exercise twenty-eight

PATIENT X WOUND INFECTION SITUATION – TESTING ANTIMICROBIALS

Antimicrobial Susceptibility Test (Kirby Bauer Method)

PATIENT SITUATION

You are testing samples from three patients. Patient B is in the burn unit and has a skin infection. Tests have determined it is caused by *Pseudomonas*. Patient S has a post-surgical infection caused by *Staphylococcus aureus*. Patient X has a surgical wound infection. For patient X, the causative organism will be identified using an Enterotube and the source of the infection determined with DNA fingerprinting. Your job is to do a culture and sensitivity test to find out which antibiotic would be best to treat each of these patients with.

SOME BACKGROUND

"Antimicrobial" is a general term for something that kills or inhibits microorganisms. An **antibiotic** is a type of antimicrobial that is made by microorganisms to kill or inhibit other microorganisms. Very few antibiotics work equally well against all types of bacteria. Some are more effective against Gram-positive bacteria, while other have a greater effect against Gram-negative bacteria. When treating an infected patient, we want to use the antibiotic that will both be the safest and most effective against their disease. In many cases, we need to test the bacteria the patient is infected with against a variety of different antibiotics to see which will work the best. As is often true, a quick, reliable, easy to do test is preferred. The goal is to find out whether the bacteria being tested is **sensitive** to the antibiotic (will be killed by it) or **resistant** to it (will be ineffective for treating the patient).

The **Kirby-Bauer method** is widely used for testing antimicrobials. It is done by covering the surface of a plate with the bacteria being tested. Paper discs containing known concentrations of different antimicrobials are then placed on the surface of the plate. The concentrations of the antimicrobial compound found in the discs are the same as those found in the body of someone taking that antimicrobial. The antimicrobial agent in the disc diffuses out of it, forming a concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. If the antimicrobial inhibits the growth of the organism a clear circular area appears

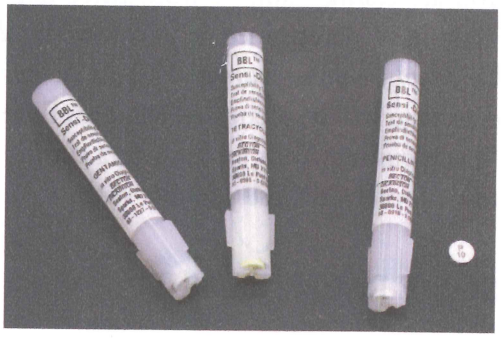
around the disc. This is called the **zone of inhibition**. The method for doing the Kirby-Bauer test is highly standardized for use in clinical laboratories. It involves the use of a special type of agar, **Mueller-Hinton agar**, which must be poured to a standard thickness. The plates are inoculated with cotton swabs rather than inoculating loops. Antibiotic discs are then placed on the surface of the plate either with tweezers or using a mechanical dispenser. After incubation, the plates are examined for zones of inhibition and the zones are measured. The measurements of the zones are then compared to standards to determine if the bacteria is sensitive to the antibiotic, resistant to it, or the measurement is between those numbers (intermediate). Your patient probably won't be happy if you treat them with an antibiotic that gives a resistant or intermediate measurement.

TIPS FOR SUCCESS

- When you swab your plates, make sure not to leave gaps, or you may not see the zones of inhibition well.
- Once the discs are all on the surface of the plates, keep them lid side down. Water dropping onto your plate surface will ruin your zones of inhibition.

