*Journal of Medical Microbiology* (2006), **55**, 1375–1380 **DOI** 10.1099/jmm.0.46558-0

# *In vitro* activity of tea-tree oil against clinical skin isolates of meticillin-resistant and -sensitive *Staphylococcus aureus* and coagulase-negative staphylococci growing planktonically and as biofilms

Aaron Brady,[1] Ryan Loughlin,[1] Deirdre Gilpin,[1] Paddy Kearney[2] and Michael Tunney[1]

[1]Clinical and Practice Research Group, School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK

[2]Microbiology Department, United Hospitals Trust, Antrim, BT41, UK

Correspondence
Michael Tunney
m.tunney@qub.ac.uk

The susceptibility of *Staphylococcus aureus* [meticillin-resistant (MRSA) and meticillin-sensitive (MSSA)] and coagulase-negative staphylococci (CoNS), which respectively form part of the transient and commensal skin flora, to tea-tree oil (TTO) was compared using broth microdilution and quantitative *in vitro* time–kill test methods. MRSA and MSSA isolates were significantly less susceptible than CoNS isolates, as measured by both MIC and minimum bactericidal concentration. A significant decrease in the mean viable count of all isolates in comparison with the control was seen at each time interval in time–kill assays. However, the only significant difference in the overall mean $\log_{10}$ reduction in viable count between the groups of isolates was between CoNS and MSSA at 3 h, with CoNS isolates demonstrating a significantly lower mean reduction. To provide a better simulation of *in vivo* conditions on the skin, where bacteria are reported to grow as microcolonies encased in glycocalyx, the bactericidal activity of TTO against isolates grown as biofilms was also compared. Biofilms formed by MSSA and MRSA isolates were completely eradicated following exposure to 5 % TTO for 1 h. In contrast, of the biofilms formed by the nine CoNS isolates tested, only five were completely killed, although a reduction in viable count was apparent for the other four isolates. These results suggest that TTO exerts a greater bactericidal activity against biofilm-grown MRSA and MSSA isolates than against some biofilm-grown CoNS isolates.

Received 8 February 2006
Accepted 19 June 2006

## INTRODUCTION

Meticillin-resistant *Staphylococcus aureus* (MRSA) is recognized as a major nosocomial pathogen that has caused problems in hospitals worldwide, with the UK having one of the highest rates of MRSA in Europe (Johnson *et al.*, 2005). By far the most important reservoir for MRSA, and hence the most important source for spread and subsequent infection, is patients who may be colonized without evidence of infection (Mulligan *et al.*, 1993). The usual sites of MRSA colonization are areas of broken skin, the anterior nares, the groin and the axillae, with MRSA infections occurring most frequently in areas of broken skin and in the bloodstream (Diekema *et al.*, 2004). It is common practice to attempt to clear MRSA colonization and infection in hospital patients with topical antimicrobials and antiseptics; mupirocin and chlorhexidine, for example, are currently employed as part of standard hospital MRSA decolonization protocols (Boyce, 2001). However, resistance to these agents is increasing, with a marked increase in antibiotic resistance recently reported for bacterial strains isolated from superficial skin wounds and leg ulcers (Colsky *et al.*, 1998; Valencia *et al.*, 2004). Alternative agents for MRSA decolonization are therefore required.

Tea-tree oil (TTO), the essential oil of *Melaleuca alternifolia*, has been suggested as a potential agent for MRSA decolonization, as it has been shown to be an effective broad-spectrum antimicrobial with good activity *in vitro* against a variety of bacteria including MRSA (Gustafson *et al.*, 1998; Hammer *et al.*, 2003; Shapiro *et al.*, 1994). Furthermore, Hammer *et al.* (1996) showed that bacteria

Abbreviations: CoNS, coagulase-negative staphylococci; ISB, IsoSensitest broth; MBC, minimum bactericidal concentration; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide; TTO, tea-tree oil.

