UK Standards for Microbiology Investigations

Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases)

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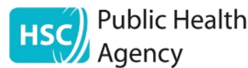

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Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	5/01.06.2022
Issue number discarded	3
Insert issue number	3.1
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Section 8: Referral to reference laboratories	Replaced ‘Anaerobic reference unit (ARU) Cardiff’ with ‘Specialist Antimicrobial Chemotherapy Unit’.

Amendment number/date	4/18.09.2020
Issue number discarded	2.1
Insert issue number	3
Anticipated next review date*	18.09.2023
Section(s) involved	**Amendment**
Whole document	Document has been transferred to a new template. Clarity on different settings when testing for carbapenemase producers added to the document. Headings changed. Clarification of use of selective media with carbapenem disc if chromogenic agar is not available. Whole document restructured for clarity
Section 6. Investigation	Investigation table amended. Updated to include new regulations regarding reporting of acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Interpreting and reporting results for culture amended.

Section 7.2.1 Controls for carbapenemase tests	Control strains producing carbapenemases available from the NCTC amended.
Appendix 1	Flowchart for the detection of carbapenemases on cultured isolates and screening samples amended.
Appendix	Appendix 2 removed. Readers are referred to detailed evaluations published to date.
References	References reviewed and updated.

*Reviews can be extended up to five years subject to resources available.

1. General information

View general information related to UK SMIs.

2. Scientific information

View scientific information related to UK SMIs.

3. Scope of document

The UK SMI gives recommendations on screening and detection of acquired 'carbapenemases' (carbapenem-hydrolysing β-lactamases). It should be used in conjunction with any local documents and PHE's framework of actions to contain carbapenemase-producing Enterobacterales.

This UK SMI should be used in conjunction with other relevant UK SMIs.

This document is intended to refer to different settings when testing for carbapenemase producers:

- Setting 1: screening of clinical samples for the presence of carbapenem resistance in particular carbapenemases
- Setting 2: preliminary detection of carbapenem resistance in cultured isolates from clinical samples.
- Formal confirmation of carbapenem resistance by susceptibility testing of suspected isolates.

We are following EUCAST recommendations for Setting 2 and formal confirmation of carbapenem resistance. There are no agreed international recommendations in place for Setting 1.

4. Background

The term 'carbapenemase' is used to mean any β-lactamase that hydrolyses carbapenems. Carbapenems (any or all of doripenem, ertapenem, imipenem and meropenem) are antimicrobials of last resort and are crucial for preventing and treating life-threatening nosocomial infections. Carbapenemases are clinically important because they destroy, and so may confer resistance to, carbapenems (and usually most other β-lactams). Carbapenemases are found naturally in a few clinically relevant bacteria, such as *Stenotrophomonas maltophilia*, *Aeromonas* species, and 'chryseobacteria', including *Elizabethkingia meningoseptica*[1]. *Acinetobacter baumannii* also has the gene for an intrinsic carbapenemase (OXA-51-like), but this confers reduced susceptibility or resistance to carbapenems only when its expression is up-regulated by genetic reorganisation[2].

In addition, non-susceptibility or resistance to specific carbapenems is an intrinsic characteristic of some Gram-negative bacteria: most non-fermenters are naturally resistant to ertapenem (but not to other carbapenems); *Serratia* species and Proteeae have intrinsic poor susceptibility or low-level resistance to imipenem (but not to other carbapenems).

Studies have shown that *Bacteroides fragilis* can harbour a chromosomally mediated metallo-β-lactamase gene called cfiA (ccrA) which confers resistance to carbapenem[3,4].

This document focuses on acquired carbapenemases. Accurate identification of bacteria to genus or species level will allow laboratories to recognise the producers of intrinsic carbapenemases detailed above.

4.1 Acquired carbapenemases

Acquired carbapenemases are diverse and include three of four Ambler's molecular classes of β-lactamases[5,6].They are detailed below:

Class A enzymes: All hydrolyse carbapenems effectively and are partially inhibited by clavulanic acid. The most widespread carbapenemases in this class are the KPC enzymes; other, less frequently-encountered class A carbapenemases include some GES types (notably GES-5), IMI/NMC-A (in *Enterobacter* species), FRI (in *Enterobacter* species) and SME (in *Serratia marcescens*)[7]. KPC enzymes have been recorded in *A. baumannii* in Central America, and in *P. aeruginosa* in Central and South America, USA, China, the Caribbean, and occasionally in the UK[8-12 13,14].

Class B enzymes: Also known as 'metallo-β-lactamases' (MBLs) or 'metallo-carbapenemases'[5,6]. These differ fundamentally from all other β-lactamases because they require zinc ions in their active sites for activity[15]. Consequently, they are inactivated by metal ion chelators, such as EDTA[16]. The major MBL families encountered in the UK are the NDM, VIM and, less commonly, IMP types. Other types include GIM, SIM, DIM and SPM-1 enzymes, which have been found at a very low frequency in *P. aeruginosa* and *Citrobacter freundii* within the UK.

Class D enzymes: This class comprises many (>400) diverse β-lactamases, a few of which are carbapenemases[5,6,17]. Important carbapenemases within the family include OXA-23, -40, -51, -58 and their variants from *Acinetobacter* species and OXA-48-like enzymes in Enterobacterales; other rarer carbapenem-hydrolysing class D types include OXA-198 in *Pseudomonas* species.

Although some are chromosomally encoded (e.g. NMC-A/IMI and SME), many acquired carbapenemases are plasmid-mediated (especially when found in Enterobacterales), giving potential for spread between strains, species and genera.

