National Department of Health

Title: Antibiotic disc susceptibility testing

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Version	
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Changes to the last Authorized Version:

Version	Date issued	Changes
A	14/9/21 reissued	Corrections to some zone size errors Enterococcus and AMP- elimination of "I" zone S. pneumoniae and SXT - - elimination of "I" zone Salmonella, Shigella and Meropenem- - elimination of "I" zone Strep Viridans – addition of I zone comments for AMP and endocarditis Enterobacterales and MEP - - elimination of "I" zone
A	26/10/21 reissued	Tetracycline breakpoints added for *S. pneumoniae* SXT breakpoints added for Strep A,B,C and G
A	17/11/21	7.4.1 Minor errors in R zones Staph AST table corrected

	Reissued	Vortex removed – not required 7.4.4 Increased dose for Strep Viridans group species added and notes regarding significance
B	6/4/22	Post review with PMGH Dr Ak during March trip
B	25/4/2022	EUCAST v12 amendments Strep pneumoniae Oxacillin now 9mm (was 8mm) Salmonella species Perfloxacin now 24mm (was 25mm) Acinetobacter sp. Gentamicin now 17mm (was 15mm) Pseudomonas sp. Amikacin now 15mm (was 19mm)

1. Purpose

Disk diffusion antibiotic susceptibility testing (AST) is suitable for testing the majority of bacterial pathogens, including the more common fastidious bacteria. It is versatile in the range of antimicrobial agents that can be tested and requires no special equipment. As with all methods, the described technique must be followed without modification in order to produce reliable results.

The zone diameter clinical breakpoints from the EUCAST method, version 11, 2021, updated annually are the basis of this protocol.

Properly conducted AST assists clinicians to optimise the choice of antibiotic(s) for treatment of infection. Collated AST data enables antibiotic resistance surveillance including production of cumulative antibiograms.

2. Scope

This procedure applies to the clinical microbiology laboratory environment within the CPHL, Animal Health and Human health Fleming Fund Country Grant partner laboratories.

It applies to the AST for routine bacterial pathogens, excluding *N. gonorrhoeae*.

3. Principle/Clinical application

A controlled quantity of antibiotic diffuses out from a disc into the agar and inhibits growth of the organism. After a controlled period of incubation, the measured zone size of inhibition provides a categorical estimate of the organism susceptibility based on reference values from EUCAST and sometimes CLSI.

Clinical breakpoints, defined by EUCAST are zone size diameter cut-points that distinguish susceptible from less susceptible (I= susceptible to an Increased dose) or resistant (R= resistant – clinical failure of treatment expected) isolates for a particular species and antibiotic.

4. Responsibilities

Role	Responsibility
Lab bench scientist	Setup, reading and data entry Identify and document AMR phenotypes that require storage/ referral
Lab manager	Validation (checking) of AST results Addition of necessary interpretative comments on the LIMS Finalise the AST record (LIMS) prior to issue of the report Identify and document AMR phenotypes that require storage/ referral Supervision and sign off of SO competency and weekly QC compliance/results
Quality officer	Weekly AST QC (see separate SOP)

5. Specimen

The starting point for disc AST is a pure agar culture of a known bacterial species derived from a clinical sample culture.

6. Equipment/Materials

- Mueller Hinton and MH-F AST agars
- Antibiotic discs
- Sterile normal (0.9%) saline
- Disc dispensers with antibiotics brought to room temperature before use
- Forceps
- Measuring ruler
- 70% alcohol
- Permanent marker or label
- 35^0C O2 incubator
- 35^0C CO2 incubator / Candle jars
- Disposable or nichrome wire loops
- Pre-prepared McFarland turbidity standard and Wickerham Card
- Sterile swabs

7. Procedure

7.1 Video instructions from EUCAST (recommended pre-reading):

Topic (duration minutes)	Link
Preparation of inoculum (2.33)	https://www.youtube.com/watch?v=M6KpdQjsgdI&t=2s
Inoculation (3.49)	https://www.youtube.com/watch?v=2fp6UORfYGg
Application of disks and incubation (2.33)	https://www.youtube.com/watch?v=rLj8BGgn45o
Reading zones (3.38)	https://www.youtube.com/watch?v=TXwPEHxjBSI

