



MICROBIOLOGY

Toward Enhanced Antibiotic Efficacy: Exploring the Synergistic Potential of Marine-Derived Lectins Against Human Pathogenic Bacteria

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Abstract: This study aimed to assess the combined antibacterial effect of lectins and antibiotics on *S. aureus* ATCC 25923, multidrug-resistant *E. coli* ATCC 11303 and *S. aureus* ATCC 700698. Using the checkerboard assay, we evaluated the antibacterial effects of eight lectins isolated from marine organisms combined with two common antibiotics, oxacillin and tetracycline, on three virulent bacterial strains. Initially, none of the tested lectins exhibited antibacterial effects when used individually. However, when combined with antibiotics, the lectins exhibited synergistic, additive, antagonistic, or no interaction. Overall, the tested lectins alone had no effect on the efficacy of oxacillin. On the other hand, different lectins in combination with tetracycline potentiated its antimicrobial effect. Lectins from red algae of the *Bryothamnium* genus, for example, exhibited the most significant synergistic effects, reducing the minimum inhibitory concentration (MIC) of tetracycline by up to 16 times. Lectins from the *Hypnea* genus also reduced the MIC of tetracycline. Our findings suggest that some lectins binding to complex carbohydrates containing fucosylated cores (α 1-6) are excellent candidates to boost the efficacy of some antibiotics.

Key words: Lectins, antibiotics, synergism, marine organisms.

INTRODUCTION

A major challenge in global public health in the 21st century is the emergence of microorganisms resistant to traditional antimicrobial agents (Ahmed et al. 2024, Coque et al. 2023). Multidrug-resistant bacteria (MDR) pose a critical threat to global health, worsening prognoses and increasing mortality among infected individuals (Hu et al. 2023). Currently, antimicrobial resistance results in approximately 1,27 million deaths annually, and if new treatments do not emerge, the World Health Organization (WHO) estimates that this number could reach 10

million by 2050 (Mancuso et al. 2021, Okeke et al. 2024).

Among the key resistance mechanisms known so far, the reduction of drug concentration within the cell has been a crucial point. Active efflux of drugs is a mechanism used by bacteria to resist drugs, and it is accomplished through the activation of efflux pumps, such as the MexAB-OprM efflux pump located in the mexR gene of *Pseudomonas aeruginosa*. Efflux pump activity permits the expulsion of a newly assimilated drug into the extracellular space, preventing both drug accumulation in the microorganism's cytoplasm and activation of its pharmacological

mechanism of action (Darby et al. 2023, Halawa et al. 2023).

The acquisition of resistance genes by susceptible bacteria has also proven to be an important resistance mechanism (Darby et al. 2023). Resistant bacterial strains can transfer genes capable of synthesizing resistant molecules to other bacteria through conjugation, transformation, or transduction, thus compounding the difficulty of combating bacteria that act collectively, such as those that form biofilm (McInnes et al. 2020, Ghai & Ghai 2018). Species like *Escherichia coli*, *Staphylococcus aureus*, and *S. epidermidis* are examples of biofilm-forming bacteria with high rates of antibiotic resistance (Shree et al. 2023).

Therefore, significant efforts have been made to discover new antimicrobial molecules and/or alternatives to increase the efficiency of traditional antibiotics to control the emergence of newly resistant strains. Among various molecules used for this purpose, lectins have gained considerable attention (Fonseca et al. 2022).

Lectins are versatile proteins with remarkable antimicrobial effects against bacteria, fungi, and protozoa. Carbohydrates comprise a major component of the bacterial surface, and since lectins bind to carbohydrates, they can cause damage to the cell wall and prevent the attachment of microorganisms to host cells (Fonseca et al. 2022). The antimicrobial activities of lectins encompass the prevention of invasion and infection, inhibition of growth and germination, and control of adhesion and movement of microbial cells (Fonseca et al. 2022, Silva & de Araújo 2021).

The potential of lectins against bacteria has long been known, either through the agglutination of strains or the antibacterial and antibiofilm effects triggered by these biomolecules (Duarte et al. 2022, Sousa et al.

