Guidance Relating to Laboratory Testing for Carbapenemase Producing Enterobacterales (CPE) and the Interpretation and Clinical Application of Results

CPE Expert Group

Scope of Guidance

This document is intended to provide guidance for healthcare workers related to laboratory testing for CPE and to guide the interpretation and clinical application of Results

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Abbreviations and Glossary of Terms

**AMRO** = Antimicrobial-Resistant Organisms

**CPE** = Carbapenemase Producing Enterobacterales

**ED** = Emergency Department

**ESBL** = Extended Spectrum Beta-lactamase Producing Enterobacterales

**EUCAST** = European Committee on Antimicrobial Susceptibility Testing

**IPC** = Infection Prevention and Control

**Isolation** = Isolation refers to accommodation of one person in a single room

**MRSA** = Methicillin-Resistant *Staphylococcus aureus*

**MIC** = Minimum Inhibitory Concentration

**NCPERL** = National Carbapenemase-Producing Reference Laboratory

**Person/People** = the terms person/people are generally used in this document and are in general interchangeable with the terms client, service user or patient.

**VRE** = Vancomycin-Resistant Enterococci

Recommendations

- Culture based and direct molecular detection methods are both suitable for detection of CPE in clinical samples.
- Laboratories should consider turnaround time, cost, workflow and timeliness of support for patient placement decisions in choosing a method.
- On-site access to very rapid turn-around molecular testing of selected individual patients for CPE is valuable to support appropriate patient placement decisions in both laboratories using culture based detection and those using direct molecular testing using high-throughput batch testing platforms.
- Laboratories should report the majority of samples on the day after receipt of the sample including those received over weekends and holiday periods.
- When using culture based methods, rapid detection of carbapenemase antigen and or rapid detection of carbapenemase genes by nucleic acid amplification technology
should be applied promptly to suspect CPE colonies to identify the more common types of CPE.

- In general there is no requirement to perform additional testing on most non Enterobacterales that grow from screening samples on CPE selective chromogenic agar.
- Periodic or selective testing of *Acinetobacter* spp. that grow from screening samples on CPE selective chromogenic agar for carbapenemase antigen/ transferrable carbapenemase genes, should be considered in samples from high risk units such as Intensive Care Units.
- Testing of all *Acinetobacter* spp. that grow from screening samples on CPE selective chromogenic agar for carbapenemase antigen/ transferrable carbapenemase genes may be appropriate for a period of time in hospitals that have identified such isolates from clinical samples or the environment.
- When using direct molecular testing methods all newly detected positive patients should be confirmed by culture of CPE if at all possible.
- People who test positive by direct molecular methods but from whom CPE cannot be cultured are not notifiable under the case definition for CPE.
- Communication with primary teams and patients regarding patients with a positive direct molecular test that is not subsequently confirmed by culture must be clear in relation to how the result should be interpreted.
- In relation to circumstances in which CPE is not confirmed by culture; people that have an unequivocal and reproducible positive molecular test on a single or multiple samples by a single molecular method should be identified as possible CPE and managed in the acute hospital as for CPE positive patients pending further evaluation.
- In relation to circumstances in which CPE is not confirmed by culture, people that have a non-reproducible positive direct molecular test on a single sample should generally not be regarded as CPE positive and should not be managed in the acute hospital as for CPE positive patients.
- In relation to meropenem susceptibility testing of isolates, the approach used should be capable of differentiating between meropenem wild type and meropenem non-wild-type Enterobacterales. Test methods that are limited to differentiation between meropenem susceptible and meropenem non-susceptible isolates are not sufficient.
- First detected CPE isolates from any patient, all CPE invasive isolates (from normally sterile body site) and suspect CPE isolates (as defined in the National CPE Reference Laboratory Service user guide) should be submitted to the reference laboratory within a week of isolation. (See reference laboratory user guide.)
- Direct molecular detection is not recommended for application to environmental samples.
Background

Antimicrobial resistance is a major challenge to healthcare delivery systems in Ireland and throughout the world. Control of antimicrobial resistance is grounded in improved use of antimicrobial agents (antimicrobial stewardship) and better control of the spread of antimicrobial resistant organisms (IPC). The website www.antibioticprescribing.ie is a very valuable support for appropriate antimicrobial prescribing in the community.

