

CHAIN ANTIMICROBIAL SUSCEPTIBILITY TESTING (CLSI)



(MASTER)

CHN58: ANTIMICROBIAL SUSCEPTIBILITY TESTING (CLSI)

1.0 PURPOSE / INTRODUCTION:

1.1 Introduction

Antimicrobial susceptibility tests are performed in order to determine whether a pathogen is likely to be susceptible or resistant to specific antibiotic treatment. Criteria for in vitro testing of isolates have been developed to provide the best guidance for clinical management, and must be used in conjunction with other variables such as drug absorption, penetration of the drug into the appropriate body compartment, and recognized in vivo limitations of some antibiotic-microbe combinations. Correctly identifying an isolate is therefore key to accurate interpretation of antibiotic susceptibility tests.

Antibiotic susceptibility testing will follow criteria provided by the Clinical Laboratory Standards Institute (CLSI) and will be based around disk diffusion methodology whenever possible. **These criteria are outlined in CLSI document M100-S25. This document and subsequent updates should always be the primary resource for interpretative criteria.**

1.2 Purpose

The purpose of this SOP is to give guidance in performing the antibiotic susceptibility testing.

2.0 SCOPE / RESPONSIBILITY:

This SOP is applicable to all trained laboratory technicians/technologists/scientists in the CHAIN study working in the microbiology laboratory.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The Study Laboratory Coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact that the Study Laboratory Coordinator through your site coordinator.

3.0 SAFETY/RISK ASSESSMENT:

NB: Standard precautions, including personal protective equipment.

3.1. Always wear personal protective equipment (PPE) such as gloves, lab coats when handling specimen and treat all isolates as potential hazards.

3.2. All used materials e.g. gloves and waste papers should be disposed off in the right biohazard bin for future incineration.

3.3. Care should be taken while handling suspensions to avoid generation of aerosols.

4.0 DEFINITIONS:

4.0 CLSI – Clinical Laboratory Standards Institute

4.1 MH – Mueller Hinton

4.2 MIC – Minimum Inhibitory Concentration

4.3 TSB - Trypticase Soy Broth

4.4 ATCC – American Type Culture Collection

4.5 ESBL- Extended Spectrum β -Lactamase

4.6 CLA - Clavulanic Acid

4.7. QA: Quality Assurance

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- 4.8. QC: Quality Control
- 4.9. AST: Antimicrobial susceptibility Testing
 - 4.9.1 AMP: Ampicillin
 - 4.9.2 AZM: Azithromycin
 - 4.9.3 C: Chloramphenicol
 - 4.9.4 CIP: Ciprofloxacin
 - 4.9.5 CRO: Ceftriaxone
 - 4.9.6 CAZ: Ceftazidime
 - 4.9.7 CN: Gentamicin
 - 4.9.8 CTX: Cefotaxime
 - 4.9.9 TMP/SMX: Trimethoprim-sulfamethoxazole
 - 4.9.10. CAZ/CLA: Ceftazidime/Clavulanate
 - 4.9.11 CTX/CLA: Cefotaxime/Clavulanate
 - 4.9.12 AMC: Amoxicillin-Clavulanate
 - 4.9.13 Cefoxitin
 - 4.9.14 IPM: Imepinem
 - 4.9.15 Cefuroxime
 - 4.9.16 AMK: Amikacin
 - 4.9.17 Meropenem

5.0 SPECIMEN:

- 5.1 Pure 24-hour isolate on a culture plate.

6.0 EQUIPMENT / MATERIALS/ REAGENTS:

6.1 EQUIPMENT

- 6.1.1 Incubators (CO₂ and O₂ at 35°C and 37°C)
- 6.1.2 Vernier calipers or ruler
- 6.1.3 Antimicrobial disks (store frozen with a desiccant)
- 6.1.4 Sterile swabs
- 6.1.5 Forceps
- 6.1.6 Disc dispenser
- 6.1.7 Bunsen burner

6.2 MATERIALS

- 6.2.1 Sterile swabs
- 6.2.2 Mueller Hinton Agar (MH agar)
- 6.2.3 Trypticase Soy Broth (TSB) or Mueller Hinton broth (MH broth)

6.3 REAGENTS

- 6.3.1 0.5 McFarland turbidity Standards
- 6.3.2 Sterile distilled water
- 6.3.3 Sterile normal Saline
- 6.3.4 ATCC control strains
- 6.3.5 Antibiotic discs

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7.0 METHODOLOGY:

7.1 PRINCIPLE

The disk diffusion method of susceptibility testing has been standardized primarily for testing of rapidly growing bacteria. To perform the test, filter paper disks containing a specific amount of antimicrobial agent are applied to the surface of an agar medium that has been inoculated with a known amount of the test organism. The drug in the disk diffuses through the agar. As the distance from the disk increases, the concentration of the antimicrobial agent decreases creating a gradient of drug concentrations in the agar medium. At the same time as the drug diffuses through the agar, the bacteria try to multiply and grow across the agar. In areas where the concentration of drug is inhibitory, no growth occurs, forming a zone of inhibition around each disk.

