Using molecular LAMP to curtail the global threat of carbapenem resistance

Molecular specialist Bradley Horn looks at the application of LAMP technology to combat the growing problem of antimicrobial resistance, particularly in Gram-negative bacteria that increasingly demonstrate multidrug resistance.

Loop-mediated isothermal amplification (LAMP) is proving to be an increasingly powerful tool for time-sensitive clinical diagnostics by offering additional benefits over traditional polymerase chain reaction (PCR) technologies.[1] Mast Group has applied LAMP to the detection of carbapenemase-producing organisms (CPO) making rapid molecular testing available to laboratories worldwide.

The problem

The increasing prevalence of Gram-negative infections exhibiting multidrug resistance has become one of the most significant threats to public health globally. Carbapenem antibiotics such as meropenem, imipenem, ertapenem and doripenem have traditionally been considered as the last resort for treating such infections; however, this is no longer the case. Over recent years there have been several outbreaks of CPOs that have caused widespread dissemination of resistance, and become endemic in numerous countries.[2,3]

As illustrated in Figure 1, the prevalence of CPOs in healthcare-associated infections has more than doubled since the turn of the century.[4] While there is an observed increase in the presence and spectrum of CPOs in the healthcare setting, the Centers for Disease Control and Prevention (CDC) has highlighted the potential for carbapenem resistance to also transmit into the community and become a frequent cause of community-acquired infections.[4,5]

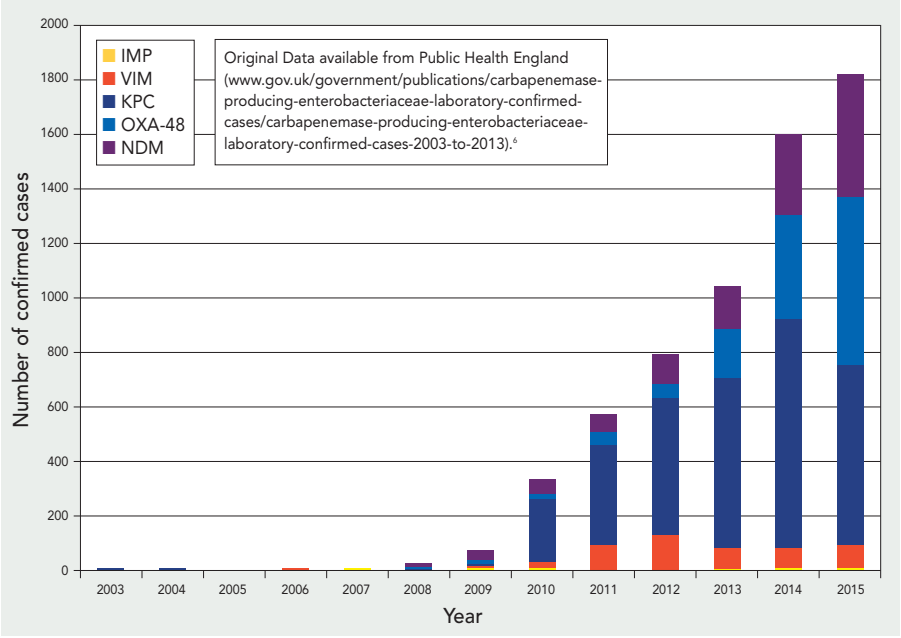


Fig 1. Public Health England-confirmed laboratory cases of carbapenemase-producing Enterobacterales from 2003 to 2015. This graph shows the extreme increase in incidences and diagnoses of the 'big five' carbapenemase-producing Enterobacterales made by the Antimicrobial Resistance and Healthcare-Associated Infections (AMRHAI) reference unit in the UK.