The most fundamental element of managing the risk of spread of microorganisms is the consistent application of Standard Precautions in all healthcare setting and with all people all the time. Standard Precautions are critical because there is no system that will immediately and consistently identify all people colonised or infected with AMRO.

Additional steps may also help to manage the risk. These include screening people to identify those carrying AMRO (including CPE) and the application of additional Transmission-Based Precautions, such as Contact Precautions in relation to people who are known to have or who are considered at high risk of having colonisation or infection with AMRO.

Transmission-Based precautions are applicable to delivery of in-patient care in the acute hospital setting. The approach taken in the acute-care setting is not applicable in the context of delivery of care in most out-patient and day-care settings, and furthermore is not considered necessary as the risks to patients are less. However, specific precautions in addition to Standard Precautions may occasionally be advised for staff delivering care in the community in very specific circumstances.

In all settings measures to manage the risk of transmission associated with AMRO must be balanced with the imperative of delivering appropriate care to people in a timely manner and respecting the right of people to visit relatives and friends in hospital.

For practical purposes it is useful to distinguish between skin and nose surface colonising AMRO (MRSA) and gut colonising AMRO (CPE, ESBL and VRE).

Skin and Nose Colonising AMRO

For those AMRO that colonise the skin and nose the risk of environmental and hand contamination is more persistently present as contact of hands with the face and nose are frequent behaviours, and they are more common when the person has a respiratory tract infection or nasal drip. In many cases it may be possible to eradicate or minimise surface colonisation with MRSA through application of a decontamination protocol if there is a clinical indication for doing so.
Gut-Colonising AMRO

This group of bacteria include a number of antibiotic resistant bacteria that have been a problem for many years including VRE and ESBLs. It also includes a major new concern (CPE). There is more detail on CPE below.

Gut colonising AMRO spread from person-to-person through the faecal-oral route, that is to say that are shed in faeces. Traces of faeces, that are often invisible, can be transferred to hands and to other surfaces by touch. The organisms can then be transferred from hand and surfaces to the mouth either directly or from contamination of food or utensils.

It follows from the above that for those with gut colonising AMRO the principal issue is about managing the risk of faecal contamination of hands and surfaces. Provided the person is continent, fully dressed, has no behavioural disturbance and is supported as necessary in performing correct hand hygiene and dressing after visiting the toilet the risk of person-to -person spread and environmental contamination is low. There is no established protocol internationally for decolonisation of the gut of people with AMRO.

Regardless of known or suspected AMRO status, a person who has diarrhoea or who is incontinent of faeces must be prioritised for immediate care in the appropriate setting, to ensure dignity and respect as well as for IPC purposes

What is CPE?

CPE is the latest major wave of antimicrobial resistant organisms that is spreading throughout the world, including in Ireland. At the moment spread of CPE is mainly a problem in the acute hospital setting.

The gut or bowel of every normal, healthy human contains bacteria including a group of bacteria called Enterobacterales. This group of bacteria includes *E. coli* and *Klebsiella pneumoniae*. When Enterobacterales get into the bladder, kidney or bloodstream, they can cause infection (cystitis, pyelonephritis, sepsis) that may sometimes be severe and life-threatening.

CPE is a particular variant of these common gut bacteria that have become resistant to a critical group of antibiotics, the carbapenems. They are often also resistant to many other antibiotics. Although they are resistant to many antibiotics, in most other respects they are like other Enterobacterales bacteria as they are harmless when they are in the gut.

What is CPE Colonisation?

A person who carries CPE in the gut but who has no clinical symptoms or illness related to the CPE is said to be colonised. People may also have asymptomatic CPE colonisation of urine, leg ulcers or indwelling devices. Colonisation with CPE (no clinical evidence of
infection) should not be treated with antibiotics. Antibiotics do not clear the colonisation from the gut and in fact are likely to make the colonisation more intense and last longer.

