Exercise 17
ANTIMICROBIAL SUSCEPTIBILITY TESTING

Student learning objectives: Upon completion of this exercise students will be able to:

a. Describe the Kirby-Bauer disc diffusion method.
b. List five factors that must be standardized for accuracy of results for antimicrobial susceptibility.
c. Define the terms susceptible, intermediate and resistant.
d. Explain why penicillin is useless in the treatment of Staphylococci.
e. Describe the test done to determine penicillin resistance.
f. Explain why it is helpful for antimicrobial susceptibility patterns to be compiled and summarized and made available to the hospital staff for the isolates.

Activities

1. Kirby-Bauer Disc Diffusion Antimicrobial Susceptibility Test.
2. Beta-lactamase Test using the Nitrocef Disc.

Materials

Work in groups of 4 per table. This is a group activity. Each group needs the following:

TSA plate cultures of:
E. coli and *Staphylococcus aureus*
Staphylococcus aureus (ATCC 43300)
Staphylococcus aureus (ATCC 25932)
Mueller-Hinton Agar
Nitrocef discs
Tube rack
Sterile swabs
Sterile 0.85% saline
0.5 McFarland standard tubes (instructor will advise)
Antibiotic impregnated discs
Forceps
Alcohol
Petri dish (empty)

Kirby Bauer Disc Diffusion Susceptibility Testing

Introduction

To determine the drug of choice for therapy for successful treatment of an infection, the physicial needs to know the susceptibility of the causative agent of this infection. This method consists of a battery of selected antimicrobial agents will assist the physician in choosing an appropriate drug for therapy. A standardized inoculum of the organism is swabbed onto the surface of a Mueller-Hinton agar plate. Filter paper discs impregnated with antimicrobial agents are placed on the agar. After overnight incubation, the diameter of the zone of inhibition around each disc is measured. By referring to standardized charts, a qualitative report of susceptible, moderately susceptible, intermediate, or resistant can be obtained.

Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH, incubation environment and length, disk content and drug diffusion rate, and measurement endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.

Procedure

1. Bring the agar plates and disks to room temperature. Disks are stored in the freezer – Remove 2 hours before use. Store jar with dessicator.

2. Using a loop or swab transfer 4 or 5 colonies grown overnight on non-selective medium (e.g. BAP or CHOC) to 0.85% sterile saline. Mix well and adjust to a 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml).

3. Within 15 minutes dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum.

4. Swab the entire surface of the agar plate three times, rotating the plate approximately 60^o between the streaking to ensure even distribution. *Avoid hitting sides of petri plate and creating aerosols.*

5. Allow inoculated plate to stand for at least 3 min. but no longer than 15 minutes before applying disks.

6. Apply disks to the agar surface by using a dispenser or sterile forceps.

7. Apply gently pressure with sterile forceps or needle to ensure complete contact of disk with agar.

8. Do not apply disks closer to each other than 24 mm from center to center.

9. Do not relocate a disk once it has made contact with the agar surface, because antimicrobial diffusion begins instantly.

10. Within 15 min. of disk application invert and incubate plates for 16 to 18 hours at 35 to 37^{o} C in an ambient-air incubator.

11. Read plates only if the lawn of growth is confluent or nearly confluent.

12. Rest plate (lid down) on, or hold plate 2 to 3 inches above a black non-reflecting surface.

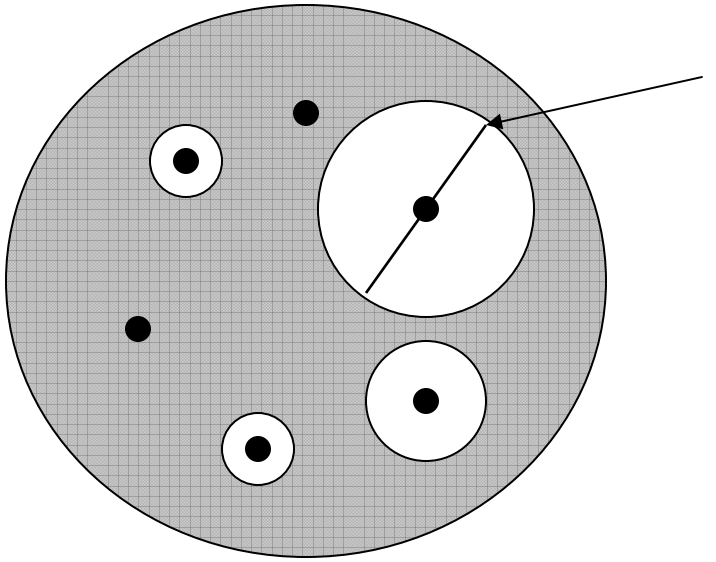
13. Illuminate plate with reflected light directly from above at a 45^{o} angle.

14. Measure diameter of inhibition zone to nearest millimeter by holding the ruler against the back of the plate. Compare to the standard antimicrobial table.

