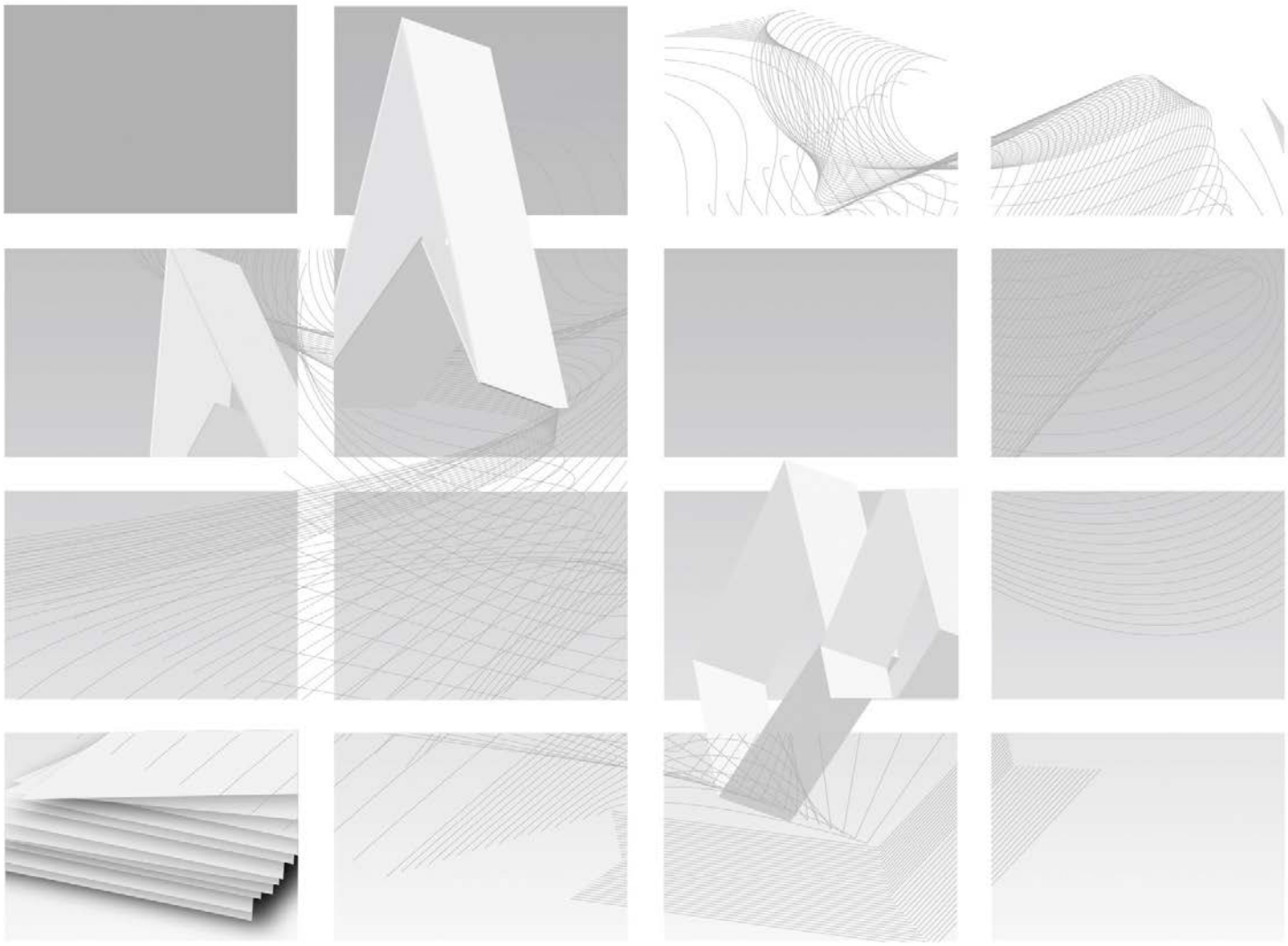
Public Health England

NHS

UK Standards for Microbiology Investigations

Laboratory Detection and Reporting of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases)



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UK Protocols | P 8 | Issue no: 1.1 | Issue date: 08.05.14 | Page: 1 of 25

Acknowledgments

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 http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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Contents

Amendment Table

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.

