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National Alert System for Critical Antimicrobial Resistances (CARAlert)

Laboratory Handbook

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Section 1: Introduction

1.1. Scope of this Handbook

The National Alert System for Critical Antimicrobial Resistances (CARAlert) was established by the Australian Commission on Safety and Quality in Health Care (the Commission) in March 2016, with funding provided by the Australian Government Department of Health and Aged Care (the Department). It is part of the Antimicrobial Use and Resistance in Australia (AURA) Surveillance System. CARAlert collects data on nationally agreed priority organisms with critical resistance to last-line antimicrobial agents (CARs). More information about CARAlert and the processes upon which it is based is included in Appendix 1.

This Handbook has been developed to provide nationally consistent guidance on the CARAlert system for originating and confirming laboratories.

This Handbook:

- Outlines the protocols and procedures relating to the operation of CARAlert
- Describes the role of participating laboratories
- Summarises current methods for use in an originating laboratory for the detection of a suspected CAR
- Outlines procedures for the referral of suspected isolates for confirmation
- Provides guidance on the tests that confirming laboratories must perform for each CAR.

The Handbook does *not address* epidemiological outbreak investigation processes. Outbreak responses are led by states and territories. CARAlert and other AURA Surveillance System data complement local surveillance data in the event of an outbreak of antimicrobial-resistant organisms.

1.2. Critical antimicrobial resistances to be reported under CARAlert

From 1 January 2023, the CARS listed in Table 1 are reported to CARAlert.

Table 1: Critical antimicrobial resistances for reporting to CARAlert

Species	Critical resistance
Acinetobacter baumannii complex	Carbapenemase-producing*
*Candida auris**	
Enterobacterales	Carbapenemase-producing, and/or ribosomal methyltransferase-producing
	Transmissible colistin resistance*
Enterococcus species	Linezolid-resistant
Mycobacterium tuberculosis	Multidrug-resistant – resistant to at least rifampicin and isoniazid
Neisseria gonorrhoeae	Ceftriaxone- or azithromycin#-nonsusceptible
	Gentamicin-resistant†
Neisseria meningiditis	Ciprofloxacin-nonsuceptible†
Salmonella species	Ceftriaxone-nonsusceptible
Shigella species	Multidrug-resistant
Staphylococcus aureus complex§	Vancomycin- or linezolid-nonsusceptible
Streptococcus pyogenes	Penicillin reduced susceptibility
Pseudomonas aeruginosa	Carbapenemase-producing*

* Reported from 1 July 2019
† Reported from 1 January 2023
§ For CARAlert, *S. aureus* complex includes *S. aureus*, *S. argenteus* and *S. schweitzeri*
\# Low level-azithromycin-nonsusceptible *N. gonorrhoeae* excluded from the Weekly Summary following review in 2018

A Weekly Summary of reports to CARAlert is issued to authorised Department staff, state and territory health authority officers and confirming laboratory users. The Weekly Summary includes the following information on confirmed CARs:

- State or territory of record*
- State or territory of patient residence
- CAR name
- CAR type
- Date of collection
- Facility type
- Patient age range
- Date of confirmation
- Confirming laboratory name.

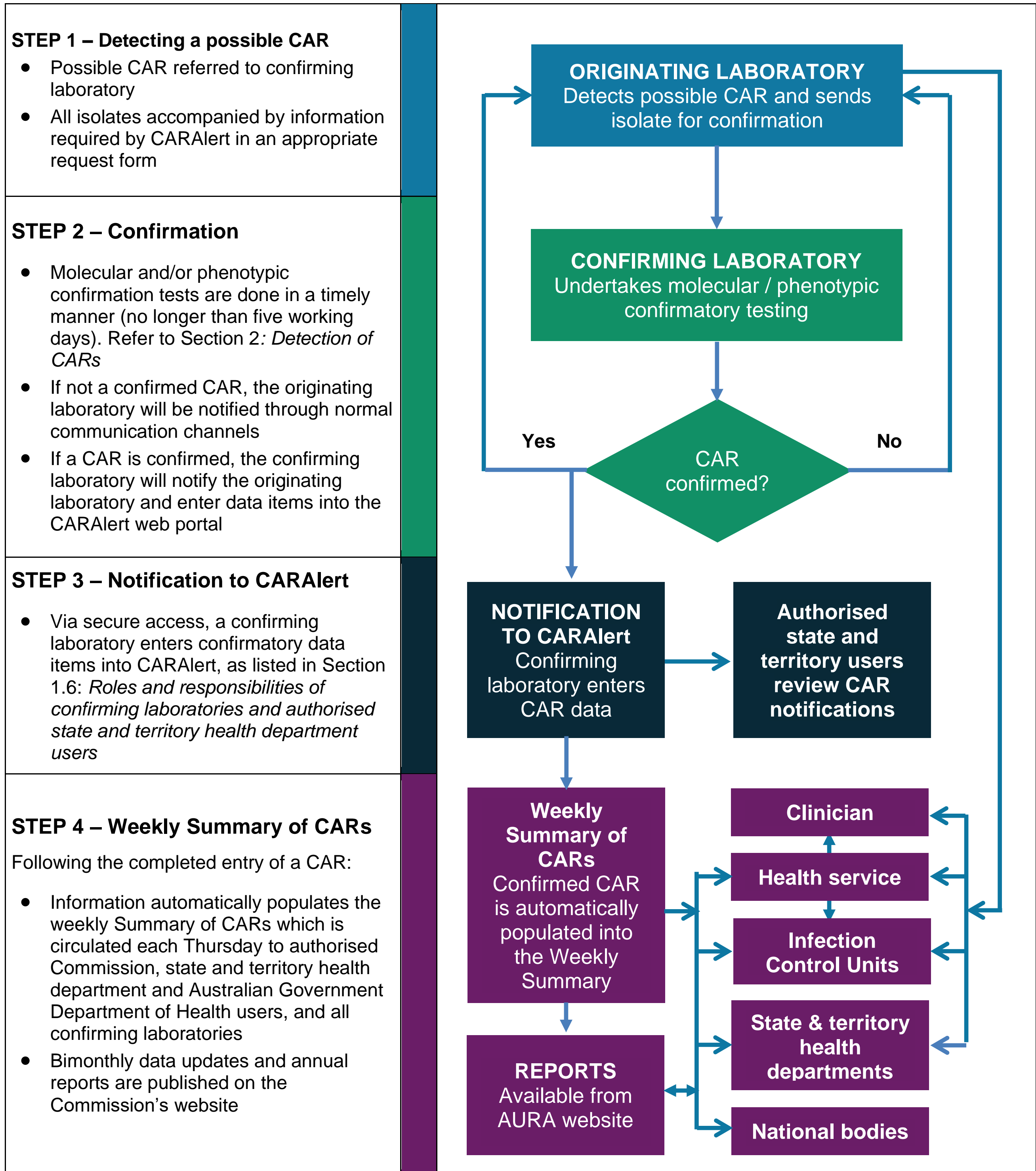
* In the first instance, this refers to the state or territory within which the hospital is located. Where this information has not been entered, or if the source of the isolate is from the community, this refers to the patient's state or territory of residence.

Quick Reference Guides are also available for authorised jurisdictional users and laboratory users which provide more detailed information about the functions of the CARAlert web portal.

1.3. The alert process

Figure 1 provides a flow chart outlining the operational model for CARAlert.

Figure 1: CARAlert alert process



1.4. Confirming Laboratory Network for CARs

The Commission consulted with state and territory health departments, the Public Health Laboratory Network (PHLN), the Australian Group on Antimicrobial Resistance (AGAR) and the private laboratory sector as part of the process of identifying confirming laboratories with the skills and capacity to confirm CARs.

Some originating laboratories also have the capability to conduct confirmatory tests for CARs. In these cases, it is not necessary to refer the isolate to another laboratory, and the confirmatory results are entered directly into CARAlert.

A list of laboratories that have been designated as confirming laboratories for each CAR monitored via CARAlert can be found in Appendix 2.

1.5. Roles and responsibilities of stakeholders

The effective operation of CARAlert relies on the cooperation and collaboration of a range of stakeholders. Tables 2 and 3 summarise the roles and responsibilities of stakeholders who provide data and who use information from CARAlert.

The Commission thanks all the participants in CARAlert for enabling this valuable national surveillance system.

Table 2: Stakeholders who provide data to the CARAlert system

Stakeholder	Roles and responsibilities
Originating laboratories	• Undertake the first routine test of isolates • Identify isolates that may have the potential to be a CAR • Notify the requesting clinician of the test results, and the suspected CAR • Send the suspected isolate onto a confirming laboratory (Appendix 2) for confirmation. Preliminary results generated during initial testing must also be provided to the confirming laboratory (e.g., CIM/Carba NP test results, MIC by MIC strip) • Ensure the following details are included on the referral form as the confirming laboratory will be required to enter this information into CARAlert if a CAR is confirmed: – name of originating laboratory (laboratory reporting on first isolation) – specimen identifier (accession number allocated by the originating laboratory, required for tracking purposes) – date of specimen collection – date specimen referred – organism name (genus and species) – clinical isolate or screen – specimen type (blood, urine, wound, screen, other) – facility type (hospital, aged care home, other, unknown), where the specimen was collected – if facility type is a hospital, name of hospital – patient demographic data – date of birth, sex and postcode of patient's residence (for an overseas patient, record as '9999'). **Note:** Date of birth is converted to an age range, prior to transmission to the CARAlert system. **Notes:** 1. In some cases, the originating laboratory may also be the confirmatory laboratory, where the necessary tests can be undertaken, and the results entered into CARAlert. These laboratories are required to register with the Commission to gain access to the CARAlert web portal. 2. The information can be provided to the confirming laboratory by completing the CARAlert Isolate Referral Form available online and included in the Handbook (Appendix 3), and including it with the isolate.

Stakeholder	Roles and responsibilities
Confirming laboratories	• Receive isolates from originating laboratories for confirmation of a CAR • Undertake the necessary confirmatory tests for a CAR • Notify the originating laboratory of test outcomes through the usual communication channels, regardless of whether a CAR is confirmed or not • Once an isolate has been confirmed as a CAR and after the originating laboratory has been notified, enter data into the CARAlert web portal. This includes data provided from the originating laboratory: – name of originating laboratory (laboratory reporting on first isolation) – specimen identifiers (accession number allocated by the originating and confirming laboratory, required for tracking purposes) – date of specimen collection – date specimen referred – organism name (genus and species) – clinical isolate or screen – specimen type (blood, urine, wound, screen, other) – facility type (hospital, residential aged care facility, other, unknown), where the specimen was collected – if facility type is a hospital, name of hospital – patient demographic data – date of birth, sex and postcode of patient's residence (for an overseas patient, record as '9999'). **Note:** Date of birth is converted to an age range prior to transmission to the CARAlert system. Please refer to the Quick Reference Guide for Laboratory Users for specific details of the CARAlert web portal and data entry system. • Confirming laboratories must also enter the following additional information on CARs: – name of confirming laboratory (laboratory confirming CAR) – date of confirmation – CARs – type or subtype if known or relevant (e.g., IMP, IMP-4). Subtyping can occur outside the five working day window • Store all confirmed CAR isolates according to usual standard operating protocol.

Table 3: Stakeholders who use information from the CARAlert system

Stakeholder	Roles and responsibilities
Clinicians	• Receive timely information on confirmed CARs from the originating laboratory, through usual notification processes. The Commission provides regular reports on analyses of trends in CARs on the Commission's website. Information from these reports can contribute to local knowledge and awareness of critical resistances, which may assist in appropriate antimicrobial prescribing.
Health Services	• Monitor confirmed CARs in facilities they manage and, where necessary implement infection prevention and control strategies, and antimicrobial stewardship programs. The Commission provides regular reports on analyses of trends in CARs on the Commission's website.
State, territory and Australian government health departments	• Receive timely information on confirmed CARs via the Weekly Summary of CARs to nominated personnel, and direct access to the CARAlert system • Provide information and advice on infection prevention and control issues related to CARs when required • In an outbreak situation, identify transmission pathways and implement action to control the spread of a CAR. The Commission provides regular reports on analyses of trends in CARs on the Commission's website.

Stakeholder	Roles and responsibilities
Consumers and other interested users	• Access publicly available information on analyses of trends in CARs in Australia via the Commission's website.
Manager of CARAlert	
Australian Commission on Safety and Quality in Health Care	Coordination and management of CARAlert, including: • Identifying priority organisms for reporting under CARAlert • Providing assistance to Originating and Confirming laboratories, state and territory health departments and private facilities where CARs are diagnosed, as required • Analysis of CARAlert data and communication of the results of analyses • Working with the Department to ensure CARAlert continues to be compatible with, and complement, other national surveillance systems.

1.6. Quality assurance

Australian laboratories are required to be accredited by the National Association of Testing Authorities (NATA) and participate in the quality assurance program of the Royal College of Pathologists Australasia (RCPA-QAP). A laboratory that performs confirmatory tests for CARs should liaise with the Commission regarding the tests to be performed and authorisation for access to CARAlert.

Specimen transportation

Isolates shipped by road transport must be packaged according to National Pathology Accreditation Advisory Council (NPAAC) requirements for packaging and transport of pathology specimens and associated materials.[6]

Isolates shipped by air must be packaged according to International Air Transport Association (IATA) regulations and sent as UN3373 Biological Substances Category B.

Any organisation sending out cultures or diagnostic specimens has a legal duty to ensure that such items are sent in a safe manner. Infectious substances that break or leak in transit can result in a major incident, putting those handling them and those in receipt of them at risk of infection.

The time taken to perform bacterial confirmation is dependent on the purity of the cultures received. Originating laboratories should take all reasonable steps to ensure that cultures that they submit to confirming laboratories are pure, but without inordinate delay in referral.

Storage of isolates

All isolates of confirmed CARs should be (according to good laboratory processes) stored at –80ºC by confirming laboratories, as part of their usual standard operating protocol These isolates will then be available for future epidemiological follow-up, translational research initiatives and may also be used as reference material for quality control.

Section 2: Detection of CARs

Information on each of the CARs contained is this Handbook is structured according to the following format:

Description – includes a short description of each CAR, including its clinical and epidemiological importance.

Detection of resistance – provides guidance on how laboratories can recognise isolates that may harbour a critical antimicrobial resistance using their standard routine susceptibility test system. These include the following systems:

- European Committee on Antimicrobial Susceptibility Testing (EUCAST)[1]
- Clinical and Laboratory Standards Institute (CLSI)[2-4]
- Calibrated Dichotomous Sensitivity (CDS) method.[5]

Note: Where EUCAST and CLSI are methodologically equivalent, tests specified in the documentation for one system have been extrapolated to the other system.

Flow chart – summarises the workflow for detecting a suspected CAR.

Confirmation – summarises the tests that confirming laboratories must perform for each CAR. Confirmation may involve molecular testing and/or phenotypic testing.

Note: Confirmatory tests and data collection processes are already in place for *Mycobacterium tuberculosis* through the Australian Mycobacterial Reference Laboratory Network (AMRLN) and for *Neisseria gonorrhoeae* by the National Neisseria Network (NNN). Laboratories that are part of these networks will be responsible for reporting CARs to CARAlert.

A summary of the screening criteria for CARs by susceptibility test method can be found in Appendix 4. The tests performed by the confirming laboratory that result in a CARAlert submission are summarised in Appendix 5.

IMPORTANT NOTE:

Some of the interpretive criteria in this Handbook differ from the breakpoints listed in the EUCAST or CLSI standards. The interpretive criteria used here are designed to capture emerging CARs at the earliest possible time. The results of confirmed CARs should not automatically result in changes to a “susceptible” result obtained with published breakpoints, but will have implications for infection prevention and control.

2.1 *Acinetobacter baumannii* complex – carbapenemase-producing

Acinetobacter baumannii complex (*A. calcoaceticus*, *A. baumannii*, *A. dijkshoorniae*, *A. nosocomialis*, *A. pittii* and *A. seifertii*) is clinically the most important of the *Acinetobacter* species. This complex is most frequently associated with hospital outbreaks. The ability to survive on inanimate surfaces and resistance to disinfectants or antimicrobials are crucial to this behaviour.

Carbapenem resistance in *Acinetobacter baumannii* complex is almost always due to production of carbapenemases. A variety of carbapenemases in *Acinetobacter* spp. have been reported worldwide. They may be plasmid or chromosomally encoded. The class D carbapenemases (oxacillinases) are by far the most prevalent carbapenemases in *A. baumannii* complex.[7] They can be grouped into those with intrinsic and acquired β-lactamases:

1. Intrinsic chromosomal
    a. OXA-51-like (over 370 variants)
2. Acquired
    a. OXA-23-like
    b. OXA-24/40-like
    c. OXA-58-like
    d. OXA-143-like
    e. OXA-235-like.

Class B metalloenzymes (MBL) such as VIM, IMP, NDM and SIM, as well as Class A carbapenemases (GES-types and KPC) have also been described. Co-occurrence of carbapenemases has been reported.

Meropenem is the recommended agent for screening for carbapenemase activity in *Acinetobacter* spp. Ertapenem resistance is inherent in the genus.

DETECTION

Meropenem is available across all susceptibility testing methods currently used in Australia.