Table 1. Carbapenemases currently known to be circulating in the UK by classification, activity and organisms

Enzyme type	Classification by ambler class	Activity spectrum	Organism(s)
KPC	A	All β-lactams	Enterobacterales; rare in *P. aeruginosa*
SME	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*S. marcescens*
NMC–A IMI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species; rare in other Enterobacterales
GES	A	Depends on enzyme variant. Some are ESBLs, others e.g. GES-5 are carbapenemases	*P. aeruginosa* and Enterobacterales
FRI	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	*Enterobacter* species

IMP **VIM** **NDM** GIM, DIM, SIM, SPM-1 (infrequently identified in the UK)	B (metallo-β-lactamases)	All β-lactams except monobactams (aztreonam)	*Pseudomonas* species; *Acinetobacter* species; Enterobacterales
OXA	D	Carbapenems (note that many OXA enzymes are NOT carbapenemases)	*A. baumannii*; Enterobacterales and rare in *P. aeruginosa*

Note: The enzyme types in bold are the five main carbapenemase families found in the UK, the so-called ‘big five’.

Delayed recognition and inappropriate treatment of severe infections caused by carbapenemase producers is associated with increased mortality[18]. Many producers are multi-resistant to non-β-lactam antibiotics including quinolones and aminoglycosides.

The carbapenem MIC ranges for Enterobacterales producing each of the ‘big five’ carbapenemases (KPC, OXA-48-like, NDM, VIM and IMP) span from below the susceptible clinical breakpoints to high-level resistance, and when combined with the diversity of carbapenemase types, this means that few, if any, strategies reliably detect all carbapenemase producers. Nevertheless, the carbapenem MICs of most carbapenemase-producing bacteria will be above the epidemiological cut-off (ECOFF) values defined by EUCAST even if some isolates are not clinically resistant (that is, MICs remain equal to or below the clinical breakpoints). ECOFF values (or breakpoints) mark the limit of the wild-type population by a statistical definition, and isolates with higher MICs/lower zone diameters represent non-wild-type isolates.

The level of carbapenem resistance displayed by some carbapenemase producers is a cause for concern. Higher MICs are observed when carbapenemase producers also lack major porins. Among strains with lower MICs and without porin loss there is potential for carbapenemase producers to spread undetected. This concern is greatest with OXA-48-like enzymes in Enterobacterales, which can give very low level carbapenem resistance[19], without cross-resistance to cephalosporins. KPC enzymes and MBLs tend to confer broader effects on the β-lactam resistance profile of the host strain.

4.2 Complexities of detection of carbapenemase production

Clinical laboratories should have a high index of suspicion while investigating isolates for carbapenemase production and should be alert to two confounders:

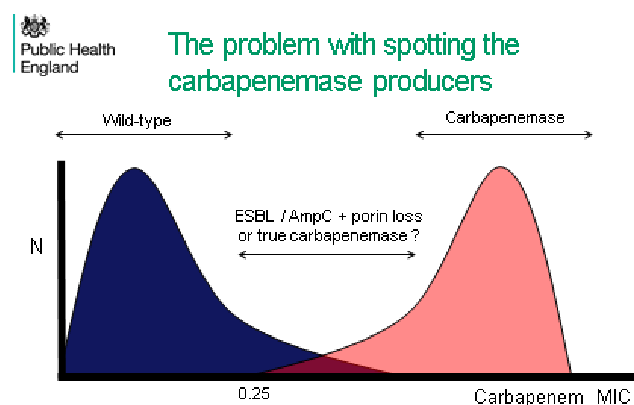
a) not all carbapenem-resistant isolates produce a carbapenemase (resistance can be mediated by other mechanisms, such as the combination of ESBL/AmpC plus impermeability, as below),

b) not all carbapenemase producers are resistant to carbapenems

Carbapenemases are not the only mechanism of acquired resistance to carbapenems but are the most important from a public health perspective. Other mechanisms include the following:

- Enterobacterales with ESBL or AmpC enzymes may lose outer membrane porins (through mutations or other disruptions in chromosomal genes), reducing carbapenem uptake[20]. In contrast to carbapenemases, these combinatorial mechanisms of carbapenem resistance are not transferable between strains (though the contributing ESBL might be) and the porin-deficient mutants may have reduced fitness and be less likely to spread in healthcare settings. This mechanism is seen most often in *Enterobacter* species and *Klebsiella* species, but also occurs in *E. coli* and other genera. It most markedly affects ertapenem; isolates may remain susceptible to other carbapenems at breakpoint concentrations, but often show some degree of reduced susceptibility or resistance, with the level contingent upon the amount of ESBL/AmpC activity and the precise nature of the porin lesion(s) (see Figure 1).

Figure 1. The problem with spotting the carbapenemase producers



Courtesy of Professor Neil Woodford, Public Health England.

- In *P. aeruginosa*, by far the commonest mode of carbapenem resistance is loss of OprD porin, and isolates only resistant to imipenem, but not other β-lactams are certain to have this mechanism. Meropenem, though not imipenem, is also affected by upregulated efflux in *P. aeruginosa*[21]. Most *P. aeruginosa* isolates that are resistant to both imipenem and meropenem will have both mutational mechanisms (perhaps also with derepressed AmpC) rather than a carbapenemase.
- Non-carbapenemase mechanisms have been claimed in *Acinetobacter*, but may reflect failure to detect weak OXA carbapenemases, rather than their absence.

In the face of the diversity of enzyme types, the considerable variation in levels of phenotypic carbapenem resistance (for example, in MIC evaluations), and the added complexity of non-carbapenemase-mediated carbapenem resistance, there is no universally applicable method to detect readily all mechanisms of carbapenem resistance.

The ideal indicator carbapenem is one to which all carbapenemases confer resistance, even when production is insufficient. No single carbapenem satisfies this criterion for all host species (Enterobacterales and non-fermenters).

