Australian Commission on **Safety and Quality** in Health Care

AURA
Antimicrobial Use and Resistance in Australia

CARAlert Laboratory Handbook

September 2026

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The Australian Commission on Safety and Quality in Health Care pays respect to the Gadigal people as the Traditional Custodians of Country where the Commission's office is located. We extend that respect to all Aboriginal and Torres Strait Islander peoples, and their deep time connections to land, water and sky.

We recognise that knowledge about healthy Country, community and culture has been developed by Aboriginal and Torres Strait Islander peoples over tens of thousands of years and has been shared for generations. We are committed to partnering with and learning from Aboriginal and Torres Strait Islander peoples through the work that we do.

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Introduction

Introduction

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 as a component of the Antimicrobial Use and Resistance in Australia (AURA) surveillance program.

Funding for CARAlert is provided by the Australian Centre for Disease Control (CDC), with contributions from the states and territories by meeting the costs of confirmatory testing and data submission processes.

CARAlert collects data on nationally agreed priority organisms with critical resistance to last-line antimicrobials (CARs). More information about CARAlert is included in Appendix 1.

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

This Laboratory 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 processes for epidemiological outbreak investigation and response as they are led by states and territories. CARAlert and other AURA surveillance program data complement local surveillance data in the event of an outbreak of antimicrobial-resistant organisms.

Review processes

The list of CARs reported to CARAlert and relevant resources, including this Laboratory Handbook are regularly reviewed and updated as appropriate by the Commission.

The CARAlert Laboratory Handbook was most recently published in December 2022 and has been reviewed and updated by the Senior Medical Advisor and Scientific Advisor for the Commission's AURA Project.

Table 1 outlines the substantive changes made to the CARAlert Laboratory Handbook on its publication in 2026. These updates are shown by:

- Purple highlight for any changes to a **zone diameter** or **minimum inhibitory concentration** (**MIC) value**
- **Bold blue font** for any substantive changes in wording.

General information about CARAlert and styling have been updated throughout this Handbook for currency but are not marked, including the recent publication of the CARAlert Data Explorer.

Table 1 Version history of the CARAlert Laboratory Handbook, 2026

Page	Section	Update/s
17	Detection of CARs	• Noted that the Calibrated Dichotomous Sensitivity (CDS) method is not supported beyond January 2026; however, CDS methodology has been maintained in this Handbook for laboratories that continue to use this method
18	ACB complex – carbapenemase-producing	• Updated naming for *Acinetobacter-calcoaceticus-baumannii* (ACB) complex • Noted that carbapenem-resistant *A. baumannii* are included in the WHO bacterial priority pathogen critical group list and CDC urgent public health threat • Updated list of intrinsic chromosomal and acquired β-lactamases groups
19	ACB complex – carbapenemase-producing	• Included information provided on the BD Phoenix™ CPO Detect test
20	ACB complex – carbapenemase-producing	• Updated Figure 2
21	*C. auris*	• Updated *Candida auris* to *Candidozyma* (*Candida*) *auris* to reflect updated taxonomy; and throughout • The only established *C. auris*–specific clinical breakpoint is for rezafungin
22	*C. auris*	• Antifungal susceptibility testing is not a requirement for submission to CARAlert
23	*Enterobacterales* – carbapenemase-producing	• Updated ECOFF definition
24	*Enterobacterales* – carbapenemase-producing	• Updated Table 9 (previously Table 2.3.2) • Updated ceftriaxone zone diameter for ESBL phenotype
25	*Enterobacterales* – carbapenemase-producing	• Information provided on BD Phoenix™ CPO Detect test
26	*Enterobacterales* – carbapenemase-producing	• Updated Table 10 (previously Table 2.3.5) • Added the NG Biotech NG-Test® CARBA-5 assay as a confirmation test • Clarified CARAlert Notification when *mcr-9* is detected by WGS
28	*Enterobacterales* – ribosomal methyltransferase-producing	• Updated Table 11 (previously Table 2.4.1) • Updated notes for Table 12 (previously Table 2.4.2)
31	*Enterococcus* species	• Updated resistance mechanisms • Updated Table 13 (previously Table 2.6.1)
32	*Enterococcus* species	• Updated Figure 5
33	*M. tuberculosis*	• Updated rifampicin critical concentration • Defined isoniazid critical concentration
35	*N. gonorrhoeae* – ceftriaxone-nonsusceptible	• Updated Table 15 (previously Table 2.9.1) • Defined extensively drug-resistant (XDR) *N. gonorrhoeae*

Page	Section	Update/s
37	*N. meningitidis* – ciprofloxacin-nonsusceptible	• Added note that confirmation is only for invasive and/or isolates with a serogroup
38	*P. aeruginosa*	• Updated list of GES-ESBL and GES-carbapenemase types • Updated Table 18 (previously Table 2.12.1)
40	*P. aeruginosa*	• Updated Figure 6
41	*Salmonella* species	• Updated Table 20 (previously Table 2.13.1)
41	*Salmonella* species	• Updated Figure 7
43	*Shigella* species	• Updated Table 21 (previously Table 2.14.1)
45	*S. aureus* complex – linezolid-nonsusceptible	• Updated Table 22 (previously Table 2.15.1) • Updated the list of resistance mechanism types and subtypes reported to CARAlert
46	*S. aureus* complex – linezolid-nonsusceptible	• Updated Figure 9
48	*S. aureus* complex – vancomycin-nonsusceptible	• Updated the list of resistance mechanism types and subtypes reported to CARAlert
50	*S. pyogenes*	• Updated Table 24 (previously Table 2.17.1) • Updated penicillin MIC • Updated Figure 11
66-67	Appendix 2	• Updated list of confirming laboratories and what CARs each laboratory can confirm
n/a	CARAlert Isolate Referral Form	• See link to CARAlert Isolate Referral Form; not included as Appendix 3 in this version
68-70	Appendix 3	• Previously Appendix 4; updated screening criteria for CARs by susceptibility testing method
71	Appendix 4	• Previously Appendix 5; updated tests performed at confirming laboratories

Critical antimicrobial resistances reported to CARAlert

The CARs reported to CARAlert are listed in Table 2. More information is included in Appendix 1.

Table 2 Critical antimicrobial resistances for reporting to CARAlert, 2026

Species	Critical resistance
Acinetobacter baumannii complex	Carbapenemase-producing*
Candidozyma (*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- and/or or azithromycin-nonsusceptible
	Gentamicin-resistant†
Neisseria meningitidis	Ciprofloxacin-nonsusceptible†
Pseudomonas aeruginosa	Carbapenemase-producing*
Salmonella species	Ceftriaxone-nonsusceptible
Shigella species	Multidrug-resistant
Staphylococcus aureus complex§	Vancomycin- or linezolid-nonsusceptible
Streptococcus pyogenes	Penicillin reduced susceptibility

* Reported from 1 July 2019
† Reported from 1 January 2023
§ For CARAlert, S. *aureus* complex includes S. *aureus*, *S. argenteus* and *S. schweitzeri*
Notes:
1. Low level-azithromycin-nonsusceptible *N. gonorrhoeae* excluded from the Weekly Summary in 2019.
2. Reporting of daptomycin-nonsusceptible *S. aureus* was suspended from 2023.

The CARAlert process

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

Figure 1 CARAlert alert process

STEP 1 – Detecting a possible CAR

- Possible CAR referred to confirming laboratory.
- All isolates accompanied by information required by CARAlert in an appropriate request form.

STEP 2 – Confirmation

- Molecular and/or phenotypic confirmation tests are done in a timely manner (no longer than five working days). Refer to *Detection of CARs*.
- If not a confirmed CAR, the originating laboratory will be notified through normal communication channels.
- If a CAR is confirmed, the confirming laboratory will notify the originating laboratory and enter data items into the CARAlert web portal.

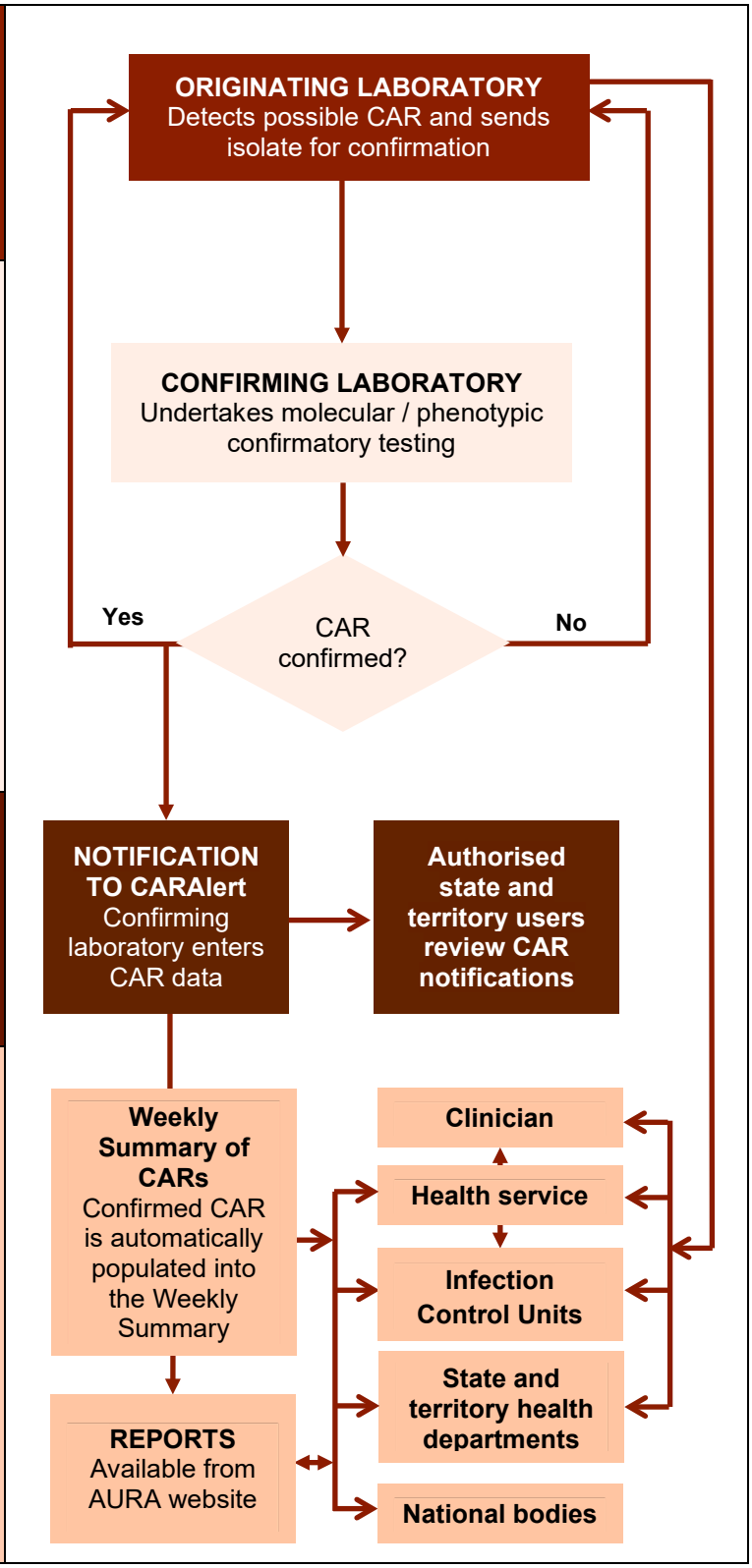
STEP 3 – Notification to CARAlert

- Via secure access, a confirming laboratory enters confirmatory data items into CARAlert. Refer to Tables 3 and 4.

