Microbiology Lab Experiment Changes

Experiment #: 7-2

Title: Antimicrobial Susceptibility Test (Kirby-Bauer Method)

Live Organisms: *E. coli, S. aureus, B. cereus*

Changes: Procedure (work in groups)

1. Obtain 3 TSA plates and label them *E. coli, S. aureus,* and *B. cereus*.

2. Using a sterile swab, inoculate the entire surface of the plate thoroughly with a different organism. Each student in the group can swab a plate with one of the organisms.

3. Apply the antibiotic discs (instructor will demonstrate) to each plate. We are using Penicillin, Tetracycline, and Streptomycin or Chloramphenicol.

4. Using a sterile loop or flamed forceps, gently press the disks onto the agar to make sure they stick.

5. Wait several minutes then invert and incubate plates at 37°C.

6. Next lab period you will measure the diameter of the zone of inhibition surrounding each disk and use the table at the bottom of the page to determine antibiotic resistance.

Take Home Lesson: The Kirby-Bauer antibiotic sensitivity method is an in vitro method used to determine the potential effectiveness of an antibiotic against specific bacteria. Note that even though there is a zone of inhibition surrounding a disk, this does not necessarily mean that the antibiotic will be effective in vivo. That is why you look at a chart. The factors affecting antibiotic effectiveness (see Lecture: Antimicrobials) account for the discrepancy between their in vitro vs. in vivo activity.

If given an antibiotic plate, a ruler and a chart of antibiotic resistance, you should be able to determine the resistance or susceptibility of an organism to a particular antibiotic.

Antibiotic (and disc identifier)	**Disk potency**	**Inhibition zone diameter to nearest mm**		
		Resistant	Intermediate	Susceptible
Chloramphenicol (C)	30 µg	12 or less	13 to 17	18 or more
Penicillin G (P)	10 units			
For staphylococci		28 or less	-	29 or more
For most other bacteria		11 or less	12 to 21	22 or more
Streptomycin (S)	10 µg	11 or less	12 to 14	15 or more
Tetracycline (TE)	30 µg	14 or less	15 to 18	19 or more