Ministry of Health

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Contents Table:

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Acronyms:

ATCC	American Type Culture Collection
ASP	Antimicrobial stewardship Program
ID	Identification
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant Staph. Aureus
SOP	Standard operating procedure
AST	Antibiotic Susceptibility Testing
CLSI	Clinical Laboratory Standards Institute
ESBL	Extended Spectrum Beta Lactamase
EUCAST	European Committee of Antimicrobial Susceptibility testing
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin resistant Staphylococci
VRE	Vancomycin Resistant Enterococci
CPE	Carbapenem Producing Enterobacteriaceae
MHA	Muller Hinton Agar
MHBA	Muller Hinton Blood Agar
MHT	Modified Hodge test
MBL	Metallo β lactamase
NS	Non susceptible

1. Purpose

This document describes the procedure for standard in-vitro disc diffusion susceptibility testing. It does not include fungal testing.

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 MIC: Minimal inhibitory concentration, is the concentration of the antibiotic at which bacteria is destroyed

3.2 Antibiogram: the overall profile of antimicrobial susceptibility testing results of a specific bacteria to a battery of antibiotics.

3.3 Breakpoint: Minimal inhibitory concentration (MIC) or zone diameter value used to categorize bacteria as susceptible, susceptible dose dependent, Intermediate, non susceptible or resistant.

3.4 Susceptible (S) – isolate is inhibited by the usually achievable concentrations, resulting in likely clinical efficacy.

3.5 Susceptible-dose dependent (SDD) – susceptibility of an isolate depends on the dosing regimen that is used in the patient. (Should be supported by the literature, widely used clinically, and/or approved)

3.6 Intermediate (I) – response rates may be lower than for susceptible isolates. Use should be guided by likely concentration at the organ/space, site & severity of infection, and clinical data.

3.7 Resistant (R) – isolate is not inhibited by the usually achievable concentrations of the agent.

3.8 Non susceptible (NS) –only a susceptible breakpoint is designated because of the absence or rare occurrence of resistant strains.

3.9 Routine test: disc diffusion or agar dilution MIC tests

3.10 Supplemental test: test to detect susceptibility or resistance to a drug or drug class by method other than routine tests (e.g. inducible clindamycin resistance, D-test)

3.11 Surrogate testing: test performed with an agent that replaces a test performed with an agent of interest. Used when the agent of interest cannot be tested due to non-availability

or performance issues.(e.g. cefoxtin testing to predict Mec-A mediated oxacillin resistance)

3.12 Screening test: test that provide presumptive results, additional test only needed for specific results (e.g. Ertapenem screen for CRE, additional test needed if resistant)

3.13 Equivalent agent testing: agent predicts results of closely related agent of the same class. (e.g. cefotaxime or ceftriaxone for enterobacterales)

4. Procedure

4.1. Clinical background:

Antibiotic susceptibility testing is an important step in patient management. It is measured by disc diffusion, broth dilution or agar dilution methods. It guides clinicians to choose appropriate antibiotics for each patient. Moreover, it very essential component of any antimicrobial stewardship program (ASP). Another point to keep in mind is that the infection preventionist nurse will rely on the results of antibiotic susceptibility testing to decide on isolating patient and labeling a patient as colonized with MDROs.

In addition, it is also used to accumulate epidemiological information on resistance pattern of isolated organisms to be used for interventions at hospital or community levels. These data also used to build an antibiogram which helps to decide about empirical choice of antibiotics.

4.2. Principle:

A standard inoculum of pure growth of an organism is uniformly inoculated in susceptibility testing media such as Muller Hinton Agar (MHA) . Filter paper discs impregnated with antibiotics are then placed at specified distances and the zone of growth inhibition around each disc is measured after overnight incubation. These zone diameters are inversely proportional to MIC (minimum inhibitory concentration). The CLSI guide is followed to interpret the zone diameters as susceptible, intermediate or resistant against each isolate.

4.3. Pre – analytical stage:

4.3.1. Sample: Young bacterial growth, 18-24 hrs. old.

Table 1- List of commonly used antibiotic

Antimicrobial class	**Agents included**
Penicillins	Penicillin (10 Units), Ampicillin (10 µg)
β-Lactam / β-lactamase inhibitor combinations	Amoxicillin-clavulanic acid (20/10 µg) Piperacillin-tazobactam (100/10 µg)
Cephalosporins	Cefazolin (30 µg) Cefuroxime (30 µg) Cefotaxime (30 µg) Ceftazidime (30 µg) Ceftriaxone (30 µg) Cefepime (30 µg)
Cephalosporins	Cefuroxime (30 µg)
Carbapenem	Ertapenem (10 µg) Imipenem (10 µg) Meropenem (10 µg)
Aminoglycosides	Amikacin (30 µg) Gentamicin (10 µg) Gentamicin (120 µg), high level resistance
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (1.25/23.75 µg)
Glycopeptides	Vancomycin (30 µg)
Lincosamides	Clindamycin(2 µg)
Polymyxins	Colistin
Macrolides	Azithromycin (15µg) Clarithromycin (15µg) Erythromycin (15µg)
Nitrofurans	Nitrofurantoin (300µg)
Nitroimidazoles	Metronidazole
Oxazolidinones	Linezolid (30µg)
Phenicols	Chloramphenicol (30µg)

