Antimicrobial Susceptibility Testing for Treatment and Control of Cholera

GLOBAL TASK FORCE ON CHOLERA CONTROL

OBJECTIVE : To provide instruction for determining in vitro susceptibility of *Vibrio cholerae* 01/0139

METHODS

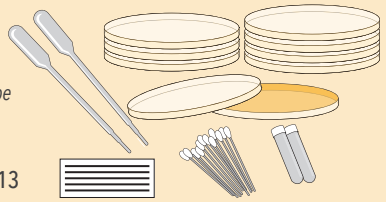
Combination of two methods:

- Agar disk diffusion method with antibiotic impregnated disks at predetermined concentrations.
- Measurement of minimum inhibitory concentration (MIC) by using test-strips impregnated with a gradient of predefined concentrations of antibiotics.

NOTE: *Test-strips are recommended for antibiotics for which no breakpoint is defined or when complementary tests are needed.*
NOTE: *Control strain(s) should always be set up in parallel with test strains.*

MATERIALS REQUIRED

- Mueller Hinton Agar (MHA) plates (4 mm ± 0,5mm deep)
- Sterile saline solution (0,85% or 0,9%) + test tubes of identical size for bacterial suspensions and the McFarland turbidity standard
- Sterile cotton tipped swabs
- Automatic disk dispenser or template with 5 or 6 disk spacing pattern and forceps
- Metric ruler *(that can measure in mm)*
- 0.5 McFarland turbidity standard
- Sheet of white paper with sharp black lines *(can be prepared by hand or printed out)*
- Control strains : *Escherichia coli* ATCC25922
  Staphylococcus aureus ATCC29213



Antibiotic to be tested and recommended method:

Initial screen test by disk diffusion	*Confirmation test by MIC mesurement*
• Erythromycin (EM), (15 mg)	• Azithromycin (AZ)
• Pefloxacin (PEF), (5 µg)	• Ciprofloxacin (CIP)
• Tetracycline (TE), (30 µg)	• Doxycycline (DO)

AZ, CIP and DOX are the three selected antibiotics recommended for treatment of cholera according to GFTCC: https://www.who.int/cholera/task_force/use-of-antibiotics-for-the-treatment-of-cholera.pdf?ua=1

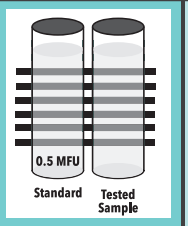
- Store antibiotic disks and test-strips between -20°C and 8°C according to manufacturer's instructions.
- Check expiration date of antibiotic disks and test strips prior use.

PROCEDURE FOR DISK AND STRIP TESTING

1. Preparation of inoculum

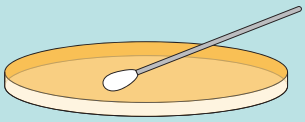
Prepare a bacterial suspension with a few well-isolated colonies from an overnight (18-24 hours at 35 ± 2°C) agar culture in sterile saline solution adjusted to 0.5 MacFarland* by comparison to the standard.

NOTE: *Ensure that the Standard is aliquoted into a tube that is the same size as the tube used to prepare the test suspension.*



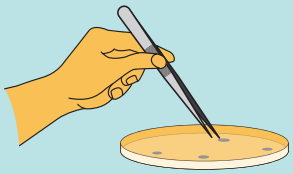
**corresponding to an inoculum of approximately 1 to 2 x 10^8 CFU/mL (Absorbance $_{600nm}$=0.08-0.1)*

2. Inoculation of MHA



- Not more than 15 minutes after preparing the inoculum suspension.
- Dip cotton swab in bacterial suspension; remove excess liquid by pressing the swab against the inside wall of the tube.
- Streak the entire surface of the plate 3 times, rotating 60 degrees each time.
- Ensure the surface is completely dry before the next step.

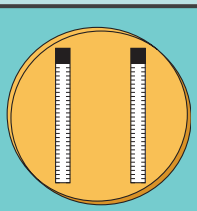
3. Application of antibiotic disks



- Not more than 15 minutes after swabbing.
- Place the disks individually with an automatic disk dispenser or sterile forceps, gently pressing down onto the agar.
- Do not move disks once deposited.
- Replace lid, invert the plates and place in the incubator.

NOTE: *Allow disks to reach ambient temperature before opening cartridge or container for storage.*

4. Application of test-strips



- Not more than 15 minutes after swabbing.
- Place the strips on the agar according to the recommendations of the manufacturer.
- Do not move strips once deposited.
- Replace lid, invert the plates and place in the incubator.

NOTE: *Test-strips must be consistently stored in a freezer at -20 C. Allow strips that will be used to reach ambient temperature before placing on the agar.*

5. Incubation: 18 ± 2 hours at 35°C ± 2°C.

6. Reading: After 18 ± 2 hours, observe the plate and measure the diameter (mm) of the inhibition ring. Read MIC value (in µg/mL) at the intersection of the lower part of the ellipse-shaped growth inhibition area with the test-strip. If a MIC value is between two fold dilutions, always round up to the highest value.

INTERPRETATION OF RESULTS

- **Quality Control: if the control strain results are unexpected or out of range,any results on *V. cholerae* strains are invalid and the laboratory should investigate the source of error.**
- Tetracycline: screening test for doxycycline sensitivity. Strains sensitive to TE can be categorized as "sensitive" to doxycycline. According to CA-SFM/EUCAST, if TE resistant, doxycycline must be tested individually by MIC mesurement.
- Pefloxacine: screening test for ciprofloxacin sensitivity testing.
- Erythromycin: screening test for azithromycin sensitivity testing.

NOTE: MIC measurement for AZ and CIP is not required for case management but is recommended for epidemiological surveillance of the strains.

NOTE1: Nalidixic acid disc-diffusion testing proved a reliable method for identifying *V. cholerae* O1 strains with decreased susceptibility or resistance to ciprofloxacin, and can be used as an alternative to the PEF screen test. If resistant to NA (based on interpretative criteria from *Enterobacteriaceae*), the isolate should be tested for susceptibility to CIP by MIC measurement. Strains showing growth up to contact with the NA disk should be considered resistant to CIP (MIC≥0.25 mg/L).

NOTE2: Additional antibiotics can be tested for the epidemiological monitoring of strains (*i.e., colistin, polymyxin B, ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, cephalosporine 1st and 3rd generations, streptomycin).*

INTERPRETATION: please refer to one of the following standards:

CLSI: https://clsi.org/all-free-resources/, CLSI M45 ED3:2016, Table 20. *Vibrio* spp. (Including *Vibrio cholerae*)
EUCAST: http://www.eucast.org/clinical_breakpoints
CA-SFM / EUCAST: https://www.sfm-microbiologie.org/wp-content/uploads/2023/06/CASFM2023_V1.0.pdf

NOTE: The standards are reviewed on a regular basis, please check you are using an up-dated version.
This document is intended for use by Reference Laboratories. Please keep isolate until testing is complete or in accordance with the laboratory sample retention policy.

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