GUIDELINE

# The important role of the clinical microbiology laboratory in the New Zealand Antimicrobial Resistance Action Plan and detection of carbapenemase-producing Enterobacteriaceae


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Antimicrobial resistance (AMR) is arguably the single biggest infectious diseases threat we currently face in New Zealand, and globally. New resistance mechanisms are emerging, which threaten our ability to treat common infectious diseases, such as urinary tract infections, and will potentially affect the ability of cancer, surgical and other services to provide safe and effective healthcare. As such, the global AMR crisis has moved to the top of government agendas worldwide. In 2011, the UK Chief Medical Officer Dame Sally Davies stated, *"Antimicrobial resistance poses a catastrophic threat. If we don't act now, any one of us could go into hospital in 20 years for minor surgery and die because of an ordinary infection that can't be treated by antibiotics. And routine operations like hip replacements or organ transplants could be deadly because of the risk of infection"* (1).

In 2015, The World Health Organisation adopted a global action plan on antimicrobial resistance. This was in recognition that, *"Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections could once again kill"* (2). New Zealand made a commitment to the World Health Assembly to have in place a national action plan on AMR by 2017. Subsequently, in December 2017, the New Zealand Ministry of Health set out the New Zealand Antimicrobial Resistance Action Plan (3).

Carbapenemase-producing Enterobacteriaceae, or CPE, harbour resistance to carbapenems such as ertapenem and meropenem, which are last line antibiotics usually reserved for serious and difficult to treat infections. Until recently in New Zealand, CPE have been mostly isolated from people with a history of overseas hospitalisation and travel to regions where CPE is now endemic, such as the Indian subcontinent and Southeast Asia. Emerging evidence now suggests that CPE infections are arising in people without prior travel history outside of New Zealand (4). This is of major concern.

Medical microbiology laboratories in New Zealand play a critical role in AMR prevention strategies, a situation acknowledged in the Ministry of Health's New Zealand Antimicrobial Resistance Action Plan. Within this plan, listed as a Year One Priority Action, is "To develop and implement an enhanced surveillance programme for multi-drug resistant gram negative micro-organisms to include laboratory identification and reporting of carbapenemase-producing *Enterobacteriaceae* (CPE)". Integral to this Priority Action is the ability of microbiology laboratories to accurately, and consistently, identify these resistance mechanisms on a day-to-day basis. This capability should not be limited to larger laboratories with access to technology and expertise in antimicrobial susceptibility testing or AMR, but rather should include every clinical microbiology laboratory across New Zealand.

We are therefore pleased to publish the New Zealand National Antimicrobial Susceptibility Testing Committee (NZNAC) CPE guideline, entitled "Minimum laboratory requirements for the detection of carbapenemase-producing Enterobacteriaceae from clinical samples and screening specimens." This document has been endorsed by the New Zealand Microbiology Network and will be included in the New Zealand CPE Action Plan, expected for publication by the Ministry of Health in 2019. IANZ have been notified about the document, and laboratories are expected to comply with the recommendations outlined within. We therefore urge all New Zealand microbiology laboratories to promptly read and implement these recommendations. Any questions or comments are gratefully received, via email to the NZNAC administrator sarah.underwood@esr.cri.nz.

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## REFERENCES


1. Chief Medical Officers report 2011, available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/138331/CMO_Annual_Report_Volume_2_2011.pdf
2. World Health Organisation Global Action Plan on Antimicrobial Resistance, available at: http://www.who.int/antimicrobial-resistance/global-action-plan/en/
3. New Zealand Antimicrobial Resistance Action Plan Ministry of Health and Ministry for Primary Industries. 2017. *New Zealand Antimicrobial Resistance Action Plan. Wellington: Ministry of Health, available at:* https://www.health.govt.nz/system/files/documents/publications/new-zealand-antimicrobial-resistance-action-plan.pdf
4. ESR 2018 data: https://surv.esr.cri.nz/antimicrobial/AccqEnterobacteriaceae.php).



