# Aim

To measure the susceptibilities of pathogenic bacteria to appropriate antimicrobials by a standardised disk diffusion method.

# Principle

Standard amounts of antibiotics impregnated onto absorbent paper are placed onto a specified agar plate seeded with a known concentration of bacteria. These plates are then incubated overnight in defined conditions (e.g. temperature and atmosphere). The antibiotic diffuses through the medium from the disk and if the bacteria are sensitive to the antibiotic then growth will be inhibited leaving a zone of clearing. The diameter of this zone is measured and compared with CLSI guidelines for zone sizes to determine whether the bacteria should be classified as sensitive, intermediately sensitive or resistant to the antibiotic.

Various factors have been identified as influencing disk diffusion susceptibility tests. These include the type of medium, excess surface moisture on the medium, agar depth, disk potency, inoculum concentration, pH, cation content, incubation atmosphere, temperature and duration, and β-lactamase production by test organisms. It is therefore of the utmost importance to follow the CLSI guidelines on testing methods precisely in order for the CLSI interpretation tables to be valid. Regular quality control is also essential.

CLSI methods are regularly reviewed and updated and so it is important that the most recent versions of CLSI standard documents are used.

In some circumstances, accurate results cannot be achieved by simple disk diffusion testing and Etest MICs are required (described in SOP MIC-002).

# Method

## Reagents and equipment

* Agar plates (3-4 mm deep)
* Antibiotic impregnated disks
* Sterile saline (2-5ml)
* Sterile cotton tipped swabs
* Automatic disk dispenser or template with 5 or 6 disk spacing pattern
* Forceps
* Incubator with correct atmosphere at appropriate temperature
* Ruler

## Inoculation

1. Subculture the organism to be tested onto a non-selective agar plate and incubate for 18-24 hours to obtain a pure growth.
2. Remove the antibiotic disks 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 disks and container in the refrigerator as soon as you have finished using them. Do not use disks past their expiry date.
3. Using a straight wire or loop, touch at least six individual colonies from the pure culture and transfer them into sterile saline solution.
4. Emulsify the colonies in sterile saline to give an equivalent turbidity of 0.5 McFarland Standard (equivalent to a growth of 1-2 X 108 CFU/mL for *E. coli* ATCC 25922). Check the turbidity by comparing with the standard by eye.
5. Within 15 minutes after adjusting the turbidity of the inoculum, 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.
6. Use the appropriate plate and incubation conditions according to Table 1.

**Table 1.** Incubation media, atmospheres and times

|  |  |  |
| --- | --- | --- |
| **Organism** | **Media** | **Incubation conditions** |
| *Aeromonas* spp.  *Enterobacteriaceae*  *Pseudomonas aeruginosa*  *Vibrio* spp. | Mueller Hinton | 35-37°C in air for 16-18h |
| *Acinetobacter* spp.  *Burkholderia cepacia*  *Burkholderia pseudomalleia*  *Stenotrophomonas maltophilia* | Mueller Hinton | 35-37°C in air for 20-24h |
| *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% CO2 for 16-18h |
| *Moraxella catarrhalis* | Mueller Hinton | 35-37°C in 5% CO2 for 20-24h |
| *Neisseria gonorrhoeae* | GC agar base and 1% defined growth supplement (CA) | 35-37°C in 5% CO2 for 20-24h |
| *Neisseria meningitidisb* | Mueller Hinton with 5% sheep blood | 35-37°C in 5% CO2 for 20-24h |
| *Streptococcus pneumoniae*, β-haemolytic streptococci and other streptococci | Mueller Hinton with 5% sheep blood | 35-37°C in 5% CO2 for 20-24h |

Notes:

1. There are no published CLSI disk diffusion interpretative criteria for *B. pseudomallei*. MIC testing is recommended by CLSI. However, local experience suggests that disk diffusion testing can be used for all antibiotics except co-trimoxazole, for which Etest MICs should be performed if the organism appears resistant by disk testing.
2. The inoculum for *N. meningitidis, N. gonorrhoeae* and *Haemophilus* spp.should be prepared from a chocolate agar plate incubated in 5% CO2 for 20-24 hours. 0.9% PBS (pH 7.0) as opposed to ordinary saline is recommended for making the suspension of *N. gonorrhoeae*. All antimicrobial susceptibility testing of *N. meningitidis* should be done in a Biosafety cabinet as manipulating heavy suspensions of this organism species outside a BSC has been associated with meningococcal disease in laboratory workers.
3. If the organism you want to test is not specifically listed in this SOP there may still be CLSI interpretative criteria for disk testing or other methods may be recommended: consult CLSI documents.
4. Inoculate the entire agar surface of the plate, either using a rotary plater or by spreading the plate 3 (or 4) times, 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.

## Application of disks

1. Place disks of the appropriate antibiotics for the species in question (Appendix 1) on the plate using the automatic disk dispenser or manually using disk spacing template. Single disks may be handled using forceps.

* Avoid placing penicillin and cephalosporin disks next to each other.
* Disks need to be applied evenly on the agar surface; press gently on the disk after application.
* Because some antibiotics diffuse almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar.
* Disks should be applied no later than 15 minutes after the plates have been inoculated. Similarly, once the disks are applied, they should be put in the incubator within a 15 minutes interval to prevent pre-diffusion of the antimicrobial at room temperature.
* Disk diffusion testing is not reliable for some organism/antibiotic combinations (e.g. *B. pseudomallei* and co-trimoxazole). In these circumstances an Etest MIC may need to be undertaken (see SOP MIC-002).

1. Invert the plates and incubate in the correct atmosphere for the appropriate time as indicated in Table 1.

* 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.

## Reading and interpreting results

1. After the incubation is complete, remove the plates from the incubator and measure the zone diameter, in mm, using a ruler. To measure zone diameter the ruler has to be held on the back of the inverted plate over a dark, non-reflecting background, and illuminated from above (except oxacillin and vancomycin, which should be read with transmitted light i.e. plate held up to light source and any discernible growth within the zone of inhibition taken as indicative of resistance).

* The diameter of the zone of inhibition includes the diameter of the disk. 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.
* 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 “oxacillin”. Isolates that are resistant to cefoxitin should be reported as resistant to all beta-lactams (i.e. penicillin, oxacillin, co-amoxiclav, ceftriaxone)..

1. Compare the measured zone size with that for the species and antibiotic combination in Appendix 1. Record the zone diameter and the category taken from the table. If the organism and zone size is not included in the table then refer to the CLSI documents for further information. Results can usually be put into one of the categories below:

* **Susceptible (S)** includes isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used.
* **Intermediate (I)** includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels, and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g. quinolones and β-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g. β-lactams). The “intermediate” category also includes a “buffer zone” which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margins.
* **Resistant (R)** includes isolates that are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms are likely (e.g. β-lactamases) and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.
* **Nonsusceptible (NS)** is used for isolates for which only a susceptible interpretative criterion has been designated because of the absence or rare occurrence of resistant strains. Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as nonsusceptible. This does not necessarily mean that the isolate has a resistance mechanism: it is possible that isolates with MICs above the susceptible breakpoint that lack resistance mechanisms may be encountered within the wild-type distribution after the susceptible only breakpoint is set.

# ESBL testing

*E. coli* and *Klebsiella* spp. that have cefpodoxime zones ≤ 17 mm or ceftriaxone zones ≤ 25 mm or ceftazidime zones ≤ 22 mm and *Proteus mirabilis* that have cefpodoxime zones ≤ 22 mm or ceftazidime zones ≤ 22 mm should be tested for ESBL production (MAST ID kit, D52C).

1. Inoculate a Mueller Hinton plate as described above.
2. Place on the inoculated plate disks of the following:
   1. Ceftazidime 30 μg
   2. Ceftazidime-clavulanic acid 30/10 μg
   3. Cefotaxime 30 μg
   4. Cefotaxime-clavulanic acid 30/10 μg
   5. Cefpodoxime 30 μg
   6. Cefpodoxime-clavulanic acid 30/10 μg
3. Incubate at 35-37°C in air for 16-18h
4. Measure zone diameters as described above
5. A ≥5mm increase in a zone diameter for either agent in the presence of clavulanic acid is a positive result for ESBL. Note: the cefpodoxime +/- clavulanic acid test does not form part of the CLSI guideline, but is a component of the MAST ID kit.