such as *S. aureus* that transiently colonize the skin were more susceptible to TTO than bacteria such as coagulase-negative staphylococci (CoNS), which are regarded as part of the normal commensal skin flora. They suggested, therefore, that TTO could be useful for removing transient skin flora while suppressing but still maintaining the resident flora, which acts as a natural defence against colonization by other pathogenic bacteria. However, as they did not compare the activity of TTO against clinical skin isolates of MRSA and CoNS, no definitive evidence was provided to show that TTO could be used for the selective decolonization of MRSA from the skin. Therefore, in this study, we compared the activity of TTO against planktonically grown clinical skin isolates of MRSA, meticillin-sensitive *S. aureus* (MSSA) and CoNS using both a modified broth microdilution method and a quantitative *in vitro* time–kill test method. Moreover, as skin bacteria are reported to grow as microcolonies encased in glycocalyx on the skin (Edwards & Harding, 2004; Marples, 1994), they may exhibit the biofilm property of decreased antimicrobial susceptibility *in situ*. Therefore, as the activity of TTO against bacteria grown as biofilms has not been studied and to provide a better simulation of the *in situ* conditions on the skin, we also compared the bactericidal activity of in-use concentrations of TTO against clinical skin isolates of MRSA, MSSA and CoNS grown as biofilms.

## METHODS

**Micro-organisms.** Thirty MRSA and 25 MSSA clinical isolates cultured from patient samples were provided by the Microbiology Department, United Hospitals Trust, Antrim, UK. A further 28 clinical CoNS isolates [*Staphylococcus epidermidis* (12), *Staphylococcus capitis* (8), *Staphylococcus lugdunensis* (3), *Staphylococcus hominis* (2), *Staphylococcus auricularis* (1), *Staphylococcus lentus* (1) and *Staphylococcus warneri* (1)] cultured from skin samples taken from patients undergoing spinal surgery were provided by the Department of Microbiology and Immunobiology, School of Medicine, Queen's University Belfast, UK. All isolates were stored on preserver beads at −70 °C and subcultured on Mueller–Hinton agar slopes before testing. All isolates had been identified initially using a range of conventional microbiological techniques. Identification of MRSA and MSSA isolates was confirmed using a multiplex PCR, based on a previously described method (Strommenger *et al.*, 2003), with primers directed against the 16S rRNA, *nuc* and *mecA* genes. The identity of each of the CoNS isolates was confirmed by amplification of the 16S rRNA gene using primers UFPL and URPL as described previously (LiPuma *et al.*, 1999). Following sequencing, nucleotide sequences were compared with previously published sequences using BLAST (Altschul *et al.*, 1997).

**Susceptibility testing.** The MIC of TTO (European pharmacopaeia grade; G. R. Lane Health Products) for all isolates was determined by the broth microdilution method according to the British Society for Antimicrobial Chemotherapy guidelines (Andrews, 2001). Serial twofold dilutions of TTO in IsoSensitest broth (ISB) were prepared in 96-well microtitre trays over the range 0·125–8 % (v/v). To enhance oil solubility, Tween 80 was included in all assays at a final concentration of 0·25 % (v/v) after inoculation. To overcome the problem of turbidity due to the solubilized oil, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), a tetrazolium salt, which is reduced by metabolically active cells to a coloured, water-soluble formazan derivative, was added to the ISB to allow visual identification of metabolic activity. The final concentration of MTT after inoculation was 0·005 % (v/v). Final test volumes of 75 µl were dispensed into microdilution wells.

The inoculum to be tested was prepared by adjusting the turbidity of an actively growing broth culture in ISB to an optical density at 550 nm equivalent to $1 \times 10^8$ c.f.u. ml$^{-1}$. The suspension was diluted further to provide a final inoculum density of $2 \times 10^5$ c.f.u. ml$^{-1}$ in ISB, which was verified by a total viable count. The final inoculum (75 µl) was then added to each well of the microdilution trays, which were incubated aerobically for 24 h at either 37 °C (MSSA and CoNS) or 30 °C (MRSA). Positive and negative growth controls were included in each assay. After incubation, growth was indicated by the development of a red colour and therefore the MIC was read as the lowest concentration of TTO at which no colour change occurred. Determination of the MIC for each isolate was carried out in triplicate and the results were taken where there was agreement in at least two out of three MIC results. After determination of the MICs, minimum bactericidal concentrations (MBCs) were determined by spreading 10 µl suspension from wells showing no growth on to IsoSensitest agar plates, which were then incubated as described previously and examined for 99·9 % killing. The results were used to determine the concentration of TTO required to inhibit 50 and 90 % of the isolates ($MIC_{50}$ and $MIC_{90}$, respectively) and to kill 50 and 90 % of the isolates ($MBC_{50}$ and $MBC_{90}$, respectively).