2021, Carneiro et al. 2017). The potential of lectins to potentiate the efficacy of commonly used antibiotics when used in combinatorial therapy has already been described. Accordingly, some lectins have already demonstrated their additive/synergistic effects on different antibiotics, including tetracycline, ampicillin, and oxacillin (Duarte et al. 2023, Santos et al. 2020a, b). The mechanisms of action underlying such effects are, however, not fully understood, but thought to be related to the recognition of carbohydrates on the bacterial surface, as noted above, and possible inhibition of efflux pumps (Santos et al. 2020a). However, whether lectins would have the same effect on MDR bacteria remains to be elucidated.

Marine-derived lectins are highly relevant in the context of antimicrobial activity due to their integral role in the innate immunity of the organisms from which they are extracted. These lectins are fundamentally involved in defense mechanisms against pathogens, which underscores their potential in combating microbial infections (Ahmmed et al. 2022). Additionally, in the realm of therapeutic applications, marine lectins exhibit low immunogenicity and high specificity for complex carbohydrates (Hatakeyama & Unno 2023, Singh & Walia 2018). This combination of properties makes them particularly promising molecules for the development of novel antimicrobial treatments. Their ability to target specific glycan structures on microbial surfaces with precision not only enhances their effectiveness but also minimizes the risk of adverse immune responses, positioning marine lectins as valuable agents in the fight against infectious diseases.

Accordingly, this study aimed to assess the effect of eight lectins of marine origin combined with antibiotics on three bacterial strains: *S. aureus* ATCC 25923, multidrug-resistant *E. coli* ATCC 11303, and *S. aureus* ATCC 700698. Our

results confirm the potential of these proteins for strengthening the effect of antibiotics, especially against Gram-positive bacteria.

MATERIALS AND METHODS

Animal and algae collection

The algae *Bryothamnium seaforthii*, *B. triquetrum*, and *Meristiella echinocarpa* were collected at Pedra Rachada Beach, Paracuru, Ceará, Brazil. The sponge *Haliclona caerulea* and algae *Hypnea cervicornis*, *H. musciformis*, and *Solieria filiformis* were collected at Pacheco Beach, Caucaia, in the metropolitan region of Fortaleza, Ceará, Brazil.

Following collection, the organisms were placed in plastic bags and transported to the laboratory where they were cleaned of epiphytes and stored at -20°C until use.

All collections were authorized by the Sistema de Autorização e Informação em Biodiversidade (SISBIO ID: 33913-12). Access to the animal's genetic heritage was authorized

according to Brazilian Biodiversity Law (SISGEN ID: ACC97AD).

Lectin purification

The lectins BSL and BTL were isolated from the algae *Bryothamnium seaforthii* (Figure 1a) and *B. triquetrum* (Figure 1b), respectively, through a combination of ammonium sulfate precipitation and ion exchange chromatography, as described by (Ainouz et al. 1995).

The lectins HCA and HML were isolated from *Hypnea cervicornis* (Figure 1c) and *H. musciformis* (Figure 1d), respectively, according to pre-established methods (Nascimento et al. 2006, Nagano et al. 2002).

The lectins MeL and SfL were isolated from the algae *Meristiella echinocarpa* (Figure 1e) and *Solieria filiformis* (Figure 1f), respectively, using previously described methods (Benevides et al. 1996, Chaves et al. 2018a).

The lectins Halilectin-2 and Halilectin-3 from the sponge *H. caerulea* (Figure 1g) were isolated as previously described (Carneiro et al. 2013a, b).

