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Article

Strain-selective *in vitro* and *in silico* structure activity relationship (SAR) of N-acyl β-lactam broad spectrum antibiotics

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SUMMARY

Different bacterial species have developed resistance to antibiotics over the years. Penicillin analogs have been developed to counter the bacteria's acquired resistance to standard antibiotics; in this study, we investigate the antibacterial efficacy of penicillin G and its analogs amoxicillin, carbenicillin, piperacillin, cloxacillin, and ampicillin, against four species of bacteria. Penicillin antibiotics mimic the D-Alanyl-D-Alanine active site region of penicillin-binding proteins (PBP). PBPs are enzymes used in the synthesis of bacterial cell walls. Through a Kirby-Bauer assay, we found that the inhibition performance of these penicillin-type antibiotics varies greatly among different bacterial species. Moreover, to investigate the structural basis for the in vivo Structure Activity Relationship observed, we also conducted *in silico* virtual screening via homology modeling, molecular docking, and density functional theory (DFT) calculations. We concluded that all six penicillin-type antibiotics inhibit *Staphylococcus epidermidis*, *Escherichia coli*, and *Neisseria sicca* with varying degrees of efficacy but exhibited no inhibition against *Bacillus cereus*. Our computational results suggest that the distance between the β-lactam ring and the active serine is more influential than the thermodynamic binding affinity for these antibiotics to interact with the PBPs. Penicillin G had the greatest broad-spectrum antibacterial activity with a high radius of inhibition against *S. epidermidis*, *E. coli*, and *N. sicca*.

INTRODUCTION

According to the Center for Disease Control (CDC), infections from antibiotic-resistant bacteria and fungi account for more than 35,000 deaths in the United States every year (1). Throughout history, pathogenic bacteria have posed a massive threat to humans due to their ability to evolve to overcome environmental pressures and grow resistant to antibiotics (2). In 1929, Sir Alexander Fleming found that a compound produced by the fungus *Penicillium notatum* inhibited the growth of bacterial cultures; this compound was later identified as penicillin G (**Figure 1A**) (3). By 1942, penicillin G was used globally as an antimicrobial agent (4). Later, it was determined that the primary targets of penicillin G in bacteria were penicillin-binding proteins (PBPs), which catalyze the transglycosylation and the transpeptidation for bacterial cell walls (5). In particular, penicillin irreversibly inactivates D,D-transpeptidase, a PBP responsible for the synthesis of cross-linked peptidoglycan in the cellular wall, by irreversibly acylating the active site nucleophile of D,D-transpeptidase. Consequently, this inhibits bacteria's ability to create and maintain their cell wall and thus their ability to survive and reproduce (6). Over time, other natural products that bear the same β-lactam core have been identified (**Table 1**). β-lactams, four-membered cyclic amides, form the structural core of many of the most important classes of antibiotics used today, including penicillins, cephalosporins, and several others (7).

However, the widespread use of β-lactam-containing antibiotics has led to the development of many species of penicillin-resistant bacteria. Generally, multidrug-resistant bacteria arise from two mechanisms: inactivity of the β-lactam by β-lactamases or target site modifications to the PBP (6). As bacteria evolve, each generation develops methods to resist conventionally-used antibiotics, such as synthesizing

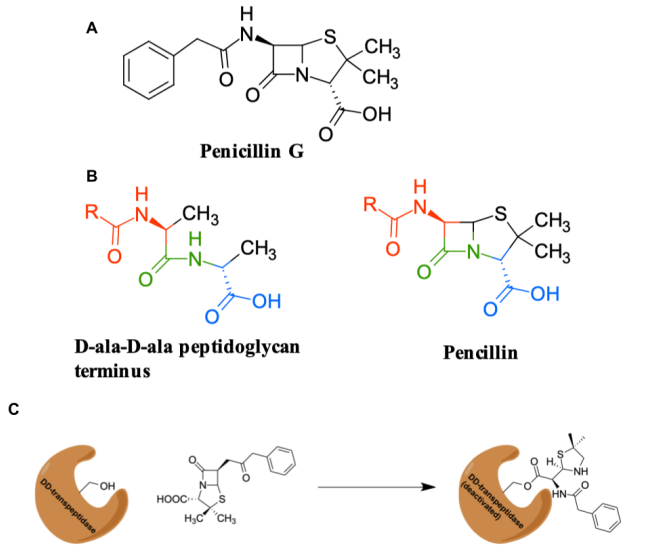
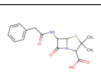
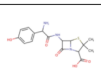
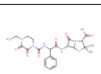


Figure 1. Structure and mechanism of action for penicillin antibiotics. A) Structure of natural product penicillin G. **B)** D-ala-D-ala active site of D,D-transpeptidase compared with the structure of the penicillin core. Red represents the similar amine and carbonyls, green represents similar active carbonyl carbon β-lactam rings, and blue represents similar carboxylic acids. **C)** Mechanism of Action for a β-lactam ring to inhibit a serine protease.

Table 1. Commercially available penicillin antibiotics used to fight various microbial infections.

Penicillin Antibiotic	Structure	Trade Name	Year Discovered/ Synthesized	Target and Usage
Penicillin G		Pfizerpen	1929	Bacterial infections (broad-spectrum).
Amoxicillin		Moxatag	1972	Infections and stomach ulcers.
Carbenicillin		Geocillin	1970	Bacterial infections.
Piperacillin		Zosyn	1974	Treatment of skin, gynecological, abdominal infections, and pneumonia (broad-spectrum).
Cloxacillin		Cloxapen, Tegopen	1960	Bacterial infections.
Ampicillin		Omnipen	1958	Broad-spectrum antibiotic; treats many types of infections.