STEP 4 – Weekly Summary of CARs

Following the completed entry of a CAR:

- Information automatically populates the Weekly Summary of CARs which is circulated each Thursday to authorised Commission, state and territory health department and Australian Centre for Disease Control users, and all confirming laboratories.
- CARAlert data updates and annual reports are published on the Commission's website.



CARAlert web portal

The CARAlert web portal is the secure platform that enables authorised representatives from confirming laboratories to submit de-identified data to CARAlert and generate local reports.

Direct access to the CARAlert web portal is also available to authorised representatives from state and territory health departments for access to data for their jurisdiction and to generate local reports.

User guides that provide more information about access to the CARAlert web portal and its functions are available on the Commission's website.

A Weekly Summary of reports to CARAlert is also issued to these authorised representatives of confirming laboratories and states and territories, as well as the Commission and the CDC. The Weekly Summary includes the following information on confirmed CARs:

- State or territory of record – 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. If place of residence is unknown or overseas, the state or territory of the originating laboratory is reported
- State or territory of patient residence
- CAR name
- CAR type
- Date of collection
- Facility type
- Patient age range
- Date of confirmation
- Confirming laboratory name.

Confirming laboratory network for CARAlert

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.

Roles and responsibilities of CARAlert stakeholders

The effective operation of CARAlert relies on the cooperation and collaboration of a range of stakeholders. Tables 3 and 4 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 3 Stakeholders who provide data to CARAlert

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 for confirmation (Appendix 2). Preliminary results generated during initial testing must also be provided to the confirming laboratory (e.g. (m)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 reported to 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 and including it with the isolate.

Table 3 *continued*

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, 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. Please refer to the user guides available on the CARAlert webpage 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 4 Stakeholders who use information from CARAlert

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 CARAlert webpage. 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 CARAlert webpage.
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 CARAlert webpage.
Consumers and other interested users	• Access publicly available information on analyses of trends in CARs in Australia via the CARAlert webpage.
Australian Commission on Safety and Quality in Health Care	Coordination and management of CARAlert in collaboration and under contract with the Australian Centre for Disease Control (CDC), 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 CDC to ensure CARAlert continues to be compatible with, and complement, other national surveillance systems.

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 the CARAlert web portal.

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.[1]

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.

Detection of a CAR

Detection of a CAR

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).[2]
- Clinical and Laboratory Standards Institute (CLSI).[3-5]

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

Flowchart – 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 testing method can be found in Appendix 3. The tests performed by the confirming laboratory that result in a CARAlert submission are summarised in Appendix 4.

Important notes:

1. 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. **The Calibrated Dichotomous Sensitivity (CDS) method of antimicrobial susceptibility testing is no longer supported beyond January 2026. However, CDS methodology has been maintained in this Handbook for laboratories that continue to use this method.**

Acinetobacter-calcoaceticus-baumannii complex – carbapenemase-producing

*Acinetobacter-**calcoaceticus**-baumannii* (ACB) complex (*A. calcoaceticus*, *A. baumannii*, *A. dijkshoorniae*, *A. nosocomialis*, *A. pittii* and *A. seifertii*) is clinically the most important of the *Acinetobacter* species.[6] 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-resistant *A. baumannii* are included in the critical group in the WHO bacterial priority pathogens list[7], and have recently been upgraded to an urgent public health threat by the CDC.[8]

Carbapenem resistance in the ACB complex is almost always due to production of carbapenemases. A variety of carbapenemases in *Acinetobacter* species have been reported worldwide. They may be plasmid or chromosomally encoded. The class D carbapenemases (oxacillinases) are by far the most prevalent carbapenemases in the ACB complex.[9] They can be grouped into those with intrinsic and acquired β-lactamases:

1. Intrinsic chromosomal
    - OXA-51-like (over **382** variants).
2. Acquired
    - OXA-23-like **(over 51 variants).**
    - OXA-24/40-like **(over 13 variants).**
    - OXA-58-like **(over 8 variants).**
    - **OXA-134-like (over 28 variants).**
    - OXA-143-like **(over 11 variants).**
    - **OXA-542.**

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* species. Ertapenem resistance is in the expected phenotype of the genus.

Detection

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

Table 5 Disc diffusion criteria for detecting carbapenemase-producing *Acinetobacter* species

Method	Meropenem disc	Zone diameter	Annular radius
EUCAST*	10 µg	< 15 mm (R)	n/a
CLSI*	10 µg	≤ 14 mm (R)	n/a
CDS†	5 µg	n/a	< 6 mm

* Mueller-Hinton agar
† Sensitest agar

Table 6 MIC criteria for detecting carbapenemase-producing *Acinetobacter* species

Method	Species	Meropenem MIC
MIC strip, Vitek 2, Phoenix NMIC-422	*Acinetobacter* species	> 8 mg/L (R)

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)[10] 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.[11, 12] Rapid (< 2 hour) tests such as the CarbaAcineto NP Test[13], Blue Carba[14, 15] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux[16, 17]; Rapid CARB Screen, ROSCO[18, 19], MAST® CARBA PAcE), are all alternatives, however these often may have poor sensitivity against isolates with Class D enzymes. **The automated BD Phoenix™ CPO Detect test, included in susceptibility panels, can eliminate delays and subjectivity in carbapenemase tests, and is able to classify most carbapenemases.**[17]

Either combined disc tests (CDT)[20] or double disc synergy tests (DDST)[21] 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* species 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 the ACB complex limit the usefulness of commercial assay systems often used for Enterobacterales (Refer to page 23, Screening agar). 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

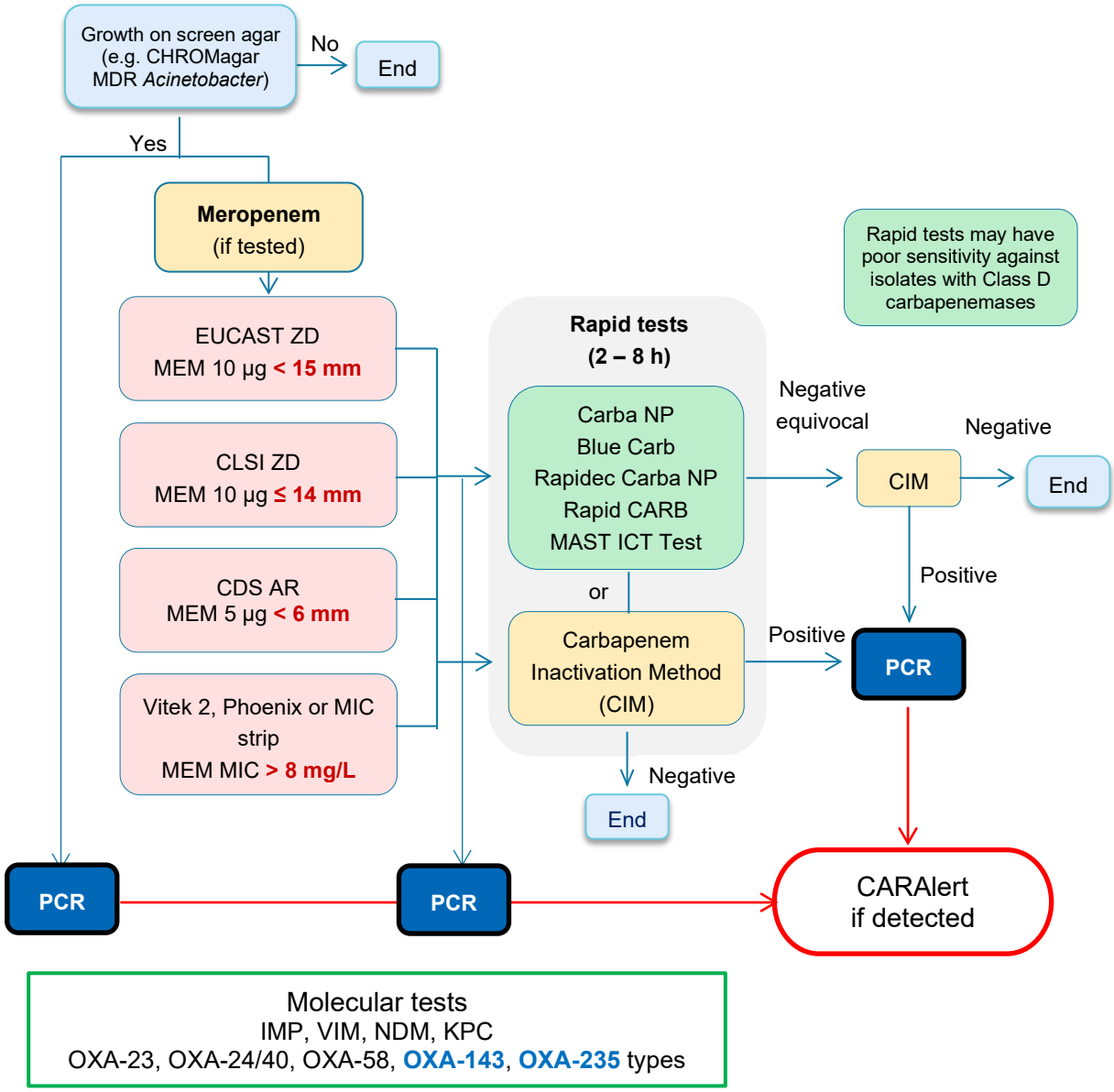
Carbapenemase-producing *Acinetobacter-calcoaceticus-baumannii* complex (*A. calcoaceticus*, *A. baumannii*, *A. dijkshoorniae*, *A. nosocomialis*, *A. pittii* or *A. seifertii*)

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-calcoaceticus-baumannii* complex flowchart



AR = annular radius; CIM = Carbapenem Inactivation Method; ICT = Indirect Carbapenemase Test; MEM = meropenem; PCR = polymerase chain reaction (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.