When people colonised with AMRO including CPE develop clinical evidence of infection more often than not the infection that they have is not caused by the AMRO. For example upper respiratory tract infection, bronchitis, pneumonia, sinusitis, skin infection and cellulitis are unlikely to be caused by CPE even in a person colonised with CPE but are likely to be caused by well recognised and common organisms that cause the same infections in those without CPE colonisation. In a person colonised with CPE just as in everyone else these are most likely due to viral infection (upper respiratory tract and bronchitis) or the usual bacterial suspects for pneumonia (pneumococcus) and cellulitis (Staphylococcus aureus or Group A Streptococcus). In most cases of people colonised with AMRO, the guidance available on www.antibioticprescribing.ie remains appropriate for patients in the community most of the time

CPE in the gut do not cause diarrhoea, vomiting or abdominal pain. In a small number of people colonised with CPE in the gut, the CPE may subsequently cause cystitis, pyelonephritis or sepsis. In this case many of the antimicrobial agents commonly used in the community do not work; however, there are some antibiotics that are effective.

If a person colonised with CPE develops clinical evidence of infection they may need treatment directed towards CPE and consultation with a Consultant Microbiologist or Infectious Disease Physician is generally appropriate. If treatment directed towards CPE is required, in so far as it is appropriate given the person’s overall care plan, transfer to an acute hospital is generally appropriate. See guidance on treatment of CPE infection at:

https://www.hpsc.ie/a-z/microbiology/antimicrobialresistance/strategyforthecontrolofantimicrobialresistanceinireland/carbapenemresistantenterobacteriaceae/cre/guidanceandpublications/

What Do We Mean by a CPE Contact?

A CPE Contact is a term used to refer to a person who has been identified by an IPC team or public health doctor as having had significant exposure to a person colonised or infected with CPE and as a result of this exposure is at higher risk of being colonized with CPE than people who are not contacts. The criteria for identifying a person as a CPE contact are as specified in National Guidance documents. Identification of a person as a CPE contact generally relates to exposure to CPE in the acute hospital setting.

CPE contacts should be given a small plastic card to show to healthcare workers to tell them that are a CPE contact. In the community Standard Precautions are generally all that is required with respect to infection prevention and control precautions for a CPE Contact.
When CPE contacts are admitted to an acute hospital they are offered testing for CPE and special precautions are taken in their care pending their test results. Additional information on CPE including Fact Sheets is available at [www.hse.ie/infectioncontrol](http://www.hse.ie/infectioncontrol).

**Laboratory Detection of CPE**

There are two general approaches to detection of organisms in clinical samples. Recovery of a viable organism in pure culture has been the long established and definitive method for establishing the presence of an organism in a sample. In recent years an alternative approach based on culture independent direct detection of specific antigens or nucleic acid has become widely used. Culture independent methods have certain advantages but also some limitations.

An evaluation of the relative merits of culture based methods compared with molecular methods as the primary test for detection of CPE in screening samples is beyond the scope of this guidance. Culture based methods are widely used as the primary method of screening CPE. Planning for implementation of the national CPE screening guidance was based on the assumption of culture based screening in most settings. However, supplementary use of rapid molecular detection of CPE for selected circumstances is valuable. Furthermore some hospitals have determined that direct use of molecular testing is appropriate as the primary method for CPE screening in their setting. Therefore direct molecular testing of samples for CPE in Ireland is relatively common and some guidance on the confirmation of results from samples subject to direct molecular testing is required.

**Detected /Not-Detected**

The ability to detect a microorganism in a sample is dependent on the quality of the sample received in the laboratory and on the methods applied to detect the organism. There is no process of testing that can guarantee detection of CPE in all people who carry CPE. Therefore test results, culture based or direct molecular, are best reported as “detected” when CPE is identified and as “not-detected” when CPE is not identified. “Not-detected” is not equivalent to negative.

There are broadly speaking two categories of clinical samples from which CPE may be detected.

**Diagnostic Samples**

Diagnostic samples are collected from a specific site because there is a concern, based on clinical features, that an organism at that site may be associated with infection. The sample collected is typically cultured on a variety of culture media including non-selective culture media. The process is intended to support the growth of a wide range of microorganisms.
After a period of incubation and an assessment of the culture media for growth, relevant organisms (colonies) may be selected for identification, rapid testing for carbapenemase enzymes/genes and for antimicrobial susceptibility testing.

CPE Screening Samples

CPE screening samples are generally collected from patients based on national guidance. The test is not requested based on a clinical suspicion of infection. The process is intended to identify patients with asymptomatic gut colonisation with CPE. This supports the implementation of additional infection prevention and control precautions and may inform choice of antimicrobial therapy if the patient subsequently develops infection.