7.2 Set-up

7.2.1 Bring all antibiotic disks to room temperature and dry sensitivity media before use.
7.2.2 Follow the 15-15-15 minute EUCAST rule (Figure 1)
7.2.3 Use the correct type of media for the organism using the table 1 below.
7.2.4 Label the sensitivity agar and 3mls normal saline tube or bottle.
7.2.5 Select a few well-isolated colonies with a sterile loop or sterile swab, and emulsify in sterile saline.
7.2.6 Adjust your suspension to a 0.5McFarland standard using the preprepared 0.5McFarland control standard and the Wickerham card as a guide (Appendix)
7.2.7 Use your suspension within 15 minutes of preparation.
7.2.8 Dip a sterile swab into the suspension, then remove as much liquid as possible from the swab by pushing it against the inside of the saline bottle.
7.2.9 Inoculate the sensitivity plate by swabbing in three directions.
7.2.10 Make sure the discs are sitting flat on the plate.
7.2.11 Do not move a disc once it has been applied
7.2.12 Put the organism in the correct incubator using table 1 as a guide.
7.2.13 Do not incubate for longer than 20 hours or less than 16 hours. ***Be mindful of the correct time the zone diameters will require reading - plates should be incubated for 18±2 hours, excepting vancomycin AST for enterococci.***

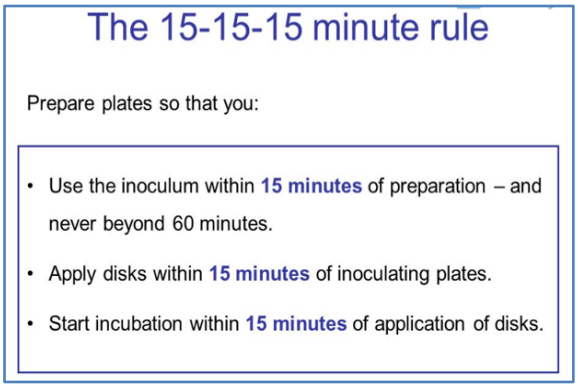


Table 1: Set up by organism group and incubation requirements

Non-fastidious organisms			
Organism	Media	Incubation conditions	Antibiotic disc panel (table 2)
Enterobacterales	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs	GNR +/- MRGN
Salmonella, Shigella	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs	SS
Pseudomonas aeruginosa	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs	MRGN
Acinetobacter spp.	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs	GNR
Staphylococcus aureus	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs	STAPH
Enterococcus spp.	Mueller Hinton agar (clear)	O^2, $35^0 \pm 1^0$ C, 18±2hrs **(except VA disc AST)**	ENC
Fastidious organisms			
Organism	Media	Incubation conditions	
Beta-haem *streptococcus* (GpA, GpB, GpC, GpG)	MH agar + 5% HB[1] & 20mg/L beta-NAD (MH-F)	CO^2, $35^0 \pm 1^0$ C, 18±2hrs	STREP
Viridans group*Streptococci including* S. Milleri group.	MH-F	CO^2, $35^0 \pm 1^0$ C, 18±2hrs	PEN1 disc only
Streptococcus pneumoniae	MH-F	CO^2, $35^0 \pm 1^0$ C, 18±2hrs	STREP
Haemophilus influenzae	MH-F	CO^2, $35^0 \pm 1^0$ C, 18±2hrs	HAEM

[1] If horse blood is unavailable then expired human blood is a suitable alternative provided that the internal media and AST QC results are as expected.

