Rapid detection of OXA-48, KPC and NDM-type carbapenemase producing organisms (CPOs) using the RESIST-3 O.K.N. assay

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BACKGROUND

Carbapenemase producing organisms (CPOs) are multi-drug resistant and have been shown to be associated with increased morbidity and mortality. Rapid detection can guide clinical management and allow implementation of appropriate infection control procedures.

The Royal Free London NHS Foundation Trust is a tertiary referral centre with a diverse, international patient population.

In April 2013, The Royal Free London NHS Foundation Trust implemented a CPO screening programme of selected universal screening in some areas (intensive care, private, renal and liver units), together with risk factor based screening in others (Haematology, oncology, stroke and infectious disease units). In addition, any patients identified with risk factors for CPO and contacts of positive cases are screened.

We most commonly detect genotype OXA-48, followed by NDM, VIM, KPC and OXA-23, respectively. In addition, we have occasionally isolated more unusual types including IMP, IMI and GES-5.

CPOs are difficult to detect in diagnostic laboratories because of the many different genotypes which can be associated with a wide range of phenotypes. Standardised methodology has not been established:

• Culture based methods are often cost effective and easy to implement, but can lack specificity (particularly for OXA-48 detection) and are often slow and laborious.

• Molecular based detection methods are rapid, but most assays available to diagnostic laboratories are not able to detect the large number of genotypes present worldwide; therefore only those deemed to be the most relevant are detected.

• Methods based on the hydrolysis of a carbapenem and the subsequent detection of hydrolysis products are rapid, cost effective and, in theory, have the ability to detect positive isolates regardless of the genotype present. However, there is debate over the most suitable carbapenem for use as the substrate, and assays have been linked with poor sensitivity for some carbapenemase types (particularly OXA-48).

The RESIST-3 O.K.N. is a lateral flow immunochromatography assay (Coris BioConcept) that detects OXA-48, KPC and NDM type carbapenemases from-isolate within 20 minutes.

AIM

To evaluate the RESIST-3 O.K.N. assay for use in the routine diagnostic laboratory for the detection of CPOs

METHOD

100 multi-drug resistant isolates were tested by RESIST-3 O.K.N. assay.

Isolates included 95 clinical isolates (22 NDM [10 E. coli, 7 Klebsiella spp., 2 A. baumannii, 1 P. aeruginosa, 1 C. freundii and 1 Enterobacter spp.], 24 OXA-48 [11 E. coli, 9 Klebsiella spp., 2 Enterobacter spp., 2 Serratia spp.], 19 VIM [16 P. aeruginosa, 2 Providencia spp., 1 E. coli], 4 OXA-23 [4 Acinetobacter spp.], 3 KPC [3 Klebsiella spp.], 4 NDM + OXA-48 [3 Klebsiella spp., 1 E. coli], 1 OXA23 + NDM [A. baumannii], 1 IMI [E. aeruginosa], 1 IMP [P. aeruginosa], 9 ESBL [7 E. coli, 2 K. pneumoniae], 3 derepressed AmpC [3 Enterobacter spp.], 4 inducible AmpC [1 K. oxytoca, 1 E. cloacae, 1 C. freundii, 1 S. marcescens]) and 5 control organisms for confirmation of CPO status.

All clinical isolates were sent to the Reference Laboratory (AMRRAI, PHE) for confirmation of CPO status.

Isolates were tested by the RESIST-3 O.K.N. following manufacturer’s instructions; - In brief, bacterial colonies from an overnight subculture on CLED agar were suspended in 10 drops of LY-A buffer, which was mixed to homogenize the solution. 3 drops of the bacterial suspension was added to the sample well of the cassette and results were read after 15 min.

REFERENCES/CONTACT

2. CarbaNP Product insert, BioMerieux
3. Contact: gemma.vanstone@nhs.net

RESULTS

The RESIST-3 O.K.N. assay correctly identified 28/28 OXA-48 (100% Sensitivity [87.7 – 100.0 CI], 100% specificity [94.6 – 100 CI]), 26/27 NDM (96.3% Sensitivity [81.0 – 99.9 CI], 100% Specificity [94.7 – 100.0 CI]), 3/3 KPC (100% Sensitivity [29.2 – 100 CI], 100% Specificity [96.0 – 100.0 CI]) positive isolates (Table 1).

Results were available within 20 minutes, with less than 2 minutes hands-on-time.

The strips were easy to read: NDM positive results were often weaker than OXA-48 and KPC results.

The assay was able to detect isolates carrying multiple carbapenemase types; the four isolates positive for OXA-48 and NDM included in this study, were all correctly identified as OXA-48 and NDM positive.

The false negative result obtained was from a NDM positive K. pneumoniae isolate that was highly mucoid (Figure 2).

No false positive results were obtained from either carbapenemase types not identified by the assay, or from carbapenemase-negative MDR organisms included in the study.

The correct result was obtained for the 5 control isolates included in the study.

<table>
<thead>
<tr>
<th>OXA-48</th>
<th>KPC</th>
<th>NDM</th>
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<tbody>
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</tr>
<tr>
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</tr>
<tr>
<td>Total</td>
<td>28</td>
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Table 1: OXA-48, KPC and NDM results obtained from the RESIST-3 O.K.N.

Figure 1: Examples of OXA-48, KPC, NDM and Negative results using the RESIST-3 O.K.N.

Figure 2: Mucoid NDM positive K. pneumoniae isolate that was negative by RESIST-3 O.K.N.

CONCLUSIONS

The RESIST-3 O.K.N. performed well for OXA-48, KPC and NDM detection, with high sensitivity and specificity for each target.

The assay was simple to perform. Results were easy to interpret and available within 20 minutes.

The NDM that was missed was from a highly mucoid K. pneumoniae isolate. Highly mucoid strains have been associated with false negative results from other CPO assays that include a lysis step. Adaptations to the methodology of some tests, to improve detection from mucoid isolates, have been described.

As not all carbapenemase types are detected by this assay (e.g. VIM, IMP, GES), users should consider the local prevalence of different genotypes, and their patient population, to decide on the suitability of this assay in their setting.

The Royal Free London NHS Foundation Trust has a diverse patient population, and has isolated CPO’s of many types; We plan to implement this assay as part of an algorithm aimed at improving detection of all carbapenemase genotypes, whilst optimising laboratory workflow.