NZ NAC New Zealand National Antimicrobial Susceptibility Testing Committee

# Minimum laboratory requirements for the detection of carbapenemase-producing Enterobacterales 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 Enterobacterales (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 mechanisms by which Enterobacterales can develop resistance to carbapenem antibiotics. Acquired carbapenemases 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.

Non-carbapenemase 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), are not readily transferable between strains and do not pose the same infection prevention and control risk.

Laboratories must therefore be able to identify and differentiate organisms with acquired carbapenemases from isolates with other mechanisms of carbapenem resistance, in order to support appropriate treatment decisions and 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 epidemiological risk factors (such as overseas travel and hospitalisation) and susceptibility testing results. A low threshold for further confirmatory testing of suspect isolates should be maintained.

This document outlines procedures required for the detection of CPE in clinical specimens (Section 4) and CPE screening samples (Section 5).

# 3 Scope

The focus of this document is on laboratory detection of acquired carbapenemases in Enterobacterales. However brief comments are also included on carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (in Section 11)

**Not included**

- Organisms with intrinsic carbapenemases, such as *Stenotrophomonas maltophilia* and *Aeromonas* spp
- ESBL or AmpC beta-lactamase detection in Enterobacterales
- Environmental and veterinary samples

# 4 Clinical isolates

## 4.1 Screening for CPE using an indicator antimicrobial

All clinically significant Enterobacterales isolates should be screened for the presence of a carbapenemase using an indicator antimicrobial as part of routine AST. Isolates where the screen (indicator antimicrobial) suggests a possible CPE require confirmatory testing .

The recommended indicator antimicrobial is meropenem since it offers the best balance of sensitivity and specificity for the detection of CPE. Where inclusion of meropenem in routine first line AST is not feasible the alternative approaches described below can be used.

**Recommended CPE screening method for all hospital and community clinical isolates:**

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacterales

*or*

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacterales that have decreased susceptibility to cefpodoxime, ceftriaxone or ceftazidime. This option is expected to have lower sensitivity for the detection of producing OXA-type carbapenemases

*or*

Test an indicator carbapenem (meropenem) against all clinically significant Enterobacterales that are resistant to cephalexin or amoxicillin-clavulanate.

### 4.1.1 Indicator antibiotic zone diameters in urine direct susceptibility testing

Laboratories performing direct susceptibility testing on urine samples should ensure that a valid inoculum is achieved before reading and interpreting indicator antimicrobial zone diameters. Any invalid results should be repeated using a controlled inoculum to avoid inaccurate results.

## 4.2 Indicator carbapenem (meropenem) interpretive criteria

Laboratories are advised to use the EUCAST carbapenemase screening criteria. Organism identification to species level is required for valid interpretation.

Confirmatory testing for carbapenemase production (Section 6) should be performed on all Enterobacterales isolates where:

Meropenem MIC >0.12 mg/L

***or***

Meropenem disc zone diameter <28 mm:

Investigation for carbapenemase production is always warranted in isolates with zone < 25 mm. Isolates with zone 25-27 mm only need further work up if resistant to piperacillin-tazobactam and/or high level resistance (MIC > 128 mg/L, disc zone < 11 mm) to temocillin (temocillin is more specific)

***or***

Automated AST system (eg, Vitek 2, Phoenix) indicates decreased susceptibility to meropenem or that a carbapenemase may be present.

Where the lowest meropenem concentration tested on an automated AST system does not allow interpretation according to the EUCAST MIC criteria outlined above, consider a manual meropenem screen for isolates with meropenem MICs ≤0.25 mg/L if they are resistant to a third-generation cephalosporin or piperacillin-tazobactam or temocillin.

**Note**:

Enterobacterales with reduced susceptibility to ertapenem, but remaining fully susceptible to meropenem, do not routinely require further testing for CPE.

For *Enterobacter cloacae, Klebsiella aerogenes, Serratia* spp*., Citrobacter freundii, Proteus vulgaris, Providencia, and Morganella* spp., 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.