Quinolones	Ciprofloxacin (5µg) Levofloxacin(5µg)
Tetracyclines	Tetracycline (30µg) Tigecycline

Note:

- the above list of antibiotic includes the most frequently used antibiotics. Each lab should keep a stock of them and be able to perform susceptibility testing
- Specific antibiotics should be tested for each type of bacteria.
- selective reporting of antibiotic will depend on overall susceptibility pattern, type of bacteria, site of infection and availability of antibiotics in the pharmacy.
- CLSI guideline should be used to guide the selection of antibiotic testing for each organism type and antibiotic reporting priority. Refer to updated CLSI guidelines M100, M45 to interpret the breakpoints.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
Muller-Hinton Agar Mueller Hinton with 5% sheep blood, Haemophilus Test Medium.	Sterile cotton tipped swabs Sterile saline (3-4ml) Antibiotic impregnated discs E-test strips	Forceps Automatic disc dispenser or template with 6 disc spacing pattern Incubator with correct atmosphere at appropriate temperature (O2, CO2, AnO2) Ruler to measure inhibition zones Moveable light source

4.3.3. Safety precaution:

- All specimens need to be treated as potentially infectious. Standard procedures for handling of biohazard material must be followed at all times. Universal Precautions must be practiced at all stages of these procedures.

4.3.4. Quality control:

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.
- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.

4.3.5. QC for Disc Diffusion method:

The quality of disc diffusion method is assured by testing ATCC control strains as recommended by CLSI.

4.3.5.1 For ATCC strains maintenance, Refer to Reference strains maintenance SOP.

4.3.5.2 For the procedure of inoculation and culture of ATCC control strains, follow sections 4.5.1 and 4.5.2.

4.3.5.3 QC Results—Documentation:

- Results from all disc diffusion QC test should be documented on a QC sheet. See annex 8.1.
- You need to document the following: Disc content (ug) , Disc Lot number, Date Opened, Expiry date, Standard QC Range (mm).
- QC ranges are provided in tables 4A, 4B, 5A, and 5B in the CLSI M100 document.
- The QC results must be counterchecked and signed by senior technician / supervisor before reporting patient's results.
- Record details of media used for testing, QC failures and corrective actions done.

4.3.5.4 QC—Frequency of Testing

Appropriate QC strains should be tested on each day the test is performed on patient isolates. Alternatively, one of two plans can be adopted to demonstrate acceptable performance to reduce the frequency of disc diffusion QC tests from daily to weekly. Either plan enables weekly QC testing once satisfactory performance with daily testing of QC strains is documented. There are instances where further testing will be required e.g. new disc shipment, modifications in the testing process etc. For guidance

on the frequency of testing in such instances, refer to tables 4C and 5F in the M100 CLSI document.

The weekly QC testing options are not applicable when disc diffusion tests are performed less than once a week.

4.3.5.5 Proceeding from Daily to Weekly QC

- Plan 1 the 20 -day or 30-day plan:
  - Test appropriate QC strains and antimicrobial agents for 20-30 consecutive test days. Record the results for each agent, each day it is tested.
  - For each drug/organism combination, no more than 1 out of 20, or 3 of 30 zone diameters (or MIC results) may be out of the acceptable CLSI-defined QC range. However, if any drug/organism result is out-of-range on two successive days, this must be investigated.
  - If the above conditions are met, you may begin weekly QC, if desired.
- Plan 2: The 15-replicate (3x5) plan:
  - Three replicates of each applicable QC strain are tested using individual inoculum preparations for five consecutive test days and the results are documented.
  - If no more than 1 out of range result is obtained for each drug/organism combination, the plan is considered successful and it is acceptable to convert to weekly testing.
  - If completion of the 15-replicate (3- × 5-day) plan is unsuccessful, corrective action should be taken, as appropriate and daily QC testing continued.

4.3.5.6 Out-of-range QC results

Once weekly QC testing is implemented, if an out-of-range QC result is obtained, try to find an identifiable error (e.g., wrong disc concentration, wrong media…etc). Tables 4D and 5G in the CLSI M100 document provides guidance on troubleshooting of out-of-range QC results. If no

identifiable error is found, this could be a random error (a one-off) or a system-related error. See Fig 1 for guidance on how to proceed if you get one out-of-range QC result

