prevalent metronidazole resistance mechanism among anaerobic bacteria in Greece, as the respective genes were not detected in a number of metronidazole non-susceptible isolates. The possible existence of alternative resistance mechanisms has been described,[4] and this may be the situation regarding the *nim*-negative metronidazole-resistant isolates reported here.

The study additionally demonstrated the presence of *nim* genes among Gram-negative anaerobic species other than *Bacteroides* spp. and indicated differences between species, MIC distribution and *nim* gene positivity. Among fully resistant isolates (MIC ≥ 32 mg/L), *nim* genes were detected mainly in *Prevotella* spp., whereas among intermediate and susceptible isolates (MIC ≤ 16 mg/L), *nim* genes were detected only among *Bacteroides* spp. Nevertheless, as only strains isolated in a particular area of Greece were investigated, further multicentre studies are needed in order to reach a definite conclusion regarding this association.

It has been reported previously that the different expression of resistance (MIC 2 to >32 mg/L) among *nim*-positive isolates may be attributed to the presence of IS elements.[3,6] In our study, IS elements were detected in two *nim*-positive isolates, which were both metronidazole non-resistant (one intermediate and one susceptible). Overall, two of the three *nim*-positive *Bacteroides* spp. were IS-positive, whereas all five *nim*-positive strains belonging to species other than *Bacteroides* spp. were IS negative. In that respect a possible species- or genus-specific resistance mechanism may exist, as other studies also have reported the presence of IS elements only among *Bacteroides* spp. isolates.[3,6] An unknown IS element, or involvement of a different *nim* gene activation pathway, as previously described,[4] may explain the situation regarding the rest of the *nim*-positive IS-negative isolates. Notwithstanding the small sample size, IS specificity regarding *nim* gene type was according to previous reports, as IS*1169* was found in a *nimD*-positive strain and IS*1168* in a *nimA*-positive strain.[7]

In conclusion, the present study confirmed the dissemination of *nim* genes among both susceptible and resistant isolates belonging to a wide range of Gram-negative anaerobic bacterial species, other than *Bacteroides* spp., and also indicated possible differences between species, MIC distribution, *nim* gene and IS element positivity, differences that need further evaluation. In that respect, the importance of periodic susceptibility testing of anaerobic bacteria is emphasized, coupled with continuous surveillance of the respective molecular resistance mechanisms.

Acknowledgements

Active contribution of the following members of the 'Hellenic Study Group for Gram Negative Anaerobic Bacteria' is acknowledged: Prof. N. J. Legakis (National and Kapodistrian University of Athens, Medical School), Dr E. Papafrangas and Dr M. Kanellopoulou ('Sismanoglion' General Hospital), Dr C. Koutsia-Karouzou and Dr D. Kairis ('Asklepeion' General Hospital), Dr C. Trikka-Graphakos ('Thriassion' General Hospital), Dr C. H. Kontou-Castellanou ('Hippokration' General Hospital), Dr H. Malamou-Ladas ('G. Gennimatas' General Hospital), Dr M. Foustoukou and Dr E. Lebessi ('P. & A. Kyriakou' Children Hospital), Dr A. Vogiatzi ('Penteli' Children Hospital) and Ass. Prof. L. Zerva ('Attikon' University General Hospital).

This study was co-funded by the European Social Fund and National Resources (grant EPEAEK II–PYTHAGORAS II).

Transparency declarations

None to declare.

References

1. Jousimies-Somer HR, Summanen P, Citron DM *et al.* Wadsworth—KTL Anaerobic Bacteriology Manual, 6th edn. Belmont, CA: Star Publishing Company, 2002.

2. Hedberg M, Nord CE. Antimicrobial susceptibility of *Bacteroides fragilis* group isolates in Europe. *Clin Microbiol Infect* 2003; **9**: 475–88.

3. Gal M, Brazier JS. Metronidazole resistance in *Bacteroides* spp. carrying *nim* genes and the selection of slow-growing metronidazole-resistant mutants. *J Antimicrob Chemother* 2004; **54**: 109–16.

