropriate patient management and for infection control purposes.
- In New Zealand, the isolation of carbapenemase-producing *Enterobacteriaceae* (CPE) is relatively infrequent and all CPE have been isolated from patients returning from overseas.[1] Currently we screen isolates that have reduced susceptibility to the carbapenems by the Modified Hodge Test (MHT) and detection of metallo-β-lactamases (MBLs) using the chelating agents dipicolinic acid (DPA) and ethylenediaminetetraacetic acid (EDTA). Confirmation requires referral to a reference laboratory.
- Recently, a number of phenotypic and molecular tests have become available, including several chromogenic agars, biochemical tests and commercial molecular systems.

Objective

- To evaluate and compare phenotypic and molecular methods for the detection of carbapenemases in *Enterobacteriaceae*.

Method

- 30 *Enterobacteriaceae* clinical isolates, including 12 characterised strains were tested in a blinded fashion. Identification was confirmed by MALDI-TOF MS (bioMérieux). All isolates had reduced susceptibility to ertapenem and/or the VITEK 2 XL (bioMérieux) indicated the presence of a carbapenemase phenotype.
- The 12 strains had been characterized by the National Reference Laboratory, ESR. see Table 1.

Phenotypic Tests

- The Modified Hodge Test (MHT) was carried out according to CLSI M100-S24 Guidelines.
- Detection of MBL using chelating agents DPA and EDTA available from Rosco Diagnostica.
- Two chromogenic screening agars:
  Brilliance™ CRE Agar (Oxoid)
  chromID® CARBA SMART Agar (bioMérieux)
- Three Biochemical Tests:
  Neo-Rapid CARB DIATABS™ (Rosco Diagnostica) – commercial assay.
  Carba NP – in-house test with phenol red solution used as the colour indicator.[2, 3]
  Blue-Carba – in-house variant of the Carba NP with bromothymol blue selected as the indicator of acidification.[4]
- Cepheid GeneXpert CARBA-R® Assay (Carba-R Assay) – Rapid detection and differentiation of the blaKPC, blaNDM, blaVIM, blaOXA-48 and blaIMP-1 gene sequences in Gram-negative bacteria.

Results

Table 1

			Chromogenic media			Biochemical tests			Molecular test
Carbapenemase producers n=12	*MHT*[a]	*MBL (DPA/EDTA)*	*Brilliance CRE*	*CARBA SMART CARBA*	*CARBA SMART OXA*	*Neo-Rapid DIATABS*	*Carba NP*	*Blue-Carba*	*Xpert Carba-R*
KPC (1) K.pneumoniae (1)	1	0	1	1	0	1	1	1	1
NDM-1 (6) E.coli (3), P.mirabilis (1), K.pneumoniae (2)	5	6	6	5	0	4	5	6	6
IMP (1) C.freundii (1)	1	1	1	1	0	1	1	1	1
IMP-27 (1) P.mirabilis (1)	0	1	0	0	0	0	0	1	0
OXA-181 (3) E.coli (2), K.pneumoniae (1)	3	0	3	(1[b])	3	0	0	0	0
Non-carbapenemase producers n=18									
C.freundii (1)	1	0	0	0	0	0	0	0	0
E.cloacae (6)	1	1	4	2	0	0	0	0	0
E.aerogenes (2)	2	0	0	0	0	0	0	0	0
S.marscecens (1)	0	0	1	0	0	0	0	0	0
E.coli (2)	0	0	1	2	0	0	0	0	0
K.pneumoniae (6)	0	0	5	2	0	0	0	0	0
Total # carbapenemase producers 12	**10/12**	**8/12**	**11/12**	**10/12**		**6/12**	**7/12**	**9/12**	**8/12**
Total # non-carbapenemase producers 18	**4/18**	**1/18**	**11/18**	**6/18**		**0/18**	**0/18**	**0/18**	**0/18**
Sensitivity %	83	67	92	83		50	58	75	67
Specificity %	78	94	39	67		100	100	100	100
PPV %	71	89	50	63		100	100	100	100
NPV %	88	81	88	86		75	78	86	82

[a] = includes weak positive results
[b] = 1 isolate grew on both CARBA and OXA media

- The MHT detected 10 (83%) CPE producing isolates, however 2 carbapenemase producing *P.mirabilis* gave indeterminate results. Four false positives (22%) were observed amongst the ESCHaPPM isolates with chromosomal AmpC.
- DPA and EDTA detected all the MBL isolates but yielded 1 false positive result.
- *Brilliance*™ CRE and chromID® CARBA SMART
  Brilliance™ CRE grew 11 (92%) of the CPE isolates.
  chromID® CARBA SMART gave similar sensitivity (83%). The bi-plate allows screening for CPE on one half and for OXA-48-like producers on the other, improving the specificity.
- Neo-Rapid CARB DIATABS™, Carba NP and Blue-Carba
  The Blue-Carba had the highest sensitivity of the three tests. However all 3 failed to detect the OXA-181 isolates. When comparing both in-house assays, the Blue-Carba was the easier to perform.
  The latest CLSI M100-S25 has introduced the Carba NP as a confirmatory test for suspected carbapenemase production in *Enterobacteriaceae*.[5]
- Xpert CARBA-R.
  Xpert CARBA-R detected all NDM-1, KPC, VIM-2 and IMP-1 isolates but its target was limited to OXA-48 and IMP-1.

Conclusion

- For *Enterobacteriaceae*, the Blue-Carba is an easy, rapid, inexpensive biochemical test for detection of diverse carbapenemase producers from bacterial cultures. However it failed to detect OXA-181 isolates.
- The Brilliance™ CRE and the chromID® CARBA SMART media detected the OXA-181 isolates. Either media in combination with the Xpert CARBA-R or the Blue-Carba would provide good sensitivity.
- Falsely positive MHT can occur with high-level AmpC producers and strains producing CTX-M.[6,7]
- At present, there isn't a single "best" detection method.[8]

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