# SUMMER INTERNSHIP 2022

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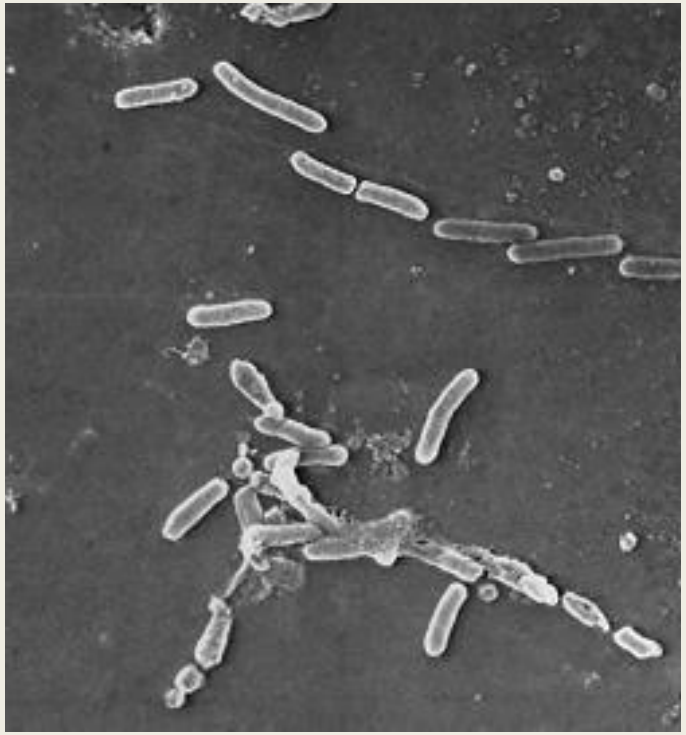
# Objective

- Characterize susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates to:
  - *Metal coupons (brass, copper, steel)*
  - *Tetracycline*
  - *$CuSO_4$*
    - Looking for possible synergistic interactions between tetracycline and $CuSO_4$

# What is *Pseudomonas aeruginosa*?

- Opportunistic Gram-negative pathogen
- Bacillus shape
- Facultative anaerobe
- Mostly affects people with large open wounds and compromised immune systems
  - *common in pulmonary infections and CF*
- Multi-drug resistant
- **1718**, 1721, 1729, 1740, 1747



# What is *Acinetobacter baumannii*?

- Opportunistic Gram-negative pathogen
- Short, almost round, rod-shaped
  - *Coccobacillus*
- Strictly aerobic
- Immunocompromised hosts
- Causes 5-10% of hospital-acquired infections
  - *CDC categorizes as top priority*

  *Copper Resistance Emerging Pathogen A. Baumannii AEM 2016*
- Multi-drug resistant
- **1719**, 1753, 1755, 1756, 1757

# Metals as Novel Therapeutic

- Antibiotic resistance is a growing concern for both pathogens → need for novel treatments
- Metals are important for normal cellular function but at higher concentrations become toxic
- High concentrations of metals are used to kill bacteria in various environments
  - *Hospital settings*
  - *Immune cells*