Culture Based Detection of CPE from CPE Screening Samples

Detection of CPE from CPE screening samples is generally based on culture on selective and differential media (usually chromogenic culture media). These culture media are designed to favour growth of CPE and discourage growth of other bacteria.

The samples may be cultured directly on selective media or may be subject to initial enrichment in a broth containing a carbapenem prior to culture on selective agar.

Culture based detection requires a minimum of 16 hours from receipt of sample to detection of colonies of microorganisms that represent suspect CPE. In some cases the colonies may not appear for 48 hours. From the time of identification of suspect colonies, most common CPE can be identified with sufficient confidence for clinical decision making within minutes to hours. This is based on confirmation that the colony is a member of the order Enterobacterales and rapid identification of suspect colonies as CPE is by testing of the colonies for carbapenemase specific antigens (immunochromographic lateral flow) or carbapenemase genes. Based on positive results by these methods a patient can be considered CPE “detected” for immediate clinical decision making and for notification as CPE.

Some less common types of CPE that grow on chromogenic agar may be undetectable by available antigen detection and rapid molecular methods Therefore suspect CPE colonies that are members of the order Enterobacterales but are not confirmed as CPE by the rapid antigen or rapid molecular methods should be tested for susceptibility to meropenem by a method that differentiates between wild-type and non-wild-type for meropenem. Isolates that are wild-type for meropenem susceptibility do not require further testing.

CPE isolates identified from patients not previously identified as CPE positive should be sent to the National CPE Reference Laboratory service for confirmation. Those isolates that are Enterobacterales and are meropenem non wild-type should be submitted to the National CPE reference laboratory service for further testing.
Culture of Bacteria Other than Enterobacterales on CPE Selective Agar

Bacteria other that members of the order Enterobacterales may grow on CPE selective agar.

In some cases these will be species that have intrinsic resistance to meropenem (for example *Stenotrophomonas maltophilia*). These isolates generally do not require further testing however it is worth noting that transferrable CPE (for example VIM) have been detected in *Stenotrophomonas maltophilia*.

In some cases these will be species such as *Pseudomonas aeruginosa* which readily acquire meropenem resistance due to porin loss and which are rarely carbapenemase producers. In general these isolates do not require further testing. *Pseudomonas* spp. producing transferrable carbapenemase have been detected in a number of hospitals in Ireland. Further testing may be appropriate in a context of suspected transmission of multi-drug resistant organism of this species.

In some cases these will be *Acinetobacter* spp. Isolates of *Acinetobacter* spp. producing transferrable carbapenemase have been detected in a number of hospitals in Ireland. These organisms are not CPE, because they are not members of the order Enterobacterales. However they can represent a very serious risk to patients.

Periodic or selective testing of certain non Enterobacterales such as *Acinetobacter* spp. and *Pseudomonas* spp. that grow from screening samples on CPE selective chromogenic agar for carbapenemase antigen/transferrable carbapenemase genes should be considered in samples from high risk units such as Intensive Care Units. Testing of all *Acinetobacter* spp. that grow from screening samples on CPE selective chromogenic agar for carbapenemase antigen/transferrable carbapenemase genes may be appropriate for a period of time in hospitals that have identified such isolates from clinical samples or the environment. *Acinetobacter* spp. that are identified as carbapenemase producers by rapid testing methods or that demonstrate high-level resistance to meropenem should be submitted to the CPE reference laboratory service. *Acinetobacter* spp. that produce a transferrable carbapenemase may be notified as novel or rare antimicrobial resistant organisms.

Direct Molecular Detection of CPE from CPE Screening Samples

Direct detection of CPE by molecular methods may allow for detection of CPE within as little as two hours of receipt of a sample in the laboratory. This is possible with testing formats that facilitate immediate processing of individual samples. Certain other formats of molecular testing are designed to test a large batch of samples. With batch processing formats the processing time may be hours but there is generally a lag time between receipt of a sample and having sufficient samples to commence running a batch.
Direct Molecular Detection

Direct molecular detection of one of the common CPE genes in a clinical sample is a sufficient basis for making immediate clinical decisions including application of Contact Precautions. Direct molecular detection is not a basis for notification of a person as CPE positive.

When a person tests positive for CPE by direct molecular methods every reasonable effort should be made to achieve confirmation by culture.

The positive sample should be cultured on selective agar plates directly or following broth enrichment. If CPE is recovered by culture from any sample the person in question is confirmed CPE positive.

