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

Paloma Rizzi • Marian High School • Mishawaka IN • United States of America

Background

Antibiotic resistance poses a major global health challenge, particularly for infections caused by Acinetobacter baumannii, a critical priority pathogen notorious for its ability to survive in hospitals and resist treatment. A key feature of gram-negative bacteria such as *A. baumannii* is the lipopolysaccharide (LPS) layer that forms part of the outer membrane. This layer 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 the LPS layer. This project will explore how the presence or absence of LPS affects *A. baumannii* susceptibility to three antibiotics: daptomycin, colistin, and clindamycin. These antibiotics normally act exclusively on gram-positive bacteria, but may show activity when the LPS layer is compromised. Understanding the effect of bacterial cell envelope structures on antibiotic resistance is crucial for designing new and effective treatment strategies.

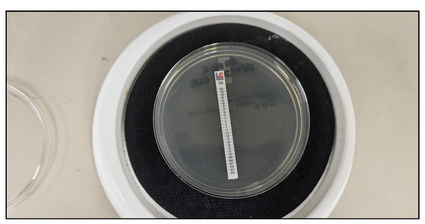
How does the absence of LPS affect antibiotic susceptibility in A.baumannii?

Hypothesis

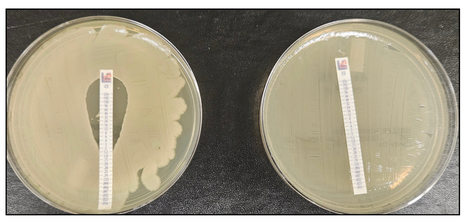
If susceptibility of the wild-type and mutant *A. baumannii* strains are tested under identical conditions, then the mutant strain will be more susceptible to daptomycin, and clindamycin because unlike the wild-type strain, the mutant membrane lacks LPS, which serves as a key barrier preventing antibiotic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

Procedure

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 of daptomycin, clindamycin, and colistin onto the agar surface (one test per plate).
6. Incubated plates at 37 °C for 18–24 hours.
7. Recorded MIC values from E-tests.
8. Repeated across six trials for both strains.

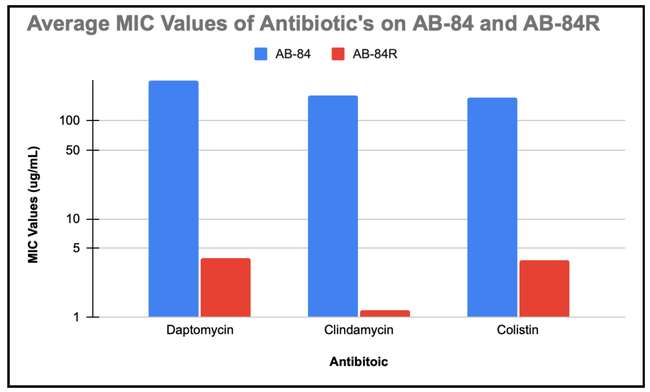


This photo depicts the trial four AB-84R strain with clindamycin before incubation. This photo was taken by the student researcher.

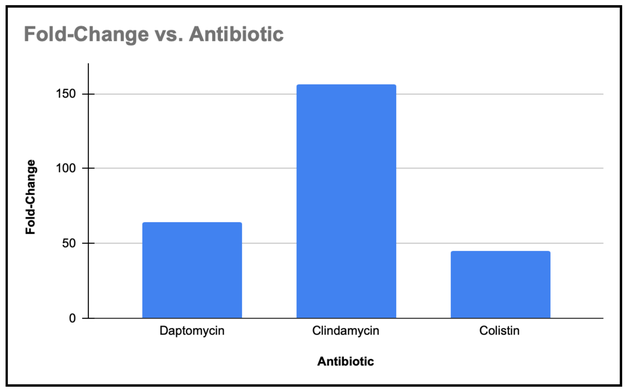


This photo shows the trial two results of the strains with colistin, the AB-84R strain on the left and AB-84 on the right. AB-84 has a MIC value >256 while AB-84R has a MIC value of 1.5. This photo was taken by the student researcher.