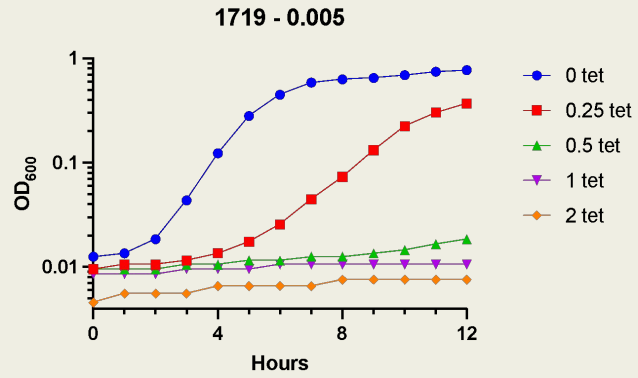
# DETERMINING ANTIBIOTICS AND STARTING OD

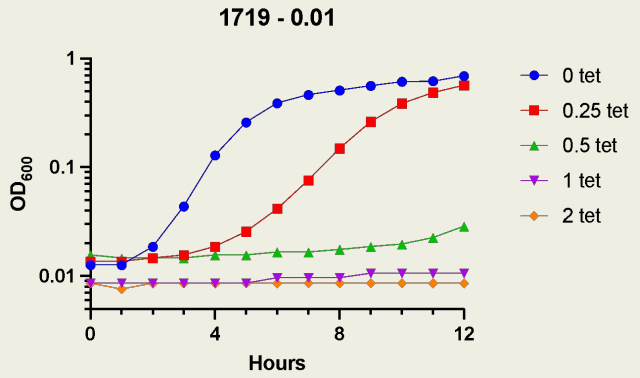
# Tetracycline MIC from CLSI Database

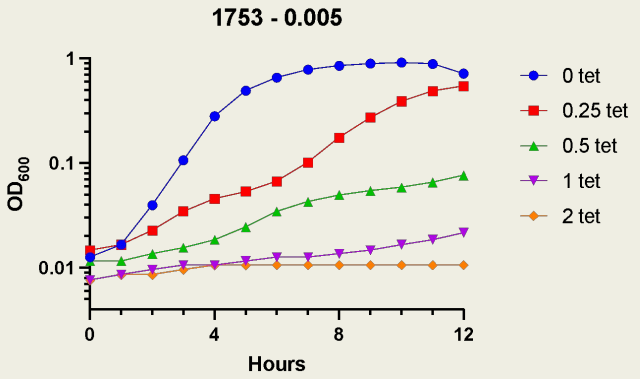
| TETRACYCLINES *Acinetobacter baumannii* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| (14) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both. | | | | | | | | | |
| B | Doxycycline | 30 µg | ≥ 13 | 10-12 | ≤ 9 | ≤ 4 | 8 | ≥ 16 | |
| B | Minocycline | 30 µg | ≥ 16 | 13-15 | ≤ 12 | ≤ 4 | 8 | ≥ 16 | |
| U | Tetracycline | 30 µg | ≥ 15 | 12-14 | ≤ 11 | ≤ 4 | 8 | ≥ 16 | |
| | | | | | | **S** | **I** | **R** | |

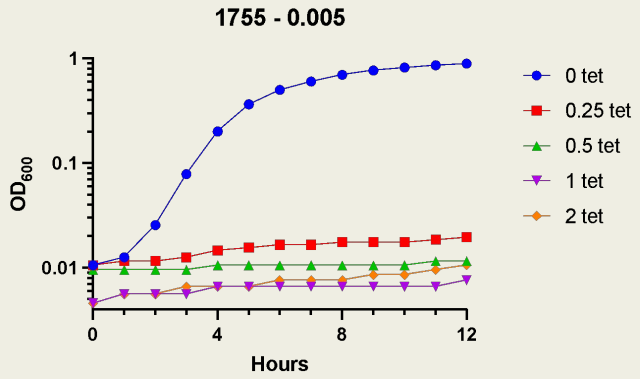
- **S** = Susceptible
- **I** = Intermediate
- **R** = Resistant

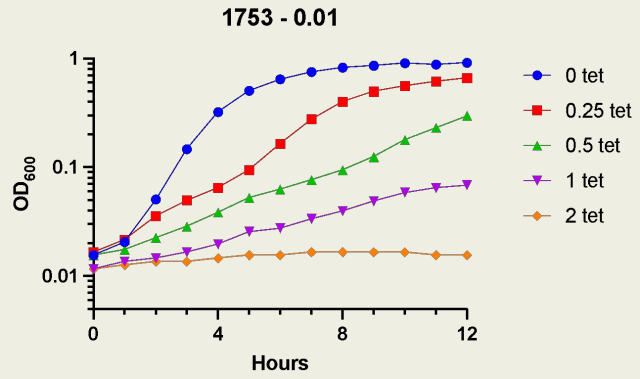
# Growth characteristics of *Acinetobacter baumannii* strains in the presence of tetracycline