If CPE is not recovered on culture and the initial direct molecular detection is not reproducible the person should be regarded as CPE “not detected”.

If CPE is not cultured from the original sample but direct molecular test is reproducible at least one and preferably two subsequent samples should be obtained from the person at intervals of at least 24 hours. Additional samples should be examined by direct molecular testing and culture with enrichment.

If CPE is not recovered on culture from any sample but samples from the patient are consistently positive on molecular testing the person should be regarded as likely CPE positive. Contact Precautions are appropriate in the acute hospital setting but the person does not meet criteria for notification.

Potential Explanations for Positive Test Result on Direct Detection of CPE in the Absence of Culture Confirmation

1. False Positive. (CPE is not present)
   Molecular detection in absence of culture confirmation is potentially explained by a positive test result in the absence of the specific gene(s) that the assay is intended to detect. This could occur in the presence of a DNA fragment that is similar in some
respects to the target nucleotide sequence but does not encode for the carbapenemase enzyme of interest.

2. True Positive (CPE is not present)
The target gene is present in a member of the Enterobacterales but culture has failed to isolate the organism. This potentially could occur because (a) the molecular test is capable of detection of CPE at lower levels than culture or (b) CPE is present at a very low level and by chance the organism is present in the material submitted to molecular testing but not in the material submitted to culture based detection (c) because CPE is present in significant numbers but the CPE is producing carbapenemase at a level that is too low to allow the organism to grow in or on selective culture media containing a carbapenem antimicrobial.

3. Detection of Target Gene in Absence of Target Organism (CPE is not present)
Molecular detection in absence of culture confirmation is potentially explained by a detection of the target gene where the gene is in an organism other than a member of the Enterobacterales. This could occur in the presence of an environmental organism for example Shewanella spp. in which a target gene may be intrinsic.

Testing of Samples from the Healthcare Environment for CPE

Testing of the healthcare environment for CPE has become increasingly important in identifying sources of spread of CPE. Environmental samples should be collected using systems intended for sampling the environment. Environmental samples should be subjected to broth enrichment before subculture to selective agar plates. Following subculture to selective agar plates the processes applied are similar to those for culture of clinical samples. Direct molecular detection should not be applied to environmental samples as the systems marketed are not validated for this application and interpretation is likely to be difficult.
References


Appendix 1. Laboratory Requirements with Respect to Detection of CPE

1.1 Conventional Culture Based Technique 1

**Note:** Commercial selective chromogenic agar that is optimised for the more common CPE types should be used. The more common CPE types present, in alphabetical order are KPC, IMP, NDM, OXA-48-like and VIM.

Day 0

This culture based system requires plating of the swab/faeces onto a CPE selective chromogenic agar plate followed by overnight incubation (36±1°C).

Samples should be set up for culture on the day of receipt 7 days per week.

Note. Some reports indicate that yield of CPE may be higher if samples are enriched in broth containing a carbapenem overnight with subsequent sub culture of the broth to agar plates. If enrichment is used the method of the CDC is appropriate. Each subsequent stage of the culture process will be delayed by 1 day if the pre-enrichment approach is used.

Day 1

Plates should be read on the day after set up seven days per week. The agar plates are inspected for colonies that morphologically resemble Enterobacterales.

- If there is NO growth of colonies that morphologically resemble Enterobacterales present and the manufacturers recommended incubation time is 18-24 hours, the laboratory may report as below, however please note that experience in some laboratories suggests that some additional CPE may be detected if plates are re-incubated and read again at 48 hours:

**Carbapenemase Producing Enterobacterales NOT detected**

- If there ARE colonies morphologically consistent with Enterobacterales present on the plate, further identification or a representative of each distinct colonial variant is required. Identification should be by a rapid method such as MALDI-ToF.
Representative growth of each species of Enterobacterales identified should be
analysed by a rapid detection assay for the more common carbapenemase specific
antigens or genes on Day 1 as soon as sufficient growth permits.

A method capable of giving results within 2-3 hours is required. These include
immunochromatographic assays (lateral flow) and nucleic acid amplification tests
(NAAT) or assays.

As outlined above a laboratory should be capable of confirming most isolates of the
common CPE types from the day-1 plates on day 1 and of reporting the cultures as
CPE-detected.