The strongest advice is for laboratory staff to have a high index of suspicion when screening clinical samples or preliminary detection of carbapenem resistance in cultured isolates for reduced carbapenem susceptibility or resistance (see Figure 1).

All suspected isolates must be followed up with confirmatory tests locally and if necessary submit to a referral laboratory following current EUCAST recommendations.

Identification to genus/species level is highly desirable for the interpretation of resistance patterns. Identify at least to genus level all isolates found resistant to any of the indicator carbapenems, to ensure that reduced susceptibility or resistance is not an intrinsic trait. Identify to species level if the genus is not known to produce intrinsic carbapenemases.

4.2.1 Detection of carbapenem resistance in screening samples (Setting:1)

Culture remains highly useful for the isolation of CPE from stool samples or rectal swabs either as a stand-alone method or as a complement to molecular methods. Molecular methods such as PCR can provide rapid results in as little as 1 hr, which can be a significant advantage, whereas culture typically requires at least 18 hr incubation. When a PCR method indicates the presence of a carbapenemase gene(s), subsequent culture is necessary to determine whether the gene is actually harboured by Enterobacterales rather than other species such as *Pseudomonas* spp., *Acinetobacter* spp. or other glucose non-fermenters[22,23]. Furthermore, isolation of CPE by culture enables antimicrobial susceptibility testing and, when necessary, epidemiological typing. When PCR is negative, culture may be useful to detect CPE with less common carbapenemase genes that may not be targeted by the PCR assay[24].

There is no 'gold standard' method for the isolation of CPE in stool samples or rectal swabs, but a wide range of different culture media has been proposed[25,26] . There are several commercially available chromogenic media designed for the isolation of CPE and/or carbapenem-resistant Enterobacterales (CRE) that are effective for isolation of CPE including those producing OXA-48-like enzymes. Chromogenic media for CPE incorporate antimicrobials for the inhibition of other microorganisms and two or more chromogenic substrates to differentiate key target species or groups of species as coloured colonies[27]. Their exact composition is often undisclosed and is subject to change over time.

It is not possible to provide firm recommendations to use a specific commercial chromogenic medium, however, a review of the published literature can help laboratory staff to make an informed choice. Readers are referred to Perry 2017, which includes a summary of detailed evaluations published to date[27].

Readers are advised to be cautious in the interpretation of study data. In all such studies, the calculation of sensitivity and specificity is based on the supposition that all isolates of CPE will be successfully detected by at least one of the methods under evaluation – although this may not actually be the case. The performance of a particular method may also be exaggerated if it is assessed alongside a relatively poor comparator. Finally, most studies are performed in a single location where a single type of carbapenemase is likely to predominate, and different types of media may show varied performances due to diverse geographical locations.

Despite these limitations, it is possible to draw some general conclusions. Producers of OXA-48-like enzymes are known to be difficult to detect because they frequently show low MICs to carbapenems. Certain media have shown limited sensitivity for

detection of such strains[25,28, 29, 30]. The problem with detection of strains with low carbapenem MICs has led some to advocate the use of chromogenic media designed for detection of ESBL producers [31]. This is not recommended for routine use owing to a lack of specificity in some studies[32], (due to the growth of ESBL-producers) and the inhibition of a proportion of strains with OXA-48-like enzymes that are susceptible to cephalosporins. Since these early studies, better options for specific detection of CPE are now available.

Media which have been evaluated specifically with KPC producers may have not been evaluated fully against certain other carbapenemase producers.

Broth media containing ertapenem or meropenem (at 2 mg/L) have been widely used following early practical recommendations made by the Centers for Disease Control and Prevention (CDC) in 2009 (and before the widespread availability of specific chromogenic media). A number of studies have since demonstrated an inferior performance in terms of both sensitivity and specificity when compared with chromogenic media for CPE[25, 31, 33,]. Furthermore, the CDC broth enrichment method requires an additional day to generate results. More promising results have been obtained by the use of other broth formulations followed by subculture onto chromogenic media. For example, an increased recovery of CPE with OXA-48-like enzymes has been demonstrated by use of an enrichment step using un-supplemented MacConkey broth[30] and nutrient broth plus 10 mg/L temocillin[29]. These findings are based on single reports and further studies are warranted.

Several reports have explored the use of non-selective media (e.g. MacConkey agar) in combination with carbapenem discs. Such methods can be compromised by overgrowth of carbapenem resistant non-carbapenemase producers and the risk of low inocula of CPE that have low carbapenem MICs appearing susceptible to a carbapenem disc. Several studies have shown equivalent performance to chromogenic agar but more recent studies have demonstrated reduced sensitivity. The use of ertapenem discs has greater sensitivity in comparison with other carbapenem discs, at a price of reduced specificity. These methods may be of value in small laboratories which would otherwise have to send samples to a referral laboratory [34,35].

It can be concluded that chromogenic media for CPE have advantages over the CDC broth method or the use of MacConkey-based agars supplemented with a carbapenem or used in conjunction with carbapenem discs. However, it is difficult to establish which, if any, chromogenic medium is optimal for detection of CPE in any particular location due to the different types of carbapenemase that may be encountered and the dominance of particular types in certain geographical regions.

There is very little evidence that extended incubation enhances the sensitivity of chromogenic media for CPE, but there is evidence to show that specificity is decreased[36].

There is only a small amount of evidence to support particular chromogenic media for the detection of carbapenemase-producing *Acinetobacter* species[27,37]. Similarly, there is no specific evidence for detecting carbapenemase-producing *Pseudomonas* species. Media containing ertapenem are not appropriate as *Acinetobacter* spp. are intrinsically resistant to this carbapenem.

