OXA-48-, KPC- & NDM-type carbapenemase-producing organism detection

Multidrug resistance exhibited by a range of microorganisms is a growing problem, a prime example being that resulting from carbapenemase production. Here, Gemma Vanstone and colleagues assess the value of the RESIST-3 O.K.N. lateral-flow immunochromatography assay.

Carbapenemase-producing organisms (CPOs) are multidrug 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 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 with identified risk factors for CPO and contacts of positive cases are screened.

The Royal Free London NHS Foundation Trust has a low prevalence of CPO (less than 1% of all patients tested in 2015), and the most commonly detected genotype is OXA-48, followed by NDM, VIM, KPC and OXA-23. In addition, occasionally more unusual types are isolated, including IMP, IMI and GES-5.

Carbapenemase-producing organisms are difficult to detect in diagnostic laboratories because of the many different genotypes that can be associated with a wide range of phenotypes. Standardised methodology has not been established, and current approaches adopted include:

- 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 about the most suitable carbapenem for use as the substrate, and assays have been linked with poor sensitivity for some carbapenemase types (particularly OXA-48).

Carbapenemases

Carbapenems are β-lactam antibiotics used mainly in hospital for the treatment of multidrug-resistant bacteria. Carbapenemases are bacterial enzymes that hydrolyse carbapenem molecules, resulting in antibiotic resistance in carbapenemase-producing organisms. Resistances conferred by the five main carbapenemases (OXA-48, KPC, NDM, IMP and VIM) continue to increase, and thus any diagnostic tool that can assist laboratory identification can improve patient safety.

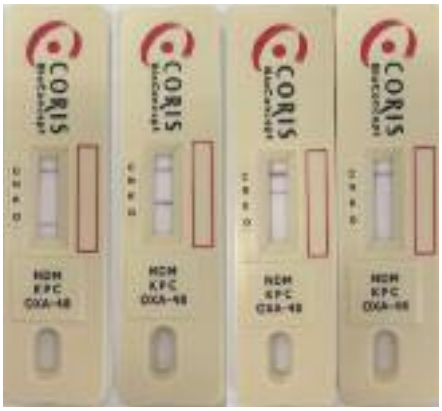

Fig 1. Examples of OXA-48, KPC, NDM and negative results using the RESIST-3 O.K.N.

The RESIST-3 O.K.N. is a lateral-flow immunochromatography assay (Coris BioConcept) that detects OXA-48-, KPC- and NDM-type carbapenemases from isolates within 20 minutes (Fig 1). Therefore, this study aims to evaluate the RESIST-3 O.K.N. assay for use in the routine diagnostic laboratory for the detection of CPOs.

Methodology

A total of 100 multidrug-resistant isolates were tested by the RESIST-3 O.K.N. assay. Samples included 95 clinical isolates (22 NDM [10 *Escherichia coli*, seven *Klebsiella* spp., two *Acinetobacter baumannii*, one *Pseudomonas aeruginosa*, one *Citrobacter freundii* and one *Enterobacter* sp.], 24 OXA-48 [11 *Escherichia coli*, nine *Klebsiella* spp., two *Enterobacter* spp.,

and two *Serratia* spp.], 19 VIM [16 *P. aeruginosa*, two *Providencia* spp., and one *Escherichia coli*], four OXA-23 [all *Acinetobacter* spp.], three KPC [all *Klebsiella* spp.], four NDM + OXA-48 [three *Klebsiella* spp. and one *E. coli*], one OXA23 + NDM [*A.baumannii*], one IMI [*Enterobacter asburiae*], one IMP [*Pseudomonas aeruginosa*], nine extended-spectrum β-lactamase [ESBL]-producing organisms [seven *Escherichia coli*, two *K. pneumoniae*], three derepressed AmpC [all *Enterobacter* spp.], four inducible AmpC [one *K. oxytoca*, one E. cloacae, one *C. freundii* and one *Serratia marcescens*]), and five control organisms.