2.1.1: Disc diffusion criteria for detecting carbapenemase-producing *Acinetobacter* spp.

EUCAST and CLSI

Method*	Meropenem disc	Zone diameter
CLSI	10 µg	≤ 14 mm
EUCAST	10 µg	< 15 mm

* Mueller-Hinton agar

CDS

Method*	Meropenem disc	Annular radius
CDS	5 µg	< 6 mm

* Sensitest agar

2.1.2: MIC criteria for detecting carbapenemase-producing *Acinetobacter* spp.

Method	Species	Meropenem MIC
MIC strip, Vitek 2, Phoenix NMIC-422	*Acinetobacter* spp.	> 8 mg/L

PHENOTYPIC TESTS

Following detection of resistance to carbapenems, phenotypic methods can be performed to indicate if the resistance is due to carbapenemase production. **The recently described carbapenem inactivation method (CIM)[8] should be performed on *all* isolates that indicate resistance to meropenem.** This test requires no specialised reagents or equipment and has shown excellent sensitivity and specificity to date. It requires at least eight hours of incubation.[9, 10] Rapid (< 2 hour) tests such as the Carba NP Test[11], Blue Carba[12, 13] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux[14, 15]; Rapid CARB Screen, ROSCO[16, 17], MAST® CARBA PAcE), are all alternatives, however these often may have poor sensitivity against isolates with Class D enzymes.

Either combined disc tests (CDT)[18] or double disc synergy tests (DDST)[19] may also be performed, but these require overnight incubation, and are of limited value for detection of Class D enzymes. If any of the phenotypic tests indicate carbapenemase activity, the isolate must be confirmed by molecular methods.

REFERRALS

Where the originating laboratory does not have the capacity to perform a CIM test, all meropenem resistant isolates must be referred to a confirming laboratory for confirmation and molecular testing. It is expected that when a positive screen result is obtained, the isolate is referred as soon as possible. A guide to the workflow is shown in Figure 2.

It is **not** necessary to refer *Acinetobacter* spp. that are:

1. CIM negative
2. Resistant to ertapenem, but susceptible to meropenem

CONFIRMATION

Molecular confirmation of carbapenemase genes is required on any CIM positive *A. baumannii* complex isolate.

The carbapenemases types detected in *A. baumannii* complex limit the usefulness of commercial assay systems often used for *Enterobacterales* (Refer to Section 2.3.3). Sequencing is required to determine the variants. Isolates that demonstrate carbapenemase activity (CIM positive) but the commercial system did not detect any gene targets, should undergo WGS.

CARAlert Notification

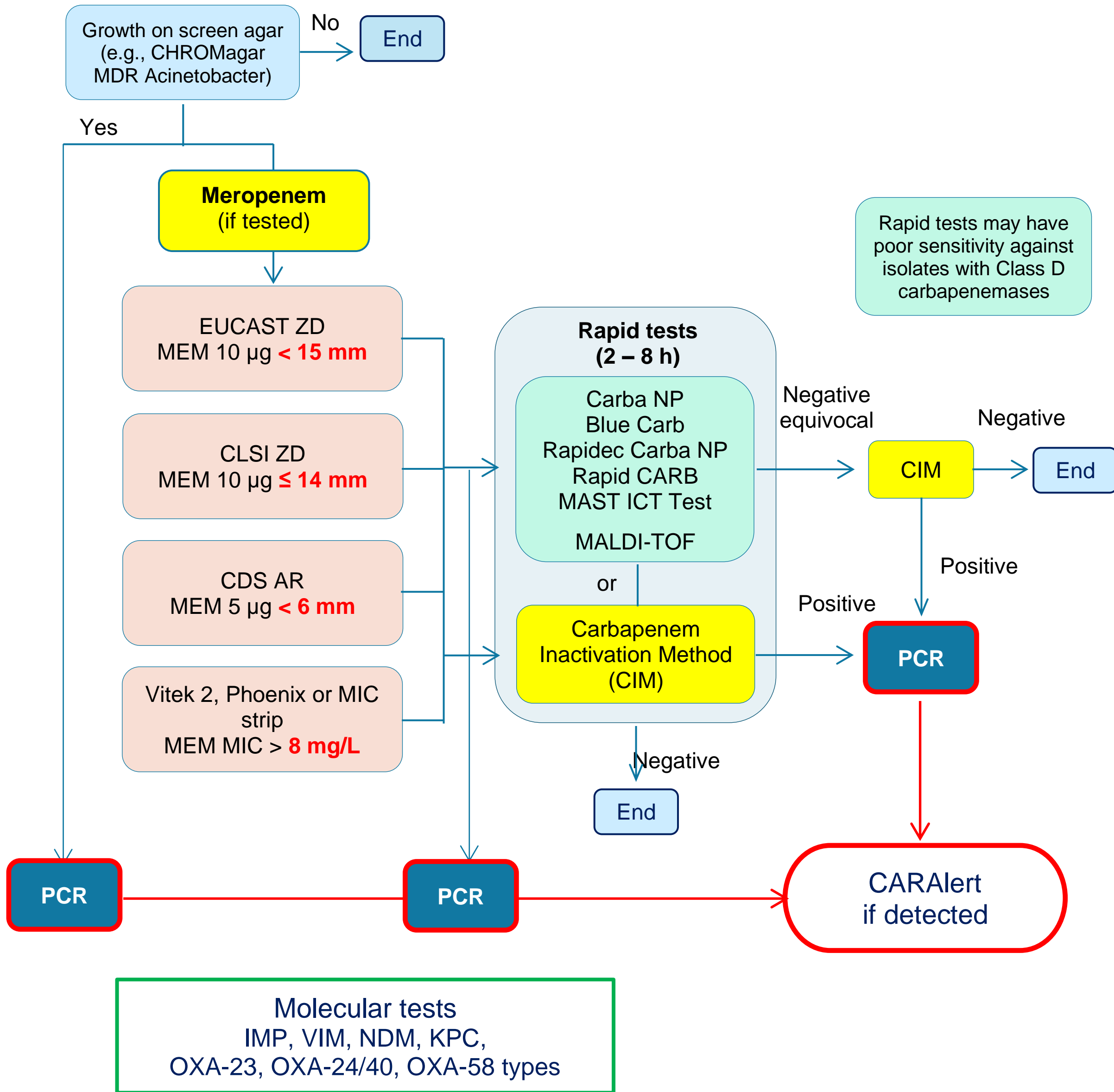
Carbapenemase-producing *Acinetobacter baumannii* complex (*A. calcoaceticus*, *A. baumannii*, *A. dijkshoorniae*, *A. nosocomialis*, *A. pittii* or *A. seifertii*).

Type: at time of confirmation (e.g., OXA-23-like, VIM, IMP etc)

Subtype: when sequencing results becomes available (e.g., OXA-23, VIM-2)

Note: Isolates that have only OXA-51-like enzymes should **not** be reported.

Figure 2: Carbapenemase-producing *Acinetobacter baumannii* complex flowchart



AR = annular radius (mm)
CIM = Carbapenem Inactivation Method
ICT = Indirect Carbapenemase Test
MEM = meropenem
PCR = refer for molecular confirmation
ZD = zone diameter

Note:
Any CIM positive isolates where carbapenemase gene targets were not detected by routine molecular assays, should be further examined using whole genome sequencing.

2.2 *Candida auris*

Candida auris was first recognised in 2009 in Japan[20] but has emerged quickly causing severe disease in hospitalised patients in many countries, including Australia.[21]

C. auris can cause invasive infections, be passed from person to person, and persist in the environment. Its severity, communicability, propensity to cause outbreaks and drug resistance make correctly identifying *C. auris* crucial to treating patients and preventing infections. However, this is challenging because traditional phenotypic methods may misidentify *C. auris*.

DETECTION

There are no reliable phenotypic characteristics that easily distinguish *C. auris* from other *Candida* species. However, clues to its presence that may assist with its differentiation with for example, *C. albicans* are its ability to grow at 42°C, absence of pseudohyphae and germ-tube negative characteristic. On traditional CHROMagar Candida, it forms white/cream to pale pink or pale purple colonies at about four days of growth. However, introduction of a novel chromogenic agar CHROMagar^{TM} Candida Plus has allowed rapid isolation and identification of *C. auris* directly from screening samples from patients suspected of being colonized with this pathogen where *C. aruis* colonies are pale cream with a distinctive blue halo that diffuses into the surrounding agar.[22] Isolates may then been reliably identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy (MALDI-TOF MS) utilising an appropriate spectral database that includes all clades of *C. auris* isolates.

Conventional phenotypic identification systems may misidentify *C. auris* as other *Candida* or *Rhodotorula* species, depending on the database version employed – these include all of the Vitek 2 YST, API 20C, API ID32C, BD Phoenix, MicroScan, and RapIDYeast Plus systems.[23] If any of the species listed in Table 2.2.1 are identified, or if species cannot be determined, further characterisation should be sought by either

1. MALDI-TOF MS (as above) (Table 2.2.1), or
2. By molecular methods based on sequencing analysis of the Internal Transcribed Region (ITS) rDNA locus and/or the D1-D2 region of the 28S rDNA locus

Although *C. auris* may be multi-drug resistant, antifungal resistance can vary widely across isolates and is dependent on region. Hence **ALL** *C. auris* isolates should be subject to antifungal susceptibility testing. There are currently no established *C. auris*–specific clinical breakpoints, although general guidance for interpretation of susceptibility testing has been provided by the Centers for Disease Control and Prevention[24], and these are supported by CLSI- and EUCAST-based ECOFFs for some antifungal agents.[25, 26] Antifungal susceptibility testing should be performed by a current approved method[27], or referred to a state reference laboratory (National Mycology Reference Centre [SA Pathology, Adelaide], the Clinical Mycology Reference Laboratory, Centre for Infectious Diseases and Microbiology Laboratory Services, ICPMR, Sydney; Victorian Infectious Diseases Reference Laboratory [Melbourne]; PathWest [Fiona Stanley Hospital], Perth, and Pathology Queensland, Royal Brisbane and Women's Hospital).

CONFIRMATION

Identification of *C. auris* must be confirmed by MALDI-TOF MS or DNA sequencing.

Note: All yeast isolates should be accurately identified to the species level when obtained from a normally sterile site.

CARAlert Notification

Any *C. auris* confirmed by MALDI-TOF or DNA sequencing.

2.2.1: Identification of *Candida auris* based on phenotypic laboratory method and initial species identification

Identification method	Database/software, if applicable	*C. auris* is confirmed if initial identification is *C. auris*	*C. auris* is possible if the following initial identifications are given. Further work-up is needed to determine if the isolate is *C. auris*
Bruker Biotyper MALDI-TOF	RUO libraries (Versions 2014 [5627] and more recent)	*C. auris*	n/a
	CA System library (Version Claim 4)	*C. auris*	n/a
bioMérieux VITEK MS MALDI-TOF	RUO library (with Saramis Version 4.14 database and Saccharomycetaceae update)	*C. auris*	*C. haemulonii* No identification
	IVD library (version 3.2)	*C. auris*	*C. haemulonii* No identification
VITEK 2 YST	Software version 8.01	*C. auris*	*C. haemulonii* *C. duobushaemulonii* *Candida* spp. not identified
	Older versions	n/a	*C. haemulonii* *C. duobushaemulonii* *Candida* spp. not identified
API 20C or ID32C		n/a	*Rhodotorula glutinis* (with characteristic red colour not present) *C. sake* *Candida* spp. not identified
BD Phoenix		n/a	*C. catenulata* *C. haemulonii* *Candida* spp. not identified
MicroScan		n/a	*C. lusitaniae** *C. guilliermondii** *C. parapsilosis** *C. famata Candida* spp. not identified
RapIDYeast Plus		n/a	*C. parapsilosis** *Candida* spp. not identified

* *C. guilliermondii*, *C. lusitaniae*, and *C. parapsilosis* isolates identified on MicroScan and any *C. parapsilosis* isolates identified on RapID Yeast Plus as possible *C. auris* isolates and further work-up should be considered

Source: National Center for Emerging and Zoonotic Infectious Diseases, CDC
https://www.cdc.gov/fungal/diseases/candidiasis/pdf/Testing-algorithm-by-Method-temp.pdf

2.3 *Enterobacterales* – carbapenemase-producing (CPE)

Carbapenemases are β-lactamases that significantly hydrolyse penicillins; in most cases cephalosporins, and to various degrees carbapenems (imipenem and/or meropenem). A variety of carbapenemases in *Enterobacterales* and *Pseudomonas* spp. have been reported worldwide. They may be plasmid or chromosomally encoded. They are grouped into three Ambler classes according to their amino acid identity:

- Class A (mostly KPC)
- Class B (metallo-β-lactamases [MBL]) of VIM, IMP, and NDM types
- Class D (mostly OXA-48-like).

Ambler class A β-lactamases are, at least partially, inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam, whereas MBLs are inhibited by divalent cation chelators such as EDTA or dipicolinic acid. Most carbapenemase-producers are resistant to extended-spectrum cephalosporins (ceftriaxone, cefotaxime, and/or ceftazidime).

For many isolates harbouring carbapenemases, the MICs of carbapenems are around the susceptible breakpoint, which can make resistance difficult to detect – particularly with automated systems. Therefore, **special zone diameter cut-offs and MICs are needed in first line screening**.

Meropenem is the recommended agent for screening for carbapenemase activity as it has the best balance of sensitivity and specificity. Imipenem is less suitable as some families such as *Proteae* and *Serratia* spp. have intrinsically higher imipenem MICs, and it is therefore harder to set a single screening breakpoint for this agent. Ertapenem has high sensitivity, but poor specificity, especially for species such as *Enterobacter*, and it therefore not recommended as an indicator of carbapenemases in *Enterobacterales.* The reason is that isolates that produce AmpC/ESBL enzymes and in addition have decreased permeability due to porin changes have higher MICs for ertapenem than for imipenem or meropenem.[19]

Detection

Meropenem is available across all susceptibility testing methods currently used in Australia. The epidemiological cut-off value (ECOFF) separates wild-type from non-wild-type populations. The ECOFF defined by EUCAST for meropenem is 0.125 mg/L or 0.25 mg/L depending on the species. As such the recommended screening criteria for detecting possible carbapenemase activity are as shown in 2.3.1 and 2.3.2.

> Epidemiological cut-off value cut-off values (ECOFFs) are the highest MIC values observed in the wild-type distribution of MICs of an antimicrobial agent. ECOFFs are specific to *individual species*.

2.3.1: Disc diffusion criteria for detecting carbapenemase-producing *Enterobacterales*

EUCAST and CLSI

Method*	Meropenem disc	Zone diameter†
ECOFF	10 µg	< 28 mm

* Mueller-Hinton agar

† EUCAST: Isolates with 25-27 mm only need to be investigate for carbapenemase-production if they are resistant to piperacillin–tazobactam and/or temocillin (temocillin contributes more to the specificity). Investigation for carbapenemases is always warranted if zone diameter of meropenem is < 25 mm

CDS

Method*	Meropenem disc	Annular radius
CDS	5 µg	< 6 mm

* Sensitest agar

Note (CDS): Resistance observed with a cefepime 10 µg disc and the absence of a synergistic zone between this disc and an adjacent amoxicillin-clavulanic acid (Augmentin) 60 µg disc is suggestive of the presence of a metallo-β-lactamase. KPC producers are often resistant to all β-lactam antimicrobials tested including the carbapenems.

2.3.2: MIC criteria for detecting carbapenemase-producing *Enterobacterales*

Method	Species	Meropenem MIC*
MIC strip, Phoenix NMIC-422†	*E. coli*, *Klebsiella*, *Enterobacter*, *Salmonella*	> 0.125 mg/L
MIC strip, Phoenix NMIC-422†	*Citrobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*	> 0.25 mg/L
Vitek-2§	*Enterobacterales*	> 0.25 mg/L
Phoenix NMIC-422§	*Enterobacterales*	> 0.25 mg/L

* Epidemiological cut-off value defined by EUCAST
† Phoenix NMIC-422: meropenem range is 0.125–16 mg/L
§ Vitek 2 AST-N246, AST-N247, AST-N435

2.3.3 Screening agar

Screening agars, most commonly chromogenic agars, may be used in selected circumstances such as during outbreaks, or for sentinel surveillance. There are numerous formulations commercially available from various manufacturers. The use of more than one medium enhances the detection of CPE, especially OXA-48 types[28]; recently a biplate formulation (chromID CARBA SMART; BioMérieux) was released that contains both chromID Carba and chromID OXA-48.

Isolates growing on selective chromogenic agar should be identified and have either meropenem susceptibility determined or be screened directly for at least the five most common carbapenemase genes (IMP, VIM, NDM, KPC, OXA-48-types).

2.3.4 Meropenem not tested initially

In situations where meropenem is not part of the routine test panel (for example routine urine specimens), *Enterobacterales* that have an ESBL phenotype or are gentamicin resistant, or both, should be tested against meropenem **before** submission for confirmation.

ESBL phenotype: Ceftriaxone or cefotaxime or ceftazidime MIC > 1 mg/L; or ceftriaxone (30 µg) zone diameter < 23 mm, or ceftazidime (10 µg) zone diameter < 22 mm; or synergy observed between amoxicillin-clavulanate and either cefotaxime/ceftriaxone or ceftazidime.