Transmission

Acquired carbapenem resistance is usually mediated by carbapenemase genes that provide a platform for transferable resistance.[5,7] These genes are located on mobile genetic elements such as transposons and plasmids that can shift within a strain through vertical gene transfer, or through unrelated naïve strains and species by horizontal gene transfer. This transference of genes can result in multiple species outbreaks and increased carbapenem resistance.[3,5,7]

Such outbreaks can be difficult to recognise and subsequently control, so early detection and characterisation of a CPO through this mechanism is essential for minimising rapid transmission and implementing effective preventative control measures.[5] A CPO can spread from patient to patient more easily than a non-producing carbapenem-resistant organism, and therefore requires more stringent infection control measures.[7]

The prevalence of acquired carbapenemases is increasing around the world, and the transfer of carbapenemase

genes via the highly transmissible mobile genetic elements is one of the focal resistance mechanisms (from a public health perspective) that underpins the concerns surrounding the spread of resistance out of the healthcare environment and into the community.[5] When combining the ease of international travel in today's society with the ability of carbapenemase genes to move between species and strains on a global scale, carbapenem-resistance becomes a great threat to public health that will require international communication and collaboration for routine surveillance and further research.[7,8] Despite the fact that the incidences of individual resistance genes vary geographically, the spread of CPO is not restricted by different locations or ethnic populations.[3]

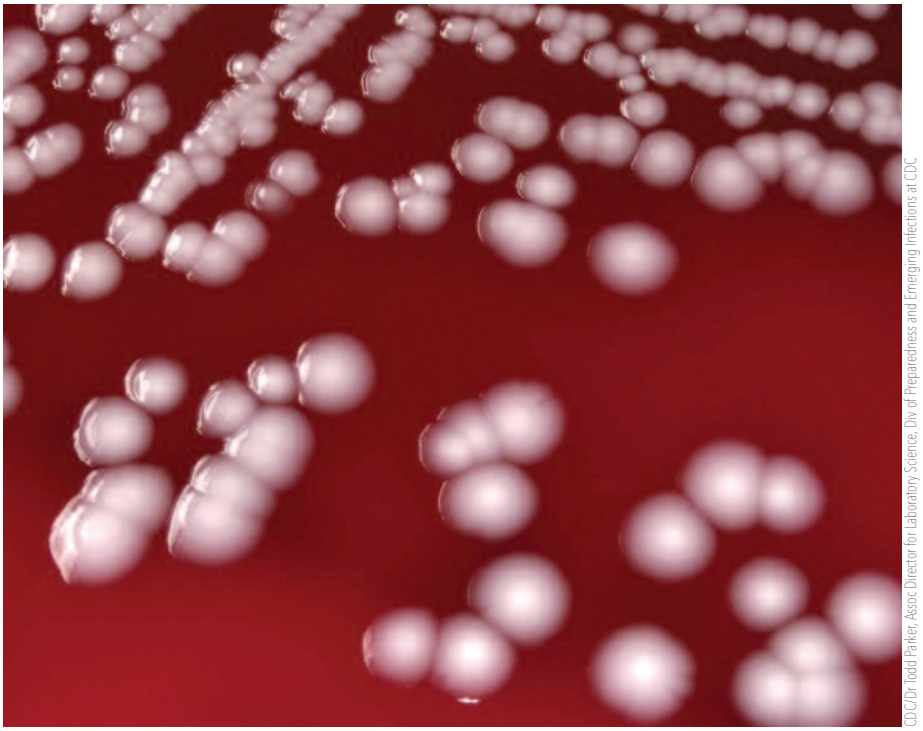
Carbapenemase families

There are several different carbapenemase families identified both in the UK and internationally, the most common of which are KPC (Ambler class A *Klebsiella pneumoniae* carbapenemases), VIM (class B Verona integron-encoded metallo-β-lactamase), NDM (class B New Dehli metallo-β-lactamase), IMP (class B Imipenem-resistant metallo-β-lactamase) and OXA-48 (class D oxacillinase).[4,5,7,8]

KPC is typically identified in Enterobacterales, but has also been identified in *Pseudomonas* spp. and *Acinetobacter* spp.[7] Following original discovery in the United States, KPCs are generally considered responsible for carbapenem-resistant outbreaks in the Western hemisphere, but have since spread into Southern Europe, Israel and China.[3] KPC is now the most frequently observed carbapenemase in both the United Kingdom and worldwide.[4,5]