β-lactamase enzymes. These β-lactamase enzymes attack and open the delicate four-membered β-lactam ring, thus rendering the antibiotic ineffective (8). Lastly, bacteria may develop resistance through mutations in the binding pocket sequence, which change the structure of the pocket. Because the alterations in the functional groups attached to the amide of an antibiotic impact its binding ability, resistance can be overcome by changing these functional groups (9). As such, analogs with varying structures are constantly being synthesized in order to counteract the rapidly evolving threat of antibiotic resistance. The structures of available analogs must also be analyzed to understand the structural basis that plays a role in pertinent antimicrobial properties against specific bacterial infections. To successfully pick an antibiotic that will be useful against a bacterial illness, it is necessary to identify species-specific effectiveness.

From Fleming's original discovery of penicillin G to the present day, researchers have made multiple natural and semi-synthetic analogs of penicillin G, creating a class of antibiotics known as penicillins. These molecules all share a core of 6-aminopenicillanic acid, or 6-APA (10). Through additions to the amide group, multiple molecules have been produced and are currently commercially used to fight a plethora of microbial-related problems. Here, we screened six of these commercially available penicillins: penicillin G, amoxicillin, carbenicillin, piperacillin, cloxacillin, and ampicillin, against four species of gram-positive and gram-negative bacteria (**Table 1**).

The bacteria examined in our study were *E. coli, B. cereus, N. sicca.,* and *S. epidermidis,* all of them being gram-negative bacteria except for *B. Cereus*. Notably, *B. Cereus* also demonstrates resistance to penicillin due to the presence of β-lactamase enzymes (11). We then tested the six penicillin derivatives in **Table 1** at four concentrations against these species.

We conducted both computational modeling and an *in vivo* antimicrobial assay to better understand the structure activity relationship (SAR) between the PBPs and the screened antibiotics based on side chain. The antibiotics were tested at concentrations of 1 mM, 0.1 mM, 0.01 mM, and 0.001 mM, and the radii of inhibition (ROI) were measured to determine the efficacy of each antibiotic to kill the bacteria at varying concentrations.

We hypothesized that piperacillin would have the strongest antimicrobial effect given its classification as an extended-spectrum antibiotic. Our data did not corroborate this initial hypothesis, instead demonstrating the importance of having multiple commercially available antibiotics and highlighting the diverse antimicrobial effects of antibiotics even within the penicillins. Moreover, given that *B. cereus* has been previously reported to be resistant to penicillin antibiotics due to the β-lactamase enzymes that they possess, we hypothesized that, regardless of predicted binding affinities from molecular docking, penicillin and its analogs will exert little to no antimicrobial activity on *B. cereus* growth, which was supported by our data (12). Our data also confirmed that diverse β-lactam penicillins allow for species-specific effectiveness against bacterial infections.

RESULTS

In order to test the effectiveness of the antibiotics, we performed Kirby-Bauer assays. The average ROI from three

duplicate Kirby-Bauer assays and the standard errors were calculated for each antibiotic-bacteria pairing (**Figure 2A-2D**).

Inhibition curves were then generated to analyze the effect of the concentration of the antibacterial activity. There were dose-dependent antibacterial effects for all of the antibiotics. A ten-fold increase in an antibiotic solution showed a constant increase in the ROI. R^2 values were indicative of a linear fit for the data values across all antibiotics and bacterial species (**Figure 3A-3D**). Statistical significance test used was an unpaired *t*-test.

S. epidermidis

All penicillin-type antibiotics had ROI that were statistically significant (all *p*-value between 0.001-0.003) for the *S. epidermidis* compared to the control for the highest concentration tested (1 mM). Specifically, penicillin G had the most potent effects at 1 mM and overall penicillin G and carbenicillin had similar and most effective antibacterial effects at all four concentrations (**Figure 2A**). Cloxacillin had a significant difference between the 1 mM concentration ($p < 0.0001$) and 0.1 mM concentration ($p < 0.0001$) and piperacillin had a significant difference between the 0.1 mM concentration ($p < 0.0001$) and 0.01 mM concentration ($p <= 0.0001$). At lower concentrations, cloxacillin and piperacillin produced no statistically significant ROI (*p*-value 0.005-1.000), as compared to the ROI of the control.

N. sicca

All the penicillin antibiotics produced statistically significant (all *p*-value between 0.001 to 0.003) ROI tested at a 1mM concentration, when compared to the control (**Figure 2B**). Piperacillin had a significantly higher ROI at 1 mM as compared to other concentrations and all other antibiotics. Piperacillin had the largest difference in concentration of all of the screened antibiotics at the 0.1 mM and 1 mM concentrations. Cloxacillin and carbenicillin did not have any inhibition below the 1 mM concentration; in comparison to the other antibiotics, they also had the lowest ROI at 1 mM. Ampicillin, amoxicillin, and penicillin G had similar ROI for all four concentrations, but overall ampicillin performed best against *N. sicca*. For all antibiotics, there was dose-dependent inhibition for bacterial growth, as the concentration increased, so did their ROI.

