Protocol for Lysine Susceptibility Testing on *Clostridium difficile NTCC 13307*
Protocol code: Lys_Sus

Materials

- Viable *C. difficile* on blood agar petri plate
- Liquid medium BHI with thioglycolate
- Disks filter paper
- Columbia agar with lysed blood petri plate
- Lysine CD27L (10 µM)
- Anaerobic chamber at 37°C

Procedure

1. Take all the colonies growth after 72 hours incubation on the blood agar plate and resuspended them in 1 mL of liquid BHI with thioglycolate.
2. Vortex the BHI tube to create a smooth suspension.
3. Dip a sterile swab into the inoculum tube.
4. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet.
5. Inoculate the dried surface of a Columbia agar plate with lysed blood by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum.
6. Drain the plate with the swab to pick up any excess liquid.
7. Discard the swab into an appropriate container.
8. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more of that, for the surface of the agar plate to dry before proceeding to the next step.
9. Place 6 disks on the surface of the agar, distributed by all the plate.
10. Inoculate different 15 µL of concentrations of the lysine CD27L on the disks.
11. Incubate the plates at 37°C by 48 hours.
12. Look for the size zones following the Kirby-Bauer method to find the inhibitory minimal concentration.

References

1. Bauer, A. W., Perry, D. M., & Kirby, W. M. (1959). Single-disk antibiotic-sensitivity testing of staphylococci: An analysis of technique and results. AMA archives of internal medicine, 104(2), 208-216.