4. Diniz CG, Farias LM, Carvalho MA *et al.* Differential gene expression in a *Bacteroides fragilis* metronidazole-resistant mutant. *J Antimicrob Chemother* 2004; **54**: 100–8.

5. National Committee for Clinical Laboratory Standards. *Methods for Antimicrobial Testing of Anaerobic Bacteria—Sixth Edition: Approved standard M11-A6*. NCCLS, Wayne, PA, 2004.

6. Löfmark S, Fang H, Hedberg M *et al.* Inducible metronidazole resistance and *nim* genes in clinical *Bacteroides fragilis* group isolates. *Antimicrob Agents Chemother* 2005; **49**: 1253–6.

7. Soki J, Gal M, Brazier J *et al.* Molecular investigation of genetic elements contributing to metronidazole resistance in *Bacteroides* strains. *J Antimicrob Chemother* 2006; **57**: 212–20.

Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkl269
Advance Access publication 20 June 2006

In vitro antimycoplasmal activity of *Melaleuca alternifolia* essential oil

Pio Maria Furneri[1]*, Donatella Paolino[2], Antonella Saija[3], Andrena Marino[3] and Giuseppe Bisignano[3]

[1]*Department of Microbiological Sciences and Gynaecological Sciences, University of Catania, Via Androne 81, 91524 Catania, Italy;* [2]*Department of Pharmacobiological Sciences, University 'Magna Grecia' of Catanzaro, Viale Europa, I-88100 Catanzaro, Italy;* [3]*Department of Pharmacobiology, University of Messina, Viale Annunziata, 98100 Messina, Italy*

Keywords: tea tree oil, TTO, *Mycoplasma*, susceptibility tests

*Corresponding author. Tel: +39-095316038; Fax: +39-095312798; E-mail: furneri@unict.it

Sir,

The essential oil of *Melaleuca alternifolia* [i.e. tea tree oil (TTO)] has a long history of use as a topical antiseptic and has been used in Australia as an antiseptic since the 1920s. It is currently enjoying resurgent popularity and it is widely available in various formulations suitable for topical use.[1]

Table 1. Susceptibilities of *M. hominis, M. fermentans* and *M. pneumoniae* to TTO

Microorganism (no. of strains)	MIC (% v/v)		
	50% of strains	90% of strains	range
M. hominis (26)	0.12	0.12	0.06–0.12
M. fermentans (6)	0.03	0.06	0.01–0.06
M. pneumoniae (2)	–	–	0.01

TTO has a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, both aerobic and anaerobic, against yeasts and fungi. It is also active against clinically isolated fluconazole-resistant *Candida* strains. The mechanism of the cytotoxic effect of the TTO against bacteria and yeasts has been reviewed recently.[1] In particular, it was demonstrated that the exposure of *Escherichia coli, Staphylococcus aureus* and *Candida albicans* to minimum inhibitory and minimum bactericidal/fungicidal concentrations of TTO inhibits respiration and increases the permeability of bacterial cytoplasmic and yeast plasma membranes; in the case of *E. coli* and *S. aureus*, TTO also caused potassium ion leakage. The observed differences in the susceptibility of the microorganisms to TTO may be interpreted in terms of variations in the rate of monoterpene penetration through the cell wall and cell membrane structures. The antimicrobial activity of TTO has been principally attributed to terpinen-4-ol.[1] Finally, TTO is relatively non-toxic when applied topically, even though cases of allergic contact dermatitis have been reported.[2]

The objective of the study described here was to determine the *in vitro* susceptibilities of *Mycoplasma pneumoniae, Mycoplasma hominis* and *Mycoplasma fermentans* to TTO.

TTO employed in our experiments was provided by Australian Botanical Products (Hallam, Australia). The oil complied with ISO 4730–1996.[3] When analysed by gas chromatography and gas chromatography–mass spectrometry, the oil appeared to be characterized by a high proportion of terpinen-4-ol (36.71%) and γ-terpinene (22.20%), and moderate levels of 1,8-cineole (2.49%), p-cymene (2.52%), α-terpinene (10.10%) and terpinolene (3.53%).[4] Stock solutions of TTO were prepared in fetal calf serum (Biokrom by Bio-Spa, Milan, Italy).

