The background features a dark blue gradient with a series of curved, parallel lines that create a sense of depth and movement. On the right side, there is a grid-like pattern of light blue lines that recedes into the distance, giving the impression of a tunnel or a futuristic architectural structure.

Azithromycin in Combination with Hamamelitannin as a Growth Inhibitor for Common Bacteria in Surgical Site Infection

THOUSAND OAKS HIGH SCHOOL
AP RESEARCH STEM

Surgical Site Infection

- 48 million surgical inpatient *procedures were performed* in the United States in 2009
- 2 to 5% of operated patients will develop SSI. SSI increases the patient's length of stay in the hospital by an average of 7.5 days

960,000 – 2,400,000 Surgical Site Infections annually in the US

Preventing the Infection

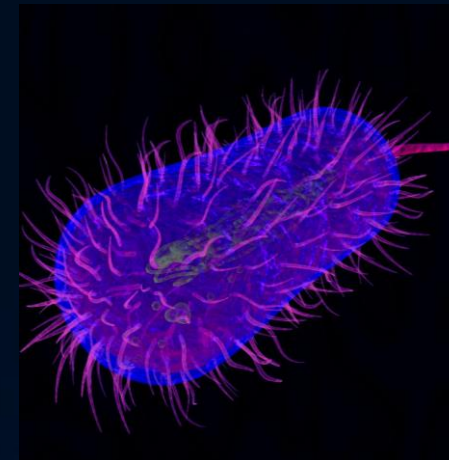
- *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* are found responsible for SSI
- Preventing Infection: Antimicrobial Suture Thread
 - FDA ban on Triclosan- New antibiotic coating needed



Ethicon's Polyglactin 910 Triclosan Coated Suture Thread



Pseudomonas aeruginosa



Escherichia coli



Staphylococcus aureus

Antibiotic Resistance

- 2 million people become infected with bacteria that are resistant to antibiotics annually
 - 23,000 people die each year as a direct result of these infections
 - Factors:
 - Overuse of antibiotics
 - Mutation
 - Quorum Sensing
- The Survival of the Fittest
-
- ```
graph LR; A[Overuse of antibiotics] --> B[The Survival of the Fittest]; C[Mutation] --> B; D[Quorum Sensing] --> B;
```



# What is Quorum Sensing?

- Bacterial cell-to-cell communication in which signaling molecules are used to coordinate gene expression among local populations
- Helps create antibiotic resistance



Quorum Sensing Inhibitors

# Azithromycin

- Inhibits the growth of bacteria
  - Attacks cell membrane
  - Quorum sensing inhibitor of *Pseudomonas* bacteria
- Not a Penicillin related antibiotic
  - Up to 10% of people report being allergy to this class of antibiotics