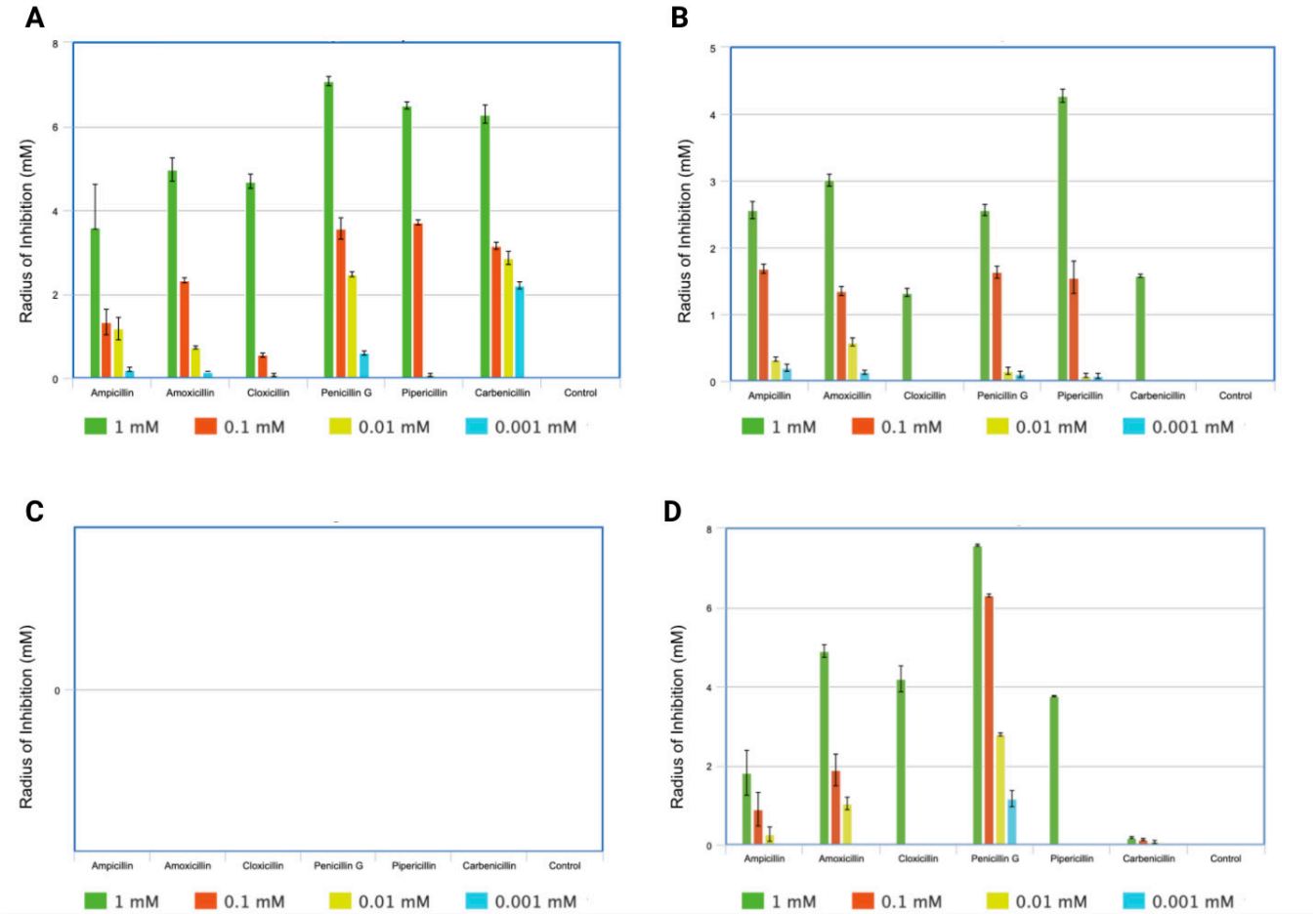


Figure 2. Average radii of inhibition for four species of bacteria with all antibiotics. The average radii of inhibition for six different penicillin antibiotics against four species of bacteria. The error bars represent ±1 standard deviation. **A)** ROI against *S. epidermidis*, **B)** ROI against *N. sicca*, **C)** ROI against *B. cereus*, **D)** ROI against *E. coli*.

