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

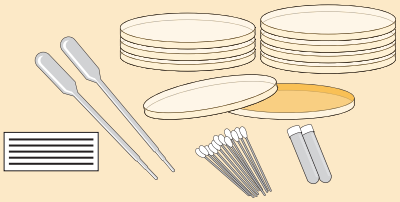
Combination of two methods:

- 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 strain : *Escherichia coli* ATCC 25922



Antibiotic to be tested and recommended method:

- Azithromycin (AZ), MIC measurement
- Nalidixic acid (NA), disk diffusion (30 µg)* *(used as an indicator of reduced fluoroquinolones susceptibility)*
- Ciprofloxacin (CIP), MIC measurement *(used to monitor the level of susceptibility to CIP for strains resistant to NA)*
- Tetracycline (TE), disk diffusion (30 µg)

AZ, CIP and TE 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

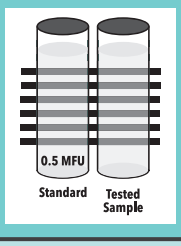
- Store antibiotic disks and test-strips between -20°C and 8°C according to manufacturer's instructions.
- Check expiration date of antibiotic disks and test strips prior use.

PROCEDURE FOR DISK AND STRIP TESTING

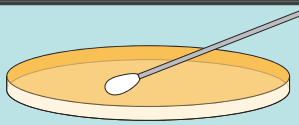
1. Preparation of inoculum

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.*

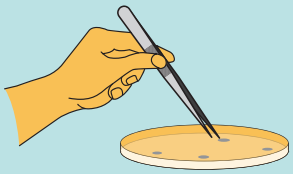


2. Inoculation of MHA



- 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.

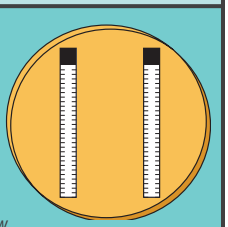
3. Application of antibiotic disks



- 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 hours at 35°C ± 2°C.

6. Reading: After 18 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

- Results from the tetracycline disk should be used to predict susceptibility to doxycycline.
- *NA susceptibility testing is an optional step but has proven to be a reliable and inexpensive first line screening tool for the detection of strains that should be individually tested for susceptibility to CIP. Strains categorized as sensitive to NA can be categorized as sensitive to CIP. If resistant to NA, the isolate should be tested for susceptibility to CIP by MIC measurement.

NOTE: *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).*

Quality Control

If the control strain results are unexpected or out of range, any results on VC strains are invalid and the laboratory should investigate the sources of error.

INTERPRETATION: please refer to one of the following standards:

EUCAST: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf *(using interpretive criteria for the Enterobacteriaceae)*

CLSI: https://clsi.org/media/1450/m45ed3_sample.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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