Candidozyma (*Candida*) *auris*

Candidozyma (*Candida*) *auris* was first recognised in 2009 in Japan[22] but has emerged quickly causing severe disease in hospitalised patients in many countries, including Australia.[23, 24]

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 42ºC, 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™ 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. auris* colonies are pale cream with a distinctive blue halo that diffuses into the surrounding agar.[25] 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.[26] 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 7), 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 multidrug 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. **Currently, the only established *C. auris*-specific clinical breakpoint is for rezafungin**[27], although general guidance for interpretation of susceptibility testing has been provided by the Centers for Disease Control and Prevention[28], and these are supported by CLSI- and EUCAST-based ECOFFs for some antifungal agents.[29, 30] Antifungal susceptibility testing should be performed by a current approved method[31], 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.

Note: Antifungal susceptibility testing is not a requirement for CARAlert submission.

Table 7 Identification of *Candidozyma* (*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. guilliermondi* 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; Algorithm to identify *C. auris* based on phenotypic laboratory method and initial species identification (cdc.gov)

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* species 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* species 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.[21]

Detection

Meropenem is available across all susceptibility testing methods currently used in Australia. The epidemiological cut-off value (ECOFF) is used to distinguish between organisms **with and without acquired resistance mechanisms**.[2] 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 Tables 8 and 9.

Epidemiological cut cut-off values (ECOFFs) are the highest MIC values **observed for an antimicrobial agent and a species devoid of phenotypically detectable acquired resistance mechanism**. ECOFFs are specific to **individual** species.

Table 8 Disc diffusion **screening cut-off values** for carbapenemase-producing *Enterobacterales*

Method	Meropenem disc	Zone diameter	Annular radius	Comment/s
EUCAST*	10 µg	< 28 mm	n/a	ECOFF
CLSI*	10 µg	< 28 mm	n/a	ECOFF
CDS†	5 µg	n/a	< 6 mm	

* Mueller-Hinton agar
† Sensitest agar
Notes:
1. 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.
2. 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.

Table 9 MIC **screening cut-off values** for detecting carbapenemase-producing *Enterobacterales*

Method	Species	Meropenem MIC*
MIC strip	**Other *Enterobacterales*†**	**> 0.125 mg/L**
MIC strip, Phoenix NMIC-422§	***E. coli*, *Klebsiella* (except *K. aerogenes*), *Proteus mirabilis***	> 0.125 mg/L
MIC strip, Phoenix NMIC-422§	***Enterobacter*, *K. aerogenes*, *Morganella*, *Proteus vulgaris***	> 0.25 mg/L
Vitek-2#	*Enterobacterales*	> 0.25 mg/L

* Epidemiological cut-off value defined by EUCAST
† ***Enterobacterales* other than species than those listed in this table.**
§ Phoenix panels: meropenem range is 0.125–16 mg/L
# Vitek® 2 cards: meropenem range is 0.25–16 mg/L

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[32]; 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).

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 **< 27 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)[10] or modified CIM (mCIM)[5] 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.[11, 12] Rapid (< 2 hour) tests such as the Carba NP Test[33], Blue Carba[14, 15] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux[16, 17]; Rapid CARB Screen, ROSCO[18, 19]), are all suitable alternatives. These rapid tests however have lower sensitivity against isolates with low carbapenemase activity such as OXA-48-like producers.

Either combined disc tests (CDT)[20] or double disc synergy tests (DDST)[21] 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.[21, 34, 35] If any of the phenotypic tests indicate carbapenemase activity, the isolate must be confirmed by molecular methods.

The automated BD Phoenix™ CPO Detect test, included in susceptibility panels, can eliminate delays and subjectivity in carbapenemase tests, and is able to classify most carbapenemases.[17]

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.[36, 37]

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* species, *Providencia* species, or *Morganella* species 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).[38-40] 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.[41] **If an isolate demonstrates carbapenemase activity (e.g. (m)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.[42]

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.

Table 10 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
NG Biotech NG-Test® CARBA-5	**KPC-1, -2, -3, -4, -5, -6, -7, -12, -14, -23, -28, -39**	**NDM-1, -2, -3, -4, -5, -6, -7, -8, -9, -11, -19**	**IMP-1, -2, -4, -5, -6, -7, -8, -10, -11, -13, -14, -15, -16, -18, -19, -22, -26, -29, -31, -37, -39, -46, -47, -56, -58, -61, -63, -71, -79**	**VIM-1, -2, -4, -5, -6, -19, -23, -26, -27, -31, -39, -46, -51, -52, -54, -56, -58, -59**	**OXA-48-like: (OXA-48, -58, -162, -181, -204, -232, -244, -245, -370, -436, -484, -515, -517, -519, -535, -793)**	

Notes:
1. The list of validated commercial tests is a guide only; is not exhaustive.
2. These commercial assays only identify the enzyme group involved. Sequencing is required to determine the variant.
3. IMP-4 is the most common IMP variant in Australia.

CARAlert Notification

Enterobacterales confirmed to contain carbapenemase genes

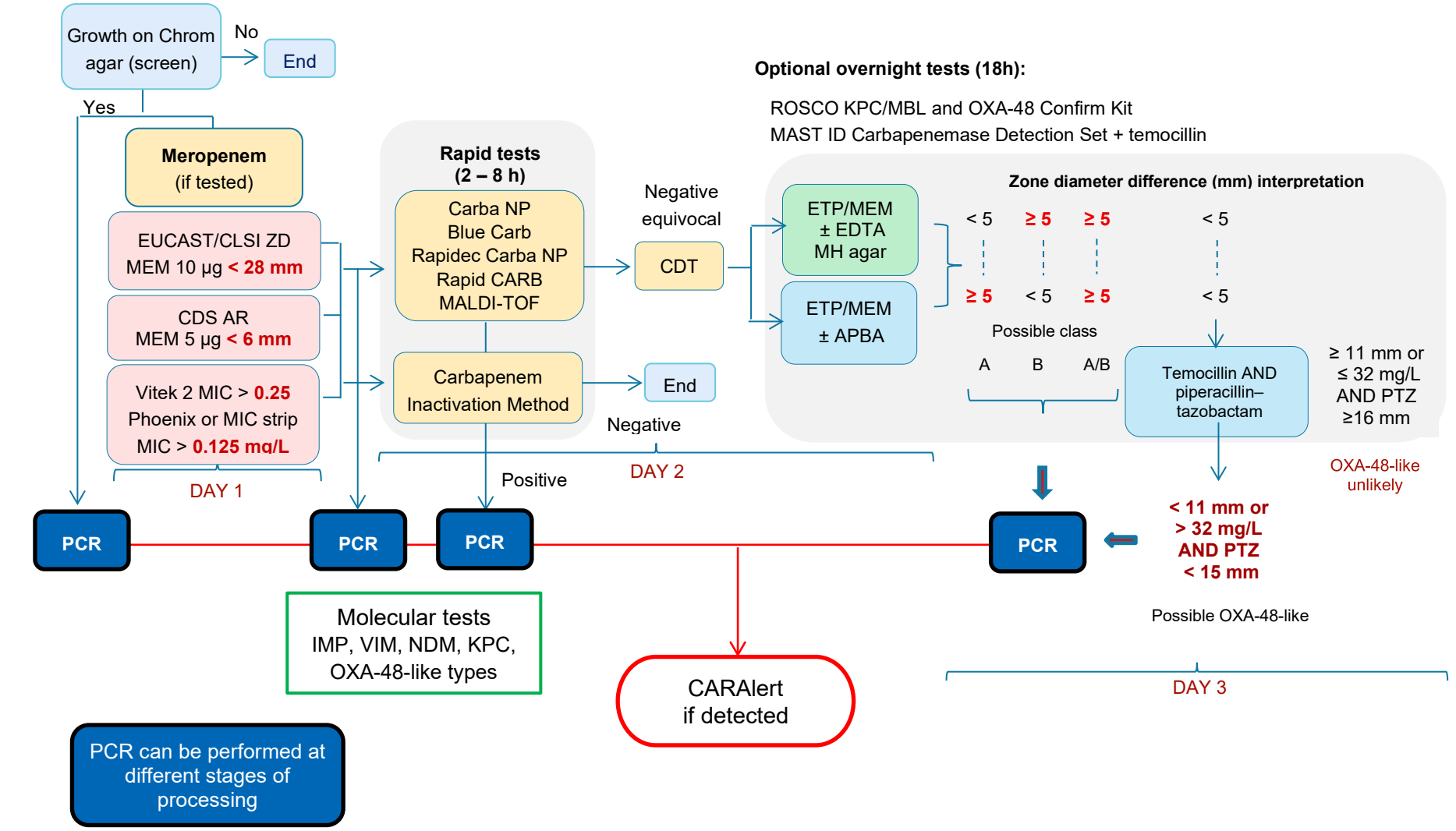
Type: at time of confirmation (e.g. IMP, NDM)

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, **report CPE but include the MCR variant along with the CPE subtype:**
   a. **Type: NDM.**
   b. **Subtype: NDM, *mcr-9*.**

Figure 3 Carbapenemase-producing *Enterobacterales* flowchart



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

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 simultaneous high-level resistance to amikacin, gentamicin and tobramycin, frequently coproduced with ESBL and carbapenemases.[43, 44]

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.[45]

Table 11 Disc diffusion criteria for **screening** *Enterobacterales* for ribosomal methyltransferases

Method	Agent	Disc	Zone diameter	Annular radius	Equivalent MIC
EUCAST*	Amikacin **and**	30 µg	< 18 mm	n/a	> 8 mg/L (R)
	gentamicin **and**	10 µg	< 17 mm	n/a	> 2 mg/L (R)
	tobramycin	10 µg	< 16 mm	n/a	> 2 mg/L (R)
CLSI*	Amikacin **and**	30 µg	≤ 16 mm	n/a	≥ 16 mg/L
	gentamicin **and**	10 µg	≤ 14 mm	n/a	≥ 8 mg/L
	tobramycin	10 µg	≤ 12 mm	n/a	≥ 8 mg/L
CDS†	Amikacin **and**	30 µg	n/a	< 4 mm	> 16 mg/L
	gentamicin **and**	10 µg	n/a	< 4 mm	> 2 mg/L
	tobramycin	10 µg	n/a	< 4 mm	> 2 mg/L

* Mueller-Hinton agar
† Sensitest agar

Table 12 MIC criteria for **screening** for *Enterobacterales* with 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

Notes:
1. Both the current Vitek 2 (AST-N246, AST-N435, AST-N470) and Phoenix (NMIC-422) cards test all three aminoglycosides, which can readily identify isolates suspected of containing ribosomal methyltransferases.
2. **Amikacin is not available on the Vitek 2 AST-N471) card.**
3. **Tobramycin is not available on the Vitek 2 AST-N410, AST-N411, AST-N434 or AST-N471) cards.**

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*, *rmtF*, *rmtG*, *rmtH* and *npmA*.

Ribosomal methyltransferases can all be readily detected using multiplex PCR[45, 46], real-time PCR[47], or by whole genome sequencing.