Table 2: Antibiotic disc panels

Panel	Antibiotics contained and disc abbreviation
STAPH	Penicillin PEN1) Cefoxitin (FOX30) Chloramphenicol (C30) Erythromycin (E15) Sulfa/trimethoprim (SXT25) Tetracycline (TE30)
ENC	Penicillin (PEN1) Ampicillin (AMP2) Nitrofurantoin (100) Vancomycin (VA5)
GNR	Amoxy+clavulanate (AMC30) Ceftriaxone (CRO30) Gentamicin (CN10) Ciprofloxacin (CIP5) Nitrofurantoin (F100) Sulfa/trimethoprim(SXT25)
MRGN	Tobramycin (TOB10) Meropenem (MEM10) Ceftazidime (CAZ10) Piperacillin/tazo (30/6) Amikacin (AK30) Chloramphenicol (C30)
SSV	Ampicillin (AMP10) Ceftriaxone (CRO30) Sulfa/trimethoprim (SXT25) Chloramphenicol (C30) Pefloxacin (PEF5) Azithromycin (15ug)
HAEM	Penicillin (PEN1) Tetracycline (TE30) Sulfa/trimethoprim (SXT25) Chloramphenicol (C30) Ceftriaxone (CRO30)
STREP	Penicillin (PEN1) Oxacillin (OX1) Erythromycin (E15) Tetracycline (TE30) Sulfa/trimeth(SXT25) Chloramphenicol (C30)

7.3 Zone diameter reading

7.3.1 Refer to the EUCAST reading guide, version 2 for instructions on how to read zone diameters.
https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Reading_guide_v_2.0_EUCAST_Disk_Test.pdf
Also review the EUCAST video above on zone reading.

7.3.2 Read to the point of complete inhibition as judged by the naked eye with the plate held at 30cm from the eye. Take into account double zones and colonies within the zone. Refer to figure 1 below.

7.3.3 Read MH (clear) susceptibility plates from the back with reflected light and the plate held above a dark background

7.3.4 Read blood susceptibility plates from the front with the lid removed and with reflected light.

7.3.5 Do not use transmitted light (plate held up to light) or a magnifying glass. Except: vancomycin (VA5) and enterococci

7.3.6 Measure the zones of inhibition to the nearest millimeter with a ruler.

7.3.7 Record measured zone size in mm for each tested antibiotic onto the paper specimen worksheet for later entry into the Laboratory Information System (LIMS).

7.3.8 Enter the zone sizes against the isolate in the LIMS after completing a quality check. The LIMS will contain the current EUCAST clinical breakpoints for disc AST and will allocate one of three interpretations – Susceptible, Susceptible at increased dose (in limited situations) and Resistant.

7.4 Result interpretation and reporting

Gram positives

7.4.1 Staphylococcus aureus

Antibiotic	**Strength**	**Zone diameter (mm)**		**Report**
STAPH SET		**Susceptible**	**Resistant**	
Penicillin	1	≥26	<26	Always report; check for sharp/fuzzy zone below
Cefoxitin (FOX)	30	≥22	<22	Record result; not reported. If R, report organism name as MRSA
Erythromycin	15	≥21	<21	Report for non-sterile site isolates only
Trim/sulfa (SXT)	1.25/23.75	≥17	<17	Report for non-sterile site isolates only
Tetracycline	30	≥22	<22	Report for non-sterile site isolates only
Chloramphenicol	30	≥18	<18	Report for sterile site isolates only
Amoxicillin	-	Extrapolate from PEN		Yes (for non-sterile site isolates)
Flucloxacillin	-	Extrapolated from FOX		Always report
Cephalexin	-			Yes (for non-sterile site isolates)
Cefazolin	-			Yes (for sterile site isolates)
Vancomycin	5	≥12	<12	**All MRSA blood isolates require testing and reporting.** Resistance is rare and requires urgent confirmation by the reference lab.

Zone ≥ 26 mm and
sharp zone edge= Resistant

Zone ≥ 26 mm and
fuzzy zone edge = Susceptible

Store all bloodstream isolates at -20 degC and forward to the National Reference Lab (NRL).

Positive blood culture comment (MSSA isolate):
"Bloodstream infection due to methicillin (flucloxacillin)-susceptible *Staphylococcus aureus* (MSSA) has high mortality and is often associated with subclinical endocarditis that will cause infection relapse if initial treatment is insufficient. Therefore high dose flucloxacillin (2g 6hrly IV) is indicated for at least 2 weeks in all adult cases. Use cefazolin 2g 8hrly IV if non-severe penicillin allergy present or chloramphenicol 1 g 6hrly IV.