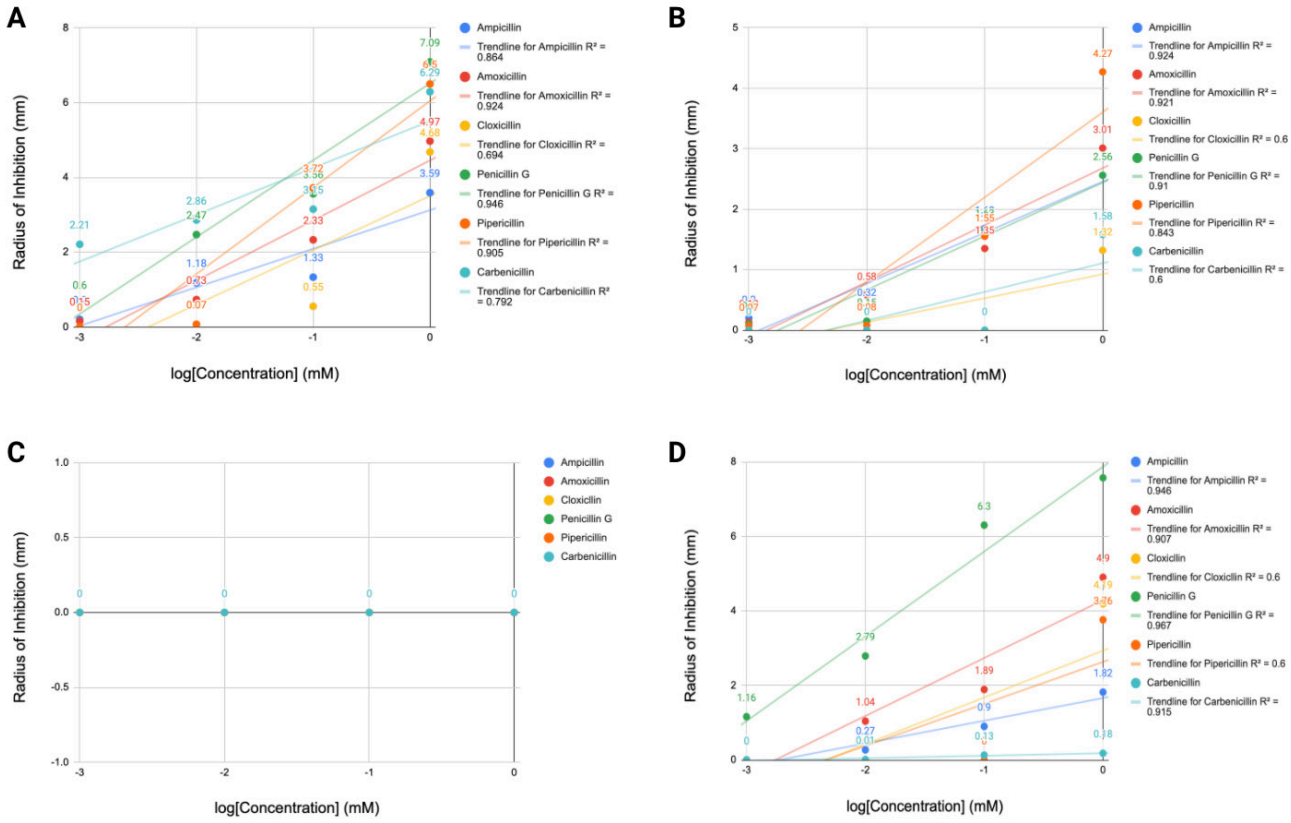


Figure 3. Inhibition curves for four species of bacteria with all antibiotics. All graphs are fit to a linear curve with R2 values shown. The figure helps to explain how higher concentrations of the antibiotic showed greater antibiotic efficacy. **A)** Inhibition curve for *S. epidermidis*; **B)** Inhibition curve for *N. sicca*; **C)** Inhibition curve for *B. cereus*; **D)** Inhibition curve for *E. coli*.

E. coli

In the study with *E. coli*, the penicillin-type antibiotics were not as potent as compared to other bacteria. Penicillin G exhibited inhibition against bacterial growth across all concentrations (**Figure 6**). Carbenicillin had almost no inhibition and only produced statistically significant inhibition at 1 mM and 0.1 mM concentrations (*p*-value between 0.001-0.002), whereas cloxacillin and piperacillin had no inhibition for concentration below 1 mM (**Figure 2C**).

B. cereus

All penicillin-type antibiotics were ineffective against *B. cereus*. There was no inhibition against the growth of *B. cereus* for all four concentrations (**Figure 2D**).

When compared to the control, all the data with a radius of inhibition was found to be statistically significant. We found that the antibiotics on average worked the best against the *S. epidermidis* species, and the *B. cereus* seems to be the most resistant to all the tested antibiotics. *E. coli* shows more resistance to the antibiotics as compared to *S. epidermidis* and *N. sicca*, but *N. sicca* shows more resistance than *S. epidermidis*. The differences in susceptibility is that all the antibiotics tested were penicillin derivatives which would cause different interactions with the penicillin binding proteins based on the difference in functional groups.

In silico studies

To provide structural insight into the biological activity of the penicillins studied, the binding affinity of each compound was predicted through computational modeling with the PBP of each bacteria species. Homology modeling was conducted to generate the desired PBP for each bacteria using base proteins of the same genus to preserve tertiary protein structure between species. The amino acid sequence of PBPs are fairly conserved except for the SXXK sequence, an SXN sequence, and a triad of KSG, KTG, HSG or HTG in the binding pocket of the penicillin-binding domain, which account for the variability between PBPs (13). Homology modeling was then conducted on the active site motifs of each protein to match the binding pocket topography based on the sequence of each species, conserving upwards of 90% of the entire protein, which means that more than 90% of the amino acids from the base protein were not changed (14). Finally, all six antibiotics were docked to the penicillin-binding domain of the four species of bacteria. The resulting binding affinities of the protein-antibiotic complex and distances between the

active serine and β-lactam core of the penicillin analogs help explain the relative effectiveness of each commercially available antibiotic.

A summary of molecular docking results performed for each antibiotic and bacteria can be found in **Table 2**. Cloxacillin showed the highest binding affinities for all the antibiotics' PBPs. Carbenicillin and penicillin G had the lowest binding affinities overall on average. Most of the antibiotics showed relatively better binding affinities when docked to *N. sicca* and *E. coli*; all antibiotics bound relatively worse to *B. cereus* and *S. epidermidis* in comparison.

Because β-lactam antibiotics are covalent inhibitors that irreversibly acylate the serine nucleophile of the target protein, we measured the distances between the carbonyl carbon of each antibiotic's β-lactam ring and the active serine in the binding site, which we envisioned would provide insight into how readily a bound penicillin molecule might react with the active site nucleophile (**Figure 4A-4D)**. *N. sicca*'s binding site serine was fairly close to the bound antibiotics, between 3 to 5 Å, while the serines in *S. epidermidis'* and *B. cereus*' binding sites were up to 10 Å away (**Table 2**) (15). Piperacillin bound to *E. coli* forms a hydrogen-bond with R198 and a parallel displaced pi-stacking interaction with Y222 (**Figure 5A**). With *S. epidermidis*, amoxicillin forms a hydrogen bond with the main-chain residue T239 and one parallel-displaced pi-stacking interaction with Y238 (**Figure 5B**). Both penicillin G and cloxacillin had the strongest binding affinity for the *N. sicca* PBP. For cloxacillin, key interactions with the PBP include hydrogen bonding with N364 and a T-shaped pi-stacking interaction with Y544 (**Figure 5C**). Unlike the other top-binding antibiotics, cloxacillin bound to *B. cereus* forms no explicit hydrogen bonds or pi-stack interactions, suggesting a binding mode that is predominantly governed by hydrophobic interactions (**Figure 5D**).