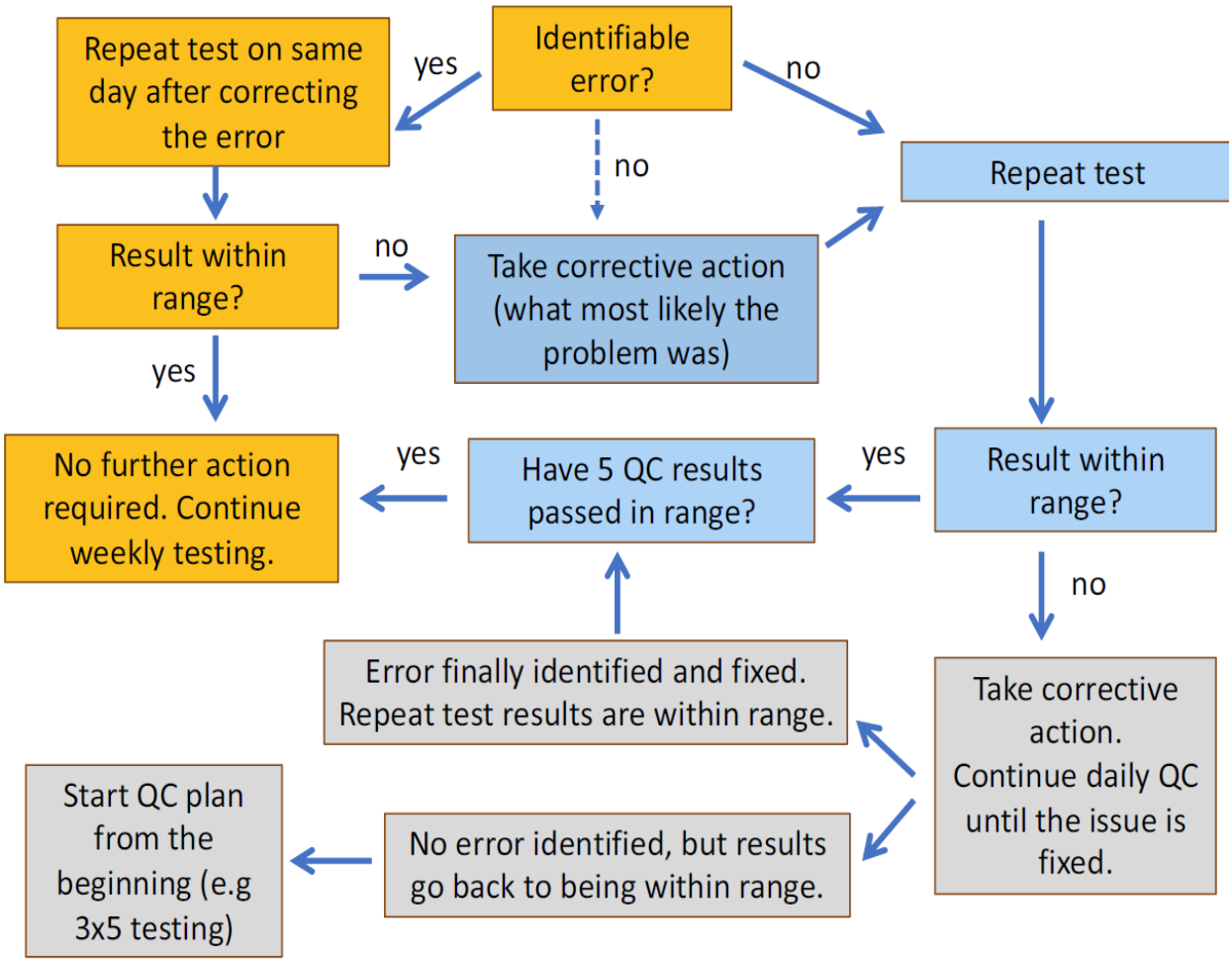

Fig 1flowchart guidance for out-of-range results

Test organism	Recommended QC
Enterobacteriaecae	*Escherichia coli* ATCC^{O} 25922 , *Escherichia coli* ATCC^{O} 35218 (for β-lactam combinations)
Pseudomonas aeruginosa	*Pseudomonas aeruginosa* ATCC^{O} 27853, *Escherichia coli* ATCC^{O} 35218 (for β-lactam combinations)
Acinetobacter species	*Escherichia coli* ATCC^{O} 25922 (for trimethoprim-sulfamethoxazole and tetracycline)
Stenotrophomonas maltophilia	*Escherichia coli* ATCC^{O} 25922 (for chloramphenicol, minocycline, and trimethoprim-sulfamthoxazole)
Staphylococcus species	*Staphylococcus aureus* ATCC^{O} 25923 (disk diffusion), *Staphylococcus aureus* ATCC^{O} 29213 (dilution methods)
Enterococcus species	Staphylococcus aureus ATCC^{O} 25923 (disk diffusion) Enterococcus faecalis ATCC^{O} 29212 (dilution methods)
Haemophilus influenzae and Haemophilus parainfluenzae	*Haemophilus influenzae* ATCC^{O} 49247 *Haemophilus influenzae* ATCC^{O} 49766 *Escherichia coli* ATCC^{O} 35218 (when testing amoxicillin-clavulanic acid)
Neisseria	*Neisseria gonorrhoeae ATC^{O} 49226*
Streptococcus pneumoniae	*Streptococcus pneumoniae ATCC^{O} 49619*
Streptococcus spp. β-Hemolytic Group	*Streptococcus pneumoniae* ATCC^{O} 49619
Streptococcus spp. Viridans Group	*Streptococcus pneumoniae* ATCC^{O} 49619
Neisseria meningitidis	*Streptococcus pneumoniae* ATCC^{O} 49619 *E. coli* ATCC^{O} 25922

Table 1 -Routine QC recommendations for each tested isolate

4.3.6. Test/Report groups:

- These antibiotic groups are classified based on CLSI guidelines. Refer to CLSI guidelines to find a specific antibiotic under each of the following group:

  Group A

  Includes antibiotics that are considered appropriate for inclusion in a routine, primary testing panel as well as for routine reporting of results for the specific organism groups.

