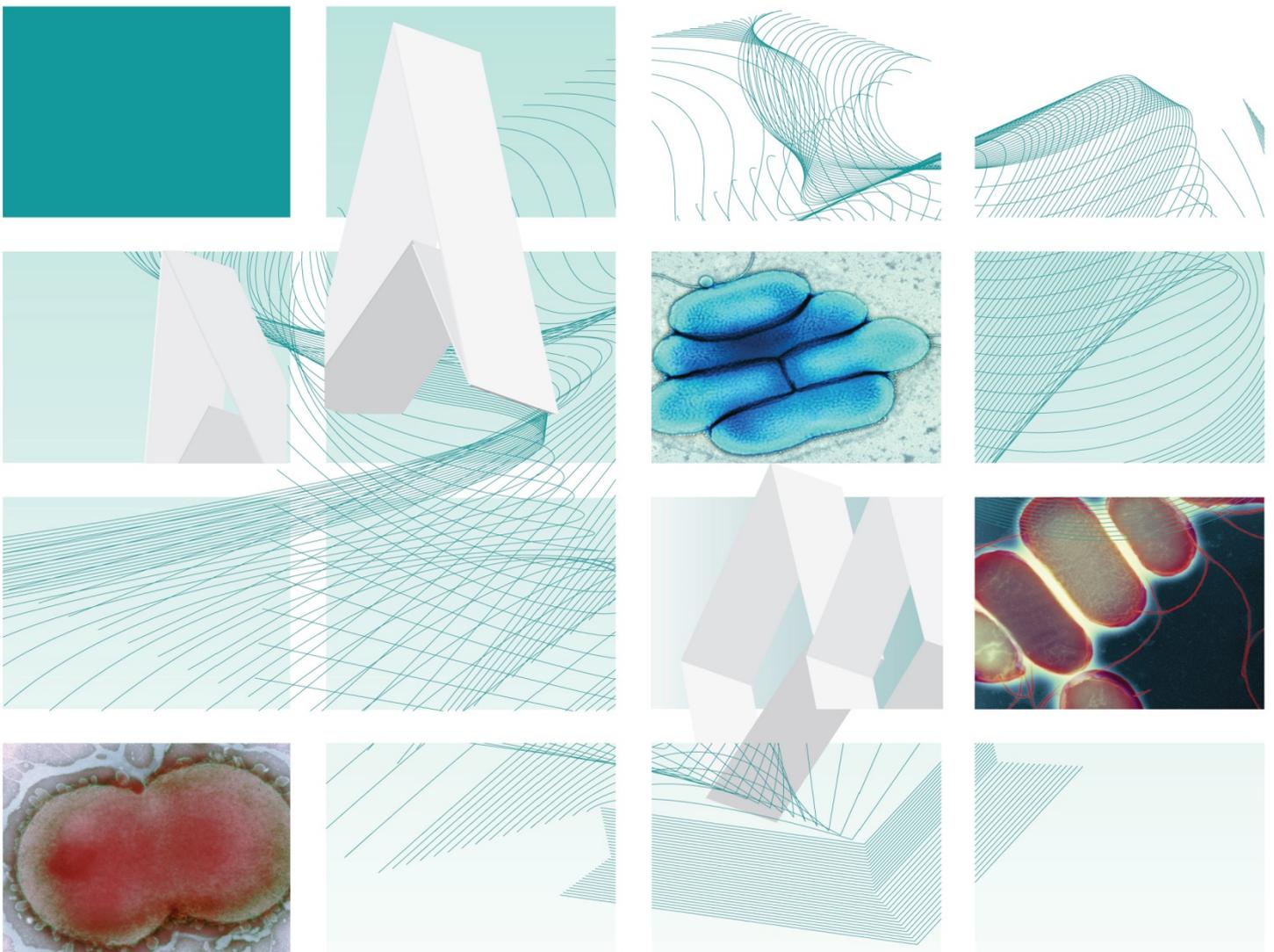




# UK Standards for Microbiology Investigations

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)



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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 60 | Issue no: 2 | Issue date: 13.09.16 | Page: 1 of 40

## Acknowledgments

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

We also acknowledge Professor Neil Woodford (AMRHAI - Colindale, PHE) for his considerable specialist input.

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PHE publications gateway number: 2015075

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

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## 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 number/date	2/13.09.16
Issue number discarded	1.1
Insert issue number	2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>B 60 formerly P 8.</p> <p>Title of document updated.</p> <p>Hyperlinks updated to gov.uk.</p> <p>Document presented in a bacteriology document format.</p> <p>The document gives recommendations on screening for and detection of 'carbapenemases' (carbapenem-hydrolysing <math>\beta</math>-lactamases).</p> <p>Scientific content updated.</p> <p>Links to different websites/articles updated.</p> <p>Technical limitations/ information section updated.</p>
Page 2.	Updated logos added.
Reporting procedure.	This has been updated with appropriate information.
Appendix.	<p>Flowchart produced for easy guidance.</p> <p>Published evaluations of media/methods for detecting carbapenemase-producing Enterobacteriaceae (CPE) compiled.</p>
References.	References reviewed and updated.

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

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### 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 good standard of practice to which all clinical and public health microbiology

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<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.

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 professionals, 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 is 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**

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, 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 by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date 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**

Public Health England. (2016). Detection of bacteria with carbapenem-hydrolysing  $\beta$ -lactamases (carbapenemases). UK Standards for Microbiology Investigations. B 60

Detection of bacteria with carbapenem-hydrolysing  $\beta$ -lactamases (carbapenemases)

Issue 2. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

## Scope of document

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### Type of specimen

Screening specimens including stool, rectal or peri-rectal swabs, any clinical specimens such as blood, wounds or urine

This SMI gives recommendations on screening for and detection of 'carbapenemases' (carbapenem-hydrolysing  $\beta$ -lactamases). This SMI document will focus solely on acquired carbapenemases. 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/329227/Acute\\_trust\\_toolkit\\_for\\_the\\_early\\_detection.pdf](http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/329227/Acute_trust_toolkit_for_the_early_detection.pdf).