This UK SMI recommends use of chromogenic agar for detection of carbapenemase producing Enterobacterales. It is recognised that some laboratories with low

throughput will not be able to maintain stocks of chromogenic media with all the necessary quality assurance. Furthermore, forwarding of samples to a referral laboratory entails a significant increase in turnaround time, which has infection control implications. In such circumstances, alternative methods may be used; however, this should be subject to local risk assessment. These include use of MacConkey or CLED with an ertapenem disc[33,38] using the screening cut-off of 27 mm[39]. Any suspect isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations.

It must be remembered that the EUCAST recommendation cut off of 25mm[40] applies to isolates that are recovered as a confluent growth. If the growth appears lighter than that stipulated for disc testing by EUCAST, colonies will require formal susceptibility testing.

4.2.2 Preliminary detection of carbapenem resistance from clinical samples (Setting 2)

This UK SMI supports the EUCAST recommendation to use meropenem as the indicator carbapenem as it offers the best compromise between sensitivity and specificity[41]. If inclusion of a meropenem disc in the routine first-line susceptibility panel is impractical, co-amoxiclav must be included in the testing panel on all such isolates. Any suspect isolates with co amoxiclav resistance or reduced susceptibility to meropenem must be subjected to full susceptibility testing in accordance with EUCAST recommendations. Although ertapenem has greater sensitivity, it is not recommended because it has poor specificity for carbapenemase producers (with the exception of screening on MacConkey/CLED). Faropenem has also been reported to show good sensitivity for detecting carbapenemase producers and the discs are commercially available[42].

4.2.3 Difficulties with detection of carbapenemase production in non-fermenters

Acquired carbapenemases are encountered in *Acinetobacter* species, *Pseudomonas* species (most commonly, though not exclusively in *P. aeruginosa)* and in other non-fermenters[5,6,17].

Consider testing meropenem, imipenem or doripenem against all clinically-significant isolates, as these have the right combination of sensitivity and specificity.

Do not use ertapenem because these species are intrinsically resistant to this carbapenem.

Carbapenem-resistant *Acinetobacter* species:

Isolates can usually be reported as likely OXA-carbapenemase producers without supplementary tests, unless the affected patient has been hospitalised overseas recently (for example, in the Middle-East or Indian subcontinent) in which case imipenem-EDTA or meropenem/dipicolinic acid (DPA) synergy[43,44] (>=8-fold) may be of value and could be sought to rule out the presence of a metallo-carbapenemase.

EDTA/DPA synergy testing in *Acinetobacter* species:

Strong EDTA or dipicolinic acid (DPA) synergy (>=8-fold) correlates well with MBL production in *Acinetobacter* species, although many OXA carbapenemase producers

show a weaker false EDTA synergy probably because metal ions are needed to maintain some OXA enzymes in an active conformation. A high false-positive rate in EDTA synergy tests may also arise due to growth inhibition of *Acinetobacter* species by EDTA alone.

Carbapenem-resistant *Pseudomonas* species:

Isolates resistant only to carbapenems can be inferred to have mutational resistance and need not be investigated further. However, isolates resistant to all relevant carbapenems (that is, imipenem, meropenem and doripenem), ceftazidime, ceftolozane/tazobactam and piperacillin/tazobactam may be tested for strong (>=8 fold) imipenem-EDTA or meropenem/DPA synergy[43,44]. Positives require further investigation using a molecular or an immunochromatographic assay. False-positive 'MBL' synergy is common and probably reflects the disorganising effects of EDTA on the outer membrane of some strains.

Aztreonam susceptibility in *Pseudomonas* species:

Susceptibility to aztreonam combined with resistance to carbapenems and other β-lactams is the 'classic' MBL phenotype, but many MBL producers are resistant to aztreonam owing to additional mechanisms meaning that the 'classic' pattern is not always seen. It should be noted that although carbapenem resistance is very common in isolates from cystic fibrosis patients, acquired carbapenemases are rare in this patient group[45].

Detection of KPC, OXA-48-like and GES-5 enzymes in non-fermenters:

GES-5, SIM, DIM-1, SPM-1 and the class D OXA-181 enzyme have also been reported in *P. aeruginosa* isolates (representing <10% of confirmed carbapenemase-producing *Pseudomonas* species referred to the AMRHAI Reference Unit, PHE unpublished data)[46,47]. At this time, it is not possible to recommend sensitive and specific phenotypic criteria to infer the presence of KPC, OXA-48-like and GES-5 non-metallo-carbapenemases in non-fermenters.

4.3 Difficulties around reporting carbapenem susceptibility for carbapenemase producing Enterobacterales

There is a division of opinion about the reporting of carbapenem susceptibility for carbapenemase producers. There has been expert opinion for several years that all carbapenemase producers should be reported resistant to all carbapenems, irrespective of susceptibility test results. However, this approach has been superseded by EUCAST recommendation of reporting susceptibility testing in accordance with breakpoints[40].

EUCAST have taken the view to adopt the low breakpoints, carbapenem susceptibility results can be taken at face value, and that carbapenems can be used as therapy so long as carbapenemase producers appear susceptible *in vitro*.

There is a need for more evidence of clinical success for carbapenems against carbapenemase producers with low MICs. Furthermore, 'susceptible' MIC and zone test results for carbapenemase producers often have poor reproducibility, with discrepant results between methods. There is a need to improve the quality of laboratory testing and reporting[48].

The best advice is to apply utmost caution if carbapenems are to be used in severe infections due to known carbapenemase producers, and to avoid using them as monotherapy[18].

New β-lactam and β-lactam/β-lactamase inhibitor combinations that have activity against some carbapenemases (principally KPC types, not MBLs) are under development or have been licensed by the European Medicines Directorate[49].