Enterobacterales, especially *E.coli,* harbouring OXA-48 enzymes can exhibit very low MICs to meropenem. High-level resistance to temocillin can be used as another tool for detection of OXA-48-producers (disc diffusion < 11 mm or MIC > 128 mg/l). This is a sensitive but not a specific marker for OXA-48-producers.

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

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

Selective culture or direct molecular detection based methods can be used for detection of CPE in screening samples

### 5.2.1 Culture-based methods

Selective culture is the most commonly used methodology for detection of CPE in screening samples.

The use of a commercially available selective chromogenic media is recommended. Media capable of detecting CPE with low carbapenem MICs (such as OXA-48/OXA-48-like producing Enterobacterales) should be used. This may require the utilisation of two different selective media. Laboratories should note that these media vary in their performance for detection of the different types of carbapenemases.

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

Any Enterobacterales 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

Commercially available molecular CPE test panels typically detect only the most common carbapenemase genes. It is recommended that laboratories use assays that at a minimum detect carbapenemase genes in the NDM, OXA-48-like, KPC, IMP, VIM families.

Less common carbapenemase types will not be detected by most assays 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 AST.

# 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.

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 of screening or community isolates may be considered less urgent but should still be available within three working days. Primary diagnostic laboratories should have agreed referral protocols in place to ensure compliance with these requirements.

Both phenotypic and molecular methods can be used for initial testing of isolates that have decreased meropenem susceptibility; however a positive result on a phenotypic test should only be considered as suggestive evidence of a CPE and this result must be confirmed with a molecular method.

## 6.1 Phenotypic methods

Phenotypic methods include:

1. Colorimetric tests, utilising pH related colour change due to hydrolysis of the indicator carbapenem (eg, CarbaNP, BlueCarba)
2. Modified carbapenem inactivation method (mCIM)
3. Combination disc testing (eg, MAST D70C)
4. Immunoassays for detection of carbapenemases (eg, Resist-3 O.K.N)

The modified Hodge test is not recommended.

In a low CPE incidence area such as New Zealand the negative predictive value of phenotypic testing is high while the positive predictive value for CPE is relatively low (for organisms with chromosomal AmpC in particular). Thus isolates with a positive phenotypic carbapenemase test 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 panels typically detect only 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 for common carbapenemases, a phenotypic carbapenemase test and/or referral to ESR is recommended.

# 7 Notification

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

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 supervising clinical microbiologist
2. The treating clinician
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
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 at this point in the laboratory process is at the discretion of the Clinical Microbiologist; taking into account the likelihood of CPE and the potential clinical risk.

## 8 Reporting

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

Terms such as carbapenem-resistant Enterobacterales (CRE) or carbapenem-resistant organism (CRO) should not be used for confirmed carbapenemase-producing Enterobacterales.

Because of implications for antimicrobial treatment the enzyme type should be reported if possible.

Carbapenem-resistant isolates with a positive phenotypic test may be reported as a 'Possible or probable carbapenemase-producing Enterobacterales (CPE), awaiting confirmation'

Non-carbapenemase-producing, carbapenem-resistant Enterobacterales (non-CP CRE) isolates should not be reported as 'Carbapenemase-producing Enterobacterales (CPE)'. Neither should they be routinely reported from screening samples.

## 9 Referral of isolates to ESR

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

1. Isolates confirmed as CPE using a molecular method
2. Isolates with a positive phenotypic carbapenemase test, but confirmatory molecular testing is negative or not done
3. Isolates with decreased carbapenem susceptibility (without an alternative explanation) from patients with a high index of suspicion for CPE, but isolate is 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 Enterobacterales (eg, *Enterobacter*).

When isolates are referred to ESR for confirmation, the following information should be supplied in addition to that requested on the ESR referral form:

1. Full antimicrobial susceptibility test results, including printout from Vitek or Phoenix if available
2. Risk factor information, in particular any details of recent overseas travel and hospitalisation for the patient or close household contacts
3. Confirmatory (phenotypic and/or molecular) CPE test results (if available).