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

Amendment No/Date.	1/06.05.14
Issue no. discarded.	1
Insert Issue no.	1.1
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Scientific content remains unchanged.

Amendment No/Date.	-/26.03.13
Issue no. discarded.	-
Insert Issue no.	1
Section(s) involved	**Amendment**

UK SMI[#]: 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 http://www.hpa.org.uk/SMI/Partnerships. 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 laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] 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.

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.

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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.

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Scope of Document

This SMI describes the gives recommendation on the detection of 'carbapenemases' (carbapenem-hydrolysing β-lactamases). It should be read in conjunction with any local documents and the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit / PHE Guidance, which is available at http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/CarbapenemResistance/GuidanceOnCarbapenamProducers/.

This SMI should be used in conjunction with other SMIs.

Introduction

The term carbapenemase is used to mean any β-lactamase that hydrolyses carbapenems ie any or all of doripenem, ertapenem, imipenem and meropenem. Of clinical concern, many carbapenemases confer resistance or reduced susceptibility to all or nearly all members of the β-lactam class, not just to carbapenems.

Carbapenemases are intrinsic (found naturally) in a few clinical bacteria, such as *Stenotrophomonas maltophilia*, *Aeromonas* sp., 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* sp. and Proteeae have intrinsically poor susceptibility or low-level resistance to imipenem.

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

Acquired carbapenemases are diverse (see http://www.lahey.org/studies) and include members of three of the four β-lactamase families[1-3].

- Class A enzymes: The most problematic here are the KPC enzymes, which are now endemic in parts of the USA, Greece, Italy, Israel and China, and are increasingly encountered elsewhere, including in the UK[2-5]. Other, less-frequently-encountered class A carbapenemases include some GES types, IMI/NMC (in *Enterobacter*), and SME (in *Serratia*). Of these, only IMI /NMC has been detected (and very rarely) in the UK during the last 10 years
- Class B enzymes: Also known as metallo-β-lactamases (MBLs) or metallo-carbapenemases[1-3]. These differ fundamentally from all other β-lactamases because they require zinc ions for activity. Consequently they are inactivated by metal ion chelators, such as EDTA. The major MBL families encountered in the UK are the NDM, VIM and, less commonly, the IMP types. Other types include AIM, DIM, GIM, SIM, and SPM enzymes, but these have not yet been detected in the UK

- Class D enzymes: This class comprises many diverse enzymes, few of which are carbapenemases[1-3,6]. Important carbapenemases within the family include OXA-23, -40, -51 and -58 and their variants from *Acinetobacter* species, OXA-48 and related enzymes in Enterobacteriaceae; other rarer carbapenem-hydrolysing 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. 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[7]. 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* sp. and *Klebsiella* spp., but also occurs in *E. coli* and other genera. It most markedly affects ertapenem; isolates may remain susceptible to other carbapenems, 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)
- *P. aeruginosa*, by far the commonest mode of carbapenem resistance is loss of OprD porin, and isolates only resistant to imipenem, but not other β-lactams are certain to have this mechanism. Meropenem, though not imipenem, is also affected by upregulated efflux in *P. aeruginosa*
- Non-carbapenemase mechanisms have been claimed in *Acinetobacter*, but may reflect failure to detect weak OXA carbapenemases, rather than their absence

Carbapenemases are clinically important because they destroy and so may confer resistance to carbapenems (and usually most other β-lactams). Delayed recognition and inappropriate treatment of severe infections caused by carbapenemase producers is associated with increased mortality[8]. Many producers are multi-resistant to non-β-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, IMP, NDM and VIM) 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.

