Microbiology for the Health Sciences Laboratory

Lab Session 6 - Antimicrobials

Introduction

Microorganisms are susceptible to a wide variety of chemical agents. Antiseptics, disinfectants and antibiotics kill or inhibit the growth of microorganisms by interacting with anatomical structures or metabolic machinery. However, the effects are complex: Different microorganisms have different susceptibilities to different substances, and, different chemicals are inherently toxic to microorganisms at different doses. There are a number of routine methods that are designed to specifically evaluate the susceptibility of an organism to an antibiotic, or the effectiveness of an antimicrobial substance. The purpose of this lab is to determine the antimicrobial activity of a variety of materials using a standard, ultra low tech, disk diffusion assay. The methods may be routine, but the practicality of the concepts to be learned are worth the effort.

A. Review the following for reference:

1. Introduction: We are starting to use BSL-2 microorganisms. Proper lab techniques are very important.
2. Text: Chapter 5 - Pages 107-116 & 120-121. Chapter 20 – Pages 458-470.
3. Pay particular attention to the following:
   -Methods used to determine the sensitivity of microorganisms to antimicrobials
   -Disk diffusion techniques.

B. Do the following during lab:

1. Work in groups of 3-4.
2. Each group will need one broth culture of each of the following m.o.'s:

	Growth media
a- *Staphylococcus aureus*** - A gram positive coccus from the nasopharynx	TSA
b- *Escherichia coli*** - A gram negative rod from the gi tract	TSA
c- *Pseudomonas aeruginosa*** - A gram negative opportunist from mucous membranes	TSA
d- *Enterococcus faecalis*** - A gram positive coccus from the gi tract	TSA

   **Caution: These are BSL-2 microorganisms and must be handled with caution.

3. Each individual will need the broth culture from your throat isolate:
   -*Unknown*** - You need to know the description of the mo (i.e. results of gram stain) TSA
4. Prepare four "lawns of growth" for each of your m.o.'s using appropriate media
   -The technique is described below (Technique D1)
   -Make sure the lawns are dry before adding any chemicals.
5. For each organism, add to each of the lawns of growth the following:
   (The Technique is described in D2 below):

Plate 1 - Antiseptics
1A = Listerine (Thymol, .064% & eucalyptol, .092%)
1B = Thimerosal (0.1%, aq.)
1C = Betadine (1% available iodine, aq)
1D = Scope (Cetylpyridinium chloride, ?)
1E = Bac Down Hand soap (0.1% Triclosan, aq)

Plate 2 - Disinfectants
2A = Lysol (1.2%, Benzyl ammonium chloride)
2B = Hydrogen peroxide (3%, aq)
2C = Isopropanol (70%, aq))
2D = Bac Down Disinfectant (0.07% BAC)
2E = Bleach (6.25% hypochlorite, aq)

Plate 3 - Antibiotics
3A = Erythromycin
3B = Tetracycline
3C = Polymyxin B
3D = S x T
3E = Rifampin

Plate 4 - Antibiotics
4A = Penicillin
4B = Cefaclor
4C = Ciprofloxicin
4D = Gentamycin
4E = Ampicillin

6. Incubate all plates at 35 C for 24-48 hours.

C. Results

1. Measure any zone of inhibition (diameter in mm) around the disks (See technique D3 below)
2. Prepare a table of results in your notebook. You will need to share data on the other 3-4 supplied microorganisms. You do not need your partner's data on their unknowns.
3. Optional: Use of 'E-Test Strips' of an antibiotic will be demonstrated.
   Low tech methods do not necessarily translate to poor quality data.

D. Techniques

1. Lawn of growth/spread plate: a) Use a sterile swab to
   b) inoculate the entire surface of an agar plate.
2. Antimicrobial: a) Carefully impregnate a sterile
   paper disc with the necessary chemical
   and transfer it to the agar plate using forceps, or
   b) Use a disc transfer device, if available.
3. Zone of Inhibition: Use a ruler to measure the
   inhibition of growth (diameter, in mm).

C. Results

Treatment	*S aureus*	*E coli*	*Ps aerug*	*E faecalis*	Your Unknow
Plate 1-Antiseptics					
1A = Listerine mouthwash					
1B = Thimerosal					
1C = Betadine					
1D = Scope mouthwash					
1E = Bac Down antiseptic					
Plate 2-Disinfectants					
2A = Lysol					
2B = Hydrogen peroxide					
2C = Isoprpyl alcohol					
2D = Bac Down disinfectant					
2E = Bleach					
Plate 3-Antibiotics					
3A = Erythromycin					
3B = Tetracycline					
3C = Polymixin B					
3D = S x T					
3E = Rifampin					
Plate 4-Antibiotics*					
4A = Penicillin					
4B = Cefaclor					
4C = Ciprofloxicin					
4D = Gentamycin					
4E = Ampicillin					

E. Discussion and Conclusions