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, that is, 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-51-like), 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.ncbi.nlm.nih.gov/projects/pathogens/submit\\_beta\\_lactamase](http://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase)) and include members of three of Ambler's four molecular classes of  $\beta$ -lactamases<sup>1-3</sup>. They are detailed below:

**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, some GES variants, 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 this document; 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 (>400) 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 the following:

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 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</b> <b>IMI</b>	A	Carbapenems and aztreonam, but not 3rd/4th G cephalosporins	<i>Enterobacter</i> species
<b>GES</b>	A	Depends on enzyme variant. Some are ESBLs, others eg GES-5 are carbapenemases	<i>P. aeruginosa</i> and Enterobacteriaceae
<b>IMP</b> <b>VIM</b> <b>NDM</b>  <b>AIM, GIM, SIM,</b> <b>(not detected in the UK yet)</b>  <b>DIM, SPM</b>	B (metallo- $\beta$ -lactamases)	All $\beta$ -lactams except monobactams (aztreonam)	<i>Pseudomonas</i> species <i>Acinetobacter</i> species Enterobacteriaceae
<b>OXA</b>	D	Carbapenems (note that many OXA enzymes are NOT carbapenemases)	<i>A. baumannii</i> , Enterobacteriaceae and rare <i>P. aeruginosa</i>

**Note:** All the enzyme types in bold 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 (that is, 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:

- 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 above),

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

b. 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.

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

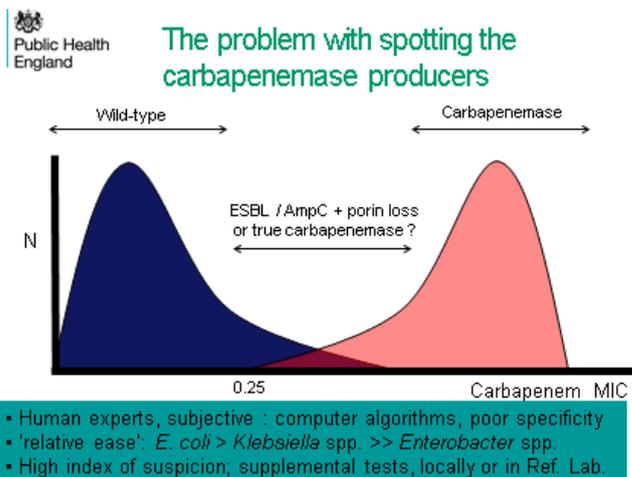
The aims are to recognise carbapenemase producers effectively; and to distinguish them from isolates that are resistant to carbapenems by virtue of other mechanisms.

In the face of the diversity of enzyme types, the considerable variation in levels of phenotypic carbapenem resistance (for example, 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).

The strongest advice is for laboratory staff to have a high index of suspicion when observing reduced carbapenem susceptibility or resistance (see figure 1) and suspected isolates should be followed up with confirmatory tests locally or in a specialist or reference laboratory (see section 4.9).

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



Courtesy of Professor Neil Woodford, Public Health England.

### Cultured isolates of Enterobacteriaceae

Ideally all clinically significant isolates of Enterobacteriaceae should be tested against a carbapenem as the UK seeks to increase the knowledge of the national distribution of carbapenemase producers. Minimum testing should include:

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

- a. isolates from 'high-risk' patients and settings in accordance with current national guidance, when the relevant information has been provided to the laboratory on the request accompanying the sample; and
- b. any isolates found grossly resistant to co-amoxiclav

At the time of writing, the criteria are: patients who have been in-patients in a hospital abroad or in a hospital in the UK known to have problems with spread of CPE or patient known to have been previously colonised or infected with CPE.

This SMI supports the recommendation of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to use meropenem as the indicator carbapenem as it offers the best compromise between sensitivity and specificity<sup>14</sup>. Although ertapenem has greater sensitivity, it is not recommended because it has poor specificity for carbapenemase producers. Faropenem has also been reported to show good sensitivity for detecting carbapenemase producers and the discs are commercially available<sup>15,16</sup>.

Perform carbapenemase confirmatory tests on isolates found resistant or to have reduced susceptibility to the indicator carbapenem (see table 3 for criteria).

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 a reference laboratory (section 4.9).

**Table 2.** Recommended EUCAST screening cut-off values for recognising possible CPE<sup>14</sup>

Carbapenem	MIC (mg/L)	Zone diameter (mm)
Meropenem <sup>a</sup>	>0.12	<25 <sup>b,c</sup>
Imipenem <sup>d</sup>	>1	<23
Ertapenem <sup>e</sup>	>0.12	<25

<sup>a</sup> Meropenem offers the best compromise between sensitivity and specificity in terms of detecting carbapenemase producers

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

<sup>c</sup> CPE with OXA-48-like enzymes are encountered sufficiently often by UK laboratories to warrant use of the <27mm criterion for meropenem

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

<sup>e</sup> Ertapenem shows high sensitivity but low specificity in terms of detecting carbapenemase producers, and so is not recommended for routine use. However, ertapenem can be used for detection in specific incidents such as in investigating of an outbreak known to have been caused by an organism that is ertapenem resistant but appears to be susceptible to meropenem on screening test method used, or where increased sensitivity is required. Another drawback with the use of ertapenem is the cost implications.

**Note:** Some laboratories in the UK still use the BSAC guidelines, which specifies a meropenem (10 $\mu$ g disc) zone diameter cut-off of 32mm (higher than the susceptibility breakpoint of 27mm by EUCAST) to enable detection of CPE. These laboratories will

be supported by BSAC to change over to the EUCAST methodology once specific BSAC guidelines cease to exist<sup>17</sup>.

### **Screening of stool samples or rectal swabs for carbapenemase-producing Enterobacteriaceae (CPE)**

There is no 'gold standard' method for the detection of CPE in stool samples or rectal swabs, but a range of different culture media have been proposed<sup>18-33</sup>. There are several commercially available chromogenic media designed for the isolation of carbapenem-resistant Enterobacteriaceae and for carbapenemase producers, including those producing OXA-48-like enzymes<sup>21-26</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 possible to provide firm recommendations to use (or avoid) specific commercial chromogenic media, a review of the published literature can help laboratory staff to make an informed choice.