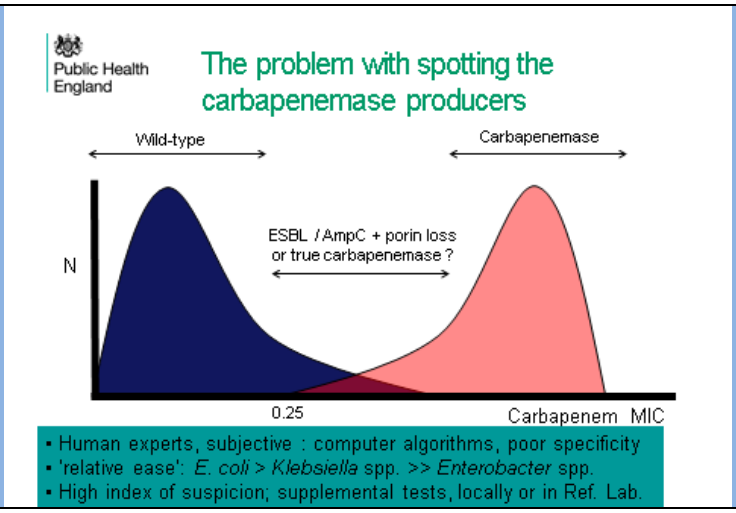
The level (or lack) of carbapenem resistance displayed by some carbapenemase producers is a genuine cause for concern. Higher MICs are observed when producers also lack major porins, but this indicates potential for carbapenemase genes to spread undetected among normally-permeable strains. This concern 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 resistance profile of the host strain.

Concerns about carbapenemases mean that all clinically-significant Gram negative bacteria should be screened routinely for susceptibility to at least one 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.

1 Overview of the Strategy for Recognizing Potential Carbapenemase Producers

- The aims are: (i) to recognize all 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
- 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 supplemental tests (see 3.2 and 3.3), locally or in a specialist or reference laboratory (eg PHE – AMRHAI – Colindale)

Figure 1. The problem with spotting the carbapenemase producers



1.1 Enterobacteriaceae

- Test a carbapenem against all clinically-significant isolates. 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
- Do carbapenemase confirmatory tests (below) on isolates found resistant 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
- Consider whether the isolate should be submitted to the reference laboratory (section 5)

1.2 Non-fermenters

- Acquired carbapenemases are also encountered in *Acinetobacter* sp, *Pseudomonas* sp (most commonly, though not exclusively in *P. aeruginosa)* and in other non-fermenters[1-3,6]
- Test imipenem, meropenem or doripenem against all clinically-significant isolates. Do not use ertapenem because these species are intrinsically resistant to this carbapenem
- Decide whether supplemental tests are needed (see 1.2.1 and 1.2.2 below)
- 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 these indicator carbapenems, and to species level if the genus is not known to produce an intrinsic carbapenemase
- Consider whether the isolate should be submitted to the reference laboratory (section 5)

1.2.1 *Acinetobacter* species

- Carbapenem resistance in *Acinetobacter* sp. 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* sp. have rarely been reported in other genera and horizontal spread to other strains, species or genera is not considered a significant risk.
- Need for supplemental tests: Carbapenem-resistant *Acinetobacter* sp. can usually be reported as likely OXA-carbapenemase producers without supplemental 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 an NDM-type metallo-enzyme.
- Strong EDTA synergy (>8-fold) correlates well with MBL production in *Acinetobacter* sp., 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 recorded in Europe[9,10].

1.2.2 *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 supplemental tests: Isolates resistant only to carbapenems can be inferred to have porin loss and need not be investigated further. However, isolates resistant to carbapenems and also to ceftazidime and piperacillin-tazobactam 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 β-lactams is the ‘classic’ MBL phenotype, but many MBL producers are resistant to aztreonam owing to additional mechanisms meaning that the ‘classic’ pattern is not always seen.

- 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, at the time of writing, in Europe[10-14].

2 Laboratory Detection: Screening, and Confirmation

The basic strategy to detect carbapenemase producers, outlined in section 2 above, is to use an indicator carbapenem to screen for resistance, and then to undertake supplementary tests (see 2.2 and 2.3) to distinguish carbapenemase producers from those that have other carbapenem resistance mechanisms.

2.1 Screening

2.1.1 Which Specimens and Isolates to Screen

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

2.1.2 How to Screen for Carbapenem Resistance

The indicator drugs should be included in primary susceptibility testing eg by the method of the British Society for Antimicrobial Chemotherapy (BSAC; http://bsac.org.uk/susceptibility/guidelines-standardized-disc-susceptibility-testing-method/) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/antimicrobial_susceptibility_testing/).

Species identification is highly desirable to allow proper interpretation of results.