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# Minimum laboratory requirements for the detection of carbapenemase-producing Enterobacteriaceae from clinical samples and screening specimens

## 1 Aim

The aim of this document is to provide a minimum requirement for the laboratory detection of carbapenemase-producing Enterobacteriaceae (CPE) in New Zealand and to ensure laboratories can identify when confirmatory testing, referral of isolates, and notification to clinical and infection prevention teams is required.

## 2 Background

There are several different mechanisms by which Enterobacteriaceae can develop resistance to carbapenem antibiotics.

Acquired carbapenemases (carbapenem-hydrolysing enzymes) are of most concern because their genetic determinants are mainly carried on plasmids and therefore can transfer between strains, species and genera. Detection of CPE can be difficult because:

- not all carbapenem resistance is due to carbapenemase production, and
- not all carbapenemase producers are phenotypically resistant to carbapenems using standard antimicrobial susceptibility testing (AST) breakpoints.

Other mechanisms of carbapenem resistance, such as extended-spectrum beta-lactamase (ESBL) or AmpC beta-lactamase production, combined with porin loss (commonly seen in *Enterobacter* spp) or efflux mechanisms, are not readily transferable between strains. Such non-carbapenemase- producing, carbapenem-resistant Enterobacteriaceae (non-CP CRE) do not pose the same infection prevention and control risk. Laboratories must therefore be able to identify organisms with acquired carbapenemases, and differentiate them from isolates with other mechanisms of carbapenem resistance, in order to support clinicians to make appropriate treatment decisions and implement appropriate infection prevention measures. Also key to improving the patient's outcome is the timely provision of accurate susceptibility data to support directive therapy.

Laboratories should maintain a high index of suspicion for CPE, based on clinical presentation, epidemiological risk factors (such as overseas travel and hospitalisation) and susceptibility testing results. A low threshold for further confirmatory testing of suspect isolates, either locally or by referral to another laboratory, should be maintained.

This document outlines procedures required for the detection of CPE in clinical specimens (section 4) and CPE screening samples (section 5). Laboratories may need to modify their testing processes or increase their testing capacity in order to meet these standards.

## 3 Scope

Laboratory detection of acquired carbapenemases in Enterobacteriaceae.[1]

[1] The authors acknowledge the recent changes in taxonomy that have resulted in several genera formerly included in the family Enterobacteriaceae now being included in other families in the order Enterobacterales. However, the term Enterobacteriaceae is used in this document, but should be considered to cover all genera now included in the order Enterobacterales.

**Not included**

- Carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*
- Organisms with intrinsic carbapenemases, such as *Stenotrophomonas maltophilia* and *Aeromonas* spp
- ESBL or AmpC beta-lactamase detection in Enterobacteriaceae
- Environmental and veterinary samples

# 4 Clinical isolates

## 4.1 Antimicrobial susceptibility testing and use of an indicator antimicrobial for CPE

All diagnostic laboratories should have the capability to perform antimicrobial susceptibility testing (AST) in accordance with methods recommended by either the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the Clinical and Laboratory Standards Institute (CLSI). The most recent versions of these methods should be used.

All clinically significant Enterobacteriaceae isolates should be screened for the presence of a carbapenemase using an indicator antimicrobial as part of routine AST. Subsequent additional confirmatory testing should be performed where the indicator antibiotic indicates it is necessary.
The suggested indicator carbapenem is meropenem since it offers the best balance of sensitivity and specificity for the detection of CPE.

**Recommended CPE screening method for all hospital and community specimens:**

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacteriaceae isolates

*or*

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacteriaceae isolates that have decreased susceptibility to cefpodoxime, ceftriaxone or ceftazidime.

*or*

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacteriaceae isolates that are resistant to cephalexin. This is the least specific option for detection of CPE, but may be considered where a carbapenem or third-generation cephalosporin is not tested as part of first-line AST.

Where there are epidemiological risk factors for CPE (such as overseas travel or hospitalisation, previous known CPE colonisation, or a household member with CPE), Enterobacteriaceae isolates resistant to amoxicillin-clavulanate, should also be considered for meropenem susceptibility testing.

