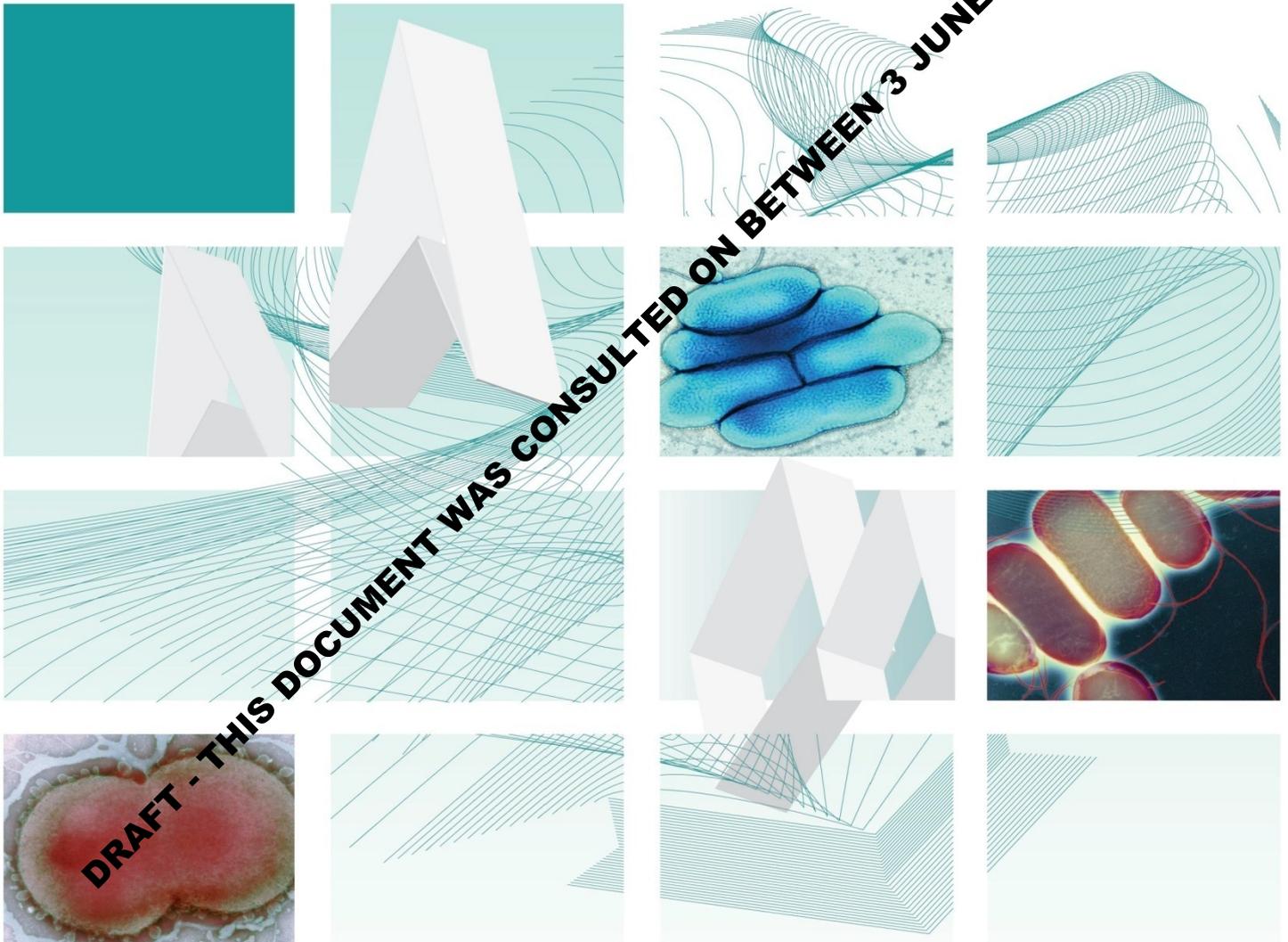




# UK Standards for Microbiology Investigations

## Screening and Detection of Bacteria with Carbapenem Hydrolysing $\beta$ -lactamases (Carbapenemases)

**DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 3 JUNE - 15 JULY 2015**



## Acknowledgments

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For further information please contact us at:

Standards Unit  
Microbiology Services  
Public Health England  
61 Colindale Avenue  
London NW9 5EQ  
E-mail: [standards@phe.gov.uk](mailto:standards@phe.gov.uk)

Website: <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

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For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment table

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Each 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](mailto:standards@phe.gov.uk).

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

Amendment No/Date.	# <tab+enter>/dd.mm.yy <tab+enter>
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<b>Section(s) involved</b>	<b>Amendment</b>

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## UK SMI<sup>#</sup>: scope and purpose

### Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

### Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post-analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

### Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

### Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a

<sup>#</sup> Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

### Patient and public involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professional scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

### Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

### Legal statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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### Suggested citation for this document

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## Scope of document

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### Type of specimen

Stool, rectal or peri-rectal swabs, clinical specimens such as blood, wounds or urine

### Scope

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This SMI describes and gives recommendations on screening for and detection of 'carbapenemases' (carbapenem-hydrolysing  $\beta$ -lactamases). It should be used in conjunction with any local documents and PHE's *Acute trust toolkit for the early detection, management and control of carbapenemase-producing Enterobacteriaceae*, which is available at

[www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/529227/Acute\\_trust\\_toolkit\\_for\\_the\\_early\\_detection.pdf](http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/529227/Acute_trust_toolkit_for_the_early_detection.pdf).

This SMI document will focus solely on acquired carbapenemases. It includes guidance on referrals to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMR/HAI) Reference Unit and its Specialist Microbiology Laboratories.

This SMI should be used in conjunction with other SMIs.

### Introduction

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The term 'carbapenemase' is used to mean any  $\beta$ -lactamase that hydrolyses carbapenems ie any or all of doripenem, ertapenem, imipenem and meropenem. These carbapenems are antimicrobial drugs of last resort and are crucial for preventing and treating life-threatening nosocomial infections. Of clinical concern, many carbapenemases confer resistance or reduced susceptibility to all or nearly all members of the  $\beta$ -lactam class, not just to carbapenems.

Carbapenemases are intrinsic (found naturally) in a few clinical bacteria, such as *Stenotrophomonas maltophilia*, *Aeromonas* species, and 'chryseobacteria', including *Elizabethkingia meningoseptica*. *Acinetobacter baumannii* also has the gene for an intrinsic carbapenemase (OXA-51like), but this confers reduced susceptibility or resistance to carbapenems only when its expression is up-regulated by genetic reorganisation.

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 Proteaeae have intrinsic poor susceptibility or low-level resistance to imipenem (but not to other carbapenems).

This document however, 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.