Table 1. *Current BSAC and EUCAST clinical breakpoints for carbapenems*

Antibiotic (10µg disc content)	**Bacteria**	**Zone breakpoints (mm)**				**MIC (mg/L)**	
		BSAC		**EUCAST**			
		R ≤	S ≥	R <	S ≥	R >	S ≤
Doripenem	Enterobacteriaceae	18	24	18	24	4	1
	Acinetobacter	14	22	15	21		
	Pseudomonas	24	32	19	25		
Ertapenem	Enterobacteriaceae	15	28	22	25	1	0.5
Imipenem	Enterobacteriaceae	16	21	16	22	8	2
	Acinetobacter	13	25	17	23		

	Pseudomonas	16	23	17	20		4
Meropenem	Enterobacteriaceae	19	27	16	22	8	2
	Acinetobacter	12	20	15	21		
	Pseudomonas	15	20	18	24		

EUCAST's ECOFFs mark the limit of the wild-type population (by a statistical definition), meaning that isolates with higher MICs/lower zone diameters represent non-wild-type isolates. EUCAST has proposed the following screening cut-off values for detecting putative carbapenemase-producing Enterobacteriaceae. It should be noted that for imipenem and ertapenem the screening cut-off MIC values have been set one-dilution step higher than the currently-defined ECOFFs to increase specificity.

Table 2. *Proposed EUCAST screening cut-off values for possible carbapenemase-producing Enterobacteriaceae**

Carbapenem	MIC (mg/L)	Zone diameter (mm)
Meropenem	>0.125	<25
Imipenem	>1	<23
Ertapenem	>0.125	<25

*Consultation document available at: http://www.eucast.org/eucast_news/news_singleview/?no_cache=1&tx_ttnews%5Btt_news%5D=54.

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

- The zone diameter around a carbapenem disc indicates non-susceptibility (in a bona fide susceptibility test or a screen using MacConkey or CLED agar)
- Colonies are obtained on any commercially-available agar for detecting carbapenem-resistant bacteria (see section 3.1 below)
- Automated systems should flag non-susceptibility to any carbapenem, irrespective of the expert interpretation given (unless it's explained by intrinsic resistance)
- In-house or commercial molecular tests yield a positive 'hit'

2.2 Confirmatory Tests for Carbapenemases: inhibitor-based

Bacterial isolates resistant to any indicator carbapenem in the screening tests above (Section 2.1) should be subjected to confirmatory tests. Many of these depend on

demonstrating synergy between an indicator carbapenem and various β-lactamase inhibitors.

Synergy is defined by a significant (>5mm) expansion of the carbapenem zone size or significant (≥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 between				Resistance to	
	Carbapenem + clavulanate*	Carbapenem + cloxacillin*	Carbapenem + boronic acid	Carbapenem + EDTA / dipicolinic acid	Aztreonam	Temocillin (MIC ≥64mg/L or no zone around 30µg disc)
ESBL or AmpC + porin loss	+/-	+/-	+/-	-	R	-
MBL (IMP, NDM, VIM)	-	-	-	+++	S	+ +
KPC	+/-	-	+++	-	R	+/-
OXA-48	-	-	-	-	S	+++

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 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*; you only need MICs. 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

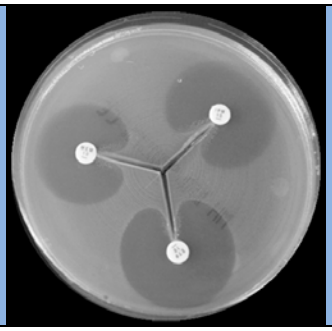
enzymes[15]. For this reason, the underlying resistance mechanisms inferred by expert algorithms should be viewed with caution; some warn of potential carbapenemase production by every carbapenem-resistant isolate (good sensitivity and poor specificity) while others attempt to distinguish true carbapenemase 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.

2.3 Confirmatory Tests for Carbapenemases: other methods

In addition to inhibitor-based supplemental tests, other methods may also be considered for detecting likely carbapenemase producers. These include:

Modified Hodge Test (MHT) or 'Cloverleaf' test: a 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 10µg discs of ETP, IPM and MEM, but the test remains subjective and 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.

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



MALDI-ToF: Increasingly available to diagnostic laboratories, MALDI-ToF also offers the potential to detect carbapenemase production[16-19]. The assay detects mass changes that follow hydrolysis of a carbapenem molecule. This assay is not yet commercially available and 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.

'Carba-NP': a recently described test in microtitre tray format. It 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* sp[20-23]. 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 β-lactamase class (class A, B or D). As with MALDI-ToF, it 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.

