Antibiotic Sensitivity by Paper-Disk Plate Method

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Introduction

Antibiotics are natural chemicals produced by microorganisms that inhibit the growth of other microorganisms. They have a primary practical application in helping to combat infectious disease. When a bacterial species is causing an infection, scientists will want to know which antibiotic will have the greatest effect on controlling the spread of the infection. This can be accomplished by isolating a pure culture of the infectious microbe, then growing it on the surface of an agar plate containing disks soaked in various antibiotics. As the bacteria grow near the antibiotic disks, their growth will be hindered most by the antibiotics they are most sensitive to. In this way, scientists can determine which antibiotics have the greatest effect on controlling bacterial growth.

Antibiotics work to inhibit microbial growth through four main pathways. First, antibiotics such as kanamycin and streptomycin can prevent protein synthesis by binding to ribosomal subunits. Ribosomes in bacteria are composed of 30s and 50s subunits, and are where proteins are made in the cell. If antibiotics bind to these sites, they can interfere with protein synthesis, and since proteins are required for growth, reproduction, and repair, blockage of protein synthesis can hinder bacterial growth. Second, antibiotics can attack the peptidoglycan layer of a cell. Peptidoglycan is a substance found in bacterial cell walls, and is necessary for the structural integrity of cells. Weakening of the peptidoglycan layer will lead to cell lysis. Third, antibiotics can alter components of the cytoplasmic membrane. This membrane contains many proteins that regulate the entry and exit of nutrients and waste products. If an antibiotic can change the composition of this membrane, exchange of nutrients and waste products may become sluggish or may stop altogether, and the cell will die due to a backup in metabolic activity. Finally, an antibiotic can bind to key bacterial enzymes. Enzymes are proteins needed to speed up the rate of chemical reactions in the

cell, and if antibiotics bind up essential enzymes, then the chemical reactions catalyzed by these enzymes will fail to proceed. The cell will ultimately die from metabolic failure.

The objective of this experiment is to determine the sensitivity of three microorganisms to various common antibiotics: bacitracin, chloramphenicol, erythromycin, kanamycin, neomycin, penicillin, streptomycin, and tetracycline. The three microorganisms that will be used in this experiment are *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium.* Of these, *E. coli* and *S. typhimurium* are gram-negative, and *S. aureus* is gram-positive. Gram (+) and gram (-) bacteria are called so because the Gram stain differentiates among two bacterial taxa with several important structural differences. One of these differences is the thickness of the cell's peptidoglycan layer. For antibiotics that affect cell wall synthesis or must penetrate the cell wall to affect protein synthesis within the cell, the thickness of this layer can be crucial.

For gram-negative *E. coli* and *S. typhimurium*, I hypothesize that all antibiotics will be effective except for bacitracin and penicillin. These antibiotics block peptidoglycan synthesis, and since gram (-) bacteria have much less peptidoglycan in their cell walls than gram (+) bacteria do, blockage of peptidoglycan synthesis will not weaken cells enough to trigger lysis. Therefore, these antibiotics should be ineffective against gram (-) bacteria. For *S. aureus*, I hypothesize that all antibiotics except kanamycin and streptomycin should be effective. These antibiotics affect protein synthesis, and must be able to pass through the cell wall to perform their job. However, because *S. aureus* is a gram (+) bacterium, it has a thick outer peptidoglycan layer that is difficult to penetrate. Therefore, these two antibiotics should be ineffective against gram (+) bacteria.

Materials and Methods

Experiments were set up as described in the BIO 103 Lab Manual (Vilgalys, 1998).

Results

The following table was formulated using data obtained from 8 groups. This data consisted of zones of inhibition (in mm) for each antibiotic, per bacterial species. While the data set for *S. typhimurium* was complete, two groups did not enter their data for both *E. coli* and *S. aureus*. Therefore, *S. typhimurium* had 8 sets of data, whereas *E. coli* and *S. aureus* only had 6. Furthermore, some groups failed to record data for one or two antibiotics. To compare antibiotic sensitivity over the three bacterial species, a useful measurement was found to account for this disparity in data. By taking the available zone of inhibition data, summing it for each antibiotic, then dividing the sum by the number of groups contributing to the sum, an average zone of inhibition can be obtained for each antibiotic. Group entries of zero were included in the calculations, but blank entries were not. Once the averages have been obtained, the Kirby-Bauer antibiotic susceptibility table can be used to assign sensitivity ratings to each bacterial species.