For isolates not yet confirmed as a CPE by the referring laboratory, ESR should aim to confirm whether a carbapenemase is present within 3 working days of receipt. Any positive results should be reported to the referring laboratory 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-Enterobacterales

Carbapenem resistance due to acquired carbapenemases may be encountered in non-fermenting Gram negatives such as *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa*. Meropenem (or imipenem) should be tested as the indicator carbapenem against all clinically significant isolates. The published clinical breakpoints should be used. There are no additional screening breakpoints for non-Enterobacterales. Ertapenem should not be used since these species are intrinsically resistant.

### 11.1 *Acinetobacter baumannii* complex

Carbapenem resistance in *A. baumannii* is mediated primarily by class D (OXA) and less frequently by class B (MBL) carbapenemases. Changes to efflux pumps and outer membrane proteins can also confer carbapenem non-susceptibility.

Phenotypic detection tests, such as mCIM and Carba-NP, do not perform well for *A. baumannii* and are not recommended. Furthermore, the OXA carbapenemases associated with *A. baumannii* (OXA-23, OXA-40, and OXA-58) are not detected by many commercial molecular CPE panels.

All carbapenem resistant *A. baumannii* require molecular testing to determine whether an OXA or MBL carbapenemase is present.

### 11.2 *Pseudomonas aeruginosa*

Carbapenem resistance in *P. aeruginosa* is mediated by a number of mechanisms including changes to efflux pumps, porin loss, de-repression of intrinsic AmpC, and the acquisition of a carbapenemase.

*P. aeruginosa* isolates resistant to carbapenems, but susceptible to piperacillin-tazobactam and/or ceftazidime do not need further investigation since isolated carbapenem resistance is almost exclusively due to mutational resistance.

*P. aeruginosa* non-susceptible to meropenem and other β-lactams also does not generally need investigation for the presence of carbapenemases unless there is a high index of suspicion (for instance hospitalisation overseas). Pan β-lactam resistance is frequently encountered due to antimicrobial therapy in patients chronically colonised with *P. aeruginosa*.

Phenotypic tests for the presence of carbapenemases may perform well in *P. aeruginosa*. The mCIM has proven to be reliable for *P. aeruginosa* and is recommended by CLSI M100 supplement document using a modified inoculum. Positive phenotypic tests should be confirmed with a genotypic method. Negative phenotypic tests should also undergo genotypic testing if there is a high clinical suspicion of a carbapenemase.

### 11.3 Referral to ESR

All the following should be referred to ESR:

- *P. aeruginosa* isolates positive for a carbapenemase by the mCIM and/or genotypic method
- *A. baumannii* resistant to meropenem.

Follow guidelines outlined in section 9.

## References


EUCAST. 2017. *EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance*. Version 2.0. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. Last accessed 24 November 2020.

Fattouh R et al. 2016. What is the appropriate meropenem MIC for screening of carbapenemase-producing Enterobacteriaceae in low-prevalence settings? *Antimicrob Agents Chemother* 60: 1556–9.

Haldorsen B, et al. 2018. Performance of the EUCAST disc diffusion method and two MIC methods in detection of Enterobacteriaciae with reduced susceptibility to meropenem: the NordicAST CPE study. *J Antimicrob Chemother* 73; 273802747

Livermore D, et al. 2020.Carbapenem-Resistant Enterobacterales, Carbapenem Resistant Organisms, Carbapenemase-Producing Enterobacterales, and Carbapenemase-Producing Organisms: Terminology Past its "Sell-By Date" in an Era of New Antibiotics and Regional Carbapenemase Epidemiology. *Clin Infect Dis* 71; 1776-82