### 4.1.1 Urine direct susceptibility testing

Laboratories performing direct susceptibility testing (DST) on urine samples should ensure that a valid inoculum is achieved before reading and reporting susceptibility results. Any invalid results should be repeated using a controlled inoculum to avoid inaccurate susceptibility results. This has particular relevance when reading the indicator antimicrobial zone diameters.

## 4.2 Indicator carbapenem interpretive criteria

Laboratories are advised to use the EUCAST carbapenemase screening criteria which offers sufficient sensitivity for CPE detection in low-prevalence settings such as New Zealand. Organism identification to species level is required for valid interpretation of AST, including CPE screening criteria.

Additional confirmatory testing (section 6) should be performed on all Enterobacteriaceae isolates where:

Meropenem MIC >0.12 mg/L

*or*

Meropenem disc zone diameter <25 mm

*or*

Meropenem disc zone diameter 25-27 mm, if also resistant to piperacillin-tazobactam (and/or temocillin)[2]

*or*
Automated AST system (eg, Vitek 2, Phoenix) indicates decreased susceptibility to meropenem or that a carbapenemase may be present. Note where the lowest meropenem concentration tested does not allow interpretation according to the criteria outlined above, an additional step may be required to meet these minimum standards. For example, laboratories using Vitek 2 AST should consider manual meropenem AST and/or additional confirmatory testing for isolates with meropenem MICs ≤0.25 mg/L that are also resistant to a third-generation cephalosporin and piperacillin-tazobactam.
Enterobacteriaceae with reduced susceptibility to ertapenem, but remaining fully susceptible to meropenem, do not routinely require further testing for CPE.

# 5 CPE screening samples

Clinical selection criteria should be applied in line with local infection prevention procedures and in accordance with national CPE guidance documents.

## 5.1 Recommended samples

A faeces specimen or rectal swab with visible faecal material are the minimum recommended sample types for CPE screening.

Additional samples types should be considered where appropriate, in line with local and national infection prevention guidance documents:

1. Urine, if symptomatic or urinary catheter/nephrostomy/stent in situ;
2. Swab from wounds and insertion sites of invasive medical devices and catheters; and
3. Lower respiratory tract specimens, if intubated.

## 5.2 Laboratory methods for detection of CPE from screening samples

### 5.2.1 Culture-based methods

Selective culture is the most commonly used methodology for detection of CPE in screening samples. There is currently no consensus best-practice culture medium for this purpose, but the use of a commercially available, selective chromogenic media is recommended. Laboratories should note that these media vary in their performance for detection of the different types of carbapenemases.

Based on the epidemiology of CPE in NZ, laboratories should utilise media capable of detecting CPE with low carbapenem MICs (such as OXA-48/OXA-48-like producing isolates). This may require the utilisation of two different selective media.

MacConkey agar with a carbapenem disc is inferior to screening with chromogenic media and is not recommended as the sole screening method.

Any Enterobacteriaceae growth on CPE screening agar should have AST (including meropenem) performed followed by confirmatory testing as required (section 6).

### 5.2.2 Molecular methods for detection of carbapenemase genes direct from screening sample

Molecular CPE test panels currently commercially available detect the most common carbapenemase genes. Less common carbapenemase types will not be detected and therefore culture-based screening may also be required where there is high clinical suspicion for a CPE genotype not included in the molecular panel available.

Clinical samples in which CPE resistance genes are detected directly should have reflex culture performed to obtain an isolate for identification and susceptibility testing.

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[2] For *Enterobacter, Serratia, Citrobacter freundii, Proteus vulgaris, Providencia and Morganella* species, the Clinical Microbiologist may exercise discretion regarding the need for further testing if resistance is likely to be due to a combination of AmpC de-repression and porin deficiency (eg, when there is no co-resistance to other antibiotic classes and resistance develops progressively during antimicrobial therapy).

## 6 Confirmatory testing for CPE

Confirmatory testing for the presence of carbapenemase or a carbapenemase gene may be performed locally or by referral to a second laboratory with the necessary expertise. Accurate organism identification to species level is mandatory for isolates which require CPE confirmatory testing.