Criteria currently recommended for interpreting zone diameters and MIC results for commonly used antimicrobial agents are published by CLSI. Results are reported categorically as Susceptible (S), Intermediate (I), or Resistant (R).

Susceptible. An infection due to the strain may be appropriately treated with the dosage of the antibiotic recommended for that type of infection.

Intermediate. Zones falling into this range may be considered equivocal. The antibiotic may be used but response will depend on doses used, the site of infection and other factors.

Resistant. Not inhibited by the usually achievable systemic concentration of the agent, or have specific microbial resistance mechanisms, e.g. β -lactamases.

7.2 PROCEDURE

7.2.1. Disk diffusion testing is one of several phenotypic assays which can be utilised to determine the antimicrobial resistance profile (antibiogramme) of an organism. Disk diffusion tests estimate *in vitro* susceptibility.

7.3 Inoculation

Note: Prior to AST testing, ensure all the antibiotic discs are removed from the fridge to attain room temperature. After testing is complete, return to the fridge immediately.

7.3.1. Standardization of inoculum

7.3.1.1. Prior to preparing the inoculum, visually examine the agar plates containing the test organism and control strain.

7.3.1.2. If culture appears mixed, a fresh sub-culture will be prepared.

7.3.1.3. With a loop or sterile swab, touch the top of at least 4 to 5 well isolated colonies.

7.3.1.4. Transfer the growth to the tube of saline. Emulsify the inoculum on the inside of the tube to avoid clumping of the cells.

7.3.1.5. Adjust the inoculum standard to a 0.5 McFarland which is equals approximately 108 CFU/mL.

7.3.2. Inoculation of Mueller-Hinton plate

7.3.2.1 Allow the discs to come to room temperature before opening the container.

7.2.2.2 Using the turbidometer or McFarland turbidity standard, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies. If there is not enough growth, inoculate the organism into TSB or MH broth, and incubate at 35°C

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for 2-4 hours or until it reaches the turbidity of a 0.5 McFarland standard. Use the suspension within 15 mins of preparing it.

7.3.2.3 Using a sterile cotton swab, inoculate the organism onto an appropriate agar plate, streaking in 3 directions over the entire agar surface. For E coli, Salmonella and Shigella organisms that grow rapidly use MH agar. Wait 5-15 mins for the suspension to adsorb into the agar, but no longer.

7.3.2.4 Using forceps or a disk dispenser, apply the appropriate antimicrobial disks onto the agar. Place the discs with an equal distance apart from each other and put no more than 6 disks on a 100mm diameter plate.

7.3.2.5 Incubate the plates aerobically at 35°C for 16-18 hours

NOTE: These suspensions should be used within 15 minutes of preparation

7.4 Interpretation

After incubation, measure the diameters of the zone of complete inhibition (as judged by the unaided eye) with calipers or ruler.

7.3.3. For MH agar:

7.3.3.1. Measure from the upper surface of the plate.

7.3.3.2 Use transmitted light (plate held up to light source).

7.3.3.3 The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye.

7.3.3.4 With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

Refer to CLSI Document M100-S25 for the zone size interpretations. Report susceptible, resistant and intermediate as appropriate. The antibiotics to report for each bacteria are listed in the tables in the appendix.

7.4 Screening for Extended Spectrum β -Lactamase (ESBL) Production

ESBL-producing Enterobacteriaceae (especially E. coli, Klebsiella pneumoniae, K. oxytoca, and Proteus mirabilis) have become increasingly widespread. ESBL-producing isolates are resistant to extended-spectrum cephalosporins, such as cefotaxime and ceftazidime, and aztreonam, as well as to penicillins and narrow spectrum cephalosporins.