### Acquired carbapenemases

Acquired carbapenemases are diverse (see <http://www.lahey.org/studies>) and include members of three of Ambler's four molecular classes of  $\beta$ -lactamases<sup>1-3</sup>. They are as follows:

## Screening and Detection of Bacteria with Carbapenem-Hydrolysing $\beta$ -lactamases (Carbapenemases)

- class A enzymes: Some of these enzymes are chromosomally encoded (NMC-A, SME, and IMI-1) and others are often plasmid-encoded (KPC, IMI-2, GES, and their derivatives), but they all hydrolyse carbapenems effectively and are partially inhibited by clavulanic acid<sup>4</sup>. The most widespread carbapenemases in this class are the KPC enzymes (with KPC-2 and KPC-3 variants being the most prevalent), which are now endemic in parts of the USA, Greece, Italy, Israel and China, and are increasingly encountered elsewhere, including in the UK<sup>2,3,5,6</sup>. Other, less-frequently-encountered class A carbapenemases include some GES types (notably GES-5), IMI/NMC-A (in *Enterobacter*), and SME (in *Serratia*).
- class B enzymes: Also known as 'metallo- $\beta$ -lactamases (MBLs) or metallo-carbapenemases'<sup>1-3</sup>. These differ fundamentally from all other  $\beta$ -lactamases because they require zinc ions in their active sites for activity<sup>7</sup>. Consequently they are inactivated by metal ion chelators, such as EDTA<sup>8</sup>. The major MBL families encountered in the UK are the NDM, VIM and, less commonly, IMP types. Other types include AIM, GIM and SIM enzymes, which at the time of writing of this BSOP; have not yet been detected in the UK. DIM-1 and SPM-1, have each been found in single isolates of *P. aeruginosa* in the UK.
- class D enzymes: This class comprises many (>100) diverse  $\beta$ -lactamases, few of which are carbapenemases<sup>1-3,9,10</sup>. Important carbapenemases within the family include OXA-23, -40, -51 and -58 and their variants from *Acinetobacter* species, OXA-48 (identified mostly in Mediterranean and European countries and in India) and related enzymes in Enterobacteriaceae; other rarer carbapenem-hydrolysing class D types include OXA-198 in *Pseudomonas* species.

Many acquired carbapenemases are plasmid-mediated (especially when found in Enterobacteriaceae), giving potential for spread between strains, species and genera.

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

- enterobacteriaceae with ESBL or AmpC enzymes may lose outer membrane porins (through mutations or other disruptions in chromosomal genes), reducing carbapenem uptake<sup>11</sup>. 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).
- 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  $\beta$ -lactams are certain to have this mechanism. Meropenem, though not imipenem, is also affected by upregulated efflux in *P. aeruginosa*<sup>12</sup>. Most *P. aeruginosa* isolates that are resistant to both imipenem and meropenem will have both of these

## Screening and Detection of Bacteria with Carbapenem-Hydrolysing $\beta$ -lactamases (Carbapenemases)

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.

**Table 1: Carbapenemases by Classification, Activity and Organisms**

Enzyme Type	Classification by Ambler Class	Activity Spectrum	Organism(s)
<b>KPC</b>	A	All $\beta$ -lactams	Enterobacteriaceae, <i>P. aeruginosa</i> , <i>A. baumannii</i>
<b>SME</b>	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	<i>S. marcescens</i>
<b>NMC-A IMI</b>	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	<i>Enterobacter</i> species
<b>GES</b>	A	Imipenem and 3rd/4th cephalosporins	<i>P. aeruginosa</i> and Enterobacteriaceae
<b>IMP VIM NDM</b>	B (metallo- $\beta$ -lactamases)	All $\beta$ -lactams except monobactams (aztreonam)	<i>Pseudomonas</i> species <i>Acinetobacter</i> species Enterobacteriaceae
<b>AIM, GIM, SIM, (not detected in the UK yet)</b>			
<b>DIM, SPM</b>			
<b>OXA</b>	D	Weakly active against carbapenems	<i>A. baumannii</i> , Enterobacteriaceae and rare <i>P. aeruginosa</i>

**Note:** All the enzyme types highlighted in red are the 5 main carbapenemase families found in the UK, the so-called 'big five'.

Carbapenemases are clinically important because they destroy (and so may confer resistance to carbapenems and usually most other  $\beta$ -lactams). Delayed recognition and inappropriate treatment of severe infections caused by carbapenemase producers is associated with increased mortality<sup>13</sup>. Many producers are multi-resistant to non- $\beta$ -lactam antibiotics including quinolones and aminoglycosides.

A simple 'Carbapenemase: Yes or No' result is sufficient for most diagnostic laboratories and infection prevention and control teams, with positive isolates referred for further investigation. All carbapenems are substrates for all carbapenemases, but resistance is often low level, complicating detection and interpretation.

The ranges of carbapenem MICs for Enterobacteriaceae producing each of the 'big five' carbapenemases (KPC, OXA-48, NDM, VIM and IMP) span from below the susceptible 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 MICs of carbapenems for most carbapenemase-producing bacteria will be above the epidemiological cut-off (ECOFF) values defined by EUCAST even if some isolates are not clinically resistant (ie MICs remain equal to or below the clinical breakpoints). ECOFFs 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.

When seeking carbapenemases, clinical laboratories should have a high index of suspicion and be alert to two confounders:

- (i) 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 above),
- (ii) not all carbapenemase producers are resistant to carbapenems

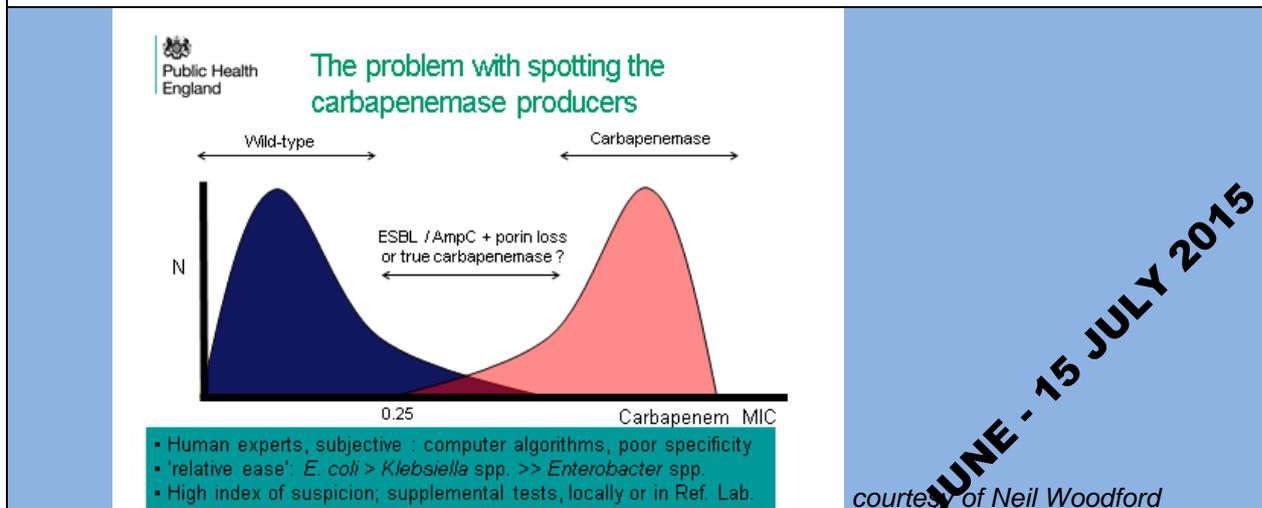
The level (or lack) of carbapenem resistance displayed by some carbapenemase producers is a genuine cause for concern. Higher MICs are observed when producers also lack major porins, but this indicates potential for carbapenemase genes to spread undetected among normally-permeable strains. This concern is greatest with OXA-48-like enzymes in Enterobacteriaceae, which can give very low level carbapenem resistance, without cross-resistance to cephalosporins. KPC enzymes and MBLs tend to confer broader effects on the  $\beta$ -lactam resistance profile of the host strain.