Appendix 2 documents referenced studies involving such media that have 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 (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 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>18-20</sup>. Others have shown that isolates of CPE with low carbapenem MICs (for example,  $\leq 2$ mg/L meropenem) may not grow on some chromogenic media<sup>21-24</sup>. Detection of isolates with OXA-48-like enzymes may be problematic (reduced sensitivity) and some agars have been designed specifically for detecting them.

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>21,24-29</sup>.

Enrichment broths supplemented with carbapenems have been advocated, for example, in guidelines from the Centers for Disease Control<sup>34</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>24-29</sup>.

In light of the limited available evidence we recommend using commercial selective chromogenic agar if stool samples or rectal swabs require screening for CPE. A

combination of two chromogenic agars may be needed to offer maximum sensitivity<sup>25,26</sup>.

For reasons of costs, if an alternative method is chosen locally, for example, MacConkey or CLED agar with a carbapenem disc, it should have been validated locally and the medium and interpretative criteria applied 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 described below.

### Cultured isolates of 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.

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.

**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 synergy should be sought to rule out presence of a metallo-enzyme.

Strong EDTA synergy ( $\geq 8$ -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.

**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) and co-amoxiclav (and usually ceftazidime) should be tested for strong ( $\geq 8$  fold) imipenem-EDTA synergy. Positives require further investigation. False-positive 'MBL' synergy is common and 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. 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<sup>35</sup>.

KPC enzymes have been recorded in *A. baumannii* in Central America, and in *P. aeruginosa* in Central and South America, USA, China, and the Caribbean, though not in Europe at the time of writing<sup>36-41</sup>. GES-5 enzyme has also been reported in *P. aeruginosa* and has been found in a few isolates in the UK; the class D enzyme,

OXA-181 has been detected in a single isolate of *P. aeruginosa* and from the UK (AMRHAI Reference Unit, PHE unpublished data). At this time it is not possible to recommend sensitive and specific phenotypic criteria to infer the presence of these non-metallo-carbapenemases in non-fermenters.

Consider whether the isolate should be submitted to a reference laboratory (section 4.9).

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

When to do confirmatory tests for carbapenemase production:

Laboratories should undertake further tests if:

- the zone diameter around an indicator 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'
- isolates resistant to co-amoxiclav if testing against carbapenems has not been performed

Enterobacteriaceae isolates resistant to the indicator carbapenem by clinical breakpoint or positive by the EUCAST screening criteria (see section 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 3.** 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	
<b>MBL</b>	+	-	-	-	Variable <sup>a</sup>
<b>KPC</b>	-	+	-	-	Variable <sup>a</sup>
<b>MBL + KPC<sup>b</sup></b>	Variable	Variable	+	-	Variable <sup>a</sup>
<b>OXA 48-like</b>	-	-	-	-	Yes
<b>AmpC + porin loss</b>	-	+	-	+	Variable <sup>a</sup>

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

<b>ESBL + porin loss</b>	-	-	-	-	No
-					

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

<sup>a</sup> Temocillin susceptibility test 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>b</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.

Table 3 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 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.

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 MIC determinations – either by agar or broth dilution or by use of gradient strip methods<sup>3,42</sup>.

The risk of onward spread may vary with underlying resistance mechanisms or combinations of those. 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>42</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 Etest and Vitek<sup>5,41,42</sup>.

### Confirmatory tests for carbapenemases: other methods

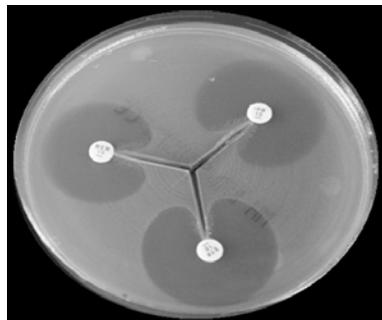
Other methods that may also be considered for detecting likely carbapenemase producers include:

**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 ETP, IPM and MEM, but the test remains subjective and is not

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

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>14,43</sup>.

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



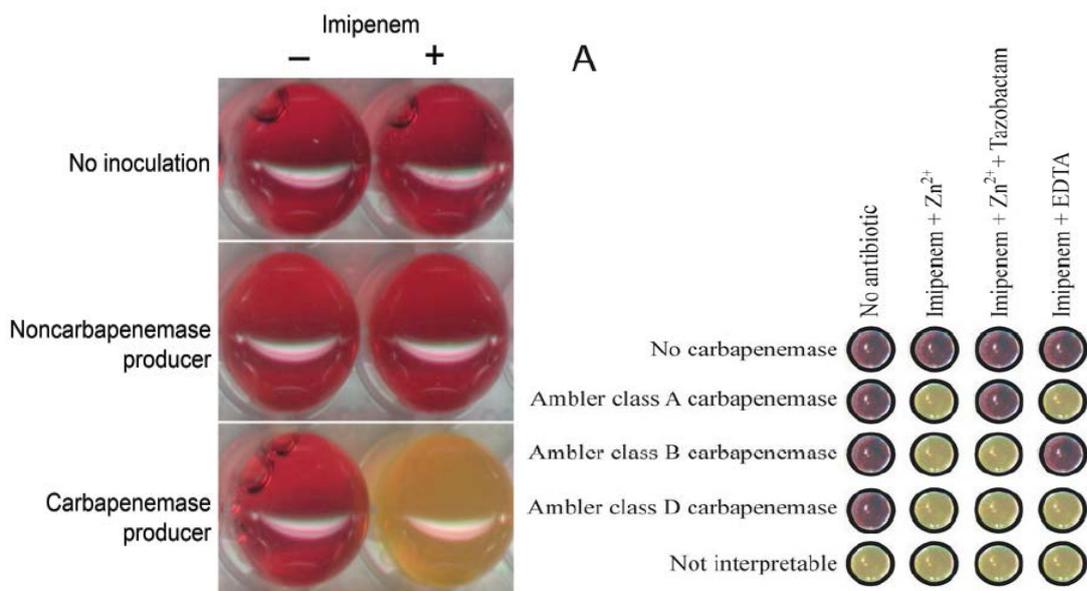
Courtesy of Professor Neil Woodford, Public Health England.

