Sesquiterpene farnesol contributing to increased susceptibility to β-lactams in strains of *Burkholderia pseudomallei* 

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**Running title:** Increased β-lactam susceptibility in *B. pseudomallei* 

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Abstract

This study aimed to evaluate the in vitro combination of farnesol with β-lactams. Two aminoglycosides were used as controls. The test was performed by standardized broth microdilution according to CLSI on 20 strains of *B. pseudomallei*, which were all inhibited by farnesol. Farnesol significantly reduced MICs for β-lactams, but not for aminoglycosides. This study opens perspectives for the use of farnesol as an inhibitor of β-lactamase in *B. pseudomallei*.

*Burkholderia pseudomallei* is a Gram-negative bacillus that causes melioidoses, a severe and usually fatal infectious disease that can be acquired by inoculation, inhalation and ingestion of the microorganism, which is distributed in the environment (2, 13).

*B. pseudomallei* is usually resistant to many antibiotics used for the treatment of melioidosis, such as ceftazidime, imipenem, meropenem, amoxixillin-clavulanate, trimethoprim-sulfamethoxazole, doxycyline and choramphenicol (17). Because of the development of resistance of *B. pseudomallei* to these antimicrobials, it is necessary to search for new effective drugs for the treatment of melioidosis (15, 18).

The sesquiterpene alcohol farnesol is present in many essential oils of plants, such as *Puchea dioscorides* and *Pittosporum undulatum*, possibly to protect against attack by predators (5, 12). Farnesol has also been detected in the supernatant of *Candida albicans*, besides being a quorum-sensing molecule of this 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 7, 8, 9). Its antimicrobial activity was also demonstrated in bacteria in biofilm (16). Some studies have shown the ability of
farnesol to increase the susceptibility of microorganisms to antimicrobials, indicating a possible adjuvant action (8). Brehm-Stecher et al. (2003) reported increased sensitivity 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.

Given the above, the objective of this study was to test the in vitro activity of farnesol, alone and in combination with β-lactam antibiotics, on strains of B. pseudomallei.

We used 20 strains of B. pseudomallei (10 environmental isolates and 10 clinical isolates), stored in the Laboratory of Emerging and Reemerging Pathogens (LAPERE) of Federal University of Ceará. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and methicillin-resistant Staphylococcus aureus (MRSA) were used as experimental controls. Susceptibility testing was performed by the broth dilution method, as standardized by the Clinical and Laboratory Standards Institute (CLSI), described in document M07-A8. The medium used was Muller-Hinton broth (Difco, USA), and the concentration ranges were from 128 to 0.25 mg/ml for amoxicillin (Roche, Brazil), 128/64-0.25/0.125mg/mL for amoxicillin-clavulanate (Roche, Brazil), 16 to 0.0312 mg/mL for imipenem (Roche, Brazil), and 1024-2 mg/mL for ampicillin (Ariston, Brazil) and oxacillin (Ariston, Brazil) (3). The aminoglycosides gentamicin and amikacin, both tested in the ranges of 64-0, 125mg/ml and purchased from Roche (Brazil), were used as controls. For farnesol (Sigma-Aldrich), a concentration of 0.585-300μM was used for all samples. The inoculums in saline solution made with 24 hours of colony growth in Muller-Hinton agar was adjusted to 0.5 on the McFarland scale of 1:100 and diluted to contain 5 x 10^5 CFU/ml cells. Reading the plates was performed after 24 hours of incubation. The MIC was defined as
the lowest concentration able to inhibit growth of 100% (3). After obtaining the MICs of the individual drugs, the antibiotics were combined with the farnesol. The initial concentrations were used in combinations of drugs and the MICs obtained for each isolated strain. Statistical analysis was performed using the Student-t test for paired samples, with significance of 5%.

To date, the antimicrobial activity of farnesol has not yet been reported in the literature for strains of *B. pseudomallei*, although studies have confirmed the inhibitory effect of farnesol on the growth of different microorganisms (4, 6, 9, 7, 8, 14). This research demonstrated sensitivity of this microorganism to this compound, with MICs ranging from 75 to 150µM. Despite the high virulence of strains of *B. pseudomallei*, the MICs found in this study are lower than those described in the literature for other bacteria, as described by Brehm-Stecher et al. (2003), where strains of *S. aureus* were shown to be sensitive to farnesol at concentrations equal to or greater than 1000 µM.