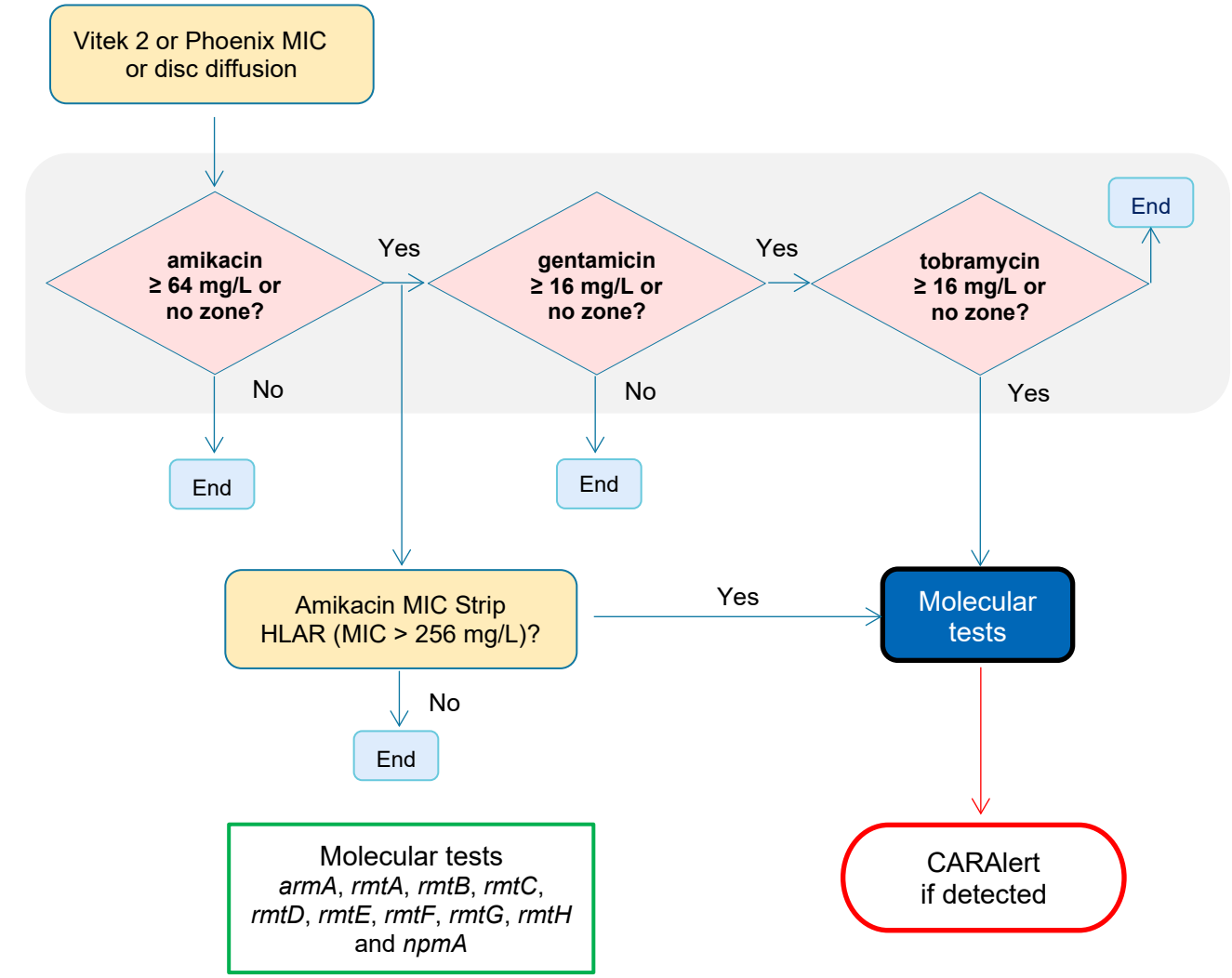
CARAlert Notification

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

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).[48] 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).[49] Since then, a number of new variants of MCR-1 have been described.[50] 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 HI2 plasmids which may carry $bla_{\text{IMP-4}}$.[51]

Proteus species, *Morganella morganii*, *Providencia* species, *Hafnia* species, 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 disc 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. **Disc 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* species, *Morganella morganii*, *Providencia* species, *Hafnia* species 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 HI2 plasmids which may carry $bla_{\text{IMP-4}}$.

Enterococcus species – linezolid-resistant

Linezolid resistance in *Enterococcus* species is commonly due to mutations in the 23S rRNA or ribosomal proteins (L3, L4, and **L22**), or acquisition of acquired resistance genes associated with mobile genetic elements such as *cfr*, *optrA* and ***poxtA***.[52-54]

Detection

Enterococcus species that test resistant to linezolid.

Table 13 Susceptibility test criteria for detecting linezolid resistant *Enterococcus* species

Method	Linezolid disc	Zone diameter	Annular radius	MIC [R]
EUCAST*	10 µg	< 20 mm	n/a	> 4 mg/L
CLSI*	30 µg	≤ 20 mm	n/a	≥ 8 mg/L
CDS†	10 µg	n/a	< 6 mm	> 4 mg/L

* Mueller-Hinton agar

† Blood Sensitest agar, CO_2, 35–37ºC

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.[55-57] Molecular methods are used to detect rRNA methyltransferases (*cfr, optrA,* ***poxtA***).

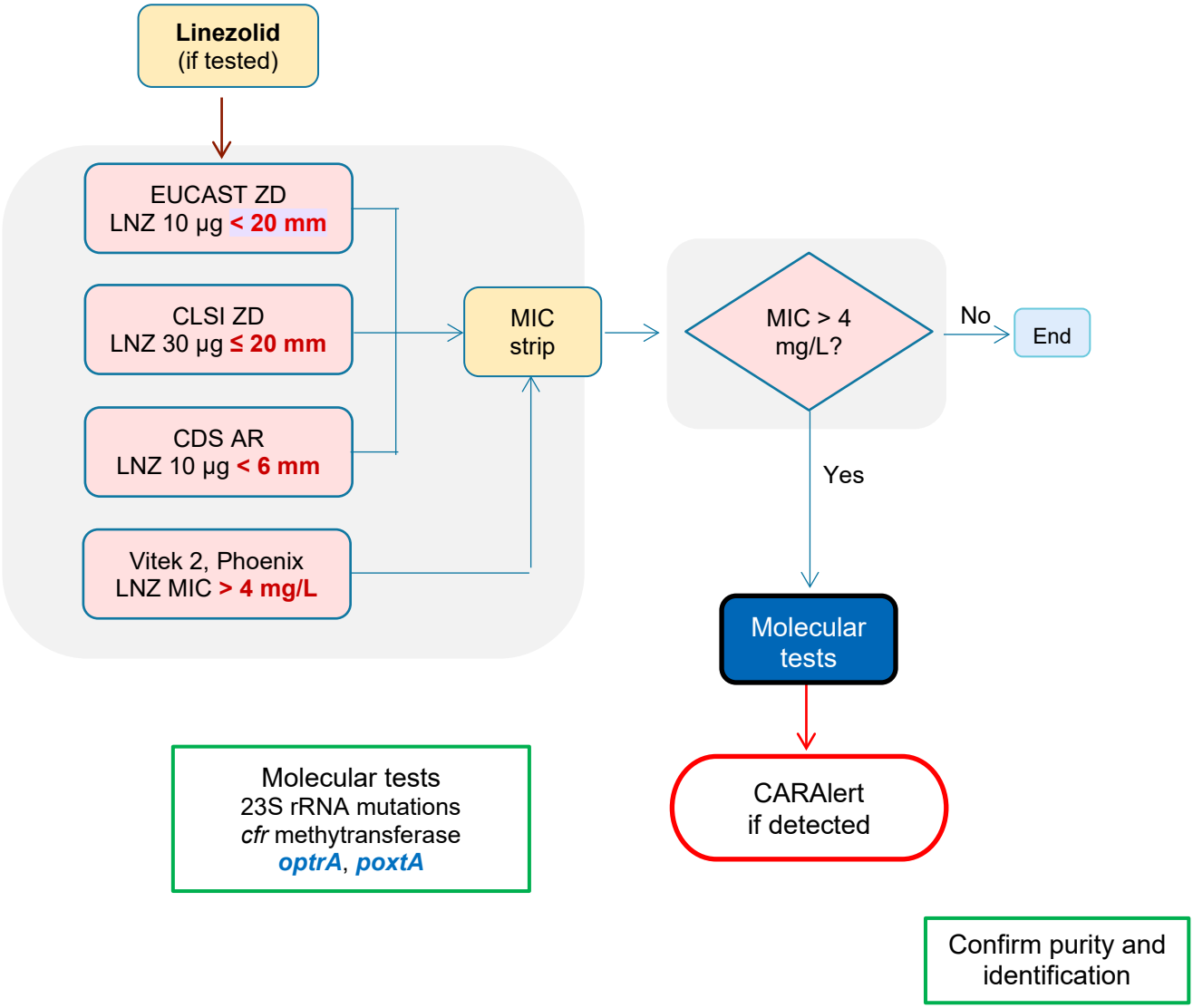
CARAlert Notification

Linezolid-resistant *Enterococcus* species with mutations detected in 23S rRNA or L3/L4/**L22** ribosomal proteins; or if *cfr* or *optrA or* ***poxtA*** has been detected.

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

Subtype: mutation or variant

Figure 5 Linezolid resistant *Enterococcus* species flowchart



AR = annular radius; LNZ = linezolid; ZD = zone diameter

Mycobacterium tuberculosis – multidrug-resistant

Mycobacterium 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.[58] Isolates with rifampicin **critical concentration** > 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 critical concentration > 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 (**critical concentration 0.1 mg/L**).

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

Neisseria gonorrhoeae – azithromycin-nonsusceptible

Antimicrobial resistance to azithromycin, benzylpenicillin, ceftriaxone or cefotaxime and ciprofloxacin in *Neisseria 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, Disability and Ageing. 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.

Table 14 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	

* 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), or azithromycin (HLR ≥256)

Notes:

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

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

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.

Table 15 Screening criteria for detecting ceftriaxone–nonsusceptible *Neisseria gonorrhoeae*

Method	Agent	MIC	Comments
EUCAST*	Ceftriaxone	≥ 0.125 mg/L	**WHO criterion for decreased susceptibility**
CLSI†	Ceftriaxone	≥ 0.125 mg/L	**WHO criterion for decreased susceptibility**
CDS§	Ceftriaxone	0.06 – 0.25 mg/L	Less susceptible; Breakpoint for resistance not yet determined

* 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.[59, 60]

CARAlert Notification

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

Type: Ceftriaxone

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

An extensively drug-resistant (XDR) *N. gonorrhoeae* phenotype is defined as displaying both high-level resistance to azithromycin (MIC ≥ 256 mg/L and decreased susceptibility to ceftriaxone (MIC ≥ 0.125 mg/L).

