Effect of Lipopolysaccharide on Antibiotic Susceptibility in *A. baumannii*

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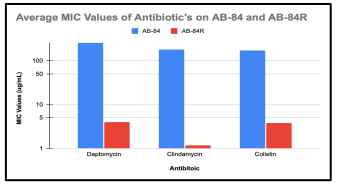
Background: Antibiotic resistance poses a major global health challenge, particularly for infections caused by *Acinetobacter baumannii*, a critical priority pathogen known for its ability to survive and resist treatment in hospital settings. The differentiating factor of gram-negative bacteria from gram-positive bacteria is the lipopolysaccharide (LPS) layer that forms in the outer membrane. LPS acts as a permeability barrier, preventing many antibiotics, particularly those targeting gram-positive bacteria, from entering the cell. Interestingly, *A. baumannii* is one of the few gram-negative species that can survive without LPS. This project will explore how the presence or absence of LPS impacts *A. baumannii's* susceptibility to three antibiotics: daptomycin, clindamycin, and colistin. Daptomycin, and clindamycin normally act on gram-positive bacteria, but may show activity on gram-negative bacteria when LPS is compromised. Colistin acts on gram-negative bacteria, but may show decreased activity since it binds directly to LPS. Understanding the influence of bacterial cell structures on antibiotic resistance is crucial for designing new and effective treatment strategies.

Research Question: How does the absence of LPS affect antibiotic susceptibility in *A.baumannii?*

Hypothesis: If susceptibility of the wild-type and mutant *A. baumannii* strains are tested under identical conditions, then the mutant strain will be more susceptible to daptomycin, and clindamycin because unlike the wild-type strain, the mutant membrane lacks LPS, which serves as a key barrier preventing antibiotic entry. Contrarily, the mutant strain will be more resistant to colistin because it binds directly to LPS.

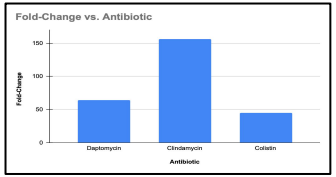
Methodology

1. Grew cultures of wild-type and mutant *A. baumannii* strains overnight prior to experimentation.
2. Prepared standardized suspensions adjusted to 0.5 McFarland turbidity.
3. Plated wild-type and mutant *A. baumannii* strains onto Mueller-Hinton agar plates.
4. Created bacterial lawns using sterile swabs.
5. Placed E-test strips for daptomycin, clindamycin, and colistin on the agar surface (one antibiotic per plate).
6. Incubated plates at 37 °C for 18–24 hours.
7. Recorded MIC values from E-tests.
8. Repeated across six trials.



This graph represents the mean MIC values of daptomycin, clindamycin , and colistin tested on strains of A. *baumannii*. Values >256 were treated as 256. The x-axis represents the antibiotics while the y-axis represents the mean MIC values measured in ug/mL. This graph was created by the student researcher.

This graph represents the fold change values which are the differences of susceptibility of the AB-84 compared to the AB-84R strains for each antibiotic tested. This graph was created by the student researcher.



Results: After six trials, MIC values were recorded for each antibiotic, averages were calculated, and results were measured in ug/mL. For calculation purposes, values >256 were treated as 256. The AB-84 mean for daptomycin was 256 ± 0.00, while the mean for AB-84R was 3.67 ± 3.42. For clindamycin, the AB-84 mean was 219 ± 91.4, while its AB-84R strain had a mean of 1.42 ± 0.74. For colistin, the AB-84 mean was 171 ± 104 and the AB-84R mean was 3.83 ± 3.24. All antibiotics displayed statistically significant MIC value differences, which were calculated with Welch's T-test: daptomycin ($p<0.001$), clindamycin ($p=0.002$), and colistin ($p=0.026$). Fold-change analysis was performed to further show the susceptibility differences between strains. This resulted in a 64x difference for daptomycin, 156x for clindamycin, and 45x for colistin.

Discussion: The results for daptomycin, and clindamycin support the hypothesis since the AB-84R strains were more susceptible to them than the AB-84 strains. In contrast, the results for colistin were inconsistent. Since colistin binds directly to lipid A in LPS (Andrade), the AB-84 strain should have been more susceptible to colistin across all trials, yet this only occurred in trials one and four. However, LPS also functions as the structural stabilizer of the membranes within gram-negative bacteria (Sabnis et al., 2021), meaning that in the absence of LPS the thin outer membrane of gram-negative bacteria (Beveridge) is left exposed. These results suggest that the compromised membrane had a greater impact on the functionality of colistin than its mechanism of action. Standard deviation values were higher in AB-84 strains due to the presence of LPS, since daptomycin, clindamycin and vancomycin normally act on gram-positive bacteria. However, standard deviations of all AB-84R strains showed little variance due to the absence of LPS, suggesting that LPS is a limiting factor in the functionality of gram-positive antibiotics. Fold-change analysis illustrated significant susceptibility differences between the strains, where daptomycin and clindamycin showing much more activity on the AB-84R strain, further supported the hypothesis. Limitations of this experiment include the number of trials and amount of antibiotics tested. This research contributes to the knowledge of bacterial structures and their influence on antibiotic resistance. Future research pursuits should focus on testing strains similar to *A. baumannii,* with the goal of discovering how to manipulate the presence or absence of LPS to develop the most effective treatments.

Conclusion: Results for daptomycin, and clindamycin across all trials supported the hypothesis since the AB-84R strains were more susceptible than the AB-84 strains due to the absence of LPS. Trials one and four for colistin also supported this hypothesis since AB-84R was more resistant to colistin in comparison to AB-84. However, the other trials of colistin refuted the hypothesis, suggesting that the compromised outer membrane had a greater effect on colistin than the absence of LPS.This research supports the development of treatments against critical-priority pathogens such as *A. baumannii.* Ultimately, future research should focus on targeting LPS manipulation which will open doors to improved antimicrobial therapies due to the role LPS plays regarding intrinsic resistance.