Zone of Inhibition Averages (in mm) of Bacterial Species per Antibiotic

Sensitivity ratings: R = resistant, I = intermediate, S = sensitive

	Zone of Inhibition Averages (mm)			
Name of Antibiotic (abbreviation)	*E.coli* gram (-)	*S. aureus* gram (+)	*S. typhimurium* gram (-)	Results
Bacitracin Peptide antibiotic; disrupts cell wall synthesis Effective against gram (+) bacteria*	3.7 **R**	17.3 **S**	0.8 **R**	Much greater effectiveness on gram (+) bacteria, as expected
Chloramphenicol Benzene derivative; protein synthesis (50s subunit) inhibitor	28.0 **S**	14.6 **I**	19.6 **S**	Appears to be slightly more effective against gram (-) bacteria.

Effective against gram (+) and (-) bacteria*				
Erythromycin Macrolide antibiotic; protein synthesis (50s subunit) inhibitor Most effective against gram (+) bacteria*	16.8 **I**	23.5 **S**	7.4 **R**	More effective on gram (+) bacteria, as expected. However, also some effectiveness on E. coli.
Kanamycin Aminoglycoside; protein synthesis (30s subunit) inhibitor Effective against gram (-) bacteria	19.8 **S**	19.8 **S**	17.9 **S**	Strong and equal effectiveness on both types of bacteria, as expected**
Neomycin Aminoglycoside; protein synthesis inhibitor* Effective against gram (+) and (-) bacteria*	19.5 **S**	18.8 **S**	16.4 **I**	Equally effective against both gram types, as expected**
Penicillin β-lactam antibiotic; disrupts cell wall synthesis by transpeptidation reaction Effective only against gram (+) bacteria Impermeable to gram (-) bacteria	13.8 **I**	10.8 **R**	11.6 **I**	Experimental numbers show slightly greater effectiveness on gram (-) bacteria
Streptomycin Aminoglycoside; protein synthesis (30s subunit) inhibitor Effective against gram (-) bacteria	15.8 **S**	12.0 **I**	14.9 **S**	More effective on gram (-) bacteria, as expected
Tetracycline Protein synthesis (30s subunit) inhibitor Effective against gram (+) and (-) bacteria	27.8 **S**	20.5 **S**	19.1 **S**	Strong and equal effectiveness on both types of bacteria, as expected**

*Source from "The Columbia Encyclopedia: Sixth Edition. 2000." http://www.bartleby.com/65/
**Although the zone of inhibition averages may vary, according to the Kirby-Bauer antibiotic susceptibility table, zone of inhibition values above a certain threshold value are assigned a rating of "susceptible." Therefore, although zones of inhibition for tetracycline vary from 19.1 to 27.8 mm, "susceptible" for tetracycline means zones of inhibition above 19. Different antibiotics have different threshold values.

Tetracycline and kanamycin produced the strongest results; both the gram (-) and gram (+) bacteria were highly sensitive to these two antibiotics. Next were chloramphenicol, neomycin, and streptomycin, to which two of the three species of bacteria were susceptible. Chloramphenicol had a slightly stronger effect on the gram-negative bacteria than on gram-positive *S. aureus*; neomycin was effective against *E. coli* and *S. aureus*, but gave an intermediate rating for *S. typhimurium.* Streptomycin was slightly more effective on gram (-) bacteria, as was hypothesized.

Bacitracin had its greatest effect on gram (+) *S. aureus*, as expected. Erythromycin was most effective on gram (+) bacteria as expected, but it also had an intermediate effectiveness on *E. coli*. Results for penicillin proved to be the most anomalous. Although I hypothesized that penicillin should impede growth of *S. aureus*, *S. aureus* exhibited penicillin resistance. Furthermore, the gram (-) bacteria showed an intermediate level of sensitivity to penicillin, which is the exact reverse of my hypothesis.