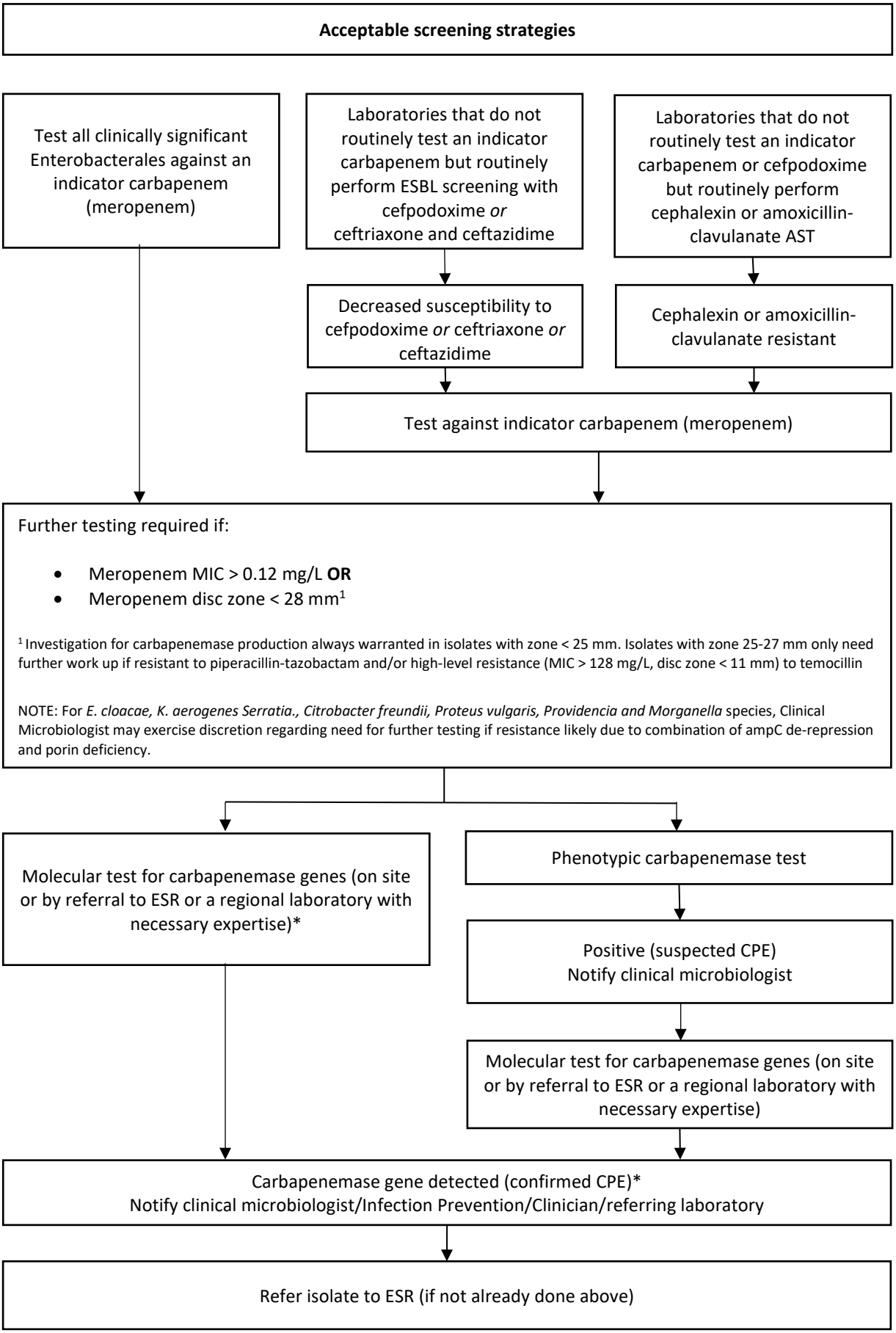
Pierce VM, et al. 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J Clin Microbiol* 55: 2321-33.

Public Health England. 2020. *Framework of actions to contain carbapenemase producing Enterobacterales*. Available at: https://www.gov.uk/government/publications/actions-to-contain-carbapenemase-producing-enterobacterales-cpe. Last accessed 24 November 2020.

Public Health England. 2020. *UK standards for microbiology investigations. Detection of bacteria with carbapenem-hydrolysing β-lactamases (carbapenemases).* London: Public Health England. Available at: https://www.gov.uk/government/publications/smi-b-60-detection-of-bacteria-with-carbapenem-hydrolysing-lactamases-carbapenemases Last accessed 24 November 2020.