Neisseria gonorrhoeae – gentamicin-resistant

The emergence of gonococcal AMR in Australia has long been influenced by the introduction of multidrug-resistant strains from overseas.[61, 62] In 2017, the first evidence of sustained spread of multidrug-resistant *N. gonorrhoeae* was reported internationally[63], followed in 2018 by coincident reports from Australia and the United Kingdom of the first extensively drug-resistant *N. gonorrhoeae* isolates.[64, 65] 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.[66] 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 0.5–8 mg/L).[67, 68]

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.[69] The spread of gonococcal resistance world-wide is well documented[63], 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.

Table 16 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

* 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.

Neisseria meningitidis – 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.[70-72] 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)[73] and in 2021 (MIC = 0.125 mg/L).[74] 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.[75]

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

Table 17 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	

* 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.
Only include invasive and/or isolates with a serogroup.

CARAlert Notification

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

Type: Not required

Notes:

1. Submission through the NNN only.
2. **Only include invasive and/or isolates with a serogroup**.

Pseudomonas aeruginosa – carbapenemase-producing

Carbapenem-resistance in *Pseudomonas 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-1-3, 7-9, 10-13, 15, 17, 19, 23, 26, 31-32, 38, 44-45, 52, 60**) are ESBLs, rather than carbapenemases (**GES-4-6, 14, 16, 18, 20-21, 24-25, 28, 30, 33, 39-41, 47-49,50-51, 53-54**).

Table 18 Disc diffusion criteria for detecting carbapenemase-producing *Pseudomonas aeruginosa*

Method	Agent	Disc	Zone diameter	Annular radius	MIC equivalent
EUCAST*	Meropenem	10 µg	< 20 mm	n/a	> 2 (I§ or R)
	Piperacillin–tazobactam	30-6 µg	< 18 mm	n/a	> 16 (R)
CLSI*	Meropenem	10 µg	< 19 mm†	n/a	> 2 (I or R)
	Piperacillin–tazobactam	100/10 µg	< 22 mm	n/a	> 16 (I or R)
CDS#	Meropenem	5 µg	n/a	< 6 mm	> 2 mg/L (R)
	Piperacillin–tazobactam	50/5 µg	n/a	< 6 mm	> 16 mg/L (R)

* Mueller-Hinton agar
† The EUCAST criterion is preferred
§ Susceptible, increased exposure
\# Sensitest agar

Table 19 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)[10] or modified CIM (mCIM)[5] 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.[11, 12] Rapid (<2 hour) tests such as the Carba NP Test[33], Blue Carba[14, 15] validated in-house test or commercial systems (RAPIDEC®CARBA NP, BioMérieux17; Rapid CARB Screen, ROSCO[18, 19]), 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)[20] or double disc synergy tests (DDST)[21] 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* species)
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

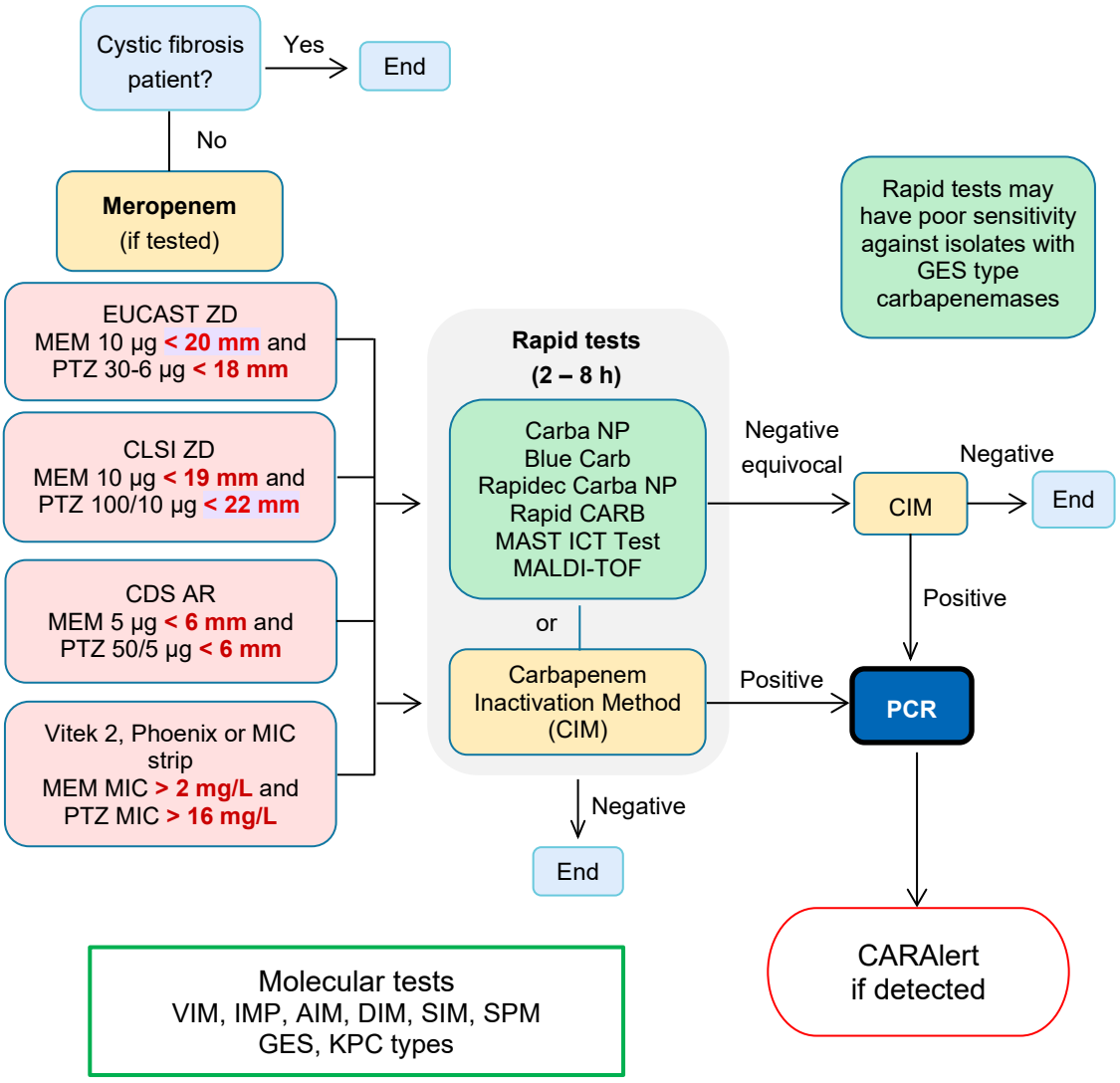
P. aeruginosa, **excluding isolates from patients with cystic fibrosis**, confirmed to contain carbapenemase genes.

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

Subtype(s): 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; CIM = carbapenem Inactivation Method; ICT = Indirect Carbapenemase Test; MEM = meropenem; PCR = refer for molecular confirmation; PTZ = piperacillin–tazobactam; 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.

Salmonella species – ceftriaxone-nonsusceptible

Salmonella species 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 ESBL and plasmid–mediated AmpC.

Detection

Table 20 Susceptibility test criteria for detecting ceftriaxone-nonsusceptible *Salmonella* species

Method	Agent	Disc	Zone diameter	MIC equivalent
EUCAST	**Cefotaxime**	5 µg	< 20 mm	> 1 mg/L (I* or R)
	Ceftriaxone	30 µg	< 27 mm	> 1 mg/L (I* or R)
CLSI	**Cefotaxime**	30 µg	< 26 mm	> 1 mg/L (I or R)
	Ceftriaxone	30 µg	< 23 mm	> 1 mg/L (I or R)
CDS	Ceftriaxone	5 µg	< 6 mm	> 1 mg/L

* Susceptible, increased exposure

Phenotypic Tests

Ceftriaxone-nonsusceptible isolates can be screened for ESBL/AmpC activity using commercially available discs (e.g.: 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).[76-78]

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 to page 23).

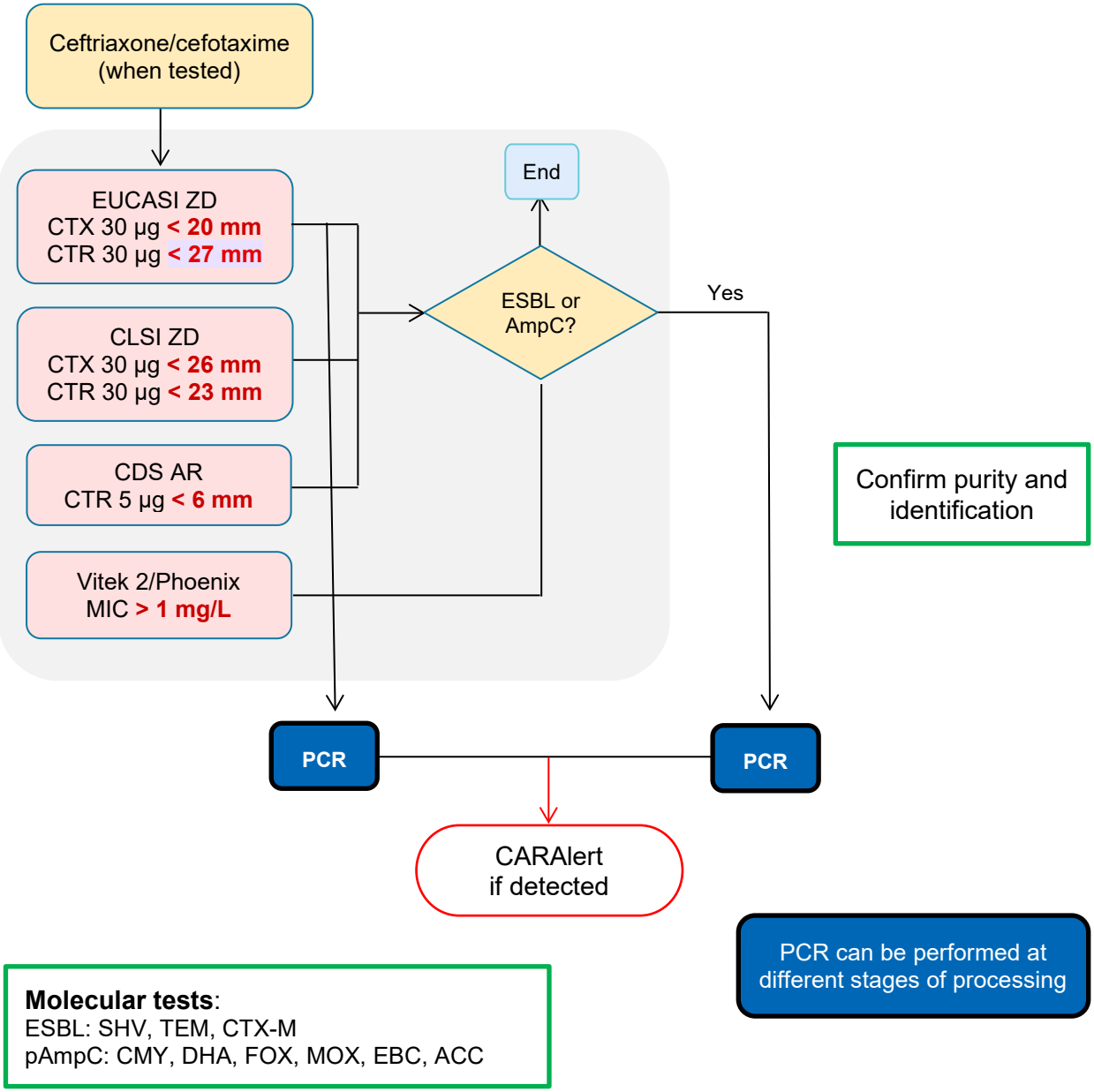
CARAlert Notification

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

Type: ESBL, AmpC

Subtype(s): CMY, CTX-M, SHV

Figure 7 Ceftriaxone-nonsusceptible *Salmonella* species flowchart



AR = annular radius; pAmpC = plasmid mediated AmpC; CTX = cefotaxime; CTR = ceftriaxone; ESBL = extended-spectrum β-lactamase; MIC = minimum inhibitory concentration; ZD = zone diameter

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.