4.4 Other methods for detection of carbapenemases

Other methods that may also be considered for detecting likely carbapenemase producers include:

Carbapenem Inactivation Method (CIM): a phenotypic test developed to detect carbapenemase activity by incubating a carbapenem disc within the test bacterial suspension. Following two hours incubation the disc is placed on an agar plate inoculated with *E. coli* ATCC 25922 and incubated for a minimum of six hours. Inactivation of the carbapenem due to carbapenemase activity will produce no zone around the disc, whereas no carbapenemase activity will produce a zone[50]. Different variants of the CIM test have been published[51], which report improvements over the original version.

Biochemical tests: Some tests provide rapid (<2hr) detection of carbapenem hydrolysis. These tests are based on the classical acidometric penicillinase test whereby the pH change arising from carbapenem hydrolysis results in a red to yellow colour change with phenol red and a blue to green/yellow change with bromophenol blue[41]. These tests have been reported to work well for detecting carbapenemases in Enterobacterales and *Pseudomonas* species[52] but can be less reliable for *Acinetobacter* species[53] depending on the version used.

Modified Hodge Test (MHT) or ‘Cloverleaf’ test: is a phenotypic bioassay to assess the ability of a test strain to hydrolyse carbapenems. However, it is not recommended by EUCAST due to concerns over its specificity and sensitivity, with several proven carbapenemase producers giving consistently negative results[41].

Synergy tests are most effective for members of the Enterobacterales. Although EDTA/dipicolinic acid-based synergy tests may also be useful for non-fermenters, EDTA-based tests give a high proportion of false-positive results for these organisms. Check individual test instructions for use to ensure that the product can be used for non-fermenters.

Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-ToF MS): MALDI-ToF MS offers the potential to detect carbapenemase production via detection of mass changes that follow hydrolysis of a carbapenem molecule[54-56]. It requires pre-incubation of a carbapenem with the test organism but can be completed in less than 2 hours and gives a ‘Yes / No’ result. Several ‘in-house’ versions have been published and the methodology has been commercialised as the MBT STAR-Carba assay[57].

Commercial assays: There are numerous commercial PCR-based and immunochromatographic assays available for detection of acquired carbapenemase genes or epitopes from bacterial cultures, screening swabs or clinical specimens. This UK SMI recommends implementation of an assay to detect at least the ‘big 4’ families (KPC, OXA-48-like, NDM and VIM). PHE has published a report to provide the

evidence base for diagnostic laboratories to facilitate an informed choice on the commercial assay to implement[58].

Automated or semi-automated systems generally can be used to detect carbapenem resistance though the ability of software to infer and warn correctly of the presence of carbapenemases is variable, especially for OXA-48-like enzymes[59]. For this reason, the underlying resistance mechanisms inferred by expert algorithms should be viewed with caution; some warn of potential carbapenemase production by every carbapenem-resistant isolate (good sensitivity and poor specificity) while others attempt to distinguish true carbapenemase producers from those with other mechanisms, which reduces their sensitivity. Studies on isolates with KPC carbapenemases indicate poor agreement between the MICs found by automated susceptibility systems[10,59,60]

4.5 Summary of UK SMI recommendations

Setting 1: Recommendations for screening of clinical samples

This UK SMI recommends the use of chromogenic agar for detection of carbapenemase producers. However, subject to local risk assessment laboratories with low throughput should consider using MacConkey or CLED with an ertapenem disc using zone size cut off of 27mm[39] (see section 4.2.1).

Setting 2: Recommendation for preliminary detection of carbapenem resistance in cultured isolates from clinical samples

This UK SMI recommends the routine use of meropenem as the indicator carbapenem. In situations where indicator carbapenem cannot be included in the primary susceptibility testing panel, any suspect isolates with co-amoxiclav resistance or reduced susceptibility to meropenem isolates must be subjected to full susceptibility testing in accordance with EUCAST recommendations (see section 4.2.2).

5. Safety considerations[61-77]

5.1 Specimen collection, transport and storage[61-66]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

5.2 Specimen processing[61-73,75-77]

Hazard Group 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[69].

Refer to current guidance on the safe handling of all organisms discussed in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

6. Investigation

6.1 Culture of carbapenemase producers

6.1.1 Specimen type

Any sample type can be used however rectal specimens (swabs with visible faecal material or discoloration) are the most sensitive for detecting CPE colonisation or a faecal specimen. If a rectal swab is not feasible or acceptable any clinical specimens such as blood, wound swab or urine is suitable.

6.1.2 Pre-laboratory processes

Specimen collection, transport and storage:

For safety considerations refer to Section 5.

Collect specimens before starting antimicrobial therapy where possible[78].

A single rectal swab is sufficient to determine CPE colonisation status on admission unless patients have previously been identified as CPE positive (in which case hospitals may wish to treat these patients as persistently colonised regardless of screening). In addition, if the patient has been hospitalised in a country with reported high prevalence of carbapenemase producers, include samples from any wounds or device-related sites[78].

In high risk situations, single swabs have poor negative predictive value. While recognising the limited sensitivity of a single swab the PHE's CPE framework recommends that a single swab is sufficient to determine the CPE colonisation status on admission in otherwise non-high risk situations.

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium[79-83].

Specimens should be transported and processed as soon as possible[78].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[78].

6.1.3 Laboratory processes (analytical stage)

Culture

Sample preparation

For safety considerations refer to Section 5.

Specimen processing

Stool samples and rectal swabs are used for screening for carbapenemases but generally rectal swabs are received.