Confirmatory testing for isolates from invasive infection should be regarded as urgent, with results available as soon as possible, and within 24 hours. Confirmatory testing for screening or community isolates may be considered less urgent but should still be available promptly and within 3 working days. Primary diagnostic laboratories should have agreed referral protocols in place to ensure compliance with these requirements. There are many available methods for confirmatory testing; extensive evaluation studies of the various methods have been undertaken elsewhere and are not included in this document.

### 6.1 Phenotypic methods

Suitable phenotypic methods include:

1. Colorimetric tests, utilising pH related colour change due to hydrolysis of the indicator carbapenem (eg, CarbaNP, BlueCarba);
2. Carbapenem inactivation method (CIM) or modified CIM (mCIM);
3. Combination disc testing (eg, MAST D70C); and
4. Immunoassays for detection of carbapenemases (eg, Resist-3 O.K.N for detection of OXA- 48-like, KPC and NDM carbapenemases).

The modified Hodge test is no longer recommended due to difficulties in interpretation, and lack of sensitivity and specificity.

Isolates with a positive phenotypic carbapenemase test will also require genotypic confirmation by a molecular method, either by local testing or referral to ESR.

Isolates with a negative phenotypic carbapenemase test but with a high clinical suspicion for CPE (due to epidemiological risk factors) may require additional testing using a molecular method.

### 6.2 Molecular methods

Commercially available molecular platforms detect the most common carbapenemase types which account for >95% of CPE. Since less common carbapenemase types will not be detected on these platforms, if a high suspicion for CPE remains despite a negative molecular test, a phenotypic carbapenemase test and/or referral to ESR is advisable.

## 7 Notification

All laboratories must have a documented procedure for notification of all suspected and confirmed CPE isolates.

For patients in a health care facility all confirmed CPE isolates must be notified as soon as possible and on the same day to:

1. The treating clinician;
2. The supervising clinical microbiologist; and
3. The Infection Prevention and Control team.

For community patients (not in a health care facility) all confirmed CPE isolates must be notified as soon as possible and on the same day to:

1. The supervising clinical microbiologist; and by the next working day to:

1. The treating clinician; and
2. The Infection Prevention and Control team.

All possible or suspected CPE isolates must be notified on the same day to the supervising clinical microbiologist whilst awaiting confirmation. Onward notification to the clinician and Infection Prevention and Control teams is at the discretion of the clinical microbiologist and should take into account the likelihood of CPE and potential clinical risk.

## 8 Reporting

Enterobacteriaceae isolates confirmed by molecular methods to carry a carbapenemase gene should be reported as a 'Carbapenemase-producing *Enterobacteriaceae* (CPE)'.

Carbapenem-resistant isolates with a positive phenotypic test may be reported as a 'Probable carbapenemase-producing *Enterobacteriaceae* (CPE), awaiting confirmation'

Non-carbapenemase-producing, carbapenem-resistant Enterobacteriaceae (non-CP CRE) isolates should not be reported as 'Carbapenemase-producing *Enterobacteriaceae* (CPE)' in order to maintain differentiation from CPE.

Similarly, terms such as carbapenemase-producing organism (CPO) and carbapenem-resistant organism (CRO) should not be used for confirmed carbapenemase-producing Enterobacteriaceae.

## 9 Referral of isolates to ESR

All suspected or confirmed CPE isolates should be referred to ESR's Antimicrobial Reference Laboratory, Kenepuru, Porirua, for confirmation and typing, as follows:

1. All isolates confirmed as CPE using a molecular method;
2. Enterobacteriaceae isolates with a positive phenotypic carbapenemase test, but confirmatory molecular testing is negative or not done; and
3. Enterobacteriaceae isolates with decreased carbapenem susceptibility from patients with risk factors for CPE, but isolate negative in phenotypic carbapenemase test and molecular test (if done).