**Acidometric test:** This test is based on the classical acidometric penicillinase test and has a colorimetric endpoint (when the indicator carbapenem is hydrolysed). This assay has been reported to work well for detecting carbapenemases in Enterobacteriaceae and *Pseudomonas* species<sup>43-46</sup>. Commercial versions of the test are available<sup>47</sup>.

The test has multiple benefits - it is relatively inexpensive, rapid, reproducible, and is reported to be highly sensitive and specific. It requires pre-incubation of a carbapenem with the test organism, but can be completed in less than 2 hours. Commercially available versions of the test can give a 'Yes / No' result. By testing carbapenems alone and in the presence of inhibitors, in-house versions 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 studies, the test has poorer sensitivity for CPE with OXA-48-like carbapenemases<sup>48-50</sup>.

The acidometric 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>43,44</sup>.

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)



This has been adapted from the CDC website (<http://wwwnc.cdc.gov/eid/article/18/9/12-0355-f1>) and Dortet<sup>44</sup>.

**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>51-55</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>56-61</sup>. Some are commercially available. Many are suitable either for screening for colonisation (that is, using rectal swabs) or for confirmation of carbapenemases in bacterial colonies. These assays vary in their scope (that is, 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>19,27,58,62</sup>. Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture<sup>27</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.

There are also commercial DNA micro-arrays to detect and distinguish the 'big five' carbapenemases, together with rarer enzymes<sup>63-67</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>14</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 EUCAST/ BSAC, or CLSI recommendations.

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 4.** Control strains producing carbapenemases available from the NCTC

<b>Class A Carbapenemase</b>		
<b>Organism</b>	<b>NCTC strain reference</b>	<b>Characteristics</b>
<i>Klebsiella pneumoniae</i>	NCTC 13438	Member of the international ST258 clone producing KPC-3 carbapenemase
<b>Class B Carbapenemases (Metallo-<math>\beta</math>-lactamases)</b>		
<b>Organism</b>	<b>NCTC strain reference</b>	<b>Characteristics</b>
<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)
<b>Class D Carbapenemases (OXA carbapenemases)</b>		
<b>Organism</b>	<b>NCTC strain reference</b>	<b>Characteristics</b>
<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 2 (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	SE Clone OXA-51-like
<i>Acinetobacter baumannii</i>	NCTC 13422	NW Clone OXA-51-like
<i>Acinetobacter baumannii</i>	NCTC 13423	T 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 (for example, 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>68,69</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 EUCAST/ BSAC, 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.

## 1 Safety considerations<sup>68-84</sup>

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### 1.1 Specimen collection, transport and storage<sup>68-73</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>68-84</sup>

Containment Level 2 organisms.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>76</sup>.

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

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

## 2 Specimen collection

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### 2.1 Type of specimens

Screening specimens including stool, rectal or peri-rectal swabs, any clinical specimens such as blood, wounds or urine

The potential for spread of acquired carbapenemases means that an indicator carbapenem should ideally be tested against all clinically-significant Gram negative bacteria. Minimum testing should include isolates from 'high-risk' patients and settings in accordance with current national guidance, such as patients who have been in-patients in a hospital abroad or in a hospital in the UK known to have problems with spread of CPE or patients known to have been previously colonised or infected with CPE, when the information has been provided on the request form accompanying the specimen and any isolates found grossly resistant to co-amoxiclav.

### 2.2 Optimal time and method of collection<sup>85</sup>

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible<sup>85</sup>.

Unless otherwise stated, swabs for bacterial culture should be placed in appropriate transport medium<sup>86-90</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>85</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 of carbapenemase producers, 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>68,69</sup>

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#### **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>85</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>85</sup>.

### **4 Specimen processing/procedure**<sup>68,69</sup>

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#### **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](#)).

### 4.5.1 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
<b>Screening:</b> Any condition + detection of carbapenem resistance/ carbapenemase production	Any sample	Chromogenic agars with carbapenem ***/ Chromogenic medium for CPE  <b>OR</b> If not available, MacConkey / CLED agar + 10µg MEM disc*	35-37	Aerobic	18-24hr	≥18hr	Carbapenemase producing Enterobacteriaceae
			35-37	Aerobic	16-48hr	≥16hr	<i>Pseudomonas</i> species# <i>Acinetobacter</i> species
			35-37	Aerobic	18-24hr	≥18hr	Carbapenemase producing Enterobacteriaceae
In certain situations where increased sensitivity is desired	Any sample	Chromogenic agars with carbapenem ***/ Chromogenic medium for CPE  <b>OR</b> If not available, MacConkey / CLED agar + 10µg ETP disc**91	35-37	Aerobic	18-24hr	≥18hr	<i>Klebsiella pneumoniae</i> <i>Escherichia coli</i>
<b>Diagnostic testing of samples:</b>  Routine Susceptibility testing of clinical samples	Any sample	Routine susceptibility testing media against co-amoxiclav## (minimum) or ideally meropenem Use Table 2 for screening criteria.	35-37	Aerobic	18-24hr	≥18hr	Carbapenemase producing Enterobacteriaceae

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

\*\*\* For chromogenic media, refer to manufacturer's instructions for recommended incubation times. Chromogenic agars are recommended. Alternative methods are not recommended, but if one is chosen locally for reasons of cost (for example MacConkey or CLED agar with a carbapenem disc) it should have been validated locally and the medium and interpretive criteria applied should have demonstrated performance at least equivalent to plating on to a commercially-prepared agar specifically recommended for this purpose.

# Isolates from patients with CF could be excluded during detection of carbapenemases in *P. aeruginosa*<sup>35</sup>.

## All co-amoxiclav resistant isolates should be tested for resistance to carbapenems.

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

All Enterobacteriaceae	species level <a href="#">ID 16 - Identification of Enterobacteriaceae</a>
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<p><a href="#">Pseudomonas species</a></p> <p><a href="#">Acinetobacter species</a></p>	<p>species level</p> <p><a href="#">ID 17 - Identification of Pseudomonas species and other non-glucose fermenters</a></p>
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Organisms may be further identified if this is clinically or epidemiologically indicated.