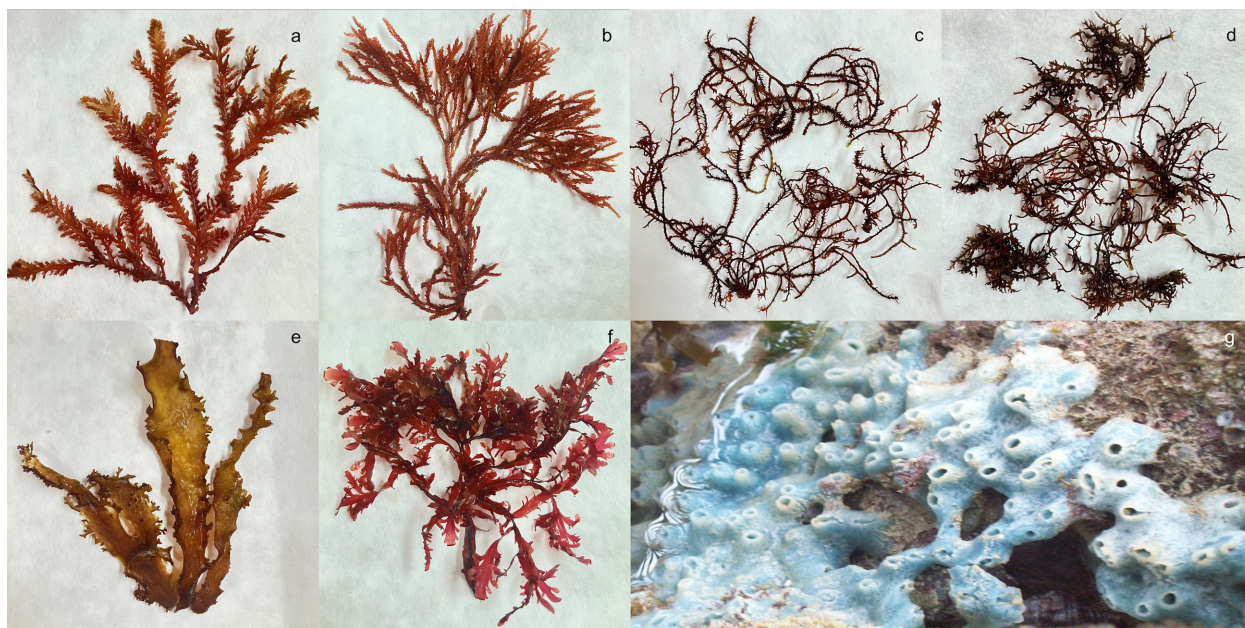


Figure 1. Marine algae and sponge used to lectin isolation. a) *Bryothamnium seaforthii*; b) *Bryothamnium triquetrum*; c) *Hypnea musciformis*; d) *Hypnea cervicornis*; e) *Meristiella echinocarpa*; f) *Solieria filiformis*; g) *Haliclona caerulea*.

Inhibition of the hemagglutination by antibiotics

To evaluate the interaction between the antibiotics Tetracycline and Oxacillin and the lectins, a hemagglutination inhibition assay was conducted. Initially, the hemagglutinating activity of the lectins was assessed using rabbit erythrocytes (Ethics Committee CEUAP 2211202101). After erythrocyte collection, the cells were washed in saline solution (0.15 M NaCl) and treated with trypsin as described by (Sampaio et al. 1998). The hemagglutinating activity was determined using the serial double dilution method, where a hemagglutination titer was defined as the inverse of the highest dilution capable of causing erythroagglutination.

Lectin solutions with 4 H.U.mL⁻¹ were then incubated for 1 hour at room temperature with varying concentrations of the antibiotics (ranging from 6.25 to 1,000 µg.mL⁻¹). Subsequently, the trypsinized erythrocyte suspension (3% v/v) was added, and hemagglutinating activity was assessed macroscopically after 1 hour.

Microorganisms and culture conditions

The following bacterial strains were used: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 700698, and *Escherichia coli* ATCC 11303. These bacteria are part of the culture collection maintained by the Laboratory of Applied Microbiology at the Integrated Laboratory of Biomolecules (LIBS), Universidade Federal do Ceará, Fortaleza, Ceará, Brazil.

The bacteria were inoculated on Tryptic Soy Agar (TSA: Himedia, India) at 37 °C for 24 hours. Isolated colonies were then inoculated in 10 mL of Tryptic Soy Broth (TSB: Himedia, India) under the same conditions as the previous step. Cells were separated by centrifugation (10 min at 9000 xg at 4 °C), suspended in TSB, adjusted to a final concentration of 1.0 x 10⁶ CFU mL⁻¹ based

on optical density at 620 nm, and stored for subsequent use.