Shigella species – multidrug-resistant

Shigella species 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.

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

Table 21 Susceptibility test criteria for detecting MDR *Shigella* species

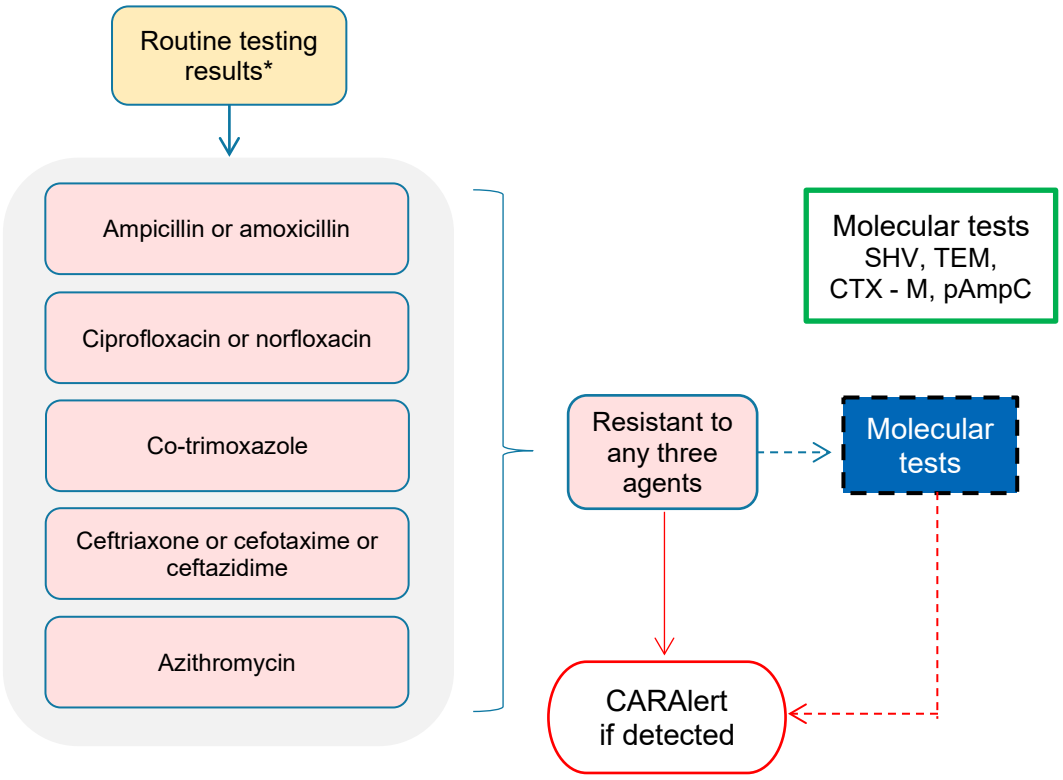
Method	Agent	Disc (µg)	Zone diameter (mm)	Annular radius (mm)	MIC (mg/L)	Comments
EUCAST	Ampicillin	10	< 14	n/a	> 8	
	Azithromycin	15	< 12*	n/a	> 16*	
	Ceftriaxone or	30	< 24	n/a	> 2	
	Cefotaxime or	5	< 17	n/a	> 2	
	Ceftazidime	10	< 19	n/a	> 4	
	Ciprofloxacin†	5	< 22	n/a	> 0.5	
	norfloxacin	10	< 24	n/a	> 0.5	Uncomplicated UTI only
	Co-trimoxazole	1.25–23.75	< 15	n/a	> 0.5	
CLSI	Ampicillin	10	≤ 13	n/a	≥ 32	
	Azithromycin	15	≤ 10	n/a	≥ 32	
	Ceftriaxone or	30	≤ 19	n/a	≥ 4	
	Cefotaxime	30	≤ 22	n/a	≥ 4	
	Ciprofloxacin†	5	≤ 21	n/a	≥ 1	
	Co-trimoxazole	1.25–23.75	≤ 10	n/a	≥ 4/76	
CDS	Ampicillin	25	n/a	< 6	> 8	
	Azithromycin	–	n/a	–	–	
	Ceftriaxone/cefotaxime or	5	n/a	< 6	> 1	
	Ceftazidime	10	n/a	< 6	> 4	
	Ciprofloxacin†	2.5	n/a	< 6	> 1	
	norfloxacin	10	n/a	< 6	> 4	Urine isolates only
	Co-trimoxazole	25	n/a	< 6	> 1/19	

– = not calibrated

* **Likely to have azithromycin resistance mechanisms**

† **The perfloxacin 1 µg screening test can be used to detect fluoroquinolone resistance (zone diameter < 24 mm)**

Figure 8 Multidrug-resistant *Shigella* species 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-27, CTX-M-15, CMY-2, SHV-12)

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

Staphylococcus aureus complex – linezolid-nonsusceptible

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

Linezolid non-susceptibility may be due to one (or more) resistance mechanisms: 23S rRNA mutations, *cfr*-, or *optrA*-mediated.[52-54, 56]

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

Detection

Table 22 Susceptibility test criteria for detecting linezolid-nonsusceptible *Staphylococcus aureus* complex

Method	Agent	Disc	Zone diameter	Annular radius	MIC
EUCAST*	Linezolid	10 µg	< 21 mm	n/a	> 4 mg/L (R)
CLSI*	Linezolid	30 µg	≤ 25 mm	n/a	> 4 mg/L (I or R)
CDS†	Linezolid	10 µg	n/a	< 6 mm	> 4 mg/L

* Mueller-Hinton agar
† Sensitest agar, air, 35–37ºC

Confirmation

S. aureus, *S. argenteus* or *S. schweitzeri* isolates that test as linezolid non-susceptible 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.

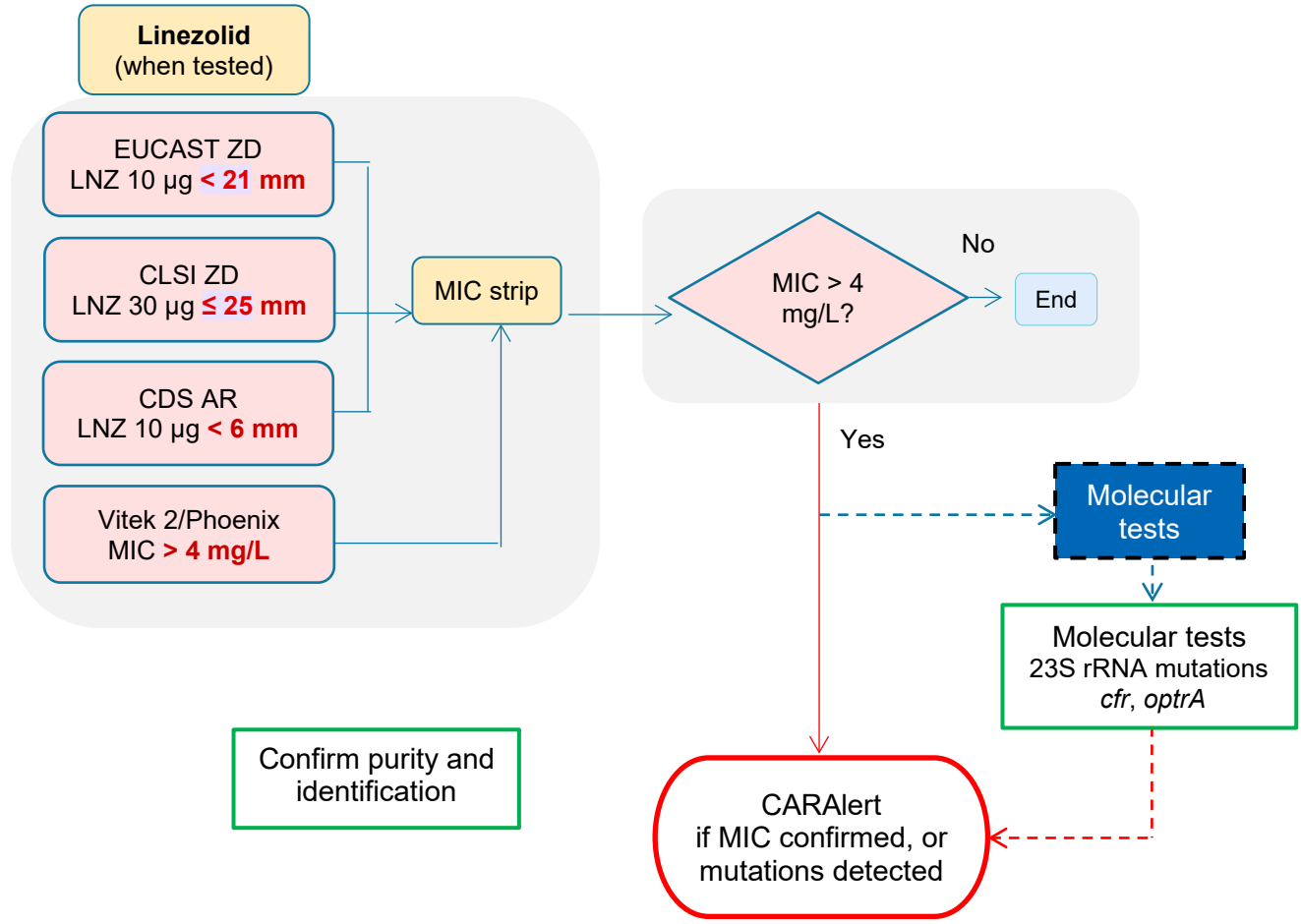
CARAlert Notification

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(s): **23S rRNA, *cfr*, *optrA*, Other**

Subtype(s) (optional): mutation or variant, or MIC

Figure 9 Linezolid-nonsusceptible *Staphylococcus aureus* complex flowchart



AR = annular radius; LNZ = linezolid; MIC = minimum inhibitory concentration; ZD = zone diameter

Staphylococcus aureus complex – vancomycin-nonsusceptible

In recent years, vancomycin breakpoints for *Staphylococcus* species 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.

