Antibiotic susceptibility assay protocol (Disk diffusion susceptibility test)

Which antibiotic works the best?

Background

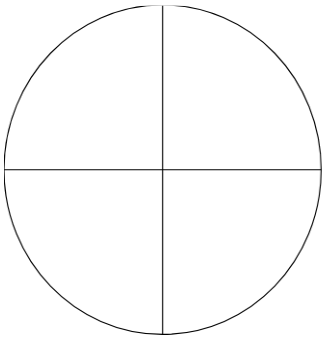
This assay, also known as the Kirby-Bauer disk diffusion susceptibility test, determines the resistance and susceptibility of bacteria to antimicrobial compounds. This test is useful in determining an effective antibiotic to prescribe to a patient. A petri dish containing bacteria inoculated with the spread plate method is used and a disk with the antimicrobial compound is placed; the antimicrobial diffuses through the plate to the surrounding bacteria. After incubation, the disks may or may not have a zone of inhibition, where bacterial growth did not occur. Based on the size of the zone of inhibition, the antimicrobial susceptibility is determined relative to other antimicrobial compounds tested. The larger the zone of inhibition, the better the antimicrobial susceptibility.

Materials

Petri dishes with LB Agar
antimicrobial disks
E. coli or other bacteria in LB broth
forceps
Drigalski spatula (cell spreader)
parafilm
incubator
ruler

Preparation of petri dishes

1. Culture *E. coli* in LB broth overnight (rehydrating bacteria from freeze-dried is fine).
2. Prepare LB agar plates as indicated on the bottle. (Bio-Rad 166-0600: 20g powder into 500mL dH2O).
   a) Sterilize agar in a way most fitting (Autoclave, microwave, hot plate, instant pot).
   b) Allow agar to cool and then pour ~ 30 mL onto a 100mm plate (~ 15mL for a 60mm plate).
   c) Let cool/solidify and store in the refrigerator for up to 4-6 weeks.
3. Dispense 50-100uL of *E. coli* culture onto LB agar plate and spread with Drigalski spatula; be sure to cover the entire surface as evenly as possible.
4. Label your plate as shown below and label each quadrant with A, B, C, and D. Using forceps, add 1-4 antimicrobial disks making sure they are evenly spread out. Fill out which antibiotic you place in each quadrant below.



Quadrant	Antibiotic Used
A	
B	
C	
D	

5. Incubate at 37°C 16-24 hours.
6. Using the ruler, determine the diameters of the zones of inhibition and determine the most effective antimicrobial agent.

Results / Analysis:

1. Report your results in the table below:

Antibiotic Nj

Diameter (mm)

2. Which antibiotic worked the best? How do you know?