Ministry of Health

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Contents Table:

Acknowledgment

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Acronyms:

BA	Blood agar
CA	Chocolate agar
MAC	macConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
QC	Quality Control
CRE	Carbapenem Resistance Enterobacteriaceae
CPE	Carbapenemase Producing Enterobacteriaceae
MDRA	Multi-Drug Resistance Acinetobacter
MDROs	Multi-Drug Resistance Organisms
SOP	Standard operating procedure
TAT	Turnaround time
GNB	Gram-Negative Bacilli
HAI	Hospital acquired infection
HAC	Hospital acquired hospitalisation
MHT	Modified Hodge test
CIM	Carbapenemase inactivation method
ETP	Ertapenum

1. Purpose

This document describes the procedure for processing specimens for MDROs screening. MDROs includes Carbapenem Resistance Enterobacteriaceae (CRE) & Multi-Drug Resistance Acinetobacter (MDRA).

2. Scope

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. Definitions

3.1 MDROs: microorganisms that are resistance to at least a drug in three antimicrobial groups that target and treat infections with those specific bacteria.

3.2 CRE: Enterobacteriaceae microorganism that is resistance to a single or all carbapenem agents by mean of production of carbapenemases or other mechanisms.

3.3 CPE: Enterobacteriaceae microorganism that is resistance to a single or all carbapenem agents by mean of production of carbapenemases .

3.4 MDRA: Acinetobacter baumanni that is resistance to at least a drug in three antimicrobial groups that target Acinetobacter baumanii species.

3.5 Carbapenemase : β-lactamase that hydrolyses carbapenems.

3.6 Carbapenems: any or all of doripenem , ertapenem, imipenem and meropenem.

3.7 Enterobacteraceae: a term used to describe groups of Gram-negative bacilli that commonly live in the gastrointestinal tract and includes organisms such as: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundii*.

4. Procedure

4.1. Clinical background:

Multidrug-resistant organisms (MDRO) have significant infection control implications and are currently affecting the clinical course of patient's healthcare institutes. Carbapenemase-producing Enterobacteriaceae (CPE) are a type of Enterobacteriaceae that are resistant to carbapenem antibiotics. These bacteria carry a gene for a carbapenemase enzyme that breaks down carbapenem antibiotics. There are different types of carbapenemases, of which KPC, OXA-48, NDM and VIM enzymes are currently the most common.

Infections caused by CPE are associated with high rates of morbidity and mortality and can have severe clinical consequences. Treatment of these infections is increasingly difficult as these organisms are often resistant to many and sometimes all available antibiotics.

Multi-Drug Resistance Acinetobacter baumannii(MDRA) is of particular importance in health care setting. The organism is widely distributed in nature and survives on moist and dry surfaces. Worldwide, multidrug-resistant *A. baumannii*(MDRA) has become a significant cause of hospital-acquired infections (HAI) and hospital-acquired colonizations (HAC) resulting in high morbidity and mortality in patients admitted to the intensive care units (ICU) and other departments as well. Strict adherence to infection control practices and environmental disinfection have been effective in controlling outbreaks. Appropriate strategies and practices must therefore be implemented to prevent the growing transmission of MDRO including weekly infection control surveillance cultures especially in ICUs.

4.2. Principle:

4.2.1 Ideally, a screening method should allow the growth of all MDROs, inhibit or differentiate other organisms, and allow direct identification tests to be performed on colonies.

4.2.2 Direct plating on selective medium like chromogenic agars has the advantage that results may be available within 24hr.

4.2.3 According to the risk assessment, MDROs screening swabs are important specimens that should be requested and processed prior to operations to rationalize the infection prevention and control measures required to contain the spread of resistance organisms.

4.2.4 Infection prevention and control team should be notified immediately for a positive preliminary result of MDROs screening specimens.

4.3.Pre – analytical stage:

4.3.1. Specimen collection, transport and storage

4.3.1.1 MDROs screening specimens are usually combination of swabs from different body sites including rectum, axilla, throat, groin or perineum. In high-risk situations, single swabs have poor negative predictive value. While recognising the limited sensitivity of a single swab it is recommended to send more than a single body swab.

4.3.1.2 Any sample type can be used however rectal specimens (swabs with visible faecal material or discoloration) are the most sensitive for detecting CPE colonisation or a faecal specimen. If a rectal swab is not feasible or acceptable any clinical specimens such as blood, wound swab or urine is suitable.

4.3.1.3 Swabs should be collected in appropriate transport medium with charcoal.

4.3.1.4 Dry swabs should not be used unless specimens can be transported immediately to the laboratory.

4.3.1.5 Swabs should be transported to the laboratory immediately through porters or pneumatic tube in sealed plastic bags.

4.3.1.6 Ensure that expired transport media is not used specimen is clearly labelled with the patient's name, number, date and time of collection, and type of sample.