OXA-48 is also commonly expressed in Enterobacterales in addition to *Pseudomonas* spp. and *Acinetobacter* spp.[7] This is often identified in Europe and North Africa, and has become the third most prevalent carbapenemase on a global scale, but the second most prevalent in the United Kingdom.[4,5,8] Throughout Europe, the rapid increase of OXA-48 is posing a serious threat to infection prevention and control.[4]

NDM has become endemic across the Asia Pacific (APAC) and Northern European regions, and is responsible for several sporadic global resistance outbreaks.[3,4] VIM appears to be localised to Europe and has previously been identified at higher incidences in Greece and Italy, whereas IMP tends to be more prevalent across Japan, Australia and China.[3]



CDC/Dr Todd Parker, Assoc Director for Laboratory Science; Div of Preparedness and Emerging Infections at CDC

OXA-23 is now considered to be one of the most common causes of carbapenem resistance in *Acinetobacter baumannii* (pictured, growing on sheep blood agar).

It is clear that previously defined geographical barriers no longer exist, as there are greater overlapping incidences between families as a consequence of the spreading of mobile genetic elements.[8] In addition to the five carbapenemase families already mentioned, there is evidence of many more groups also spreading resistance. OXA-23 is being observed at an increased frequency globally in Enterobacterales, *Pseudomonas* spp. and *Acinetobacter* spp., and is now considered to be one of the most common causes of carbapenem resistance in *A. baumannii*.[9–11] OXA-24/40 was only discovered in 2000 in Spain, but is already becoming more prevalent on a global scale.[9,12,13] In addition to an increasing number of significant families, many more isolates are being identified that are co-producers and testing positive for multiple carbapenemases. For example, the first three identifications of NDM and OXA-48 co-producers in the UK were all made in 2012. Between 2013 and 2015, there was another 71 confirmed cases of this multiple variant.[6]

The increasing prevalence of Gram-negative infections exhibiting multidrug resistance has become one of the most significant threats to public health globally

Importance of testing

As a result of the increasing prevalence of CPOs on a global scale, it is currently recommended by Public Health England (PHE) and the CDC that laboratories should implement a testing strategy for the identification of the most common carbapenemases.[5,14] The rapid transmission of carbapenem resistance is an ever-growing threat to public health, and the ability to detect CPOs reliably is the main ingredient in a recipe that will allow management of the situation with early identification, optimised treatments and minimal onward transmission.[5,7]

The severity of infections caused by CPOs and the limited therapeutic treatment options have resulted in associated mortality rates of greater than 50%, so it is critical to have a rapid diagnosis allowing clinical intervention.[2,4,5,7]

Characterisation at a local level is essential to inform patient-specific treatment options because different antibiotics will exhibit different activities against a specific carbapenemase.[3,5,7] The ability to differentiate between the different carbapenemase families is useful for surveillance by allowing the distribution to be monitored globally. This improved CPO characterisation will in turn enable the identification of novel treatments, which are required urgently to prevent the rise of CPO-associated mortality and reduce the economic burden caused by prolonged hospital admissions.[3,5]

The ready-to-use LAMP reagents are provided as lyophilised pellets that contain the necessary components for a successful amplification

The solution

Mast ISOPLEX *CRE-ART* is an *in vitro* diagnostic that uses loop-mediated isothermal amplification (LAMP) technology to provide a rapid solution for the molecular identification and differentiation of the seven most prevalent CPO families, including over 230 family members.

The LAMP technology was developed in 2000 by the Eiken Chemical Company, with the potential to replace and provide an alternative technology to PCR. It achieves high specificity by using four to eight specifically designed primers that target six to eight regions of the genome, and achieves high sensitivity due to the extremely high efficiency of the reaction. It allows femtogram quantities of DNA to be amplified to 10^9 copies in less than 60 minutes.[1]

The technique relies upon the strand displacement activity from a DNA polymerase, which in turn no longer requires the denaturation of DNA, allowing LAMP to be performed in equipment capable of maintaining a temperature of 60–65˚C, and removing the need to have access to thermal cyclers capable of different temperature transitions for denaturing, annealing and extending.

