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Sesquiterpene Farnesol Contributes to Increased Susceptibility to β-Lactams in Strains of *Burkholderia pseudomallei*

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This study aimed to evaluate the *in vitro* combination of farnesol and β-lactams against *Burkholderia pseudomallei*. A total of 12 β-lactamase-positive strains were tested according to CLSI standards. All strains were inhibited by farnesol, with MICs ranging from 75 to 150 μM. The combination of this compound with β-lactams resulted in statistically significant β-lactam MIC reduction ($P \leq 0.05$). This study provides new perspectives for the use of farnesol combined with β-lactam antibiotics against strains of *B. pseudomallei*.

Burkholderia pseudomallei is a Gram-negative bacillus that causes melioidosis, a severe and potentially fatal disease endemic to Southeast Asia and hyperendemic to Northern Australia (2, 17).

Some antibiotics currently recommended for the treatment of melioidosis are ceftazidime, imipenem, meropenem, amoxicillin-clavulanate, cefoperazone-sulbactam, trimethoprim-sulfamethoxazole, doxycycline, and chloramphenicol (17). However, *B. pseudomallei* has developed resistance to these drugs (14, 15, 17, 18); hence, it is necessary to search for new agents that are effective against this microorganism.

The sesquiterpene alcohol farnesol is present in many essential oils of plants, such as *Pluchea dioscoridis* and *Pittosporum undulatum*, possibly to protect against attack by predators (5, 12). Farnesol has also been detected in the supernatant of *Candida albicans* broth cultures, as it is a quorum-sensing molecule of this fungal species (11).

Moreover, farnesol is able to inhibit some microorganisms, such as *Staphylococcus aureus*, *Streptococcus mutans*, and *Paracoccidioides brasiliensis*, indicating its potential antimicrobial activity (4, 6–9), which has also been demonstrated against bacterial biofilms (16). Some studies have shown the ability of farnesol to increase the susceptibility of microorganisms to antimicrobials, indicating a possible applicability as an adjuvant drug (7). Brehm-Stecher et al. (1) reported increased susceptibility of *S. aureus* to ciprofloxacin, clindamycin, erythromycin, gentamicin, tetracycline, and vancomycin, as well as of *Escherichia coli* to polymyxin B, when these drugs were combined with farnesol. Thus, the objective of this study was to evaluate the *in vitro* activity of farnesol, alone and in combination with β-lactams, against strains of *B. pseudomallei*.

We used 12 strains of β-lactamase-producing *B. pseudomallei*, stored in the Laboratory of Emerging and Reemerging Pathogens (LAPERE) of the Federal University of Ceará. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, methicillin-resistant *Staphylococcus aureus* (MRSA), and β-lactamase-negative *S. aureus* were used as experimental controls. Susceptibility testing was performed by the broth dilution method, as standardized by CLSI, described in document M07-A8 (3). The medium used was Mueller-Hinton broth (Difco, USA), and the concentration ranges were from 0.25 to 128 mg/ml for amoxicillin (Roche, Brazil), from 0.25/0.125 to 128/64 mg/ml for amoxicillin-clavulanate (Roche, Brazil), from 0.0312 to 16 mg/ml for imipenem (Roche, Brazil), and from 2 to 1,024 mg/ml for ampicillin (Ariston, Brazil) and oxacillin (Ariston, Brazil) (3). The aminoglycosides gentamicin and amikacin (Roche, Brazil) were tested in the ranges of 0.125 to 64 mg/ml and were used as controls. For farnesol (*E,E*-farnesol; Sigma-Aldrich, Brazil), a concentration of 0.585 to 300 μM was used for all samples. The inocula were prepared with saline solution after 24 h of colony growth in Mueller-Hinton agar, and they were adjusted to 0.5 on the McFarland scale and diluted to 1:100, resulting in 5×10^5 CFU/ml. Plates were read after 24 h of incubation. The MIC was defined as the lowest concentration able to inhibit 100% of growth (3). After obtaining the MICs of the individual drugs, the antibiotics were combined with farnesol. For the combination assay, sub-MIC concentrations of farnesol and antibacterial drugs were used. The initial concentration of drugs in combination was the MIC obtained for each individual isolate. Then, successive drug dilutions were performed, reaching a final concentration that was 256 times lower than the final concentration of the drug alone. Statistical analysis was performed using Student's *t* test for paired samples, with 5% significance.