Table 2: Investigation

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Setting 1: Screening of clinical samples[a]: Any condition + detection of carbapenem resistance/ carbapenemase production	Any sample	Chromogenic agar with carbapenem [b]/ Chromogenic medium for CPE **OR**	Refer to manufacturer's instructions [b]				Carbapenemase producing Enterobacterales
		If chromogenic agar not available, MacConkey / CLED agar + 10µg ETP disc [c] [39]	35-37	Aerobic	16-48hr	≥16hr [d]	Any carbapenemase producing Gram negative organism [e]
Setting 2: Preliminary detection of carbapenem resistance in cultured isolates from clinical samples: Routine Susceptibility testing of clinical samples	Any sample	Routine susceptibility testing media against co-amoxiclav [f] (minimum) or ideally meropenem.	35-37	Aerobic	18-24hr	≥18hr	Any carbapenemase producing Gram negative organisms

a Any suspect carbapenem resistant isolate should be followed up with susceptibility testing in accordance with EUCAST recommendations.
b For chromogenic media, refer to manufacturer's instructions for recommended incubation times.
c Ertapenem = ETP

d This UK SMI recommends zone size cut off of 27mm. Any suspect isolates should be submitted for full sensitivity testing in accordance with EUCAST recommendations.

e When investigating an outbreak of *Acinetobacter* species the incubation may have to be increased to 48 hours. Carriage of *Acinetobacter* using this method may be reduced.

f All co-amoxiclav resistant isolates should be screened for resistance or reduced susceptibility to carbapenems in accordance with EUCAST recommendations (if meropenem has not been tested).

Identification

Refer to UK SMI mentioned below for organism identification.

Minimum level of identification in the laboratory

All Enterobacterales	species level ID 16 - Identification of Enterobacterales
Pseudomonas species *Acinetobacter* species	species level ID 17 - Identification of *Pseudomonas* species and other non-glucose fermenters

Note: From 1 October 2020 diagnostic laboratories in England will have a duty to report acquired carbapenemase-producing Gram-negative bacteria identified in human samples to PHE. Further to this, diagnostic laboratories in England will have a duty to report the results of any antimicrobial susceptibility test and any carbapenem resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations[84], where this is known to the operator.

Technical limitations

Specimen containers: UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

Quality control: The carbapenem discs that are used should be quality control tested, using disc diffusion methods and quality control strains, as described in accordance with EUCAST recommendations guideline[85]. Follow guidelines for frequency of disc quality control testing and corrective action if results are out of range.

Chromogenic media: Chromogenic media are affected by light and plates should be stored in the dark and not left in the light before or after inoculation. Inoculate culture media with a rectal swab or other sample (refer to Q 5 – Inoculation of culture media in bacteriology). Incubation times for chromogenic media should be as recommended by the manufacturers.

Microscopy

N/A

6.1.4 Post-laboratory processes (reporting procedures)

Culture

Interpreting and reporting results

Setting 1: Screening of clinical samples report culture result as:

Positive report

“Carbapenem-resistant / non-susceptible organism isolated. Further report on susceptibility mechanism to follow”.

Negative report

“Carbapenem-resistant / non-susceptible organism not isolated”

Setting 2: Preliminary detection of carbapenem resistance in cultured isolates report as usual following confirmation of susceptibility testing.

Culture reporting time

Interim or preliminary results should be issued on detection of clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Microscopy

Interpreting and reporting results

N/A

Gram stain

N/A

Microscopy reporting time

N/A

7. Antimicrobial susceptibility testing

Use the media recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and refer to the respective guidelines.

Species identification is highly desirable to allow interpretation of results. Recommended clinical breakpoints for the carbapenems may be updated annually and should be sought from the links above.

Variations in enzyme expression and interplay with other host strain factors, mean that not all carbapenemase producers will show phenotypic resistance, that is, MICs for some or all carbapenems may lie below the clinical breakpoints or zone size diameters may be larger. Hence reliance on these values for detection of producers lacks sensitivity. EUCAST has therefore recommended screening cut-off values for detecting putative CPE[41].

7.1 Cultured isolates of Enterobacterales

Ideally all clinically significant isolates of Enterobacterales should be tested against a carbapenem. As a minimum testing for carbapenem resistance should be undertaken on all isolates from 'high-risk' patients and settings in accordance with current national guidance and any Enterobacterales isolates found resistant to co-amoxiclav or *Pseudomonas* isolates resistant to piperacillin-tazobactam.

Perform formal susceptibility testing in accordance to EUCAST recommendations. Refer to EUCAST guidelines for breakpoint information.

Laboratories should undertake further tests if automated systems flag any non-susceptibility to a carbapenem, irrespective of the expert interpretation given (unless it is explained by intrinsic resistance). Enterobacterales isolates resistant to the indicator carbapenem by clinical breakpoint or otherwise positive by the EUCAST screening criteria (see section 7) should be subjected to confirmatory tests[41].

Perform a molecular or immunochromatographic assay for at least the detection of KPC, OXA-48-like, NDM and VIM carbapenemase families on isolates.

If required, perform carbapenemase confirmatory tests as recommended by EUCAST on isolates found resistant or to have reduced susceptibility to the indicator carbapenem.

EUCAST advocate that supplemental tests to confirm carbapenemase production are unnecessary for individual patient management; the only test needed is MIC determination – either by agar or broth dilution, or by use of gradient strip methods[6,59]. The risk of onward spread may vary with underlying resistance mechanisms. EUCAST indicate the value of supplemental testing for infection prevention and control purposes, and for local epidemiological investigations.

7.2 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

7.2.1 Controls for carbapenemase tests

Quality control of the carbapenem discs used in the screening should follow standard EUCAST recommendations.

Positive controls should be used to ensure the performance of carbapenemase confirmatory tests. Various strains, including strains that express known carbapenemases and EUCAST recommended control strains are available from Public Health England's National Collection of Type Cultures (NCTC) (https://www.phe-culturecollections.org.uk/media.aspx?pid=182182). The NCTC online catalogue can be searched using the NCTC numbers listed below. Alternatively, some may be obtained commercially from other suppliers.

