

## Letter to the Editor

### In Vitro Susceptibilities of Lactobacilli and Organisms Associated with Bacterial Vaginosis to *Melaleuca alternifolia* (Tea Tree) Oil

Tea tree oil, an essential oil from the Australian plant *Melaleuca alternifolia*, has broad antimicrobial activity and is incorporated into a diverse range of pharmaceutical and cosmetic products (2, 3). Blackwell (1) described a patient with typical signs and symptoms of bacterial vaginosis (BV) who treated herself with tea tree oil vaginal pessaries. After treatment, the patient was symptom free and the vaginal flora comprised predominantly gram-positive bacilli. It was suggested that the use of tea tree oil for the treatment of BV be further assessed. Therefore, the aim of this study was to evaluate the activities of tea tree oil against lactobacilli and a range of organisms associated with BV.

Reference ( $n = 9$ ) and stored clinical ( $n = 24$ ) isolates were obtained from the culture collections of the Western Australian Centre for Pathology and Medical Research (PathCentre) and the Department of Microbiology at The University of Western Australia. Recent clinical isolates ( $n = 69$ ) were recovered from vaginal swabs submitted to PathCentre and identified according to the Wadsworth Anaerobic Bacteriology Manual (5). Three isolates of *Gardnerella vaginalis* were kindly provided by Helen MacDonald of the Women's and Children's Hospital, Adelaide, Australia.

Organisms were tested against doubling dilutions of tea tree oil (batch 93/04, Australian Plantations Pty. Ltd., Wyrallah, Australia) prepared in agar or broth medium. *Bacteroides*, *Prevotella*, fusobacteria, and anaerobic gram-positive cocci were tested in supplemented brucella laked-blood agar (5), with 0.5% (vol/vol) Tween 20 added to enhance oil solubility. *G. vaginalis* and *Mobiluncus* spp. were tested in Columbia agar base supplemented with 1% Proteose Peptone 3 and 5% chocolate sheep blood. Lactobacilli ( $n = 26$ ) were tested by the broth microdilution method using de Man-Rogosa-Sharp (MRS) broth (Unipath Ltd., Basingstoke, United Kingdom). Selected BV isolates ( $n = 13$ ) were tested by a broth macrodilution method (5) using brain heart infusion broth (BHIB) supplemented with 1 µg of vitamin K and 5 µg of hemin per ml, with a final test volume of 2 ml. A final concentration of 0.001% (vol/vol) Tween 80 was added to both MRS and supplemented BHIB, to enhance oil solubility. Preparation of inocula, inoculation of susceptibility tests, incubations, and determination of MICs and/or MBCs were carried out as described elsewhere (5).

Table 1 lists MIC data obtained by the agar dilution assay. In addition, for five *G. vaginalis* isolates tea tree oil MICs were 0.06%, and for three of the four *Mobiluncus* isolates the MICs were 0.03%, with 0.06% for the fourth. By broth macrodilution, MICs for all six *Bacteroides* isolates were 0.06%. The

MBCs for five isolates were 0.06%, while the MBC for the remaining isolate was 0.12%. MICs and MBCs for two *Prevotella* isolates were 0.03%. The MICs for four of the five *Peptostreptococcus anaerobius* isolates were 0.06%, with 0.03% for the fifth. MBCs were 0.03% for one isolate, 0.06% for three isolates, and 0.12% for the remaining isolate. MICs for the lactobacilli ranged from 0.12 to 2.0% and the MIC at which 90% of isolates were inhibited (MIC<sub>90</sub>) was 2.0%. MBCs for lactobacilli ranged from 0.25 to 2.0%, and the MBC<sub>90</sub> was 2.0%.

These MIC data are similar to those obtained in previous studies which indicated that a variety of anaerobic and aerobic bacteria were susceptible to tea tree oil (2–4). The data also show that all lactobacilli tested were appreciably more resistant to tea tree oil than organisms known to be associated with BV, with at least a twofold difference in MIC<sub>90</sub> results. Therefore, the clinical success reported by Blackwell (1) may be due, in part, to the susceptibility of BV-associated organisms to tea tree oil and the relative resistance of commensal lactobacilli. This difference in susceptibility may allow formulation of products that will selectively kill or inhibit certain organisms while having a minimal effect on the commensal lactobacilli. Appropriate trials are now urgently needed to determine whether these theoretical benefits will translate into clinical practice.

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TABLE 1. Tea tree oil MICs for organisms associated with BV

Organism (no. of isolates)	MIC (% vol/vol)		
	Range	50%	90%
<i>Bacteroides</i> (12)	0.03–0.5	0.25	0.5
<i>Prevotella</i> (24)	0.03–0.25	0.12	0.25
<i>Fusobacterium</i> (10)	0.06–0.25	0.12	0.25
<i>P. anaerobius</i> (12)	0.06–0.25	0.25	0.25
Other gram-positive anaerobic cocci (12)	0.03–0.25	0.06	0.12