PHENOTYPIC TESTS

Following detection of reduced susceptibility to carbapenems, phenotypic methods can be performed to indicate if the reduced susceptibility is due to carbapenemase production. **The carbapenem inactivation method (CIM)[8] or modified CIM (mCIM)[4] are the most sensitive methods, and should be performed on *all* isolates that indicate reduced susceptibility to meropenem**. This test requires no specialised reagents or equipment and has shown excellent sensitivity and specificity to date. It requires at least eight hours of incubation.[9, 10] Rapid (< 2 hour) tests such as the Carba NP Test[11], Blue Carba[12, 13] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux[14, 15]; Rapid CARB Screen, ROSCO[16, 17]), are all suitable alternatives. These rapid tests however have lower sensitivity against isolates with low carbapenemase activity such as OXA-48-like producers. Matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) – has recently shown promise as a practical method for rapid detection of carbapenemases in the clinical setting.[29-33]

Either combined disc tests (CDT)[18] or double disc synergy tests (DDST)[19] may also be performed, but these require overnight incubation. Temocillin susceptibility has been utilized in various algorithms for carbapenemase characterisation as high-level temocillin resistance serves as a phenotypic OXA-48 marker.[19, 34, 35] If any of the phenotypic tests indicate carbapenemase activity, the isolate must be confirmed by molecular methods.

REFERRAL

Where the originating laboratory does not have the capacity to undertake molecular confirmation, isolates must be referred to a designated confirming laboratory. It is expected that when a positive screen result is obtained, the isolate is referred as soon as possible. A guide to the CPE workflow is shown in Figure 3.

It is not necessary to refer *Proteus* spp. *Providencia* spp. or *Morganella* spp. that are resistant to imipenem, but susceptible to meropenem.

CONFIRMATION

Detection of these gene targets can be achieved using either gel-based and/or real-time polymerase chain reactions (PCR).[36-38] For maximum sensitivity, molecular assays must include probes or primers to detect all known gene variants. Family coverage is relatively straight forward for KPC and NDM; harder for OXA-48-like; but much harder for IMP and VIM due to the large number of gene variants.

There are increasing numbers of commercial assays now available for the detection of carbapenemases. Their coverage for the ‘top five’ enzymes (IMP, VIM, KPC, NDM, and OXA-48-like), however, varies, and the results vary from ‘yes/no’ tests to ‘full’ group differentiation.[39] **If an isolate demonstrates carbapenemase activity (e.g. CIM or Carba NP positive) but the commercial system did not detect any enzymes, the isolate would need to be tested or referred to a laboratory that utilises methods capable of detecting the whole family.** Sequencing is required to determine the variants. The use of in-house assays (single or multi-plex; gel-based or real-time PCR) remains common due to their flexibility, and lower cost. However, these protocols must be *validated* against well characterised strains according to NPAAC guidelines.[40]

To aid with epidemiology, especially in outbreak investigations, whole genome sequencing (WGS) may be performed. WGS is useful for detecting all resistance genes (resistome) as well as establishing clonality.

2.3.5: Commercial assay systems for confirming carbapenemase-producing *Enterobacterales*

Company	KPC	NDM	IMP	VIM	OXA-48-like	Other
Cepheid Xpert® Carba-R	1-19	1–12	IMP-1-3, 4, 6, 8, 9, 10, 11, 19, 20-22, 24, 25, 27, 28, 30, 31, 33, 37, 40, 42 Limitations: IMP-7, -13, -14	1–40	OXA-48-like (-162, -163, -181, -204, -232, -244, -245, -247, -370)	
AusDiagnostics CRE (16-well), Catalogue number: 21098, Version: 03	All	1–8	IMP-1, 4, 5, 6, 7, 10, 13, 26, 29, 34, 40 and 42 Limitations: IMP-14	VIM-1-7, 11, -19, and 36-40	OXA-48-like (-181, -204, -232, -244, -245, -484)	IMI, SME, GES, OXA-23-like, OXA-51-like, OXA-58-like CMY, CTX-M group 1, CTX-M group 9
AusDiagnostics CRE EU (16-well), Catalogue number: 21099, Version: 02	All	1–8	IMP-1, 4, 5, 6, 7, 8, 10, 13, 14a, 26, 29, 34, 40 and 42	VIM-1-7, 11, -19, and 36-40	OXA-48, -181, -204, -232, -244, -245, -484	IMI, SME, FRI-1, GES, SPM, SIM, GIM mcr-1

Notes:

1. These commercial assays only identify the enzyme group involved. Sequencing is required to determine the variant.
2. IMP-4 is the most common variant in Australia.
3. OXA-181 and -232 are so far the commonest variants in Australia.

CARAlert Notification

Enterobacterales confirmed to contain carbapenemase genes.

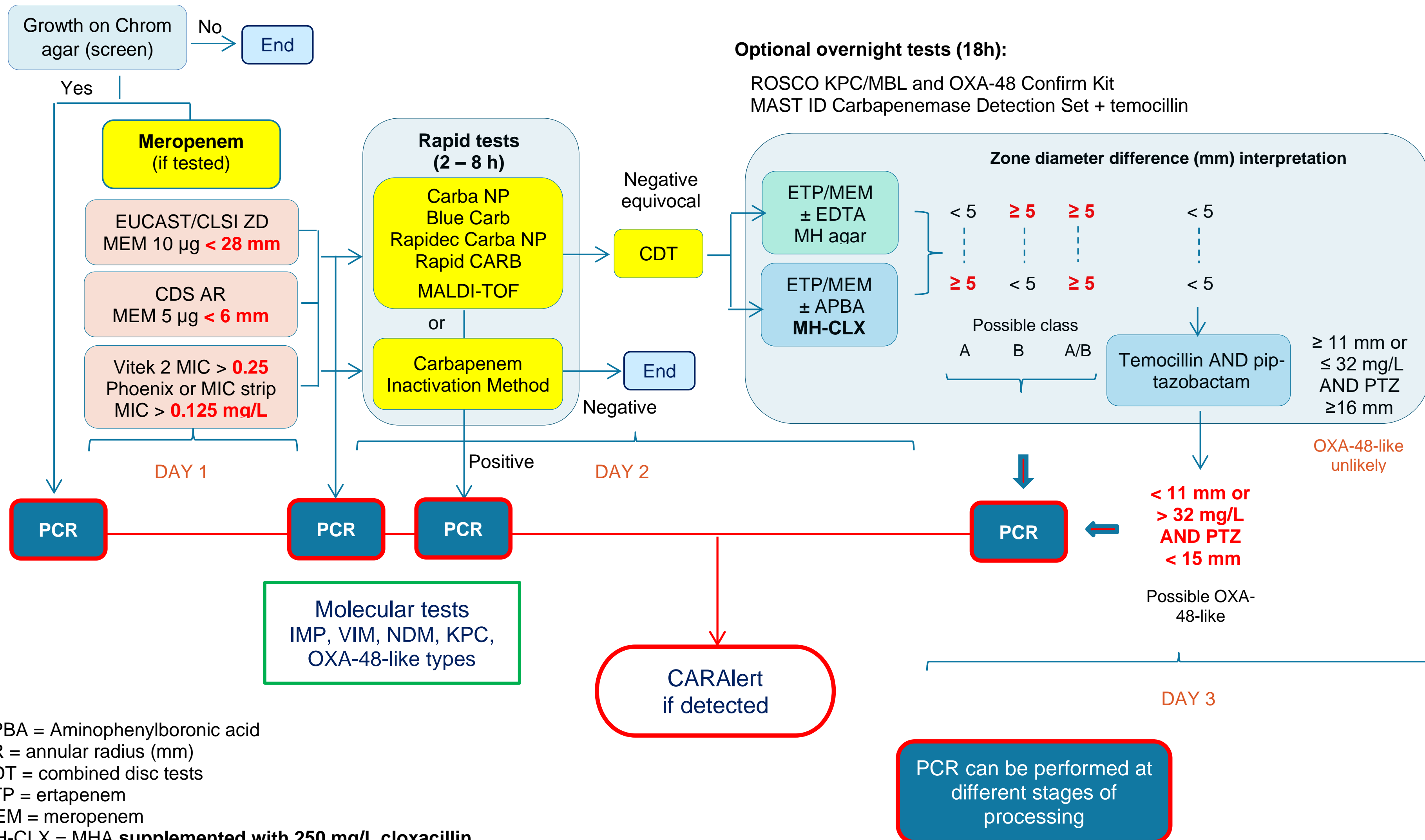
Type: at time of confirmation (e.g., IMP, NDM)

Subtype: when sequencing results becomes available (e.g., IMP-4, NDM-5)

Notes:

1. Submit the CAR as soon as the type is known; do not wait until the subtype is available.
2. If *mcr-9* is detected by WGS, include the variant along with the CPE subtype.

Figure 3: Carbapenemase-producing *Enterobacterales* flowchart



APBA = Aminophenylboronic acid
AR = annular radius (mm)
CDT = combined disc tests
ETP = ertapenem
MEM = meropenem
MH-CLX = MHA **supplemented with 250 mg/L cloxacillin**
PCR = refer for molecular confirmation
PTZ = piperacillin–tazobactam
ZD = zone diameter (mm)

2.4 *Enterobacterales* – ribosomal methyltransferase-producing

High-level resistance to amikacin (MIC > 256 mg/L) is an indication that ribosomal methyltransferases (RMT) may be the cause of the aminoglycoside resistance. The typical phenotype of isolates containing RMT is high-level resistance to amikacin, gentamicin *and* tobramycin.[41, 42]

Detection

Amikacin resistant isolates should be tested for gentamicin and tobramycin resistance and screened for high-level amikacin resistance (HLAR) (MIC > 256 mg/L) using a MIC strip. If the isolate is confirmed resistant to all three aminoglycosides **and** has HLAR refer to confirming laboratory for molecular confirmation. RMT are often associated with carbapenemases, especially NDM-types.[43]

2.4.1: Disc diffusion criteria for detecting *Enterobacterales* with ribosomal methyltransferases

EUCAST/CLSI

Method*	Agent	Disc	Zone diameter	MIC
EUCAST	Amikacin	30 µg	< 15 mm	> 16 mg/L
	Gentamicin	10 µg	< 14 mm	> 4 mg/L
	Tobramycin	10 µg	< 14 mm	> 4 mg/L
CLSI	Amikacin	30 µg	≤ 14 mm	≥ 64 mg/L
	Gentamicin	10 µg	≤ 12 mm	≥ 16 mg/L
	Tobramycin	10 µg	≤ 12 mm	≥ 16 mg/L

* Mueller-Hinton agar

CDS

Method*	Agent	Disc	Annular radius	MIC
CDS	Amikacin	30 µg	< 4 mm	> 16 mg/L
	Gentamicin	10 µg	< 4 mm	> 2 mg/L
	Tobramycin	10 µg	< 4 mm	> 2 mg/L

* Sensitest agar

2.4.2: MIC criteria for detecting *Enterobacterales* with ribosomal methyltransferases

Both the current Vitek 2 (AST-N246, AST-N247, AST-N435) and Phoenix (NMIC-422) cards test all three aminoglycosides, which can readily identify isolates suspected of containing ribosomal methyltransferases

Agent	Vitek 2	Phoenix
Amikacin	≥ 64 mg/L	> 32 mg/L
Gentamicin	≥ 16 mg/L	> 8 mg/L
Tobramycin	≥16 mg/L	> 8 mg/L

2.4.3: Amikacin and/or tobramycin not tested

Gentamicin may be the only agent tested routinely for particular specimens. If the isolate has only been tested against gentamicin, there is no requirement for further testing. However, if gentamicin and tobramycin have been tested, and both are resistant, amikacin should be tested. If such strains test as susceptible to amikacin, no further testing or referral is required.

CONFIRMATION

Only isolates that are resistant to all three aminoglycosides (amikacin, gentamicin and tobramycin), **and** show high-level amikacin resistance (MIC > 256 mg/L), should be referred for molecular confirmation.

Ten acquired ribosomal methyltransferase-encoding genes have been identified, including *armA*, *rmtA, rmtB*, *rmtC*, *rmtD*, *rmtE*, r*mtF*, *rmtG, rmtH* and *npmA*.

Ribosomal methyltransferases can all be readily detected using multiplex PCR[43, 44], real-time PCR[45], or by whole genome sequencing.

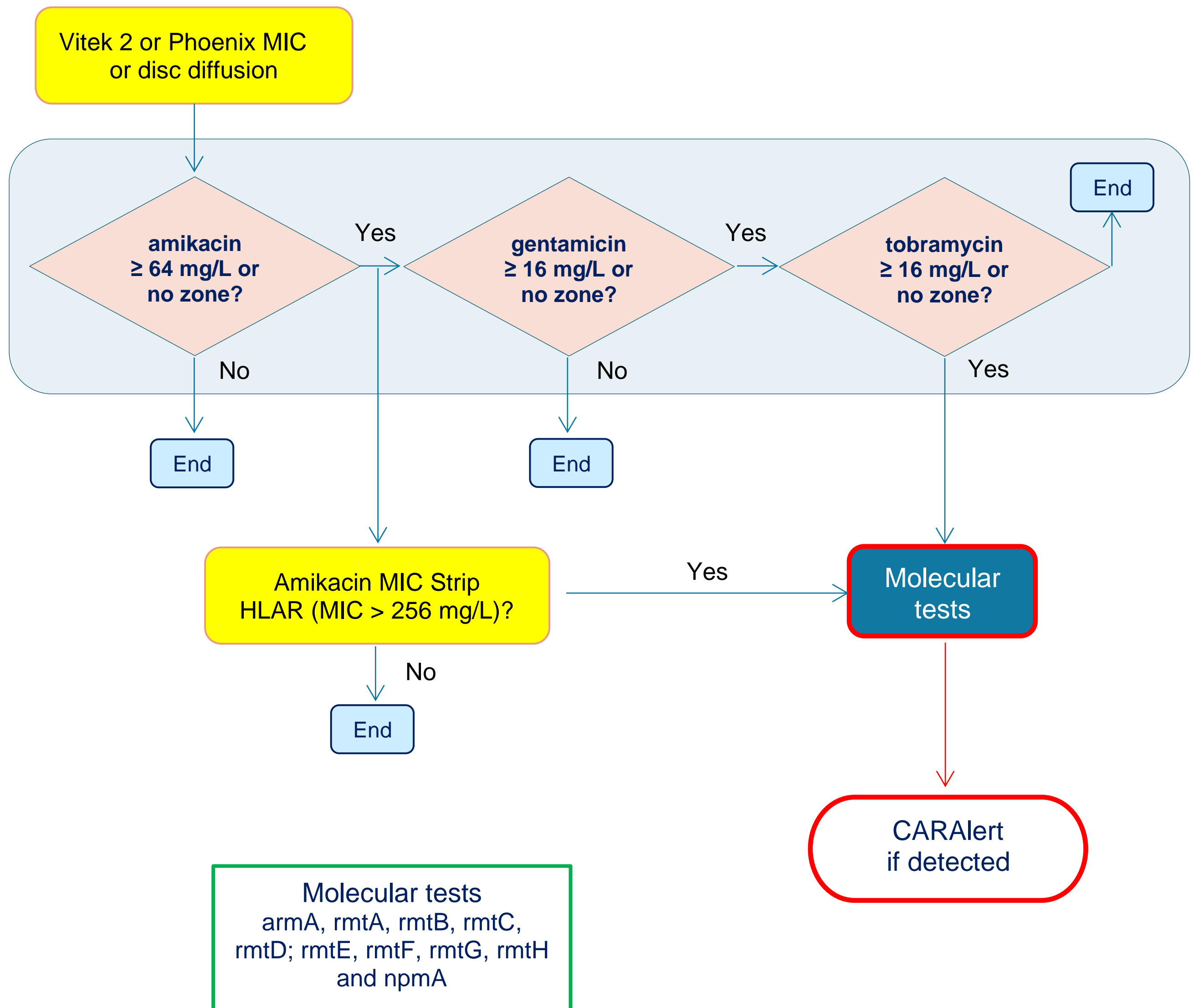
CARAlert Notification

Enterobacterales confirmed to contain ribosomal methyltransferase genes.

Type: at time of confirmation (e.g., *rmtB*, *armA*)

Subtype: when sequencing results becomes available (e.g., *rmtB1*)

Figure 4: Ribosomal methyltransferases flowchart



HLAR = high-level amikacin resistance

2.5 *Enterobacterales* – transmissible colistin resistance

Previously, polymyxin resistance was reported always to be chromosomally mediated and usually related to mutations in several genes included in the two-component regulatory system for biosynthesis of lipid A, and thereby regulation of charge in the lipopolysaccharide (LPS).[46] A plasmid-borne gene encoding a phosphoethanolamine transferase conferring resistance to colistin was reported in 2015 in China and named *mcr-1* (mobile colistin resistance).[47] Since then, a number of new variants of MCR-1 have been described.[48] The worldwide emergence of transmissible colistin resistance in animals, food products and humans is especially worrisome, since it has a great propensity for horizontal dissemination.