The LAMP technology is less susceptible to inhibiters than PCR, which negates the need for potential post-extraction purification steps. Amplification can be seen in as little as five minutes compared to the significantly longer PCR cycles that typically take 90–180 minutes. The products from a LAMP reaction can be interpreted in real-time with no post-PCR modifications required.

LAMP testing for CPOs

In February 2017 the World Health Organization (WHO) published its first list of antibiotic 'priority pathogens' that pose the greatest threat to human health. These include *Pseudomonas aeruginosa* and *A. baumannii*, emphasising the need for laboratories and hospitals to have access to rapid, easy-to-use technologies. Many current technologies are under-utilised, so it is essential to encourage the development of next-generation technologies, as well as the uptake of technologies such as LAMP that are now available.

Mast Group has developed a LAMP-based Mast ISOPLEX *CRE-ART* kit that simultaneously detects OXA-48, OXA-23, OXA-24/40, KPC, NDM, VIM and IMP from bacterial cultures, and includes an inhibition control for each sample. The inhibition control gives confidence to the end user and confirms the integrity of the assay reagents. For each carbapenemase family targeted by the assay, multiple family members can be detected, as follows:

- OXA-48 – 31 members detected
- OXA-23 – 39 members detected
- OXA-24/40 – 10 members detected
- KPC – 45 members detected
- VIM – 64 members detected
- NDM – 26 members detected
- IMP – 16 members detected.

The ready-to-use LAMP reagents are provided as lyophilised pellets that contain the necessary components for a successful amplification. The assay comprises an eight-tube strip, whereby each of the seven specific carbapenemase targets occupies its own tube, as does the inhibition control. Extracted DNA is

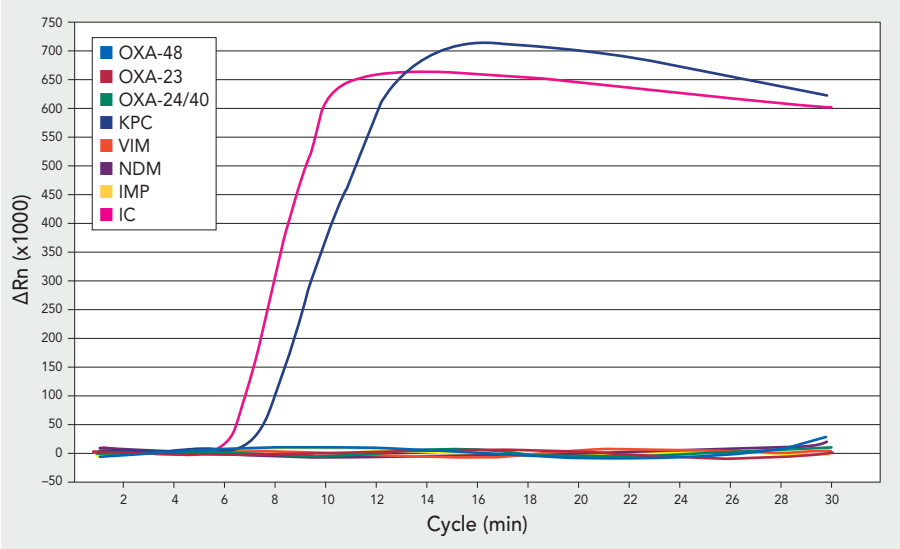


Fig 2. Positive result. This results graph shows a positive amplification profile for KPC (dark blue) and the inhibition control (pink) using the MAST ISOPLEX *CRE-ART* assay.