Twenty-five low-passage clinically isolated strains of *M. hominis* (from vagina, urethra and cervix) and one reference strain (PG 21) of *M. hominis*, one clinically isolated strain of *M. pneumoniae* and one reference strain (FH) of *M. pneumoniae*, four low-passage strains (from vagina) and two reference strains (PG18 and K7) of *M. fermentans* were investigated. Mycoplasmas were grown in SP-4 broth[5] and maintained frozen (–80°C) until used. The presence of arginine in SP-4 broth was avoided for cultivation of *M. fermentans*; the pH of SP-4 medium was decreased to 6.0 when used with *M. hominis*.

The MIC was determined by a broth microdilution assay as described previously.[6]

The MIC was defined as the lowest concentration of the essential oil that inhibited a colour change in the broth by a given strain of mycoplasma at the time when the colour of the control tube changed, that is when the pH of the medium decreased from 7.5 to 7.0 (*M. pneumoniae* and *M. fermentans*) or increased from 6.0 to 6.5 (*M. hominis*). The required incubation times were 24–48 h for *M. hominis* and *M. fermentans* and 3–5 days for *M. pneumoniae*. Further incubations were not carried out. Standard control procedures were done as previously described.[6] Moreover, *S. aureus* ATCC 29213 was included as control; the MIC of the essential oil obtained in Mueller–Hinton broth (Beckton Dickinson & C., Sparks, MD) (MH) was compared with that obtained in SP-4.

The results of the *in vitro* susceptibility tests are given in Table 1. TTO inhibited mycoplasmas at concentrations from 0.01% to 0.12% (v/v). *M. pneumoniae* showed MIC values of 0.01% (v/v). *M. fermentans*, showed MIC_{90} values of 0.06% (v/v). *M. hominis* was the least susceptible with MIC_{90} values of 0.12% (v/v). As regards reproducibility no variations among the results of MIC values were observed in the separate assays. *S. aureus* retained its susceptibility to TTO in both MH broth and SP-4 broth (MIC 0.12% v/v).

TTO has broad antimicrobial activity and is incorporated into a diverse range of pharmaceutical and cosmetic products. Therefore, TTO and products containing the oil have been evaluated *in vivo* for the treatment of superficial fungal infections such as onychomycosis and oral candidiasis, with some favourable clinical outcomes. It is difficult to explain the observed differences of MIC values among mycoplasmas, though differences in the susceptibility might be interpreted in terms of variations in the rate of monoterpene penetration through cell membranes; furthermore, the cytotoxic activity might be impaired by the fact that a more rapid growth accounts for a minor time of contact with cell membranes.[1]

Appropriate studies are now needed to determine whether this *in vitro* activity will translate into *in vivo* activity and also to clarify the mechanism of antimycoplasmal activity, given that mycoplasmas could be an interesting tool in order to characterize the interaction of TTO with bacterial membranes.

Transparency declarations

None to declare.

References

1. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev* 2006; **19**: 50–62.

2. Fritz TM, Burg G, Krasovec M. Allergic contact dermatitis to cosmetics containing *Melaleuca alternifolia* (tea tree oil). *Ann Dermatol Venereol* 2001; **128**: 123–6.

3. Shellie R, Marriott P, Zappia G *et al.* Interactive use of linear retention indices on polar and apolar columns with an MS-library for reliable characterisation of Australian tea tree and other *Melaleuca* sp. oils. *J Essent Oil Res* 2003; **15**: 305–12.

4. International Organisation for Standardisation. *Essential Oils—Oil of Melaleuca, terpinen-4-ol type (tea tree oil).* ISO-4730. Geneva, Switzerland: ISO, 1996.

5. Tully JG, Rose DL, Whitcomb RF *et al.* Enhanced isolation of *Mycoplasma pneumoniae* from throat washings with a newly-modified culture medium. *J Infect Dis* 1979; **139**: 478–82.

6. Furneri PM, Piperno A, Sajia A *et al.* Antimycoplasmal activity of hydroxytyrosol. *Antimicrob Agents Chemother* 2004; **48**: 4892–4.