  Group B

  Includes antimicrobial agents that may warrant primary testing but they may be reported only selectively, such as when the organism is resistant to agents of the same class, as in Group A. Other indications for reporting the result might include a selected specimen source (e.g., a third-generation cephalosporin for enteric bacilli from cerebrospinal fluid or trimethoprim-sulfamethoxazole for urinary tract isolates); a polymicrobial infection, infections involving multiple sites; cases of patient allergy, intolerance, or failure to respond to an agent in Group A; or for purposes of infection control.

  Group C

  Includes Alternative or supplemental antimicrobial agents that may require testing in those institutions that harbor endemic or epidemic strains resistant to several of the primary drugs (especially in the same class, e.g., beta-lactams); for treatment of patients allergic to primary drugs; for treatment of unusual organisms (e.g., chloramphenicol for extra-intestinal isolates of *Salmonella* spp.); or for reporting to infection control as an epidemiological aid.

  Group U (“urine”)

  Includes, antimicrobial agents (e.g., nitrofurantoin and certain quinolones) that are used only or primarily for treating urinary tract infections. These agents should not be routinely reported against pathogens recovered from other sites of infection. Other agents with broader indications may be

included in Group U for specific urinary pathogens (e.g., *P. aeruginosa* and ofloxacin).

Group O ("other")

Includes antimicrobial agents that have a clinical indication for the organism group, but are generally not candidates for routine testing and reporting.

4.4. Storage and handling:

4.4.1 Storage

- Do not use the product beyond the stated expiry date.
- Once the cartridge is open ensure it is stored in an opaque desiccated environment to prevent degradation of the antibiotic.
- If the disc does not produce the expected zone sizes with recommended control organisms, check the entire procedure
- Unopened cartridge must be stored at -20c to 8 c until required
- Unopened cartridges should be allowed to come to room temperature before removing them from the packaging to minimize condensation as it may reduce antimicrobial potency
- Expiry date is valid only for unopened pack stored under proper condition.
- Once the cartridge open they need to be stored in the dispenser with container provided

4.4.2 Handling:

- After removing unopened containers of discs from freezer or refrigerator, allow them to equilibrate to room temperature (requires at least 1 hr.) prior to opening to minimize condensation.
- Bring agar plates to room temperature before use to avoid condensation.

4.5. Inoculum preparation

4.5.1 Direct colony suspension method:

- Prepare the inoculum by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate (use a nonselective medium, such as blood agar).
- Adjust the suspension to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 × 10^8

colony-forming units (CFU)/mL for *Escherichia coli* ATCC® 25922. To perform this step accurately, use either a photometric device or, if performed visually, use adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

4.5.2 Growth enhancement method (alternative in case of tiny growth or inadequate colonies or to preserve the colony for different purposes):

- Touch the top of the colony with a loop or sterile swab and transfer the growth into a tube containing 4 to 5 mL of a suitable enriched broth medium, such as BHI broth.
- Incubate the broth culture at 35 ± 2°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually two to six hours).
- Adjust the turbidity of the actively growing broth culture with same broth to achieve a turbidity equivalent to that of a 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 × 10^8CFU/mL. To perform this step accurately, use either a photometric device or, if performed visually, use adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

4.6. Inoculation into sensitivity media:

4.6.1 Subculture the organism to be tested onto a non-selective appropriate agar plate and incubate for 18-24 hours to obtain a pure growth.

4.6.2 Remove the antibiotic discs from the fridge so they reach room temperature before the container is open (to avoid condensation and subsequent deterioration). Containers must contain active desiccant. Replace the discs and containers in the refrigerator as soon as you have finished using them .Do not use discs past their expiry date.

4.6.3 Within 15 minutes after adjusting the turbidity of the inoculums, immerse a sterile cotton swab into the emulsion. Press the swab against the inner side of the tube, above the fluid level, to remove excess fluid.

4.6.4 Use the appropriate incubation conditions and plates for both purity and testing.

4.6.5 Inoculate the entire agar surface of the plate, either using a plate rotator or by spreading the plate 3 or 4times, rotating the plate 60° (or 90°) between the streaks and then swabbing the rim of the agar surface.

- The plate to be inoculated should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the Petri dish covers. If so, the plate and its lid should be left between 10-30 minutes in the Biosafety cabinet to dry.
- Care should be taken not to mark the agar by too much pressure when streaking and that there is evenness of spread, particularly at the edge.
- The plate may be left to dry for 3-5 minutes (no more than 15 minutes) after streaking to allow for any excess surface moisture to be absorbed.

4.6.6 Place discs of the appropriate antibiotics for the species on the plate using the automatic disc dispenser or manually using disc placing template. Single discs may be handled using sterile forceps.

- Avoid placing penicillin and cephalosporin discs next to each other.
- Discs need to be applied evenly on the agar surface; press gently on the disc after application.
- Because some antibiotics diffuse almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
- Discs should be applied no later than 15 minutes after the plates have been inoculated. Similarly, once the discs are applied, they should be put in the incubator within a 15 minutes interval to prevent pre-diffusion of the antimicrobial at room temperature.