Other species should not undergo ESBL testing using the double disk methods, since alternative mechanisms may be responsible for cephalosporin resistance and ESBL disk test results may not be reliable.

In accordance with current CLSI guidelines, ceftriaxone (CRO) and ceftazidime (CAZ) disc results may be reported prior to EBSL testing, and should no longer be amended to “resistant” is an organism is found to be ESBL positive.

# Quality assurance

Regular testing of the correct quality control strains is essential to provide assurance that the media and disks being used most frequently are performing satisfactorily. Test the organisms and agents shown in Appendix 2 every week (preferably on a Monday) and record the results on the sheets in Appendix 2. If any of the results fall outside the ranges given on the sheets, inform the Laboratory Manager. If testing an unusual antibiotic which is not on this list, check the relevant CLSI documents and perform relevant quality control at the same time as doing the test.

If ESBL testing is being performed regularly, *K. pneumoniae* ATCC 700603 should be used for QC: see CLSI documents for further details. If testing large numbers of isolates of *Haemophilus* sp. or *S. pneumoniae*, additional QC strains may need to be obtained and tested (e.g. *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766, *S. pneumoniae* ATCC 49619).

Etest QC procedures are described in SOP MIC-002.

# Limitations

Although the quality control carried out during manufacture of antibiotic disks is usually of a high standard, disk content may deteriorate during storage. This is one reason why regular quality control is essential. Correct storage and rotation of disk stocks is essential to maintain this quality. The main stock of disks should be stored at -20°C with a small quantity for current use being kept at 4°C. The disks required for the day’s work should be brought to room temperature before opening the container. Desiccant should be changed regularly and kept in sealed containers. The oldest disks must be used first and always before their expiry date.

Tests on mixed cultures (as judged by different zone patterns or colonial types) may be unreliable and should be repeated.

There are a number of characteristics of particular organisms that mean that their zones must be read and interpreted with caution. It is difficult to list all of these but some of the commoner issues that can cause problems are listed below. For further details see the CLSI documents.

* **Penicillin & *Staphylococcus aureus***

When a staphylococcus produces the enzyme penicillinase it will be resistant to penicillin. This phenomenon can be observed by examining the zone edge. If there is evidence of a built up or 'heaped up' edge to the zone of inhibition, regardless of the zone size, then the strain should be called resistant. Equivocal strains should be tested for β-lactamase (penicillinase) production.

* **Sulphonamides and Trimethoprim**

These drugs must only be tested on PABA free media as this sulphonamide inhibitor is present in other media such as blood agar. Heavy inoculation may invalidate tests with sulphonamides as enough PABA is contained in the inoculum to inactivate the drug. Disregard any slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

* ***Proteus* species**

Swarming Proteus can cause problems if too heavily inoculated or insufficiently dried plates are used. There is frequently swarming back on the chloramphenicol and trimethoprim zones. When carefully examined a zone edge can be discerned with swarming growth inside it. Measurement should be from the zone edge and the swarming growth disregarded.

* **Enterococci**

Lancefield Group D streptococci such as *E. faecalis* are only moderately sensitive (MIC 2μg/ml) to penicillin and ampicillin and will therefore give no zone to 1μg or a small zone to a 1.5μg disk so l0μg disks are used. They are always resistant to cephalosporins and aminoglycosides and should be reported as such (although enterococci that do not have high level resistance to aminoglycosides may exhibit synergy with cell wall acting agents and may be used in combination with them for severe infections such as endocarditis).

* **Streptococci**

Aminoglycosides (e.g. gentamicin) have only moderate activity against streptococci (MIC 4-6μg/ml) and is therefore not recommended clinically. These should be reported as resistant irrespective of zone diameter.