Isolates do not require referral to ESR where there is a low index of suspicion and the carbapenemase confirmatory test is negative, such as AmpC beta-lactamase-producing Enterobacteriaceae (eg, *Enterobacter*,) with decreased susceptibility to meropenem and/or ertapenem.
When isolates are referred to ESR for confirmation, the following information should be supplied in addition to that requested on the standard ESR referral form (see http://www.esr.cri.nz/assets/Test-Forms/ESR0039-Single-Human-Source-Specimen.pdf):

Based on the epidemiology of CPE in NZ, laboratories should utilise media capable of detecting CPE with low carbapenem MICs (such as OXA-48/OXA-48-like producing isolates). This may require the utilisation of two different selective media.

MacConkey agar with a carbapenem disc is inferior to screening with chromogenic media and is not recommended as the sole screening method.

Any Enterobacteriaceae growth on CPE screening agar should have AST (including meropenem) performed followed by confirmatory testing as required (section 6).

1. Full antimicrobial susceptibility test results for the isolate, including printout from Vitek or similar if available; and
2. Risk factor information, in particular any details of recent overseas travel and hospitalisation for the patient or close household contacts; and
3. Molecular testing results (if available).

ESR should aim to confirm the isolate is a CPE within 3 working days of receipt. Any positive results should be reported to the referring laboratory as soon as possible and on the same day that results are available.

## 10 Storage of isolates

All confirmed CPE isolates will be stored by ESR on referral. Primary diagnostic laboratories are also advised to store isolates for surveillance purposes for a minimum of 6 months.

## 11 Carbapenemases in non-Enterobacteriaceae

Detection of carbapenemases in non-Enterobacteriaceae isolates is beyond the scope of this document. However, laboratories must be aware that transferable carbapenemases do also occur in species such as *P. aeruginosa* and *A. baumannii*. As such, laboratories are advised to follow EUCAST or CLSI guidance to determine when further confirmatory testing or referral to ESR should be performed.

## References


CDC. Facility guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). Atlanta (GA): Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases; 2015. Available at: https://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html. Last accessed 6 March 2018.

EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 2.0; 2017 Jul. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. Last accessed 6 March 2018.

Fattouh R, Tijet N, McGeer A, Poutanen SM, Melano RG, Patel SN. What is the appropriate meropenem MIC for screening of carbapenemase-producing Enterobacteriaceae in low-prevalence settings? Antimicrob Agents Chemother 2016; 60: 1556–9.

Health Protection Scotland. Toolkit for the early detection, management and control of carbapenemase-producing Enterobacteriaceae in Scottish acute settings. Health Protection Scotland. Version 1.1; 2017 Jul. Available at: http://www.hps.scot.nhs.uk/resourcedocument.aspx?id=6083. Last accessed 6 March 2018.

Parker VA, Logan CK, Currie B. Carbapenem-resistant Enterobacteriaceae (CRE) control and prevention toolkit. Rockville (MD): Dept. of Health and Human Services (US), Agency for Healthcare Research and Quality; 2014 Apr. AHRQ Publication No. 14-0028.

Pierce VM, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. J Clin Microbiol 2017; 55: 2321-33.