4.3.1.7 Compliance with transport and storage regulations is essential.

4.3.1.8 If processing of swabs is delayed, refrigeration is preferable to storage at ambient temperatures. Delays of over 48 hours are undesirable.

4.3.1.9 Specimens for molecular methods should follow manufacturer's instructions. Specimens should be transported and processed as soon as possible.

4.3.1.10 All specimens are stored for one week (+/-) after processing or according to lab storage capacity.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
- Gram stain reagents - Blood agar - Chromogenic agars - Mueller Hinton agar 0.85% sterile saline - 5mL of BHI containing 10-ug carbapenem disk - CLED containing g Ciprofloxacin & g Vancomycin plate.	- 10 ul disposable loops - Cleaned glass slides - Sterile Pasteur pipettes - Bunsen burner - Staining rack	- Class 2 Biosafety cabinet - Hot plate - Light Microscope - Automated ID machine - Aerobic Incubator (35 - 37°C) - 0.5 McFarland standard

4.3.3. Safety precaution:

4.3.3.1 Laboratory procedures must be conducted in a microbiological safety cabinet Class 2.

4.3.3.2 Disinfect all work surfaces with 70% alcohol or a freshly prepared 10% bleach solution prior to testing and after processing.

4.3.3.3 All samples and reagents should be properly discarded according to the current standards for disposal of hazardous waste.

4.3.4. Quality control:

4.3.4.1 All reagents, kits, stains and media must pass the quality check before use.

4.3.4.2 Results of all tests are invalid if the QC test results are not as expected.

4.3.4.3 Ensure that expiry date of the swabs and plates are not used beyond the expiry date.

4.3.4.4 All media used should be examined visually just before use to ensure that there is no contamination or deterioration appearing as lysis, discoloration, drying, shrinkage, or cracking of the media.

4.3.4.5 The ability to recover MDRA using this procedure could be assessed as follows:

1. Inoculate 5mL of BHI containing the 10-ug carbapenem disk with a known CRE- / MDRA negative specimen swab.
2. Inoculate the BHI with 0.5 mL of a 1 x 10^5 CFU/mL suspension of carbapenemase-producing K. pneumoniae ATCC BAA-1).
3. Proceed with Step 2 of the procedure.
4. The carbapenemase-producing K. pneumonia should be recovered on the CLED containing g Ciprofloxacin & g Vancomycin plate.
5. Quality control testing of carbapenem disc using disk diffusion methods and quality control strains as described in the CLSI guideline documents M2 and M100 (1).

4.4.Analytical stage:

4.4.1. Swabbing

- Pooled swabs or direct swabbing methods may be used.
- Use of correct swabbing technique has been shown to improve bacterial recovery rate.

4.4.1.1 Direct method using chromogenic agar (CRE / MDRA commercial chromogenic agars)

1. Inoculate media and streak for individual colonies.
2. Incubate aerobically at 35 - 37°C for 48 hours.
3. Reading should be after 18-24 hrs. If negative, re-incubate the plates and take reading after 48 hrs.
4. Check for specific-coloured colonies on chromogenic agar or according to manufactured recommendations.

4.4.1.2 Direct culture method using MacConkey / CLED agar:

1. Inoculate swabs or pooled swabs in to MacConkey or CLED agars and streak for individual colonies using a sterile loop.
2. After streaking, Place the ertapenum (ETP) or carbapenem disc in the inoculated MacConkey or CLED agars.
3. Incubate aerobically at 35 - 37°C for 48 hours.
4. Reading should be after 18-24 hrs. If negative, re-incubate the plates and take reading after 48 hrs.

5. Check for typical colonies around the carbapenem disc and the presence or absence of inhibition zone around the disc.
6. Perform Gram stain and Oxidase test to confirm Gram-Negative Bacilli.
7. For typical GNB, send for full ID either conventionally or through automated ID machines.

4.4.1.3 Indirect Enrichment culture method (pooling method):

1. Aseptically, inoculate pre prepared 5 ml Brain Heart Infusion Broth (BHI) with carbapenem discs with the (rectal, perineum, or throat) swab.
2. Incubate overnight at 35 ± 2ºC, ambient air.
3. Next day, Vortex and subculture the incubated broth culture onto CLED Ciprofloxacin & Vancomycin agar.
4. Streak for isolation and incubate overnight at 35 ± 2ºC, ambient air
5. Examine plates for CRE:
   - Lactose-fermenting (pink-red) colonies. More than one colony morphology may represent different species of Enterobacteriaceae .
   - It may be necessary to subculture representative colonies of each morphology type to a non-selective media for isolation and/or for susceptibility testing.
6. Perform follow up with the suspected isolate.
7. Identify all Lactose fermenters to species level and perform sensitivity testing.
8. Confirm CRE with Modefied Hodge Test (MHT) and Metalo-Beta- Lactamase (MBL) E test.
9. Examine plates for MDRA
   - Non-Lactose-fermenter (pale) colonies.
   - perform gram stain (gram negative coccobacilli) and oxidase test.
   - If oxidase positive: ignore
   - If oxidase negative: Perform follow up with the suspected isolate.
10. Reading should be after 18-24 hrs. If negative, re-incubate the plates and take reading after 48 hrs.