Table 23 Susceptibility test criteria for detecting vancomycin-nonsusceptible *Staphylococcus aureus* complex

Method	Agent	Disc	Zone diameter	Annular radius	MIC	Comments
EUCAST*	Vancomycin	–†	n/a	n/a	> 2 mg/L	MIC strips: > 3 mg/L
CLSI*	Vancomycin	–†	n/a	n/a	> 2 mg/L	MIC strips: > 3 mg/L
CDS§	Vancomycin	5 µg	n/a	< 2 mm	> 4 mg/L	

* Mueller-Hinton agar
† MIC method only
§ 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-hour 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-hour 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

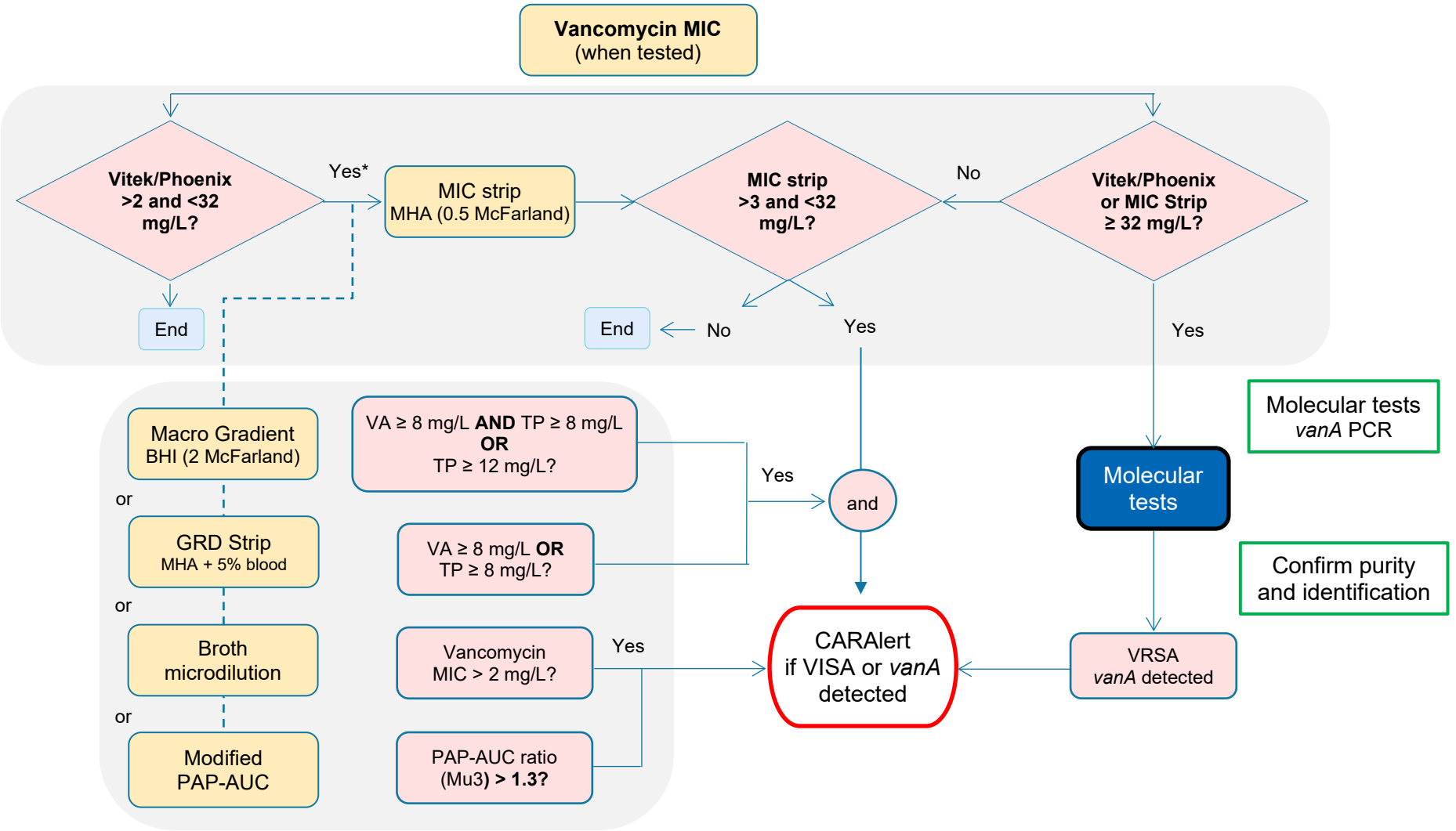
S. aureus with vancomycin MIC > 2 mg/L by broth microdilution, > 3 mg/L by standard MIC strip, GRD Strip indicative of VISA; or *vanA* gene detected (VRSA).

Notification of hVISA strains is not required for CARAlert.

Type: **VISA, *vanA*, Other**

Subtype(s) (optional): MIC

Figure 10 Vancomycin-nonsusceptible *Staphylococcus aureus* complex flowchart



BHI = Brain Heart Infusion agar; MHA = Mueller-Hinton agar; TP = teicoplanin; VA = vancomycin
* Additional tests may be performed as indicated by the dashed line; Results are interpreted with MIC strip

Streptococcus pyogenes – reduced penicillin susceptibility

Streptococcus 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.

The resistance mechanism is not known.

Detection

Table 24 Susceptibility test criteria for detecting *Streptococcus pyogenes* with reduced penicillin susceptibility

Method	Penicillin disc	Zone diameter	Annular radius	MIC
EUCAST	1 U	< 23 mm	n/a	> 0.03 mg/L
CLSI	10 U	< 24 mm	n/a	> 0.125 mg/L
CDS	0.5 U	n/a	< 6 mm	> 0.125 mg/L

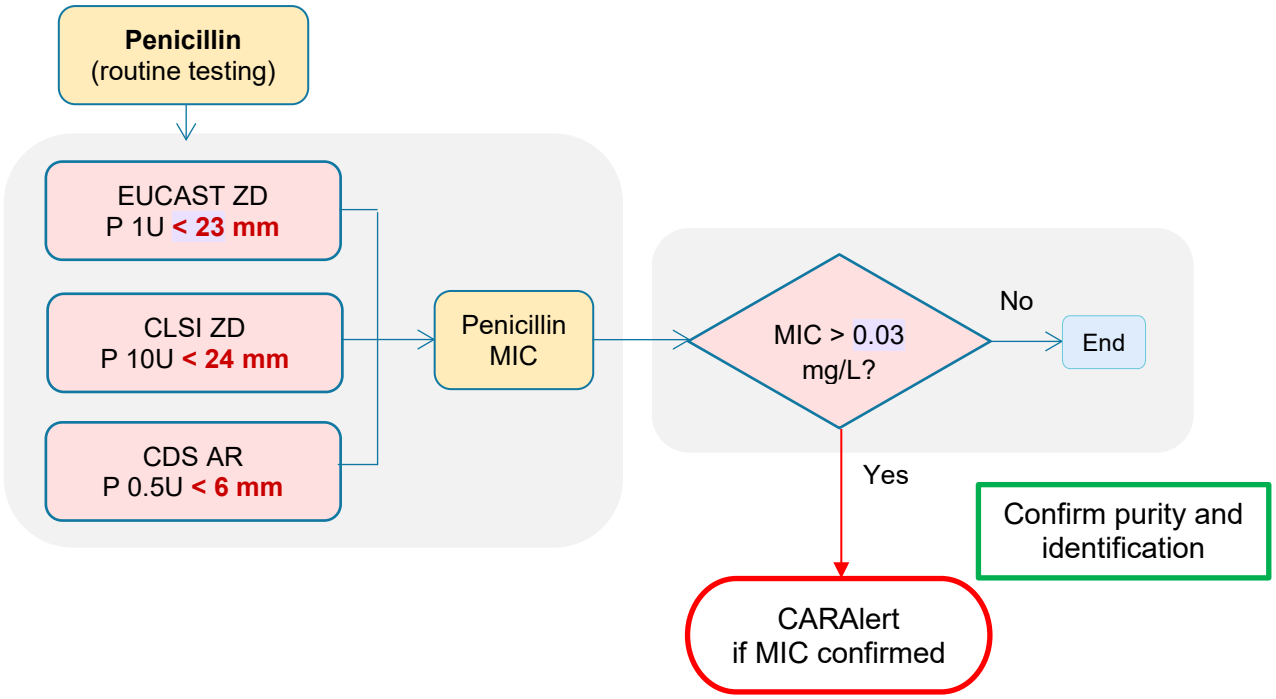
Confirmation

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 > 0.03 mg/L confirmed.

Figure 11 Penicillin-nonsusceptible Streptococcus *pyogenes* flowchart



AR = annular radius; MIC = minimum inhibitory concentration; ZD = zone diameter

Considerations before submission of a CAR

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.

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.

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.

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.

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.

Abbreviations and terminology

Abbreviations and terminology

Acronym	Definition
ACB complex	*Acinetobacter-calcoaceticus-baumannii* complex
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
CDC	Australian Centre for Disease Control
CDS	Calibrated Dichotomous Sensitivity
CDT	combined disc tests
CIM	carbapenem inactivation 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 ≤ 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
IATA	International Air Transport Association
LNZ	linezolid
MALDI-TOF MS	matrix-assisted laser desorption ionization – time of flight mass spectrometry

Acronym	Definition
MBL	metallo-β-lactamase
MCR	mobile colistin resistance
MDR	multidrug-resistant
MIC	minimum inhibitory concentration
MTB	*Mycobacterium tuberculosis* complex
NATA	National Association of Testing Authorities
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 Australasia
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
WHO	World Health Organization
XDR	Extensively drug-resistant **Note: *N. gonorrhoeae* isolates displaying both high-level resistance to azithromycin (MIC ≥ 256 mg/L and decreased susceptibility to ceftriaxone (MIC ≥ 0.125 mg/L) are defined as extensively drug-resistant.**

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References

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Appendices

Appendices

Appendix 1: About CARAlert

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 as a component of the Antimicrobial Use and Resistance in Australia (AURA) surveillance program.

Funding for CARAlert and the Commission's AURA Project is provided by the Australian Centre for Disease Control (CDC) and previously the Australian Government Department of Health, Disability and Ageing (the Department), with contributions from the states and territories by meeting the costs of confirmatory testing and data submission processes.

CARAlert data on confirmed cases of critical antimicrobial resistances (CARs) can be used to identify seasonal, geographic and national trends. The potential for CARAlert to act as an early warning system for CAR outbreaks to enable timely infection prevention and control responses is dependent on timely reporting of CARs by confirming laboratories.