Concerns about carbapenemases mean that all clinically-significant Gram negative bacteria on those patients deemed to be from a high risk area or in contact with known carriers or multi resistant isolates should be screened routinely for susceptibility to at least one indicator carbapenem. Although ertapenem is the most sensitive indicator of likely carbapenemase production, it is also the analogue most affected by porin-mediated mechanisms and so is the least specific; it is also inappropriate for use with non-fermenters.

### Overview of the strategy for recognising potential carbapenemase producers

- the aims are: (i) to recognise carbapenemase producers effectively; and (ii) to distinguish them from isolates that are resistant to carbapenems by virtue of other mechanisms
- in the face of the diversity of enzyme types, the considerable variation in levels of phenotypic carbapenem resistance (eg in MIC evaluations), and the added complexity of non-carbapenemase-mediated carbapenem resistance, there is no universally applicable method able to realize these aims
- the ideal indicator carbapenem is one to which all carbapenemases confer resistance, even when production is scanty. No single carbapenem satisfies this criterion for all host species (Enterobacteriaceae and non-fermenters)
- this SMI seeks to document current opinion and best options available, however imperfect. The strongest advice is for laboratory staff to have a high index of suspicion when observing reduced carbapenem susceptibility or resistance (see figure 1)
- as a general principle, frontline diagnostic methods must have high sensitivity (ability to detect carbapenem resistance), even at the expense of specificity (ability to distinguish true carbapenemase producers)
- recognition of carbapenem resistance should be followed up with supplementary tests locally or in a specialist or reference laboratory (eg AMRHAI, PHE Colindale)

**Figure 1.** The problem with spotting the carbapenemase producers



## Laboratory detection: screening and confirmation

### Screening

Screening should be undertaken in accordance with current guidance from the appropriate public health authorities. At the time of writing of this SOP, this includes all patients who have been in-patients in a hospital overseas or in a hospital in the UK known to have had problems with transmission of carbapenemase-producing organisms in the last 12 months. UK regions where problems have been noted in some hospitals include North West England, especially Manchester, and London. Updates on affected areas should be sought from the PHE acute trust toolkit<sup>14</sup>. Furthermore, any patients or their close contacts who have been colonised with carbapenemase producing organisms should also be screened, regardless of the time since the last proven documented colonisation.

#### Enterobacteriaceae

- test a carbapenem against all clinically-significant isolates in high risk settings in accordance with current national guidance. Ertapenem has the best sensitivity among the available analogues, but poor specificity for carbapenemase producers. Meropenem and imipenem may have better specificity, but reduced sensitivity. Faropenem has also been reported to show good sensitivity for detecting carbapenemase producers<sup>15,16</sup>. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommends using meropenem as it offers the best compromise between sensitivity and specificity
- perform carbapenemase confirmatory tests (below) on isolates found resistant or to have reduced susceptibility to the indicator carbapenem
- identification to genus/species level is highly desirable for the interpretation of resistance patterns. Identify all isolates found resistant to the indicator

carbapenem to ensure that reduced susceptibility or resistance to the tested carbapenem is not an intrinsic trait

- consider whether the isolate should be submitted to the reference laboratory (section 4.9)

## Screening of stool samples or rectal swabs for carbapenemase-producing enterobacteriaceae

### Selective culture media

There is no 'gold standard' method for detection of carbapenemase-producing Enterobacteriaceae in stool samples or rectal swabs, but a range of different culture media has been proposed<sup>17-32</sup>. Such media incorporate antimicrobials for the inhibition of other microorganisms and biochemical markers to differentiate species or groups of species using either chromogenic substrates or fermentable carbohydrates with a pH indicator. Their exact composition is often undisclosed. Due to a lack of published studies, it is not yet possible to provide firm recommendations to use (or avoid) specific media but a review of the published literature can help laboratory staff to make an informed choice.

Table 6 documents referenced studies involving such media that has been published either in print or online in English up until 2014. 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 carbapenemase-producing Enterobacteriaceae 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 may predominate, and different media may show different performances in different geographical locations. It is likely that most methods have been optimised for KPC carbapenemases, as these predominate in several of the larger markets.

Some commercially-available chromogenic media have been designed specifically for the isolation of carbapenem-resistant Enterobacteriaceae. Such media have generally shown good performance when compared with in-house preparations of MacConkey agar incorporating imipenem (or MacConkey with carbapenem discs)<sup>17-19</sup>. Others have shown that isolates of carbapenemase-producing Enterobacteriaceae (CPE) with low carbapenem MICs (eg  $\leq 2$ mg/L meropenem) may not grow on some chromogenic media<sup>20,21</sup>. Detection of isolates with OXA-48-like enzymes may be problematic (reduced sensitivity) and some agars have been designed specifically for detecting them. A combination of two commercial agars may be needed to offer maximum sensitivity<sup>24,25</sup>.

Chromogenic media that have been developed for detection of ESBL-producers, are likely to have poor specificity when screening for CPE, particularly in areas where ESBL producers are commonplace and no advantage has yet been demonstrated in trials with clinical samples<sup>20,23-28</sup>.

Enrichment broths supplemented with carbapenems have also been advocated, eg in guidelines from the Centers for Disease Control<sup>33</sup>. However, the current limited evidence suggests an inferior performance to commercially available chromogenic

agars with the added disadvantage that an extra day is required to obtain colonies for further testing<sup>23-28</sup>.

In light of the limited available evidence we recommend that if stool samples or rectal swabs require screening for CPE, the method chosen should have demonstrated performance at least equivalent to plating on to a commercially-prepared agar specifically recommended for this purpose. It is essential that suspect colonies are then subjected to confirmatory tests as described further below.

**Note:** Chromogenic agar media should be recommended for isolation as it is more sensitive and gives results more quickly than MacConkey agar with a disc, which is cheaper but not very sensitive and will require further confirmation. However, these two agar media should be used in conjunction for maximum isolation. This is optional because not many laboratories can afford the cost of both media. For more information on Chromogenic agars, see Appendix 2 on published evaluations.