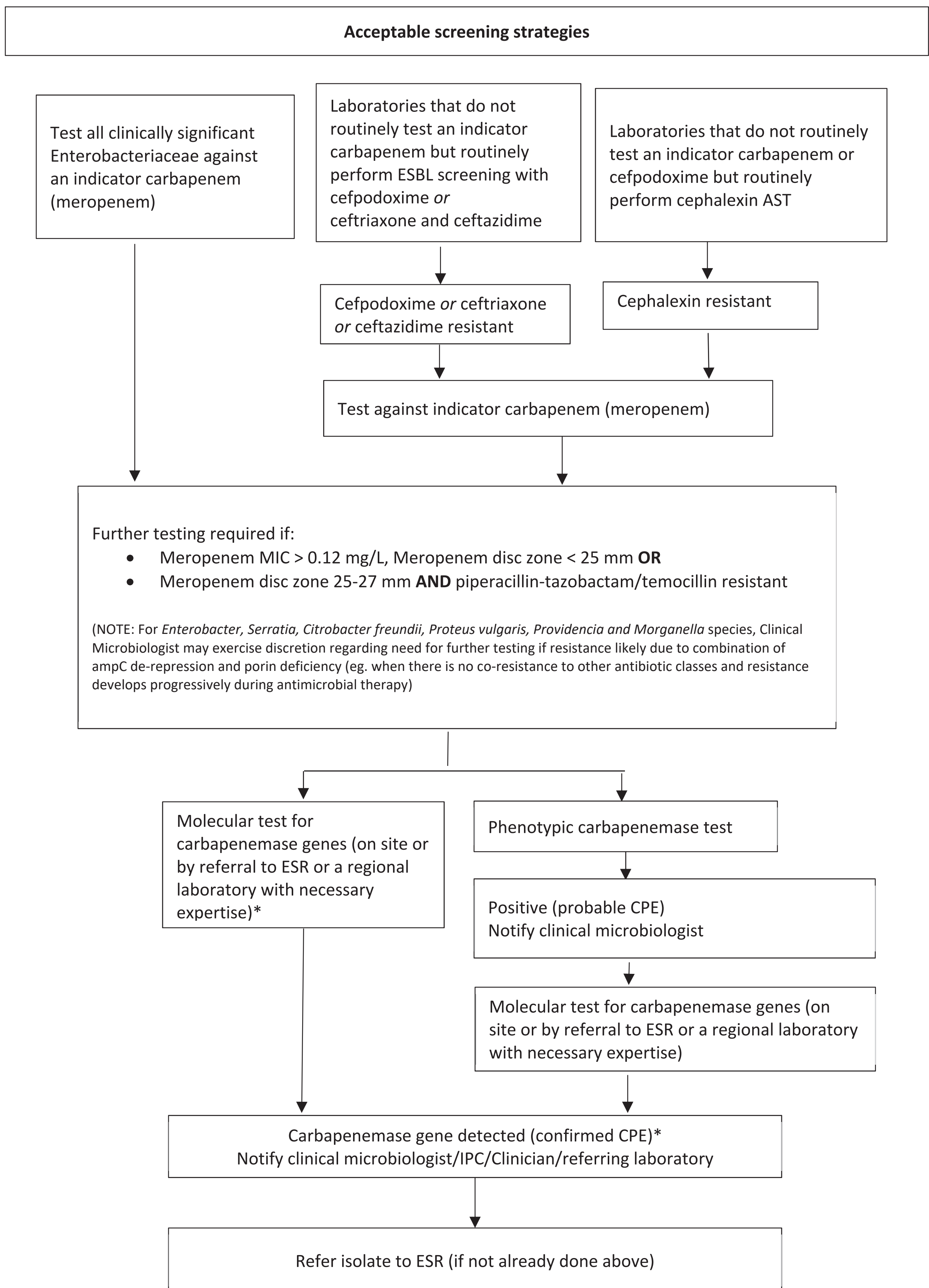
Public Health England. Acute trust toolkit for the early detection, management and control of carbapenemase-producing Enterobacteriaceae. London: Public Health England; 2013. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/329227/Acute_trust_toolkit_for_the_early_detection.pdf. Last accessed 6 March 2018.

Public Health England. UK standards for microbiology investigations. Laboratory detection and reporting of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases). London: Public Health England; 2014. Available at: http://webarchive.nationalarchives.gov.uk/20140714032104/http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317138520481. Last accessed 6 March 2018.

State Government of Victoria. Victorian guideline on carbapenemase-producing Enterobacteriaceae. Version 2. Victoria, Australia: State of Victoria, Department of Health and Human Services; 2017 Apr. Available at: https://www2.health.vic.gov.au/public-health/infectious-diseases/infection-control-guidelines/carbapenemase-producing-enterobacteriaceae-management. Last accessed 6 March 2018.