Lectin activity on the planktonic growth of microorganisms

The effect of lectins on planktonic growth was measured using a broth microdilution assay in wells of a 96-well polystyrene plate following standards suggested by CLSI (Clinical and Laboratory Standards Institute) with some modifications. Initially, lectins were diluted in 150 mM NaCl at concentrations ranging from 7.8 to 500 µg.mL⁻¹. Subsequently, 100 µL of the protein solutions and 100 µL of bacterial cells (2.0 x 10⁶ CFU.mL⁻¹) were added to each well. The plates were incubated at 37 °C with constant agitation. Bacterial growth was evaluated by measuring turbidity through the optical density of each well, using a microplate reader (SpectraMax® I3) at a wavelength of 620 nm according to (Vasconcelos et al. 2014) with modifications.

Lectin activity combined with antibiotics

The checkerboard assay (Rosato et al. 2007) is a commonly used methodology for *in vitro* assessment of different phenotypic effects, such as synergism, additivity, antagonism or indifference, between substances with antibacterial action. The process involves multiple dilutions of two antibacterial agents at concentrations either above or below the minimum inhibitory concentrations (MICs) for the bacteria under study. The method further consists of columns containing different concentrations of substance “A” diluted along the x-axis and rows containing substance “B” along the y-axis.

To evaluate the effect(s) of lectins in combination with antibiotics, we followed (Duarte et al. 2023). To accomplish this, lectins and antibiotics were separately diluted in microdilution plates and then mixed in a new

plate containing 50 µL of the antibiotic, 50 µL of the lectin, and 100 µL of the previously adjusted bacterial inoculum. After this process, the plate was incubated for 24 hours at 37 °C. Finally, to determine the new combined inhibitory concentration (CIC), optical density was checked at a wavelength of 620 nm using a microplate reader (Spectramax®). Values equal to $\frac{1}{2} \times \text{MIC}$ were considered additive, values between $\frac{1}{4}$ and $\frac{1}{16}$ were considered synergistic.

RESULTS

Antibacterial activity

When assessed individually against the bacterial strains *S. aureus* ATCC 25923, *S. aureus* ATCC 700698, and *E. coli* ATCC 11303, the lectins alone exhibited no antibacterial effect (data not shown).

Inhibition of hemagglutination by antibiotics

All lectins evaluated exhibited hemagglutinating activity against the tested rabbit erythrocytes. However, none of the lectins had their hemagglutinating activity inhibited by the antibiotics tetracycline and oxacillin. These findings suggest that the antibiotics do not interact with the carbohydrate recognition domains of the lectins.

Effect of Lectins MeL and SfL combined with antibiotics

MeL and SfL exhibited similar effects when combined with the tested antibiotics. That is, when combined with oxacillin, the lectins showed either antagonistic effects or no interaction at all, as observed across the three tested bacterial strains (Table I).

Conversely, when MeL and SfL were combined with tetracycline, both synergistic and additive effects were observed for both lectins against *S. aureus* ATCC 25923 and *S. aureus* ATCC 700698, respectively. MeL and SfL were able to reduce the MIC of tetracycline by 8 and 4 times, respectively, against the *S. aureus* ATCC 25923 strain. Against the *E. coli* ATCC 11303 strain, MeL exhibited an additive effect, while SfL did not alter the MIC of the antibiotic.

Effect of Lectins BSL and BTL combined with antibiotics

The impact of BTL and BSL in combination with tetracycline against the tested bacterial strains was similar. Both lectins exhibited a synergistic effect, reducing the MIC of tetracycline by 16 and 4 times for *S. aureus* ATCC 25923 and *S. aureus* ATCC 700698, respectively. Against *E. coli* ATCC 11303, BTL and BSL showed an additive effect, reducing the antibiotic's MIC by 2 times (Table II).

Table I. Effect of OAAH lectins (MeL and SfL) combined with tetracycline and oxacillin against bacteria strains.

