Antibiotic Sensitivity Testing

The disc-plate method of determining which of a wide variety of antibiotics is antagonistic to an organism is a rapid, accurate, and inexpensive diagnostic tool. This procedure gives physicians information regarding effective microbial drugs to use in the chemotherapy of infectious diseases. This is important because treating an infection with an inappropriate antibiotic will not only not help the situation, it may make things worse by destroying competitors of the infectious agent. In addition, if there are individuals within the bacterial population that are resistant to the antibiotic being used, these organisms will be selected for, through elimination of organisms that are sensitive. If organisms are only marginally sensitive, it may take higher concentrations or longer durations to completely destroy the population. It is for this last reason that it is important to complete an antibiotic regimen once started, in order to avoid selecting for antibiotic resistance.

In the disc-plate method, a number of small discs impregnated with an antibiotic of known concentration are placed on the surface of an agar plate that has been inoculated with the organism to be tested. After proper incubation, the plate is observed and zones of inhibition are measured to determine the susceptibility of the test organism to particular antibiotics. Based on the size of the zone of inhibition the particular microbial agent will be designated as resistant, intermediate, or sensitive to the test antibiotic. The chart accompanying this exercise lists the appropriate zone sizes for each antibiotic tested.

In this exercise, the disc-plate method of antibiotic sensitivity testing will be used to determine the antimicrobial ability of eight antibiotics on a variety of bacteria and one species of yeast. The eight antibiotics have been selected so as to represent different modes of antibiotic action. These modes of action can be summarized as follows:

A. Inhibition of Cell Wall Synthesis:
   1. Penicillin G (P 10): interferes with deposition of muramic acid into the growing cell wall, and with final cross-linking of wall components.

B. Inhibition of Cell Membrane Activity:
   1. Polymyxin B (PB): binds to phospholipids in prokaryotic membranes, thus contributing to osmotic stress.

C. Inhibition of Nucleic Acid Synthesis:

D. Inhibition of Protein Synthesis:
   1. Chloramphenicol (C 30): binds to 50S subunit of the ribosome, inhibiting protein synthesis
   2. Kanamycin (K 30): binds to 30S subunit of ribosome, slowing down protein synthesis and inducing misreading of mRNA
   3. Streptomycin (S 10): irreversibly binds with 30S ribosomal sub-units, causing misreading of mRNA and blockage of protein synthesis initiation.
   4. Tetracycline (TE 5): binds to ribosome and inhibits incoming aminoacyl-tRNA from binding to the ribosome.

E. Inhibition of Enzymatic Activity:
   1. Trimethoprim (TMP 5): competitive inhibitor of p-aminobenzoic acid, a needed metabolite in folic acid synthesis.
Materials:

1. Broth cultures of organism(s) to be tested:
2. Sterile cotton tipped swabs
3. Mueller Hinton Agar plates
4. Forceps
5. Coplin jar of ethyl alcohol
6. Antibiotic discs
7. Millimeter ruler (second period)

Procedure:

1. For each organism to be tested, you will need two Mueller Hinton Agar Plates. Draw lines on the bottom of the plates, dividing each of them into fourths. Moisten a sterile swab in the broth culture of your organism and inoculate the entire surface of each plate. Discard the swab into the biohazard beaker.

2. Dip the ends of the forceps into the jar of alcohol. Carefully pass the tip of the forceps through the flame of a Bunsen burner to ignite the alcohol and allow the alcohol to burn off. **DO NOT HEAT THE FORCEPS IN THE OPEN FLAME.** This procedure will flame sterilize the forceps.

3. Use the forceps to obtain an antibiotic disc and place the disc approximately one centimeter in from the edge of the plate, centered between the lines dividing the plate in fourths. Re-sterilize the forceps.

4. Repeat steps 2-3 for each antibiotic to be tested.

5. Within 3 to 5 minutes the discs will have absorbed enough moisture to adhere firmly to the agar surface. At that time carefully invert the plate and incubate in the incubator until the next laboratory session.

6. At the next laboratory meeting, note the efficacy of each antibiotic tested by observing and measuring the zone of inhibition around each disc. **Use the zone chart on the next page to determine whether your test organism is resistant, intermediate, or sensitive to each antibiotic.** Compare your results with those obtained by the rest of the class.
<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - Chloramphenicol</td>
<td>12</td>
<td>13-17</td>
<td>18 or more</td>
</tr>
<tr>
<td>K – Kanamycin</td>
<td>13</td>
<td>14-17</td>
<td>18 or more</td>
</tr>
<tr>
<td>NB – Novobiocin</td>
<td>17 or less</td>
<td>18 – 21</td>
<td>22 or more</td>
</tr>
<tr>
<td>P – Penicillin G</td>
<td>20 or less</td>
<td>21 – 28</td>
<td>29 or more</td>
</tr>
<tr>
<td>Other Bacteria</td>
<td>11 or less</td>
<td>12 – 21</td>
<td>22 or more</td>
</tr>
<tr>
<td>PB – Polymyxin B</td>
<td>9 or less</td>
<td>9 – 11</td>
<td>12 or more</td>
</tr>
<tr>
<td>S – Streptomycin</td>
<td>11 or less</td>
<td>12 – 14</td>
<td>15 or more</td>
</tr>
<tr>
<td>TE – Tetracycline</td>
<td>14 or less</td>
<td>15 – 18</td>
<td>19 or more</td>
</tr>
<tr>
<td>TMP - Trimethoprim</td>
<td>10</td>
<td>11-15</td>
<td>16 or more</td>
</tr>
</tbody>
</table>