Evaluating Four Commercial Tests to Detect Carbapenemase in Enterobacteriaceae

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AIM

To examine the performance of four commercial tests to determine an improved and cost-effective way to allow for in-house confirmation of Carbapenemase activity in Enterobacteriaceae. The assays will include PCR (Xpert® Carbra-R), disc diffusion (MASTDISCS® combi Carba Plus D73C), lateral flow (NG-CARBA5) and a paper test device (MAST® ICT—Indirect Carbapenemase Test).

BACKGROUND

Carbapenemase refers to β-lactamases that hydrolyse carbapenem. They work by binding to penicillin-binding proteins and preventing cell wall synthesis. There are many clinical connections concerning carbapenem resistance as they are used to treat infections caused by multi-drug resistant bacteria. They also have a selective advantage in hospital settings (1) therefore screening and treatment is important for infection control. Carbapenemases are commonly hosted by Enterobacteriaceae (namely Klebsiella sp. and Escherichia. coli) and are known as Carbapenemase-producing Enterobacteriaceae (CPE). There are three main classes of Carbapenemases and they are distinct in their molecular features (1).

RESULTS

The evaluation showed that all four commercial tests were reliable in identifying Carbapenemases in unknown CPE isolates and have potential to be incorporated into the screening process (Table 3). Caution must be taken due to the small sample size and the inability to test negatives to eliminate the possibility of false positives. Other evaluation research is available and can substantiate our claims. The Associated French National Reference Centre assessed over 296 isolates and showed a sensitivity of 100% and specificity 100% for the NG-Carba 5 (2). While another study evaluating MAST® ICT showed 100% sensitivity for CPEs but this can drop to 70.3% due to its ability to detect non-carbapenemases confirmation (3).

METHOD

21 isolates included in this study comprised of 5 NCTC control organisms containing the 5 main Carbapenemases, 16 unknown isolates and a patient sample. All isolates except for the patient sample were cultures from ~80°C freezer storage onto blood agar. The patient sample was taken from Muller-Hinton incubated for 18 hours. Each kit were tested and interpreted as per manufacturer's instructions (Table 2).

Table 2: Methods for each of the four tests used

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Image of Test</th>
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<tbody>
<tr>
<td>NG Carba 5 (11 tests carried out including controls)</td>
<td>A colony from blood agar was suspended in buffer dilution and 100ul used in the cassette and interpreted after 15 minutes.</td>
<td></td>
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<tr>
<td>MASTDISCS® combi Carba plus D73C (10 tests carried out including controls)</td>
<td>0.5 McF is placed into Muller Hinton with 5 antibiotic discs placed prior to incubation for 24 hours.</td>
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<tr>
<td>MAST® ICT—Indirect Carbapenemase Test (4 tests carried out)</td>
<td>0.5 McF of reporter organism (E. coli ATCC 25322) was spread onto Muller Hinton. Control organism (negative) was placed on the underside of number 4 and test organism on the underside of number 3.</td>
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<tr>
<td>Xpert® Carba-R (10 tests carried out including controls)</td>
<td>A 10 µl aliquot of a 0.5 McF of cell suspension was diluted 1 in 2 in 20µl of PBS, 10ul of this dilution was mixed into a well of sample reservoir approximately 1.7 µl was added to the Xpert® Carba-R assay cartridge.</td>
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Prior to this study all samples that were deemed to be presumptive CPE by BD Phoenix had their carbapenem resistance confirmed by MIC and were then sent to Public Health England to determine the presence and type of Carbapenemase present, the process taking 11-15 days.

NG-CARBA 5 and MASTDISCS® combi were unable to detect a CPE gene in PMK32. This was due to the Carbapenemase present not being one of the main 5. This would be a problem if new and emerging Carbapenemases were to breakthrough into the UK.

CONCLUSION

Following the evaluation this project there has been a change in procedure at Microbiology. After presenting the work to the senior staff and medical team it was decided that the MAST® ICT would be implemented as an initial screening following isolation of a presumptive CPE. Alongside the ICT Meropenem and Ertapenem MICs will also be setup. If the ICT is positive, on the same day a NG-Carba 5 test can be setup and the type of Carbapenemase will be reported within 15 minutes. Any presumptive CPE that is positive by the ICT but negative on the lateral flow will be sent to the reference lab to capture any potential emerging Carbapenemases. This reduces turn around time from 11-15 day to 72-96 hours impacting patient care as novel treatment and drug combinations can be delivered sooner and Infection Control measures can be implemented.

REFERENCES