Bacteria		<i>S. aureus</i> (ATCC 25923)			<i>S. aureus</i> (ATCC 700698)			<i>E. coli</i> (ATCC 11303)		
Lectin + Antibiotic		MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio
SfL	tetracycline	0.250	0.0625	$\frac{1}{4}$ (S)	128	64	$\frac{1}{2}$ (AD)	1	0.5	$\frac{1}{2}$ (AD)
	oxacillin	0.250	0.250	1 (NE)	128	256	2 (AN)	256	512	2 (AN)
MeL	tetracycline	0.250	0.0625	$\frac{1}{4}$ (S)	128	64	$\frac{1}{2}$ (AD)	1	0.5	$\frac{1}{2}$ (AD)
	oxacillin	0.250	0.250	1 (NE)	128	256	2 (AN)	256	512	2 (AN)

MIC^a = MIC value of the antibiotic alone; MIC^b = novel MIC value of antibiotic combined to lectins; All values were expressed in µg.mL⁻¹. Combination Effects: (S) Synergistic, (AD) Additive, (AN) Antagonistic, (NE) No effect.

While BTL in combination with oxacillin exhibited a synergistic effect on *S. aureus* ATCC 25923 strain, BSL exhibited an additive effect. For the other strains, results indicated either no interaction or antagonism.

Effect of Lectins HCA and HML combined with antibiotics

When combined with the antibiotic oxacillin, HCA and HML exhibited a synergistic and additive effect, respectively, against the *S. aureus* ATCC 25923 strain. For the other strains, the lectins demonstrated either antagonistic effects or no interaction (Table III).

When these lectins were combined with tetracycline, the data reveal a synergistic effect of both lectins against *S. aureus* ATCC 25923.

While HML exhibited a synergistic effect against *S. aureus* ATCC 700698, HCA exhibited an additive effect. Both lectins exhibited an additive effect against *E. coli* ATCC 11303.

Effect of Halilectin-2 and -3 combined with antibiotics

Lectins from the sponge *H. caerulea* exhibited different effects on *S. aureus* ATCC 25923 when combined with oxacillin. Halilectin-2 demonstrated antagonism, while Halilectin-3 showed synergy. For the other strains, both lectins displayed antagonistic effects (Table IV).

In combination with tetracycline, both lectins demonstrated synergy against *S. aureus* ATCC 25923 and additive effects against the other strains.

Table II. Effect of *Bryothamnium* lectins (BSL and BTL) combined with tetracycline and oxacillin against bacteria strains.

Bacteria		<i>S. aureus</i> (ATCC 25923)			<i>S. aureus</i> (ATCC 700698)			<i>E. coli</i> (ATCC 11303)		
Lectin + Antibiotic		MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio
BSL	tetracycline	0.250	0.0156	1/16 (S)	128	32	1/4 (S)	1	0.5	1/2 (AD)
	oxacillin	0.250	0.125	1/2 (AD)	128	256	2 (AN)	256	512	2 (AN)
BTL	tetracycline	0.250	0.0156	1/16 (S)	128	32	1/4 (S)	1	0.5	1/2 (AD)
	oxacillin	0.250	0.0156	1/16 (S)	128	256	2 (AN)	256	256	1 (NE)

MIC^a = MIC value of the antibiotic alone; MIC^b = novel MIC value of antibiotic combined to lectins; All values were expressed in µg.mL⁻¹. Combination Effects: (S) Synergistic, (AD) Additive, (AN) Antagonistic, (NE) No effect.

Table III. Effect of *Hypnea* lectins (HCA and HML) combined with tetracycline and oxacillin against bacteria strains.

Bacteria		<i>S. aureus</i> (ATCC 25923)			<i>S. aureus</i> (ATCC 700698)			<i>E. coli</i> (ATCC 11303)		
Lectin + Antibiotic		MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio
HCA	tetracycline	0.250	0.0625	1/4 (S)	128	64	1/2	1	0.5	1/2 (AD)
	oxacillin	0.250	0.125	1/2 (AD)	128	256	2 (AN)	256	512	2 (AN)
HML	tetracycline	0.250	0.0156	1/16 (S)	128	32	1/4 (S)	1	0.5	1/2 (AD)
	oxacillin	0.250	0.0625	1/4 (S)	128	256	2 (AN)	256	256	1 (NE)

MIC^a = MIC value of the antibiotic alone; MIC^b = novel MIC value of antibiotic combined to lectins; All values were expressed in µg.mL⁻¹. Combination Effects: (S) Synergistic, (AD) Additive, (AN) Antagonistic, (NE) No effect.