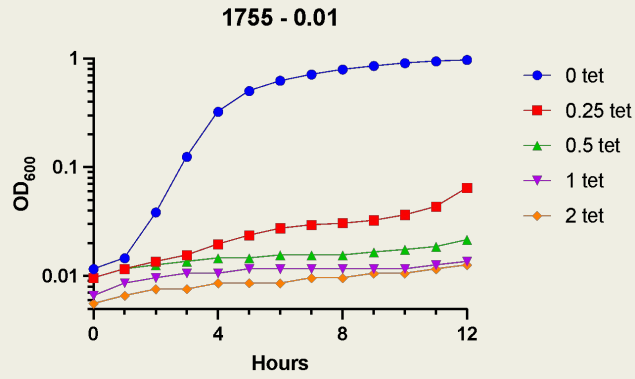


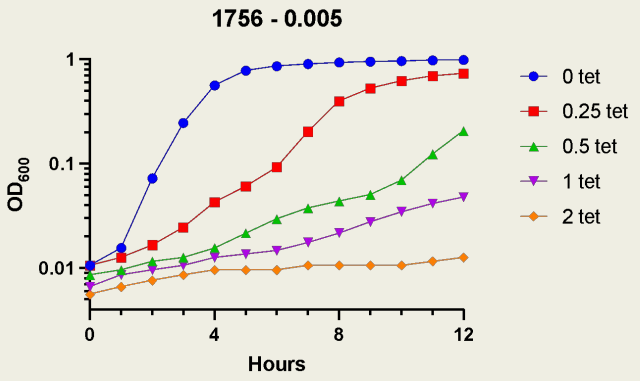



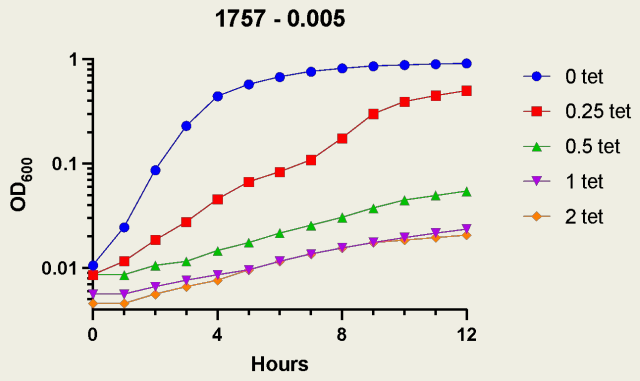



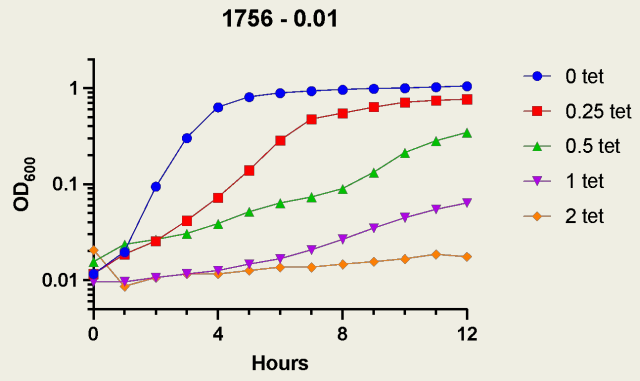



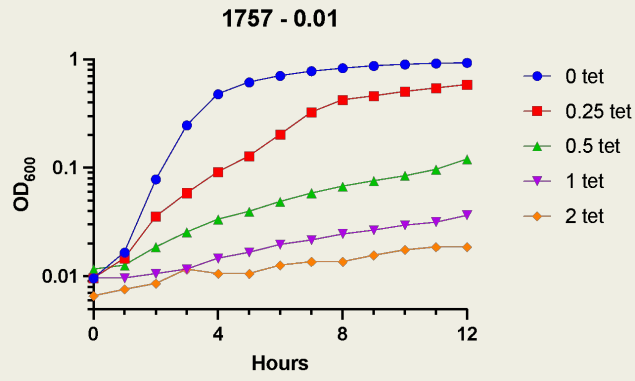



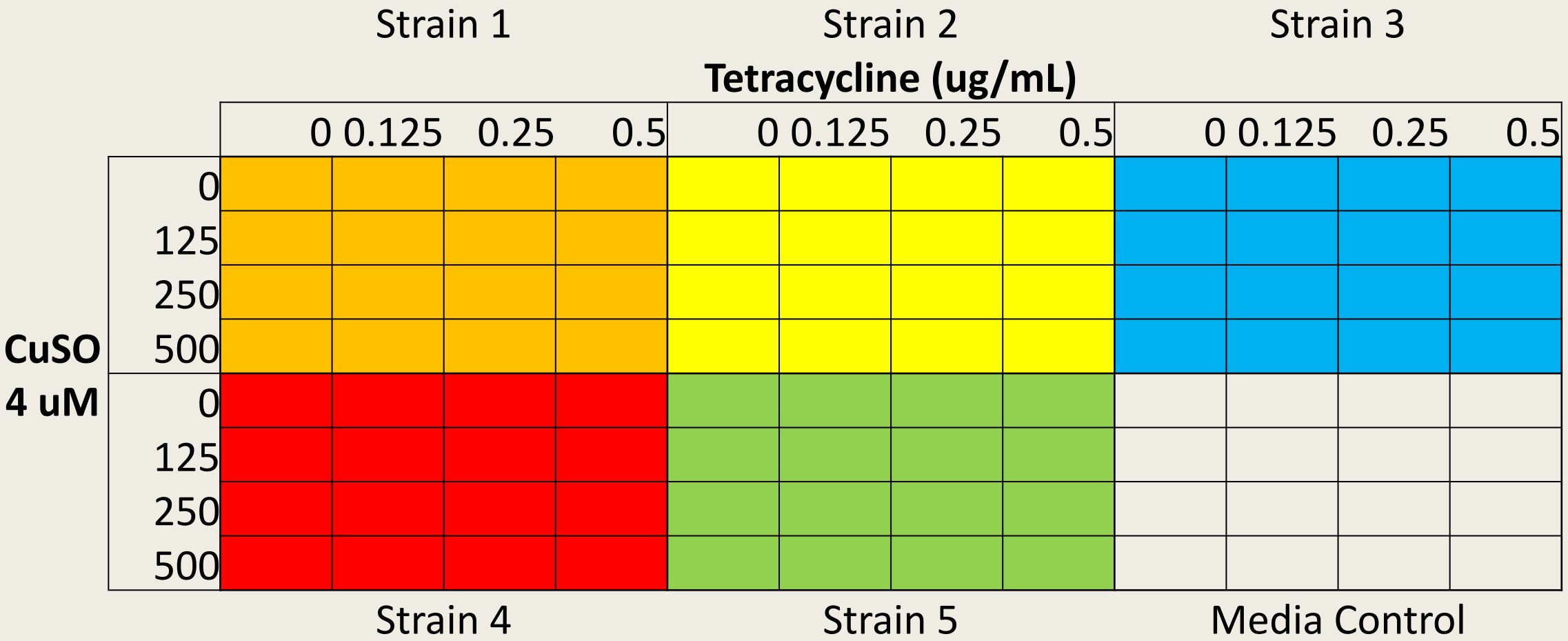



# DOES COPPER ENHANCE THE KILLING OF TETRACYCLINE?

# Protocol

- Set up liquid cultures night before in M9 and incubate at 37 degrees Celsius shaking at 190 RPM for 16-19 hours
  - *4 single colonies*
- Make stocks for various concentrations of Tetracycline and CuSO4
- Pipette 5uL of each concentration in 96-well plate
- Take OD600 of overnight cultures
- Calculate sub-culture to pipette 5uL into 96-well plate
- Pipette 5uL of inoculum into 96-well plate
- Add M9 so that the final volume of each well is 200 uL
- Place in BioTek for 12 hours
  - *Set Temp to 37 degrees Celsius*
  - *Shake for 30 seconds (orbital, fast)*
  - *Read 600 (3x3 but only used fifth reading)*
  - *Shake for 1 hour (orbital, fast)*
  - *Repeat previous 2 steps until T = 12*