E. coli, K. pneumoniae, K. oxytoca, and Proteus mirabilis considered significant enough for susceptibility testing should be screened for ESBL by using a cefotaxime (30 μ g) disk or ceftazidime (30 μ g) disk. Zone sizes ≤ 27 mm for cefotaxime or ≤ 22 mm for ceftazidime may indicate ESBL production and should be confirmed an additional phenotypic test.

The confirmatory procedure (outlined in CLSI Document M100-S25 Table 2A-S25) involves testing the strain against ceftazime, ceftazidime-clavulanic acid, cefotaxime, cefotaxime-clavulanic acid using standard disk diffusion recommendations. After 16-18 hours incubation at 35 \pm 2°C in ambient air, a ≥ 5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid compared to its zone when tested alone indicates an ESBL

7.5 Quality Assurance / Quality Control

7.5.1 Test the following organisms each time a new batch of MH agar is prepared and once weekly. Subculture the organisms to sheep blood agar the day before setting up the QC.

- E. coli ATCC 25922
- P. aeruginosa ATCC 27853
- E.coli NCTC 13351

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See CLSI Document M100-S24 Table 4A for acceptable QC results.

For troubleshooting out-of range QC results, see CLSI Document M100-S25 Table 4D.

8.0 REFERENCES

- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 26th Informational Supplement. M100-S24. CLSI, Wayne, Pennsylvania, 2014.
- Performance standards for antimicrobial testing (twelve int. supplement) Vol 22 No 1. 2002.
- P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed), *Manual of Clinical Microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
- Monica Cheeseborough (ed). *District Laboratory Practice in Tropical countries-part 2*. Cambridge University Press, United Kingdom.
- B.A. Forbes, D.F. Sahm, A.S. Weissfeld. (ed). *Bailey and Scott's Diagnostic Microbiology*. 10th ed. Morsby

9.0 APPENDICES:

Antibiotic Susceptibility Panels for CLSI

The following antibiotics will be tested for isolates recovered from clinical and research specimens.

- **Enterobacteriaceae**

ANTIMICROBIAL AGENT	DISK CONTENT	Sensitive ranges	Intermediete ranges	Resistance ranges
Ampicilin AMP	10ug	≥17	14-16	≤13
Azthromicine AZM	15ug	≥13	-	≤12
Chloramphenicol C	30ug	≥18	13-17	≤12
Ciprofloxacin CIP a	5ug	≥31	21-30	≤20
Ciprofloxacin CIP b	5ug	≥21	16-20	≤15
Cetrixone CRO	30ug	≥23	20-22	≤19
Cefotaxime CTX	30ug	≥26	23-25	≤22
Ceftazidime CAZ	30ug	≥21	18-20	<18
Gentamicin CN	10ug	≥15	13-14	≤12
Cotrimoxazole TMP-SMX	1.25/23.75µg	≥16	11-15	≤10
Augmentin AMC	20/10ug	≥18	14-17	≤13
Imipenem IMP	10ug	≥23	20-22	≤19
Cefuroxime	30ug	≥23	15-22	≤14
Amikacin AMK	30ug	≥17	15-16	≤14
Meropenem	10ug	≥23	20 - 22	≤19
Cefoxitin FOX	30ug	≥18	15-17	≤14

Notes:

CIP a For reporting against salmonellae SPP.

CIP b For reporting against Enterobacteriaceae other than Salmonellae.

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Notes:

1. *Salmonella* species should be interpreted as resistant to Gentamicin and Amikacin irrespective of their susceptibility testing, as it is inactive *in vivo*.
2. *E. coli*, *Salmonella* and *Shigella* which are resistant to ceftazidime, ceftriaxone, or cefotaxime need to be tested for the presence of an Extended Spectrum Beta-Lactamase (ESBL) before the results of susceptibility to any β -lactam drugs can be used. If an ESBL is confirmed, the isolate will be reported as resistant to all penicillins and cephalosporins regardless of the initial disc diffusion result for each drug.
3. Cefoxitin is not a drug that is used in patient care but it can be a marker for an *AmpC* cephalosporinase.
4. Ciprofloxacin zone size for *Salmonella* is categorized as ≥ 31 (sensitive), 21-30 (intermediate) and ≤ 20 (resistant)

Document history

Version	Author	Approved by	Dated
1.01 (MASTER) ANTIMICROBIAL SUSCEPTIBILITY TESTING (CLSI) CHN	Katana Karisa	Caroline Tigoi	10/10/2016
1.02			

Site training record

All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

Document History				
Version No.	Trained staff initials	Signature of trained staff	Date	Trainer's Initials
1.01	KDT	Example row	1 st Jan 2016	DM