4.4.2. Culture

Table 1. The culture conditions:

<table>
<tr><th>Clinical details/ conditions</th><th>Specimens</th><th>Media</th><th colspan="3">Incubation</th><th>Cultures read</th><th>Target organisms</th></tr>
<tr><th></th><th></th><th></th><th>Temp ºC</th><th>Atmos</th><th>Time</th><th></th><th></th></tr>
<tr><td rowspan="2">Direct culture</td><td rowspan="3">MDROs screening specimens</td><td>Chromogenic selective mediums</td><td colspan="4">Refer to manufacturer’s instructions</td><td>CRE/CPE</td></tr>
<tr><td>MacConkey / CLED agar +
10µg ETP disc</td><td>35-37</td><td>Aerobic</td><td>16-48hr</td><td>≥16hr</td><td>MDRA</td></tr>
<tr><td colspan="8">OR</td></tr>
<tr><td rowspan="3">Enrichment culture (Pooling method)</td><td rowspan="3">MDROs screening specimens</td><td>Pool samples in BHI with ETP disc</td><td>35-37</td><td>Aerobic</td><td>18-24hr</td><td>NA</td><td rowspan="3">CRE/CPE
MDRA</td></tr>
<tr><td>CLED</td><td colspan="4"></td></tr>
<tr><td>Ciprofloxacin & g Vancomycin agar.</td><td>35-37</td><td>Aerobic</td><td>16-48hr</td><td>≥16hr</td></tr>
<tr><td colspan="8">* Molecular methods may be considered if a rapid result is required</td></tr>
</table>

4.4.3. Identification

- Identification can be performed by automated identification systems or conventional biomedical tests. It is essential that the organism is identified to species level to enable correct interpretation of test results.

- Following identification and sensitivity, CRE confirmation tests should always be done using one of the following tests:
    - **Modified Hodge Test (MHT) or 'Cloverleaf' test:** is a phenotypic bioassay to assess the ability of a test strain to hydrolyse carbapenems. However, it is not recommended due to concerns over its specificity and sensitivity, with several proven carbapenemase producers giving consistently negative results.
    - **Synergy tests** are most effective for members of the Enterobacterales. Although EDTA/dipicolinic acid-based synergy tests may also be useful for non-fermenters, EDTA-based tests give a high proportion of false-positive results for these organisms.
    - **Carbapenem Inactivation Method (CIM):** a phenotypic test developed to detect carbapenemase activity by incubating a carbapenem disc within the test bacterial suspension. Following two hours' incubation the disc is placed on an agar plate inoculated with *E. coli* ATCC 25922 and incubated for a minimum of six hours. Inactivation of the carbapenem due to carbapenemase activity will produce no zone around the disc, whereas no carbapenemase activity will produce a zone. Different variants of the CIM test have been published, which report improvements over the original version (mCIM).
    - **Molecular methods:** Different commercial kits use different combinations of genetic specific targets. Assessment of available methods indicated good performance and results in 2-3hr even using in house methods.
    - **Automated or semi-automated systems** generally can be used to detect carbapenem resistance though the ability of software to infer and warn correctly of the presence of carbapenemases is variable, especially for OXA-48-like enzymes. For this reason, the underlying resistance mechanisms inferred by expert algorithms should be viewed with caution.

4.4.4. Antimicrobial susceptibility testing:

- The susceptibility of MDROs strains from screening samples should follow the CLSI guidelines.

- Susceptibility should be reported if required.
- The susceptibility can be done either in automated ID machines when available or manual using disc diffusion methods as follows:

4.5.Post – analytical stage:

4.5.1. Reporting:

- Negatives: “MDROs (CRE & MDRA) are not detected.
- Positives: “MDROs (CRE & MDRA) are detected”, along with the susceptibility report if required and a comment mentioning the importance of notifying and adhering to infection prevention and control precautions

5. Responsibilities

5.1.Responsible staff:

- To ensure the results are verified by microbiologist or pathologist.
- To ensure adherence to critical result communication procedure.
- To facilitate the alternative communication channels once needed.

5.2.Quality manager /officer

- To follow up the implementation of the procedure.
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended.
- To report test failures or incident.

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
1. 1_cpe-acute-toolkit-v1.3.pdf [Internet]. [cited 2022 Oct 24]. Available from: https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2531/documents/1_cpe-acute-toolkit-v1.3.pdf			
1. B_60i3.1.pdf [Internet]. [cited 2022 Oct 24]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1080594/B_60i3.1.pdf			