Computational experiments suggest that the relative strength of binding interactions do not factor as significantly in the inhibition of the bacterial growth compared to the orientation of the reactive β-lactam ring relative to the active serines of the PBPs.

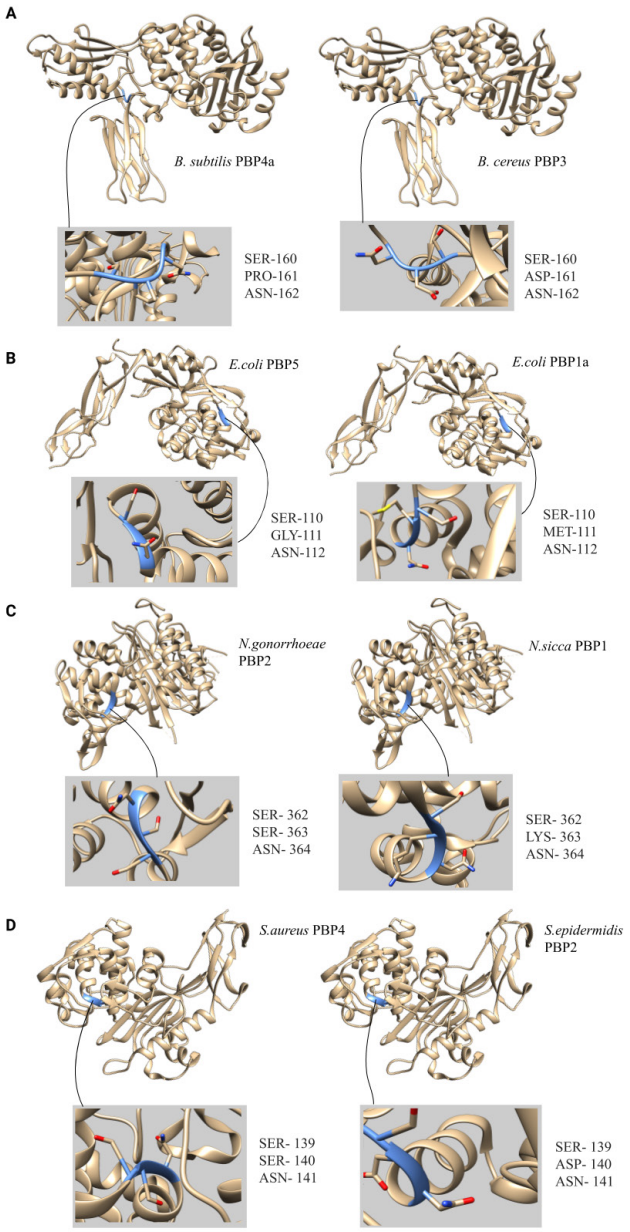
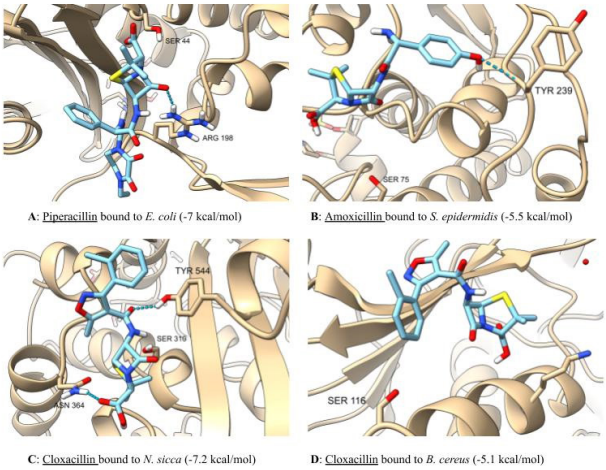


Figure 4. The differing amino acid residues on the *B. cereus*, *E. coli*, *N. sicca*, *S. epidermidis* base PBP and homology-modeled PBPs. The blue residues represent the SXN sequence that was changed in all four bacteria. The resulting homology-modeled proteins were then docked against. **A)** Amino acids on the crystal structure *B. subtilis* (PDB code: 1NZO) were replaced with the respective residues using the Rotamers tool on UCSF Chimera. **B)** Amino acids on the crystal structure *E. coli* (PDB code: 1W5D) were replaced with the respective residues using the Rotamers tool on UCSF Chimera. **C)** Amino acids on the crystal structure *N. gonorrhoeae* (PDB code: 3EQV) were replaced with the respective residues using the Rotamers tool on UCSF Chimera. **D)** Amino acids on the crystal structure *S. aureus* (PDB code: 1TVF) were replaced with the respective residues using the Rotamers tool on UCSF Chimera.

Table 2. Heat map of binding affinities (kcal/mol) of penicillin antibiotics docked on *E. coli*, *S. epidermidis*, *N. sicca*, and *B. cereus*.

	E. coli	Distance to S44 (Å)	*S. epidermidis*	Distance to S75 (Å)	*N. sicca*	Distance to S310 (Å)	*B. cereus*	Distance to S116 (Å)
Penicillin G	-5.7	6.631	-4.3	8.532	-7.2	3.048	-5	7.134
Ampicillin	-5.6	6.693	-5.3	8.635	-6.4	3.009	-4.4	10.152
Amoxicillin	-5.9	8.031	-5.5	8.742	-6.7	3.04	-4.5	6.944
Carbenicillin	-5.5	7.233	-3.8	8.859	-4.3	4.708	-4.9	6.492
Cloxacillin	-6.7	7.29	-3.2	8.715	-7.2	4.742	-5.1	7.042
Piperacillin	-7	6.741	-1.1	10.702	1.2	5.287	-4.2	7.09
KEY	> -3.5	-3.5 to -4.2	-4.2 to -4.9	-4.9 to -5.5	-5.5 to -6.2	-6.2 to -6.9	< -6.9	

NOTE: Distances (Å) between each bound antibiotic and the active serine are listed.