Molecular tests: There are numerous block-based or real-time PCR assays in the literature, either using simple or multiplex formats[24-29]. Some are commercially available. These vary in their scope (ie the range of genes sought) and the extent to which they can be customized by the end-user. There are also commercial micro-arrays to detect and distinguish the 'big five' carbapenemases[30-34]. 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.

2.4 Controls for Carbapenemase Tests

Quality control of the carbapenem discs used in the primary screening should follow standard 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 (http://www.hpacultures.org.uk/media/793/06/M015.20121119.v2_AntimicrobResMech_A4.pdf).

Table 4. Control strains producing carbapenemases available from the NCTC

4.1 Class A Carbapenemase

Organism	NCTC® Strain Reference	Characteristics
Klebsiella pneumoniae	NCTC 13438	Member of the international ST258 clone producing KPC-3 carbapenemase

4.2 Class B Carbapenemases (Metallo-β-lactamases):

Organism	NCTC® Strain Reference	Characteristics
Pseudomonas aeruginosa	NCTC 13437	VIM-10; VEB-1
Klebsiella pneumoniae	NCTC 13439	VIM-1; QnrS1 (outbreak strain)
Klebsiella pneumoniae	NCTC 13440	VIM-1; QnrS1 (sporadic)
Klebsiella pneumoniae	NCTC 13443	New Delhi Metallo-β-lactamase (NDM-1)
Escherichia coli	NCTC 13476	IMP-type (unsequenced)

4.3 Class D Carbapenemases (OXA carbapenemases):

Organism	NCTC® Strain Reference	Characteristics
Acinetobacter baumannii	NCTC 13301	OXA-23 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13302	OXA-25 (OXA-24/40-like) (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13303	OXA-26 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13304	OXA-27 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13305	(A 15) OXA-58 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13421	OXA-23 Clone 2 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13424	OXA-23 Clone 1 (also with OXA-51-like)
Acinetobacter baumannii	NCTC 13420	SE Clone OXA-51-like
Acinetobacter baumannii	NCTC 13422	NW Clone OXA-51-like
Acinetobacter baumannii	NCTC 13423	T strain (UK3) OXA-51-like
Klebsiella pneumoniae	NCTC 13442	Sequence type 353 with OXA-48

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

3 Screening of Stool Samples or Rectal Swabs for Carbapenemase-producing Enterobacteriaceae

3.1 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. 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 5 documents studies involving such media that were published either in print or online in English up until the end of 2012. Only studies that have included clinical samples from colonized patients are included. Readers are advised to be cautious in the interpretation of these 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.

CHROMagar KPC (also available as pre-poured plates under the 'Colorex' brand) was the first commercially available chromogenic medium designed for isolation of carbapenem-resistant Enterobacteriaceae. In three early studies it showed good performance when compared with in-house preparations of MacConkey agar incorporating imipenem (or MacConkey with carbapenem discs)[35-37]. Others have shown that isolates of carbapenemase– producing Enterobacteriaceae (CPE) with low carbapenem MICs (eg ≤ 2mg/L meropenem) may not grow on this medium[38,39]. chromID CARBA (or its prototype ID CARBA) has been the subject of two evaluations in Pakistan and one in Greece[39-41]. In the first study in Pakistan, chromID CARBA detected significantly more isolates of Enterobacteriaceae with NDM-1 than Colorex KPC – but despite this, only one additional colonized patient was detected[39]. The second study in Pakistan compared chromID CARBA with *Brilliance* CRE, and significantly more colonized patients were detected using chromID CARBA[40]. However, the authors speculate that the relatively poor performance of *Brilliance* CRE may have been due to deterioration of selective agents during transport of media to Pakistan, so further studies are required[40]. Nordmann *et al*., have advocated the use of SUPERCARBA medium for isolation of all carbapenemase producers including those producing OXA-48, which may be particularly problematic to detect[42,43]. However, there are no published evaluations of SUPERCARBA with samples from colonized patients at this time.

Other media that have been recommended include chromogenic media developed for detection of ESBL-producers e.g. CHROMagar ESBL and chromID ESBL[38,44,45], 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[41-46]. Enrichment broths supplemented with carbapenems have also been advocated, eg in guidelines from the Centers for Disease Control[47]. 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[41-46].