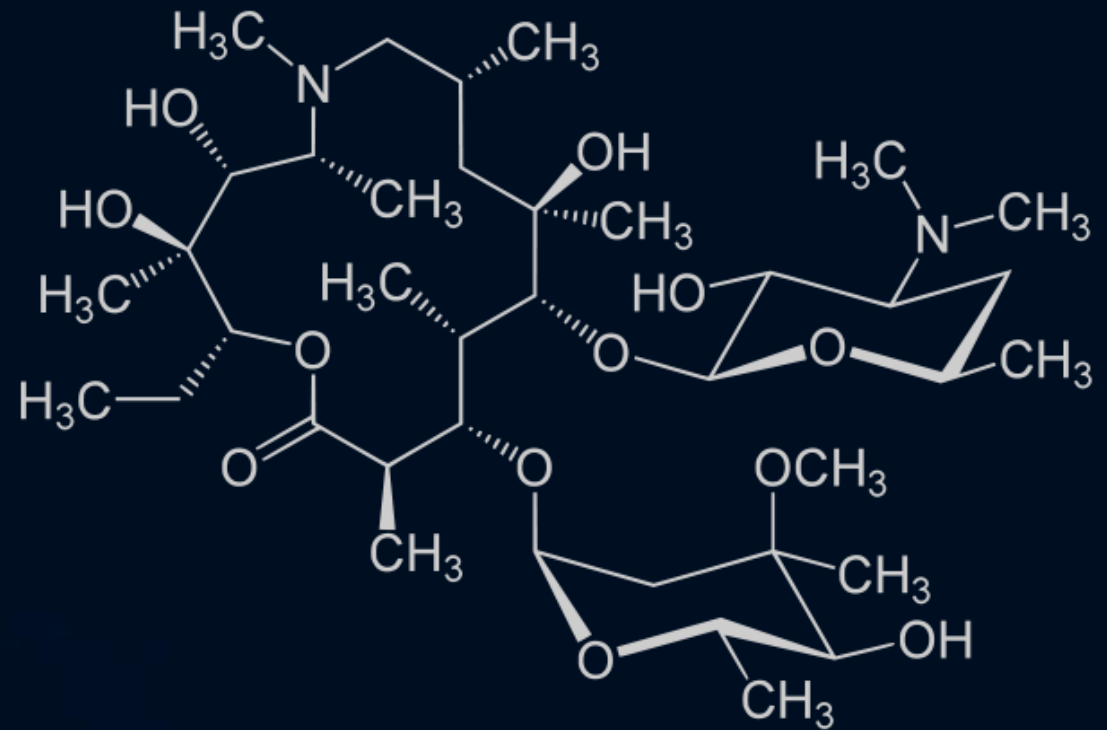


Figure 4. The 2D Structure of Azithromycin

# Hamamelitannin

- Major active component in the bark of witch hazel
- Quorum sensing inhibitor *Staphylococcus* bacteria
  - Inhibits RNAIII in *Staphylococcus*
- Not expected to inhibit growth on its own
  - Increases Antibiotic susceptibility of antimicrobial substances

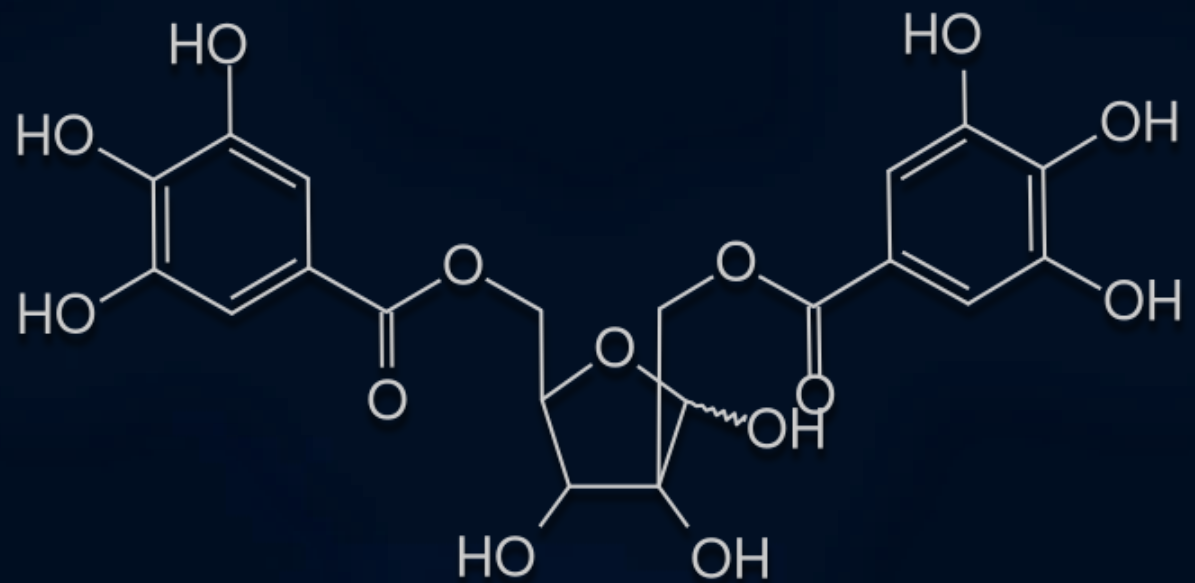


Figure 5. The 2D Structure of Hamamelitannin

# *Staphylococcus epidermidis*

- Safety Issues with *Staphylococcus aureus* → *Staphylococcus epidermidis*
- Thousand Oaks Lab: Biosafety Level 1
  - *Staphylococcus epidermidis*: Biosafety Level 1
- Characteristics:
  - Gram-Positive
  - Facultative anaerobe
  - Used as model organism for *Staphylococcus aureus*