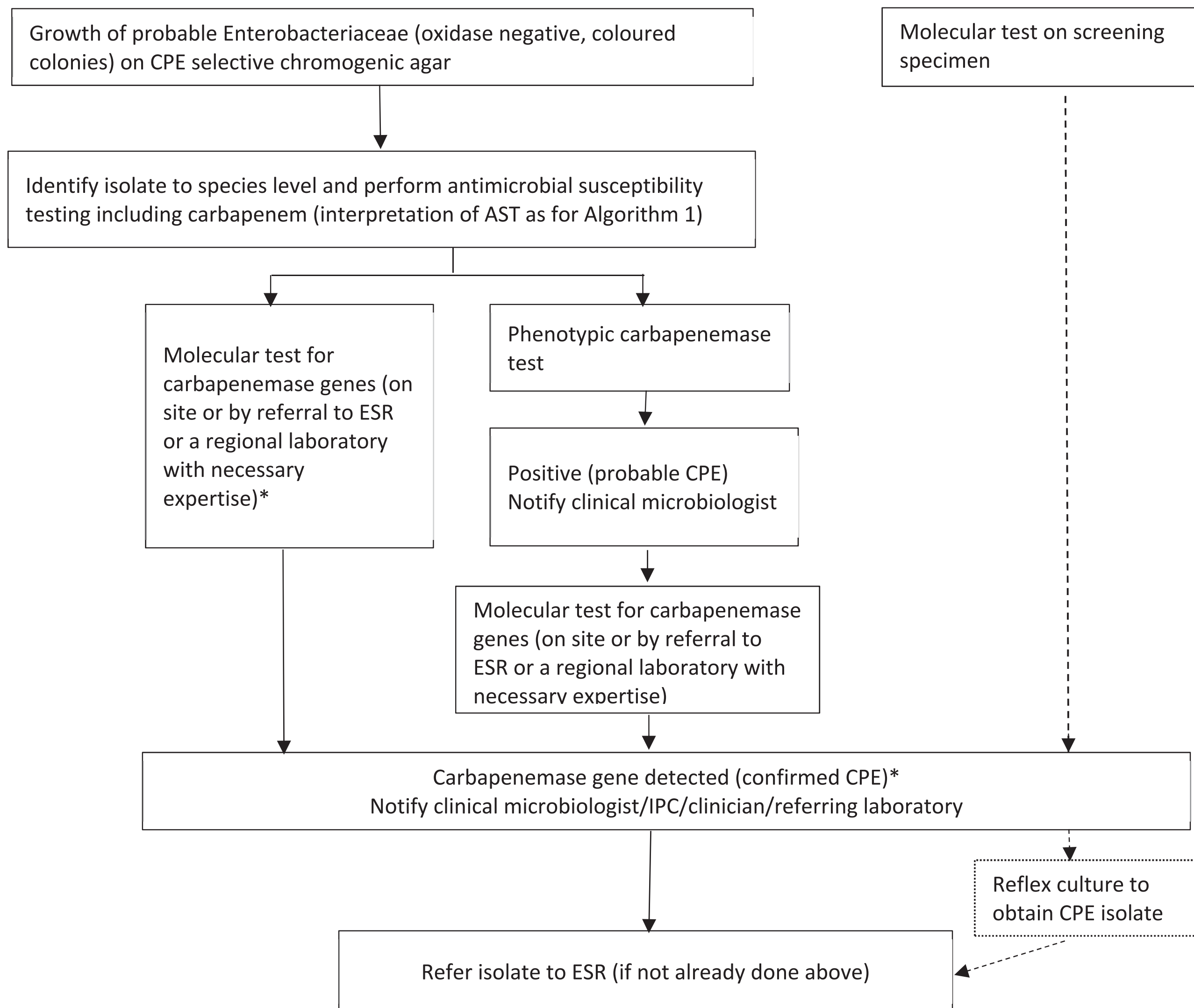
Tzouvelekis LS, et al. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Micro Rev 2012; 25: 682-707.

**Algorithm 1. Clinical specimens**




*phenotypic carbapenemase test or referral to ESR may still be required if molecular test negative but significant clinical suspicion for CPE type not included in molecular assay

**Algorithm 2. Screening specimens**




*phenotypic carbapenemase test or referral to ESR may still be required if molecular test negative but significant clinical suspicion for CPE type not included in molecular assay