In light of the limited available evidence we would currently 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 chromogenic agar medium specifically recommended for this purpose. It is essential that suspect colonies are then subjected to confirmatory tests as previously described (eg see sections 2.2 and 2.3).

3.2 Molecular Methods

PCR has been successfully utilized for the detection of single or multiple carbapenemase genes directly from clinical samples[26,36,44,48]. Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture[44]. Disadvantages include a higher cost for processing samples and the need for specialized equipment and/or expertise. 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. Even then, this approach will not detect new or rare carbapenemases and will not provide information regarding the host species or its susceptibility.

Table 5. *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
35	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			
36	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			
37	CHROMagar KPC	97.8	ND[a]	Greece	46 / 126	Predominant carbapenemases were KPC and VIM
	MacConkey plus imipenem (1 mg/L)	78.3	ND			
39	Colorex KPC	97	96	Pakistan	37 / 200	Prototype version of chromID CARBA tested. All CPE had NDM-1
	chromID CARBA	100	93			
40	chromID CARBA	100	98	Pakistan	32 / 175	All CPE had NDM-1
	Brilliance CRE	62.5	34			
41	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			
44	CHROMagar ESBL	77.3	100	USA	66 / 95	All isolates of CPE had KPC enzyme
	VACC[b]	77.3	100			
	PCR for bla_{KPC}	97	96.6			

[a]Not determined or not reported.

[b]Selective agar with vancomycin, amphotericin B, ceftazidime, and clindamycin (VACC).

4 Reporting of Carbapenemase Producers

4.1 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[49].

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 low-MIC carbapenemase producers. 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[49].

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[8].

New β-lactamase inhibitors (avibactam, MK-7655, RPX7009) are under development and have activity against some carbapenemases (principally KPC types, not MBLs). None is currently licensed 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 β-lactam(s).

4.2 Other antibiotics

Many carbapenemase producers are multi-resistant to fluoroquinolones and aminoglycosides, but these are options if the patient's isolate is susceptible.

Most carbapenemase producers (c. 90%) are susceptible to polymyxins (eg colistin), although there are notable reports of resistance eg in some variants of the ST258 *K. pneumoniae* clone with KPC enzyme.

Tigecycline may remain active against carbapenemase-producing Enterobacteriaceae, at least *in vitro*, but *Pseudomonas* sp. are intrinsically resistant, there are no specific breakpoints vs. *Acinetobacter* sp., and there are cautions about the drug's efficacy in severe infection (see: http://www.ema.europa.eu/docs/en_GB/document_library/Medicine_QA/human/000644/WC500102228.pdf).

Colistin and/or tigecycline may be considered as combination therapy alongside a carbapenem.

Nitrofurantoin and fosfomycin are active against most carbapenem-resistant *E. coli* isolates, but have variable activity against other genera. Fosfomycin is not marketed in the UK and requires importation by a pharmacist. They are suitable only for lower UTIs.

Empirical treatment strategies and antibiotic policies may need to be re-thought in settings and locales where carbapenemase producers are prevalent (see ARHAI/PHE guidance on the Infection Prevention and Control issues in such circumstances:

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/CarbapenemResistance/GuidanceOnCarbapenamProducers/. Empirical treatment strategies may need to be re-thought where patients are considered to be 'high risk' (eg previous colonization or infection with a carbapenemase producer, history of recent travel to a country with an endemic problem, prior hospitalization in a UK centre with a known carbapenemase problem).

5 Which Carbapenem-Resistant Bacteria to Send to the Reference Laboratory?

The reference laboratory at PHE, Colindale seeks:

- All Enterobacteriaceae suspected to produce a carbapenemase.
- All *Pseudomonas* sp. suspected to produce a carbapenemase ie isolates resistant to carbapenems, ceftazidime and piperacillin-tazobactam AND with strong imipenem-EDTA synergy (irrespective of susceptibility or resistance to aztreonam). There is no need to send isolates resistant only to carbapenems and susceptible to other β-lactams.
- All *Acinetobacter* sp. suspected to produce a metallo-carbapenemase i.e. with strong imipenem-EDTA synergy.
- Microbiology laboratories are encouraged to have a high index of suspicion, at least for Enterobacteriaceae, and, for reasons outlined in this SMI, it is accepted that the reference laboratory will not find a carbapenemase in all referred isolates.
- In addition, we 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.