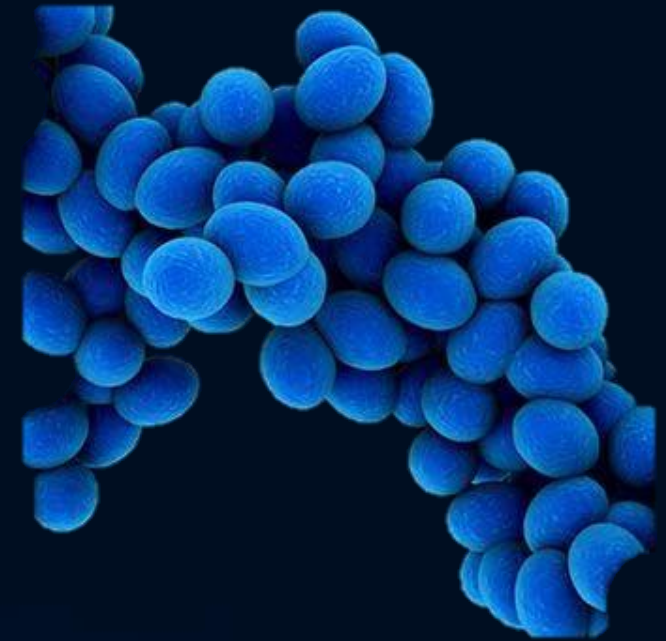


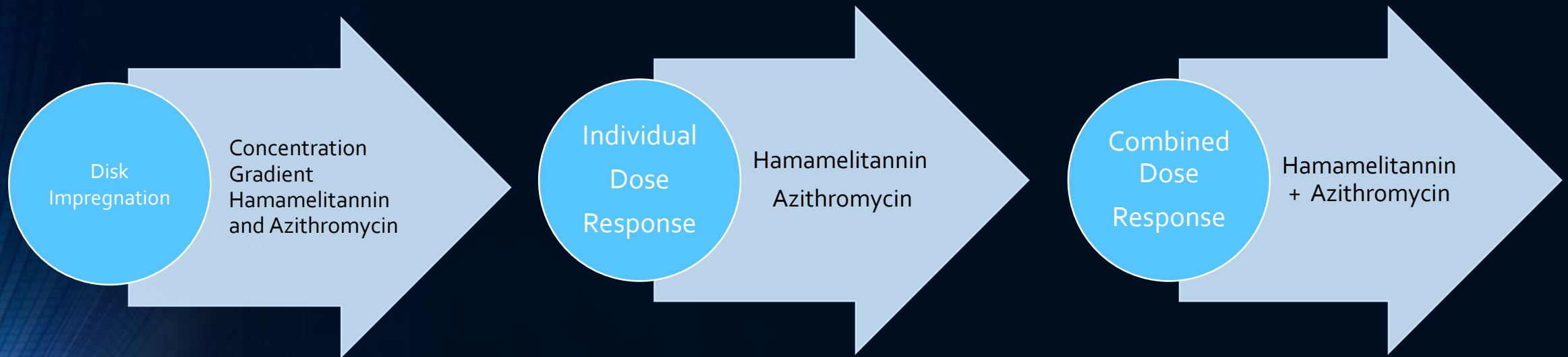
Figure 4. *Staphylococcus aureus*



# Materials

- Incubator
- Autoclave
- Blank Susceptibility Disks
- Azithromycin
- Hamamelitannin
- *Staphylococcus epidermidis*
- Tryptic Soy Broth
- Mannitol Salt Agar

# Methods Overview



# Antibiotic Concentrations used

## AZITHROMYCIN (AZI)

Concentration  
Gradient: AZI

0.05 µg/mL diH<sub>2</sub>O

0.10 µg/mL diH<sub>2</sub>O

0.50 µg/mL diH<sub>2</sub>O

1.0 µg/mL diH<sub>2</sub>O

1.5 µg/mL diH<sub>2</sub>O

## HAMAMELITANNIN (HAM)

Concentration  
Gradient: HAM

1.0 µg/ml diH<sub>2</sub>O

5.0 µg/ml diH<sub>2</sub>O

10.0 µg/ml diH<sub>2</sub>O

20.0 µg/ml diH<sub>2</sub>O

30.0 µg/ml diH<sub>2</sub>O

## COMBINED (AZI+HAM)

Concentration gradient

(1/2 of the most effective  
concentration AZI /mL  
diH<sub>2</sub>O + \_\_\_ ) :