#### 4.7 Antimicrobial susceptibility testing

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

Species identification is highly desirable to allow proper interpretation of results. BSAC and EUCAST recommended clinical breakpoints for the carbapenems 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, 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 (see Table 2) for detecting putative CPE, with meropenem the preferred screening agent since it offers the best compromise between sensitivity and specificity.

#### 4.8 Referral for outbreak investigations

See section 4.9.

#### 4.9 Referral to reference laboratories

Any isolate which appears to be resistant to the indicator carbapenem through an acquired resistance mechanism should be referred for confirmation. Most NHS laboratories in England submit their isolates to AMRHAI, PHE<sup>92</sup>. Some PHE Specialist Laboratories offer referral services at a regional level, and then refer selected isolates onwards to AMRHAI. 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://cro.phe.nhs.uk/>) when submitting isolates of Gram negative bacteria suspected to produce an acquired carbapenemases<sup>93</sup>.

At the time of issue, the AMRHAI Reference Unit at PHE Colindale seeks:

- all Enterobacteriaceae suspected to produce a carbapenemase
  - a. isolates of *Enterobacter* that have borderline resistance to ertapenem, but remain fully susceptible to other carbapenems should not be sent
  - b. 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 to produce a carbapenemase (that is, a transferable carbapenem resistance mechanism). Such isolates should be resistant to all relevant carbapenems (that is, imipenem, meropenem and

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

meropenem) and usually ceftazidime; susceptibility or resistance to aztreonam is variable because most carbapenemase-producing pseudomonads have metallo-enzymes, usually VIM-types

- a. isolates of *Pseudomonas* species resistant only to carbapenems and susceptible to other  $\beta$ -lactams should not be sent
  - b. 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
- all *Acinetobacter* species suspected to produce a metallo-carbapenemase, that is, with strong imipenem-EDTA synergy
    - a. isolates of *Acinetobacter* that are resistant to ertapenem, but susceptible to other carbapenems should not be sent. Ertapenem resistance is inherent in the genus
  - isolates of *Stenotrophomonas maltophilia*, *Aeromonas* species and 'chryseobacteria' for investigation of carbapenem resistance should not be sent for confirmation of either carbapenem resistance or carbapenemase production because metallo-carbapenemase production is an intrinsic characteristic of these bacteria

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.

In addition, the AMRHAI Reference Unit seeks 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 (AMRHAI) Reference Unit

Bacteriology Reference Department

National Infections 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 3276511/ 7877

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

<http://www.wales.nhs.uk/sites3/page.cfm?orgid=457&pid=28768>

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

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### 5.1 Microscopy

N/A

#### 5.1.1 Microscopy reporting time

N/A

### 5.2 Culture

#### Screening samples

##### 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

Interim or preliminary results should be issued on detection of potentially 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.

### 5.3 Antimicrobial susceptibility testing

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

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>92</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>92</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>94,95</sup>, or equivalent in the devolved administrations<sup>96-99</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.

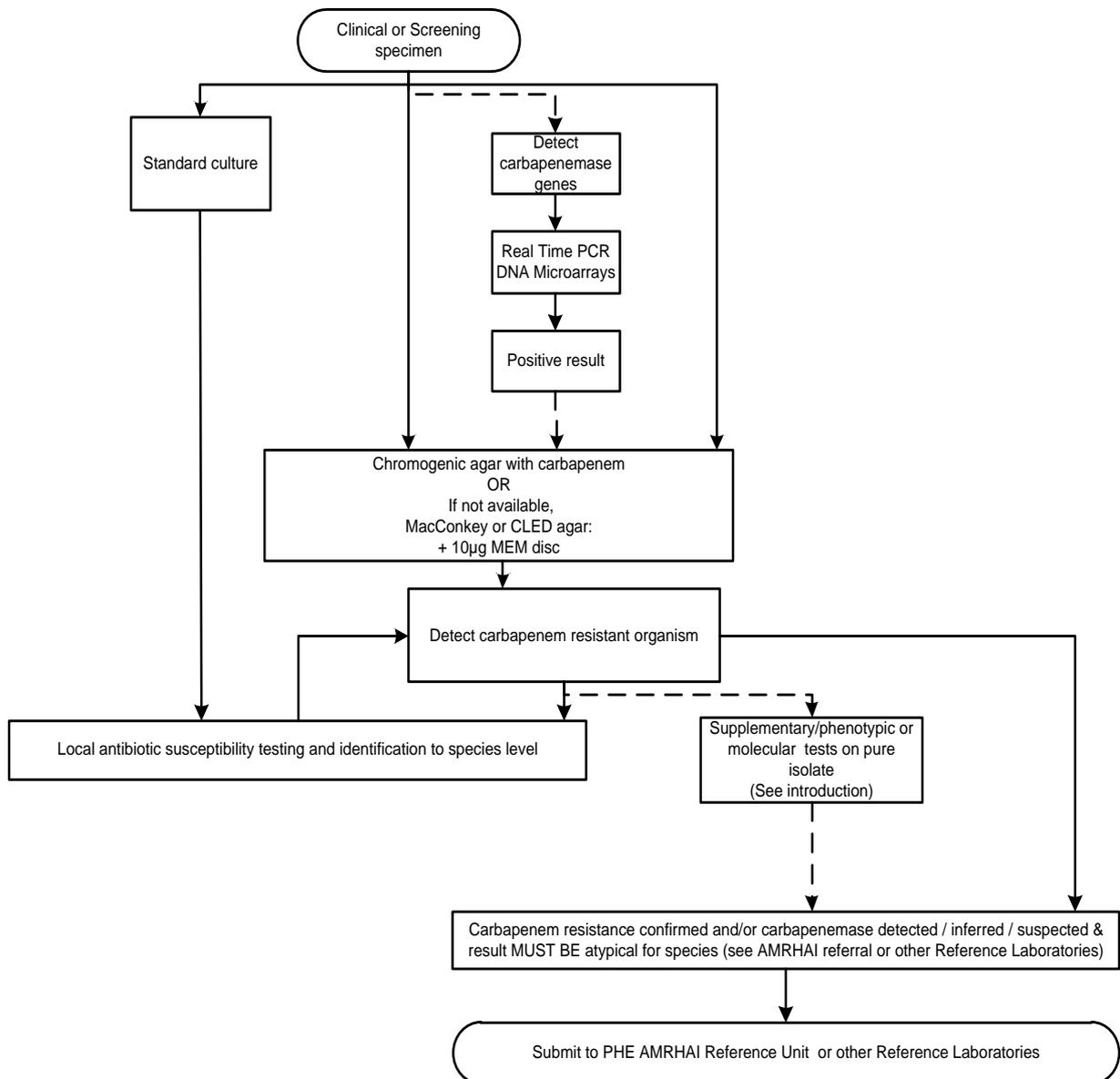
**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it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