# References

1. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Tenth Edition. M02-A11. Clinical and Laboratory Standards Institute, January 2012.
2. Performance Standards for Antimicrobial Disk Susceptibility Tests; Twenty-Third Informational Supplement. M100-S23. Clinical and Laboratory Standards Institute, January 2013.
3. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. M45-2A. Clinical and Laboratory Standards Institute 2010.
4. Standard Operating Procedures (SOP), Wellcome-Mahidol-Oxford Melioidosis project laboratory, Sapasitthiprasong Hospital, Ubon Ratchatani. Version: 1.1, June 2003.
5. Bridson EY (2006). The Oxoid Manual, 9th Edition. Oxoid Ltd.
6. Murray P.R., Baron E.J., Pfaller M.A., Tenover F.C., Yolken R.H. Manual of Clinical Microbiology 7th Edition (1999) Am. Soc. Micro.

# Risk assessment

|  |  |
| --- | --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Determination of bacterial antimicrobial susceptibilities by disk diffusion | **Substances used**  1. Cultured bacteria  2. Antimicrobial impregnated disks |
| **Quantities of chemicals used**  5ml of bacterial suspensions | **Frequency of SOP use**  Daily |
| **Hazards identified**  1. Autoclaved liquid  2. Potentially infectious material in sample  Potentially pathogenic bacteria | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  1. Training in good laboratory practices (GLP)  2. Appropriate PPE (lab coat, gloves, eye protection)  3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) | |
| **Checks on control measures**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements**  1. GLP  2. Specific training in this SOP |
| **Emergency procedures**  1. Report all incidents to Safety Adviser  2. Use eyewash for splashes  3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**  1. Sharps discarded into appropriate rigid containers for incineration  2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration  3. Chemical waste disposed of according to manufacturer’s instructions |