1.0 µg/ml diH<sub>2</sub>O

5.0 µg/ml diH<sub>2</sub>O

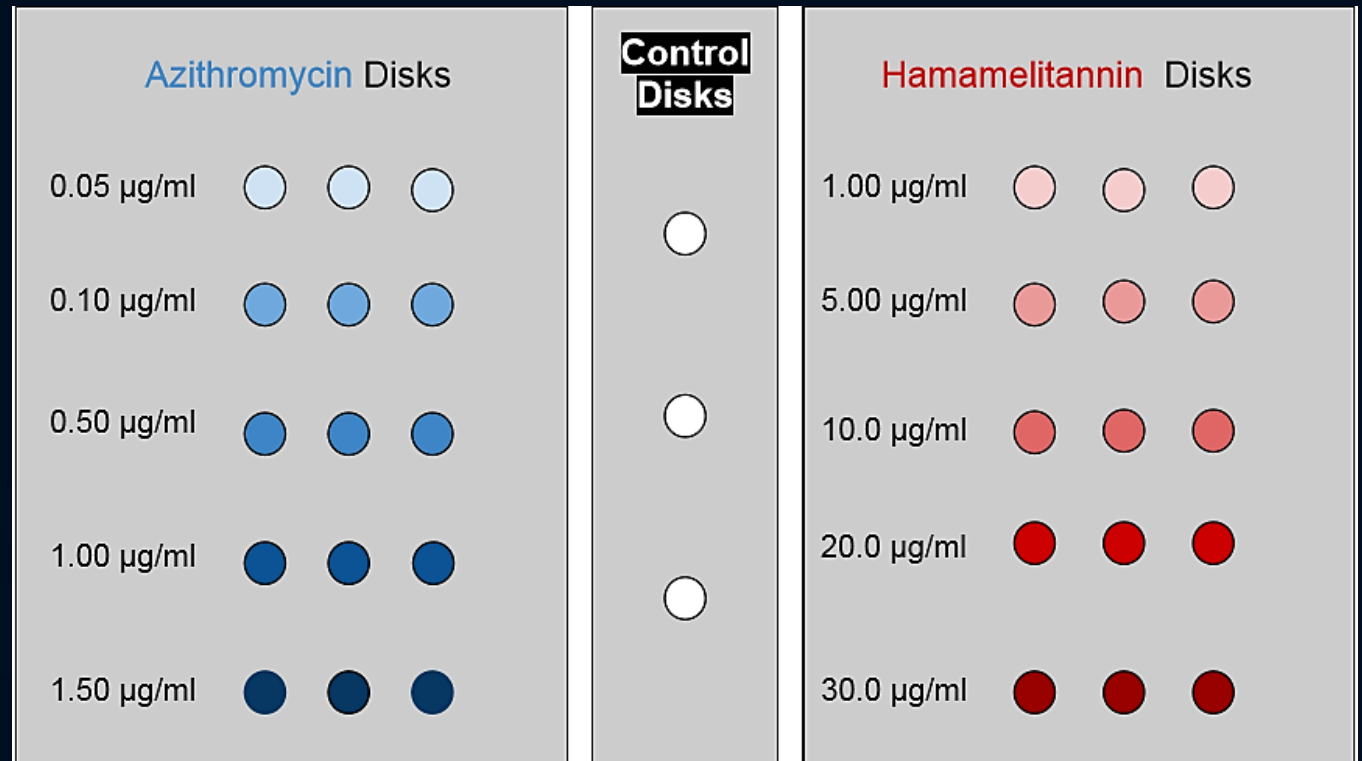
10.0 µg/ml diH<sub>2</sub>O

20.0 µg/ml diH<sub>2</sub>O

30.0 µg/ml diH<sub>2</sub>O

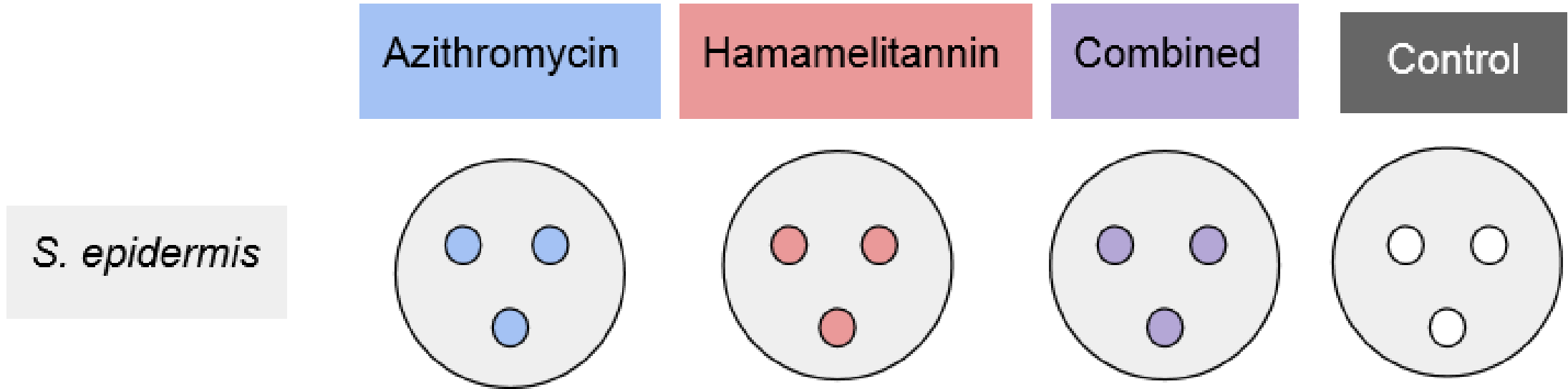
# Disk Impregnation

- Disks were found to fully absorb 30  $\mu\text{L}$  of solution
- Completed in Triplicate
- Steps for Sterility:
  - Completed in a Laminar Flow Hood
  - Solutions, Pipette tips, and Disks were autoclaved
  - Gloves





# Kirby-Bauer Diffusion Assay



- Performed in Fume Hood
- Incubated at 37°C for 24 Hours
- Zones' diameters were measured with a ruler in millimeters

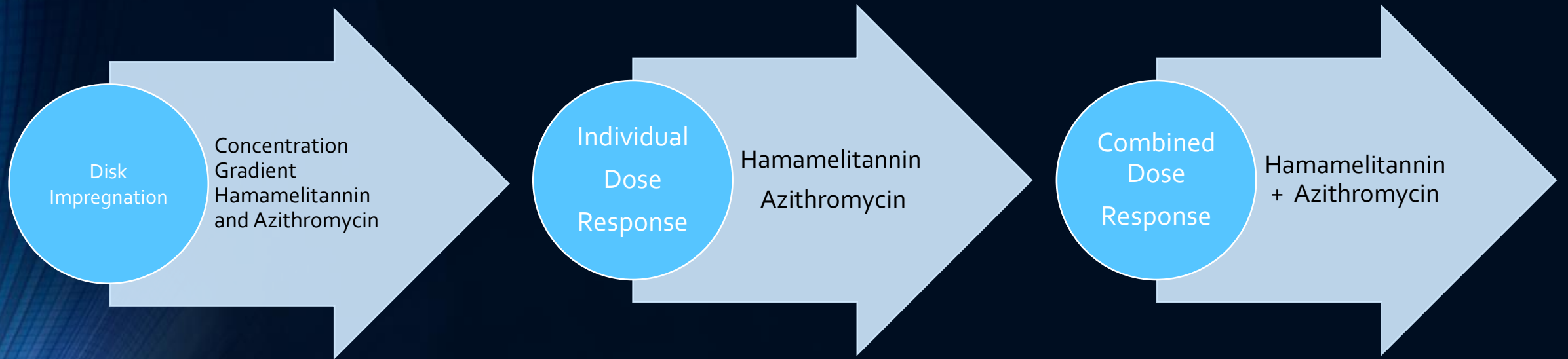
## Hypothesis

Adding hamamelitannin to azithromycin will increase the antibiotic susceptibility of *Staphylococcus epidermidis*.

# Individual Dose Response

| Concentration $\mu\text{g}$<br>Azithromycin /ml<br>$\text{H}_2\text{O}$ | Trial 1 | Trial 2 | Trial 3 | Average |
|-------------------------------------------------------------------------|---------|---------|---------|---------|
| 0.05                                                                    | 6 mm    | 7 mm    | 6 mm    | 6.33 mm |
| 0.1                                                                     | 7 mm    | 8 mm    | 7 mm    | 7.33 mm |
| 0.5                                                                     | 9 mm    | 8 mm    | 7 mm    | 8 mm    |
| 1                                                                       | 11 mm   | 13 mm   | 12 mm   | 12 mm   |
| 1.5                                                                     | 10 mm   | 8 mm    | 9 mm    | 9 mm    |