### Non-fermenters

- acquired carbapenemases are also encountered in *Acinetobacter* species, *Pseudomonas* species (most commonly, though not exclusively in *P. aeruginosa*) and in other non-fermenters<sup>1-3,9</sup>
- test meropenem, or imipenem or doripenem against all clinically-significant isolates. Do not use ertapenem because these species are intrinsically resistant to this carbapenem
- decide whether supplementary tests are needed
- 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
- consider whether the isolate should be submitted to the reference laboratory (section 4.9)

### *Acinetobacter* species

- carbapenem resistance in *Acinetobacter* species most often results from the production of one or more OXA-type carbapenemases (eg OXA-23-like, OXA-40-like, OXA-58-like, OXA-143 like). These can only be distinguished by molecular tests. In addition, all isolates of *A. baumannii* have the gene for an intrinsic OXA-type carbapenemase (OXA-51-like), which can confer reduced susceptibility or resistance to carbapenems (usually low-level) only if its expression is up-regulated by genetic reorganisation
- the OXA enzymes of *Acinetobacter* species have rarely been reported in other genera and horizontal spread to other strains, species or genera is not considered a significant risk
- need for supplementary tests: Carbapenem-resistant *Acinetobacter* species can usually be reported as likely OXA-carbapenemase producers without supplementary tests, unless the affected patient has been hospitalized overseas recently (eg in the Middle-East or Indian subcontinent) in which case

imipenem-EDTA synergy should be sought to rule out presence of a metallo-enzyme

- strong EDTA synergy (>8-fold) correlates well with MBL production in *Acinetobacter* species, although many OXA carbapenemase producers show a weaker false synergy probably because metal ions are needed to maintain some OXA enzymes in an active conformation
- KPC has also been recorded in *A. baumannii* in Central America, though not in Europe<sup>34,35</sup>

### ***Pseudomonas* species**

- carbapenem resistance in *P. aeruginosa* arises most often through mutation. Loss or reduced expression of the OprD (D2) porin leads to imipenem resistance, while up-regulation of the MexAB-OprM efflux pump combined with OprD loss leads to meropenem resistance
- isolates with broader resistance most often have OprD loss combined with other mutational mechanisms (up-regulated efflux and derepressed AmpC), but may have acquired carbapenemases
- need for supplementary tests: 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 (ie imipenem, meropenem and doripenem) and piperacillin-tazobactam (and usually ceftazidime) should be tested for imipenem-EDTA synergy. Most will be negative. However false-positive 'MBL' synergy results are common. These probably reflect the disorganising effects of EDTA on the outer membrane of some strains
- susceptibility to aztreonam combined with resistance to carbapenems and other  $\beta$ -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
- most acquired carbapenemases in the genus are MBLs; KPC has also been recorded in *P. aeruginosa* in Central and South America, USA, China, and the Caribbean though not in Europe at the time of writing<sup>35-39</sup>. GES-5 enzyme has also been reported in the species and has been found in a few isolates in the UK; the class D enzyme, OXA-181 has been detected a single isolate from the UK (AMRHA1 Reference Unit, unpublished data)

at this time it is not possible to recommend sensitive and specific phenotypic criteria to infer the presence of non-metallo-carbapenemases in the genus

Laboratories should have a high index of suspicion and should undertake further tests if:

- the zone diameter around a carbapenem disc indicates non-susceptibility
- colonies of an appropriate colour are obtained on any commercially-available agar for detecting carbapenem-resistant bacteria
- automated systems flag non-susceptibility to any carbapenem, irrespective of the expert interpretation given (unless it's explained by intrinsic resistance)

- in-house or commercial phenotypic or molecular tests yield a positive 'hit'

### Confirmatory tests for carbapenemases: inhibitor-based tests

Bacterial isolates resistant to the indicator carbapenem by clinical breakpoint or positive by the EUCAST screening criteria (see 4.7) should be subjected to confirmatory tests. Many of these depend on demonstrating synergy between an indicator carbapenem and various  $\beta$ -lactamase inhibitors.

Synergy is defined by a significant (>5mm) expansion of the carbapenem zone size or significant ( $\geq 8$ -fold) reduction in carbapenem MIC in the presence of the inhibitor. Synergy is sought using in-house or commercially-available methods (including combination disc tests, gradient tests or automated systems).

**Table 2: Interpretation of inhibitor-based phenotypic tests**

Carbapenem resistance mechanism	Synergy observed as increase in zone diameter (mm) with 10 $\mu$ g meropenem disc/tablet				Temocillin MIC >64mg/L or zone diameter <11mm around 30 $\mu$ g disc
	DPA/EDTA	APBA/PBA	DPA+APBA	CLX	
MBL	+	-	-	-	Variable <sup>1</sup>
KPC	-	+	-	-	Variable <sup>1</sup>
MBL + KPC <sup>2</sup>	Variable	Variable	+	-	Variable <sup>1</sup>
OXA 48-like	-	-	-	-	Yes
AmpC + porin loss	-	-	-	+	Variable <sup>1</sup>
ESBL + porin loss	-	-	-	-	No

DPA=dipicolinic acid, EDTA=ethylenediaminetetraacetic acid, APBA= aminophenyl boronic acid, PBA= phenyl boronic acid, CLX=cloxacillin

<sup>1</sup> Temocillin susceptibility tests is recommended only in cases where no synergy is detected, in order to differentiate between ESBL + porin loss and OXA-48-like enzymes. When other enzymes are present the susceptibility is variable and does not provide any further indication of the  $\beta$ -lactamase present.

<sup>2</sup> There is one report supporting the use of commercial tablets containing double inhibitors (DPA or EDTA plus APBA or PBA). This combination confers high-level resistance to carbapenems and is rare outside Greece.

#### Caveats:

- this table illustrates 'classic' phenotypic patterns, but Gram negative clinical isolates are becoming more complex and co-resident mechanisms lead to exceptions. There is an increasing need for molecular methods (PCR, arrays) to detect and identify any carbapenemase present. In particular many isolates with MBLs are resistant to aztreonam owing to coproduction of ESBLs or AmpC, and many with OXA-48-like enzymes are resistant to cephalosporins for the same reason.
- synergy tests are most effective for members of the Enterobacteriaceae.

## Screening and Detection of Bacteria with Carbapenem-Hydrolysing $\beta$ -lactamases (Carbapenemases)

- although EDTA/dipicolinic acid synergy tests may also be useful for non-fermenters, they give a high proportion of false-positive results for these organisms.
- EUCAST and CLSI advocate that supplemental tests to confirm carbapenemase production are unnecessary for individual patient management; the only test needed is the MICs – either agar or broth dilution or by use of gradient strip methods. This stance is contentious.
- the risk of onward spread may vary with underlying resistance mechanisms or combinations of those (see also ‘Reporting for Carbapenemase Producers’ below). Hence, both EUCAST and CLSI indicate the value of supplemental testing for infection prevention and control purposes, and for local epidemiological investigations.
- 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<sup>40</sup>. 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 Test and Vitek.