See:

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/CarbapenemResistance/GuidanceOnCarbapenamProducers/.

References

1. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents 2010;36 Suppl 3:S8-14.

2. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 2012;18:413-31.

3. Woodford N. Fighting the rising tide of carbapenemases in *Enterobacteriaceae*. Be S M A R T with Resistence International Newsletter 2012.

4. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228-36.

5. Chen LF, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of Klebsiella pneumoniae carbapenemases (KPC) resistance. Infect Drug Resist 2012;5:133-41.

6. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 2010;54:24-38.

7. 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.

8. Akova M, Daikos GL, Tzouvelekis L, Carmeli Y. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clin Microbiol Infect 2012;18:439-48.

9. Robledo IE, Aquino EE, Sante MI, Santana JL, Otero DM, Leon CF, et al. Detection of KPC in Acinetobacter spp. in Puerto Rico. Antimicrob Agents Chemother 2010;54:1354-7.

10. 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. Antimicrob Agents Chemother 2011;55:2968-70.

11. 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. Antimicrob Agents Chemother 2011;55:5350-3.

12. Poirel L, Nordmann P, Lagrutta E, Cleary T, Munoz-Price LS. Emergence of KPC-producing Pseudomonas aeruginosa in the United States. Antimicrob Agents Chemother 2010;54:3072.

13. Ge C, Wei Z, Jiang Y, Shen P, Yu Y, Li L. Identification of KPC-2-producing Pseudomonas aeruginosa isolates in China. J Antimicrob Chemother 2011;66:1184-6.

14. Akpaka PE, Swanston WH, Ihemere HN, Correa A, Torres JA, Tafur JD, et al. Emergence of KPC-producing Pseudomonas aeruginosa in Trinidad and Tobago. J Clin Microbiol 2009;47:2670-1.

15. 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. J Clin Microbiol 2010;48:2999-3002.

16. 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. J Clin Microbiol 2011;49:3321-4.

17. 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. J Clin Microbiol 2012;50:2441-3.

18. 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. J Clin Microbiol 2012;50:927-37.

19. 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. Clin Microbiol Rev 2013;26:103-14.

20. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 2012;18:1503-7.

21. Dortet L, Poirel L, Nordmann P. Rapid identification of carbapenemase types in Enterobacteriaceae and Pseudomonas spp. by using a biochemical test. Antimicrob Agents Chemother 2012;56:6437-40.

22. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2013;68:487-9.

23. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. J Clin Microbiol 2012;50:3773-6.

24. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. J Antimicrob Chemother 2007;59:321-2.

25. 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. J Clin Microbiol 2007;45:544-7.

26. 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. J Microbiol Methods 2010;83:185-7.

27. 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. Int J Antimicrob Agents 2011;38:35-8.

28. Kaase M, Szabados F, Wassill L, Gatermann SG. Detection of carbapenemases in Enterobacteriaceae by a commercial multiplex PCR. J Clin Microbiol 2012;50:3115-8.

29. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119-23.

30. 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. J Clin Microbiol 2010;48:2618-22.

31. Stuart JC, Voets G, Scharringa J, Fluit AC, Leverstein-Van Hall MA. Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray. J Med Microbiol 2012;61:809-12.

32. 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. J Clin Microbiol 2011;49:1608-13.

33. Woodford N, Warner M, Pike R, Zhang J. Evaluation of a commercial microarray to detect carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2011;66:2887-8.

34. 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) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother 2012;67:1865-9.

35. 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.

36. 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.

37. 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.

38. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol 2011;49:718-21.

39. 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.

40. 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.

41. 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. J Clin Microbiol 2012;50:1841-6.

42. Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium. J Clin Microbiol 2012;50:2761-6.

43. 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. Diagn Microbiol Infect Dis 2013;75:214-7.

44. 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. J Clin Microbiol 2012;50:2596-600.

45. 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. J Clin Pathol 2013.

46. Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae. J Clin Microbiol 2012;50:3102-4.

47. 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.

48. Naas T, Cotellon G, Ergani A, Nordmann P. Real-time PCR for detection of blaOXA-48 genes from stools. J Antimicrob Chemother 2013;68:101-4.

49. 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 Antimicrob Chemother 2012;67:1569-77.