Identifying the 'Big Four' carbapenemases in the routine laboratory

The continued increase in the incidence of CPEs is creating workload issues for reference laboratories, but the diagnostics industry is responding with simple, more affordable identification methods that can be adopted by front-line microbiology laboratories, speeding confirmation and reporting.

In 2006, four carbapenemases were referred to Public Health England (PHE) Colindale for identification; in 2015 a total of 1893 carbapenemases were referred to PHE Colindale (now AMRHAI) for identification. When this increase in workload at AMRHAI is added to significant increases in other aspects of combating the rise in antimicrobial resistance (AMR), it is not surprising that there is pressure to encourage front-line laboratories to consider increasing their own in-house capability.

The recently published issue of PHE *AMRHAI News* makes it clear that identification of the 'Big Four' carbapenemases should be done in front-line microbiology laboratories and not referred to AMRHAI, except under circumstances outlined in the newsletter. To encourage this change (or discourage referrals), charges for some testing will be implemented from 1 April 2018. *AMRHAI News* makes similar comments about colistin minimum inhibitory concentration (MIC) testing, pointing out that commercial products are available for carbapenemase identification and colistin MIC by broth microdilution. Both carbapenemase identification and colistin MIC products should be more widely adopted.

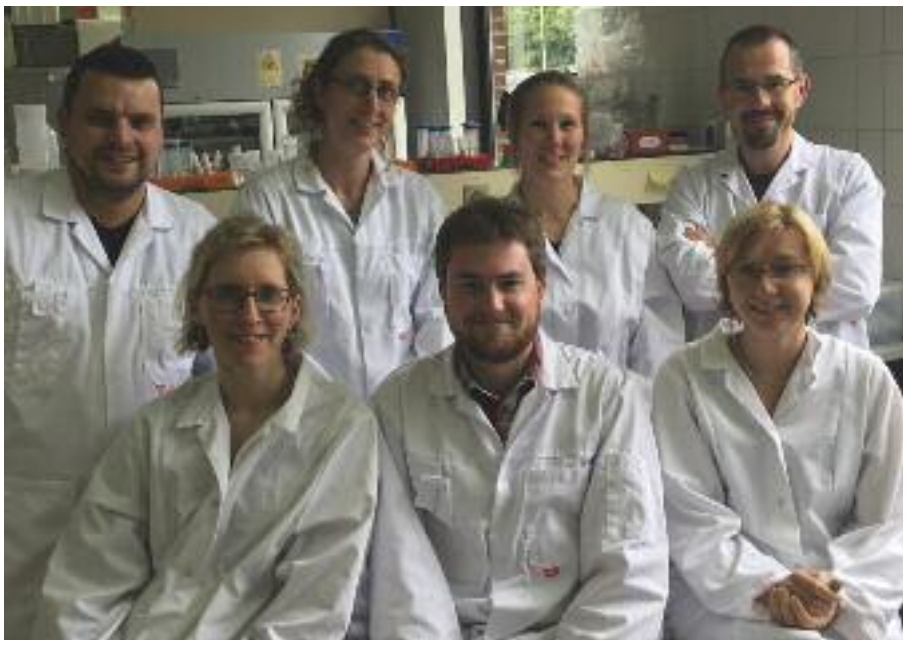

Fig 1. The Coris BioConcept team responsible for the first immunochromatography test for carbapenemase identification and winner of a Longitude Prize Discovery Award.

Bringing testing in-house: the options

So, it is time to start looking at how to bring carbapenemase identification in-house? This requires a test with accuracy as close as possible to 100%, which rules out phenotyping, and, until recently, leaves only molecular options with their associated cost implications.