A: Piperacillin bound to *E. coli* (-7 kcal/mol) **B**: Amoxicillin bound to *S. epidermidis* (-5.5 kcal/mol)

C: Cloxacillin bound to *N. sicca* (-7.2 kcal/mol) **D**: Cloxacillin bound to *B. cereus* (-5.1 kcal/mol)

Figure 5. Binding poses of top four antibiotics bound to four species of bacteria with potential interactions to amino acids displayed. A) *E. coli*; **B)** *S. epidermidis*; **C)** *N. sicca*; **D)** *B. cereus*.

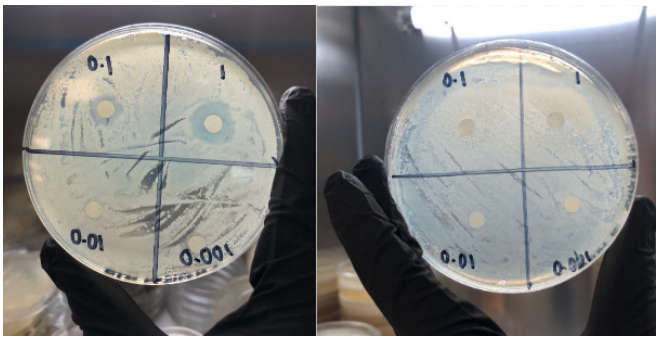


Figure 6. Petri dishes to show radii of inhibition. Petri dishes inoculated with *E. coli*, with the radii of inhibition as the result of penicillin G (left) and *B. cereus* with the radii inhibition as a result of penicillin G (right) at four different concentrations: 1 mM, 0.1 mM, 0.01 mM, and 0.001 mM. Inhibition of the bacterial growth is seen in the area around the filter paper. No inhibition is visible in the *B. cereus* Petri dish (right) whereas significant inhibition is shown in the *E. coli* Petri dish (left).

DISCUSSION

Though β-lactam antibiotics have been used as antimicrobial agents since the 19th century, there is a growing demand for new β-lactam antibiotics as more drug-resistant bacteria emerge (16). In our *in vitro* screening, we found that although the six antibiotics screened inhibit *N. sicca, E. coli,* and *S. epidermidis* bacteria, some antibiotics are more effective than others; for example, penicillin G outperformed other penicillin antibiotics in inhibition of these three bacteria species. Further, consistent with our initial hypothesis, *B. cereus* was not inhibited by any of the antibiotics.

In silico studies showed that cloxacillin had the strongest binding affinities against *N. sicca* and *E. coli*, whereas carbenicillin had the worst binding affinities on average. Strong binding affinities between penicillin G and *N. sicca*, cloxacillin and *N. sicca*, and piperacillin and *E. coli* indicate that these antibiotics might bind in a manner where the β-lactam core is well poised to engage the active site serine nucleophile. We would assume this results in a higher level of inhibition *in vitro*, as the antibiotics would be able to interact with the active site more easily. However, penicillin G and cloxacillin did not inhibit *N. sicca* at the computationally-predicted caliber as opposed to the other antibiotics. By contrast, piperacillin inhibited *E. coli* at a similar level when compared to the molecular docking studies. While binding affinities may correlate with biological activity, it is important to distinguish inhibition from simply binding. For the reaction between the serine and the carbonyl carbon of the β-lactam to occur, each antibiotic must bind in the correct orientation with enough energy because the antibiotics are classified as covalent inhibitors.

In addition, there are many factors that could occur in a biological setting that are not modeled computationally. The permeability for each compound to enter the bacteria could play a role into how they are able to affect the penicillin-binding proteins (17). If the compounds are not able to enter the cell, they will not be able to covalently inhibit the protein, or even come in contact with the proteins. In addition, the reactivity of the compounds to bind to the serine active site would affect how they are able to inhibit the penicillin-binding proteins and other transpeptidases. Lastly, although the compounds might be thermodynamically favorable, they might not be in the right binding pose to interact with the serine active site correctly, meaning they cannot inhibit the proteins (18).

Notably, penicillin G, ampicillin, and amoxicillin had the shortest distances (Å) between the carbonyl carbon and the serine nucleophile of *N. sicca*. These shorter distances correlate with the comparatively high antimicrobial activity of these antibiotics, which may be a result of the antibiotics being covalent inhibitors. The distance between the active atoms would play a larger role in reactivity than the spontaneity of the system, especially since β-lactam are covalent inhibitors, and would thus be a better value to determine projected efficacy of the antibiotics.

Various factors, such as transport and metabolism, may have contributed to the discrepancy between the *B. cereus* computational and *in vitro* screening results, as computational analyses do not effectively take these factors into account. *B. cereus* is known to possess β-lactamases which hydrolyze the core of all penicillin-type antibiotics, thereby marking these antibiotics ineffective against *B.cereus* (19). In order to further investigate the effectiveness and selectivity of the tested penicillin antibiotics, we plan on conducting the same screening against more bacterial species, which would allow us to be able to compare results between species. Moreover, the resulting SAR developed from this work potentially informs the future design and development of β-lactam antibiotics towards targeting antibiotic-resistant bacteria.

METHODS

In vitro testing

Six penicillins: amoxicillin (Vega Pharma, tech. grade), ampicillin sodium (ACTGene, 91%), penicillin G sodium salt (Sigma-Aldrich, 96.0-102.0%), cloxacillin sodium monohydrate (AK Scientific, 95%), carbenicillin disodium salt (AK Scientific, 90%), and piperacillin sodium salt (AK Scientific, 98%), were compared using ROI values acquired through a Kirby-Bauer assay.