Discussion

For gram-negative *E. coli* and *S. typhimurium*, all antibiotics were effective except for bacitracin, erythromycin, and penicillin. Bacitracin was the least effective of the three, while erythromycin and penicillin both had intermediate ratings of effectiveness. The results for bacitracin supported my hypothesis, which was expected because bacitracin's effect on peptidoglycan synthesis should lead to cell lysis in gram (-) bacteria. However, penicillin did not have a similar effect on gram (-) bacteria as expected. Penicillin is impermeable to the membranes of gram (-) bacteria, yet the results show that these bacteria were intermediately sensitive to penicillin. This anomalous result could be due to the type of penicillin used in this experiment, since new semisynthetic penicillins have been developed that are effective against gram (-) bacteria (Madigan et al, 2000). Also, the results show that *S. aureus* was resistant to penicillin. Although this antibiotic should be effective against gram (+) bacteria like *S. aureus*, this bacterium could have carried genes for penicillin resistance.

The results for erythromycin also unexpectedly supported my hypothesis, but for a different reason. Erythromycin functions by inhibiting protein synthesis, and therefore must be able to penetrate the cell wall. Because *S. aureus* was susceptible and the gram (-) bacteria were either resistant or only intermediately susceptible, these results could be due to erythromycin's inability to get past the chemically complex, thick outer membrane of gram (-) cells. If *S. typhimurium* has a larger capsule than *E.coli*, then perhaps this can explain why *S. typhimurium* was resistant and *E. coli* was intermediately susceptible.

For the gram (+) bacterium *S. aureus*, I had hypothesized that all antibiotics but kanamycin and streptomycin should be effective. However, the results did not support this hypothesis, since these two antibiotics were clearly effective in controlling microbial growth. *S. aureus* was sensitive to kanamycin, and intermediately sensitive to streptomycin. From this observation alone, it is unclear how *S. aureus* can be sensitive to these protein synthesis inhibitors, because they would have to be able to pass through the bacterium's thick peptidoglycan layer to perform their job. However, if we return to the original zone of inhibition data collected by the eight groups, we find that one group lacked streptomycin data for all three species. If this data had been recorded, perhaps this result might have come out differently.

From this experiment, I learned that an antibiotic's effectiveness cannot be predicted solely from the way it works. For example, *S. aureus*, a gram (+) bacterium, was relatively unaffected by penicillin. Penicillin is designed to hinder growth of gram (+) microorganisms, but it had very little effect on the *S. aureus* used in this experiment. A primary reason for this might be the presence of penicillin resistance genes in the *S. aureus* population. Also, bacterial cells in a population might be at different stages of growth, and antibiotics affecting protein or cell wall synthesis will have their greatest effect when cells are in log phase rather than stationary phase. Depending on when a species enters log phase, zones of inhibition for a particular antibiotic might be larger or smaller. This can affect the sensitivity ratings assigned to each species.

Conclusion

The main purpose of this experiment is to determine the sensitivity of three microorganisms to various antibiotics. The three organisms used in this test were *E. coli, S. aureus, and S. typhimurium.* These three organisms were all examined for their sensitivity to various antibiotics commonly used in medicine and biology. I hypothesized that the gram (-) bacteria *E. coli* and *S. typhimurium* would be sensitive to all antibiotics tested except bacitracin and penicillin, since these antibiotics would be unable to disrupt cell wall

synthesis in gram (-) bacteria. I also hypothesized that the gram (+) bacterium *S. aureus* would be sensitive to all antibiotics except kanamycin and streptomycin, which are protein synthesis inhibitors that must pass through the bacterium's thick peptidoglycan layer to disrupt bacterial growth. The results of this experiment supported only my first hypothesis.

Works Cited

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Vilgalys, Rytas. Laboratory Exercises for BIO 103: General Microbiology. Fall 2000.

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