# Methods Overview





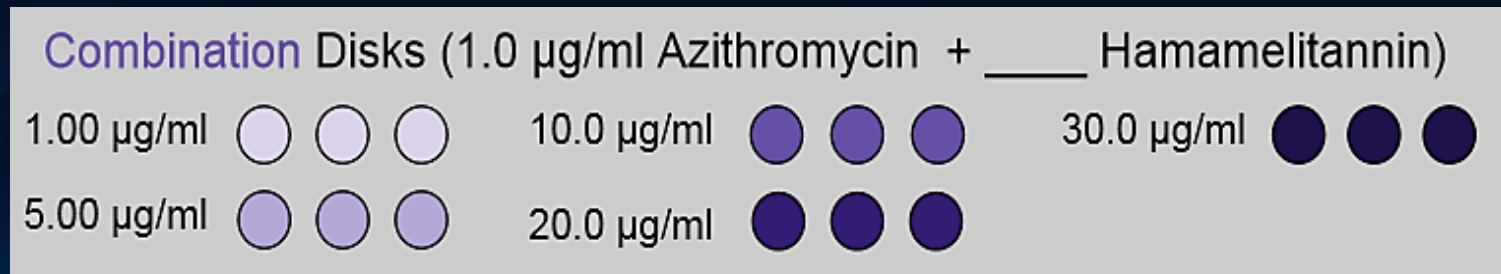
# Individual Dose Response

Hamamelitannin did not inhibit the growth of *S. epidermidis*.

1.0  $\mu\text{g}$  Azithromycin /mL diH<sub>2</sub>O provided the largest zones of inhibition