# Appendix 1: Antimicrobial disk testing panels and interpretations

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Staphylococcus* spp.** | | | | | | | | | | | |
| **Drug** | | **Details** | | | **Zone (mm) / MIC (µg/ml)** | | | | | | **Comment** |
| **Code** | | **µg** | **S** | **I** | | **R** | | |
| **Plate 1** | | | | | | | | | | | |
| **Cefoxitin** | | | | | | | | | | | Report as oxacillin |
| *S. aureus* | | FOX | | 30 | ≥22 | - | | ≤21 | | |
| CoNS | | FOX | | 30 | ≥25 | - | | ≤24 | | |
| **Ciprofloxacin** | | CIP | | 5 | ≥21 | 16-20 | | ≤15 | | |  |
| **Erythromycin** | | E | | 15 | ≥23 | 14-22 | | ≤13 | | |  |
| **Gentamicin** | | CN | | 10 | ≥15 | 13-14 | | ≤12 | | |  |
| **Penicillin** | | P | | 10 | ≥29 | - | | ≤28 | | |  |
| **Co-trimoxazole** | | SXT | | 25 | ≥16 | 11-15 | | ≤10 | | |  |
| **Plate 2**  Only set up if ≥3 drugs on plate 1 are resistant (or if cefoxitin resistant) | | | | | | | | | | | |
| **Drug** | **Details** | | | | **Zone (mm) / MIC (µg/ml)** | | | | | **Comment** | |
| **Code** | | **µg** | | **S** | | **I** | | **R** |
| **Chloramphenicol** | C | | 30 | | ≥18 | | 13-17 | | ≤12 |  | |
| **Clindamycin** | DA | | 2 | | ≥21 | | 15-20 | | ≤14 |  | |
| **Rifampicin** | RD | | 5 | | ≥20 | | 17-19 | | ≤16 | Do not report | |
| **Tetracycline** | TE | | 30 | | ≥19 | | 15-18 | | ≤14 | Do not report | |
| **Vancomycin** | | | | | | | | | | Should use Etest | |
| *S. aureus* | VA | | | 30 | ≥7 | - | | - | |
|  | VA | | | Etest | ≤2 | 4-8 | | ≥16 | |
| CoNS | VA | | | Etest | ≤4 | 8-16 | | ≥32 | |
| ***Streptococcus pneumoniae*** | | | | | | | | | | | |
| **Drug** | | **Details** | | | **Zone diameter (mm)** | | | | | | **Comment** |
| **Code** | | **µg** | **S** | **I** | | **R** | | |
| **Plate 1** | | | | | | | | | | | |
| **Chloramphenicol** | | C | | 30 | ≥21 | - | | ≤20 | | |  |
| **Clindamycin** | | DA | | 2 | ≥19 | 16-18 | | ≤15 | | | Do not report |
| **Erythromycin** | | E | | 15 | ≥21 | 16-20 | | ≤15 | | |  |
| **Oxacillin** | | OX | | 1 | ≥20 | - | | - | | | See below |
| **Co-trimoxazole** | | SXT | | 25 | ≥19 | 16-18 | | ≤15 | | |  |
| **Tetracycline** | | TE | | 30 | ≥28 | 25-27 | | ≤24 | | | Do not report |
| **Plate 2**  Only set up PG and TX Etests if oxacillin zone <20mm (can put both on same agar plate) | | | | | | | | | | | |
| **Drug** | | **Details** | | | **MIC (µg/ml)** | | | | | | **Comment** |
| **Code** | | **µg** | **S** | **I** | | **R** | | |
| **Ceftriaxone** | | | | | | | | | | |  |
| Meningitis | | TX | | Etest | ≤0.5 | 1 | | ≥2 | | |
| Non-meningitis | | TX | | Etest | ≤1 | 2 | | ≥4 | | |
| **Penicillin** | | | | | | | | | | |  |
| Meningitis | | PG | | Etest | ≤0.06 | - | | ≥0.12 | | |
| Non-meningitis | | PG | | Etest | ≤2 | 4 | | ≥8 | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Alpha- and Beta-haemolytic streptococci** | | | | | | |
| **Drug** | **Details** | | **Zone (mm) / MIC (µg/ml)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ceftriaxone** | | | | | |  |
| Alpha-haem | CRO | 30 | ≥27 | 25-26 | ≤24 |  |
| Beta-haem | CRO | 30 | ≥24 | - | - |  |
| **Chloramphenicol** | C | 30 | ≥21 | 18-20 | ≤17 |  |
| **Clindamycin** | DA | 2 | ≥19 | 16-18 | ≤15 | Do not report |
| **Erythromycin** | E | 15 | ≥ 21 | 16-20 | ≤15 |  |
| **Penicillin** | | | | | | Only do PG Etest if significant blood culture isolate |
| Alpha-haem | PG | Etest | ≤0.12 | 0.25-2 | ≥4 |
| Beta-haem | P | 10 | ≥24 | - | - |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Enterococcus* spp.** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ampicillin** | AMP | 10 | ≥17 | - | ≤16 |  |
| **Chloramphenicol** | C | 30 | ≥18 | 13-17 | ≤12 |  |
| **Ciprofloxacin** | CIP | 5 | ≥21 | 16-20 | ≤15 |  |
| **Vancomycin** | VA | 30 | ≥17 | 15-16 | ≤14 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Enterobacteriaceae (coliforms)** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Plate 1** | | | | | | |
| **Ampicillin** | AMP | 10 | ≥17 | 14-16 | ≤13 |  |
| **Cefpodoxime\*** | CPD | 10 | ≥21 | 18-20 | ≤17 | Do not report |
| **Ciprofloxacin** | CIP | 5 | ≥21 | 16-20 | ≤15 |  |
| **Gentamicin** | CN | 10 | ≥15 | 13-14 | ≤12 |  |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |
| **Imipenem** | IPM | 10 | ≥23 | 20-22 | ≤19 |  |
| **Plate 2**  Set up on all isolates | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Co-amoxiclav** | AMC | 30 | ≥18 | 14-17 | ≤13 |  |
| **Ceftazidime\*** | CAZ | 30 | ≥21 | 18-20 | ≤17 | Do not report |
| **Ceftriaxone\*** | CRO | 30 | ≥23 | 20-22 | ≤19 |  |
| **Chloramphenicol** | C | 30 | ≥18 | 13-17 | ≤12 | Do not report if urine |
| **Nitrofurantoin** | F | 300 | ≥17 | 15-16 | ≤14 |  |