"Complicated" cases require at least 4 weeks of IV therapy. Complicated means any of a) persistent bacteraemia > 72 hrs after treatment start, b) persistent fever > 72 hrs after rx start, c) evidence of endocarditis or abnormal valvular morphology on echocardiogram, d) metastatic foci or e) intravascular prosthetic material that cannot be removed. "

Positive blood culture comment (MRSA isolate):
Bloodstream infection due to methicillin (flucloxacillin) resistant *Staphylococcus aureus* (MRSA) has high mortality and is often associated with subclinical endocarditis that will cause relapse if initial treatment is insufficient. Intravenous vancomycin (see guideline for dosing) or chloramphenicol 1g 6hrly is indicated for at least 2 weeks in all cases.

"Complicated" cases require at least 4 weeks of IV therapy. Complicated means any of a) persistent bacteraemia > 72 hrs after treatment start, b) persistent fever > 72 hrs after rx start, c) evidence of endocarditis or abnormal valvular morphology on echocardiogram, d) metastatic foci or e) intravascular prosthetic material that cannot be removed. "

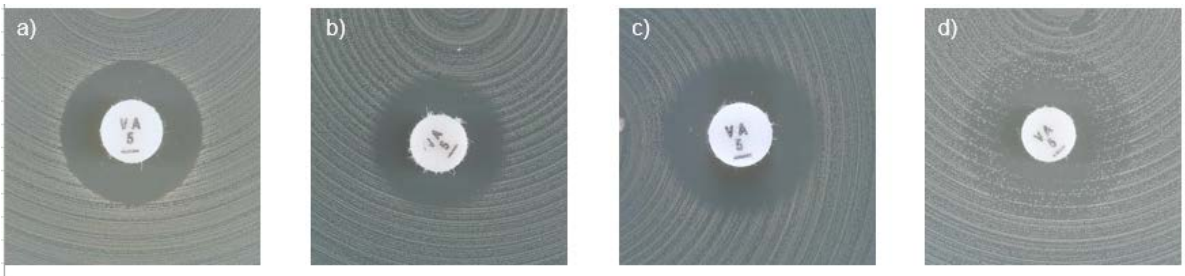
7.4.2 Streptococcus groups A, B, C and G (beta-haemolytic species)

Antibiotic	**Strength**	**Zone diameter**		**Report**
STREP SET		**Susceptible**	**Resistant**	
Penicillin	1	≥18	<18	Always report
Erythromycin	15	≥21	<21	Report for non-sterile site isolates only
Tetracycline	30	≥23	<23	Report for non-sterile site isolates only
Trim/sulfa (SXT)	1.25/23.75	≥18	<18	Report for non-sterile site isolates only
Chloramphenicol	30	≥19	<19	Report for sterile site isolates only

Positive blood culture comment:
Total treatment duration for bacteraemia due to Strepococcus pyogenes (group A) or other beta haemolytic species (groups B ,C or G) is usually 7-10 days. Benzylpenicillin or flucloxacillin is suitable (use cefazolin for patients with non-immediate penicillin allergy). Switch to oral amoxycillin when sepsis source control and clinical response attained. Endocarditis is rare with these species and echocardiography is not required routinely.

7.4.3 Enterococcus species *(E. faecalis* and *E. faecium)*

Antibiotic	Strength	Zone diameter		Report
ENC Set		Susceptible	Resistant	
Ampicillin	2	≥10	<10	Always report
Vancomycin **(read after 24hrs) see below**	5	≥12	<12	If R, report organism name as Vancomycin-resistant *Enterococcus* species
Nitrofurantoin	100	≥15	<15	Report for urine isolates of AMP susceptible *Enterococcus* species only
Amoxicillin	-	Extrapolated from AMP		Report as oral therapy for urinary infection only



Examples of inhibition zones for *Enterococcus* spp. with vancomycin.
a) Sharp zone edge **and** zone diameter ≥ 12 mm. Report susceptible.
b-d) Fuzzy zone edge or colonies within zone. Perform confirmatory testing with PCR or report resistant even if the zone diameter ≥ 12 mm.

