Correspondence

In-vitro activity of the essential oil of *Melaleuca alternifolia* against *Streptococcus* spp.


Sir,

The essential oil of *Melaleuca alternifolia*, or tea tree oil, is a widely used naturally occurring antimicrobial which has been recommended in the treatment of many cutaneous conditions, including acne, eczema, furunculosis, onychomycosis and tinea (Carson & Riley, 1993). The oil exhibits a broad spectrum of antimicrobial activity in vitro although its efficacy in vivo remains relatively unsubstantiated. Recently we demonstrated the antibacterial activity of tea tree oil against *Staphylococcus aureus*, both methicillin-susceptible and -resistant (Carson *et al.*, 1995a; Carson, Hammer & Riley, 1995b). *S. aureus*, along with *Streptococcus pyogenes*, are the main aetiological agents of the common childhood infection impetigo (Shriner, Schwartz & Janniger, 1995). In light of suggestions that the oil may be useful in the topical treatment of impetigo, data regarding the susceptibility of *Streptococcus* spp. were sought.

Clinical isolates of *S. pyogenes* (*n* = 15) were obtained from the Infection Control Unit of The Western Australian Centre for Pathology and Medical Research. Other streptococci (*n* = 10) were obtained from the culture collection of the Department of Microbiology, The University of Western Australia and were as follows: *Streptococcus dysgalactiae* NCTC 4669; *Streptococcus equisimilis* NCTC 5371 and ATCC 35666; *S. pyogenes* NCTC 8191, NCTC 8302, ATCC 19615 and ATCC 10389; *Streptococcus zooepidemicus* NCTC 6176; and Lancefield's group G streptococcus NCTC 9603. *S. aureus* NCTC 6571 was included in each test as a control.

Tea tree oil (batch 93/04) was obtained from Australian Plantations Pty. Ltd., Wyrallah, New South Wales, Australia. In accordance with the Australian Standard (AS 2782-1985), the terpinen-4-ol level exceeded 30% (37.1%) and the 1,8-cineole level was less than 15% (3.2%) as measured by gas liquid chromatography.

A broth microdilution method was used to determine MICs and MBCs of tea tree oil against the test organisms. All tests were performed in Todd-Hewitt broth (THB) (Unipath Ltd, Basingstoke, Hampshire, England) supplemented with 0.5% yeast extract (Unipath) and 0.001% Tween 80 detergent (Tw) (Sigma Chemical Co., St. Louis, MO, USA).

Test organisms were inoculated on to blood agar and incubated overnight at 35°C in a 5% CO$_2$ atmosphere. Serial doubling dilutions of tea tree oil in THB/Tw over the range 0.03-8.0% (v/v) were prepared in a 96-well tray (Falcon, Becton-Dickinson and Co., Lincoln Park, New Jersey, USA). Suspensions of streptococci were prepared in THB and adjusted so that the final concentration in each well following inoculation of the microtitre tray was approximately 5.0 x 10$^5$ cfu/mL. Viable counts on blood agar were used to confirm the concentration of each inoculum. Positive and negative growth controls were included in every test together with *S. aureus* NCTC 6571. Trays were incubated in a 5% CO$_2$ atmosphere at 35°C for 24 h. MICs were confirmed and MBCs established by subculture. Five µL of broth were removed from each well and spot inoculated on to blood agar (Unipath). The number of surviving organisms was determined after incubation at 35°C overnight in a 5% CO$_2$ atmosphere. The lowest concentration which resulted in the maintenance or reduction of inoculum viability was deemed the MIC, while the MBC was the concentration where 99.9% or more of the initial inoculum was killed. Tests were initially performed in duplicate and repeated if the results were inconsistent or if the MICs of the control strain varied by more than one serial dilution in either direction. Modal MIC and MBC values were selected.

For the 19 *S. pyogenes* isolates, the MIC$_{50}$ was 0.12%, while the MBC$_{50}$ was 0.25%. The most susceptible organisms were *S. dysgalactiae* and one of the *S. pyogenes* isolates with an
MIC and MBC of 0.03%. S. equi, S. equisimilis and the Lancefield’s group G streptococcus had an MIC and MBC of 0.12%. The MIC for S. zooepidemicus was 0.06% while the MBC was 0.12%.

Several reports on the in-vitro susceptibility of bacteria and fungi to tea tree oil have been published in recent years (Altman, 1988; Carson et al., 1995a), however, none has given data on the susceptibility of S. pyogenes to tea tree oil except for Altman’s reported MICs of about 1% (v/v). The MIC of 0.12% demonstrated in our study suggests that tea tree oil may be effective against streptococci when used topically as a wound disinfectant.

Although systemic treatment is usually indicated for impetigo, topical treatment may also be of value (Barnett & Frieden, 1992; Shriner et al., 1995). Mupirocin has been used successfully for this problem and offers the advantage that it is effective against both S. aureus and S. pyogenes (Barnett & Frieden, 1992). However, the development of mupirocin-resistant S. aureus poses a problem (Riley et al., 1994). Tea tree oil has already demonstrated antibacterial activity against S. aureus, MRSA and mupirocin-resistant S. aureus (Carson et al., 1995a, b) and development of resistance to the oil has not been observed. Given that tea tree oil exhibits antibacterial activity against both of the organisms in impetigo, it may prove useful in topical treatment. Careful product formulation and clinical trials are now required to evaluate the potential of this natural product.

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Anaerobic activity of levofloxacin and metronidazole separately and in combination


Sir,

The fluoroquinolone, levofloxacin, has a broad spectrum of antibacterial activity against aerobic and facultatively anaerobic microorganisms (Une et al., 1988; Fu et al., 1992; Pfäffer, Barry & Fuchs, 1993) and it has moderate activity against some anaerobic bacteria (Marshall & Jones, 1993). The variety of microorganisms that are associated with intraabdominal infections or pelvic inflammatory disease should be susceptible to levofloxacin and a second agent that is more active against the anaerobes, e.g. metronidazole.

Clinical studies to evaluate such combined therapy are being considered. We first carried out in-vitro studies to determine whether there may be some unexpected antagonism or synergism against commonly encountered anaerobic bacteria.

Microdilution susceptibility tests were performed with a broth version of Wilkins-Chalgren medium without added blood or blood products. The inocula were 8 x 10^5 to 8 x 10^6 cfu/mL and MICs were recorded after 48 h at 35°C in anaerobic jars. The test panels contained serial dilutions of levofloxacin (0.06-64 mg/L) and metronidazole (0.12-8.0 mg/L) separately and combined in a checkerboard fashion. Fractional Inhibitory Concentrations (FICs) were calculated for different drug combinations. Synergy was defined as an FIC <0.5 and combinations with FIC >4.0 were deemed antagonistic (Eliopoulos & Moellering, 1991). Bactericidal endpoints were also determined with 50 selected isolates. After MICs were recorded, the trays were shaken and two 10 μL samples were removed from each well with no visible turbidity. The samples were transferred to blood agar plates and the number of viable cells was determined. The MBCs were defined...