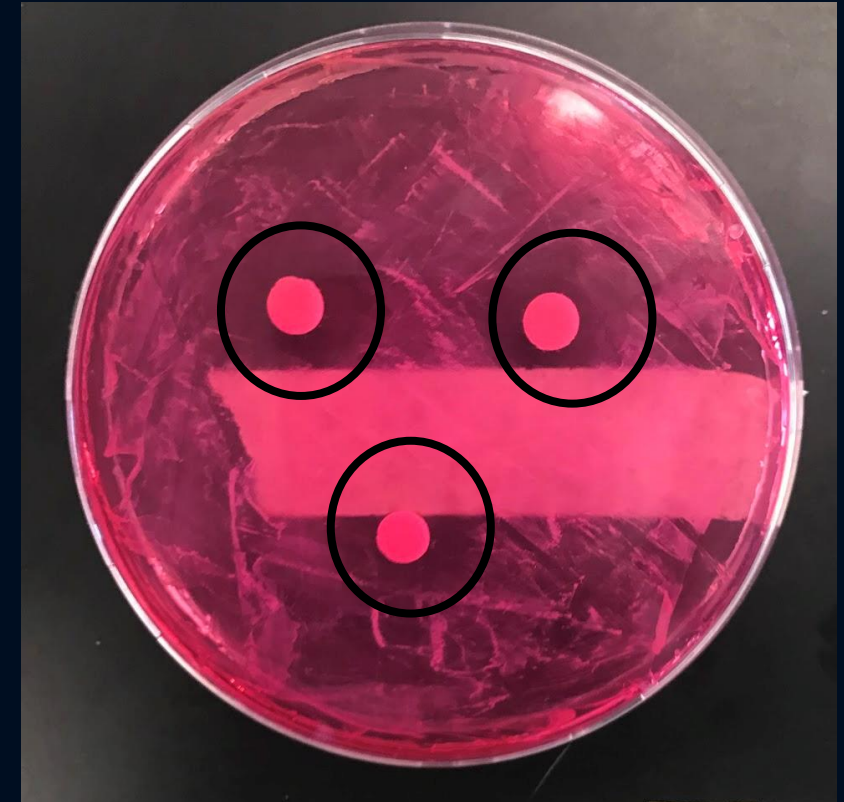


Used for Synergistic Dose Response



# Combined dose response

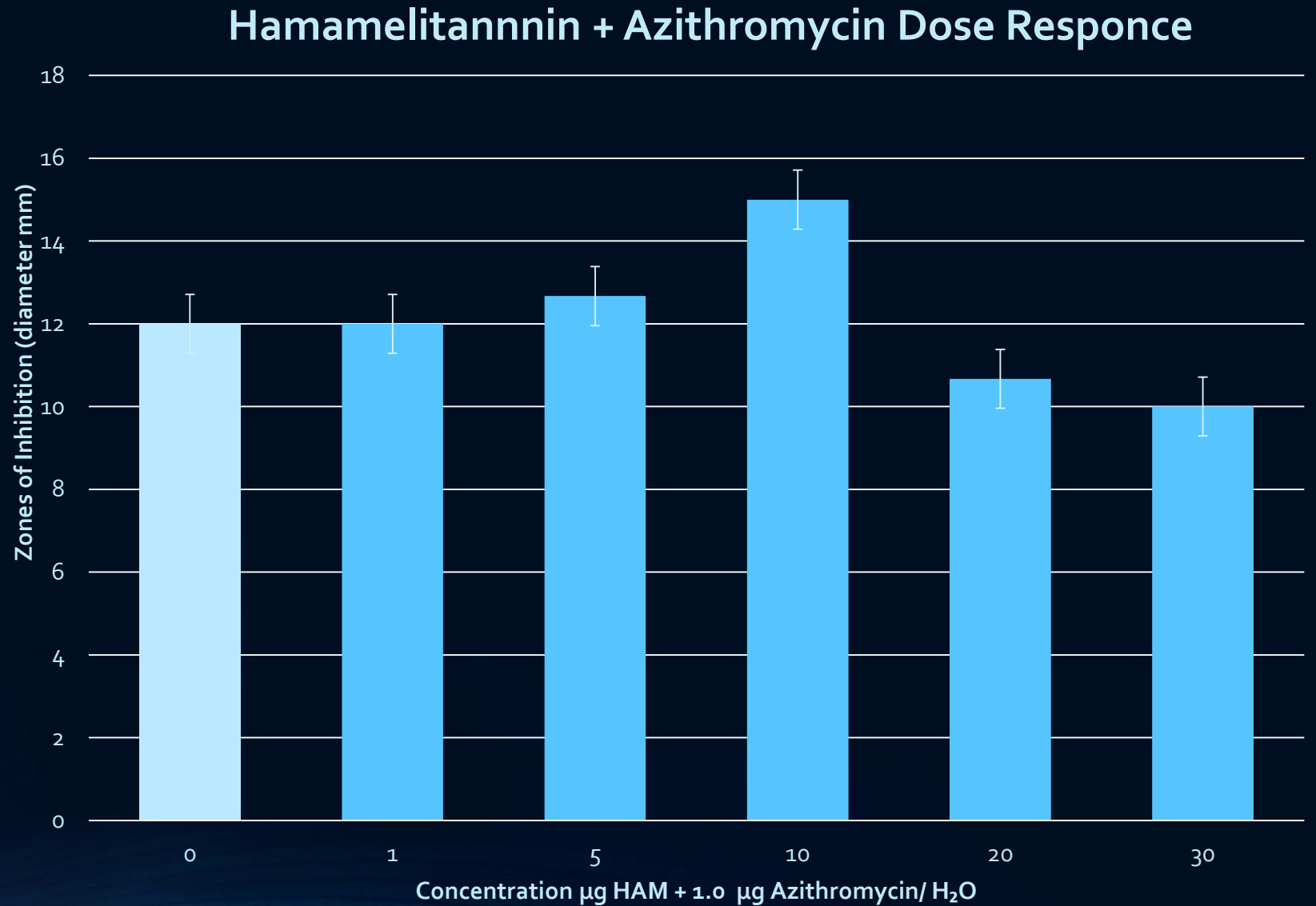
| Concentration $\mu$<br>Hamamelitannin<br>/ml H <sub>2</sub> O + 1.0 $\mu$ g<br>Azithromycin /ml<br>H <sub>2</sub> O | Trial 1 | Trial 2 | Trial 3 | Average  |
|---------------------------------------------------------------------------------------------------------------------|---------|---------|---------|----------|
| 0                                                                                                                   | 11 mm   | 12 mm   | 13 mm   | 12 mm    |
| 1                                                                                                                   | 12 mm   | 14 mm   | 11 mm   | 12 mm    |
| 5                                                                                                                   | 13 mm   | 13 mm   | 12 mm   | 12.67 mm |
| 10                                                                                                                  | 14 mm   | 15 mm   | 16 mm   | 15 mm    |
| 20                                                                                                                  | 11 mm   | 10 mm   | 11 mm   | 10.67 mm |
| 30                                                                                                                  | 9 mm    | 11 mm   | 10 mm   | 10 mm    |



# Results

## Best Combination

1.0  $\mu\text{g}$  Azithromycin  
/ml  $\text{H}_2\text{O}$  +10  $\mu\text{g}$   
Hamamelitannin/  
ml  $\text{H}_2\text{O}$



# Discussion- What does this mean?

- The addition of Hamamelitannin increases the antimicrobial effect of Azithromycin by 25 %
- My null hypothesis can be rejected
  - T-test score: 0.011



# Addressing the Limitations

- The size of the zone may be affected by:
  - Viscosity of the culture medium
  - The rate of diffusion of the antibiotic
  - Concentration of the antibiotic on the filter disc
  - May have adverse effects in the system for which it is intended

provides the basis for defining a sensitivity spectrum of the organism

## Conclusion

The results of the experiment indicate that the synergistic effect of Azithromycin and Hamamelitannin greater than the antimicrobial effect of either agents individually.