For the Kirby-Bauer assay, live bacterial cultures of *B. cereus*, *E. coli*, *N. sicca*, and *S. epidermidis* were obtained from Carolina Biological. Overnight cultures of the bacteria were grown in falcon tubes of 10-15 mL of LB media (1% tryptone, 1% NaCl, 0.5% protein media, 97.5% water) and were kept incubated at 37 °C. Then, antibiotic solutions were prepared in four different concentrations (1 mM, 0.1 mM, 0.01 mM, 0.001 mM) in Tris buffer at pH 7.4. All antibiotics were used without further purification. A solution of deionized water was used as the negative control and no positive control was used.

Bacteria from the overnight cultures were inoculated on Petri dishes plated with Mueller-Hinton Agar (Sigma-Aldrich). Each Petri dish was first inoculated with one species of bacteria and was then split into four sections. The antibiotic solutions were administered through filter paper discs that were saturated with the solution. Four filter paper discs with the same antibiotic solution of varying concentrations were placed on quarters of the same Petri dish. Petri dishes were then incubated at 37 °C for 24-25 hours in the absence of light. This was done in three different trials, in which the cultures used were biological replicates.

Statistical analysis

Radius of inhibition measurements were measured in millimeters through the use of an electronic caliper. An average radius was calculated for three biological replicates. The sample standard deviation (s) and sample variance (s^2) were then calculated. Graph error bars were created based on standard deviation.

Because sample variance for all trials were close to 0, we were able to use unpaired t-testing. The equation used for unpaired *t*-testing is as follows: $t=(x_1-x_2)/(s^2(1/n_1+1/n_2))^{1/2}$, where $s^2=(\Sigma^{n1}_{i=1}\ (x_i-x_1)^2-\Sigma^{n2}_{j=1}(x_j-x_2)^2)/(n_1+n_2-2)$, $n1=n_1$, $n2=n_2$, and x_1 and x_2 represent sample means, s^2 represents pooled sample variance, n_1 and n_2 represent sample size and *t* is a Student T quantile. Degrees of freedom were determined by the equation n_1+n_2-2. Sample variance was calculated by the following equation. After calculating t, the *t*-distribution critical values table was used to calculate the *p*-value. Statistical significance was determined by a p-value < 0.05.

In silico

Homology modeling was conducted to generate PBPs for each of the bacterial species on UCSF Chimera. Although the amino acids that form PBPs vary between species, three motifs are conserved amongst the active site region of all PBPs: an active-site serine in an SXXK sequence, an SXN sequence, and a triad of KSG, KTG, HSG or HTG (12, 20). The motifs present in the amino acid sequence of each base protein (PDB codes: 1NZO for *E. coli*, 1W5D for *B. subtilis*, 3EQV for *N. gonorrhoeae*, and 1TVF for *S. aureus*) were found from PDB and were then compared to the motifs in the amino acid sequences of the desired protein. The *E. coli* PBP1a, *B. cereus* PBP3, and *S. epidermidis* PBP2 sequences were obtained from NCBI Nucleotide Sequence, while the *N. sicca* PBP1 was found through UniProt. Specific amino acids were then altered to match the corresponding amino acids using the rotamers tool in UCSF Chimera (21, 22). Possible interactions between amino acids were determined using the find clashes/contacts tool and were then mitigated using the minimization tool.

Density Functional Theory (DFT) calculations were performed on each antibiotic to optimize their chemical structures in solvent. Three-dimensional models of the penicillin antibiotics were visualized using Avogadro, a cross-platform molecular editor and visualizer (23). Each compound was optimized by molecular mechanics using the Merck Molecular Forcefield (MMFF94) to 10,000 steps. Input files for all compounds were created using Avogadro. DFT was used to quantum mechanically calculate the molecular geometries of all the compounds. ORCA, an *ab initio* quantum chemistry program (computational chemistry methods based on quantum chemistry taking only inputs that are physical contstants), was used to compute quantum mechanically rigorous thermodynamically optimized geometries of penicillin analogs (24). An implicit conductor-like polarizable continuum (CPCM) solvation model of water, and the hybrid functional B3LYP and 6-31G basis set were used for the calculations. All DFT calculations were carried out using normal convergence thresholds performed on a Dell Poweredge 710 server with a 24 core Intel Xeon X5660 processor @ 2.80GHz and 32GB RAM.

Molecular docking was then performed on the optimized structures using AutoDockVina (ADV), an open-source molecular docking program. AutoDockTools (ADT) was used to prepare and generate coordinate files for use with ADV (32, 33) (25). To prepare the PBPs for the docking procedure, polar hydrogens were added to the target proteins in ADT. We determined coordinates of each grid box to narrow the scope of the docking algorithm, based on where the antibiotics were bound in their crystal structures. Each ligand's .pdb file was converted in ADT to a .pdbqt file for use in ADV for docking.

Dockings were run through the command prompt using ADV with configuration files as input and 9 potential binding modes of the protein-ligand complex as output. Configuration files were prepared with the receptor and ligand files' information, the center coordinates and dimensions of the grid box, and an exhaustiveness value of 8. Results were visualized using UCSF ChimeraX, a molecular visualization program. The binding mode with the lowest binding affinity from the log output file was analyzed and its binding affinity

recorded.