Table 3. Selected control strains producing carbapenemases available from the NCTC

Class A Carbapenemases		
Organism	**NCTC strain reference**	**Characteristics**
Klebsiella pneumoniae	NCTC 13438	Member of the international ST258 clone producing KPC-3 non-metallo-carbapenemase
	NCTC 14327	KPC-3 non-metallo-carbapenemase
	NCTC 14384	KPC-33 non-metallo-carbapenemase
Escherichia coli	NCTC 13919	GES-5 non-metallo-carbapenemase
	NCTC 14320	KPC non-metallo-carbapenemase IMP metallo-carbapenemase OXA-48-like non-metallo-carbapenemase

	NCTC 14321	KPC non-metallo-carbapenemase OXA-48-like non-metallo-carbapenemase
Serratia marcescens	NCTC 13920	SME-4 non-metallo-carbapenemase
Enterobacter cloacae / complex	NCTC 13922	NMC-A non-metallo-carbapenemase
	NCTC 13925	IMI-2 non-metallo-carbapenemase
	NCTC 14322	KPC-4 non-metallo-carbapenemase
	NCTC 14336	KPC-2 non-metallo-carbapenemase
Enterobacter asburiae	NCTC 14055	FRI-2 non-metallo-carbapenemase
Class B Carbapenemases (Metallo-β-lactamases)		
Organism	**NCTC strain reference**	**Characteristics**
Pseudomonas aeruginosa	NCTC 13437	VIM-10 metallo-carbapenemase; VEB-1 ESBL
	NCTC 13921	SPM-1 metallo-carbapenemase
	NCTC 14361	SIM metallo-carbapenemase
Pseudomonas guariconensis	NCTC 14056	DIM-1 metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13439	VIM-1 metallo-carbapenemase
	NCTC 13440	VIM-1 metallo-carbapenemase
	NCTC 13443	NDM-1 metallo-carbapenemase
	NCTC 14323	NDM-1 metallo-carbapenemase; OXA-48 non-metallo-carbapenemase
	NCTC 14331	NDM-1 metallo-carbapenemase

	NCTC 14332	NDM-1 metallo-carbapenemase; OXA-232 non-metallo-carbapenemase
	NCTC 14334	IMP-4 metallo-carbapenemase
	NCTC 14337	IMP-1 metallo-carbapenemase
Escherichia coli	NCTC 13476	IMP-type metallo-carbapenemase
	NCTC 14320	IMP metallo-carbapenemase; KPC non-metallo-carbapenemase; OXA-48-like non-metallo-carbapenemase
	NCTC 14325	NDM-7 metallo-carbapenemase
	NCTC 14333	NDM-4 metallo-carbapenemase
	NCTC 14339	NDM-5 metallo-carbapenemase
Salmonella Seftenberg	NCTC 13953	NDM-1 metallo-carbapenemase
Enterobacter cloacae	NCTC 14326	VIM-1 metallo-carbapenemase
	NCTC 14328	VIM-4 metallo-carbapenemase
Citrobacter freundii	NCTC 14089	GIM-1 metallo-carbapenemase
Class D Carbapenemases (OXA carbapenemases)		
Organism	**NCTC strain reference**	**Characteristics**
Acinetobacter baumannii	NCTC 13301	OXA-23-like (and intrinsic OXA-51-like)
	NCTC 13302	OXA-25-like (OXA-24/40-like) (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13303	OXA-26 (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13304	OXA-27 (and intrinsic OXA-51-like) non-metallo-carbapenemases

	NCTC 13305	OXA-58-like (and intrinsic OXA-51-like) non-metallo-carbapenemases
	NCTC 13420	OXA-51-like non-metallo-carbapenemase
Klebsiella pneumoniae	NCTC 13442	Sequence type 353 with OXA-48-like
	NCTC 14323	OXA-48 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14330	OXA-181 non-metallo-carbapenemase
	NCTC 14332	OXA-232 non-metallo-carbapenemase; NDM-1 metallo-carbapenemase
	NCTC 14335	OXA-232 non-metallo-carbapenemase
Escherichia coli	NCTC 14320	OXA-48-like non-metallo-carbapenemase; IMP metallo-carbapenemase; KPC non-metallo-carbapenemase9
	NCTC 14321	OXA-48-like non-metallo-carbapenemase; KPC non-metallo-carbapenemase
	NCTC 14324	OXA-484 non-metallo-carbapenemase
	NCTC 14329	OXA-244 non-metallo-carbapenemase
	NCTC 14338	OXA-48 non-metallo-carbapenemase
Salmonella Typhimurium	NCTC 13954	OXA-48 non-metallo-carbapenemase, as mediated by the pOXA-48a-like plasmid

Note: Either *Escherichia coli* NCTC 10418 or NCTC 12241 (equivalent to ATCC 25922) should be used as a negative control in confirmation tests.

A lenticule® disc including NCTC positive controls for the 'big 5' carbapenemases is available from NCTC (https://www.phe-culturecollections.org.uk/products/bacteria/antimicrobial-resistance-gene-controls.aspx). To assist with local validation of in-house or commercial molecular and

immunochromatographic assays a panel of CPE isolates (NCTC 14320 – NCTC 14339 consecutively, and listed above) representing common variants of KPC, OXA-48-like, NDM, VIM and IMP carbapenemases known to be circulating in the UK can also be obtained from the NCTC.

8. Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see PHE user manuals and request forms.