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.

The CARAlert Data Explorer, a publicly accessible and interactive data dashboard, was first published in June 2025. The Data Explorer offers customised analytics and trends for CARs and is complementary to CARAlert data updates and annual reports that are regularly published on the Commission's website.

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 CARAlert – 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.

Isolates collected from patients are reported to CARAlert as either a clinical isolate, that is a specimen (e.g., from blood, urine, wound) taken to guide clinical diagnosis, or as a screen for infection prevention and control purposes. No patient-level data are held in the CARAlert system.

The results of confirmatory testing are provided to the originating laboratory as soon as possible after confirmation. Generally, confirming laboratories submit a CAR report within seven days of the isolate being confirmed as a CAR.

Australian 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 Centre for Disease Control (CDC) and 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, including the CARAlert Data Explorer.

Secure access to the CARAlert web portal allows designated state and territory health authorities to view records for their own jurisdiction at any time, as well as authorised confirming laboratory users for their laboratory. All de-identified information submitted to CARAlert is available 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 via the CARAlert web portal. Over time, the data will become increasingly useful to inform a broader range of safety and quality improvement programs.

It is intended that states and territories use CARAlert 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 jurisdictions have made selected CARs, such as carbapenemase-producing *Enterobacterales* (CPE) and *Candidozyma* (*Candida*) *auris*, notifiable either using their public health legislation or by policy. Some states and territories have standalone systems for monitoring selected CARs, which complement CARAlert, but these are not widespread. Over time, CARAlert data have the potential to inform a broad range of safety and quality improvement programs.

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 AURA surveillance program. The scope of organisms and CARs are regularly reviewed, based on the latest evidence on CARs that emerge in Australia and overseas.

The Commission reviewed the CARs reported to CARAlert in 2016, 2018 and 2022, in conjunction with the states and territories and a range of clinical experts. Respective changes implemented were:

- Four new CARs were reported to CARAlert from July 2019: Carbapenemase-producing *Acinetobacter baumannii* complex, *C. auris*, transmissible resistance to colistin in *Enterobacterales* and carbapenemase-producing *Pseudomonas aeruginosa*.
- Two new CARs were reported to CARAlert from January 2023: Gentamicin-resistant *Neisseria gonorrhoeae* and ciprofloxacin-nonsusceptible *N. meningitidis*.
- One CAR was suspended from reporting to CARAlert from January 2023: Daptomycin-nonsusceptible *Staphylococcus aureus*. Reporting of this CAR will be reconsidered when more reliable phenotypic testing methods are available.

Appendix 2: CARAlert Confirming Laboratories

State or Territory	Confirming laboratory / CAR	*Acinetobacter baumannii* complex	*Candidozyma (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 (Children's Hospital Westmead)**	–	–	**Yes**	–	–	–	**Yes**
	NSWHP (Concord Hospital)	Yes	Yes	Yes	–	–	–	Yes
	NSWHP (Gosford Hospital)	–	**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**	Yes	**Yes**	–	–	–	**Yes**
	NSWHP (Westmead Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	NSWHP (Wollongong Hospital)	–	**Yes**	**Yes**	–	–	–	–
	St Vincent's Pathology (SydPath)	Yes	Yes	Yes	–	–	–	Yes
NT	Territory Pathology (Royal Darwin Hospital)	Yes	Yes	Yes	–	–	–	Yes
Qld	**Mater Pathology Queensland**	–	**Yes**	**Yes**	–	–	–	**Yes**
	Pathology QLD (Central Laboratory†)	Yes	Yes	Yes	Yes	–	–	Yes
	Pathology QLD (Forensic & Scientific Services)	–	–	–	–	–	–	–
	QML Pathology	–	Yes	–	–	–	–	–
	Sullivan Nicolaides Pathology	Yes	Yes	Yes	**Yes**	–	–	Yes
SA	SA Pathology (Royal Adelaide Hospital)	Yes	Yes	Yes	Yes	**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	–	**Yes**	–	–	–	–	–
	Melbourne Pathology	–	Yes	–	–	–	–	–
	Monash Pathology	–	Yes	–	–	–	–	–
WA	PathWest (Fiona Stanley Hospital)	–	Yes	–	–	–	Yes	–
	PathWest (QEII Medical Centre)	Yes	Yes	Yes	Yes	Yes	–	Yes

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

* MCR variants other than *mcr-9*

† Royal Brisbane and Women's Hospital

Appendix 2: CARAlert Laboratories (continued)

State or Territory	Confirming laboratory / CAR	*Salmonella* species	*Shigella* species	*Staphylococcus aureus* complex		*Streptococcus pyogenes*	*Mycobacterium tuberculosis*	*Neisseria gonorrhoeae*	*Neisseria meningitidis*
		Ceftriaxone-nonsusceptible (ESBL/AmpC)	Multidrug-resistant	Vancomycin-nonsusceptible (MIC)	Linezolid-nonsusceptible (MIC)	Penicillin reduced susceptibility	Multidrug-resistant	Azithromycin-or ceftriaxone-nonsusceptible; gentamicin-resistant	Ciprofloxacin-nonsusceptible
ACT	ACT Pathology	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes
NSW	**NSWHP (Children's Hospital Westmead)**	**–**	**Yes**	**–**	**–**	**Yes**	**–**	**–**	**–**
	NSWHP (Concord Hospital)	Yes	Yes	Yes	Yes	Yes	–	–	–
	NSWHP (Gosford Hospital)	**–**	**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	Yes
	NSWHP (Westmead Hospital)	Yes	Yes	Yes	Yes	Yes	Yes	–	–
	NSWHP (Wollongong Hospital)	**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	**Mater Pathology Queensland**	**–**	**–**	**–**	**Yes**	**Yes**	**–**	**–**	**–**
	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	–	–

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

* Royal Brisbane and Women's Hospital

Appendix 3: Screening criteria for CARs by susceptibility testing method

Table A3.1 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
	Meropenem	10 µg	< 28	> 0.125*	CPO
		10 µg	< 28	> 0.25†	
Enterobacterales	Amikacin and	30 µg	< 18	> 8	Resistant to all three agents
	gentamicin and	10 µg	< 17	> 2	
	tobramycin	10 µg	< 16	> 2	
***Enterococcus* species**	Linezolid	10 µg	< 20	> 4	Linezolid-nonsusceptible
Pseudomonas aeruginosa	Meropenem	10 µg	< 20	> 2	CPO
	Piperacillin–tazobactam	30-6 µg	< 18	> 16	
***Salmonella* species**	Cefotaxime or	5 µg	< 20	> 1	Ceftriaxone-nonsusceptible
	Ceftriaxone	30 µg	< 27	> 1	
***Shigella* species**	Ampicillin	10 µg	< 14	> 8	Multidrug-resistant strains: Resistant to any three antimicrobial categories
	Azithromycin	n/a	n/a	> 16	
	Ciprofloxacin	5 µg	< 22	> 0.5	
	Norfloxacin (urine isolates)	10 µg	< 24	> 0.5	
	Co-trimoxazole	1.25–23.75	< 15	> 4/76	
	Ceftriaxone or	30 µg	< 24	> 2	
	Cefotaxime or	5 µg	< 17	> 2	
	ceftazidime	10 µg	< 19	> 4	
***Staphylococcus aureus* complex**	Vancomycin§	n/a	n/a	> 2#	Vancomycin-NS
	Linezolid	10 µg	< 21	> 4	Linezolid-nonsusceptible
Streptococcus pyogenes	Penicillin	1 U	< 23	> 0.03	Penicillin-NS

CPO = carbapenemase-producing organism; n/a = not applicable; NS = non-susceptible

* Card limitation (Vitek 2 AST-N246, AST-N247, AST-N435, **AST-N710, AST-N711**) meropenem range is 0.25–16 mg/L

† *Citrobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia*

§ MIC method only

\# MIC strips: > 3 mg/L

Table A3.2 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†	
	Amikacin	30 µg	≤ 16	≥ 16	Resistant to all three agents
	Gentamicin	10 µg	≤ 14	≥ 8	
	Tobramycin	10 µg	≤ 12	≥ 8	
***Enterococcus* species**	Linezolid	30 µg	≤ 20	≥ 8	Linezolid-nonsusceptible
Pseudomonas aeruginosa	Meropenem	10 µg	< 19	> 2	CPO
	Piperacillin–tazobactam	100/10 µg	< 22	> 16	
***Salmonella* species**	Cefotaxime or	30 µg	< 26	> 1	Ceftriaxone-nonsusceptible
	Ceftriaxone	30 µg	< 23	> 1	
***Shigella* species**	Ampicillin	10 µg	≤ 13	≥ 32	Multidrug-resistant strains: Resistant to any three antimicrobial categories
	Azithromycin	15 µg	≤ 10	≥ 32	
	Ciprofloxacin	5 µg	≤ 21	≥ 1	
	Co-trimoxazole	1.25–23.75	≤ 10	≥ 4/76	
	Ceftriaxone or	30 µg	≤ 19	≥ 4	
	Cefotaxime	30 µg	≤ 22	≥ 4	
***Staphylococcus aureus* complex**	Vancomycin§	–#	–#	> 2**	Vancomycin-NS
	Linezolid	30 µg	≤ 25	> 4	Linezolid-nonsusceptible
Streptococcus pyogenes	Penicillin	10 U	< 24	> 0.125	Penicillin-NS

CPO = carbapenemase-producing organism; n/a = 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

Table A3.3 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
	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; NS = non-susceptible

* Blood Sensitest agar, CO_2, 35–37oC

† Not calibrated

Appendix 4: Tests performed at confirming laboratories

Organism	CAR	Confirmed MIC	CARAlert	Molecular targets
***Acinetobacter baumannii* complex**	Carbapenemase-producing		Molecular confirmation	OXA-23-like, 24/40-like, 58-like, 134-like, 143-like, 542; IMP, VIM, KPC, NDM
Candidozyma* (*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)
	16S rRNA methyltransferases		Molecular confirmation	At least *armA, rmtA, rmtB, rmtC, rmtF.* Other types (*rmtD, rmtE, rmtG, rmtH, npmA)* optional
	Colistin mobile resistance		Molecular confirmation	*MCR* **(genes other than *mcr-9*)**
***Enterococcus* species**	Linezolid-nonsusceptible	MIC > 4 mg/L	Molecular confirmation	Mutations in 23S rRNA (especially G2576T); ***cfr, optrA, poxtA* resistance genes**
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* species**	Ceftriaxone-nonsusceptible	MIC > 1 mg/L	ESBL or AmpC phenotype confirmed	SHV, TEM, CTX-M types, plasmid-mediated AmpC
***Shigella* species**	Multidrug-resistant		ESBL or AmpC phenotype confirmed	**If ceftriaxone resistant**, SHV, TEM, CTX-M types, plasmid-mediated AmpC
***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 biosynthesis/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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