Store all isolates at -20 degC and forward to NRL for confirmatory ID and AST.

Speciate significant isolates from sterile fluids and blood if possible. *E. faecium* is AMP R whereas *E. faecalis* is AMR S.

If there is concern that the patient could have endocarditis (e.g. multiple positive cultures over time or culture positive relapse post treatment), please refer the clinician to Dr Ak at PMGH to obtain treatment guidance and share the case on the WHATSAPP lab group for discussion.

Sterile site isolate (including blood) comment :
Enterococcus species are intrinsically resistant to cephalosporins (i.e. ceftriaxone, cephalexin) and aminoglycosides (gentamicin).

7.4.4 *Streptococcus* Viridans group, including *S. anginosus* (milleri) group

Only do AST on significant sterile site (blood) isolate (patient with clinical evidence of endocarditis and/or isolation from more than one set or from blood culture set and a tissue site), set up PEN1 and AMP2 discs. See note below.

This group of bacteria includes many species, which can be grouped as follows:
S. *anginosus* group: *S. anginosus, S. constellatus, S. intermedius*
S. *mitis* group: *S. australis, S. cristatus, S. infantis, S. mitis, S. oligofermentans, S. oralis,S. peroris, S. pseudopneur*
S. *sanguinis* group: *S. sanguinis, S. parasanguinis, S. gordonii*
S. *bovis* group: *S. equinus, S. gallolyticus (S. bovis), S. infantarius*
S. *salivarius* group: *S. salivarius, S. vestibularis, S. thermophilus*
S. *mutans* group: *S. mutans, S. sobrinus*

Antibiotic	**Strength**	**Zone diameter (mm)**		Report
ENC Set		**Susceptible**	**Resistant**	
Penicillin	1	≥18	<12	Report for blood culture/sterile site only if contamination unlikely – see notes. Sterile sites, report 12-18mm as "I, susceptible at increased dose (see below)
Ampicillin	2	≥21	<15	Record, don't report
Vancomycin	5	≥15	<15	If R, check ID (most probably a VRE) and make sure isolate is referred to reference for confirmation

Store all isolates at -20 degC and forward to NRL for confirmatory ID and MIC testing.

Note that many blood culture events that isolate *Streptococcus* Viridans group species represent contamination and treatment is not indicated. Significance is more likely if one of the following criteria are satisfied:

- High pre-test clinical probability of endocarditis
- More than 1 blood culture sample positive with the same species
- Repeat blood culture sample positive prior to treatment
- If there is concern that the patient could have endocarditis, please refer the clinician to Dr Ak at PMGH and/or discuss the case on the relevant lab WHATSAPP group.

Note also that even a single dose of penicillin often is sufficient to make a subsequent blood culture sample negative, even if the patient has streptococcal endocarditis.

"I" category result (EUCAST):
For significant isolates (e.g. endocarditis), use this comment:
Use benzylpenicillin 2400 mg every 4 hours IVI as the higher dose.

For *Streptococcus* Milleri (anginosus group) wounds and non-sterile body sites, no AST is required; report presumptive ID only with the following comment:
S. anginosus group is predictably susceptible to penicillin, ampicillin or amoxycillin

7.4.5 Streptococcus pneumoniae

Antibiotic	**Strength**	**Zone diameter (mm)**		**Report**
STREP set		**Susceptible**	**Resistant**	
Oxacillin	1	≥9	<9	Record result; not reported
Chloramphenicol	30	≥21	<21	Otherwise record & report for CSF only
Erythromycin	15	≥22	<22	Yes- non sterile site specimen
Trim/sulfa (SXT)	1.25/23.75	≥13	<13	Yes- non sterile site specimen
Tetracycline	30	≥25	<25	Yes- non sterile site specimen
Penicillin	-	Extrapolate from OX		Report all specimens
Amoxicillin	-			Report for non sterile site specimen
Ceftriaxone	-			Report for CSF only or blood isolates where meningitis likely

Store all CSF isolates at -20 degC and refer isolates to NRL (NRL to perform penicillin and ceftriaxone MIC testing where oxacillin screen was < 9mm).