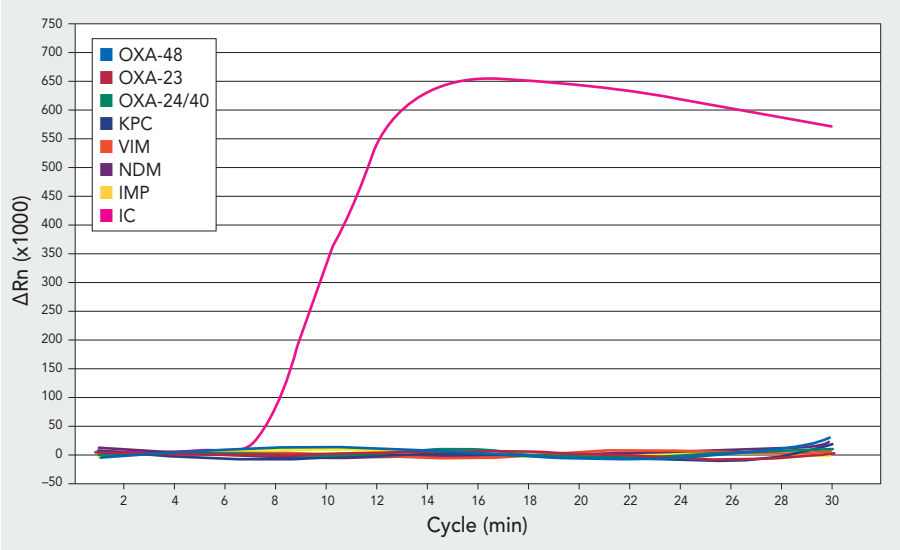


Fig 3. Negative result. This results graph shows a negative result for all carbapenemase targets using the MAST ISOPLEX *CRE-ART* assay. The inhibition control is positive indicating there has been no inhibition of the sample.

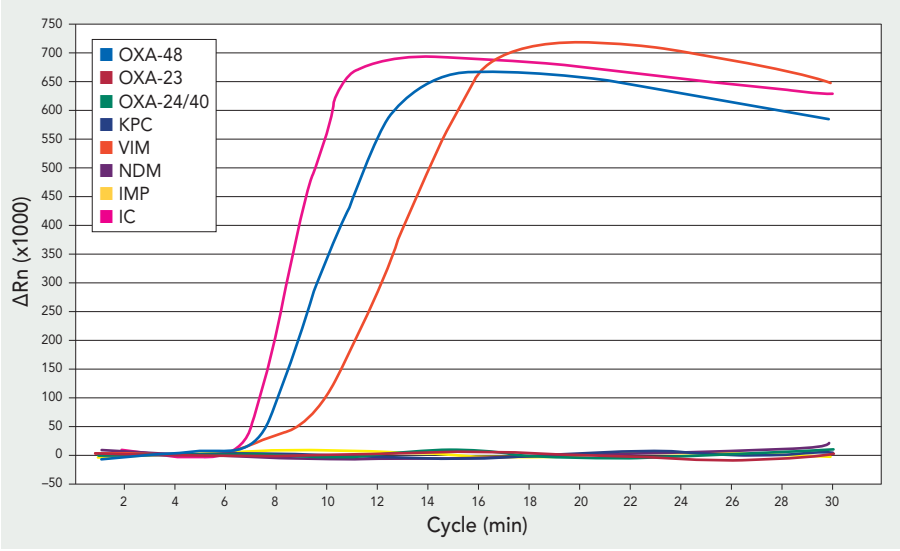


Fig 4. Co-producing positive result. This graph shows a positive amplification profile for OXA-48 (light blue) and VIM (orange) using the MAST ISOPLEX *CRE-ART* kit. The inhibition control (red) is also positive as expected.

Molecular diagnostic techniques bring enhanced specificity and sensitivity, and can offer a quicker and more accurate diagnosis than traditional methods

added to each tube in the strip to allow testing of all seven carbapenemase families. Owing to the high specificity of LAMP, amplified products can indicate the presence of genes encoding carbapenemase target DNA within 30 minutes. The kit satisfies the WHO 'priority pathogens' as it is able to test for resistance in Enterobacterales, *Pseudomonas* spp. and *Acinetobacter* spp.

Workflow

Mast ISOPLEX *CRE-ART* removes any complexity associated with molecular assays as reagents are ready to use. DNA can be extracted from an isolated bacterial colony by a simple 'boil and spin' step in the buffer provided. This extracted solution is added directly to the LAMP pellets in the eight-tube strip in order to run the assay and to determine if the sample being tested is a CPO. Amplification is very easy. Any strip/tube/plate-format thermal cycler can be used, with amplification detected via the FAM filter. In order to aid workflow in the laboratory and reduce pressure on already stretched thermal cycling resources, Mast Group will soon be introducing the TubeScanner platform for rapid amplification and detection on the bench.