In this study, all tested *B. pseudomallei* strains were susceptible to farnesol, with MICs ranging from 75 to 150 μM. To our knowledge, the antimicrobial activity of farnesol against *B. pseudomallei* has not been reported previously, although studies have confirmed the inhibitory effect of farnesol on the growth of different microorganisms (4, 6–9, 13). Despite the high virulence of *B. pseudomallei*, the farnesol MICs found in this study are lower than those reported for other bacteria, such as *S. aureus*, which was

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TABLE 1 Increased antimicrobial susceptibility of *Burkholderia pseudomallei* and MRSA strains treated with farnesol[a]

Strain	MIC (μg/ml) without/with farnesol (fold ratio of increased susceptibility by farnesol) for:						
	AMOX	AMOX + CLA	OXA	AMP	IMI	AMI	GEN
B. pseudomallei							
03-6-033	>128/16 (>8)	8/4, 1/0.5 (8)	256/64 (4)	64/16 (4)	0.25/0.125 (2)	16/16	32/32
03-6-034	>128/16 (>8)	32/16, 0.5/0.25 (64)	256/64 (4)	128/32 (4)	0.5/0.25 (2)	32/32	32/32
03-6-035	64/16 (4)	8/4, 0.5/0.25 (16)	64/16 (4)	64/16 (4)	0.5/0.25 (2)	32/32	32/32
03-6-036	>128/16 (>8)	8/4, 0.5/0.25 (16)	128/64 (2)	128/64(2)	0.5/0.25 (2)	32/32	32/32
03-6-037	128/16 (8)	8/4, 1/0.5 (8)	128/32 (4)	128/64 (2)	0.5/0.25 (2)	32/32	32/32
03-6-038	128/16 (8)	16/8, 2/1 (8)	1,024/16 (64)	1,024/128 (8)	1/1	32/32	32/32
05-3-008	64/16 (4)	8/4, 1/0.5 (8)	1,024/16 (64)	1,024/256 (4)	0.25/0.25	16/16	16/16
05-3-009	64/16 (4)	4/2, 0.5/0.25 (8)	1,024/16 (64)	1,024/256 (4)	0.5/0.5	16/16	32/32
05-3-010	128/16 (8)	16/8, 1/0.5 (16)	256/8 (32)	256/256 (1)	0.5/0.5	64/64	64/64
03-6-039	128/32(4)	8/4, 2/1 (4)	1,024/64 (16)	1,024/256 (4)	0.25/0.25	32/32	8/8
03-6-040	>128/32 (>4)	8/4, 2/1 (4)	512/32 (16)	512/256 (2)	0.5/0.5	128/128	8/8
03-6-041	128/8 (16)	8/4, 2/1 (4)	1,024/128 (8)	1,024/512 (2)	1/1	16/16	16/16
MRSA	1,024/32(32)	32/16, 16/8 (2)	1,024/32 (32)	1,024/64 (16)	1,250/1,250		
β-Lactamase-negative *S. aureus*	2/2	0.5/0.25, 0.5/0.25	0.25/0.25	0.25/0.125 (2)	4/4		

[a] AMOX, amoxicillin; CLA, clavulanic acid; OXA, oxacillin; AMP, ampicillin; IMI, imipenem; AMI, amikacin; GEN, gentamicin.

shown to be susceptible to farnesol at concentrations equal to or greater than 1,000 μM (1).

Another important finding of this study was a statistically significant reduction in the MICs for amoxicillin ($P = 0.0001$), ampicillin ($P = 0.0026$), and oxacillin ($P = 0.0001$) when combined with farnesol, which decreased the MICs up to eight times for amoxicillin and up to three times for ampicillin and oxacillin. The triple combination of amoxicillin-clavulanate ($P = 0.0005$) and farnesol resulted in MIC reductions of up to five times. The combination of farnesol and imipenem ($P = 0.0105$) showed a more discrete reduction compared to the other β-lactams, possibly because imipenem already has strong antimicrobial activity against strains of *B. pseudomallei* and its association with other drugs contributes little to its effectiveness. In contrast, the MIC values of the aminoglycosides amikacin and gentamicin against *B. pseudomallei* did not change when they were combined with farnesol. Additionally, the combination of farnesol with β-lactams against the MRSA strains resulted in reductions of up to six times in the MICs of amoxicillin and oxacillin and up to five times in the MICs of ampicillin. On the other hand, there was no reduction in β-lactam MICs after combination with farnesol against strains of β-lactamase-negative *S. aureus* (Table 1).

Regarding the mechanism of action of farnesol against *B. pseudomallei*, these results show that it may act by inhibiting the secretion of β-lactamases or impairing their activity or by interfering with the synthesis of cell wall, as demonstrated by Kuroda et al. (10). These authors observed a lower production of β-lactamases by MRSA strains previously incubated with farnesol. Additionally, they also demonstrated the effects of farnesol on the bacterial cell wall, in which the compound interferes with the biosynthesis of peptidoglycan by inhibiting the synthesis of a lipid carrier (undecaprenyl-C55) responsible for the transport of murein, which is a peptidoglycan monomer precursor (10). Therefore, through the interference with cell wall biosynthesis, farnesol may potentiate the effect of β-lactams, requiring lower antibacterial concentrations to promote cell death.

This study provides new perspectives for the use of farnesol combined with β-lactam antibiotics against strains of *B. Pseudomallei*, but it is necessary to perform further *in vivo* studies to evaluate the effectiveness of these combinations.

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