Regarding the mechanism of action of farnesol, Kuroda et al. (2007) demonstrated its effect on the bacterial cell wall, in which the compound interferes with the biosynthesis of peptidioglycan by inhibiting the synthesis of a lipid carrier (undecaprenyl-C55) responsible for the transport of murein, which is a peptidioglycan monomer precursor. Farnesol has a chemical structure similar to farnesyl pyrophosphate, an essential substrate for the synthesis of undecaprenyl (C55). Thus, farnesol through this pathway may inhibit the biosynthesis of terpenoid, interfering with bacterial cell wall biosynthesis (10). This study demonstrated MIC’s reduction the combination of farnesol with amoxillin, amoxillin-clavulanate, ampicillin and oxacillin against the strains of methicillin-resistant *S. aureus*. These combinations with farnesol
reduced the MICs up to six times for oxacillin and amoxillin and up to five times for ampicillin.

Another important finding of this study was a statistically significant reduction presented by the combination of farnesol with amoxicillin (p=0.0001), ampicillin (p=0.0026) and oxacillin (p=0.0001), since these drugs alone have high MICs against the strains of *B. pseudomallei*. These combinations with farnesol reduced 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 showed reduced MIs of up to five times. The combination of farnesol and imipenem (p = 0.0105) showed a smaller reduction compared to the other β-lactams. This result is possibly due to the fact that imipenem already has strong antimicrobial activity against strains of *B. pseudomallei*, and its association with other drugs possibly contributes little to their effectiveness. This study also demonstrated that the MIC values of the aminoglycosides amikacin and gentamicin are not changed when they are combined with farnesol. These results confirm the possible mechanism of farnesol via β-lactamase inhibition, as these antimicrobials are not sensitive to this enzyme.

These results suggest that farnesol act on a mechanism related to inhibition of β-lactamases, as evidenced by Kuroda et al. (2007), who showed that farnesol inhibited the production of β-lactamase enzyme activity in *S. aureus*. Moreover, these authors observed a lower production of β-lactamases by MRSA strains previously incubated with farnesol.

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


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Table 1. Minimum inhibitory concentration of the drugs amoxicillin, ampicillin, oxacillin, amoxicillin-clavulanate, imipenem, amikacin, and gentamicin alone and in combination with farnesol.

<table>
<thead>
<tr>
<th>Species</th>
<th>Antimicrobial</th>
<th>MIC</th>
<th>Antimicrobial</th>
<th>MIC Combination</th>
<th>1st drug</th>
<th>2nd drug</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Burkholderia pseudomallei</em> (n= 20)</td>
<td>FNZ</td>
<td>75-150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMOX</td>
<td>64-128</td>
<td>FNZ+AMOX</td>
<td>18.75-75</td>
<td>8-64</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>AMOX + CLA</td>
<td>8-4-32</td>
<td>FNZ+AMOX+CLA</td>
<td>4.8875-9.375</td>
<td>0.5-0.25</td>
<td>1-0.5</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>AMP</td>
<td>64-128</td>
<td>FNZ+AMP</td>
<td>37.5-75</td>
<td>32-64</td>
<td></td>
<td>0.0026</td>
</tr>
<tr>
<td></td>
<td>OXA</td>
<td>256-64</td>
<td>FNZ+OXA</td>
<td>37.5-75</td>
<td>16-64</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>0.25-0.5</td>
<td>FNZ+IMI</td>
<td>75-150</td>
<td>0.125-0.25</td>
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</tr>
<tr>
<td></td>
<td>AMI</td>
<td>16-128</td>
<td>FNZ+AMI</td>
<td>75-150</td>
<td>16-128</td>
<td></td>
<td>&gt;0.005</td>
</tr>
<tr>
<td></td>
<td>GEN</td>
<td>0.8-64</td>
<td>FNZ+GEN</td>
<td>75-150</td>
<td>8-64</td>
<td></td>
<td>&gt;0.005</td>
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<td><em>Methicillin-resistant Staphylococcus aureus</em></td>
<td>FNZ</td>
<td>300</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>AMOX + CLA</td>
<td>32/16</td>
<td>FNZ+AMOX+CLA</td>
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<td>16/8</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>AMP</td>
<td>1024</td>
<td>FNZ+AMP</td>
<td>37.5</td>
<td>64</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>OXA</td>
<td>1024</td>
<td>FNZ+OXA</td>
<td>9.375</td>
<td>32</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>IMI</td>
<td>1250</td>
<td>FNZ+IMI</td>
<td>300</td>
<td>1250</td>
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<tr>
<td></td>
<td>AMOX</td>
<td>1024</td>
<td>FNZ+AMOX</td>
<td>9.375</td>
<td>32</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

FNZ: Farnesol (µM); AMOX: Amoxicillin (µg/mL); CLA: Clavulanic acid (µg/mL); AMP: Ampicillin (µg/mL); OXA: Oxacillin (µg/mL); IMI: Imipenem (µg/mL); AMI: Amikacin (µg/mL); GEN: Gentamicina (µg/mL).