Lab Exercise: Antibiotics- Evaluation using Kirby Bauer method.

OBJECTIVES

1. Compare the antimicrobial capabilities of different antibiotics.
2. Compare effectiveness of with different types of bacteria.
3. Utilize aseptic techniques.

INTRODUCTION

You may have heard of Methicillin-Resistant *Staphylococcus aureus*, or MRSA. This strain of *S. aureus* is resistant to most used antibiotics and therefore a dangerous concern. This lab will look at how resistant different bacteria are to some known antibiotics. The **Kirby-Bauer test for antibiotic susceptibility**, otherwise known as the **disc diffusion test**, is a standard that has been used for years. It may be used even before a clinical sample is identified. A sample from an infected patient can be swabbed and plated. Antibiotics can be used to determine a preferred course of treatment against this infection. One trouble with this test is that different antimicrobial compounds have different sizes and solubilities. A standard for comparison is required. We will use a standardized media, Mueller Hinton agar. We will compare the **zones of inhibition** to standards from a table for each antibiotic. This will give us results of "Resistant," "Intermediate," or "Susceptible" to each antibiotic. See page 104 – 107 in the Alexander atlas for more on this procedure.

LAB EXERCISES

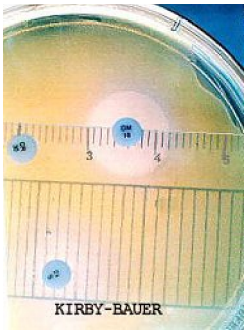
Table supplies	Team supplies	Individual supplies
Antibiotic disc dispenser	Culture of *Escherichia coli*	1 Mueller Hinton (MH) agar plate
	Culture of *Pseudomonas aeruginosa*	
	Culture of *Bacillus cereus*	
	Culture of *Staphylococcus epidermidis*	
	Pipet and pipet tips	
	Metal spreader, turntable, and alcohol	

Protocol:

Day 1

1. Assign each member of your team one of the test organisms above. Label your plate with the species used.
2. Transfer 100 μl of the test organism to your plate and top spread.

3. Each table will have one dispenser. **Record** the antibiotics loaded into the dispenser.
4. Place the dispenser over your open plate.
5. Gently but firmly press down on the handle, do not force. Several antibiotic disks should eject down onto your plate. No need to press down on the individual discs, they will adhere to the inoculated plate.
6. Invert plates and incubate at the appropriate temperature.



Day 2

1. Take your plates and measure the **diameter** of the zone of inhibition for each antibiotic.
2. Record your results in the below data table.
3. Check the below Table of standards at end of this lab, record whether your test organism is resistant, intermediate or sensitive for your organism.
4. Compare your results for others.

Antibiotic	**Code**	**Disk Potency µg**	**Resistant**	**Intermediate**	**Sensitive**
Amoxicillin	AMC 30	30			
Staphylococcus aureus			≤19		≥20
Enterobiaceae			≤13	14-17	≥18
Ampicillin	AM 10	75			
Gram – Enterics			≤13	14-16	≥17
Staphylococci			≤28		≥29
Enterococci			≤16		≥17
Streptococci (not *S. pneumoniae*)			≤21	22-29	≥30
Haemophilus spp.			≤18	19-21	≥22
Listeria monocytogenes			≤19		≥20
Bacitracin	B10	10	≤8	9-12	≥13
Carbenicillin	CB100	100	≤19	20-22	≥23
P. aeruginosa			≤13	14-16	≥17
Cephalothin	CF30	30	≤14	15-17	≥18
Chloramphenicol	C30	30	≤12	13-17	≥18
S. pneumoniae			≤20		≥21
Ciprofloxacin	CIP-5	5	≤15	16-20	≥21
Erythromycin	E15	15	≤13	14-22	≥23
S. pneumoniae			≤15	16-20	≥21
Gentamicin	GM 10 / GM120	10	≤12	13-14	≥15
Kanamycin	K30	30	≤13	14-17	≥18
Neomycin	N-30	30	≤12	13-16	≥17
Novobiocin	NB-5	5	≤16		>16
Optochin	OPT	5	≤7	7-13	≥14
Penicillin (Staphylococci)	P10	10	≤28		≥29
Enterococci			≤14		≥15
Streptococci (not *S. pneumoniae*)			≤19	20-27	≥28
Neisseria gonorrhoeae			≤26	27-46	≥47
Listeria monocytogenes			≤19		≥20
Streptomycin	S-10	10 units	≤10	11-14	≤15
Tetracycline	Te30	30	≤14	15-18	≥19
S. pneumoniae			≤17	18-21	≥22
Tobramycin	NN10	10	≤12	13-15	≥15
Trimethoprim/Sulfamethoxazole (SXT)	SXT	1.25/23.75	≤10	11-15	≥16

DATA AND OBSERVATIONS

1. Record the results for the various chemicals used in this exercise.

Organism	Antibiotic	Zone in mm	Rating R, I, S
E. coli			
P. aeruginosa			
B. cereus			
S. epidermidis			

2. Use the above data to answer the following:

a. The most effective antibiotic I used was _____.

b. The least effective antibiotic I used was _____.

c. The organism I used was _____.

3. Compare your results with others in your team. Were your results the same or different for the same antibiotics, explain.

4. Which antibiotics were most effective against each of the bacteria we used?

*Escherichia coli*_____

*Pseudomonas aeruginosa*_____

*Bacillus cereus*_____

*Staphylococcus epidermitis*_____

5. Is there a difference in levels of susceptibility with Gram + and Gram - bacteria? Give examples with species and antibiotics.

DISCUSSION

1. Define **narrow** and **broad spectrum** antibiotics. Give an example of each from our results.

2. Why might Gram (+) and Gram (-) respond differently to the same antibiotic?

3. How can drug resistance in bacteria, like that is MRSA be stopped or reduced?