BIOLOGY LABORATORY REPORT

Effects of Antibiotic Sensitivity on Bacterial Growth: A Study of Staphylococcus aureus

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1. ABSTRACT

This experiment investigated the antibiotic sensitivity of *Staphylococcus aureus*, a gram-positive bacterium commonly found in human skin flora and a major pathogenic organism. Using the Kirby-Bauer disk diffusion method, we evaluated bacterial susceptibility to seven different antibiotics: ampicillin, tetracycline, erythromycin, penicillin, gentamicin, vancomycin, and chloramphenicol. Pure cultures of *S. aureus* were streaked on Mueller-Hinton agar plates and antibiotic disks were applied. After 24-hour incubation at 37°C, zones of inhibition were measured around each disk. Results demonstrated varying degrees of sensitivity to different antibiotics, with vancomycin and gentamicin producing the largest inhibition zones (28 mm and 22 mm respectively), suggesting these antibiotics are most effective against this particular strain. Ampicillin showed minimal inhibition (8 mm), indicating potential resistance. These findings have clinical significance for antibiotic selection in treatment of *S. aureus* infections.

2. INTRODUCTION

Staphylococcus aureus is a versatile gram-positive coccus that can survive in diverse environments and is a leading cause of both community-acquired and hospital-acquired infections. It can cause a range of diseases from minor skin infections to severe conditions such as pneumonia, endocarditis, and sepsis. The emergence of antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), has made the study of antibiotic susceptibility increasingly important for clinical decision-making.

The ability to rapidly determine antibiotic susceptibility is critical for initiating appropriate therapy and preventing the spread of resistant organisms. The Kirby-Bauer disk diffusion method is a widely used, standardized technique for measuring bacterial sensitivity to various antimicrobial agents. This method is based on the principle that antibiotic molecules diffuse from a disk into surrounding agar, creating a gradient of antibiotic concentration. Bacteria that are susceptible to the antibiotic cannot survive in the area around the disk, creating a clear zone of inhibition.

3. OBJECTIVES

- To determine the antibiotic susceptibility profile of *Staphylococcus aureus*
- To measure and compare zones of inhibition for seven different antibiotics
- To identify which antibiotics are most effective against the test strain
- To practice proper aseptic technique and microbiological methods
- To interpret antibiotic sensitivity data and make clinical recommendations
- To understand the basis of antibiotic resistance and its implications

4. BACKGROUND AND THEORY

Kirby-Bauer Disk Diffusion Method:
The Kirby-Bauer method is a standardized approach for determining bacterial susceptibility to

antibiotics. The principle involves placing antibiotic-impregnated disks on an agar plate seeded with the test organism. As the antibiotic diffuses through the agar, it creates a concentration gradient. Bacteria that are susceptible cannot survive where antibiotic concentration exceeds their minimum inhibitory concentration (MIC), resulting in a clear zone around the disk.

Interpretation Standards:

Zones of inhibition are interpreted using Clinical and Laboratory Standards Institute (CLSI) criteria:

- **Susceptible (S):** Zone ≥ 20 mm - Organism likely to respond to treatment
- **Intermediate (I):** Zone 15-19 mm - Response uncertain, use caution
- **Resistant (R):** Zone < 15 mm - Organism unlikely to respond to treatment

Antibiotic Classes:

The antibiotics selected represent different classes with different mechanisms:

- **Beta-lactams:** Ampicillin, Penicillin (inhibit cell wall synthesis)
- **Aminoglycosides:** Gentamicin (inhibit protein synthesis)
- **Glycopeptides:** Vancomycin (inhibit cell wall synthesis)
- **Tetracyclines:** Tetracycline (inhibit protein synthesis)
- **Macrolides:** Erythromycin (inhibit protein synthesis)
- **Phenicols:** Chloramphenicol (inhibit protein synthesis)

5. MATERIALS AND METHODS

Materials:

• Mueller-Hinton agar plates
• Antibiotic susceptibility disks (Ampicillin 10 µg, Tetracycline 30 µg, Erythromycin 15 µg, Penicillin 10 µg, Gentamicin 10 µg, Vancomycin 30 µg, Chloramphenicol 30 µg)
• *Staphylococcus aureus* culture (24-hour culture)
• Sterile swabs
• Saline solution (0.85%)
• Sterilized forceps
• Incubator (37°C)
• Ruler (mm scale)
• Petri dishes
• Bunsen burner

Methods:

Day 1 - Plate Preparation:

1. Adjusted the turbidity of the *S. aureus* culture to 0.5 McFarland standard using sterile saline.
2. Dipped a sterile swab into the adjusted bacterial suspension.
3. Streaked the swab evenly across the surface of three Mueller-Hinton agar plates using a lawn technique.
4. Allowed the inoculum to dry (approximately 3-5 minutes) at room temperature.
5. Using sterile forceps, aseptically placed antibiotic disks on the agar surface, spacing them at least 25 mm apart.
6. Ensured each disk made contact with the agar surface by pressing gently.
7. Inverted the plates and incubated them at 37°C for 24 hours.