Add comment for meningitis (CSF isolates or blood isolate where clinical or LP evidence of meningitis present):
Ceftriaxone 50mg/kg up to 2g 12-hrly IV is standard treatment for meningitis for 10-14 days.

If OX < 9mm, then seek treatment advice from Dr Ak at PMGH and discuss the case on the WHATSAPP lab group. Options – continue ceftriaxone alone if good clinical response. If not, then repeat CSF culture if possible to assess response and add chloramphenicol IV, provided it tests as S.

Comment for other isolates when meningitis not present:
Benzylpenicillin or amoxicillin is the preferred treatment for pneumonia- usual total duration is 5-7 days total, switch to oral as soon as patient is clinically stable. Add gentamicin 5mg/kg/d for severe pneumonia (if renal function normal, give a maximum

of 3 x daily doses. If abnormal renal function (estimated GFR < 60mLs) give only a single dose).

Gram Negatives

7.4.6 *Enterobacterales (E. coli, Klebsiella*, etc.)

Antibiotic	Strength	Zone diameter (mm)		Report
GNR disc set		**Susceptible**	**Resistant**	
Amoxi/clav	30	≥19mm	<19	Yes*
Ceftriaxone	30	≥25mm	R<25	Yes*
Gentamicin	10	≥17mm	<17	Yes
Ciprofloxacin	5	≥25mm	R < 25	Yes
Nitrofurantoin	100	≥11mm	<11	*E.coli* in urine sample Otherwise record & don't report
Sulfa/trimethoprim	25	≥14mm	<14	Yes
MRGN disc set				
Tobramycin	10	≥16mm	<16	Report If GENT Resistant
Meropenem	10	≥22mm	R<22	Report if ceftriaxone resistant or ESCHAPPM species
Ceftazidime	10	≥22mm	R<22	Record, don't report
Piperacillin/tazobactam	30/6	≥20mm	<20	Record, don't report
Amikacin	30	≥18mm	<18	Record, don't report
Chloramphenicol	30	≥17mm	<17	Record, don't report

* If ESCHAPPM **always** report these antibiotics as resistant.

"I" category result (EUCAST):
I (susceptible at increased dose) category not implemented – instead report Meropenem, CRO, CIP and CAZ with revised conservative R breakpoint as above and use usual doses.

Store all isolates at -20 degC and refer to NRL.

ESCHAPPM Organisms:

Klebsiella aerogenes	*Citrobacter (except C. koseri* or *C. amalonaticus)*	
Enterobacter	*Proteus (except P. mirabilis)*	
Serratia	*Providencia*	*Morganella*
Hafnia	*Aeromonas*	*Acinetobacter*

Positive blood culture comment :

Total treatment duration for Gram negative bacteraemia is usually 5-10 days but varies according to the site of the infection. Use a single antibiotic (based on the bacterial antibiogram) at an appropriate dose. (NB. Cefazolin adult dose is 2g 8-hourly IV). Switch from IV to oral antibiotic is indicated when sepsis source control is attained and sustained clinical response has occurred (e.g. normotensive and normothermic for 2 consecutive days).

For noting:

Common sources for GNR sepsis include:

- Renal tract – check for obstruction, check for urinary catheter – remove if possible; generally short IV period needed (1-2 days) and follow-up with oral antibiotic out to at least 7 days total rx; 14 days for pregnant woman
- Biliary tract – often due to obstruction or cholecystitis; may need surgical, percutaneous or endoscopic release of obstruction; with relief of obstruction, BSI settles quickly and short course only required.
- Central line associated BSI – note that line exit site may look ok; if no other apparent source, get the line out!
- -GIT – Salmonella, Campylobacter, E coli usually; only short course treatment required unless Salmonella Typhi.
- For latter (S. Typhi), early convert from ceftriaxone to cipro oral 500mg bd provided the pefloxacin result is S
- -Intraabdominal infection- generally a perforated viscus or post op infection; needs surgical source control etc
- -Liver abscess (uncommon) – generally needs drainage unless small/multifocal; need to consider amoebic abscess as well and treat with metronidazole as well

Risk factors for poor outcome:

- Wrong antibiotic – ensure change to susceptible agent ASAP following AST result
- Delay in antibiotic and/or source control

7.4.7 *Salmonella* and *Shigella* species

Antibiotic	Strength	Zone diameter (mm)		Report
SSV Set		Susceptible	Resistant	
Ampicillin	10	≥14mm	<14	Yes
Ceftriaxone	30	≥25mm	<25	Record, report blood isolates only **If any isolate R, set up meropenem (below)**
Sulph/trimethoprim (SXT)	25	≥14mm	<14	Yes
Chloramphenicol	30	≥17mm	<17	Record, don't report
Perfloxacin (PEF)	5	≥24mm	<24	Record, don't report
Azithromycin	15	≥12mm	<12	Yes if SXT and CIP R
Meropenem	10	≥22mm	R<22	Record, don't report
Amoxicillin		extrapolate from AMP		Yes
Ciprofloxacin		extrapolate from PEF		Yes

"I" category result (EUCAST):
I (susceptible at increased dose) category not implemented –report meropenem with revised conservative R breakpoint as above and use usual doses.

Store all isolates at -20 degC and refer to NRL for confirmatory ID and AST.

Comment for all: Salmonella and Shigella species are intrinsically resistant to cefazolin and gentamicin.

Comment for positive blood culture with non-typhoidal *Salmonella* species: Salmonella bacteraemia may be complicated by metastatic infection of bone or vascular tissue and can lead to splenic or other organ infarction.

Comment for stool isolates of non-typhoidal *Salmonella* species: Antibiotic therapy is indicated only for significant systemic symptoms (e.g. shock) or prolonged illness, particularly in neonates or elderly patients.

7.4.8 *Acinetobacter* species from normally sterile site including blood and from endotracheal specimen (ICU only)

Antibiotic	Strength	Zone diameter (mm)		Report
GNR disc set		**Susceptible**	**Resistant**	
Gentamicin	10	≥17mm	<17	Yes
Ciprofloxacin	5	≥50mm	<21	Yes; IF ≥ 21mm, report as "I" see below
Sulfa/trimethoprim	25	≥14mm	<14	Yes
MRGN disc set				
Tobramycin	10	≥17mm	<17	Yes if GENT resistant
Meropenem	10	≥21mm	R < 21	Yes if GENT and CIP R
Amikacin	30	≥19mm	<19	Yes if GENT and CIP R

"I" category result (EUCAST)

I (susceptible at increased dose) category not implemented for meropenem– instead report with revised conservative R breakpoint as above and use usual doses.

N.B. Ciprofloxacin increased dose recommendation – adult 750mg 12hry oral

Species identification should be done by either API20NE or MALDI when available.

Store presumptive *Acinetobacter* species isolates at -20 degC and refer to the NRL for confirmatory ID and AST.

Comment for all reported isolates: Acinetobacter species are intrinsically resistant to amoxicillin-clavulanate, ceftriaxone and chloramphenicol.

7.4.9 *Pseudomonas* sp. from normally sterile site including blood and from endotracheal specimen (ICU only)

Antibiotic	Strength	Zone diameter (mm)		Report
GNR disc set		**Susceptible**	**Resistant**	
Gentamicin	10	≥15	<15	Yes *
Ciprofloxacin	5	≥50mm	<26	Yes; ≥ 26mm, report as "I" see below
MRGN disc set				
Tobramycin	10	≥18mm	<18	Yes
Meropenem	10	≥24mm	R < 24	Yes if TOB R and CIP R
Ceftazidime	10	≥50mm	<17	Yes; IF ≥ 17mm, report as "I" see below
Piperacillin/tazobactam	30/6	≥50mm	<18	Record, don't report
Amikacin	30	≥15mm	<15	Record, don't report

* Gentamicin breakpoint above is derived from EUCAST version 8.

"I" category result (EUCAST) :
I (susceptible at increased dose) category not implemented for meropenem– instead report with revised conservative R breakpoint as above and use usual doses.
N.B. Ciprofloxacin increased dose recommendation – adult 750mg 12hry oral
Ceftazidime increased dose recommendation 2g 8-hrly IV

Store isolates of *Pseudomonas* species from blood at -20deg C and refer to NRL.