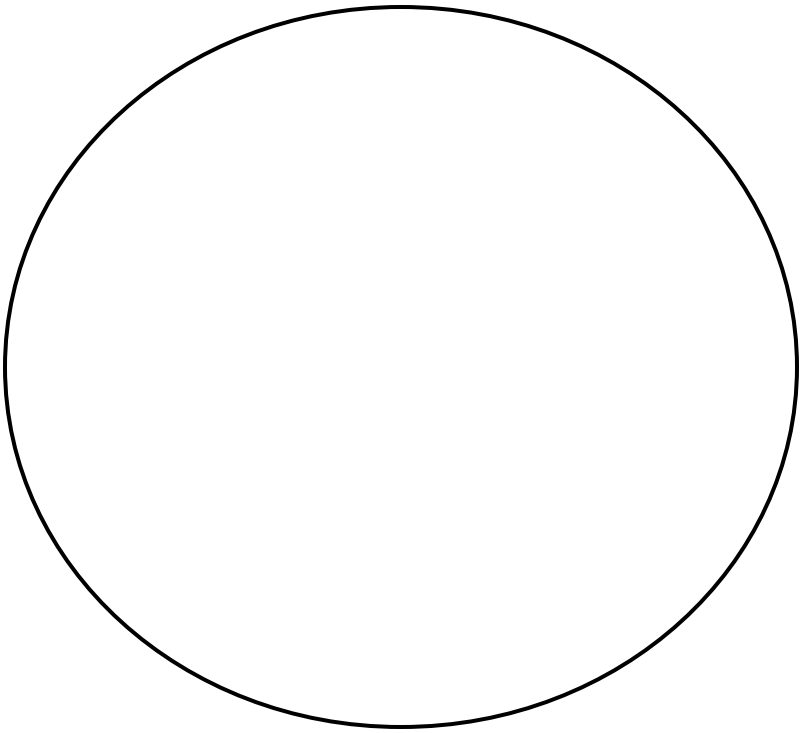
We will be using *Escherichia coli* and *Staphylococcus aureus* as our test organisms.

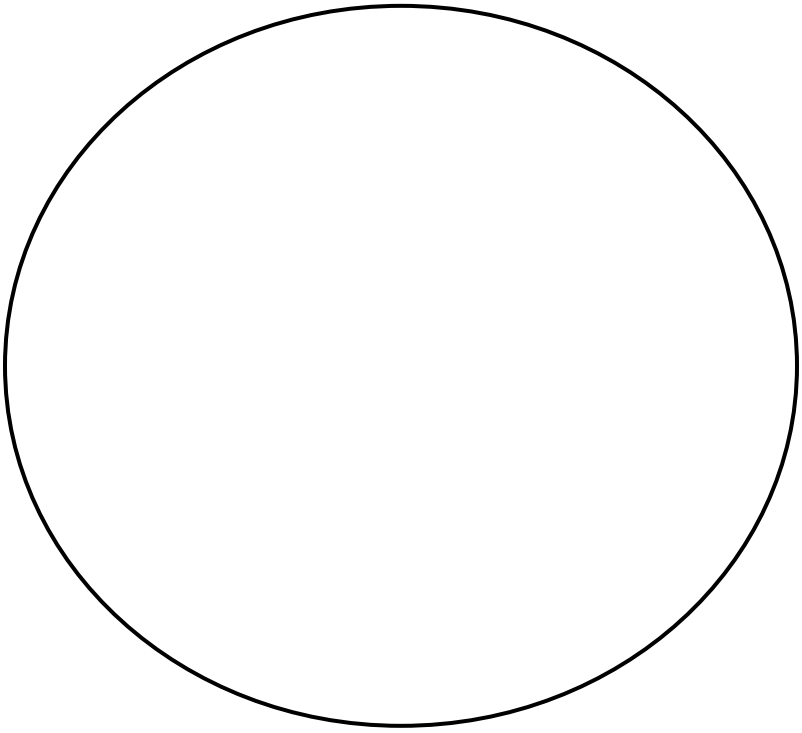
After incubation, measure the zone of inhibition for each antibiotic with a ruler and record results.

Error!



Draw your MH plates below and show the zones of inhibition. Shade the confluent growth with a pencil.





Fill in the results of your Kirby Bauer tests

Antibiotic	Results		Conclusion/ Interpretation (use Atlas)	
	Staphylococcus aureus	*Escherichia coli*	**S. aureus**	**E. coli**
Ampicillin (AM10)				
Chloramphenicol (C30)				
Erythromycin (E15)				
Gentamicin (GM10)				
Penicillin G (P10)				
Polymyxin B (PB300)				
Streptomycin (S10)				
Tetracycline (Te30)				

1. Are there differences in antimicrobial susceptibility patterns between E. coli (Gram negative) and S. aureus (Gram positive)? Why?

_____

_____

2. List another method for determining the antimicrobial resistance patterns.

_____

3. What is MRSA? What is the significance of finding this in community-acquired infections, compared to hospital acquired infections?

_____

_____

Beta-Lactamase Test Using Nitrocef Discs

Nitrocef discs are intended for use in rapid testing of isolated colonies of Neisseria gonorrhea, Staphyloccus species, Haemophilus influenzae and anaerobic bacteria for the production of beta-lactamase. Many of these organisms are known to be resistant to penicillin.
The disc is impregnated with a compound that changes color when the beta lactam ring is broken by the enzyme beta lacatamase. When a bacterium produces this enzyme in significant quantities, the yellow-colored disc turns red in the area where isolate is smeared.

Procedure

Place a Nitrocef disk on the lid of the petri dish of the organism being tested. Moisten disk with 1 drop of water. With a sterile toothpick or an applicator stick remove several well-isolated colonies and smear onto surface of the disc.

Interpretation of Results

A positive reaction will show a yellow to red color change on the area where culture was applied.

Note: Color change does not usually develop over entire disc.
A negative result will show no color change on disc.

For most bacterial strains a positive result will develop within 5 minutes. However, positive reactions for some staphylococci may take up to 1 hour to develop.

Run this test on a beta lactamase positive organism *Staphylococcus aureus* (ATCC 43300) and a beta lactamase negative organism *Staphylococcus aureus* (ATCC 25932). Do the results agree?

Results

Organism Strain	Reaction color	Beta lactamase Positive or Negative