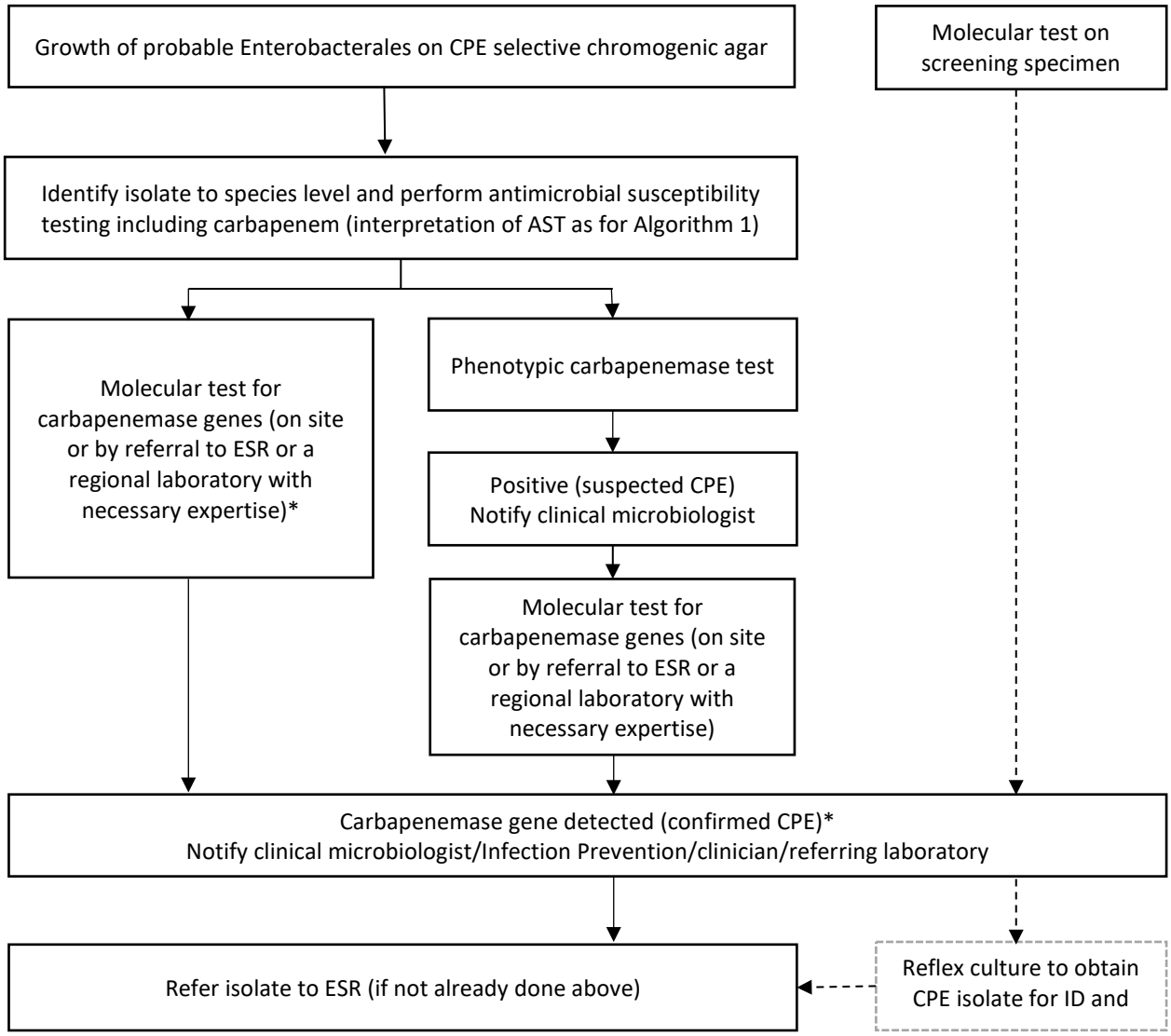
Tamma PD, Simner PJ. 2018. Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. *J Clin Microbiol*. 56 (11) e01140-18; DOI: 10.1128/JCM.01140-18

**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