Table IV. Effect of *Haliclona caerulea* lectins (Halilectin-2 and -3) combined with tetracycline and oxacillin against bacteria strains.

Bacteria		S. aureus (ATCC 25923)			S. aureus (ATCC 700698)			E. coli (ATCC 11303)		
Lectin + Antibiotic		MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio	MIC ^a	MIC ^b	MIC ratio
H-2	tetracycline	0.250	0.0625	1/4 (S)	128	64	1/2 (AD)	1	0.5	1/2 (AD)
	oxacillin	0.250	0.5	2 (AN)	128	256	2 (AN)	256	512	2 (AN)
H-3	tetracycline	0.250	0.0312	1/8 (S)	128	64	1/2 (AD)	1	0.5	1/2 (AD)
	oxacillin	0.250	0.125	1/2 (AD)	128	256	2 (AN)	256	512	2 (AN)

MIC^a = MIC value of the antibiotic alone; MIC^b = novel MIC value of antibiotic combined to lectins; All values were expressed in $\mu\text{g.mL}^{-1}$. Combination Effects: (S) Synergistic, (AD) Additive, (AN) Antagonistic.

DISCUSSION

In this study, we evaluated the combined effect of lectins and antibiotics against three bacterial strains: *S. aureus* ATCC 25923, the multidrug-resistant *S. aureus* ATCC 700698 and *E. coli* ATCC 11303.

The eight assessed lectins were isolated from marine organisms and exhibited diverse structural and binding characteristics. For instance, lectins MeL and SfL, isolated from red algae *Meristiella echinocarpa* and *Solieria filiformis*, respectively, belong to the OAAH family (*Oscilathoria agardhii* agglutinin homologues). Both MeL and SfL have four domains, totaling a mass of approximately 28 kDa, and recognize yeast mannan (Chaves et al. 2018a, b, 2023).

Lectins BTL and BSL share similar features in that both are proteins composed of a single polypeptide chain of 9 kDa, containing four cysteines, with an affinity restricted to complex carbohydrates containing a fucosylated core (α 1-6) (Nascimento-Neto et al. 2012, 2015, Calvete et al. 2000).

Hypnea genus lectins, HCA and HML, are 9 kDa proteins, and, like *Bryothamnium* genus lectins, they also exhibit specificity for complex carbohydrates with a fucosylated core (α 1-6),

beyond several cysteine residues (Nagano et al. 2005). However, when compared to BTL and BSL, HCA and HML have distinct amino acid sequences.

Lastly, lectins from the sponge *H. caerulea* do not share sequence similarity; instead, they have a common affinity for mucins, O-type glycoproteins (Carneiro et al. 2013a, b).

Initially, the antibacterial potential of the lectins was assessed. In these assays, it was observed that none of the tested lectins exhibited antibacterial effects when used individually, noting that only a few lectins reported in the literature typically show such effects (Sadanandan et al. 2022, Silva & de Araújo 2021, Hung & Trang 2021). Nonetheless, many lectins can recognize and agglutinate bacteria and other microorganisms, as well as demonstrate antibiofilm effects (Carneiro et al. 2017, 2019, Marques et al. 2018).

On the other hand, when combined with different antibiotics in this study, the lectins exhibited various interactions against bacteria that could be characterized as additive, synergistic, antagonistic or indifferent.

Overall, the tested lectins were unable to modulate the effect of oxacillin. Indeed, for *E. coli* ATCC 11303 and *S. aureus* ATCC 700698, all

eight lectins tested in combination with oxacillin showed either antagonism or no interaction. However, lectins BSL and HML did show slight synergism with oxacillin against *S. aureus* ATCC 25923, while BTL, HCA, and Halilectin-3 demonstrated an additive effect. The remaining lectins were either antagonistic or showed no interaction.

These findings stand in contrast to those observed by Duarte et al. (2023) who reported that the lectin from the marine sponge *Aplysina lactuca* (ALL) showed both synergistic and additive effect when combined with oxacillin against *S. aureus* ATCC 25923, *E. coli* ATCC 11303, and *S. aureus* ATCC 700698.

