KIRBY-BAUER TEST for Antibiotic Susceptibility

The Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. It has been superceded in clinical labs by automated tests. But the K-B is still used in some some labs, or used with certain bacteria that automation does not work well with.

The basics are easy: The bacterium is swabbed on the agar and the antibiotic discs are placed on top. The antibiotic diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or inhibited by the concentration of the antibiotic, there will be NO growth in the immediate area around the disc: This is called the zone of inhibition. The zone sizes are looked up on a standardized chart to give a result of sensitive, resistant, or intermediate. Many charts have a corresponding column that also gives the MIC (minimal inhibitory concentration) for that drug.

Each student will need to subculture his/her Staph (from ear, nose, or throat) into a fresh TSB PRIOR to this lab in order to have a young culture to run the antibiotic sensitivity test. Have you made sure that the culture is a G+ coccus, catalase +? Is it growing well in the TSB broth?

The interesting aspect of this particular lab is that you will all be running the same genus of bacterium---Staphylococcus. However, you will see that there can be a lot of variation between species of Staph, and even strains within one species. The Mueller-Hinton medium being used for the K-B is very high in protein, in particular.

OBJECTIVES:

Determine the susceptibility of the strains of Staph species to various antibiotics.

MATERIALS NEEDED: in pairs

- a Mueller-Hinton agar plate/student
- the Staph isolate (in a broth culture) from EACH pair of students (if both of you have a Staph isolate, choose 1 to run)
- sterile swabs
- antibiotics
- ethanol
- good forceps
- Pseudomonas aeruginosa Kirby-bauer plate for demo
- guidelines chart for interpretation of antibiotic susceptibility
THE PROCEDURES:

1. Swab a Mueller-Hinton plate with each of the bacteria. Dip a sterile swab into the broth and express any excess moisture by pressing the swab against the side of the tube.
2. Swab the surface of the agar completely (you do not want to leave any unswabbed agar areas at all). In the pictures above and below, you can see what happens when the plate is not swabbed correctly with even coverage of the bacterium over the entire agar.
3. After completely swabbing the plate, turn it 90 degrees and repeat the swabbing process. (It is not necessary to re-moisten the swab.) Run the swab around the circumference of the plate before discarding it in the discard bag.
4. Allow the surface to dry for about 5 minutes before placing antibiotic disks on the agar.
5. THE ANTIBIOTIC DISKS:
   - The antibiotic dispensers have 8 antibiotic cartridges in them. If you do not see 8 disks come out onto your agar plate, you will have to manually remove the antibiotic from a free cartridge (see line below).
   - Each free antibiotic cartridge should have a little metal arm that allows you to dispense the disc right onto the agar. Even so, sometimes the discs pop out and fall in a place on the agar that you do not want it to be. Just quickly pick up the disc and move it to the appropriate place with the sterile forceps.
   - Lightly touch each disc with your sterile inoculating loop to make sure that it is in good contact with the agar surface. Incubate upside down and incubate at $37^\circ C$.

INTERPRETATION:

1. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. HOLDING THE PLATE UP TO THE LIGHT MIGHT HELP.
2. The disc diameter will actually be part of that number. If there is NO zone at all, report it as 0---even though the disc itself is around 7 mm.
3. Zone diameter is reported in millimeters, looked up on the lab chart (available in the lab), and result reported as S (sensitive), R (resistant), or I (intermediate).
4. Record the results for everyone on your table in the table below.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>isolate 1</th>
<th>isolate 2</th>
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<tbody>
<tr>
<td></td>
<td>zone diameter</td>
<td>S, R, or I</td>
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Your instructor may want the class to compare antibiotics sensitivities of the various Staph isolates.

QUESTIONS:
1. The larger the zone size, the more ______________ the bacterium is to that antibiotic.
2. What measurement units are used to measure the zone sizes?
3. How does the sensitivity of the Staph compare with the sensitivity of the Pseudomonas?

LAB MANUAL: TABLE OF CONTENTS

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