Detection of bacteria with carbapenem-hydrolysing  $\beta$ -lactamases (carbapenemases)

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

Other arrangements exist in [Scotland](#)<sup>96,97</sup>, [Wales](#)<sup>98</sup> and [Northern Ireland](#)<sup>99</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

<i>Published evaluations of media / methods for detecting carbapenemase-producing Enterobacteriaceae in various patient populations</i>						
Reference	Media / Method tested	Sensitivity (%)	Specificity (%)	Study location	No. of positive Samples/Total Samples	Comments
18	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			
19	CHROMagar KPC	100	98.4	Israel	41 / 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			
20	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			
22	Colorex KPC	97	96	Pakistan	37 / 200	Prototype version of chromID CARBA tested. All CPE had NDM-1
	chromID CARBA	100	93			
23	chromID CARBA	100	98	Pakistan	32 / 175	All CPE had NDM-1
	Brilliance CRE	62.5	34			
24	TSB plus ertapenem (2 mg/L)	89.1	86.4	Greece	73 / 200	Predominant carbapenemases were KPC and VIM
	chromID ESBL	92.4	93.3			
	chromID ESBL (plus enrichment)	92.4	84.7			
	chromID CARBA	92.4	96.9			
	MacConkey plus meropenem (1 mg/L)	89.1	85.2			
27	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<sub>KPC</sub></i>	97	96.6			
30	CHROMagar KPC	76.6	75.7	USA	47 / 150	All isolates of CPE had KPC enzyme
	Remel SPECTRA CRE	97.8	86.4			

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	MacConkey plus ertapenem disc	83	73.8			
31	chromID OXA-48	91	100	France	53/117	OXA-48 producers were easily detected
	chromID CARBA	21	68			
	SUPERCARBA	91	53			
32	chromID ESBL <sup>c</sup>	90	68.6	Morocco	10 / 77	All CPE had OXA-48
	<i>Brilliance</i> CRE <sup>c</sup>	80	86.6			
	SUPERCARBA <sup>c</sup>	80	98.5			
33	chromID CARBA	96.5	91.2	Greece	86/177	KPC was the predominant carbapenemase
	MacConkey plus imipenem (1 mg/L)	89.5	31.9			
	TSB plus ertapenem (2 mg/L)	98.8	80.2			
100	chromID CARBA	57.6	98.9	Turkey	33/302	All CPE had OXA-48
	chromID OXA-48	75.8	99.3			
	TSB plus ertapenem (2 mg/L)	57.6	95.2			

### Footnotes

- a) Not determined or not reported.
- b) Selective agar with vancomycin, amphotericin B, ceftazidime, and clindamycin (VACC).
- c) All media showed 100% sensitivity after enrichment.

## References

### Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation		Quality of evidence	
A	Strongly recommended	I	Evidence from randomised controlled trials, meta-analysis and systematic reviews
B	Recommended but other alternatives may be acceptable	II	Evidence from non-randomised studies
C	Weakly recommended: seek alternatives	III	Non-analytical studies, eg case reports, reviews, case series
D	Never recommended	IV	Expert opinion and wide acceptance as good practice but with no study evidence
		V	Required by legislation, code of practice or national standard
		VI	Letter or other

- Walsh TR. Emerging carbapenemases: a global perspective. *IntJAntimicrobAgents* 2010;36 Suppl 3:S8-14. **B, III**
- Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *ClinMicrobiolInfect* 2012;18:413-31. **B, III**
- Woodford N. Fighting the rising tide of carbapenemases in *Enterobacteriaceae*. Be SMART with ResistanceInternational Newsletter 2012. **C, III**
- Walther-Rasmussen J, Hoiby N. Class A carbapenemases. *JAntimicrobChemother* 2007;60:470-82. **B, III**
- Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet InfectDis* 2009;9:228-36. **B, III**
- Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. *InfectDrug Resist* 2012;5:133-41. **B, III**
- Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *ClinMicrobiolRev* 2007;20:440-58, table. **B, III**