All lectins combined with tetracycline exhibited a synergistic effect against *S. aureus* ATCC 25923. The lectins from *Bryothamnium* and HML showed a synergistic effect against *S. aureus* ATCC 700698, while the other lectins demonstrated an additive effect. All lectins, except SfL, exhibited an additive effect against *E. coli* ATCC 11303.

These data collectively suggest that the combination of lectins with tetracycline can potentiate antimicrobial effect, even against multidrug-resistant strains and Gram-negative bacteria, which are naturally more resistant owing to the complexity of their cell walls. Interestingly, Duarte et al. (2023) observed only additive effects when combining ALL with tetracycline.

The variability in the interaction patterns between lectins and antibiotics, such as synergism or antagonism, can be attributed to several structural factors inherent to the lectins. One critical aspect is the specificity and affinity of the lectin's CRDs, lectins with similar specificity yield similar association results. Moreover, lectins with multiple CRDs or those capable of binding complex carbohydrates might facilitate the attachment and aggregation of

bacterial cells, potentially altering the bacterial cell surface properties and permeability. This can enhance or inhibit the uptake of antibiotics, leading to synergistic or antagonistic effects.

For instance, lectins like MeL and SfL, with four CRDs and high affinity for yeast mannan (Chaves et al. 2018a, 2023), might cause conformational changes in the bacterial cell wall, affecting the efficacy of certain antibiotics. Additionally, the presence of specific amino acid residues in lectin structure can influence the stability and binding interactions with antibiotics, modulating their antibacterial activity. Furthermore, the overall structural stability and flexibility of the lectins, determined by factors such as disulfide bonds in HML and HCA (Nagano et al. 2005), can also play a role in their interaction with antibiotics. These structural characteristics underscore the complex interplay between lectins and antibiotics, contributing to the observed variations in their combined antibacterial effects. Nevertheless, it is important to note that possible binding between lectins and antibiotics did not involve lectin's CRD, which remains free to bind bacterial cells.

The selected antibiotics for the assays, tetracycline and oxacillin, exhibit distinct mechanisms of action. While tetracycline acts by blocking protein synthesis, oxacillin, as a beta-lactam antibiotic, inhibits bacterial cell wall synthesis (Nguyen et al. 2014, Bozdogan & Appelbaum 2004, Rusu & Buta 2021).

Possible mechanisms for the observed synergistic and antagonistic effects could be attributed to how the structural features of the lectins interact with different antibiotics. For instance, the synergistic effects seen with tetracycline might be due to lectins enhancing the permeability of bacterial cell walls, thereby facilitating greater uptake of the antibiotic. Tetracycline works by inhibiting

protein synthesis, and increased intracellular concentrations could amplify its effectiveness.

On the other hand, the antagonistic effects observed with oxacillin might arise from competition between the lectins and the antibiotic for binding sites on the bacterial cell surface, or potential interference with the mechanism of action of oxacillin, which targets cell wall synthesis. This competition could reduce the antibiotic's efficacy, leading to antagonism.

Based on the data obtained in this study, it can be asserted that lectins binding to complex carbohydrates containing fucosylated cores ($\alpha 1-6$), such as BTL and BSL, are excellent candidates for use in combination with antibiotics. However, the synergistic effect, or lack thereof, may depend on complex interactions between the mechanisms of action of lectins and specific antibiotics yet to be elucidated in our future studies, but outside the scope of the current work.

Moreover, differences in cell wall structures among bacterial strains can influence the response to combinatorial lectin/antibiotic therapy. As suggested above, we will work toward a more precise understanding of these interactions in future studies, including specific assays to elucidate the mechanisms of action involved in different lectin/antibiotic combinations against specific bacterial strains.

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RFC compiled the data and wrote the original manuscript (Conceptualization, Writing - Review & Editing). PAST, JGSC and PAVRJ performed chromatography aiming at the accumulation of proteins for biological assays (Investigation). VPPM and AA performed microbiological assays (Investigation). MAV revised the original draft (Writing - Original Draft). EHT carried out the antibiofilm experiments (Resources). CSN carried out laboratory experiments and revised the manuscript (Resources, Supervision). AHS carried out protein purification and characterization and revised the manuscript (Resources, Supervision). All authors read and approved the final manuscript.