Frontline diagnostic laboratories are strongly recommended to implement a PCR or immunochromatographic assay for detection of the 'big 4' carbapenemase families[58] in any isolate that appears to be resistant to the indicator carbapenem. Some PHE Specialist Laboratories offer referral services at a regional level, and then refer selected isolates onwards to the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit in Colindale. Laboratories using this regional service should not submit isolates to AMRHAI directly. All carbapenemase producers confirmed by diagnostic laboratories or PHE Specialist Laboratories from normally sterile sites only should be referred to AMRHAI for inclusion in the national strain archive. Refer to PHE's Bacteriology Reference Department user manual for up-to-date guidance on bacterial isolates that should be referred to the AMRHAI Reference Unit, turnaround times, transport procedure and the other requirements.

From 1 October 2020, diagnostic laboratories in England will have the duty to report the following to PHE:

- Acquired carbapenemase-producing Gram-negative bacteria isolated from human samples
- The results of any antimicrobial susceptibility test and any resistance mechanism identified in any of the causative agents listed in Schedule 2 of the Regulations.

Contact the appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Bacteriology Reference Department
National Infection Service
Public Health England
61 Colindale Avenue
London
NW9 5EQ

https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services

Telephone: +44 (0) 208 3277887

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Wales

Specialist Antimicrobial Chemotherapy Unit
Public Health Wales Microbiology Cardiff
University Hospital of Wales
Heath Park
Cardiff
CF14 4XW

Specialist Antimicrobial Chemotherapy Unit (SACU) 2021 - Public Health Wales (nhs.wales)

Scotland

https://www.hps.scot.nhs.uk/a-to-z-of-topics/reference-laboratories/#publications

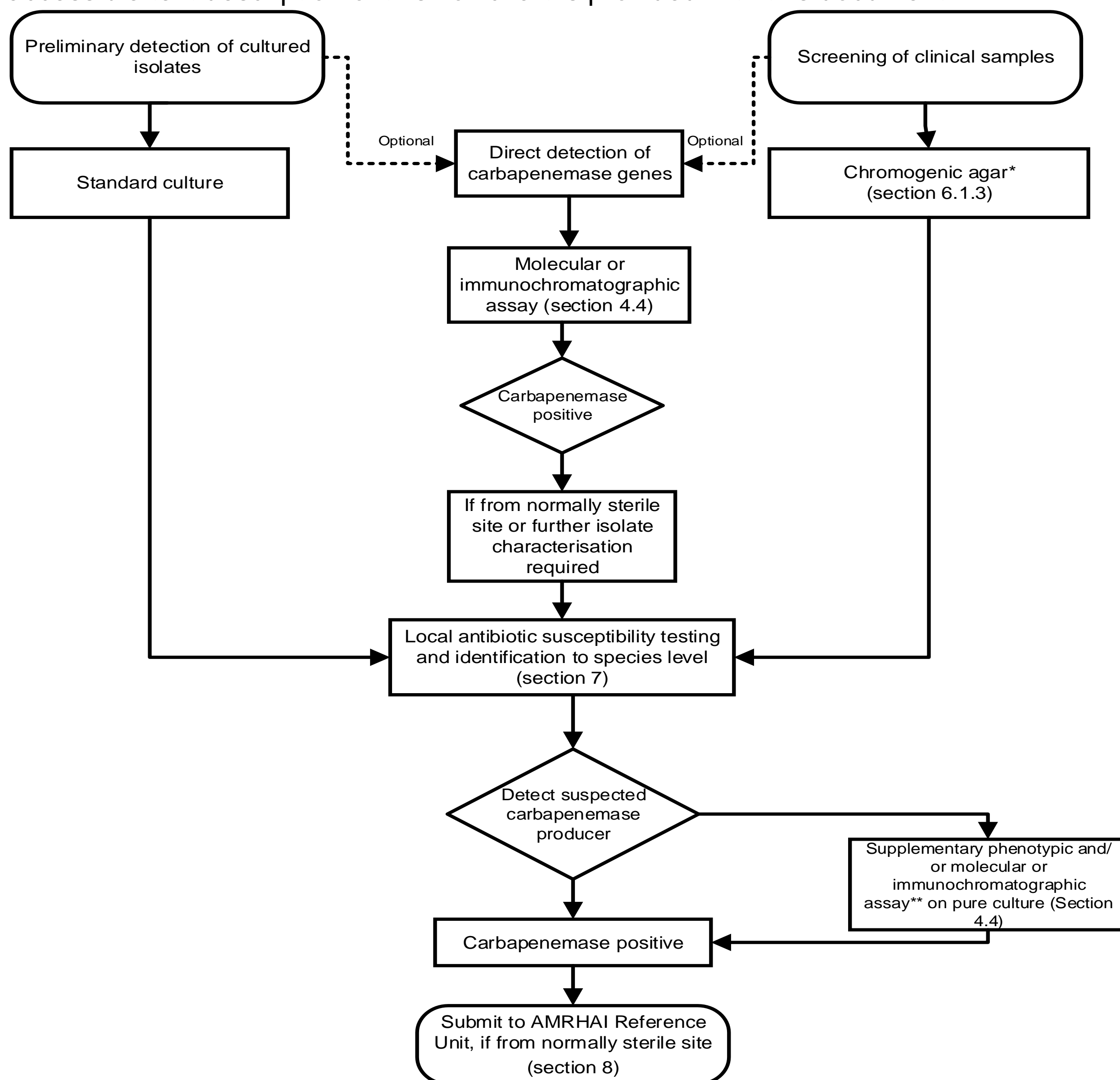
Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

Appendix 1: Flowchart for the detection of carbapenemases on cultured isolates and screening samples

An accessible text description of this flowchart is provided with this document



*If not available use MacConkey / CLED agar + 10µg ETP disc.
**As a minimum perform molecular or immunochromatographic detection of the 'big 4' (KPC, OXA-48-like, NDM and VIM). Isolates that are negative for the 'big 4' carbapenemase families should also be referred to the AMRHAI Reference Unit to rule out presence of rarer carbapenemase families.

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