4.6.7 Invert the plates and incubate in the correct atmosphere for the appropriate time.

Note: Agar plates should not be placed in stacks of more than 10 because the middle plates will take longer to reach the incubator temperature. This delay could cause overlarge zones.

Table 2: Media and incubation conditions

Organism	Media	Incubation conditions
*Aeromonas*spp. *Enterobacteriaceae* *Pseudomonas aeruginosa* *Vibrio* spp.	Mueller Hinton	35-37°C in air for 18-24h
*Acinetobacter*spp. *Burkholderiacepacia* *Burkholderiapseudomallei*[a] *Stenotrophomonasmaltophilia*		
Staphylococcus aureus	Mueller Hinton	35-37°C in air for 16-18h (**35°C for 24 hours for cefoxitin**)
Coagulase negative staphylococci	Mueller Hinton	35-37°C in air for 24 hours
Enterococci	Mueller Hinton	35-37°C in air for 16-18h **(24h for vancomycin)**
Haemophilus spp.	Haemophilus Test Medium	35-37°C in 5% CO_2 for 16-18h
Moraxella catarrhalis	Mueller Hinton	35-37°C in 5% CO_2 for 20-24h
Neisseria gonorrhoeae	GC agar base and 1% defined growth supplement (CA)	35-37°C in 5% CO_2 for 20-24h
Neisseria meningitides[*]	Mueller Hinton with 5% sheep blood	35-37°C in 5% CO_2 for 20-24h
Streptococcus pneumoniae, β-haemolytic streptococci and other streptococci	Mueller Hinton with 5% sheep blood	35-37°C in 5% CO_2 for 20-24h

*To be send to CPHL or processed in Class11, containment level III.

4.7. Reading antibiotic sensitivity culture plate:

- The diameter of the zone of inhibition includes the diameter of the disc. The end of the zone should be taken as the area showing no obvious visible growth that can be detected with unaided eyes. Ignore faint growth of tiny colonies that can only be detected with a magnifying lens at the edge of the zone of inhibited growth.
- When measuring zones on Mueller-Hinton plates with blood, the zone of growth inhibition should be measured NOT the zone of haemolysis inhibition. The zones should be measured from the upper surface of the agar, illuminated with reflected light, with the cover removed.
- In case of existence of a double zone, take the reading of the inner most zone provided the growth is pure (check the purity plate).
- The growth on the plates must be even and near confluent. If there are only isolated colonies, the test must be repeated.
- For staphylococci, the cefoxitin result should be reported as for “Cloxacillin”. Isolates that are resistant to cefoxitin should be reported as resistant to all beta-lactams (i.e. penicillin, oxacillin, co-amoxiclav, ceftriaxone..
- Results can usually be put into one of the following categories: susceptible, intermediate, resistant or non-susceptible.
- The susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution after the susceptible only breakpoint is set.

Table 3: Screening for antibiotics resistance:

Organism group	Resistance phenotype or mechanism	Screening test	Required confirmatory testing
Enterobacteriacae	ESBL production	Cefotaxime and ceftazidime	Inhibition by clavulanic acid
	Carbapenemase production	Ertapenem or Meropenem	Modified Hodge test and metallobetalactamase test modified carbapenem inactivation method
Staphylococcus aureus	Beta lactamase production	Penicillin disc diffusion zone edge test	No confirmatory test
	Mec A mediated Oxacillin resistance	Disc diffusion with cefoxitin	Non
	Vancomycin MIC ≥ 8ug/ml	Agar dilution, BHI with 6ug/ml of vancomycin	Vancomycin MIC
	Inducible clindamycin resistance	Disc diffusion with clindamycin and erythromycin	D- test
	High level mupirocin resistance	Disc diffusion with mupirocin	Non
Enterococci	Vancomycin MIC ≥ 8ug/ml	Agar dilution, BHI with 6ug/ml of vancomycin	Vancomycin MIC
	HLAR (high level aminoglycoside resistance)	Disc diffusion with gentamicin and streptomycin	If result is not conclusive: Agar dilution or broth dilution

Streptococcus pneumonia	Penicillin resistance	Disc diffusion with oxacillin	If oxacillin ≤19mm to do penicillin E-test
	Inducible clindamycin resistance	Disc diffusion with clindamycin and erythromycin	D- test
Beta haemolytic streptococci	Inducible clindamycin resistance	Disc diffusion with clindamycin and erythromycin	D-test

Note: Refer to CLSI guideline for procedure method of screening tests

4.8. Verification of tests results:

4.8.1 Where the antibiotics tested against isolates showed phenotype that:

- Have never been documented.
- Uncommon.
- Represent results that could easily occur from technical errors which may have significant clinical consequences.

4.8.2 Verify the result as follows:

- Check purity plate.
- Check previous reports on the patient.
- Confirm the identification of the isolate from the original isolation medium.
- Repeat susceptibility test to confirm the result.
- If the result is confirmed, notify the microbiologist/ In-Charge Technologist.
- When necessary, save the isolate and send to CPHL for confirmation.