\*Do ESBL tests on the following organisms:

* *E. coli* and *Klebsiella* spp. that have
  + cefpodoxime zones ≤ 17 mm OR
  + ceftriaxone zones ≤ 25 mm OR
  + ceftazidime zones ≤ 22 mm
* *Proteus mirabilis* that have
  + cefpodoxime zones ≤ 22 mm OR
  + ceftazidime zones ≤ 22 mm
* Report CRO / CAZ disc results before ESBL testing is completed and DO NOT edit to “R” if ESBL positive

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Salmonella* spp.** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Plate 1** | | | | | | |
| **Ampicillin** | AMP | 10 | ≥17 | 14-16 | ≤13 |  |
| **Ceftriaxone** | CRO | 30 | ≥23 | 20-22 | ≤19 |  |
| **Chloramphenicol** | C | 30 | ≥18 | 13-17 | ≤12 |  |
| **Ciprofloxacin** | CIP | 5 | ≥31 | 21-30 | ≤20 |  |
| **Nalidixic acid** | NAL | 30 | ≥19 | 14-18 | ≤13 | Do not report |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |
| **Plate 2**  Set up ciprofloxacin Etest on all isolates | | | | | | |
| **Drug** | **Details** | | **MIC (µg/ml)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ciprofloxacin** | CI | Etest | ≤0.06 | 0.12-0.5 | ≥1 |  |

In addition, azithromycin disk (AZM 15) and Etest (AZ) may be requested by the medical staff: note that CLSI breakpoints are not available for these tests.

**For *Shigella* spp. isolates set up *Salmonella* spp. plate 1 only but note that the ciprofloxacin zones sizes should be interpreted using the Enterobacteriaceae criteria below**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Shigella* spp.** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ciprofloxacin** | CIP | 5 | ≥21 | 16-20 | ≤15 |  |
| ***Acinetobacter* spp.** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ceftazidime** | CAZ | 30 | ≥18 | 15-17 | ≤14 |  |
| **Ceftriaxone** | CRO | 30 | ≥21 | 14-20 | ≤13 |  |
| **Ciprofloxacin** | CIP | 5 | ≥21 | 16-20 | ≤15 |  |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |
| **Gentamicin** | CN | 10 | ≥15 | 13-14 | ≤12 |  |
| **Imipenem** | IPM | 10 | ≥16 | 14-15 | ≤13 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Burkholderia cepacia*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ceftazidime** | CAZ | 30 | ≥21 | 18-20 | ≤17 |  |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Burkholderia pseudomallei*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Plate 1** | | | | | | |
| **Co-amoxiclav** | AMC | 30 | ≥18 | 14-17 | ≤13 |  |
| **Ceftazidime** | CAZ | 30 | ≥18 | 15-17 | ≤14 |  |
| **Chloramphenicol** | C | 30 | ≥16 | 13-15 | ≤12 |  |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 | See below |
| **Doxycycline** | DO | 30 | ≥16 | 13-15 | ≤12 |  |
| **Imipenem** | IPM | 10 | ≥18 | 14-17 | ≤13 |  |
| **Plate 2**  Only set up if co-trimoxazole intermediate or resistant by SXT disk | | | | | | |
| Drug | Details | | MIC (µg/ml) | | | Comment |
| **Code** | **µg** | **S** | **I** | **R** |
| **Co-trimoxazole** | TS | Etest | ≤2 | - | - |  |

