

Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy.

Carson CF, Mee BJ, Riley TV.

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Department of Microbiology, The University of Western Australia, Crawley.
ccarson@cyllene.uwa.edu.au

The essential oil of *Melaleuca alternifolia* (tea tree) has broad-spectrum antimicrobial activity. The mechanisms of action of tea tree oil and three of its components, 1,8-cineole, terpinen-4-ol, and alpha-terpineol, against *Staphylococcus aureus* ATCC 9144 were investigated. Treatment with these agents at their MICs and two times their MICs, particularly treatment with terpinen-4-ol and alpha-terpineol, reduced the viability of *S. aureus*. None of the agents caused lysis, as determined by measurement of the optical density at 620 nm, although cells became disproportionately sensitive to subsequent autolysis. Loss of 260-nm-absorbing material occurred after treatment with concentrations equivalent to the MIC, particularly after treatment with 1,8-cineole and alpha-terpineol. *S. aureus* organisms treated with tea tree oil or its components at the MIC or two times the MIC showed a significant loss of tolerance to NaCl. When the agents were tested at one-half the MIC, only 1,8-cineole significantly reduced the tolerance of *S. aureus* to NaCl. Electron microscopy of terpinen-4-ol-treated cells showed the formation of mesosomes and the loss of cytoplasmic contents. The predisposition to lysis, the loss of 260-nm-absorbing material, the loss of tolerance to NaCl, and the altered morphology seen by electron microscopy all suggest that tea tree oil and its components compromise the cytoplasmic membrane.