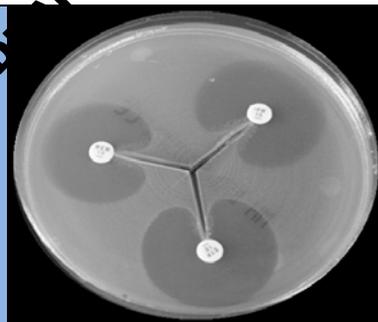
### Confirmatory tests for carbapenemases: other methods

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

#### Non-chromogenic methods

**Modified Hodge Test (MHT) or ‘Cloverleaf’ test:** a phenotypic bioassay to assess the ability of a test strain to hydrolyse carbapenems, as judged by indentations of the inhibition zones for an indicator strain of *E. coli*. Maximum sensitivity is achieved by using 10 $\mu$ g discs of TP, IPM and MEM, but the test remains subjective and is not recommended for routine use in clinical laboratories as results are difficult to interpret and the test lacks specificity (especially with AmpC producers, which show weak positive results). Concerns have also been raised over its sensitivity, with several proven carbapenemase producers giving consistently negative results<sup>41,42</sup>.

Figure 2. Example of a Modified Hodge test (MHT) or clover-leaf test



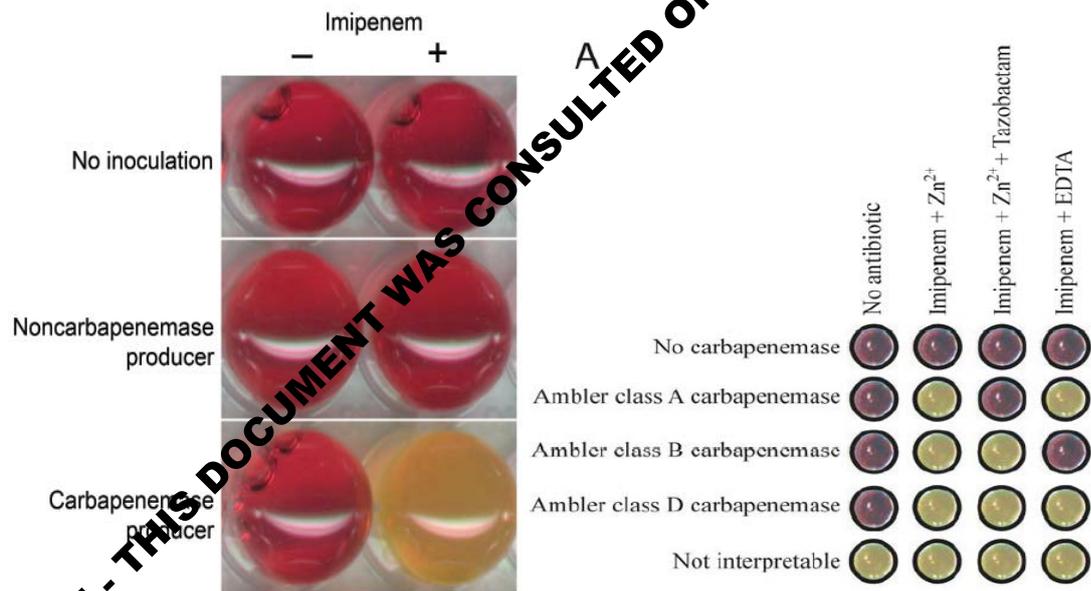
courtesy of Neil Woodford

## Chromogenic methods

**'Carba-NP' test:** This test is based on the classical acidometric penicillinase test and has a colorimetric endpoint (phenol red indicator turns yellow if the indicator carbapenem is hydrolysed). This assay has been reported to work well for detecting carbapenemases in Enterobacteriaceae and *Pseudomonas* species<sup>41,43-45</sup>. Commercial versions of the test are available.

The Carba NP test has multiple benefits - It is inexpensive, rapid, reproducible, and is reported to be highly sensitive and specific. However, it requires pre-incubation of a carbapenem with the test organism, but can be completed in less than 2 hours. The test can give a 'Yes / No' result or, by testing carbapenems alone and in the presence of inhibitors, can also be used to assign any detected carbapenemase to its appropriate  $\beta$ -lactamase class (class A, B or D). Most published evidence on this test has originated from the centre where it was developed. It has been recommended as a diagnostic method by CLSI. Questions remain about 'user-friendliness', potential for subjective interpretation, its sensitivity vs. all carbapenemase types and its specificity vs. isolates with large amounts of AmpC enzyme. In some more recent studies, they have reported poorer sensitivity for CPE with OXA-48-like carbapenemases<sup>46-48</sup>.

**Note:** The Carba NP test has been validated with bacterial colonies grown on Mueller-Hinton agar plates, blood agar plates, trypticase soy agar plates, and most selective media used in screening for carbapenemase producers. However, this test cannot be performed with bacterial colonies grown on Drigalski or MacConkey agar plates<sup>41,43</sup>.



This has been adapted from the CDC website (<http://wwwnc.cdc.gov/eid/article/18/9/12-0355-f1>) and Artet<sup>43</sup>.

**Commercial agars:** As stated previously, there are several commercially available chromogenic media designed for isolation of carbapenem-resistant Enterobacteriaceae and for carbapenemase producers, including those producing OXA-48-like enzymes<sup>20-25</sup>. Chromogenic media have also been developed for detection of ESBL-producers, but these are likely to be less specific, particularly in areas where ESBL producers are commonplace and no advantage has yet been demonstrated in trials with clinical samples<sup>20,23-28</sup>. Media designed for ESBL producers may show reduced sensitivity for some carbapenemase producers (eg those with

OXA-48-like enzymes but lacking co-resident ESBLs). Two chromogenic agars may be needed to maximise sensitivity.

## Rapid methods

**Matrix-Assisted Laser Desorption/Ionisation - Time of Flight (MALDI-ToF):** This is increasingly available to diagnostic laboratories; MALDI-ToF also offers the potential to detect carbapenemase production<sup>49-53</sup>. The assay detects mass changes that follow hydrolysis of a carbapenem molecule. It requires pre-incubation of a carbapenem with the test organism, but can be completed in less than 2 hours. The test gives a 'Yes / No' result, but needs validation before it can be recommended as a diagnostic method, to determine its sensitivity vs. all carbapenemase types and its specificity vs. isolates with large amounts of AmpC enzyme. This assay is not yet commercially available, but is under development.

**Molecular tests:** There are numerous block-based or real-time amplification (PCR or LAMP) assays in the literature, either using simplex or multiplex formats<sup>54-59</sup>. Some are commercially available. Many are suitable either for screening for colonisation (ie using rectal swabs) or for confirmation of carbapenemases in bacterial colonies. These assays vary in their scope (ie the range of genes sought) and the extent to which they can be customised by the end-user. PCR has been successfully utilized for the detection of single or multiple carbapenemase genes directly from clinical or screening samples<sup>18,26,56,60</sup>. Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture<sup>26</sup>. Disadvantages include a higher cost for processing samples and the need for specialised equipment and/or expertise and so molecular tests might be considered expensive in some settings. Given the range of carbapenemases that may be encountered in the UK it would be necessary to target a range of genes to rule out the presence of carbapenemase-producing Enterobacteriaceae.

There are also commercial DNA micro-arrays to detect and distinguish the 'big five' carbapenemases, together with rarer enzymes<sup>61-65</sup>.

Some commercial systems will give a 'Yes / No' result, while others identify the carbapenemase type (KPC, OXA-48, IMP, NDM or VIM). Molecular tests are the only reliable means of detecting production of multiple carbapenemases by an isolate.