Transmissible resistance to colistin refers to the presence of *mcr* genes other than *mcr-9*. This variant is not associated with a colistin-resistant phenotype but is typically found on H12 plasmids which may carry *bla*$_{IMP-4}$.[49]

Proteus spp. *Morganella morganii*, *Providencia* spp. *Hafnia* spp. and *Serratia marcescens* all have expected resistant phenotypes to polymixins.

DETECTION

There are currently no extensively evaluated methods for phenotypic characterization of the different polymyxin resistance mechanisms other than the MIC itself determined by broth microdilution (gradient diffusion and disk diffusion are unreliable for this drug class).

Laboratories are advised to always use broth microdilution for susceptibility testing of colistin, and to always use colistin sulfate. **Disk diffusion and gradient tests should not be used**, as they are associated with high-risk of both very major and major AST errors. Currently available gradient tests underestimate colistin MIC values and under call resistance, and should be avoided, even when quality control results are within range.

Commercially available colistin broth microdilution panels include MICRONAUT-S, MIC-Strip Colistin (Merlin) and Sensititre™ (ThermoFisher Scientific).

If colistin has been tested, isolates with colistin MIC > 2 mg/L (BMD) should be referred for molecular testing.

As colistin may not be routinely tested, isolates with MCR may be detected by whole genome sequencing of isolates referred for confirmation of carbapenemase genes.

CONFIRMATION

Molecular confirmation of MCR-genes.

CARAlert Notification

Enterobacterales (excluding *Proteus* spp., *Morganella morganii*, *Providencia* spp., *Hafnia* spp. and *Serratia marcescens*) with confirmed transmissible colistin resistance (MCR).

Type: at time of confirmation (MCR)

Subtype: when sequencing results becomes available (e.g., *mcr-1*, *mcr-1.3*)

Note: Transmissible resistance to colistin refers to the presence of *mcr* genes other than *mcr-9*. This variant is not associated with a colistin-resistant phenotype but is typically found on H12 plasmids which may carry *bla*$_{IMP-4}$.

2.6 *Enterococcus* species – linezolid-resistant

Linezolid resistance in *Enterococcus* spp. is commonly due to mutations in the 23S rRNA or ribosomal proteins (L3 and L4), or acquisition of a plasmid-borne ribosomal methyltransferase, *cfr*, or *optrA*-mediated.[50, 51]

Detection

Enterococcus spp. that test resistant to linezolid.

2.6.1: Susceptibility test criteria for detecting linezolid-resistant *Enterococcus* species

EUCAST/CLSI

Method*	Linezolid disc	Zone diameter	MIC
EUCAST	10 µg	< 19 mm	> 4 mg/L
CLSI	30 µg	≤ 20 mm	≥ 8 mg/L

* Mueller-Hinton agar

CDS

Method*	Disc	Annular radius	MIC
CDS	10 µg	< 6 mm	> 4 mg/L

* Blood Sensitest agar, CO_2, 35–37°C

Confirmation

Enterococcus species isolates that test as linezolid-resistant by Vitek 2/Phoenix should have the MIC determined using either a linezolid MIC strip or by broth microdilution.

Confirmation of the common mutation (G2576T) to the 23S rRNA responsible for linezolid resistance in enterococci can be done by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) using *NheI* restriction enzyme, real-time PCR or by sequencing.[52-54] Molecular methods are used to detect rRNA methyltransferases (*cfr* or *optrA*).

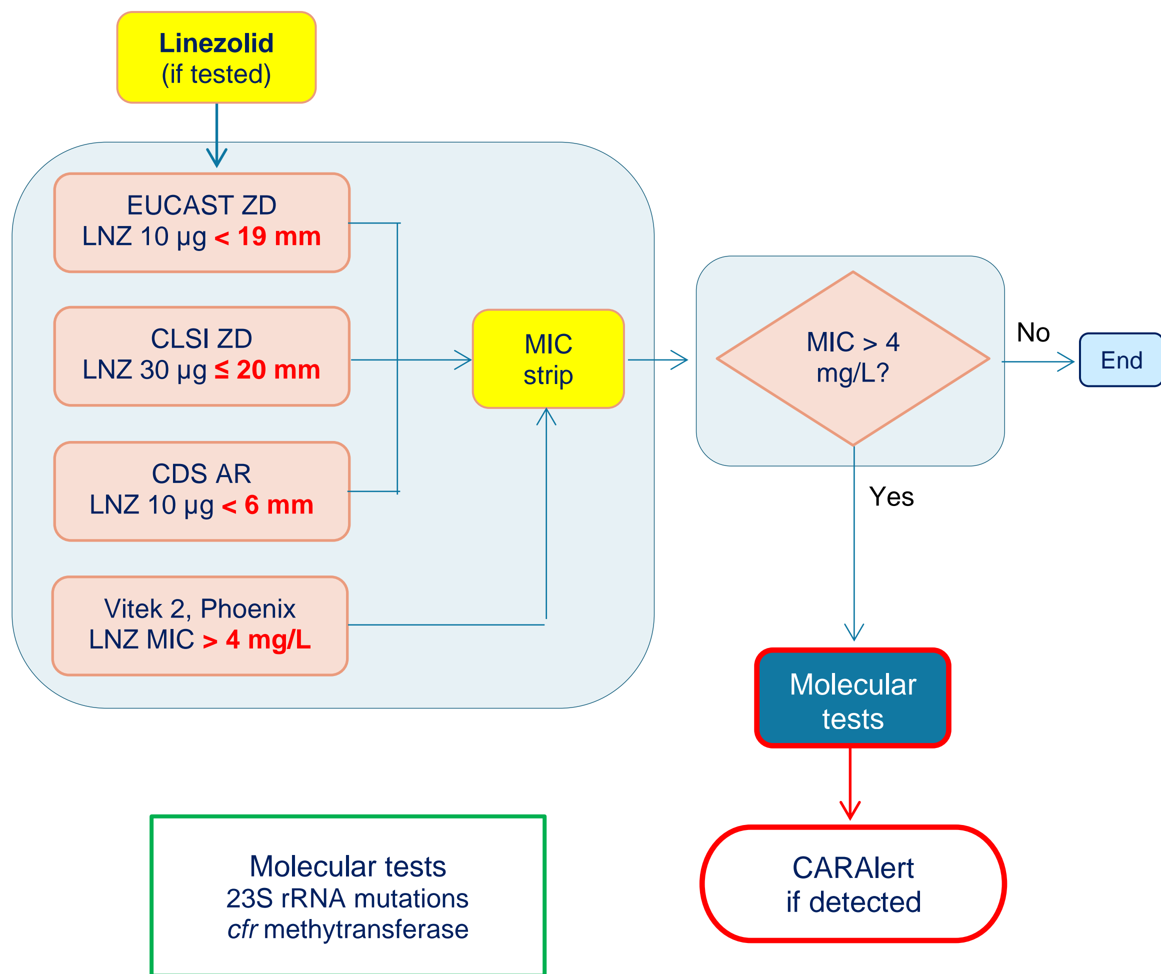
CARAlert Notification

Enterococcus spp. with mutations detected in 23S rRNA or L3/L4 ribosomal proteins; or if *cfr* or *optrA* has been detected.

Type: 23S rRNA, *cfr*, *optrA*, Ribosomal proteins, Other

Subtype: mutation or variant

Figure 5: Linezolid-resistant *Enterococcus* spp. flowchart



Confirm purity and identification

AR = annular radius (mm)
LNZ = linezolid
ZD = zone diameter (mm)

2.7 *Mycobacterium tuberculosis* – multidrug-resistant

M. tuberculosis complex (MTB) is tested against isoniazid, rifampicin, ethambutol and pyrazinamide through the Australian Mycobacterial Reference Laboratory Network (AMRLN). Rifampicin-resistant strains are regarded as presumptively multidrug-resistant. A commercial liquid medium based Mycobacteria Growth Indicator system (BACTEC MGIT 960; Becton Dickinson) is used for susceptibility testing by the AMRLN. The Cepheid Xpert® MTB/RIF molecular test can detect MTB and rifampicin resistance mutations. This test will be monitored by the ARMLN for suitability of reporting to CARAlert. WHO recently announced a change to the rifampicin breakpoint for MTB in MIGIT.[55] Isolates with rifampicin MIC > 0.5 mg/L in the MGIT system are considered to be phenotypically resistant.

CONFIRMATION

Rifampicin-resistant strains are associated with various *rpoB* mutations.

CARAlert Notification

MTB with rifampicin MIC > 0.5 mg/L in the MGIT system and/or *rpoB* mutation recognised as encoding rifampicin resistance clinically, and resistant in addition to at least isoniazid.

Note: Submission through the **AMRLN** only.

2.8 *Neisseria gonorrhoeae* – azithromycin-nonsusceptible

Antimicrobial resistance to azithromycin, benzylpenicillin, ceftriaxone or cefotaxime and ciprofloxacin in *N. gonorrhoeae* is monitored by the Australian Gonococcal Surveillance Programme (AGSP) through the National Neisseria Network (NNN). The NNN is coordinated by the World Health Organization Collaborating Centre for STI and AMR, Sydney, and supported by the Australian Government Department of Health and Aged Care. Isolates with azithromycin MIC > 1 mg/L are to be reported to CARAlert.

Detection

A EUCAST method is not fully developed for susceptibility testing of *N. gonorrhoeae*. Reference laboratories report using CLSI methods. Disc susceptibility testing by the routine laboratory is not recommended.

2.8.1: Susceptibility test criteria for detecting azithromycin–nonsusceptible *Neisseria gonorrhoeae*

Method	Agent	MIC	Comments
EUCAST*	Azithromycin	> 1 mg/L	ECOFF
CLSI[†]	Azithromycin	> 1 mg/L	
CDS[§]	Azithromycin	≥ 1 mg/L	

– = not yet defined
* Commercial MIC method; follow the manufacturer's instructions
† GC Agar Base supplemented with 1% defined growth supplement
§ Chocolate agar (Columbia Agar Base supplemented with 8% defibrinated horse blood)

Confirmation

N. gonorrhoeae with azithromycin MIC > 1 mg/L confirmed by the NNN laboratories.

CARAlert Notification

N. gonorrhoeae with azithromycin MIC > 1 mg/L confirmed by NNN laboratories.

Type: Azithromycin (LLR <256), azithromycin (HLR ≥256)

Notes:
1. Submission through the **NNN** only.
2. *N. gonorrhoeae* with MIC ≤ 256 mg/L are excluded from the weekly digest.

LLR = low-level resistance, MIC < 256 mg/L; HLR = high-level resistance, MIC ≥ 256 mg/L

2.9 *Neisseria gonorrhoeae* – ceftriaxone-nonsusceptible

Antimicrobial resistance to azithromycin, benzylpenicillin, ceftriaxone or cefotaxime and ciprofloxacin in *N. gonorrhoeae* is monitored by the AGSP through the NNN. Isolates with ceftriaxone MIC ≥ 0.125 mg/L are to be reported to CARAlert.

DETECTION

A EUCAST method is not fully developed for susceptibility testing of *N. gonorrhoeae*. Reference laboratories report using CLSI methods. Disc susceptibility testing by the routine laboratory is not recommended.

2.9.1: Susceptibility test criteria for detecting ceftriaxone–nonsusceptible *Neisseria gonorrhoeae*

Method	Agent	MIC	Comments
EUCAST*	Ceftriaxone	> 0.125 mg/L	
CLSI†	Ceftriaxone	> 0.25 mg/L	
CDS§	Ceftriaxone	0.06 – 0.25	Less susceptible. Breakpoint for resistance not yet determined

– = not yet defined

* Commercial MIC method; follow the manufacturer's instructions

† GC Agar Base supplemented with 1% defined growth supplement

§ Chocolate agar (Columbia Agar Base supplemented with 8% defibrinated horse blood)

CONFIRMATION

N. gonorrhoeae with ceftriaxone MIC ≥ 0.125 mg/L confirmed by the NNN laboratories. The mosaic structure of the *penA* gene (encoding penicillin-binding protein 2, PBP2) will be determined by the NNN on all isolates with ceftriaxone MIC ≥ 0.125 mg/L.[56, 57]

CARAlert Notification

N. gonorrhoeae with ceftriaxone MIC ≥ 0.125 mg/L confirmed by NNN laboratories.

Type: Ceftriaxone

Note: Submission through the **NNN** only.

2.10 *Neisseria gonorrhoeae* – gentamicin-resistant

The emergence of gonococcal AMR in Australia has long been influenced by the introduction of multidrug-resistant strains from overseas.[58, 59] In 2017, the first evidence of sustained spread of multidrug-resistant *N. gonorrhoeae* was reported internationally[60], followed in 2018 by coincident reports from Australia and the United Kingdom of the first extensively drug-resistant *N. gonorrhoeae* isolates.[61, 62] These data are captured by the AGSP and azithromycin- and ceftriaxone-nonsusceptible *N. gonorrhoeae* have been reported to CARAlert since its inception.

Clinical breakpoints for gentamicin have not been established however, on the basis of clinical correlate data, isolates are considered to have acquired resistance to gentamicin when the MIC value is > 16 mg/L on GC Agar base supplemented with 1% IsoVitaleX.[63] Gentamicin resistance in *N. gonorrhoeae* has not yet been reported in Australia. In New South Wales, a recent study with results applicable to other states and territories, found that gonococcal resistance to gentamicin was not demonstrated in clinical isolates from 2015 to 2020, and there was no detectable increase in gentamicin MIC with median MIC of 4 mg/L (range of 0.5–8 mg/L).[64, 65]

There are limited treatment options for multidrug-resistant *N. gonorrhoeae*, and gentamicin is now recommended in Australian guidelines as part of the treatment strategy for multidrug- and extensively drug-resistant gonorrhoea in Australia.[66] The spread of gonococcal resistance world-wide is well documented[60], however data on gentamicin resistance in *N. gonorrhoeae* are limited.

Antimicrobial resistance to azithromycin, benzylpenicillin, ceftriaxone or cefotaxime, ciprofloxacin and gentamicin in *N. gonorrhoeae* is monitored by the AGSP through the NNN. Isolates with gentamicin MIC > 8 mg/L are to be reported to CARAlert.

Detection

A EUCAST method is not fully developed for susceptibility testing of *N. gonorrhoeae*. Reference laboratories report using CLSI methods. Disc susceptibility testing by the routine laboratory is not recommended.

2.10.1: Susceptibility test criteria for detecting gentamicin-resistant *Neisseria gonorrhoeae*

Method	Agent	MIC
EUCAST*	Gentamicin	> 8 mg/L
CLSI†	Gentamicin	> 8 mg/L
CDS§	Gentamicin	> 8 mg/L

– = no breakpoints
* Commercial MIC method; follow the manufacturer's instructions
† GC Agar Base supplemented with 1% defined growth supplement
§ Chocolate agar (Columbia Agar Base supplemented with 8% defibrinated horse blood)

Confirmation

N. gonorrhoeae with gentamicin MIC > 8 mg/L confirmed by the NNN laboratories

CARAlert Notification

N. gonorrhoeae with gentamicin MIC > 8 mg/L confirmed by NNN laboratories.

Type: Not required

Note: Submission through the **NNN** only.

2.11 *Neisseria meningiditis* – ciprofloxacin-nonsusceptible

In the Asia-Pacific, North America, and Europe, there is evidence of the emergence of novel strains of *N. meningitidis* resistant to ciprofloxacin and penicillin.[67-69] In Australia, these data are captured by routine processes for the Australian Meningococcal Surveillance Programme (AMSP). To date, ciprofloxacin resistance in *N. meningitidis* was reported in Australia in 2018 (MIC = 0.25 mg/L)[70] and in 2021 (MIC = 0.125 mg/L).[71] Whilst the incidence of resistant strains is currently infrequent, there is potential for the importation and spread of resistant isolates, that has implications for the future treatment of prophylaxis recommendations for invasive meningococcal disease. In view of this situation, continued targeted surveillance of ciprofloxacin resistance in *N. meningitidis* is essential to maintain successful treatment regimens for invasive meningococcal disease and chemoprophylaxis regimens.

Antimicrobial resistance to benzylpenicillin, ceftriaxone, ciprofloxacin and rifampicin in *N. meningitidis* is monitored by the AMSP through the NNN. Isolates with ciprofloxacin MIC > 0.016 mg/L are to be reported to CARAlert.

Detection

Perform all AST of *N. meningitidis* in a biological safety cabinet. All laboratory staff who routinely work with *N. meningitidis* should be up to date with the Meningococcal (MenACWY and MenB) vaccine.[72]

EUCAST disk diffusion criteria for AST of *N. meningitidis* have not yet been defined, and an MIC method should be used.

2.11.1: Susceptibility test criteria for detecting ciprofloxacin-nonsusceptible *Neisseria meningitidis*

Method	Agent	MIC	Comments
EUCAST*	Ciprofloxacin	> 0.016 mg/L	Meningitis breakpoints
CLSI†	Ciprofloxacin	> 0.03 mg/L	

– = not yet defined

* Commercial MIC method; follow the manufacturer's instructions

† Mueller-Hinton Agar with 5% sheep blood agar

Confirmation

N. meningitidis with ciprofloxacin MIC > 0.016 mg/L confirmed by the NNN laboratories.

CARAlert Notification

N. meningitidis with ciprofloxacin MIC > 0.016 mg/L confirmed by NNN laboratories.

Type: Not required

Note: Submission through the **NNN** only.