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

8. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011;17:1791-8. **B, III**
9. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrob Agents Chemother* 2010;54:24-38. **C, III**
10. Marsik FJ, Nambiar S. Review of carbapenemases and AmpC-beta lactamases. *Pediatr Infect Dis J* 2011;30:1094-5. **B, III**
11. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. *J Antimicrob Chemother* 2009;63:659-67. **B, I**
12. Livermore DM. Of Pseudomonas, porins, pumps and carbapenems. *J Antimicrob Chemother* 2001;47:247-50. **B, III**
13. Akova M, Daikos GL, Tzouveleki L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. *Clin Microbiol Infect* 2012;18:439-48. **C, III**
14. Giske CG, Martinez-Martinez L, R C, Stefani S, Skov R, Y G et al. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0. Testing) EECAS 2013. 1-40. **B, V**
15. Day KM, Pike R, Winstanley TG, Lanyon C, Cummings SP, Raza MW et al. Use of faropenem as an indicator of carbapenemase activity in the Enterobacteriaceae. *J Clin Microbiol* 2013;51:1881-6. **B, I**
16. Hu F, Ahn C, O'Hara JA, Doi Y. Faropenem disks for screening of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol* 2014;52:3501-2. **B, II**
17. BSAC. Susceptibility Testing News BSAC. 2015. **A, V**
18. Adler A, Navon-Venezia S, Moran-Gilad J, Marcos E, Schwartz D, Carmeli Y. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant Enterobacteriaceae from surveillance rectal swabs. *J Clin Microbiol* 2011;49:2239-42. **C, II**
19. Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. *J Clin Microbiol* 2008;46:3110-1. **C, II**
20. Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H. Evaluation of CHROMagar KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures. *Int J Antimicrob Agents* 2011;37:124-8. **C, I**
21. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. *J Clin Microbiol* 2011;49:718-21. **B, III**
22. Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S et al. Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. *J Antimicrob Chemother* 2011;66:2288-94. **C, I**
23. Day KM, Ali S, Mirza IA, Sidjabat HE, Silvey A, Lanyon CV et al. Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media. *Diagn Microbiol Infect Dis* 2013;75:187-91. **B, I**

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

24. Vrioni G, Daniil I, Voulgari E, Ranellou K, Koumaki V, Ghirardi S et al. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. *JClinMicrobiol* 2012;50:1841-6. **C, I**
25. Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium. *JClinMicrobiol* 2012;50:2761-6. **C, I**
26. Girlich D, Poirel L, Nordmann P. Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of Enterobacteriaceae with reduced susceptibility to carbapenems. *DiagnMicrobiolInfectDis* 2013;75:214-7. **C, II**
27. Singh K, Mangold KA, Wyant K, Schora DM, Voss B, Kaul KL et al. Rectal screening for *Klebsiella pneumoniae* carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. *JClinMicrobiol* 2012;50:2596-600. **C, II**
28. Hornsey M, Phee L, Woodford N, Turton J, Meunier D, Thomas C et al. Evaluation of three selective chromogenic media, CHROMagar ESBL, CHROMagar CTX-M and CHROMagar KPC, for the detection of *Klebsiella pneumoniae* producing OXA-48 carbapenemase. *JClinPathol* 2013. **C, II**
29. Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae. *JClinMicrobiol* 2012;50:3102-4. **C, I**
30. Vasoo S, Lolans K, Li H, Prabaker K, Hayden MK. Comparison of the CHROMagar KPC, Remel Spectra CRE, and a direct ertapenem disk method for the detection of KPC-producing Enterobacteriaceae from perirectal swabs. *DiagnMicrobiolInfectDis* 2014;78:356-9. **B, II**
31. Girlich D, Anglade C, Zambardi G, Nordmann P. Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae. *DiagnMicrobiolInfectDis* 2013;77:296-300. **B, II**
32. Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P. High rate of faecal carriage of extended-spectrum beta-lactamase and OXA-48 carbapenemase-producing Enterobacteriaceae at a university hospital in Morocco. *ClinMicrobiolInfect* 2014;20:350-4. **B, I**
33. Papadimitriou-Olivgeris M, Bartzavali C, Christofidou M, Bereksi N, Hey J, Zambardi G et al. Performance of chromID(R) CARBA medium for carbapenemase-producing Enterobacteriaceae detection during rectal screening. *EurJClinMicrobiolInfectDis* 2014;33:35-40. **B, II**
34. Centers for Disease Control and Prevention. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp. and *E. coli* from rectal swabs. 2009. **B, III**
35. Tai AS, Kidd TJ, Whiley DM, Ramsay KA, Buckley C, Bell SC. Molecular surveillance for carbapenemase genes in carbapenem-resistant *Pseudomonas aeruginosa* in Australian patients with cystic fibrosis. *Pathology* 2015;47:156-60. **B, II**
36. Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF et al. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. *Antimicrobial agents and chemotherapy* 2010;54:1354-7. **B, III**
37. Robledo IE, Aquino EE, Vazquez GJ. Detection of the KPC gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-based nosocomial surveillance study in Puerto Rico. *AntimicrobAgents Chemother* 2011;55:2968-70. **B, III**

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

38. Cuzon G, Naas T, Villegas MV, Correa A, Quinn JP, Nordmann P. Wide dissemination of *Pseudomonas aeruginosa* producing beta-lactamase blaKPC-2 gene in Colombia. *AntimicrobAgents Chemother* 2011;55:5350-3. **C, III**
39. Poirel L, Nordmann P, Lagrutta E, Cleary T, Munoz-Price LS. Emergence of KPC-producing *Pseudomonas aeruginosa* in the United States. *AntimicrobAgents Chemother* 2010;54:3072. **B, III**
40. Ge C, Wei Z, Jiang Y, Shen P, Yu Y, Li L. Identification of KPC-2-producing *Pseudomonas aeruginosa* isolates in China. *JAntimicrobChemother* 2011;66:1184-6. **B, III**
41. Akpaka PE, Swanston WH, Ihemere HN, Correa A, Torres JA, Tafur JD et al. Emergence of KPC-producing *Pseudomonas aeruginosa* in Trinidad and Tobago. *JClinMicrobiol* 2009;47:2670-1. **B, III**
42. Woodford N, Eastaway AT, Ford M, Leanord A, Keane C, Quayle RM et al. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mechanisms responsible for carbapenem resistance in Enterobacteriaceae. *JClinMicrobiol* 2010;48:2999-3002. **B, III**
43. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *EmergInfectDis* 2012;18:1503-7. **B, I**
44. Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in Enterobacteriaceae and *Pseudomonas* spp. by using a biochemical test. *AntimicrobAgents Chemother* 2012;56:6437-40. **B, II**
45. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. *JAntimicrobChemother* 2013;68:487-9. **B, III**
46. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing *Pseudomonas* spp. *JClinMicrobiol* 2012;50:3773-6. **B, I**
47. Pires J, Novais A, Peixe L. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. *Journal of clinical microbiology* 2013;51:4281-3. **B, II**
48. Huang TD, Berhin C, Bogaerts P, Glupczynski Y. Comparative evaluation of two chromogenic tests for rapid detection of carbapenemase in Enterobacteriaceae and in *Pseudomonas aeruginosa* isolates. *JClinMicrobiol* 2014;52:3060-3. **B, I**
49. Knox J, Jadhav S, Sevier D, Agyekum A, Whipp M, Waring L et al. Phenotypic detection of carbapenemase-producing Enterobacteriaceae using MALDI-TOF MS and the Carba NP test. *JClinMicrobiol* 2014. **B, II**
50. Tijet N, Boyd D, Patel SN, Mulvey MR, Melano RG. Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and *Pseudomonas aeruginosa*. *AntimicrobAgents Chemother* 2013;57:4578-80. **B, II**
51. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. *JClinMicrobiol* 2011;49:3321-4. **B, III**
52. Hrabak J, Studentova V, Walkova R, Zemlickova H, Jakubu V, Chudackova E et al. Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *JClinMicrobiol* 2012;50:2441-3. **B, II**