Molecular methods will not identify producers of rare or new carbapenemases (unlike, for example, a test that detects carbapenem hydrolysis), and do not provide information on the host species if performed directly on clinical specimens; culture of positive samples would be required to obtain the carbapenemase producer for susceptibility testing and identification<sup>42</sup>.

Commercial molecular assays offer a reliable means of detecting bacteria with the most clinically-significant carbapenemases, especially KPC, NDM and VIM types.

Coverage of some assays has been expanded to detect OXA-181 producers and so maximize the sensitivity for OXA-48-like carbapenemases. Choice will ultimately depend on preferred gene coverage, intended throughput, cost, and ability to fit into local workflows.

## Controls for carbapenemase tests

Quality control of the carbapenem discs used in the primary screening should follow standard BSAC, EUCAST or CLSI recommendations.

## Screening and Detection of Bacteria with Carbapenem-Hydrolysing $\beta$ -lactamases (Carbapenemases)

Positive controls should be used to ensure the performance of carbapenemase confirmatory tests. Various strains with known carbapenemases are available from NCTC ([https://www.phe-culturecollections.org.uk/media/63614/m01520130827v4\\_antimicrobresmech-a4.pdf](https://www.phe-culturecollections.org.uk/media/63614/m01520130827v4_antimicrobresmech-a4.pdf)). Alternatively, some may be obtained commercially from other suppliers.

**Table 3.** Control strains producing carbapenemases available from the NCTC

4.1 Class A Carbapenemase		
Organism	NCTC <sup>c</sup> Strain Reference	Characteristics
<i>Klebsiella pneumoniae</i>	NCTC 13438	Member of the international ST258 clone producing KPC-3 carbapenemase
4.2 Class B Carbapenemases (Metallo- $\beta$ -lactamases):		
Organism	NCTC <sup>c</sup> Strain Reference	Characteristics
<i>Pseudomonas aeruginosa</i>	NCTC 13437	VIM-10; VEB-1
<i>Klebsiella pneumoniae</i>	NCTC 13439	VIM-1; QnrS1 (outbreak strain)
<i>Klebsiella pneumoniae</i>	NCTC 13440	VIM-1; QnrS1 (sporadic)
<i>Klebsiella pneumoniae</i>	NCTC 13443	New Delhi Metallo- $\beta$ -lactamase (NDM-1)
<i>Escherichia coli</i>	NCTC 13476	IMP-type (unsequenced)
4.3 Class D Carbapenemases (OXA carbapenemases):		
Organism	NCTC <sup>c</sup> Strain Reference	Characteristics
<i>Acinetobacter baumannii</i>	NCTC 13301	OXA-23 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13302	OXA-25 (OXA-24/40-like) also with OXA-51-like
<i>Acinetobacter baumannii</i>	NCTC 13303	OXA-26 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13304	OXA-27 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13305	(A 15) OXA-58 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13421	OXA-23 Clone 1 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13424	OXA-23 Clone 1 (also with OXA-51-like)
<i>Acinetobacter baumannii</i>	NCTC 13420	Clone OXA-51-like
<i>Acinetobacter baumannii</i>	NCTC 13422	W Clone OXA-51-like
<i>Acinetobacter baumannii</i>	NCTC 13423	strain (UK3) OXA-51-like
<i>Klebsiella pneumoniae</i>	NCTC 13442	sequence type 353 with OXA-48

**Note:** Either *E. coli* NCTC 10418 or ATCC 25922 should be used as a negative control in confirmation tests.

## Technical information/limitations

### Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

### Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

### Specimen containers<sup>66,67</sup>

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 the BSAC, EUCAST or CLSI guideline documents. 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. Incubation times for chromogenic media should be as recommended by the manufacturers.

**DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 3 JUNE - 15 JULY 2015**

## 1 Safety considerations<sup>66-82</sup>

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### 1.1 Specimen collection, transport and storage<sup>66-71</sup>

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.

### 1.2 Specimen processing<sup>66-82</sup>

Containment Level 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>74</sup>.

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

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

## 2 Specimen collection

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### 2.1 Type of specimens

Stool, rectal or peri-rectal swabs – sent specifically for screening, clinical specimens such as blood, wounds or urine.

The potential for spread of acquired carbapenemases means that an indicator carbapenem should be tested against all clinically-significant Gram negative bacteria.

### 2.2 Optimal time and method of collection<sup>83</sup>

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible<sup>83</sup>.

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium<sup>84-88</sup>.

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

### 2.3 Adequate quantity and appropriate number of specimens<sup>83</sup>

There should be visible faecal material on the rectal or peri-rectal swabs taken.

In patients who fulfil the criteria to be considered to be a suspected case of colonisation or infection, a rectal swab or stool sample is taken and if result is negative, a further two consecutive samples are taken 48 hours apart. In addition, if patient has been hospitalised in a country with reported high prevalence, include samples from any wounds or device-related sites.

Numbers and frequency of specimen collection are dependent on clinical condition of patient or for screening specimens, on local policies and practices.

### 3 Specimen transport and storage<sup>66,67</sup>

#### 3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible<sup>83</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>83</sup>.

### 4 Specimen processing/procedure<sup>66,67</sup>

#### 4.1 Test selection

N/A

#### 4.2 Appearance

N/A

#### 4.3 Sample preparation

For safety considerations refer to Section 1.2

#### 4.4 Microscopy

##### 4.4.1 Standard

N/A

##### 4.4.2 Supplementary / preparation of smears

N/A

#### 4.5 Culture and investigation

##### Direct culture

Inoculate culture media with swab or other sample (refer to [Q 5 – Inoculation of culture media in bacteriology](#)).

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 3 JUNE - 15 JULY 2015

### 4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atoms	Time		
<b>Detection:</b> Any condition + detection of carbapenemase resistance/ carbapenemase production	Any sample	MacConkey agar or CLED agar + 10 $\mu$ g MEM disc <sup>*89</sup>	35-37	Aerobic	18-24hr	$\geq$ 18hr	Carbapenemase producing Enterobacteriaceae
		OR	35-37	Aerobic	16-48hr	$\geq$ 16hr	<i>Pseudomonas</i> species <i>Acinetobacter</i> species
		Chromogenic agar with carbapenem <sup>***</sup>	35-37	Aerobic	18-24hr	$\geq$ 18hr	Carbapenemase producing Enterobacteriaceae

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
If increased sensitivity is desired	Any sample	MacConkey agar or CLED agar + 10 $\mu$ g ETP disc <sup>**</sup>  Enrichment broths <sup>****</sup> (eg Trypticase Soy broth) + 10 $\mu$ g ETP or MEM disc and then subculture on MacConkey agar (and incubate under the same conditions)	35-37	Aerobic	18-24hr	$\geq$ 18hr	<i>Klebsiella pneumoniae</i> <i>Escherichia coli</i>

\* Meropenem = MEM \*\* Ertapenem = ETP

\*\*\* For chromogenic media refer to manufacturer's instructions for recommended incubation times.