The commonly available lateral flow assays and NAATs cannot definitively exclude
carbapenemase production. If Enterobacterales, in particular *E. coli* and *Klebsiella
pneumoniae*, are cultured on the day 1 plate but carbapenemase is not confirmed by
the rapid assays, it is appropriate to consider a requirement for precautionary
application of contact precautions taking account of local experience with previous
similar isolates.

**Day 1 Colonies, Enterobacterales, Not Confirmed as CPE by Rapid Testing**

- Susceptibility to meropenem and/or ertapenem should be performed by the
  EUCAST disc diffusion method or by a MIC method capable of differentiating
  between wild type and non-wild type isolates. (see table 1 for screening cut-off)²
- Susceptibility testing can generally be set-up on Day 1 direct from the chromogenic
  agar plate if there is sufficient growth however validation of direct susceptibility in the
  laboratory is required.
- Enterobacterales that are not confirmed as CPE by rapid method but which meet the
criteria specified in the user’s guide of the National CPE Reference Laboratory
Service should be submitted to the National CPE Reference Laboratory Service.
- With respect to Enterobacterales that are not confirmed as CPE by a rapid method
  but which do require submission to the National CPE Reference Laboratory Service
  it is appropriate to issue an interim report. The content of the interim report should
take account of the characteristics of the isolate (for example the species and how
high is the MIC), resistance to other antimicrobial agents and recent experience of
the laboratory in question with such isolates. In general it may be appropriate to
report the isolate as provisional MDRO Enterobacterales as most such isolates are
not subsequently confirmed as CPE.
The first confirmed CPE of each Enterobacterales species from each person and all isolates from normally sterile body sites should be submitted to the National CPE Reference Laboratory Service within 7 days of isolation.

Suspect CPE should be submitted to the National CPE Reference Laboratory Service within 7 days of isolation.

1.2 Direct molecular testing for CPE technique

Day 0/1

- There are a variety of platforms for direct molecular testing with differences in format.
- Some are designed primarily for testing large batches together. Others are designed primarily for testing individual samples as they are received (random access).
- An assessment of the relative merits of the different systems for direct molecular detection is beyond the scope of this document.
- Samples for direct molecular detection should be processed within 24 hours of receipt 7 days per week.
Appendix 2.0. Antimicrobial Susceptibility Testing of Enterobacterales
Which Require Testing for Susceptibility to Carbapenems

Enterobacterales which require testing for susceptibility to carbapenems may be tested by
the EUCAST disc diffusion method or by an MIC method capable of differentiating between
isolates with an MIC of less than or equal to 0.125 mg/L of meropenem (wild-type) and
those with a meropenem MIC of greater than 0.125 mg/L (non-wild type).

Table 1. Clinical breakpoints and screening cut-off values for carbapenemase-
producing
Enterobacterales

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>MIC (mg/L)</th>
<th>Disc diffusion zone diameter (mm) with 10 µg disks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/I breakpoint</td>
<td>Screening cut-off</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤ 2</td>
<td>&gt;0.125</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤ 0.5</td>
<td>&gt;0.125</td>
</tr>
</tbody>
</table>

If a commercial automated antimicrobial susceptibility testing (AST system is in place it is
important that user defined alerts are added in order to alert to a requirement for
supplementary testing in isolates that test as susceptible but which are non-wild type.

As per EUCAST\(^2\), meropenem is deemed to be the best balance between sensitivity and
specificity in terms of detecting carbapenemase-producers. The NCPCRRLs recommends
that meropenem is the carbapenem of choice for screening for CPE.

It is acknowledged that some laboratories may use ertapenem for screening of CPE, these
isolates will be accepted by the NCPCRRLs for analysis.