# Application to the Real world

- With the application of this agent in the medical field, the rate of Surgical Site Infections could decrease
  - Coating suture thread
- If a quorum sensing inhibitor is designed for the most threatening bacteria, doctors can lower their patient's likelihood of infection substantially

# Further Works

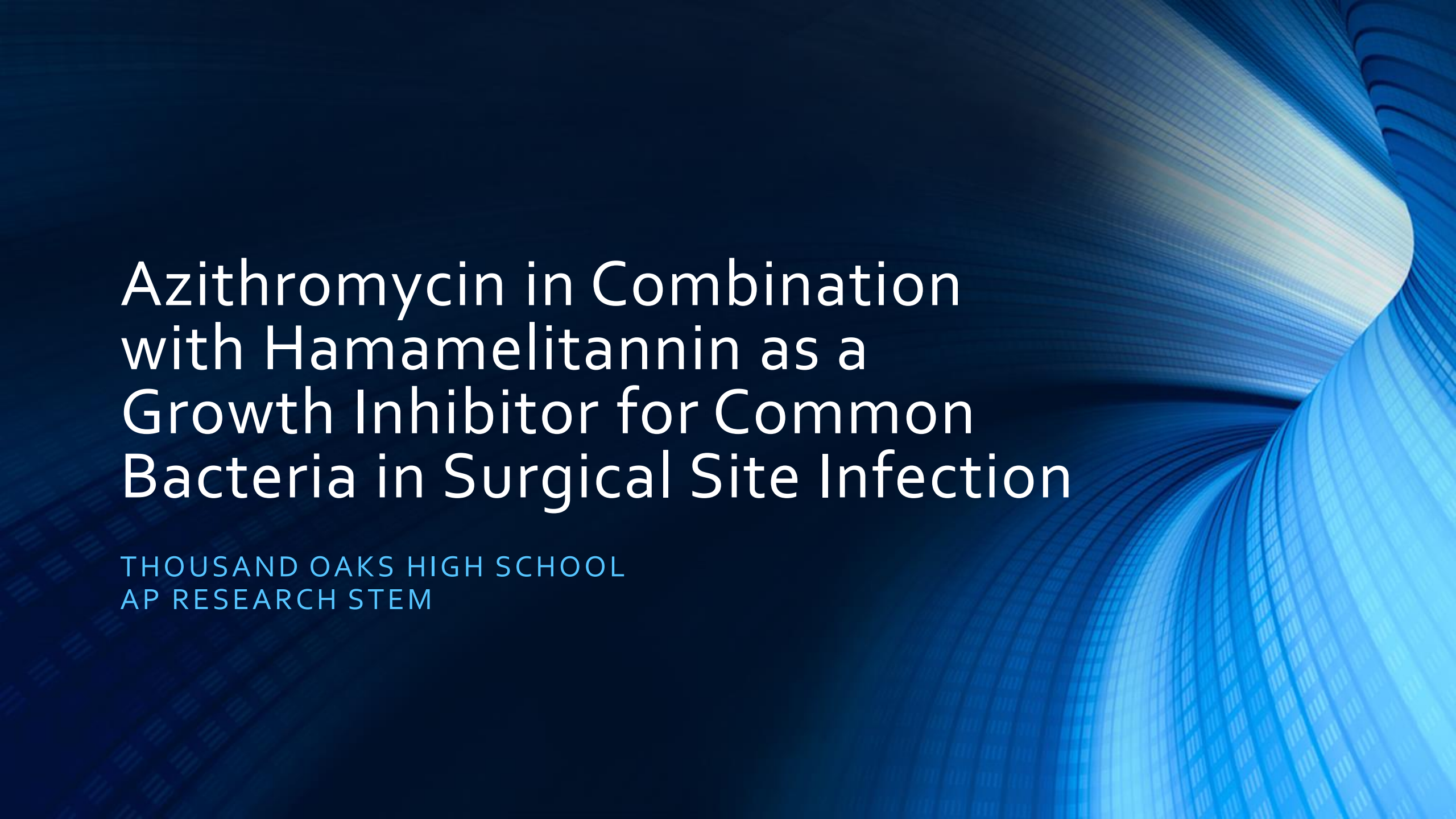
- To test Azithromycin against the main three bacteria in Surgical Site Infection
  - *Pseudomonas aeruginosa*
  - *Staphylococcus aureus*
  - *Escherichia coli*



# Acknowledgements

- Dr. Nikki Malhotra
- Mr. Jeff Lewis

# Works Cited

The background features a dark blue field with a grid of thin, light blue lines. A bright, glowing blue light source at the top right creates a perspective effect, making the grid lines appear to recede into a tunnel that curves towards the bottom right.

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