Table 4: Examples of uncommon finding that needs verifications

Organism or Group	**Uncommon results**
Any organism	Resistant to all agents routinely tested
Gram-negative organisms	
Enterobacteriaceae	Carbapenem – I or R
Citrobacter freundii *Enterobacter* species	Ampicillin, Cefazolin – S

Organism or Group	Uncommon results
Serratia marcescens	
Klebsiella species *Proteus vulgaris* *Providencia* species	Ampicillin – S
Escherichia coli, Klebsiella species, *Proteus* species	Extended –spectrum cephalosporin –I or R
Salmonella and Shigella spp	Cephalosporin III –I or R Fluoroquinolone-I or R
Acinetobacter baumannii	Colistin – I or R Carbapenem- I or R
Stenotrophomonas maltophilia	Imipenem, Meropenem – S Trimethoprim-sulfamethoxazole-I or R
Pseudomonas aeruginosa	Colistin-I or R Amikacin, gentamicin and tobramycin –R Carbapenem-I or R
Haemophilus influenzae	Imipenem, Meropenem – not S 3rd generation cephalosporin – not S Fluoroqinolone – not S
Neisseria gonorrhoeae	3rd generation cephalosporin – R Flouroquinolone –I or R
Gram-positive organisms	
Enterococcus faecalis	Ampicillin or Penicillin – R Linezolid – I or R Vancomycin –R
Enterococcus faecium	Linezolid – I or R Vancomycin -R
Staphylococcus aureus	Linezolid – not S Vancomycin – I or R
Coagulase-negative *Staphylococcus*	Linezolid – not S

Organism or Group	Uncommon results
	Vancomycin – I or R
Streptococcus pneumoniae	3rd generation cephalosporin – R Fluoroquinolone–I or R Linezolid – not S Vancomycin – not S
B-heamolytic streptococci	Penicillin -R

4.9. Post analytical:

4.9.1 Reporting of antibiotics for Enterobacteriaceae:

Enterobacteriaceae [9,10,11]		
1ST Line	2ND Line	3rd line
Ampicillin Augmintin[1] Cefuroxime [2] Cephalothin[3] Gentamycin Co-Trimoxazole Ciprofloxacin Nitrofurantoin[4]	Amikacin [5] Ceftriaxone[6] Ceftazidime[7] Tazocin[7] Cefipime [7] Meropenem[7] Imipenem[7]	Colistin[8]

- Report If Ampicillin Resistant
- Report If Cephradine /Cephalothin Resistant
- Cephalothin interpretive criteria should only be used to predict results of the oral agents, cefadroxil, cefpodoxime,cephalexin, and loracarbef
- Report for urine samples only.
- Report if gentamicin resistant
- Ceftriaxone Predicts Susceptibility to Cefotaxime
- Report only if Resistant first line or as requested

- No disc diffusion breakpoints, only E-test., report for CRE isolates
- For ESBL Report All Cephalosporins and Augmentin as Resistant
- AmpC isolates are *Citrobacter SPP (*except *C.koseri), ,Enterobacter* spp., *Hafnia* spp., *Morganella morganii,, Proteus penneri, Proteus vulgaris, Providencia* species, *Serratia* species
- CRE isolates are R to carbapenem antibiotics

4.9.2 Reporting antibiotics for Pseudomonas aeruginosa

Pseudomonas aeruginosa[4]	
1st Line	2nd Line
Gentamicin Cefipime Ceftazidime Tazocin Ciprofloxacin Tobramycin[1]	Amikacin[2] Colistin[3] Imipenem Meropenem

- Report for patients with cystic fibrosis
- Report If Gentamycin Resistant
- No disc diffusion breakpoints, only E-test. Report only for MDR isolates
- MDR Pseudomonas: At least three classes the result showed Resistance or intermediate to all tested antibiotic of the same class:
  - Aminoglycoside: CN/AK
  - Penicillin: TZP
  - Cephalosporins: CAZ, CEP
  - Quinolones: CIP
  - Carbapenems: MEM, IMP

4.9.3 Reporting antibiotics for Acinetobacter Species

Acinetobacter Species[5]	
1st line	2nd
Gentamicin Cefipime[1] Ceftazdime Ceftriaxone Tazocin Ciprofloxacin Co-trimoxazole	Amikacin[2] Colistin[4] Imipenem[3] Meropenem [3]

- Report If ceftriaxone or ceftazidime are Resistant
- Report If Gentamycin Resistant
- Not routinely reported
- No disc diffusion breakpoints, only E-test. Report only for MDR isolates
- MDR Acinetobacter: At least three classes the result showed Resistance or intermediate to all tested antibiotic of the same class:
  - Aminoglycoside: CN/AK
  - Penicillin: TZP
  - Cephalosporins: CRO, CAZ, CEP
  - Quinolones: CIP
  - Carbapenems: MEM, IMP

4.9.4 Reporting antibiotics for streptococcus species

Streptococcus spp [1]	
1st	2nd
Penicillin Ceftriaxone[2] Erythromycin Clindamycin	Vancomycin[3]

- The viridans group of streptococci includes the following five groups, with several species within each group: *mutans* group, *salivarius* group, *bovis* group, *anginosus* group (previously "*S. milleri*" group), and *mitis* group. The *anginosus* group includes small colony–forming β-hemolytic strains with Groups A, C, F, and G antigens.
- Report If pencillin R
- Report only if pencillin and ceftriaxone R or if the patient is allergic to B-lactam.