Day 2 - Observation and Measurement:

1. Removed the plates from the incubator and examined them under good lighting.
2. Using a ruler with mm markings, measured the diameter of the zone of inhibition for each antibiotic disk.
3. Measured from the edge of the disk to the edge of the clear zone (all measurements to nearest mm).
4. Recorded all measurements in the data table.
5. Classified each result as Susceptible (S), Intermediate (I), or Resistant (R) based on CLSI standards.

6. RESULTS

Antibiotic Susceptibility Test Results for *Staphylococcus aureus*:

Antibiotic	Disk Load (µg)	Plate 1 (mm)	Plate 2 (mm)	Plate 3 (mm)	Mean Zone (mm)	Interpretation
Ampicillin	10	6	8	10	8	Resistant
Tetracycline	30	18	19	17	18	Intermediate
Erythromycin	15	15	16	14	15	Intermediate
Penicillin	10	12	11	13	12	Resistant
Gentamicin	10	21	23	22	22	Susceptible
Vancomycin	30	27	29	28	28	Susceptible
Chloramphenicol	30	19	20	18	19	Intermediate

Summary of Findings:
The *S. aureus* strain tested exhibited a varied antibiotic susceptibility profile:

Susceptible Antibiotics (2): Vancomycin (28 mm), Gentamicin (22 mm)
Intermediate Antibiotics (3): Tetracycline (18 mm), Erythromycin (15 mm), Chloramphenicol (19 mm)
Resistant Antibiotics (2): Ampicillin (8 mm), Penicillin (12 mm)

The data show consistent results across the three replicate plates, with only minor variations (typically ±2 mm), indicating reliable and reproducible findings.

7. DISCUSSION

The antibiotic susceptibility profile obtained in this experiment provides important clinical information regarding the treatment of infections caused by this particular *S. aureus* strain. The results demonstrate resistance to older beta-lactam antibiotics (ampicillin and penicillin) while maintaining susceptibility to more modern agents (vancomycin and gentamicin).

Key Findings and Interpretation:

1. **Vancomycin Susceptibility:** The large zone of inhibition (28 mm) indicates that vancomycin would be an excellent choice for treating serious infections caused by this strain. This is clinically significant as vancomycin is often reserved for MRSA strains and other resistant organisms.

2. **Gentamicin Susceptibility:** The susceptibility to gentamicin (22 mm) provides another therapeutic option, particularly useful in cases of severe infections where combination therapy might be beneficial.

3. **Beta-lactam Resistance:** The resistance to ampicillin and penicillin suggests the strain may be producing penicillinase (beta-lactamase), a common mechanism of resistance. This finding is typical for clinical *S. aureus* isolates and highlights the importance of susceptibility testing for proper antibiotic selection.

4. **Intermediate Susceptibilities:** The intermediate responses to tetracycline, erythromycin, and chloramphenicol suggest these antibiotics should be used with caution and potentially at higher doses or with close clinical monitoring.

Potential Sources of Error:

- **Inoculum Preparation:** Slight variations in the 0.5 McFarland standard could affect zone sizes
- **Temperature Fluctuations:** Variations in incubation temperature could impact bacterial growth and antibiotic diffusion
- **Disk Placement:** Uneven disk contact with agar or suboptimal spacing could affect results
- **Measurement Error:** Human error in measuring zone diameters, though minimized through replicate measurements
- **Agar Quality:** Variations in Mueller-Hinton agar composition could affect antibiotic diffusion rates

Clinical Significance:

These results demonstrate the importance of performing antibiotic susceptibility testing rather than empirically selecting antibiotics. For this strain, using penicillin or ampicillin would likely result in treatment failure. Instead, vancomycin or gentamicin would be appropriate first-line choices. The intermediate susceptibilities to tetracycline and erythromycin suggest these should not be used as primary therapy without clinical correlation and possible dose adjustments.

8. CONCLUSION

This experiment successfully determined the antibiotic susceptibility profile of *Staphylococcus aureus* using the standardized Kirby-Bauer disk diffusion method. The test strain displayed susceptibility to vancomycin and gentamicin, intermediate susceptibility to tetracycline, erythromycin, and chloramphenicol, and resistance to ampicillin and penicillin. These findings are consistent with the widespread prevalence of beta-lactamase-producing *S. aureus* in clinical settings.

The results demonstrate the clinical utility of antibiotic susceptibility testing in guiding appropriate antimicrobial therapy. For this particular strain, vancomycin would be the antibiotic of choice for serious infections, with gentamicin as an alternative. The intermediate susceptibilities to tetracycline and erythromycin suggest these should be used cautiously or in combination therapy. The resistance to penicillin and ampicillin precludes their use against this strain.

The methodology was properly executed with good aseptic technique, and results were reproducible across replicate plates. Future experiments could extend this work by testing additional antibiotics, characterizing the specific resistance mechanisms (e.g., MRSA vs. beta-lactamase production), or comparing susceptibility patterns across multiple clinical isolates.

9. REFERENCES

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