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REFERENCES

1. "Biggest Threats and Data." *Centers for Disease Control and Prevention*, 28 Oct. 2020, www.cdc.gov/drugresistance/biggest-threats.html.
2. "Antibiotic Resistance." *ScienceDaily*, ScienceDaily, www.sciencedaily.com/terms/antibiotic_resistance.htm#:~:text=Antibiotic%20resistance%20is%20the%20ability,evolutionary%20stress%20on%20a%20population.
3. Fleming, Alexander. "On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ." *British Journal of Experimental Pathology,* vol. 10, no. 3, 1929, pp. 226–236.
4. Ligon, B Lee. "Penicillin: Its Discovery and Early Development." *Seminars in Pediatric Infectious Diseases* vol. 15,1 (2004): 52-7. doi:10.1053/j.spid.2004.02.001
5. Sauvage, Eric *et al*. "The Penicillin-Binding Proteins: Structure and Role in Peptidoglycan Biosynthesis." *FEMS Microbiology Reviews* vol. 32,2 (2008): 234-58. doi:10.1111/j.1574-6976.2008.00105.x
6. Knowles, J. *Accounts of Chemical Research*. 1985, Vol. 18, No. 4, 97-104
7. Tooke, Catherine L., and Philip Hinchliffe. "β-Lactamases and β-Lactamase Inhibitors in the 21st Century." *Journal of Molecular Biology*, Academic Press, 5 Apr. 2019, www.sciencedirect.com/science/article/pii/S0022283619301822.
8. Lerminiaux, Nicole A, and Andrew D S Cameron. "Horizontal transfer of Antibiotic Resistance Genes in Clinical Environments." *Canadian Journal of Microbiology* vol. 65,1 (2019): 34-44. doi:10.1139/cjm-2018-0275
9. Waxman , David J, and Jack L Strominger. "Penicillin-Binding Proteins and the Mechanism of Action of Beta-Lactam Antibiotics." *Annual Reviews*, www.annualreviews.org/doi/abs/10.1146/annurev.bi.52.070183.004141?journalCode=biochem.
10. Ford, Jeffrey, *et al*. "Resistance and the Background Conversations of Change." *Journal of Organizational Change Management*, MCB UP Ltd, 1 Apr. 2002, www.emerald.com/insight/content/doi/10.1108/09534810210422991/full/html.
11. Rolinson, G.N., and A.M. Geddes. "The 50th Anniversary of the Discovery OF 6-AMINOPENICILLANIC Acid (6-Apa)." *International Journal of Antimicrobial Agents*, Elsevier, 29 Nov. 2006, www.sciencedirect.com/science/article/abs/pii/S0924857906003797.
12. Fenselau, Catherine, *et a*l. "Identification of β-Lactamase in Antibiotic-resistant Bacillus Cereus Spores." *Applied and Environmental Microbiology* 74.3 (2008): 904-906.
13. Bounaga, Sakina, et al. "The Mechanism of Catalysis and the Inhibition of the Bacillus Cereus Zinc-Dependent β-Lactamase." *Portland Press*, Portland Press, 1 May 1998, portlandpress.com/biochemj/article-abstract/331/3/703/34381/The-mechanism-of-catalysis-and-the-inhibition-of.
14. Martin, C *et al*. "Nucleotide sequences of genes encoding penicillin-binding proteins from Streptococcus pneumoniae and Streptococcus oralis with high homology to Escherichia coli penicillin-binding proteins 1a and 1b." *Journal of Bacteriology* vol. 174,13 (1992): 4517-23. doi:10.1128/jb.174.13.4517-4523.1992
15. Hackbarth, C J *et al*. "Point mutations in Staphylococcus aureus PBP 2 gene affect penicillin-binding kinetics and are associated with resistance." *Antimicrobial Agents and Chemotherapy* vol. 39,1 (1995): 103-6. doi:10.1128/aac.39.1.103
16. Bauer, Renato A. "Covalent Inhibitors in Drug Discovery: From Accidental Discoveries to Avoided Liabilities and Designed Therapies." *Drug Discovery Today*, Elsevier Current Trends, 19 May 2015, www.sciencedirect.com/science/article/abs/pii/S135964461500183X.
17. Kong, Kok-Fai, *et al*. "Beta-Lactam Antibiotics: From Antibiosis to Resistance and Bacteriology." *Wiley Online Library*, John Wiley & Sons, Ltd, 14 Dec. 2009, onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-0463.2009.02563.x.
18. Zervosen, Astrid, *et al*. "Development of New Drugs for an Old Target - the Penicillin Binding Proteins." *MDPI*, Multidisciplinary Digital Publishing Institute, 24 Oct. 2012, www.mdpi.com/1420-3049/17/11/12478.
19. Uehara, Shota, and Shigenori Tanaka. "AutoDock-GIST: Incorporating Thermodynamics of Active-Site Water into Scoring Function for ACCURATE PROTEIN-LIGAND Docking." *MDPI*, Multidisciplinary Digital Publishing Institute, 23 Nov. 2016, www.mdpi.com/1420-3049/21/11/1604.
20. Micetich, R. G., *et al*. "β-lactamases and their inhibitors: an update." *Current Medicinal Chemistry-Anti-Infective Agents* 1.3 (2002): 193-213.
21. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. "UCSF Chimera -A Visualization System for Exploratory Research and Analysis." J. Comput. Chem. 25(13):1605-1612 (2004).
22. UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Goddard TD, Huang CC, Meng

EC, Pettersen
23. Hanwell, Marcus D., *et al.* "Avogadro: an advanced semantic chemical editor, visualization, and analysis platform." Journal of cheminformatics 4.1 (2012): 17.
24. Angeli, Celestino, *et al.* "Third-order Multireference Perturbation Theory: The n-electron Valence State Perturbation-Theory Approach." *The Journal of Chemical Physics* 124.5 (2006): 054108.
25. Trott, Oleg, and Arthur J. Olson. "AutoDock Vina: Impr 3oving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading." *Journal of Computational Chemistry* 31.2 (2010): 455-461.