All clinical isolates were sent to the reference laboratory (AMRHAI, Public Health England [PHE]) for confirmation of CPO status.

Isolates were tested by the RESIST-3 O.K.N. following the manufacturer's instructions. In brief, bacterial colonies from an overnight subculture on cysteine lactose electrolyte-deficient (CLED) agar were suspended in 10 drops of LY-A buffer, which was mixed to homogenise the solution. Three drops of the bacterial suspension were added to the sample well of the cassette, and the results were read after 15 minutes.

Results

The RESIST-3 O.K.N. assay correctly identified 28/28 OXA-48 (100% sensitivity, 87.7–100 confidence interval [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), and all three KPC (100% sensitivity, 29.2–100 CI; 100% specificity, 96.0–100 CI) -positive isolates (Table 1).

The results were available within 20 minutes, with less than two minutes' hands-on time. The strips were easy to read, although 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.

Overview of RESIST-3 O.K.N.

Method

The test is performed from a fresh culture in a Petri dish or in a blood culture bottle. Following a standard immunochromatography protocol, the test requires three minutes' hands-on time and 15 minutes running time. No equipment is needed.

Validation

Compared to molecular methods and in various evaluations, the first two RESIST kits (OXA-48 and KPC) consistently gave 100% sensitivity and specificity. Now combining these two products and adding NDM in a single test, sensitivity and specificity remain at 100%

Presentation

Each RESIST kit contains 20 tests. The cassettes are packaged individually and dilution buffer and tubes are included. The product is stored at room temperature and has a shelf life of 12 months from the date of manufacture.

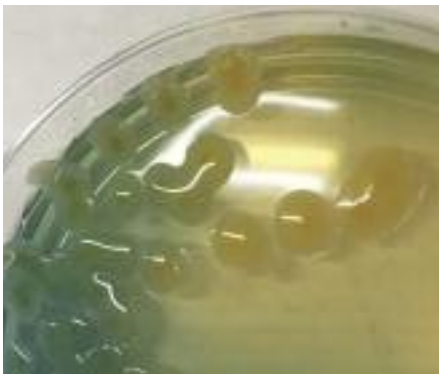

Fig 2. Mucoid NDM-positive *K. pneumoniae* isolate that was negative by RESIST-3 O.K.N.

The false-negative result obtained was from an NDM-positive *K. pneumoniae* isolate that was highly mucoid (Fig 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 five control isolates included in the study.

Discussion and 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, and results were easy to interpret and available within 20 minutes.

The missed NDM 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. However, adaptations to the methodology of some tests, in order to improve detection from mucoid isolates, have been described.

As not all carbapenemase types are detected by this assay (e. 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 serves a diverse patient population, and currently has a low prevalence of CPO. The RESIST-3 O.K.N. has now been implemented into the routine diagnostic laboratory as part of an algorithm aimed at improving detection of all carbapenemase genotypes, while optimising laboratory workflow.

Table 1. OXA-48, KPC and NDM results obtained with the RESIST-3 O.K.N.

	RESIST3-O.K.N.								
	OXA-48			KPC			NDM		
Reference laboratory result	Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
Positive	28	0	28	3	0	3	26	1	27
Negative	0	67	67	0	92	92	0	68	68
Total	28	67	95	3	92	95	26	69	95

Further reading

- Meunier D, Vickers A, Pike R, Hill RL, Woodford N, Hopkins KL. Evaluation of the K-SeT R.E.S.I.S.T. immunochromatographic assay for the rapid detection of KPC and OXA-48-like carbapenemases. *J Antimicrob Chemother* 2016; **71** (8): 2357–9.
- Wareham DW, Abdul Momin MH. Rapid detection of carbapenemases in Enterobacteriaceae: evaluation of the RESIST-3 O.K.N (OXA-48, KPC, NDM) multiplexed lateral flow assay. *J Clin Microbiol* 2017; **55** (4): 1223–5.

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