2.12 *Pseudomonas aeruginosa* – carbapenemase-producing

Carbapenem-resistance in *P. aeruginosa* may be due to multiple chromosomal mechanisms, such as active efflux, porin alteration or deficiencies. Isolates resistant to all relevant carbapenems (imipenem and meropenem) and piperacillin–tazobactam should be tested for carbapenemase activity.

Although carbapenem resistance is very common in isolates from cystic fibrosis patients, acquired carbapenemases are rare in this patient group. As such, for CARAlert, isolates from cystic fibrosis patients are excluded.

Class B carbapenemases (VIM, IMP, AIM, DIM, SIM, SPM) are the dominate enzymes found in *P. aeruginosa* isolates. Class A (GES-5, KPC) and Class D (OXA-181) types have also been reported.

DETECTION

Meropenem and piperacillin-tazobactam are available across all susceptibility testing methods currently used in Australia.

Some GES types (GES-1, GES-3, GES-7, GES-9) are ESBLs, rather than carbapenemases (GES-2, GES-4, GES-5, GES-6, GES-8, GES-24)

2.12.1: Disc diffusion criteria for detecting carbapenemase-producing *Pseudomonas aeruginosa*

EUCAST and CLSI

Method*	Agent	Disc	Zone diameter	MIC equivalent
CLSI	Meropenem	10 µg	< 19 mm†	> 2 (I or R)
	Piperacillin-tazobactam	100/10 µg	< 21 mm	> 16 (R)
EUCAST	Meropenem	10 µg	< 24 mm	> 2 (I or R)
	Piperacillin-tazobactam	30-6 µg	< 18 mm	> 16 (I or R)

* Mueller-Hinton agar
† The EUCAST criterion is preferred

CDS

Method*	Agent	Disc	Annular radius	MIC equivalent
CDS	Meropenem	5 µg	< 6 mm	> 2 mg/L (R)
	Piperacillin-tazobactam	50/5 µg	< 6 mm	> 16 mg/L (R)

* Sensitest agar

2.12.2: MIC criteria for detecting carbapenemase-producing *Pseudomonas aeruginosa*

Method	Agent	Meropenem MIC
MIC strip, Vitek 2, Phoenix NMIC-422	Meropenem	> 2 mg/L
	Piperacillin-tazobactam	> 16 mg/L

Phenotypic tests

Following detection of reduced susceptibility to carbapenems, phenotypic methods can be performed to indicate if the reduced susceptibility is due to carbapenemase production. **The recently described carbapenem inactivation method (CIM)[8] or modified CIM (mCIM)[4] should be performed on *all* isolates that indicate reduced susceptibility to meropenem plus piperacillin–tazobactam.** This test requires no specialised reagents or equipment and has shown excellent sensitivity and specificity to date. It requires at least eight hours of incubation.[9, 10] Rapid (< 2 hour) tests such as the Carba NP Test[11], Blue Carba[12, 13] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux[14, 15]; Rapid CARB Screen, ROSCO[16, 17]), are all suitable alternatives, however these often may have lower sensitivity against isolates with GES-type carbapenemases commonly found in *Pseudomonas* species.

Either combined disc tests (CDT)[18] or double disc synergy tests (DDST)[19] may also be performed, but these require overnight incubation. If any of the phenotypic tests indicate carbapenemase activity, the isolate must be confirmed by molecular methods.

Referrals

Where the originating laboratory does not have the capacity to undertake molecular confirmation, isolates must be referred to a designated confirming laboratory for molecular testing. It is expected that when a positive screen result is obtained, the isolate is referred as soon as possible.

It is **not** necessary to refer *P. aeruginosa* that are:

1. Resistant only to carbapenems and susceptible to other β-lactams (inferred to have mutational resistance)
2. Resistant to ertapenem, but susceptible to meropenem. Ertapenem resistance in intrinsic in *Pseudomonas*
3. From cystic fibrosis patients
4. CIM negative.

Confirmation

Molecular confirmation of carbapenemase genes is required on any CIM positive *P. aeruginosa* isolate.

Most commercial assay systems are designed to detect carbapenemase genes commonly found in *Enterobacterales*, and may not detect those seen in *P. aeruginosa*, such as GES, AIM, DIM, SIM, and SPM types (refer to Section 2.3.5). Isolates that demonstrate carbapenemase activity (CIM positive) but where gene targets were not detected by the commercial assay system, should undergo WGS. Sequencing is required to determine the variants.

CARAlert Notification

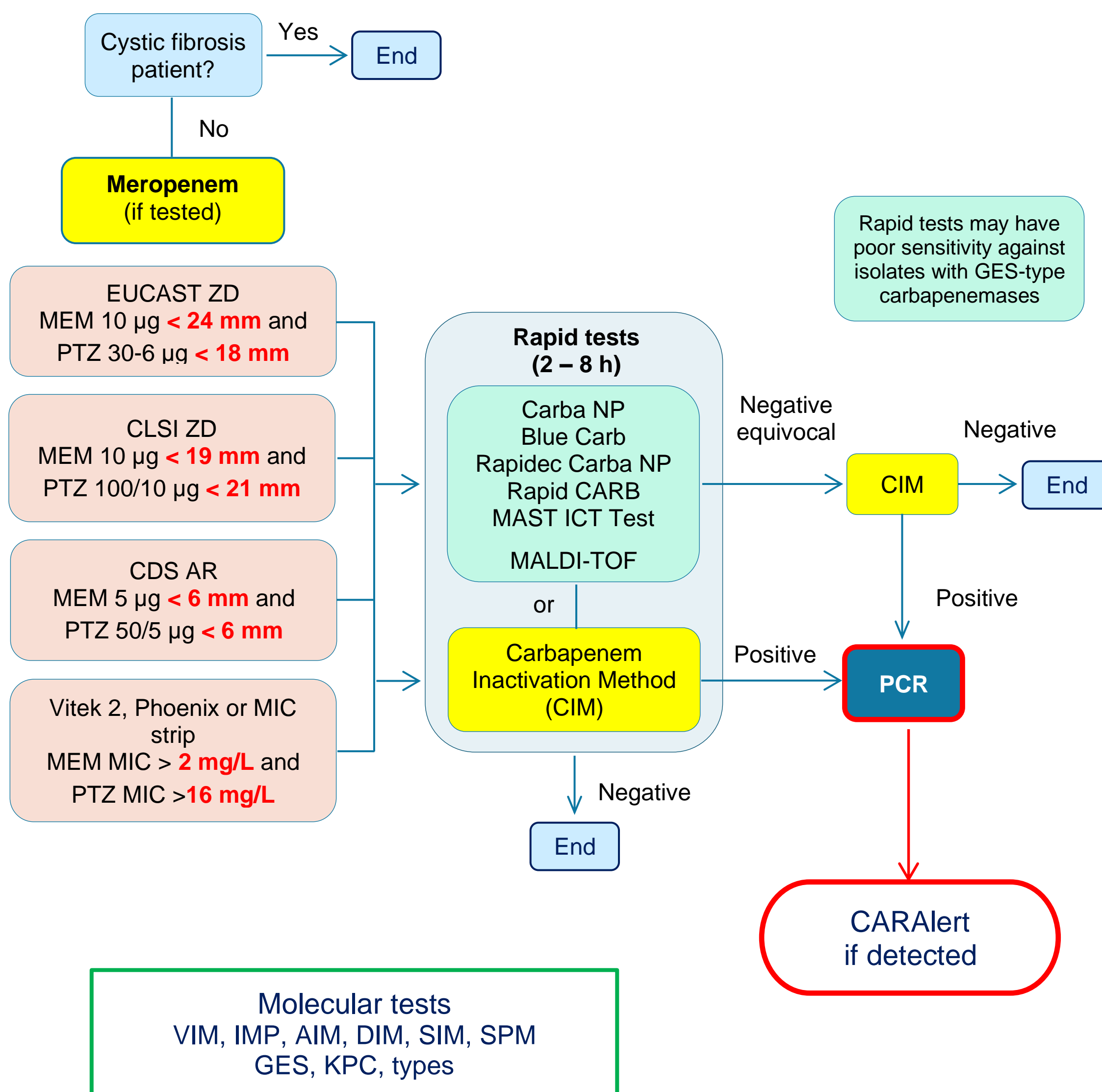
Pseudomonas aeruginosa, **excluding isolates from patients with cystic fibrosis**, confirmed to contain carbapenemase genes.

Type: at time of confirmation (e.g., GES, NDM)

Subtype: when sequencing results becomes available (e.g., GES-5, NDM-5)

Note: GES types that are CIM negative should not be reported.

Figure 6: Carbapenemase-producing *Pseudomonas aeruginosa* flowchart



AR = annular radius (mm)
CIM = Carbapenem Inactivation Method
ICT = Indirect Carbapenemase Test
MEM = meropenem
PCR = refer for molecular confirmation
PTZ = piperacillin–tazobactam
ZD = zone diameter (mm)

Note:
Any CIM positive isolates where carbapenemase gene targets were not detected by routine molecular assays, should be further examined using whole genome sequencing.

2.13 *Salmonella* species – ceftriaxone-nonsusceptible

Salmonella spp. associated with gastroenteritis are frequently not tested or have limited antimicrobial susceptibility tests performed at the time of isolation. Extra-intestinal isolates should be routinely tested against ampicillin, ceftriaxone/cefotaxime and ciprofloxacin. Isolates that test as ceftriaxone-nonsusceptible should be tested for the presence of extended-spectrum β-lactamases (ESBL) and plasmid–mediated AmpC.

Detection

2.13.1: Susceptibility test criteria for detecting ceftriaxone-nonsusceptible *Salmonella* species

EUCAST/CLSI

Method	Ceftriaxone disc	Zone diameter	MIC
EUCAST	30 µg	< 23 mm	> 1 mg/L
CLSI	30 µg	< 23 mm	> 1 mg/L

CDS

Method	Ceftriaxone disc	Annular radius	MIC
CDS	5 µg	< 6 mm	> 1 mg/L

Phenotypic Tests

Ceftriaxone-nonsusceptible isolates can be screened for ESBL/AmpC activity using commercially available discs (for example ESBL/AmpC discs; MAST, ROSCO).

Confirmation

Molecular methods are required to determine the ESBL (SHV, TEM, CTX-M types) and plasmid-mediated AmpC genes (CMY, DHA, FOX, MOX, EBC, ACC).[73-77]

Notes:

1. Some PCR assays do not discriminate between genes for narrow spectrum β-lactamases (TEM-1/2, SHV-1, SHV-11) and those that encode ESBLs.
2. Ceftriaxone-nonsusceptible isolates with no ESBL or pAmpC genes detected should be tested for possible carbapenemases (refer Section 2.3).

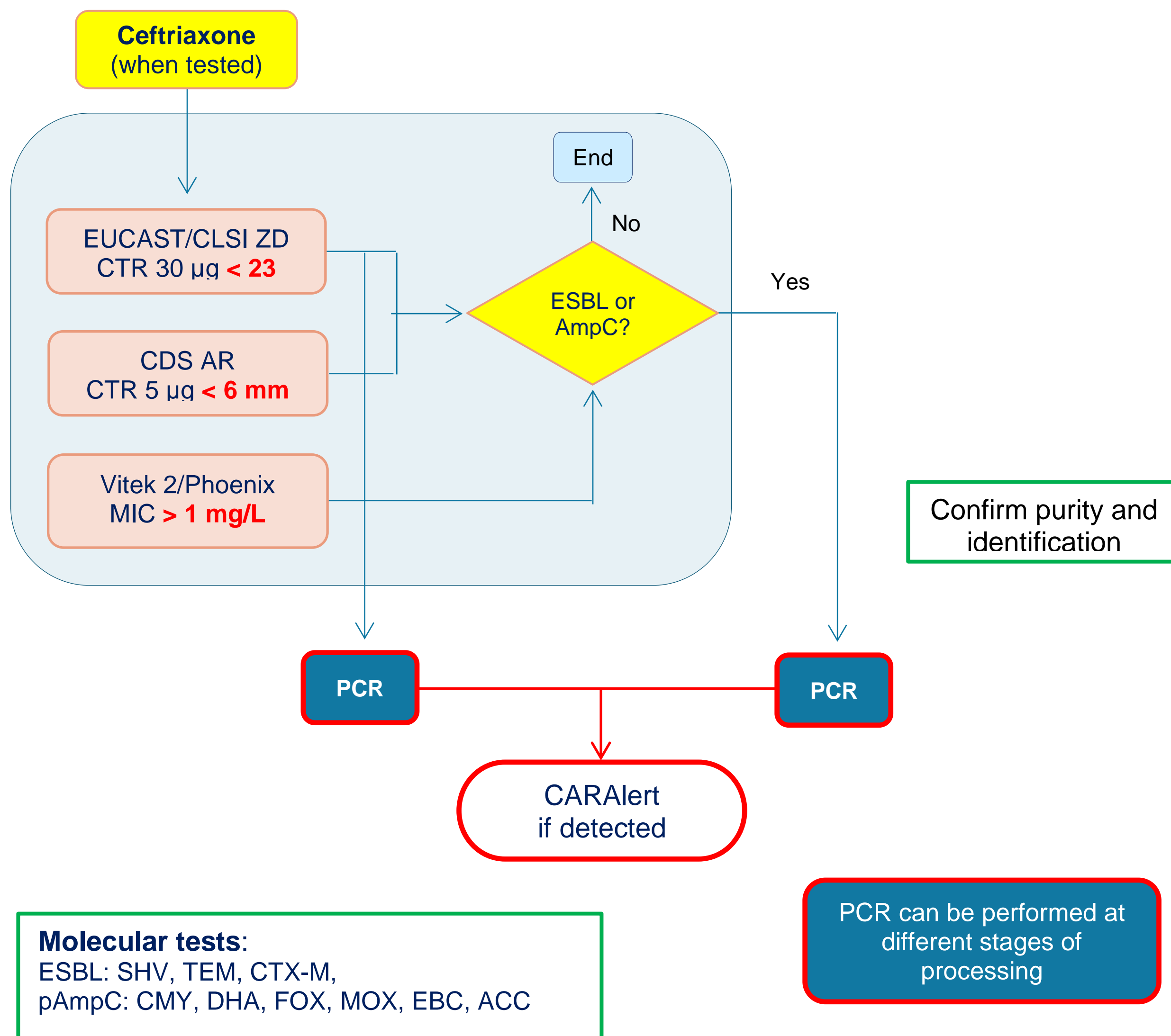
CARAlert Notification

Salmonella spp. (including *S.* Typhi / *S.* Paratyphi) with ceftriaxone MIC > 1 mg/L with SHV, TEM, CTX-M or plasmid-mediated AmpC types detected.

Type: ESBL, AmpC

Subtype: CMY, CTX-M, SHV

Figure 7: Ceftriaxone-nonsusceptible *Salmonella* spp. flowchart



Molecular tests:
ESBL: SHV, TEM, CTX-M,
pAmpC: CMY, DHA, FOX, MOX, EBC, ACC

PCR can be performed at different stages of processing

pAmpC = plasmid mediated AmpC
AR = annular radius (mm)
ESBL = extended-spectrum β-lactamase
MIC = minimum inhibitory concentration
ZD = zone diameter (mm)

Note:

Some PCR assays do not discriminate between genes for narrow spectrum β-lactamases (TEM-1/2, SHV-1, SHV-11) and those that encode ESBLs.

2.14 *Shigella* species – multidrug-resistant

Shigella spp. should have antimicrobial susceptibility tests performed at time of isolation. Reportable agents include ampicillin/amoxicillin, ciprofloxacin/norfloxacin, co-trimoxazole, third generation cephalosporins (ceftriaxone/cefotaxime/ceftazidime). Azithromycin is becoming more commonly used to treat multidrug-resistant (MDR) strains and should be considered for routine testing. CLSI has guidelines for two common species (*S. flexneri* and *S. sonnei*), which are included below in 2.8.1.

Of concern for CARAlert are MDR strains. For this purpose, MDR refers to isolates resistant to three or more of the following agents: ampicillin/amoxicillin, ciprofloxacin/norfloxacin, co-trimoxazole, ceftriaxone/cefotaxime/ceftazidime or azithromycin.