The National Institute for Health and Care Excellence (NICE) produced a document in 2016 (Medtech innovation briefing; nice.org.uk/guidance/mib52), which looked at a molecular test, Xpert Carba-R, for carbapenemase identification. Xpert Carba-R can be used as a direct screen, giving results from a rectal swab in about one hour, or can be used to identify a carbapenemase direct from a culture plate. The document puts the cost per test for direct use at £35 and the cost of equipment (GeneXpert) starting at £18,000 with an ongoing annual service charge upwards of £2000

There is now a viable option to inaccurate phenotyping and expensive molecular tests. In 2016, Coris BioConcept patented the first immunochromatography

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

	RESIST-3 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

test for carbapenemase identification (Fig 1). The first product (RESIST-OXA) detected OXA-48-like carbapenemases direct from a culture in 15 minutes using a classical immunochromatography procedure. Evaluations both in the UK and throughout Europe have shown the test to be 100% sensitive and specific. The second product (RESIST-KPC) soon followed and was shown to identify KPC also with 100% sensitivity and specificity. Although welcomed, both products prompted the request for a more comprehensive product.

RESIST-3 OKN

In December 2016, RESIST-3 OKN was launched. The three carbapenemases OXA-48-like, KPC and NDM account for 91% of the carbapenemases confirmed by PHE AMRHAI. This product is now in use or under validation in over 30 UK hospitals (Table 1).

RESIST-4 OKNV

RESIST-4 OKNV has just been launched, adding VIM to the three carbapenemases in RESIST-3 OKN, so we now have a product that covers the four major carbapenemases that account for 98% of referred isolates.

RESIST: the science

The process of developing the RESIST immunochromatography assay began with the production of monoclonal antibodies for the OXA-48 test using a DNA immunisation strategy. Mice were immunised with a DNA construct, combining a booster sequence and the *OXA-48* gene. Hybridomas resulting from fusion were screened for reaction on OXA-48-purified recombinant protein. Resulting monoclonal antibodies were tested in a sandwich immunochromatographic assay to map their reactivities towards OXA-48 and other oxacillinases. Monoclonals for KPC were obtained by classical immunisation. Mice were immunised with a recombinant protein. Western blots were performed on recombinant OXA-48 and KPC proteins.

Epitope mapping was initially performed by competition experiments and was then refined by testing the antibodies on overlapping peptides. Monoclonals were then characterised by competition experiments to select the best antibodies for use as conjugate or coating reagent.

Immunochromatographic sandwich tests were finally developed by using antibodies in both capture and detection after coupling to colloidal gold particles. The best antibody pairs were selected to build prototype tests that were evaluated on recombinant OXA-48 or KPC protein and on lysates of OXA-48- or KPC-producing strains.

The process for producing immunochromatography assays for other carbapenemases follows a similar pathway where a selection of high-quality antibodies is produced, then screening those antibodies to select the best antibody pairs to build prototype tests. Exhaustive in-house testing with collection strains of Enterobacteriaceae and *Pseudomonas* spp. producing different carbapenem-resistant Enterobacteriaceae (CPE) variants are used to select the best prototypes for all the targets (Fig 2). These strains have been characterised phenotypically (identification and antimicrobial susceptibility testing) and genotypically (polymerase chain reaction [PCR] sequencing) for their mechanisms of resistance to β-lactam agents. Customer evaluation is always performed before a product is released for sale.

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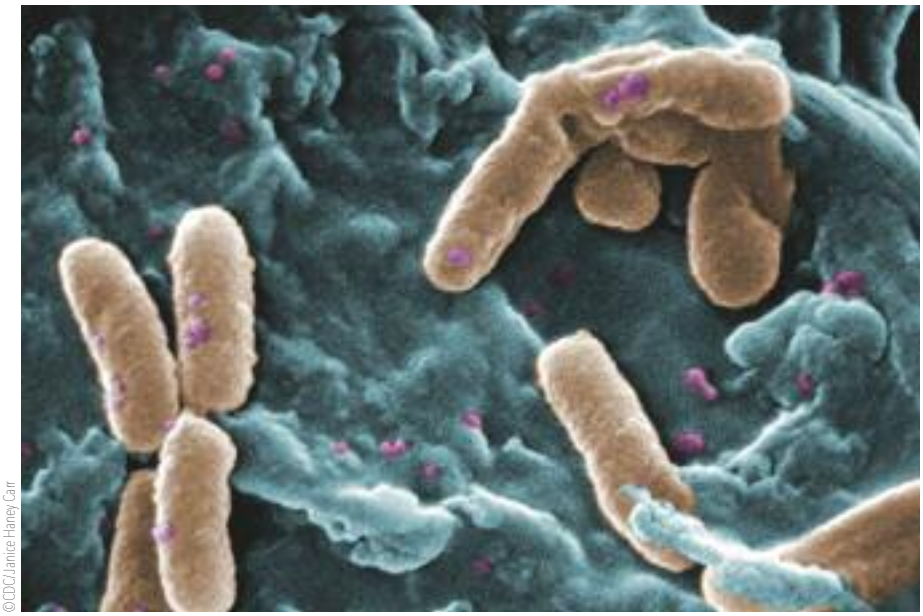