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

53. Sparbier K, Schubert S, Weller U, Boogen C, Kostrzewa M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against beta-lactam antibiotics. *JClinMicrobiol* 2012;50:927-37. **B, II**
54. Hrabak J, Chudackova E, Walkova R. Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *ClinMicrobiolRev* 2013;26:103-14. **B, III**
55. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. *ClinMicrobiolRev* 2013;26:547-603. **B, III**
56. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *JAntimicrobChemother* 2007;59:321-2. **B, II**
57. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC et al. Rapid detection and identification of metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. *JClinMicrobiol* 2007;45:544-7. **B, II**
58. Avlami A, Bekris S, Ganteris G, Kraniotaki E, Malamou-Lada E, Orfanidou M et al. Detection of metallo-beta-lactamase genes in clinical specimens by a commercial multiplex PCR system. *JMicrobiolMethods* 2010;83:185-7. **B, I**
59. Swayne RL, Ludlam HA, Shet VG, Woodford N, Curran MD. Real-time TaqMan PCR for rapid detection of genes encoding five types of non-metallo- (class A and D) carbapenemases in Enterobacteriaceae. *IntJAntimicrobAgents* 2011;38:35-8. **B, I**
60. Kaase M, Szabados F, Wassill L, Gatermann SG. Detection of carbapenemases in Enterobacteriaceae by a commercial multiplex PCR. *JClinMicrobiol* 2012;50:3115-8. **B, II**
61. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *DiagnMicrobiolInfectDis* 2011;70:119-23. **B, I**
62. Naas T, Cotellon G, Ergani A, Nordmann P. Real-time PCR for detection of blaOXA-48 genes from stools. *JAntimicrobChemother* 2013;68:101-4. **B, II**
63. Endimiani A, Hujer AM, Hujer KM, Gatta JA, Schriver AC, Jacobs MR et al. Evaluation of a commercial microarray system for detection of SHV-, TEM-, CTX-M-, and KPC-type beta-lactamase genes in Gram-negative isolates. *JClinMicrobiol* 2010;48:2618-22. **B, II**
64. Stuart JC, Voets G, Scharringa J, Fluit AC, Leverstein-Van Hall MA. Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray. *JMed Microbiol* 2012;61:809-12. **B, II**
65. Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. *JClinMicrobiol* 2011;49:1608-13. **B, II**
66. Woodford N, Warner M, Pike R, Zhang J. Evaluation of a commercial microarray to detect carbapenemase-producing Enterobacteriaceae. *JAntimicrobChemother* 2011;66:2887-8. **B, II**
67. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX)

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). *JAntimicrobChemother* 2012;67:1865-9. **B, II**

68. European Parliament. UK Standards for Microbiology Investigations (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". 1998.
69. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices 1998. 1-37.
70. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009.
71. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
72. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
73. Home Office. Anti-terrorism, Crime and Security Act. 2001.
74. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2013. 1-32.
75. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office 2003.
76. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive 2005.
77. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive 2008.
78. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. *MMWR Surveill Summ* 2012;61:1-102.
79. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed.: HSE Books; 2002.
80. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
81. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
82. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books 2003.
83. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000.

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

84. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. 1-14.
85. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Jr. et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *ClinInfectDis* 2013;57:e22-e121.
86. Rishmawi N, Ghneim R, Kattan R, Ghneim R, Zoughbi M, Abu-Diab A et al. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. *JClinMicrobiol* 2007;45:1278-83.
87. Barber S, Lawson PJ, Grove DI. Evaluation of bacteriological transport swabs. *Pathology* 1998;30:179-82.
88. Van Horn KG, Audette CD, Sebeck D, Tucker KA. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. *JClinMicrobiol* 2008;46:1655-8.
89. Nys S, Vijgen S, Magerman K, Cartuyvels R. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*. *EurJClinMicrobiolInfectDis* 2010;29:453-6.
90. Tano E, Melhus A. Evaluation of three swab transport systems for the maintenance of clinically important bacteria in simulated mono- and polymicrobial samples. *APMIS* 2011;119:198-203.
91. Lolans K, Calvert K, Won S, Clark J, Hayden MK. Direct ertapenem disk screening method for identification of KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* in surveillance swab specimens. *JClinMicrobiol* 2010;48:836-41. **B, II**
92. Livermore DM, Andrews JM, Hawkey PM, Ho PL, Keness Y, Doi Y et al. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? *J AntimicrobChemother* 2012;67:1569-77. **C, III**
93. Freeman R, Ironmonger D, Puleston R, Woodford N, Johnson A. Implementation of an Electronic Reporting System for the enhanced surveillance of carbapenemase-producing Gram-negative bacteria. 2015/032. England PH 2015. **A, V**
94. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories 2013. 1-37.
95. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010.
96. Scottish Government. Public Health (Scotland) Act. 2008.
97. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.
98. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
99. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967.
100. Zarakolu P, Day KM, Sidjabat HE, Kamolvit W, Lanyon CV, Cummings SP et al. Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey. *European journal of clinical*

## Detection of bacteria with carbapenem-hydrolysing $\beta$ -lactamases (carbapenemases)

microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 2015;34:519-25. **B, II**