Detection

2.14.1: Susceptibility test criteria for detecting MDR *Shigella* species

EUCAST/CLSI

Method	Agent	Disc	Zone diameter, mm	MIC, mg/L	Comments
EUCAST	Ampicillin	10 µg	< 14	> 8	
	Azithromycin	–*	–*	> 16	ECOFF
	Ceftriaxone or	30 µg	< 22	> 2	
	Cefotaxime or	5 µg	< 17	> 2	
	Ceftazidime	10 µg	< 19	> 4	
	Ciprofloxacin	5 µg	< 22	> 0.5	
	norfloxacin (urine isolates only)	10 µg	< 19	> 1	
	Co-trimoxazole	1.25–23.75	< 13	> 4/76	
CLSI	Ampicillin	10 µg	≤ 13	≥ 32	
	Ciprofloxacin	5 µg	≤ 21	≥ 1	
	Co-trimoxazole	1.25–23.75	≤ 10	≥ 4/7L	
	Ceftriaxone or	30 µg	≤ 19	≥ 4	
	Cefotaxime or	30 µg	≤ 22	≥ 4	
	Ceftazidime	30 µg	≤ 17	≥ 16	
	Azithromycin (*S. flexneri*)	15 µg	≤15	≥ 16	
	Azithromycin (*S. sonnei*)	15 µg	–*	≥ 32	

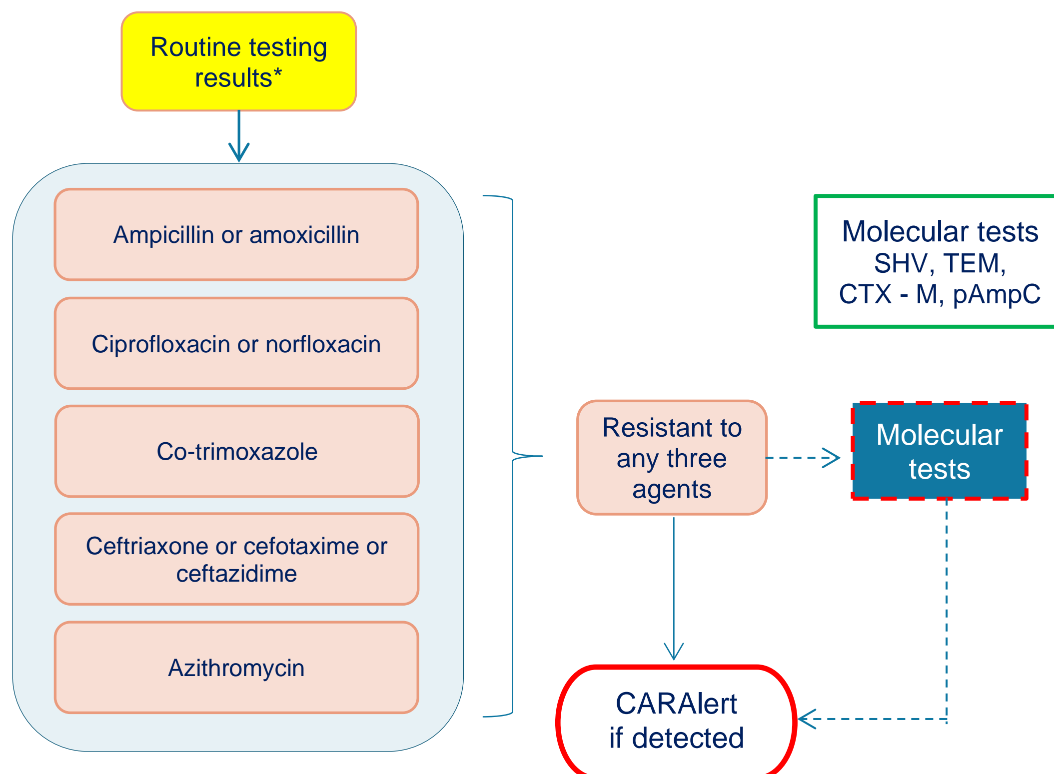
* At this time is has not been possible to establish a zone diameter cut-off due to methodological issues

CDS

Method	Agent	Disc	Annular radius, mm	MIC, mg/L	Comments
CDS	Ampicillin	25 µg	< 6	> 8	
	Azithromycin	–	–	–	
	Ceftriaxone/cefotaxime or	5 µg	< 6	> 1	
	Ceftazidime	10 µg	< 6	> 4	
	Ciprofloxacin	2.5 µg	< 6	> 1	
	norfloxacin (urine isolates only)	10 µg	< 6	> 4	
	Co-trimoxazole	25 µg	< 6	> 1/19	

–, not calibrated

Figure 8: MDR *Shigella* spp. flowchart



* Not all agents may have been tested

Note: Include if plasmid-borne AmpC enzymes detected.

CONFIRMATION

Molecular methods are recommended (but not required for notification) to determine the β-lactamase gene (ESBL [SHV, TEM, CTX-M types], or plasmid-mediated AmpC [CMY, DHA, FOX, MOX, EBC, ACC]).

CARAlert Notification

Shigella species resistant to any three of ampicillin/amoxicillin, azithromycin, ceftriaxone/cefotaxime/ceftazidime, ciprofloxacin/norfloxacin, or co-trimoxazole.

Type: If ceftriaxone-resistant: ESBL, AmpC, ESBL/AmpC not detected

If ceftriaxone-susceptible: Not determined

Subtype (optional): gene(s) detected (e.g., CTX-M Group 1, CTX-M-15, CMY-2)

Note: Notify if plasmid-borne AmpC enzymes are confirmed.

2.15 *Staphylococcus aureus* complex – linezolid-nonsusceptible

Linezolid is also considered as an alternative agent to treat patients with severe *S. aureus* infection when the vancomycin MIC is > 2 mg/L or if therapeutic failure is suspected, and sometimes for follow-on oral treatment.

Linezolid-nonsusceptibility may be due to one (or more) resistance mechanisms: 23S rRNA mutations, *cfr*-, or *optrA*-mediated.[51, 53, 78]

Note: For CARAlert *S. aureus* complex includes *S. aureus*, *S. argenteus* and *S. schweitzeri*.

Detection

2.15.1: Susceptibility test criteria for detecting linezolid-nonsusceptible *Staphylococcus aureus* complex

EUCAST/CLSI

Method *	Agent	Disc	Zone diameter	MIC
EUCAST	Linezolid	10 µg	< 19 mm	> 4 mg/L
CLSI	Linezolid	30 µg	≤ 20 mm	> 4 mg/L

* Mueller-Hinton agar

CDS

Method *	Agent	Disc	Annular radius	MIC
CDS	Linezolid	10 µg	< 6 mm	> 4 mg/L

* Sensitest agar, air, 35–37°C

Confirmation

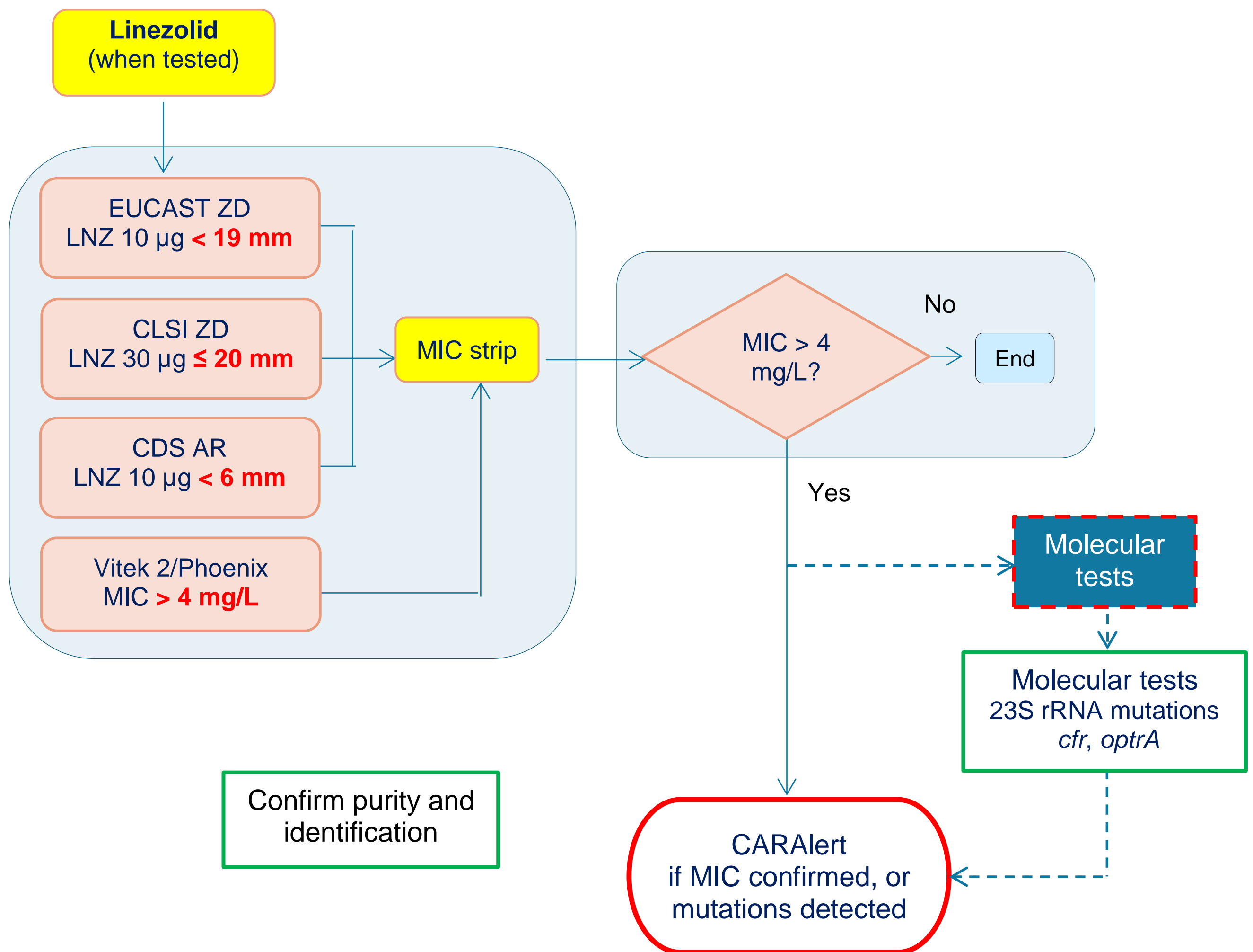
S. aureus, *S. argenteus* or *S. schweitzeri* isolates that test as linezolid-nonsusceptible by Vitek 2/Phoenix should have the MIC determined using either a linezolid MIC strip or by broth microdilution. Molecular methods can be used to detect the resistance mechanisms involved.

CARAlert Notification

S. aureus, *S. argenteus* or *S. schweitzeri* with linezolid MIC > 4 mg/L confirmed (broth microdilution or MIC strip), regardless of whether mutations in 23S rRNA or methyltransferases (*cfr* or *optrA*) were detected.

Type: Linezolid

Figure 9: Linezolid-nonsusceptible *Staphylococcus aureus* complex flowchart



AR = annular radius (mm)
LNZ = linezolid
MIC = minimum inhibitory concentration
ZD = zone diameter (mm)

2.16 *Staphylococcus aureus* complex – vancomycin-nonsusceptible

In recent years, vancomycin breakpoints for *Staphylococcus* spp. have been lowered. However, there are important differences between the mechanism of resistance in *vanA*-mediated high-level vancomycin-resistant *S. aureus* (VRSA) and non-*van*A mediated low-level resistant isolates.[79] The terms vancomycin-intermediate *S. aureus* (VISA) and heteroresistant vancomycin-intermediate *S. aureus* (hVISA) have been applied to isolates with non-*vanA*-mediated low-level resistance to vancomycin.

For both VISA and hVISA isolates the resistance is endogenous (i.e., chromosomal mutations) and the mechanism is highly complex, with no single genetic change being responsible. The VISA/hVISA phenotype has been linked to a thickening of the bacterial cell wall, with hyperproduction of glycopeptide binding targets. The hVISA phenotype is often unstable in the laboratory, but hVISAs have the capacity to develop into VISA *in vivo*.[79]

For the purpose of this Handbook, the following definitions are used:

Phenotype	Definition
VRSA: vancomycin-resistant *S. aureus*:	*S. aureus* isolates with high-level resistance to vancomycin (MIC > 8 mg/L)
VISA: vancomycin-intermediate *S. aureus*	*S. aureus* isolates with low-level resistance to vancomycin (MIC > 2 – 8 mg/L)
hVISA: Heterogeneous vancomycin-intermediate *S. aureus*	*S. aureus* isolates susceptible to vancomycin (MICs ≤ 2 mg/L) but with minority populations (1 in 10^6 cells) with vancomycin MIC > 2 mg/L, as judged by population analysis profile investigation

Note: For CARAlert *S. aureus* complex includes *S. aureus*, *S. argenteus* and *S. schweitzeri*.

DETECTION

Notes:

1. MIC strips will generally produce values about one half-step dilution higher than those obtained using broth micro dilution (BMD). For example, a BMD MIC of 1 mg/L will often yield an MIC strip value of 1.5 mg/L.[80-82]
2. Commercial MIC systems also tend to either under call (Vitek 2) or overcall (Phoenix) vancomycin resistance.[83] *S. aureus* with vancomycin MIC > 2 mg/L obtained by the Vitek 2 or Phoenix MIC systems must have identification confirmed and MIC determined using a MIC strip.
3. Vancomycin discs cannot be used for susceptibility tests on *S. aureus* isolates in the EUCAST and CLSI systems.

2.16.1: Susceptibility test criteria for detecting vancomycin-nonsusceptible *Staphylococcus aureus* complex

EUCAST/CLSI

Method*	Agent	Disc	Zone diameter	MIC	Comments
EUCAST	Vancomycin	–†	–	> 2 mg/L	MIC strips: > 3 mg/L
CLSI	Vancomycin	–†	–	> 2 mg/L	MIC strips: > 3 mg/L

* Mueller-Hinton agar
† MIC method only

CDS

Method*	Agent	Disc	Annular radius	MIC	Comments
CDS	Vancomycin	5 µg	< 2 mm	> 4 mg/L	

* Sensitest agar, air, 35–37ºC

Macro Gradient Test[84]

A gradient strip with both vancomycin (VA) and teicoplanin (TP) is placed onto Brain Heart Infusion agar, inoculated with 100 µl 2.0 McFarland inoculum. As no discrimination can be made between hVISA/VISA, a standard vancomycin MIC must be performed. Increasing the inoculum to 200 µl 2.0 McFarland inoculum has been shown to increase the sensitivity and specificity of this method.[85]

Phenotype interpretation: after 48 h incubation at 35 ± 2ºC
VISA/hVISA: VA ≥ 8 mg/L *and* TP ≥ 8 mg/L, or TP ≥ 12 mg/L (alone)

GRD Gradient Test[86]

A MIC strip with both vancomycin (VA) and teicoplanin (TP) is placed onto Mueller-Hinton agar supplemented with 5% blood, inoculated with a 0.5 McFarland inoculum.

Phenotype interpretation: after 48 h incubation at 35 ± 2ºC
hVISA: VA or TP ≥ 8 mg/L *and* standard VA MIC ≤ 2 mg/L
VISA: VA or TP ≥ 8 mg/L *and* standard VA MIC > 2 mg/L

CONFIRMATION

Vancomycin non-susceptibility

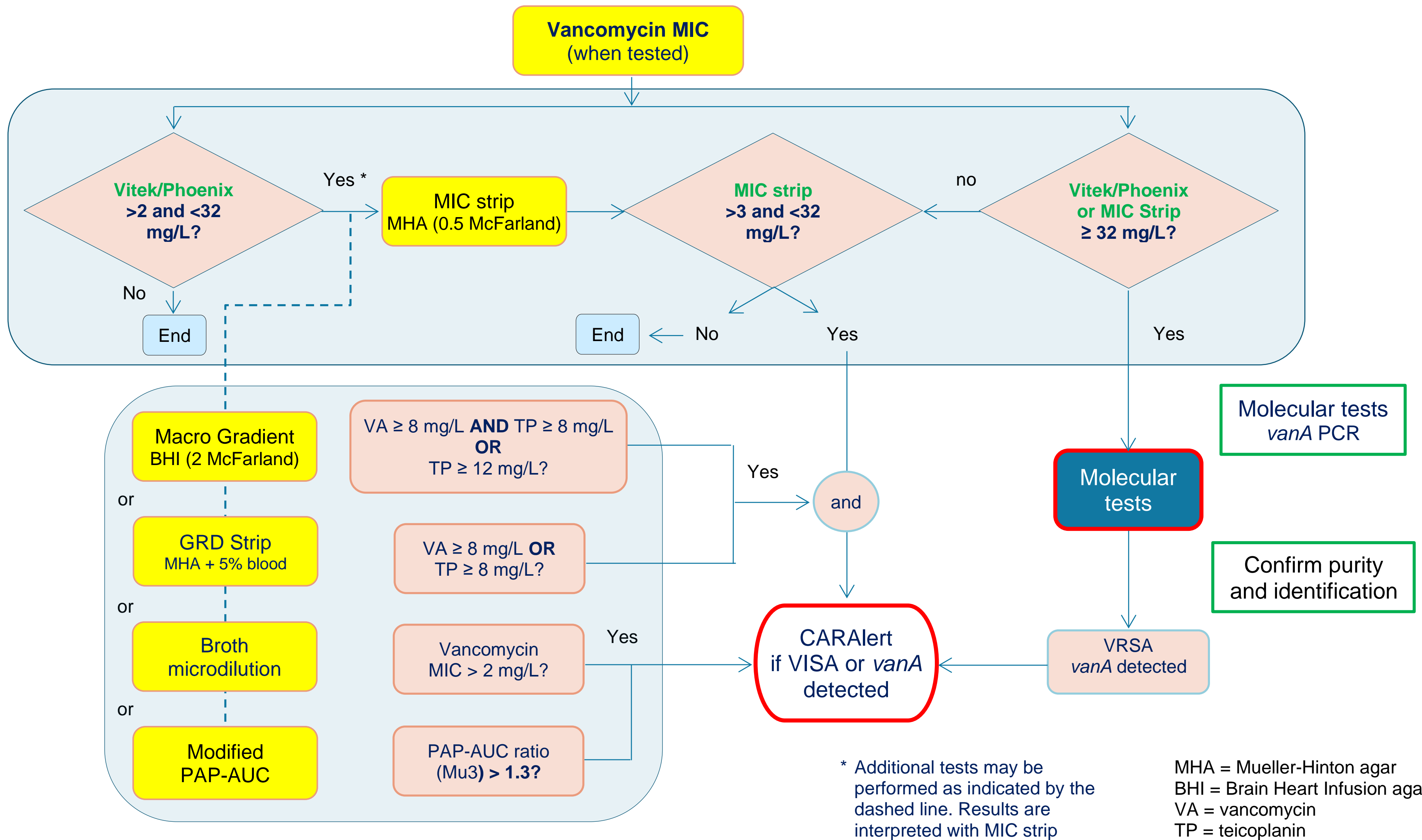
With vancomycin-resistant *S. aureus* (VRSA) the resistance is mediated by the *vanA* gene exogenously acquired from enterococci. This can be detected by PCR or WGS.