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Fig 2. Strains of Enterobacteriaceae and *Pseudomonas* spp. are used to select the best prototypes for all the targets.

Lean and easy

Customer feedback on the performance of RESIST kits has verged on the enthusiastic, with comments such as "it works beautifully", "super easy, super good" and "we may have missed it if we didn't have the kit".

When used in conjunction with a chromogenic medium or picking off

The RESIST range of carbapenemase identification tests has shown to be accurate, easy to use and to implement

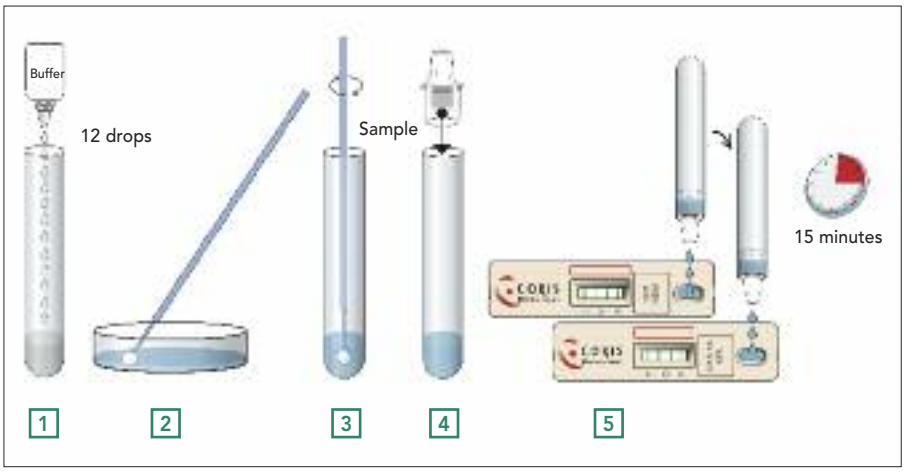

Fig 3. The five steps of the RESIST immunochromatographic assay.

colonies growing near a carbapenem disk, the microbiologist selects a colony and emulsifies it in the 10 drops of buffer previously dispensed into one of the disposable tubes supplied. The dropper cap is attached and three drops of the diluted sample are added to the cassette well. The test is run at room temperature for up to 15 minutes, and the result is then read (Fig 3).

The whole process is simple; no equipment, incubation or walking around the laboratory are required. Positive results may be reported sooner than 15 minutes if both the test and control lines become visible.

Conclusions

The RESIST range of carbapenemase identification tests has shown to be accurate, easy to use and to implement, allowing laboratories to confirm the identity of a carbapenem within 24 hours of a faecal sample arriving in the laboratory. The number of isolates that need referring to AMRHAI can be greatly reduced, resulting in cost savings and the freeing up of time both in the front-line laboratory and at the reference laboratory.

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): 2637–9.
- Public Health England. *AMRHAI News* Issue 9 Winter 2018, PHE gateway number 2017757.
- Vanstone GL, Wey E, Smith R, Mack D, Balakrishnan I. OXA-48-, KPC- & NDM-type carbapenemase-producing organism detection. *Pathology in Practice* 2017 Aug; **18** (3) 46–7.
- Wareham DW, 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.