Week 9: Antibiotic Sensitivity

Materials

- 1 tube of overnight culture of your sample

- Sterile swabs

- Forceps/Lighter

- Appropriate (LB and BHI) agar plates

- Antibiotic disks:

· Ampicillin disks

· Chloramphenicol disks

· Tetracycline disks

· Penicillin disks

· Gentamicin disks

· Enrofloxacin disks

· Neomycin disks

· Streptomycin disks

**Procedure**

Set up modified Kirby Bauer test

- The Kirby Bauer test allows microbiologists to test the efficacy of multiple antibiotics on one culture. After the antibiotic disk is placed on the agar, it begins to diffuse into the agar so that the farther away from the disk (center of the zone of diffusion), the lower the concentration of antibiotic. For each type of antibiotic, there must be a set minimum zone of clearing for the bacteria to be considered susceptible to the antibiotic. Limitations to this method includes the potential variation in amount of bacteria initially inoculated onto the plate (we aren’t going to normalize for this). The minimum diameter of the zone of inhibition will be measured next week in class.

1) Gently shake your overnight culture to resuspend the bacteria into the broth. This is your stock culture.

2) Dip a sterile swab into the liquid culture, use the swab to spread the liquid culture **evenly** over the entire plate.

3) Allow the liquid to dry

4) Using flame sterilized forceps, aseptically place 4 different antibiotics disks onto each labeled quadrant of a plate. FLAME THE FORCEPS BETWEEN EACH DISK. Press down \*gently\* to make sure the disks stick to the agar and don’t fall off when the plate is inverted. Repeat for second plate

5) Plate your perfectly labeled plates into the appropriate pile