# Checkerboard Assay Setup



# % Inhibition

## ***Pseudomonas aeruginosa***

Concentration of Tetracycline (ug/mL)

| | 0 | 0.125 | 0.25 | 0.5 | |
|---|---|---|---|---|---|
| 1718 | 0 | 5 | 10 | 8 | 0 CuSO4 |
| | 20 | 23 | 25 | 25 | 125 CuSO4 |
| | 25 | 27 | 25 | 24 | 250 CuSO4 |
| | 31 | 32 | 34 | 36 | 500 CuSO4 |
| 1721 | 0 | 7 | 14 | 18 | 0 CuSO4 |
| | 8 | 25 | 26 | 28 | 125 CuSO4 |
| | 8 | 14 | 17 | 14 | 250 CuSO4 |
| | 21 | 30 | 29 | 32 | 500 CuSO4 |
| 1729 | 0 | 2 | 0 | 1 | 0 CuSO4 |
| | 1 | -3 | 10 | 7 | 125 CuSO4 |
| | 27 | 31 | 36 | 29 | 250 CuSO4 |
| | 41 | 44 | 42 | 35 | 500 CuSO4 |
| 1740 | 0 | -2 | -1 | -3 | 0 CuSO4 |
| | 22 | 25 | 29 | 27 | 125 CuSO4 |
| | 22 | 28 | 27 | 34 | 250 CuSO4 |
| | 43 | 33 | 36 | 40 | 500 CuSO4 |
| 1747 | 0 | 8 | 13 | 10 | 0 CuSO4 |
| | 41 | 39 | 39 | 35 | 125 CuSO4 |
| | 47 | 48 | 49 | 45 | 250 CuSO4 |
| | 49 | 46 | 49 | 49 | 500 CuSO4 |

0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

## ***Acinetobacter baumannii***

Concentration of Tetracycline (ug/mL)

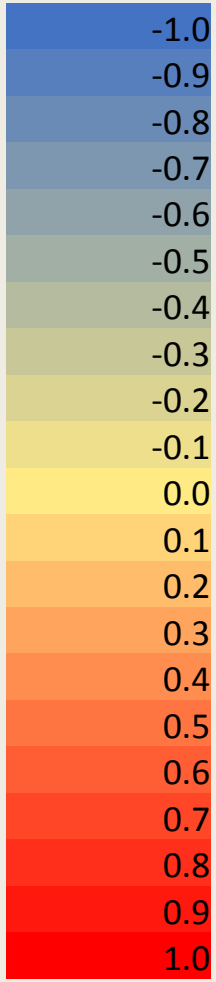
| | 0 | 0.125 | 0.25 | 0.5 | |
|---|---|---|---|---|---|
| 1719 | 0 | 19 | 38 | 49 | 0 CuSO4 |
| | 55 | 35 | 38 | 56 | 125 CuSO4 |
| | 83 | 78 | 59 | 72 | 250 CuSO4 |
| | 89 | 94 | 95 | 92 | 500 CuSO4 |
| 1753 | 0 | 37 | 62 | 67 | 0 CuSO4 |
| | 17 | 49 | 67 | 73 | 125 CuSO4 |
| | 39 | 52 | 68 | 77 | 250 CuSO4 |
| | 91 | 94 | 89 | 95 | 500 CuSO4 |
| 1755 | 0 | 30 | 71 | 88 | 0 CuSO4 |
| | 17 | 43 | 64 | 84 | 125 CuSO4 |
| | 14 | 43 | 60 | 81 | 250 CuSO4 |
| | 11 | 36 | 60 | 70 | 500 CuSO4 |
| 1756 | 0 | 24 | 39 | 55 | 0 CuSO4 |
| | 16 | 27 | 42 | 45 | 125 CuSO4 |
| | 63 | 55 | 45 | 59 | 250 CuSO4 |
| | 93 | 92 | 88 | 92 | 500 CuSO4 |
| 1757 | 0 | 16 | 37 | 55 | 0 CuSO4 |
| | 7 | 21 | 27 | 48 | 125 CuSO4 |
| | 17 | 25 | 31 | 54 | 250 CuSO4 |
| | 37 | 38 | 37 | 43 | 500 CuSO4 |