4.9.5 Reporting antibiotics for Beta hemolytic streptococcus

Beta Haemolytic Streptococci(group A,B,C,G)	
Ist line	2nd
Pencillin [1] Clindamycin [2] erythromycin[2]	Vancomycin [3]

- Notify microbiologist if penicillin R
- Not reported for urine.
- Report for penicillin allergic patient or if requested.

4.9.6 Reporting antibiotics for Enterococcus spp

Enterococcus spp[5]	
Ampicillin [1] Ciprofloxacin [2] Nitrofurantion[2] Vancomycin[3] Gentamycin-synergy[4]	Linezolid[6]

- Ampicillin results predict susceptibility to amoxicillin-clavulanic acid, piperacillin, tazobactam among non β-lactamase producing enterococci
- Report for urine sample only

- Report only if ampicillin R or the patient is allergic to B-lactam .
- Verify and confirm vancomycin resistance by Etest
- for blood culture iso;ates, use high potency disc
- E.gallinarum and E. casseliflavus are intrinsically R to Vancomycin
- Release only for VRE isolates

4.9.7 Reporting antibiotics for pneumococcus

Strept.pneumoniae	
1st line	2nd line
Penicillin [1] [2] Ceftriaxone[1] [2] [3] Erythromycin[4] Clindamycin Levofloxacin	Meropenem [5] Vancomycin[6]

- For CSF and blood isolates report PEN and CRO sensitivity based on E test. Report according to meningitis and non-meningitis breakpoints.
- For isolates other than blood/ CSF, report according to oxacillin disk diffusion test. If OXA zone ≥20mm report as sensitive to penicillin and ceftriaxone. If OXA zone is ≤ 19 mm then Do PEN , CRO E- test
- Report only if Pen I or R
- Report Erythromycin for respiratory specimens only
- Report if requested only
- Report if R to ceftriaxone or B lactam allergy or if pneumococcal meningitis cannot be ruled out clinically.

4.9.8 Reporting antibiotics for S.aureus and S.ludgunensis

First line	Second line
Cloxacillin [1] Trimethoprim/Sulphamethoxazole Erythromycin	Ciprofloxacin Tetracycline2 Gentamicin 4

Clindamycin Rifampicin3	Nitrofurantion5 Vancomycin6 Linezolid4

- Base on Oxacillin MIC /Cefoxitin MIC or Disc Diffusion result
- Tetracycline predicated activity of Doxycycline ,Tetracycline sensitive isolates can be regarded as Doxycycline sensitive
- Rifampicin Must not be given alone for treatment
- Report if requested.
- Report only for urinary isolate.
- Report Only if Oxacillin/ Cefoxitin is R Or B lactam allergy

4.9.9 Reporting antibiotics for Coagulase negative staphylococcus[1]

First line	Second line
Vancomycin Clindamycin Rifampicin Trimethoprim/Sulphamethoxazole	Teicoplanin Linezolid Cloxacillin

- Report antibiotics If requested only.

4.9.10 Reporting antibiotics for Salmonella/Shigella, Spp

Salmonella/Shigella, Spp[1'2]	
1ST Line	2ND Line
Ampicillin Ciprofloxacin Trimethoprim/Sulphamethoxazole Ceftrioxone[3]	Meropenem[4]

- For Salmonella and shigella spp. first and second –generation cephalosporins, aminoglycoside and cephamycin may appear active in vitro, but are not effective clinically and should not be reported as susceptible

- For intestinal isolates of *Salmonella* and *Shigella* spp. Only ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole should be reported routinely. For extraintestinal isolates of *Salmonella* spp report ampicillin, a fluoroquinolone, and trimethoprim-sulfamethoxazole and ceftriaxone.
- If ceftriaxone resistant strain, repeat to confirm and ESBL confirmation
- Report if ceftriaxone R.

4.9.11 Reporting antibiotics for Haemophilusinfluenzae and Parainfluenza

First line	Second line[4]
Ampicillin[1] Ceftrioxone[2] Cotrimoxazole Ciprofloxacin[3]	Meropenem Rifampicin

- For Ampicillin resistant isolates.
  - B-lactamase Positive: Report Augmentin & Cefuroxime sensitive
  - B-lactamase Negative: Report Augmentin and Cefuroxime resistant.
- Report if R to Ampcillin and B-lactmase negative.
- Report if requested.
- Report when requested by microbiologist only.

4.9.12 Reporting antibiotics for Stenotrophomonas maltophilia[1]

First line	Second line
Trimethoprim-sulfamethoxazole Ceftazidime	Levofloxacin[2] Minocycline[2]

- intrinsically resistant to carbapenams, aminoglycosides and cephalosporins
- only report if R to Trimethoprim-sulfamethoxazole

4.9.13 Reporting antibiotics for Burkholderia cepacia[1]

Fiest line	Second line
Trimethoprim-sulfamethoxazole Ceftazidime Levofloxacin	Meropenem[2]

- intrinsically resistant to colistin
- report if Ceftazidime resistant.