For identification, also set up a plate with gentamicin (CN 10) and colistin (CT 10) discs: *B. pseudomallei* is sensitive to AMC but resistant to CN and CT.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Pseudomonas aeruginosa*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ceftazidime** | CAZ | 30 | ≥18 | 15-17 | ≤14 |  |
| **Ciprofloxacin** | CIP | 5 | ≥21 | 16-20 | ≤15 |  |
| **Colistin** | CT | 10 | ≥11 | - | ≤10 | Do not report |
| **Gentamicin** | CN | 10 | ≥15 | 13-14 | ≤12 |  |
| **Imipenem** | IPM | 10 | ≥19 | 16-18 | ≤15 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Stenotrophomonas maltophilia*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Haemophilus influenzae* and *Haemophilus parainfluenzae*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ampicillin** | AMP | 10 | ≥22 | 19-21 | ≤18 |  |
| **Co-amoxiclav** | AMC | 30 | ≥20 | - | ≤19 |  |
| **Ceftriaxone** | CRO | 30 | ≥26 | - | - |  |
| **Chloramphenicol** | C | 30 | ≥29 | 26-28 | ≤25 |  |
| **Ciprofloxacin** | CIP | 5 | ≥21 | - | - |  |
| **Co-trimoxazole** | SXT | 25 | ≥16 | 11-15 | ≤10 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Moraxella catarrhalis*** | | | | | | |
| **Drug** | **Details** | | **Zone (mm) / MIC (µg/ml)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Co-amoxiclav** | AMC | 30 | ≥24 | - | ≤23 |  |
| **Ceftriaxone** | If clinically required (e.g. blood culture isolate, do TX Etest (S ≤2) | | | | | |
| **Co-trimoxazole** | SXT | 25 | ≥13 | 11-12 | ≤10 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Neisseria meningitidis*** | | | | | | |
| **Drug** | **Details** | | **Zone (mm) / MIC (µg/ml)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ceftriaxone** | CRO | 30 | ≥34 | - | - |  |
| **Chloramphenicol** | C | 30 | ≥26 | 20-25 | ≤19 |  |
| **Ciprofloxacin** | CIP | 5 | ≥35 | 33-34 | ≤32 |  |
| **Penicillin** | PG | Etest | ≤0.06 | 0.12-0.25 | ≥0.5 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Neisseria gonorrhoeae*** | | | | | | |
| **Drug** | **Details** | | **Zone diameter (mm)** | | | **Comment** |
| **Code** | **µg** | **S** | **I** | **R** |
| **Ciprofloxacin** | CIP | 5 | ≥41 | 28-40 | ≤27 |  |
| **Ceftriaxone** | CRO | 30 | ≥35 | - | - |  |
| **Penicillin** | P | 10 | ≥47 | 27-46 | ≤26 |  |
| **Tetracycline** | TE | 30 | ≥38 | 31-37 | ≤30 | Do not report |

# Appendix 2: Antimicrobial disk QC templates

## Quality control for non-fastidious organisms

**Antimicrobial disk / Mueller-Hinton agar (MH) QC sheet**

|  |  |
| --- | --- |
| **QC strains:** | *E. coli* ATCC 35218; *E. coli* ATCC 25922  *S. aureus* ATCC 25923; *P. aeruginosa* ATCC 27853 |
| **Date MH prepared:** |  |
| **Date zones measured:** |  |
| **QC performed by:** |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Disk details** | | **Zone diameter (mm)** | | | | | | | |
| ***E. coli***  **ATCC 35218** | | ***E. coli***  **ATCC 25922** | | ***S. aureus***  **ATCC 25923** | | ***P. aeruginosa***  **ATCC 27853** | |
| **Code** | **µg** | **Expected** | **Measured** | **Expected** | **Measured** | **Expected** | **Measured** | **Expected** | **Measured** |
| **AMC** | 30 | 17-22 |  | 18-24 |  | - | - | - | - |
| **AMP** | 10 | 6 |  | 16-22 |  | - | - | - | - |
| **AZM** | 15 | - | - | - | - | 21-26 |  | - | - |
| **C** | 30 | - | - | 21-27 |  | 19-26 |  | - | - |
| **CAZ** | 30 | - | - | 25-32 |  | - | - | 22-29 |  |
| **CPD** | 10 | - | - | 23-28 |  | - | - | - | - |
| **CIP** | 5 | - | - | 30-40 |  | 22-30 |  | 25-33 |  |
| **CRO** | 30 | - | - | 29-35 |  | - | - | - | - |
| **CN** | 10 |  |  | 19-26 |  | 19-27 |  | 17-23 |  |
| **CT** | 10 | - | - | 11-17 |  | - | - | 11-17 |  |
| **DA** | 2 | - | - | - | - | 24-30 |  | - | - |
| **DO** | 30 | - | - | 18-24 |  | - | - | - | - |
| **E** | 15 | - | - | - | - | 22-30 |  | - | - |
| **F** | 300 | - | - | 20-25 |  | - | - | - | - |
| **FOX** | 30 | - | - | - | - | 23-29 |  | - | - |
| **IPM** | 10 | - | - | 26-32 |  | - | - | 20-28 |  |
| **NA** | 30 | - | - | 22-28 |  | - | - | - | - |
| **P** | 10 | - | - | - | - | 26-37 |  | - | - |
| **RD** | 5 | - | - | - | - | 26-34 |  | - | - |
| **SXT** | 25 | - | - | 23-29 |  | 24-32 |  | - | - |
| **TE** | 30 | - | - | - | - | 24-30 |  | - | - |
| **VA** | 30 | - | - | - | - | 17-21 |  | - | - |