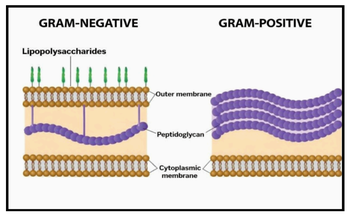
Data



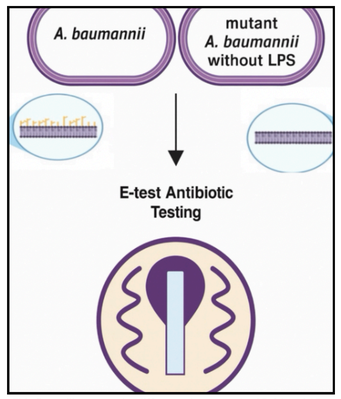
This graph represents the average MIC values of daptomycin, clindamycin, and colistin tested on Ab-84 and AB-84R strains of *A. baumannii*. The x-axis represents the antibiotics while the y-axis on a log-scale represents the average MIC values measured in ug/mL. This graph was created by the student researcher.



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



This diagram illustrates the structural differences of the membranes of gram-posotive and gram-negative bacteria. This diagram was created by the *Checmical & Engineering News (C&en)*



This figure illustrates the difference between the AB-84 and AB-84R strains: the presence of the LPS. This figure also represents the procedure. This figure was created by Michael McConnell, the mentor.

Minimum Inhibitory Concentration Values (ug/mL)

Antibiotic	Strain	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Dapt.	AB-84	>256	>256	>256	>256	>256	>256
Dapt.	AB-84R	1.5	1.5	9	1.5	1.5	7
Co.	AB-84	1.5	>256	>256	1.5	>256	>256
Co.	AB-84R	8	2	1.5	8	1.5	2
Clinda.	AB-84	32	>256	>256	>256	>256	>256
Clinda.	AB-84R	0	1.5	2	1.5	2	1.5

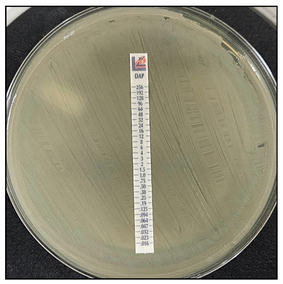
This table represents the minimum inhibitory concentration (MIC) values for daptomycin, colistin, and clindamycin tested on wild-type AB-84 strains and mutant AB-84R strains of *A. baumannii.* Lower MIC values indicate greater susceptibility while higher MIC values indicate strong resistance to these antibiotics. This table was created by the student researcher.

Statistical Analysis

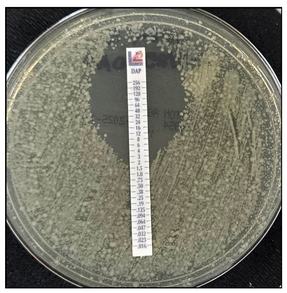
Mean MIC values and Variability of AB-84 and AB-84R (ug/mL)

Antibiotic	AB-84 mean+SD	AB-84R mean+SD	Fold Change	P-Value
Daptomycin	256 ± 0.00	4.00 ± 4.33	64x	<0.001
Clindamycin	181 ± 129	1.16 ± 1.04	156x	0.002
Colistin	171 ± 146	3.83 ± 3.62	45x	0.026

This table represents the average MIC values, standard deviations (SD), fold-change, and p-values for AB-84 and AB-84R strains measured in (ug/mL). MIC values >256 were treated as 256 for calculations. This table was created by the student researcher.



This photo shows the results from trial one of the AB-84 strain with daptomycin where the MIC value is >256. This photo was taken by the student researcher.



This photo shows the results from trial one of the mutant AB-84R strain with daptomycin, where the MIC value is 1.5. This photo was taken by the student researcher.

Results

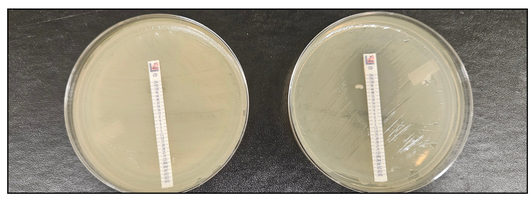
After six trials, MIC values were recorded for each antibiotic, averages were calculated, and results were measured in ug/mL. For calculation purposes, values >256 were treated as 256. The AB-84 mean for daptomycin was 256 ± 0.00, while the mean for AB-84R was 3.67 ± 3.42. For clindamycin, the AB-84 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 and clindamycin 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 showed much more activity on the AB-84R strain, further supporting the hypothesis. Limitations of this experiment include the number of trials and the 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.



This photo shows the trial four results of the strains with clindamycin, the AB-84 strain on the left and AB-84R on the right. AB-84R has a MIC value of 1.5 while AB-84 has a value >256. This photo was taken by the student researcher.