# Synergy

## ***Pseudomonas aeruginosa***

Concentration of Tetracycline (ug/mL)

| | 0.125 | 0.25 | 0.5 | |
|---|---|---|---|---|
| 1718 | -1.0 | -1.0 | -1.0 | 125 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 250 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 500 CuSO4 |
| 1721 | -1.0 | -1.0 | -0.9 | 125 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 250 CuSO4 |
| | -1.0 | -0.9 | -0.9 | 500 CuSO4 |
| 1729 | -1.0 | -1.0 | -1.0 | 125 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 250 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 500 CuSO4 |
| 1740 | -1.0 | -1.0 | -1.0 | 125 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 250 CuSO4 |
| | -1.0 | -1.0 | -1.0 | 500 CuSO4 |
| 1747 | -1.0 | -0.9 | -1.0 | 125 CuSO4 |
| | -1.0 | -0.9 | -0.9 | 250 CuSO4 |
| | -1.0 | -0.9 | -0.9 | 500 CuSO4 |




## ***Acinetobacter baumannii***

Concentration of Tetracycline (ug/mL)

| | 0.125 | 0.25 | 0.5 | |
|---|---|---|---|---|
| 1719 | -1.0 | -0.9 | -0.8 | 125 CuSO4 |
| | -0.9 | -0.8 | -0.7 | 250 CuSO4 |
| | -0.9 | -0.7 | -0.6 | 500 CuSO4 |
| 1753 | -0.9 | -0.7 | -0.7 | 125 CuSO4 |
| | -0.9 | -0.7 | -0.6 | 250 CuSO4 |
| | -0.8 | -0.6 | -0.6 | 500 CuSO4 |
| 1755 | -1.0 | -0.7 | -0.4 | 125 CuSO4 |
| | -1.0 | -0.7 | -0.5 | 250 CuSO4 |
| | -1.0 | -0.7 | -0.6 | 500 CuSO4 |
| 1756 | -0.9 | -0.8 | -0.8 | 125 CuSO4 |
| | -0.9 | -0.8 | -0.7 | 250 CuSO4 |
| | -0.8 | -0.7 | -0.6 | 500 CuSO4 |
| 1757 | -1.0 | -0.9 | -0.8 | 125 CuSO4 |
| | -1.0 | -0.9 | -0.8 | 250 CuSO4 |
| | -0.9 | -0.9 | -0.8 | 500 CuSO4 |