CARAlert Notification

S. aureus with vancomycin MIC > 2 mg/L by broth microdilution, > 3 mg/L by standard MIC strip, GRD Strip indicative of VISA; or *vanA* gene detected (VRSA). ***Notification of hVISA strains is not required for CARAlert.***

Type: Vancomycin

Figure 10: Vancomycin-nonsusceptible *Staphylococcus aureus* complex flowchart



* Additional tests may be performed as indicated by the dashed line. Results are interpreted with MIC strip

MHA = Mueller-Hinton agar
BHI = Brain Heart Infusion agar
VA = vancomycin
TP = teicoplanin

2.17 *Streptococcus pyogenes* – reduced penicillin susceptibility

S. pyogenes should be routinely tested for benzylpenicillin susceptibility by disc diffusion methods. To date, no *S. pyogenes* isolates have been confirmed anywhere in the world with elevated penicillin MIC.

Resistance Mechanism

Not known.

Detection

2.17.1: Susceptibility test criteria for detecting *Streptococcus pyogenes* with reduced penicillin susceptibility

EUCAST/CLSI

Method	Penicillin disc	Zone diameter	MIC
EUCAST	1 U	< 18 mm	> 0.25 mg/L
CLSI	10 U	< 23 mm	> 0.125 mg/L

CDS

Method	Penicillin disc	Annular radius	MIC
CDS	0.5 U	< 6 mm	> 0.125 mg/L

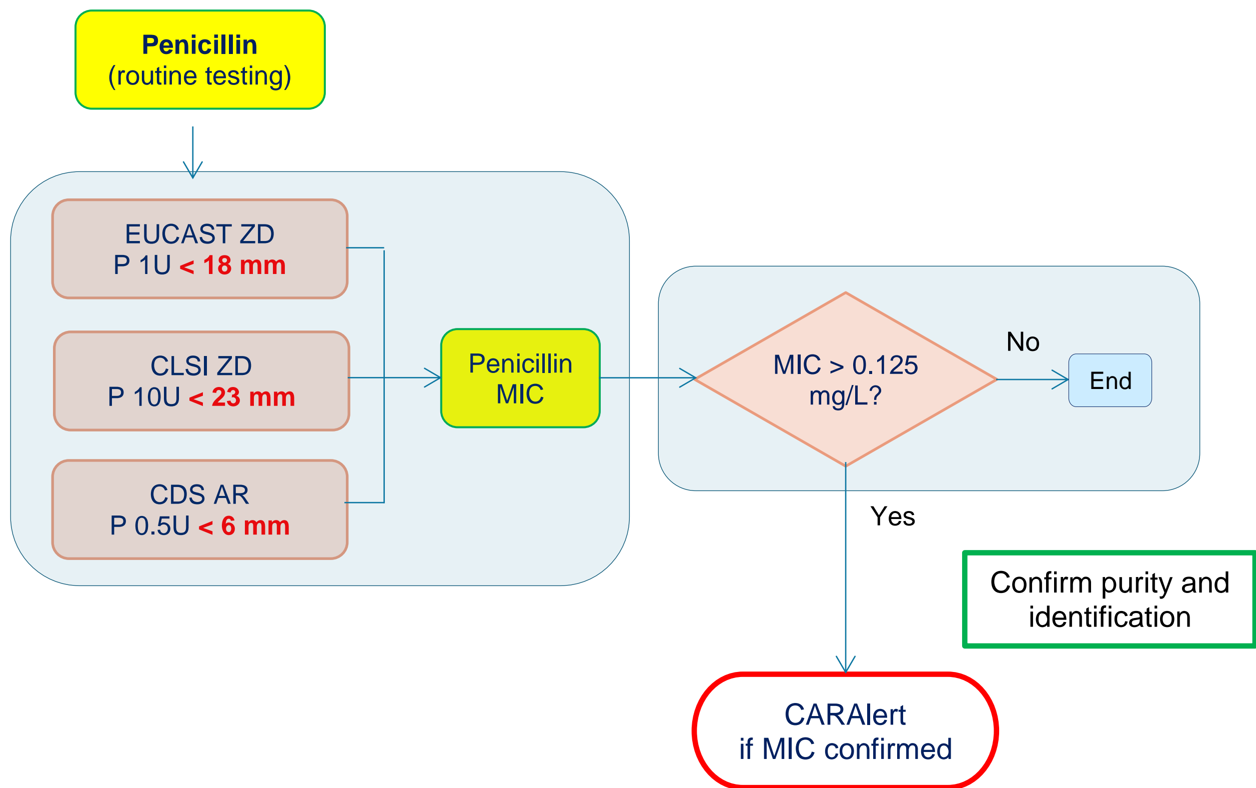
Confirmation

Isolates with MIC values above the breakpoint have not yet been reported. Any β-haemolytic streptococcus found to be non-susceptible should be re-identified, and a penicillin MIC determined. Non-susceptible isolates confirmed in the originating laboratory should be sent to a *S. aureus* vancomycin MIC confirming laboratory (see Appendix 2) to ensure reproducibility.

CARAlert Notification

S. pyogenes with penicillin MIC > 0.125 mg/L confirmed.

Figure 11: Penicillin-nonsusceptible *Streptococcus pyogenes* flowchart



AR = annular radius (mm)
MIC = minimum inhibitory concentration
ZD = zone diameter (mm)

Section 3: Considerations before submission of a CAR

To improve the accuracy of the CARAlert data, the following situations should be considered before the submission of a new CAR.

3.1. Duplicate records

The CARAlert web portal will indicate if the new submission is a possible duplicate record if the following fields are the same:

- Confirming lab name
- Specimen identifier
- CAR
- Type
- Organism name

Patient demographic data

- Age range
- Gender
- State or territory of patent residence

All information should be carefully checked before submitting a possible duplicate CAR.

3.2. Multiple samples from the same patient

1. The **same CAR/type/species** should **not** be entered where the sample originated from the same patient who had the previous CAR, and the isolate was:
    a. collected on the same day; or
    b. collected in the same admission or within three months
2. **Different** CARs from the same patient should always be entered, regardless of time between collection.

Note: Isolates from a clinical specimen take preference to those from a screen. All blood isolates should be submitted, unless a previous blood isolate meeting the criteria above has already been entered into CARAlert.

3.3. Multiple organisms with the same CAR

If the same CAR/Type was detected in multiple organisms from the same patient, then all should be submitted.

3.4. Additional information about a submitted CAR

If additional information becomes available from an isolate that has already been submitted, please contact the Commission via CARAlert@safetyandquality.gov.au to advise the details. The Commission will arrange for the CARAlert web portal to be updated.

Do not add another record with the additional CAR.

Review processes

The list of CARs reported to CARAlert and relevant resources, including this Handbook will be regularly reviewed, and updated as appropriate by the Commission. The most recent review occurred in 2022. More information about reviews of CARAlert is included in Appendix 1.

Abbreviations and terminology

Acronym	Definition
AGAR	Australian Group on Antimicrobial Resistance
AGSP	Australian Gonococcal Surveillance Programme
AMR	Antimicrobial resistance
AMRLN	Australian Mycobacterial Reference Laboratory Network
AR	Annular radius
ATCC	American Type Culture Collection
AU	Antimicrobial use
AURA	Antimicrobial Use and Resistance in Australia
BMD	Broth micro dilution
CAR	Critical antimicrobial resistance
CDS	Calibrated Dichotomous Sensitivity Method
CDT	Combined disc tests
CIM	Carbapenem inhibition method
Clinical isolate	Specimen taken to guide clinical diagnosis (blood, urine, wound, or other)
CLSI	Clinical and Laboratory Standards Institute
Commission	Australian Commission on Safety and Quality in Health Care
CPE	Carbapenemase-producing *Enterobacterales*
CPO	Carbapenemase-producing organisms
DDST	Double disc synergy tests
ECOFF	Epidemiological cut-off value
ESBL	Extended-spectrum β-lactamase
ETP	Ertapenem
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GRD	Glycopeptide resistance detection
hVISA	Heterogeneous vancomycin-intermediate *Staphylococcus aureus* *S. aureus* isolates susceptible to vancomycin (MICs ≤ 2mg/L) but with minority populations (1 in 10^6 cells) with vancomycin MIC > 2 mg/L, as judged by population analysis profile investigation
IATA	International Air Transport Association
LNZ	Linezolid
MALDI-TOF MS	Matrix-assisted laser desorption ionization – time of flight mass spectrometry
MBL	Metallo-β-lactamase
MCR	Mobile colistin resistance
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration

Acronym	Definition
MTB	*Mycobacterium tuberculosis* complex
NATA	National Association of Testing
NNN	National Neisseria Network
NPAAC	National Pathology Accreditation Advisory Council
PAP-AUC	Population analysis profiling with area under the curve analysis
PBP2	Penicillin-binding protein 2
PCR	Polymerase chain reaction
PHLN	Public Health Laboratory Network
PTZ	Piperacillin-tazobactam
QAP	Quality assurance program
RCPA	Royal College of Pathologists Australia
RFLP-PCR	Restriction fragment length polymorphism polymerase chain reaction
RMT	ribosomal methyltransferase
Screen	Any specimen taken for infection prevention and control purposes
SNPs	Single nucleotide polymorphisms
TP	Teicoplanin
VA	Vancomycin
VISA	Vancomycin-intermediate *S. aureus* *S. aureus* isolates with low-level resistance to vancomycin (MIC > 2 – 8 mg/L)
VRSA	Vancomycin-resistant *S. aureus* *S. aureus* isolates with high-level resistance to vancomycin (MIC > 8 mg/L)
WGS	Whole genome sequencing

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Appendix 1: About CARAlert

Why was CARAlert established?

The National Alert System for Critical Antimicrobial Resistances (CARAlert) was established by the Australian Commission on Safety and Quality in Health Care (the Commission) to provide timely advice to state and territory health authorities on the occurrence of critical antimicrobial resistances (CARs) in their hospitals and communities, to provide a national picture of selected CARs and to standardised guidance on processes for confirming CARs.

Although some data on CARs are captured through local surveillance programs where they exist, CARAlert is the only nationally coordinated system that supports both collection and communication of information on confirmed CARs and potential CAR outbreaks, as close as possible to the time of confirmation.

How does CARAlert work?

The CARAlert system is based on routine processes used by pathology laboratories for identifying and confirming potential CARs:

- Collection and routine testing – the isolate is collected from the patient and sent to the originating laboratory for routine testing
- Confirmation – if the originating laboratory suspects that the isolate is a CAR, the isolate is sent to a confirming laboratory that has the capacity to confirm the CAR
- Reporting to clinicians in accordance with usual laboratory processes – the confirming laboratory reports back to the originating laboratory, which in turn reports to the clinician who initially requested the microbiological testing
- Submission to the CARAlert system – the confirming laboratory advises the originating laboratory of the result of the test, and the originating laboratory reports back to the health service that cared for the patient from whom the specimen was collected; the confirming laboratory then submits the details of the resistance and organism into the secure CARAlert web portal.

Public and private pathology laboratories that have the capacity to confirm CARs were identified through consultation with state and territory health authorities, the Public Health Laboratory Network (PHLN) and the Australian Group on Antimicrobial Resistance (AGAR).

The CARAlert system generates a weekly summary email alert to report information on confirmed CARs to state and territory health authorities, the Australian Government Department of Health and Aged Care (the Department) and the confirming laboratories. The information collected through CARAlert allows health service providers, laboratories, public health units and policymakers at local, state and territory, and national levels to receive timely reports and analyses of national data, which complement current local reporting to the providers of patient care. In addition, regular reports, with analyses of data on CARs, are published on the Commission's website.

Since October 2016, secure access to the CARAlert system has enabled designated state and territory health authorities to view records for their own jurisdiction at any time, including the name of the public hospital where the patient who had the infection was being cared for at the time the specimen was collected. This enables timely monitoring of the geographic distribution of CARs, and liaison with hospitals, as appropriate, to confirm that infection prevention and control action has been taken in the event of an outbreak. These authorities can also generate their own reports from CARAlert. Over time, the data will become increasingly useful to inform a broader range of safety and quality improvement programs. No patient level data is held by the CARAlert system.

It is intended that states and territories will use this data to identify local issues, and respond to potential and proven multi-site outbreaks of CARs. Primary responsibility for clinical response to CARs lies with local health organisations, and state and territory health departments. Some states have made carbapenemase-producing *Enterobacterales* (CPE) notifiable, and others have implemented local surveillance of CPE. CARAlert complements this local data.

How was the list of CARs reported to CARAlert determined?

The organisms reported under CARAlert are drawn from the list of high-priority organisms and antimicrobials that are the focus of the Antimicrobial Use and Resistance in Australia (AURA) Surveillance System. The scope of organisms and CARs are regularly reviewed, based on the latest evidence on CARs that emerge in Australia and overseas.

Reviews of CARs reported to CARAlert were conducted in 2016, 2018 and 2022. No changes were made after the 2016 review, which also considered operational processes and notifications.

The 2018 review considered CARs, and laboratory reporting processes including capacity to confirm and submit CARs. Four additional CAR were added – transferrable colistin resistance in *Enterobacterales*, carbapenemase-producing *Acinetobacter baumannii* complex, carbapenemase-producing *Pseudomonas aeruginosa* and *Candida auris*.

In response to the 2022 review, two CARs were added to CARAlert – ciprofloxacin-nonsusceptible *Neisseria meningiditis* and gentamicin-resistant *N. gonorrhoeae*. Reporting of daptomycin-nonsusceptible *Staphylococcus aureus* was suspended and will be considered for reintroduction when more reliable phenotypic testing methods are available.

Appendix 2: CAR Designated Confirming Laboratories

State or Territory	Confirming laboratory / CAR	*Acinetobacter baumannii* complex	*Candida auris*	*Enterobacterales*			*Enterococcus* species	*Pseudomonas aeruginosa*
		Carbapenemase -producing (molecular)	Identification (MALDI-ToF)	Carbapenemase -producing (molecular)	Ribosomal methylase -producing (molecular)	Transferrable resistance to colistin* (molecular)	Linezolid -resistant (molecular)	Carbapenemase -producing (molecular)
ACT	ACT Pathology	Yes	Yes	Yes	–	–	–	Yes
NSW	NSWHP (Concord Hospital)	Yes	Yes	Yes	–	–	–	Yes
	NSWHP (Liverpool Hospital)	Yes	Yes	Yes	–	–	–	Yes
	NSWHP (John Hunter Hospital)	–	Yes	Yes	–	–	–	–
	NSWHP (Royal North Shore Hospital)	Yes	Yes	Yes	–	–	–	Yes
	NSWHP (Royal Prince Alfred Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	NSWHP (St George Hospital)	Yes	Yes	Yes	Yes	Yes	–	Yes
	NSWHP (The Prince of Wales Hospital)	–	Yes	–	–	–	–	–
	NSWHP (Westmead Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	St Vincent's Pathology (SydPath)	Yes	Yes	Yes	–	–	–	Yes
NT	Territory Pathology (Royal Darwin Hospital)	Yes	Yes	Yes	–	–	–	Yes
Qld	Pathology QLD (Central Laboratory†)	Yes	Yes	Yes	Yes	–	–	Yes
	Pathology QLD (Forensic & Scientific Services)	–	–	–	–	–	–	–
	QML Pathology	–	Yes	–	–	–	–	–
	Sullivan Nicolaides Pathology	Yes	Yes	Yes	–	–	–	Yes
SA	SA Pathology (Royal Adelaide Hospital)	–	Yes	Yes	Yes	–	–	–
Tas	Royal Hobart Hospital	Yes	Yes	Yes	–	–	–	Yes
Vic	Alfred Pathology Service	–	Yes	–	–	–	–	–
	Dorevitch Pathology	–	Yes	–	–	–	–	–
	MDU Public Health Laboratory	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Victorian Infectious Diseases Reference Laboratory	–	–	–	–	–	–	–
	Melbourne Pathology	–	Yes	–	–	–	–	–
	Monash Pathology	–	Yes	–	–	–	–	–
WA	PathWest (Fiona Stanley Hospital)	–	Yes	–	–	–	Yes	–
	PathWest (QEII Medical Centre)	Yes	Yes	Yes	Yes	Yes	–	Yes