ORGANISMS: (Broth cultures)

~~Patient X (has a Gram-negative infection)~~
~~Patient S (has a *Staphylococcus* infection)~~
~~Patient B (has a *Pseudomonas* infection)~~
Patient sample

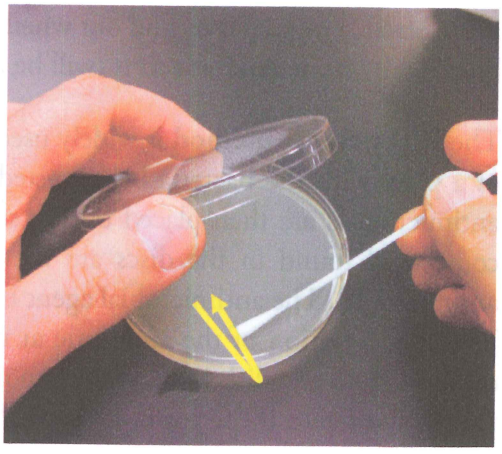


MATERIALS

Sterile swabs for inoculating the plates
Antibiotic discs (BBL Sensi-Discs) Chloramphenicol (30 µg), Gentamycin (10 µg), Penicillin (10 units), Streptomycin (10 µg), Tetracycline (30 µg), Vancomycin (30 µg) (Codes are printed on the top and bottom of the discs to tell you which antibiotic it contains)
Tweezers
Alcohol beakers for sterilizing tweezers
Metric rulers (period 2)

MEDIA

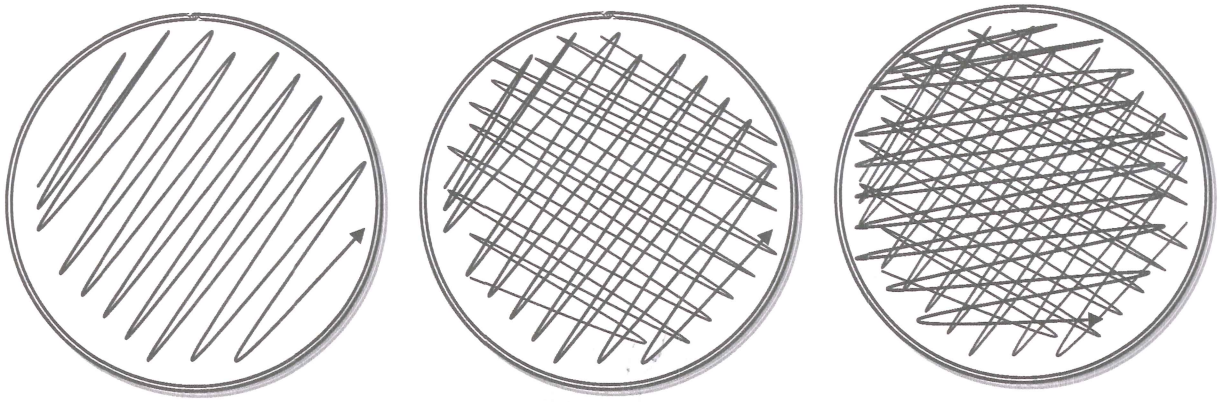
1 ~~3~~ Mueller-Hinton Plates



PROCEDURE

PERIOD 1

1. Use a sterile swab to make a "lawn" of bacteria on each plate (completely cover the plate as shown in the figure).



Use your swab to make parallel streaks in one direction that are close together

Rotate the plate 90° and make another set of parallel streaks

Rotate the plate 45° and make a final set of parallel streaks

2. Discard your swabs in the biohazardous waste.
3. You will use sterile tweezers (or a disc dispenser if available) to place the 6 different antimicrobial discs on the plates.
4. Sterilize your tweezers by dipping them in alcohol and lighting the alcohol with the flame of your Bunsen burner. **Hold the tip of the tweezers below your hand** to avoid injury. Leave the tweezers in the burner flame only long enough to light the alcohol – do not hold them in the flame.
5. Pick up a cartridge (container) of discs.
6. Push against one edge of the disc at the opening of the cartridge so that it sticks out slightly from the other side.
7. Grasp the disc with the tweezers and carefully place it on the surface of the plate.
8. Tap the disc down gently with your tweezers to make sure it is in good contact with the plate surface.
9. Sterilize the tweezers again and repeat until all three plates have six different antimicrobial discs on them (as shown in the diagram).
10. Place the plates in the incubator lid side down.