\*\*\*\* In selected situations where maximal sensitivity is required, enrichment broths can be used.

**Note:** Molecular methods such as PCR or LAMP assays and MALDI-TOF MS can be used for detection.

### 4.6 Identification

Refer to individual SMIs for organism identification.

#### 4.6.1 Minimum level of identification in the laboratory

<a href="#">Klebsiella species</a> <a href="#">Enterobacter species</a> <a href="#">Escherichia species</a> <a href="#">Serratia species</a> <a href="#">Morganella species</a> <a href="#">Proteus species</a>	species level <a href="#">ID 16 - Identification of Enterobacteriaceae</a>
<a href="#">Pseudomonas species</a> <a href="#">Acinetobacter species</a>	species level <a href="#">ID 17 - Identification of Pseudomonas species and other non-glucose fermenters</a>

Organisms may be further identified if this is clinically or epidemiologically indicated.

#### 4.7 Antimicrobial susceptibility testing

As the media used are not as recommended in BSAC/EUCAST/CLSI methodology, a cut-off value of within 20mm is recommended, taking due regard of inoculum density or mixed cultures. Any isolates that shows suspected resistance should be identified and submitted for formal susceptibility testing.

If using the recommended media by [British Society for Antimicrobial Chemotherapy \(BSAC\)](#), [European Committee on Antimicrobial Susceptibility Testing \(EUCAST\)](#) or [Clinical and Laboratory Standards Institute \(CLSI\)](#) methodology, refer to the respective guidelines.

Species identification is highly desirable to allow proper interpretation of results. BSAC and EUCAST recommended breakpoints for the carbapenems advocated are 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 ie MICs for some or all carbapenems may be 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 (Table 4) for detecting putative carbapenemase-producing Enterobacteriaceae, with meropenem the preferred screening agent since it offers the best compromise between sensitivity and specificity.

Table 4. Recommended EUCAST screening cut-off values for recognising possible carbapenemase-producing Enterobacteriaceae\*

Carbapenem	MIC (mg/L)	Zone diameter (mm)
Meropenem <sup>1</sup>	>0.12	<25 <sup>2</sup>
Imipenem <sup>3</sup>	>1	<23
Ertapenem <sup>4</sup>	>0.12	<25

<sup>1</sup> Meropenem offers the best compromise between sensitivity and specificity in terms of

detecting carbapenemase producers

<sup>2</sup> In some cases zone diameters for OXA-48-producers are up to 26 mm, so <27 mm may be used as a screening cut-off in countries where OXA-48 is endemic, but at the expense of lower specificity

<sup>3</sup> Imipenem is not recommended for use as a stand-alone screening test compound because it is relatively poor for separating wild-type organisms and carbapenemase producers.

<sup>4</sup> Ertapenem shows high sensitivity but low specificity in terms of detecting carbapenemase producers, and so is not recommended for routine use.

\*The EUCAST guidelines document is available at:

[http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Resistance\\_mechanisms/EUCAST\\_detection\\_of\\_resistance\\_mechanisms\\_v1.0\\_20131211.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf) OR  
[http://www.eucast.org/resistance\\_mechanisms/](http://www.eucast.org/resistance_mechanisms/).

## 4.8 Referral for outbreak investigations

See 4.9.

## 4.9 Referral to reference laboratories

Any isolate which appears to be resistant to the indicator carbapenem should be referred for confirmation. Most NHS laboratories in England submit their isolates to AMRHAI. Some PHE Specialist Laboratories offer referral services at a regional level, and then refer selected isolates onwards to AMRHAI<sup>90</sup>. Laboratories using this regional service should not submit isolates to AMRHAI directly.

NHS microbiology laboratories are now required to use the web-based Electronic Reporting System (ERS) (<https://crp.phe.nhs.uk/>) when submitting isolates of Gram-negative bacteria suspected to produce an acquired carbapenemase.

The AMRHAI Reference Unit at PHE Colindale seeks:

- all Enterobacteriaceae suspected to produce a carbapenemase.
  - isolates of *Enterobacter* that have borderline resistance to ertapenem, but remain fully susceptible to other carbapenems should not be sent.
  - isolates of *Serratia*, *Morganella* or *Proteus* species that are borderline resistant to imipenem, but susceptible to other carbapenems should not be sent.
- all *Pseudomonas* species suspected validly to produce a carbapenemase (ie a transferable carbapenem resistance mechanism). Such isolates should be resistant to all relevant carbapenems (ie imipenem, meropenem and doripenem) and piperacillin-tazobactam and usually ceftazidime; susceptibility or resistance to aztreonam is variable because most carbapenemase-producing pseudomonads have metallo-enzymes, usually VIM-types.
  - isolates of *Pseudomonas* species resistant only to carbapenems and susceptible to other  $\beta$ -lactams should not be sent.
  - isolates of *Pseudomonas* species that are resistant to ertapenem, but susceptible to other carbapenems should not be sent. Ertapenem resistance is inherent in the genus.

## Screening and Detection of Bacteria with Carbapenem-Hydrolysing $\beta$ -lactamases (Carbapenemases)

- all *Acinetobacter* species suspected to produce a metallo-carbapenemase, ie with strong imipenem-EDTA synergy.
  - isolates of *Acinetobacter* that are resistant to ertapenem, but susceptible to other carbapenems should not be sent. Ertapenem resistance is inherent in the genus.
- despite all of the above, microbiology laboratories are encouraged to have a high index of suspicion, at least for Enterobacteriaceae, and Reference laboratories to also accept that they would not find a carbapenemase in all referred carbapenem-resistant isolates. There is no penalty charge when the AMRHAI Reference Unit don't (unless isolates turn out to be fully susceptible).
- isolates of *Stenotrophomonas maltophilia*, *Aeromonas* species and 'chryseobacteria' for investigation of carbapenem resistance (though note final bullet below) should not be sent because metallo-carbapenemase production is an intrinsic characteristic of these bacteria.
- in addition, the AMRHAI Reference Unit seek representatives of any carbapenem-resistant strains (irrespective of suspected mechanism, and including species with intrinsic carbapenem resistance) that are associated with clusters or outbreaks of infection or colonization.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

Organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

Contact 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 Reference Unit (AMRHAI)

Bacteriology Reference Department

Microbiology Services Division

Public Health England

61 Colindale Avenue

London

NW9 5EQ

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

Telephone: +44 (0) 208 3276511/ 7877

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

England and Wales

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

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

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

## 5 Reporting procedure

### 5.1 Microscopy

N/A

### 5.2 Culture

#### Negatives

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

#### Positives

“Carbapenem – resistant / non-susceptible organism isolated” It may produce a carbapenemase; further investigations are being undertaken

#### 5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically when available. Written report within 72hr stating, if appropriate, that a further report will be issued.

### 5.3 Antimicrobial susceptibility testing

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

#### 5.3.1 Carbapenems

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, the merit of this approach is not as clear as for cephalosporins vs. ESBL producers, since there is no obvious ‘next’ drug vs. carbapenemase producers<sup>91</sup>.