– = not able to confirm CAR; MALDI-TOF = matrix-assisted laser desorption ionization – time of flight mass spectrometry; Yes = able to confirm CAR

* MCR variants other than *mcr-9*
† Royal Brisbane and Women's Hospital

Appendix 2: CAR Designated Confirming Laboratories (continued)

State or Territory	Confirming laboratory / CAR	*Salmonella* species	*Shigella* species	*Staphylococcus aureus* complex		*Streptococcus pyogenes*	*Mycobacterium tuberculosis*	*Neisseria gonorrhoeae*	*Neisseria meningitidis*
		Ceftriaxone-non susceptible (ESBL/AmpC)	Multidrug-resistant	Vancomycin-non susceptible (MIC)	Linezolid-non susceptible (MIC)	Penicillin reduced susceptibility	Multidrug-resistant	Azithromycin- or ceftriaxone-non susceptible; gentamicin-resistant	Ciprofloxacin-non susceptible
ACT	ACT Pathology	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes
NSW	NSWHP (Concord Hospital)	Yes	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (Liverpool Hospital)	Yes	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (John Hunter Hospital)	–	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (Royal North Shore Hospital)	Yes	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (Royal Prince Alfred Hospital)	–	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (St George Hospital)	Yes	Yes	Yes	Yes	–	–	–	–
	NSWHP (The Prince of Wales Hospital)	–	–	Yes	Yes	Yes	–	Yes	Yes
	NSWHP (Westmead Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	–	–
	St Vincent's Pathology (SydPath)	Yes	Yes	Yes	Yes	Yes	–	–	–
NT	Territory Pathology (Royal Darwin Hospital)	Yes	Yes	–	–	–	–	Yes	Yes
Qld	Pathology QLD (Central Laboratory*)	Yes	Yes	Yes	Yes	Yes	Yes	–	–
	Pathology QLD (Forensic & Scientific Services)	–	–	–	–	–	–	Yes	Yes
	QML Pathology	–	Yes	Yes	Yes	Yes	–	–	–
	Sullivan Nicolaides Pathology	Yes	Yes	Yes	Yes	Yes	–	–	–
SA	SA Pathology (Royal Adelaide Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tas	Royal Hobart Hospital	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes
Vic	Alfred Pathology Service	–	–	Yes	Yes	Yes	–	–	–
	Dorevitch Pathology	–	–	Yes	Yes	Yes	–	–	–
	MDU Public Health Laboratory	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes
	Victorian Infectious Diseases Reference Laboratory	–	–	–	–	–	Yes	–	–
	Melbourne Pathology	–	–	Yes	Yes	Yes	–	–	–
	Monash Pathology	–	–	Yes	Yes	Yes	–	–	–
WA	PathWest (Fiona Stanley Hospital)	–	–	Yes	Yes	Yes	–	Yes	Yes
	PathWest (QEII Medical Centre)	Yes	Yes	Yes	Yes	Yes	Yes	–	–

– = not able to confirm CAR; Yes = able to confirm CAR

* Royal Brisbane and Women's Hospital

AUSTRALIAN COMMISSION ON SAFETY AND QUALITY IN HEALTH CARE

AURA

Appendix 3: CARAlert Isolate Referral Form

This form must accompany the referred isolate

Fields with red text indicate mandatory data.
On completion, print the form and include a copy with the shipment. A copy of the form can be saved for your records.
A copy of the request form may also be included.

Laboratory Details

Name of referring laboratory: Click here to enter text.

Contact details:

Name: Click here to enter text. Email: Click to enter text. Telephone: Click here to enter text.

Date referred: Click to enter a date.

Patient Demographics

Name (optional): First name: Click to enter text. Surname: Click to enter text.

Date of birth: Click enter a date. or age (if DOB not known): Click here to enter text.

Sex: ◯ Female ◯ Male ◯ Unknown

Postcode of residence: Click here to enter text. (Use 8888 if overseas, 3999 if unknown)

Facility type (where specimen collected): ◯ Hospital ◯ Aged care home ◯ Unknown ◯ Other

Hospital name: Click here to enter text.

Isolate Details

Reason for referral (*select from list*): Choose an item.

Specimen identifier: Click to enter text. Organism (*genus and species*): Click to enter text.

Date of collection: Click to enter a date.

Clinical isolate or screen: ◯ Blood ◯ Urine ◯ Wound ◯ Other ◯ Screen

Laboratory Findings

Record as many laboratory test results as possible.

Agent	MIC (mg/L)	Disc diffusion zone/radius (mm)	Agent	MIC (mg/L)	Disc diffusion zone/radius (mm)
Amikacin			Daptomycin		
Ampicillin			Gentamicin		
Azithromycin			Lincomycin		
Ceftriaxone			Meropenem		
Ciprofloxacin			Tobramycin		
Colistin		–	Vancomycin		
Co-trimoxazole					

CIM / Carba NP: ◯ Positive ◯ Negative ◯ Equivocal ◯ Not performed

ESBL: ☐ AmpC: ☐

Comments (include any molecular results): Click here to enter text.

Appendix 4: Screening criteria for CARs by susceptibility test method

A4.1 European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Organism	Agent	Disc	Zone diameter (mm)	MIC (mg/L)	Possible CAR
Acinetobacter baumannii complex	Meropenem	10 µg	< 15	> 8	CPO
Enterobacterales	Meropenem	10 µg	< 28	> 0.125[*]	CPO
		10 µg	< 28	> 0.25[†]	
Enterobacterales	Amikacin	30 µg	< 15	> 16	Resistant to all three agents
	Gentamicin	10 µg	< 14	> 4	
	Tobramycin	10 µg	< 14	> 4	
Enterococcus species	Linezolid	10 µg	< 19	> 4	Linezolid-NS
Pseudomonas aeruginosa	Meropenem	10 µg	< 24	> 2	CPO
	Piperacillin-tazobactam	30-6 µg	< 18	> 16	
Salmonella species	Ceftriaxone	30 µg	< 23	> 1	Ceftriaxone-NS
Shigella species	Ampicillin	10 µg	< 14	> 8	Multidrug-resistant strains: Resistant to any three antimicrobial categories
	Azithromycin	na	na	> 16	
	Ciprofloxacin	5 µg	< 22	> 0.5	
	Norfloxacin (urine isolates)	10 µg	< 19	> 1	
	Co-trimoxazole	1.25–23.75	< 13	> 4/76	
	Ceftriaxone or	30 µg	< 22	> 2	
	Cefotaxime or	5 µg	< 17	> 2	
	ceftazidime	10 µg	< 19	> 4	
Staphylococcus aureus complex	Vancomycin[§]	na	na	> 2[#]	Vancomycin-NS
	Linezolid	10 µg	< 19	> 4	Linezolid–NS
Streptococcus pyogenes	Penicillin	1 U	< 18	> 0.25	Penicillin-NS

CPO = carbapenemase-producing organism; na = not applicable; NS = non-susceptible

* Card limitation (Vitek 2 AST-N246, AST-N247, AST-N435) meropenem range is 0.25–16 mg/L.
† *Citrobacter, Serratia, Proteus, Morganella, Providencia*
§ MIC method only
# MIC strips: > 3 mg/L

A4.2 Clinical and Laboratory Standards Institute (CLSI)

Organism	Agent	Disc	Zone diameter, mm	MIC, mg/L	Possible CAR
Acinetobacter baumannii complex	Meropenem	10 µg	≤ 14	> 2	CPO
Enterobacterales	Meropenem	10 µg	< 28	>0.125*	CPO
		10 µg	< 28	>0.25[†]	
Enterobacterales	Amikacin	30 µg	≤ 14	≥ 64	Resistant to all three agents
	Gentamicin	10 µg	≤ 12	≥ 16	
	Tobramycin	10 µg	≤ 12	≥ 16	
Enterococcus species	Linezolid	30 µg	≤ 20	≥ 8	Linezolid-NS
Pseudomonas aeruginosa	Meropenem	10 µg	< 19	> 2	CPO
	Piperacillin-tazobactam	100/10 µg	< 21	> 16	
Salmonella species	Ceftriaxone	30 µg	< 23	> 1	Ceftriaxone-NS
Shigella species	Azithromycin *S. flexneri*	15 µg	≤ 15	≥ 16	Multidrug-resistant strains: Resistant to any three antimicrobial categories
	S. sonnei	15 µg	na	≥ 32	
	Ampicillin	10 µg	≤ 13	≥ 32	
	Ciprofloxacin	5 µg	≤ 21	≥ 1	
	Co-trimoxazole	1.25–23.75	≤ 10	≥ 4/76	
	Ceftriaxone or	30 µg	≤ 19	≥ 4	
	Cefotaxime or	30 µg	≤ 22	≥ 4	
	Ceftazidime	30 µg	≤ 17	≥ 16	
Staphylococcus aureus complex	Vancomycin[§]	–[#]	–[#]	> 2**	Vancomycin-NS
	Linezolid	30 µg	≤ 20	> 4	Linezolid-NS
Streptococcus pyogenes	Penicillin	10 U	< 23	> 0.125	Penicillin-NS

CPO = carbapenemase-producing organism; na = not applicable; NS = non-susceptible

* Card limitation (Vitek 2 AST-N246, AST-N247, AST-N435); meropenem range is 0.25–16 mg/L.
† *Citrobacter, Serratia, Proteus, Morganella, Providencia*
§ MIC method only
\# Not calibrated
** MIC strips: > 3 mg/L

A4.3 Calibrated Dichotomous Sensitivity Method (CDS)

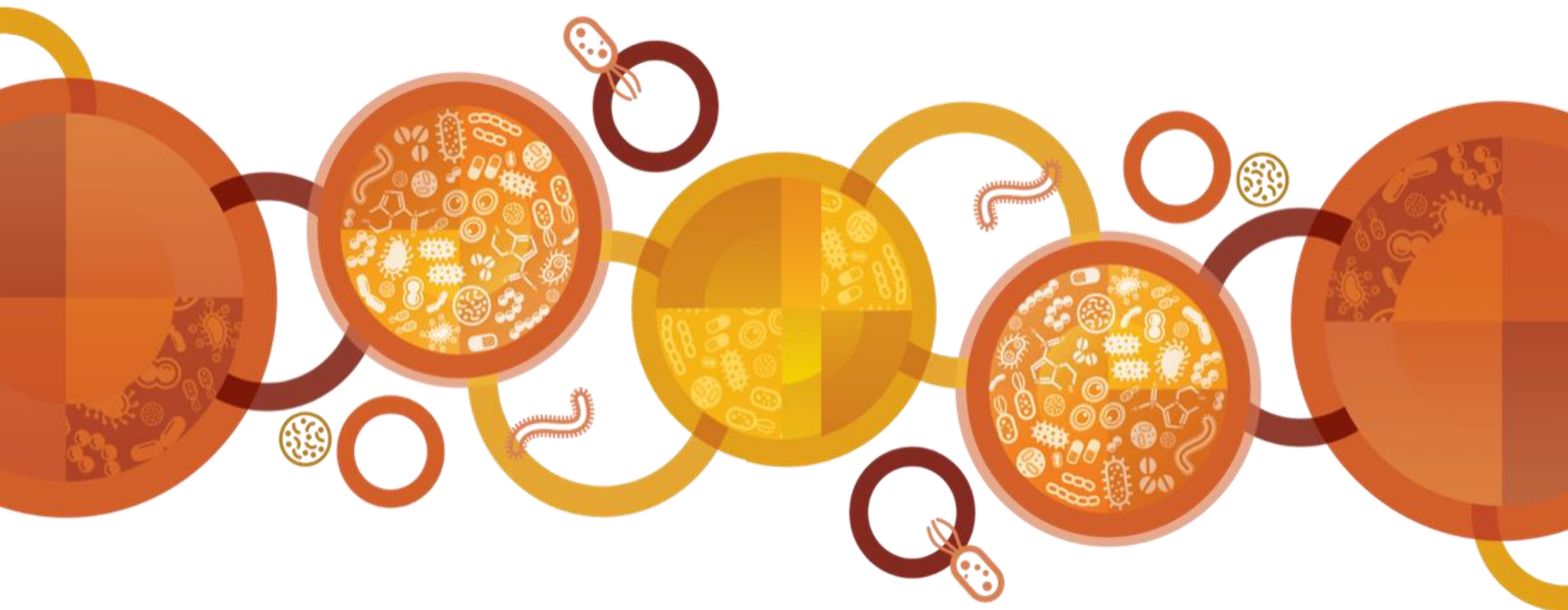
Organism	Agent	Disc	Annular radius, mm	MIC, mg/L	Possible CAR
Acinetobacter baumannii complex	Meropenem	5 µg	< 6	> 2	CPO
Enterobacterales	Meropenem	5 µg	< 6	> 0.125	CPO
Enterobacterales	Amikacin	30 µg	< 4	> 16	Resistant to all three agents
	Gentamicin	10 µg	< 4	> 2	
	Tobramycin	10 µg	< 4	> 2	
Enterococcus species*	Linezolid	10 µg	< 6	> 4	Linezolid-NS
Pseudomonas aeruginosa	Meropenem	5 µg	< 6	> 2	CPO
	Piperacillin-tazobactam	50/5 µg	< 6	> 16	
Salmonella species	Ceftriaxone	5 µg	< 6	> 1	Ceftriaxone-NS
Shigella species	Azithromycin	–†	–†	–†	Multidrug-resistant strains: Resistant to any three antimicrobial categories
	Ampicillin	25 µg	< 6	> 8	
	Ciprofloxacin	2.5 µg	< 6	> 1	
	Norfloxacin (urine isolates)	10 µg	< 6	> 4	
	Co-trimoxazole	25 µg	< 6	> 1/19	
	Ceftriaxone or cefotaxime or ceftazidime	5 µg	< 6	> 1	
Staphylococcus aureus complex	Vancomycin	5 µg	< 2	> 4	Vancomycin-NS
	Linezolid	10 µg	< 6	> 4	Linezolid-NS
Streptococcus pyogenes	Penicillin	0.5 U	< 6	> 0.125	Penicillin-NS

CPO = carbapenemase-producing organism; na = not applicable; NS = non-susceptible

* Blood Sensitest agar, CO_2, 35–37ºC
† Not calibrated

Appendix 5: Tests performed at confirming laboratories

Organism	CAR	Confirmed MIC	CARAlert	Molecular targets
Acinetobacter baumannii complex	Carbapenemase-producing		Molecular confirmation	OXA-23 like, -24, 58 IMP, VIM, KPC, NDM
Candida auris	Identification		MALDI-TOF confirmed	
Enterobacterales	Carbapenemase-producing		Molecular confirmation	IMP-, VIM-, KPC-, NDM-, and OXA-48-like types (investigate for other types if top five not detected)
Enterobacterales	16S rRNA methyltransferases		Molecular confirmation	At least *armA, rmtA, rmtB, rmtC, rmtF.* Other types (*rmtD, rmtE, rmtG, rmtH, npmA)* optional
Enterobacterales	Colistin mobile resistance		Molecular confirmation	*MCR*
Enterococcus spp.	Linezolid-nonsusceptible	MIC > 4 mg/L	Molecular confirmation	Mutations in 23S rRNA (especially G2576T)
Mycobacterium tuberculosis	Rifampicin-resistant plus at least isoniazid-resistant		Molecular confirmation	*rpoB* mutations
Neisseria gonorrhoeae	Azithromycin-nonsusceptible	MIC ≥ 1 mg/L MIC ≥ 256 mg/L	**Low-level** MIC confirmed between 1 and 256 mg/L **High-level**: MIC ≥ 256 mg/L	
	Ceftriaxone-nonsusceptible	MIC ≥ 0.125 mg/L	MIC confirmed	Mosaic structure of *penA* encoding PBP2
	Gentamicin-resistant	MIC > 8 mg/L	MIC confirmed	
Neisseria meningitidis	Ciprofloxacin-nonsusceptible	MIC > 0.016 mg/L	MIC confirmed	
Pseudomonas aeruginosa	Carbapenemase-producing		Molecular confirmation	IMP-, VIM-, NDM-, KPC-, GES-types (investigate for other types if top five not detected)
Salmonella spp.	Ceftriaxone-nonsusceptible	MIC > 1 mg/L	ESBL or AmpC phenotype confirmed	SHV, TEM, CTX-M types, plasmid-mediated AmpC
Shigella spp.	Multidrug-resistant		ESBL or AmpC phenotype confirmed	SHV, TEM, CTX-M types, plasmid-mediated AmpC (**optional**)
Staphylococcus aureus complex	Vancomycin-nonsusceptible	MIC > 2 mg/L; (> 3 mg/L MIC strip) Macro Etest/GRD Strip	**VISA**: MIC confirmed and positive Macro Etest/GRD Strip **VRSA**: VanA PCR	Mutations in genes involved in biosysthesis/metabolism of the staphylococcal cell wall (**optional**); *vanA* (if MIC > 8 mg/L)
	Linezolid-nonsusceptible	MIC > 4 mg/L	MIC confirmed	Mutations in 23S rRNA, *cfr* (*optrA* if negative for 23S rRNA and *cfr*)
Streptococcus pyogenes	Penicillin-nonsusceptible	MIC > 0.125 mg/L	MIC confirmed	–



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