Supplementary Testing

- If automated AST systems are used- and the method is not capable of differentiating
  between wild-type and non-wild-type – use EUCAST disc diffusion or manual M.I.C.
  for meropenem/ertapenem
- Rapid molecular analysis or immunochromatography assays

Note rapid assays do not detect all CPE types.
Appendix 3: Algorithm for Carbapenemase Producing Enterobacterales Screening Using Culture Technique

Meropenem Screening Criteria for Enterobacterales Isolates testing as "Carbapenemase not detected:

- Enterobacterales Isolated but carbapenemase not detected on rapid assay
  - EUCAST screening criteria Meropenem (MIC/DD)
    - M.I.C. > 0.125 or DD<28mm – Refer to Reference Laboratory
    - M.I.C. < 0.125 or DD≥28 mm - Report as CPE NOT Detected
Appendix 4: Algorithm for Carbapenemase Producing Enterobacterales Screening Using Direct Molecular Analysis

Enrichment Step required:

1) Rectal swab inoculated into TSB incubated 24hrs at 37 °C
2) Subculture (heavy inoculum of broth using a swab onto selective chromogenic agar 24hrs at 37 °C and follow up all suspect colonies

*Enrichment may be set up on day 1 of the PCR result, in order to ensure more rapid reporting of results.
Appendix 5: Technical Note

The following note is relevant to the interpretation of findings of direct molecular positive results that cannot be confirmed by culture of CPE.

The OXA-48 progenitor has been widely acknowledged to be *Shewanella* spp. A number of class D oxacillinase variants have been identified on the organism’s chromosome. It is hypothesised that these chromosomal genes, from this environmental organism had been mobilised by addition of an insertion sequence and have been transmitted to Enterobacterales in the form of plasmids. The presence of these chromosomal progenitors has implications regarding direct molecular testing. One study, Ceccarelli, et al. (2017) tested 4,440 faecal samples from animals, fish and environment. Isolates containing blaOXA-48-like variants were detected in 92 samples. No Enterobacterales were isolated. *Shewanella* spp. (n=21) were isolated, all of which were confirmed with blaOXA-48-like genes. Plasmid transformation and conjugation experiments were not successful leading the authors to conclude that the genes were likely to be chromosomal.

There have been reports of human intestinal carriage of *Shewanella* spp. simulating carriage of OXA-48 producing Enterobacterales. A patient with previous OXA-48 *Klebsiella pneumoniae* was tested on subsequent admission, with direct real-time PCR blaOXA-48-like genes detected. This sample did not yield CPE on selective chromogenic media. Non-lactose fermenting colonies were isolated on McConkey agar within 25mm of the meropenem disc. These identified as *Shewanella putrefaciens* and had the blaOXA-48-like genes detected on real time PCR. Transformation and conjugation experiments were not successful, suggesting chromosomal location of the gene. Subsequent CPE screens from this patient did not detect CPE genes. Transient carriage of *Shewanella* spp. may have potential for causing “false positives” on direct molecular CPE screening assays.
Appendix 6: Communication With People With Molecular Detection of CPE Not Confirmed by Culture

Information generally consistent with the following should be provided to people who have a positive direct molecular test result:

1. Direct Molecular Positive – Culture Confirmation Pending
   There is a rapid test that shows that you probably are carrying CPE bacteria. This test is usually reliable but we want to do some additional tests to be sure that you have CPE. For the time being we need to work as if you have CPE but we will come back to you in a few days to let you know more and we might need to ask you for another sample.
   Other information regarding CPE can be provided as per information leaflets.

2. Direct Molecular Positive – Culture Confirmed
   The extra work done in the lab shows that you definitely have CPE.
   Other information regarding CPE can be provided as per information leaflets.

3. Direct Molecular Positive – Unconfirmed by Culture but reproducible positive by a second method or by the same method in a second sample.
   With the additional work in the laboratory we have not been able to grow CPE from your sample but the tests for CPE genes are definitely positive. Even though we are not sure you have CPE we think it is safer to take it for the time being that you have CPE and we will offer you some follow up testing once a week for 3 weeks if you have to stay in hospital. If we can’t grow the CPE in any of the three/four follow tests we will make decision that you don’t have CPE and stop taking extra infection prevention and control precautions during your stays in hospital. You will still be offered testing for CPE though anytime you have to come back into hospital.
   Other information regarding CPE can be provided as per information leaflets.

4. Direct Molecular Positive – Not reproducible and not culture confirmed.
   Based on the extra work done in the lab we have not been able to find CPE in your sample so the result of the first test we talked about was a false alarm (sometimes referred to as a false positive). False alarms sometimes cause upset for people but no test is perfect and almost all tests do give false alarms sometimes. So for now we are making a decision that you don’t have CPE. We will stop taking extra infection prevention and control precautions during your stays in hospital. You will still be offered testing for CPE though anytime you have to come back into hospital.
   Other information regarding CPE can be provided as per information leaflets.