Recently, EUCAST and CLSI have taken the view that, with the low breakpoints now adopted by both organisations, 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 need to improve the quality of laboratory testing and reporting<sup>91</sup>.

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<sup>13</sup>.

New  $\beta$ -lactamase inhibitors (avibactam, MK-7655, RPX7009) are under development and have activity against some carbapenemases (principally KPC types, not MBLs).

None is currently licensed in Europe (though ceftazidime-avibactam is licensed in the US by FDA) and, while these may offer future options, their ability to 'cover' the diversity of acquired carbapenemases and range of host species will depend on the partnering  $\beta$ -lactam(s).

## 6 Notification to PHE<sup>92,93</sup> or equivalent in the devolved administrations<sup>94-97</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

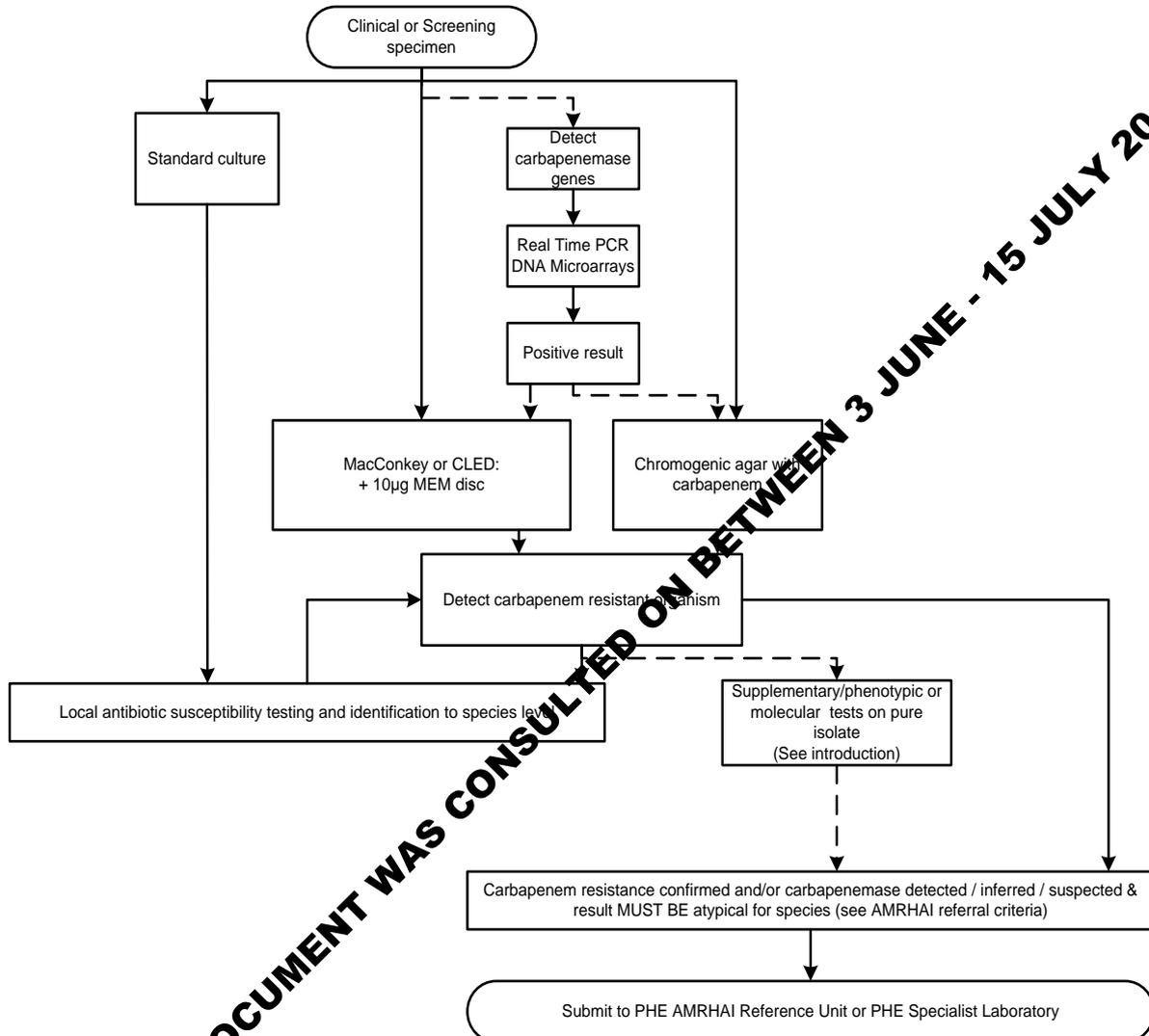
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>94,95</sup>, [Wales](#)<sup>96</sup> and [Northern Ireland](#)<sup>97</sup>.

## Appendix 1: Flowchart for the screening and detection of carbapenemases



Note: The branch with the dotted lines in this flowchart is optional but useful for diagnostic laboratories that have advanced into using molecular methods.

The flowchart is for guidance only.

## Appendix 2: Published evaluations of media / methods for detecting carbapenemase-producing enterobacteriaceae

**Table 6.** Published evaluations of media / methods for detecting carbapenemase-producing enterobacteriaceae in various patient populations

Reference	Media / Method tested	Sensitivity (%)	Specificity (%)	Study location	No. of positive Samples/Total Samples	Comments
17	CHROMagar KPC	84.9	88.7	Israel	33 / 139	All isolates of CPE had KPC enzyme.
	MacConkey plus carbapenem discs	75.8	89.6			
	MacConkey plus imipenem (1 mg/L)	84.9	94.3			
18	CHROMagar KPC	100	98.4	Israel	122	Sensitivity and specificity of both media were calculated relative to PCR testing of samples. All isolates of CPE had KPC-3 enzyme
	MacConkey plus carbapenem discs.	92.7	95.9			
19	CHROMagar KPC	97.8	ND <sup>a</sup>	Greece	46 / 126	Predominant carbapenemases were KPC and VIM
	MacConkey plus imipenem (1 mg/L)	78.3	ND			
21	Colorex KPC	97	96	Pakistan	37 / 200	Prototype version of chromID CARBA tested. All CPE had NDM-1
	chromID CARBA	100	93			
22	chromID CARBA	100	98	Pakistan	32 / 175	All CPE had NDM-1
	Brilliance CRE	62.5	94			
23	TSB plus ertapenem (2 mg/L)	89.1	86.4	Greece	73 / 200	Predominant carbapenemases were KPC and VIM
	chromID ESBL	92	93.3			
	chromID ESBL (plus enrichment)	94	84.7			
	chromID CARBA	92.4	96.9			
	MacConkey plus meropenem (1 mg/L)	89.1	85.2			
26	CHROMagar ESBL	77.3	100	USA	66 / 95	All isolates of CPE had KPC enzyme
	VACC <sup>b</sup>	77.3	100			
	PCR for <i>bla</i> <sub>KPC</sub>	97	96.6			

## Footnotes

<sup>a</sup> Not determined or not reported.

<sup>b</sup> Selective agar with vancomycin, amphotericin B, ceftazidime, and clindamycin (VACC).

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