Comment for all: "Pseudomonas aeruginosa is intrinsically resistant to amoxicillin-clavulanate, ceftriaxone, sulfamethoxazole/trimethoprim, nitrofurantoin and chloramphenicol."

Comment for blood isolates:
In systemic infections, the aminoglycoside must be supported by another susceptible antibiotic.

7.4.10 Haemophilus influenzae

Antibiotic	**Strength**	**Zone diameter**		**Report**
HAEM set"		**Susceptible**	**Resistant**	
Penicillin	1	≥12mm	<12	Yes - sputum only
Ceftriaxone	30	≥32mm	<32	PEN R or CSF isolates
Sulfa/trimethoprim	1.25-23.75	≥23mm	R<23	Yes - sputum only
Tetracycline	30	≥25mm	R<25	Yes - sputum only
Chloramphenicol	30	≥28mm	<28	Record, don't report
Amoxicillin		Extrapolate from PEN1		Yes - sputum only

"I" category result (EUCAST) :
I (susceptible at increased dose) category not implemented for SXT and TET – instead report with revised conservative R breakpoint as above and use usual doses.

Store all CSF and blood isolates at -20deg C and refer to National Reference Lab.

CSF comment (CSF or Blood isolate with and clinical or LP evidence of meningitis present);
Ceftriaxone 50mg/kg up to 2g 12-hrly IV for 7 days is the standard treatment for meningitis.

8. Safety

8.1 PPE required as per standard precautions – eye protection and gloves
8.2 Setup and reading– can be performed on the open bench
8.3 Wipe down benches with 70% alcohol after completion of AST setup

9. Quality Control

QC is to be done weekly and subject to documented management review.
Refer to G_90_SOP_3-A Disc diffusion AST QC.

10. Reference and Related documents

10.1 Related documents

For access, refer to https://path-png.org/microbiology-sops-fleming-fund/

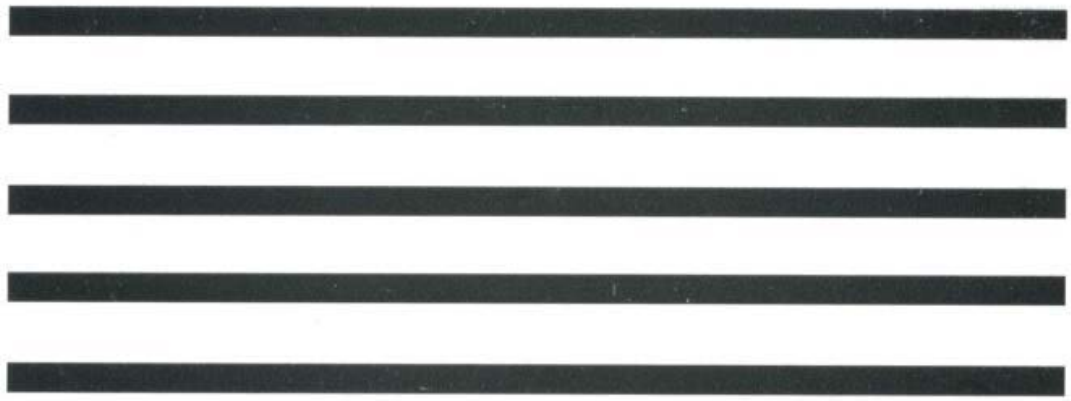
Weekly AST QC summary	G_90_WS_3
Weekly AST QC Worksheets	G_90_WS_4
Setup of disc antibiotic susceptibility tests	G_90_J_1
Disk AST Testing Competency assessment	G_90_COMP_1
Disc diffusion quality control	G_90_SOP_3
Maintenance of cultures used for quality control testing	G_90_SOP_2

10.2 EUCAST Clinical Breakpoint tables
https://www.eucast.org/clinical_breakpoints/

10.3 EUCAST AST reading guide:
https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Reading_guide_v_2.0_EUCAST_Disk_Test.pdf

Appendix A – Wickerham Cards

Wickerham Card



Wickerham Card