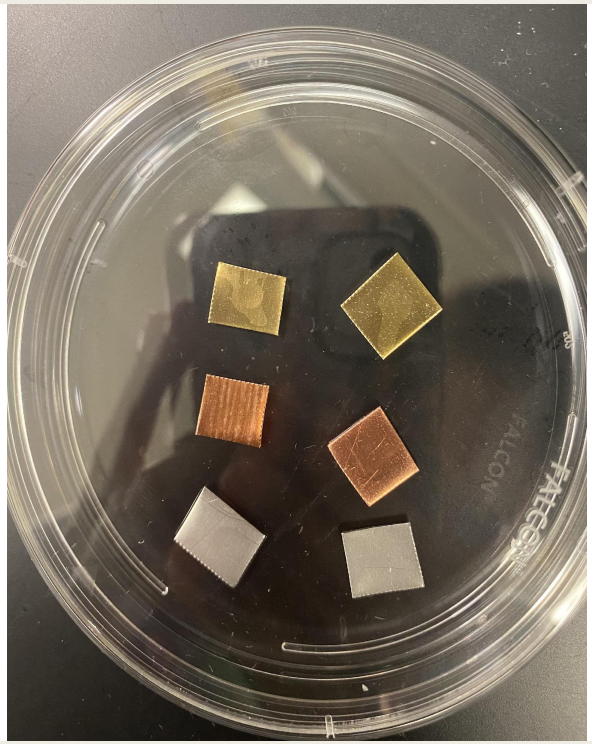
# Conclusions for Combination Therapy

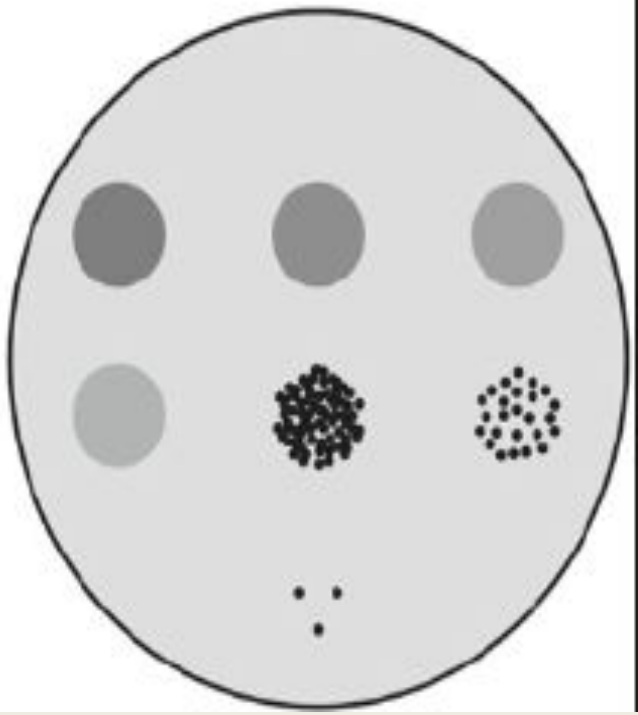
*Acinetobacter baumannii* more susceptible to tetracycline and $CuSO_4$ than *Pseudomonas aeruginosa*