Results

Determining the presence of target DNA is simple and easy. A positive result is indicated by a rapid increase in the fluorescence intensity within the 30-minute assay time (Fig 2), whereas a negative result shows no rapid increase in fluorescence intensity over the same period of time (Fig 3). Co-producers will also be identified using this assay and will be indicated by the presence of multiple targets (Fig 4). For all samples tested and for a result to be valid, the inhibition control should show a positive profile. An invalid result caused by a negative control profile would suggest that there has been some inhibition with the sample, and the results may be compromised.

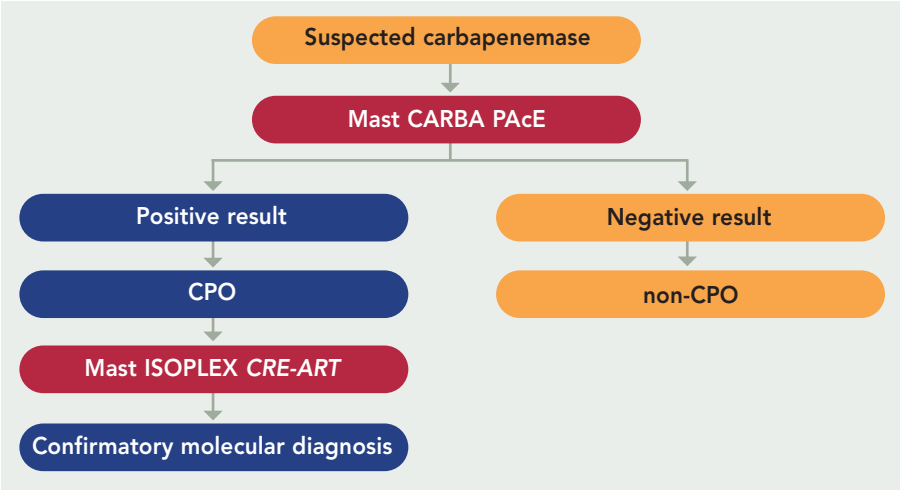


Fig 5. Using LAMP to enhance a laboratory workflow. Suspected carbapenemase isolates should be tested initially against a carbapenem before performing confirmatory tests as per current UK Standards for Microbiology Investigations. Rapid indications can be achieved through products such as MAST CARBA PAcE, which is a colorimetric test used to show the presence of a carbapenemase. MAST ISOPLEX *CRE-ART* can then be used for accurate confirmation and characterisation in order to determine the carbapenemase family.

Applications

As previously mentioned, laboratories are being encouraged to routinely test and identify carbapenemase-producing organisms. Current testing pathways that include bacterial culture and other antibiotic susceptibility tests are able to indicate the presence of a carbapenemase; however, supplementary phenotypic methods for identifying and characterising the carbapenemase type are time-consuming with limited accuracy. Molecular diagnostic techniques bring enhanced specificity and sensitivity, and can offer a quicker and more accurate diagnosis than traditional methods. This test offers laboratories the opportunity to bring CPO detection and characterisation in-house in a cost-effective manner (Fig 5). The kit can be stored at 2–30˚C and is stable at temperatures of up to 50˚C and >90% relative humidity, making this an ideal product for laboratories worldwide.

In summary

Mast ISOPLEX *CRE-ART* uses LAMP technology to provide a rapid solution for the detection and characterisation of carbapenemase-producing organisms. The technology overcomes some of the restrictions of PCR, making molecular testing accessible to all, especially those with limited resources and molecular experience. In addition to detecting the 'big five' carbapenemase families (KPC, NDM, OXA-48, VIM, IMP), this assay will detect OXA-23 and OXA-24/40, the prevalence of which is increasing on a global scale. *CRE-ART* removes some of the technical complexity associated with the more cumbersome molecular assays and provides accurate results that are easy to read and interpret. Early detection of CPOs will aid appropriate therapeutic decision-making in order to improve patient prognosis, prevent further transmission and reduce hospital costs. With such testing, it is hoped that future sporadic outbreaks can be prevented and the mortality rate from carbapenem-resistant infections can be reduced.

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