4.9.14 Reporting antibiotics for Aeromonas Hydrophila &Plesiomonas spp[1]

1st line	2nd line
Augmentin Ceftazidime Cefotaxime Ceftriaxone Ciprofloxacin Gentamicin Cotrimoxazole Cefipime Tazocin	Amikacin[2] Imipenem[3] Meropenem[3]

- Aeromonasspp are uniformly resistant to Ampicillin.Plesiomonasspp are resistant to Penicillins. Testing with Ampicillin NOT indicated
- *Report if I/R to gentamycin* .
- Report Meropenem/ Imipenem Only if Tazocin, Cefotaxime or Cefipime Resistant or if requested.

4.9.15 Reporting antibiotics for Campylobacter jejuni/coli:

Campylobacter jejuni/coli
Erythromycin Ciprofloxacin

- Agents for primary testing are erythromycin and ciprofloxacin
- E-test done if there is evidence of any zone of inhibition around the erythromycin and ciprofloxacin disc

4.9.16 Reporting antibiotics for Pasteurella species

Pasteurella species
Penicillin [1]
Amoxicillin-clavulanic acid
Ceftriaxone
Levofloxacin
Tetracycline
Erythromycin

- If B-lacatamase test Positive, regard the isolate as R to Pen and Amox.

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative 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**
Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. M100.	Clinical and Laboratory Standards Institute (CLSI)	2022	
National antimicrobial susceptibility testing	MOH	2017	
"The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org	European Committee on Antimicrobial Susceptibility Testing	2022	

8. Annex 1: Disc diffusion QC sheet

			Month			
QC organism / Media	Antibiotic disk	Acceptable range (mm)	Date ……	Date ……	Date ……	Date ……
E.coli ATCC 25922 MH	AMP 10	16-22				
	AMC 30	18-24				
	CAZ 30	25-32				
	CTX 30	29-35				
	ERTAP 10	29 - 36				
E.coli ATCC 25922 MH	CXM 30	20-26				
	CRO 30	29-35				
	SXT 25	23-29				
	CIP 5	30-40				
	NA 30	22-28				
E.coli ATCC 25922 MH	IMP 10	26-32				
	MEM 10	28-34				
	CN 10	19-26				
	AK 30	19-26				
	TZP 110	24-30				
S. aureus ATCC 25923 MH	PEN 10 Units	26-37				
	OX 1	18-24				
	CN 10	19-27				
	E 15	22-30				
	SXT 25	24-32				
	VAN 30	17-21				
	CIP 5	22-30				
	NTT 300	18-22				
S.Pneumoniae ATCC 49619 MH+5% sheep blood	OX 1	≤12				
	PEN 10 Units	24-30				
	E 15	25-30				
H. influenzae ATCC 49247	AMP 10	13-21				
	CRO 30	31-39				
	CIP 5	34-42				
	SXT 25	24-32				
Staff initial						
Supervisor initial						

Annex 2: -Antibiotic abbreviations

ANTIBIOTIC	CODE	ANTIBIOTIC	CODE
AMIKACIN	AK	FOSFOMYCIN	FOS
AMOXICILLIN	AML	GENTAMICIN	CN
AMOXICILLIN* / CLAVULANIC ACID (2/1)	AUG		
AMPICILLIN	AMP	IMIPENEM	IMI
AMPICILLIN*/ SULBACTAM (2/1)	AMS	LEVOFLOXACIN	LEV
AZITHROMYCIN	AZM	LINEZOLID	LNZ
AZTREONAM	ATM	MEROPENEM	MRP
CEFACLOR	CEC	METRONIDAZOLE	LZ
CEFEPIME	FEP	MOXIFLOXACIN	MXF
CEFIXIME	CFM	NALIDIXIC ACID	NA
CEFOTAXIME	CTX	NETILMICIN	NET
CEFOTAXIME	CTX	NORFLOXACIN	NOR
CEFOXITIN	FOX	OFLOXACIN	OFX
CEFTAZIDIME	CAZ	OXACILLIN	OX
CEFTRIAXONE	CRO	PENICILLIN G	P
CEFTRIAXONE	CRO	PENICILLIN G	P
CEFUROXIME	CXM	PIPERACILLIN	PIP
CEPHALOTHIN	KF	PIPERACILLIN* / TAZOBACTAM (4 g/mL)	TZP
CHLORAMPHENICOL	C	RIFAMPICIN	RD
CIPROFLOXACIN	CIP	STREPTOMYCIN	S
CLARITHROMYCIN	CLR	TEICOPLANIN	TEC
CLINDAMYCIN	CD	TETRACYCLINE	TE
COLISTIN	CS	TICARCILLIN* / CLAVULANIC	TTC

		ACID (2 g/mL)	
DAPTOMYCIN	DAP	TIGECYCLIN	TGC
DOXYCYCLINE	DX	TOBRAMYCIN	TOB
ERTAPENEM	ETP	TRIMETHOPRIM* / SULFAMETHOXAZOLE (1/19)	SXT
ERYTHROMYCIN	E	VANCOMYCIN	VA
		CEFEPIME + CLAVULANIC ACID	FEP/FEL
GENTAMICIN	CN	CEFOTAXIME + CLAVULANIC ACID	CTX/CTL
GENTAMICIN	CN	CEFTAZIDIME + CLAVULANIC ACID	CAZ/CAL

Annex 3 – **Workflow for subculturing and using reference strains**