No synergy observed between these two compounds

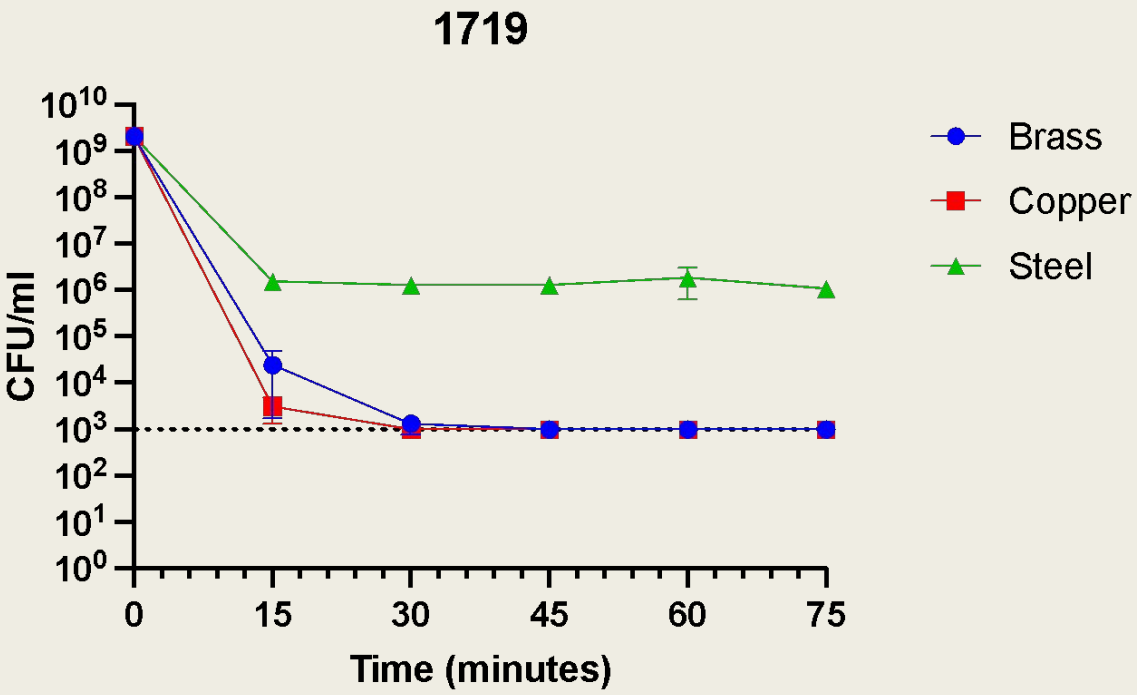
# BACTERIAL SURVIVAL ON METAL SURFACES

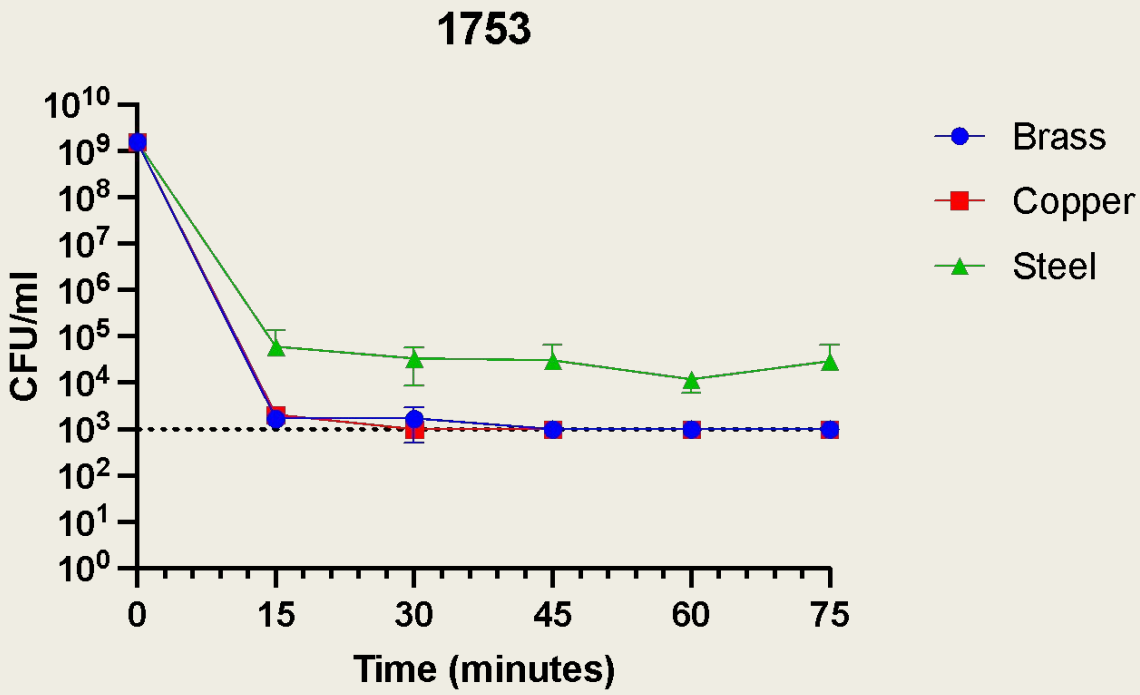
# Protocol

# Results


1719
CFU/ml
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
$10^{4}$
$10^{3}$
$10^{2}$
$10^{1}$
$10^{0}$
0
15
30
45
60
75
Time (minutes)
Brass
Copper
Steel


1753
CFU/ml
$10^{10}$
$10^{9}$
$10^{8}$
$10^{7}$
$10^{6}$
$10^{5}$
$10^{4}$
$10^{3}$
$10^{2}$
$10^{1}$
$10^{0}$
0
15
30
45
60
75
Time (minutes)
Brass
Copper
Steel

# Metal Coupons Conclusions

*Acinetobacter baumannii* most susceptible to copper and brass

*Acinetobacter baumannii* the least susceptible to steel

# CONCLUSIONS AND FUTURE DIRECTIONS

# Personal Conclusions

- Multi-pipetting skills
- Reading papers
- Lab up-keep
- Processing and analyzing data
- Liquid cultures
- Streaking bacteria
- Measuring OD and plating for CFU
- Patience
- Save everything

# Acknowledgements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- Marina Wylie