**ESBL disk / Mueller-Hinton agar (MH) QC sheet**

|  |  |
| --- | --- |
| **QC strain:** | *E. coli* ATCC 25922; *K. pneumoniae* ATCC 700603 |
| **Date MH prepared:** |  |
| **Date zones measured:** |  |
| **QC performed by:** |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Disk details** | | **Zone diameter (mm)** | | **Zone diameter (mm)** | |
| ***E. coli***  **ATCC 25922** | | *K. pneumoniae* ATCC 700603 | |
| **Code** | **µg** | **Expected** | **Measured** | **Expected** | **Measured** |
| **CAZ** | 30 | 25-32 |  | 10-18 |  |
| **CAZ/CLAV** | 30/10 | ≤ 2mm increase from CAZ alone |  | ≥ 5mm increase from CAZ alone |  |
| **CPD** | 30 | Not defined by CLSI\* |  | Not defined by CLSI\* |  |
| **CPD/CLAV** | 30/10 | Not defined by CLSI\* |  | Not defined by CLSI\* |  |
| **CTX** | 30 | 29-35 |  | 17-25 |  |
| **CTX/CLAV** | 30/10 | ≤ 2mm increase from CTX alone |  | ≥ 3mm increase from CTX alone |  |

\*CLSI protocol uses CPD10 disk

## Quality control for fastidious organisms

**Antimicrobial disk / Haemophilus Test Medium (HTM) QC sheet**

|  |  |
| --- | --- |
| **QC strain:** | *H. influenzae* ATCC 49247 |
| **Date HTM prepared:** |  |
| **Date zones measured:** |  |
| **QC performed by:** |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Disk details** | | **Zone diameter (mm)** | |
| **Code** | **µg** | **Expected** | **Measured** |
| AMC | 30 | 15-23 |  |
| AMP | 10 | 13-21 |  |
| C | 30 | 31-40 |  |
| CIP | 5 | 34-42 |  |
| CRO | 30 | 31-39 |  |
| SXT | 25 | 24-32 |  |

**Antimicrobial disk / Mueller-Hinton agar + 5% sheep blood (MHB) QC sheet**

|  |  |
| --- | --- |
| **QC strain:** | *S. pneumoniae* ATCC 49619 |
| **Date MHB prepared:** |  |
| **Date zones measured:** |  |
| **QC performed by:** |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Disk details** | | **Zone diameter (mm)** | |
| **Code** | **µg** | **Expected** | **Measured** |
| C | 30 | 23-27 |  |
| CRO | 30 | 30-35 |  |
| DA | 2 | 19-25 |  |
| E | 15 | 25-30 |  |
| OP | - | ≥14 |  |
| OX | 1 | ≤12 |  |
| P | 10 | 24-30 |  |
| SXT | 25 | 20-28 |  |
| TE | 30 | 27-31 |  |

\*Better assessed using *S. aureus* ATCC 25923 (see *non-fastidious organisms*)