**Time–kill studies.** Time–kill studies were performed for ten MRSA, ten MSSA and 11 CoNS isolates [*S. epidermidis* (6), *S. capitis* (2), *S. lugdunensis* (1), *S. hominis* (1) and *S. warneri* (1)] in McCartney bottles using a method based on the European Standard quantitative suspension test (European Standard EN 1040; European Committee for Standardization, 1997). An initial inoculum of $5 \times 10^8$ c.f.u. ml$^{-1}$ was prepared as described previously for each isolate for use in time–kill studies by diluting an actively growing culture in ISB with the inoculum used for each isolate verified by a total viable count. Samples (1 ml) of the initial inoculum were then added to 9 ml sterile water containing either TTO and Tween 80 (test) or Tween 80 only (control). The final concentrations of TTO and Tween 80 were 5 and 0·5 %, respectively. The McCartney bottles for all isolates were shaken (100 r.p.m.) at 37 °C and samples (1 ml) were taken in triplicate at 0, 1, 2 and 3 h and serial tenfold dilutions were made and plated on IsoSensitest agar. The total viable count was determined after overnight incubation at 37 °C.

**Biofilm susceptibility.** Biofilm susceptibility tests were performed for 12 MRSA, ten MSSA and nine CoNS isolates [*S. epidermidis* (5), *S. capitis* (2), *S. lugdunensis* (1) and *S. warneri* (1)]. An initial inoculum of $5 \times 10^9$ c.f.u. ml$^{-1}$ was prepared for each isolate for use in biofilm susceptibility studies by diluting an actively growing culture in ISB as described above. Samples of the initial inoculum (0·05 ml) for each isolate were inoculated on to the surface of six glass discs, which were dried at 37 °C in an incubator for 1 h. After the discs had been washed gently with sterile PBS to remove any non-adherent bacteria, they were placed in two sterile Petri dishes (three discs per dish) containing 20 ml ISB and incubated at 37 °C for 24 h. After gently washing with sterile PBS to remove any non-adherent bacteria, the discs were transferred to separate conical flasks containing 10 ml of test (5 % TTO with 0·5 % Tween 80 in sterile distilled water) or control (0·5 % Tween 80 in sterile distilled water) suspension. The flasks were shaken (100 r.p.m.) at 37 °C in an orbital incubator for 1 h. Following incubation, the discs were washed and placed in 5 ml PBS in sterile McCartney bottles and bacteria retained on the surface were dislodged by mild ultrasonication (5 min) in a 150 W ultrasonic bath operating at a nominal frequency of 50 Hz, followed by rapid vortex mixing (30 s). Serial tenfold dilutions were performed and total viable counts determined as

described above for the time–kill studies. All experiments were performed in triplicate with three discs tested with TTO suspension and three discs tested with control suspension for each isolate.

**Statistical analysis.** Statistical analysis (Kruskal–Wallis, Mann–Whitney and Bonferroni tests) was performed with the SPSS software package, with $P$ values of $<0{\cdot}05$ considered to be significant.

## RESULTS AND DISCUSSION

Eradication of MRSA from the skin and nose of colonized and infected patients is becoming increasingly important as a strategy to prevent the potentially devastating consequences that arise from uncontrolled spread of MRSA in hospitals. With resistance to routinely used topical antibiotics and antiseptics increasing (Colsky *et al.*, 1998; Valencia *et al.*, 2004), TTO has been suggested as an alternative topical decolonization agent (Carson & Riley, 2001; Carson *et al.*, 1998, 2006; Hammer *et al.*, 1996). It is recognized that two distinct bacterial populations exist on the skin surface, namely resident and transient bacteria. The resident bacteria, with CoNS such as *S. epidermidis* predominating, multiply and persist on the skin of healthy individuals without causing infection (Kloos & Musselwhite, 1975; Leeming *et al.*, 1984). Transient bacteria such as *S. aureus*, which are thought to be deposited on the skin surface from environmental sources or from reservoirs within the body, are present in smaller numbers and it is generally presumed that they are unable to multiply on healthy skin and become part of the resident flora (Higaki *et al.*, 2000). As previous reports have suggested that bacteria such as *S. aureus* that transiently colonize the skin are more susceptible to TTO than bacteria such as CoNS, which are regarded as part of the resident skin flora (Chan & Loudon, 1998; Hammer *et al.*, 1996), we compared the susceptibility to TTO of MRSA, MSSA and CoNS isolates cultured from the skin of patients.

The MIC and MBC results for all of the isolates tested are shown in Table 1. Although there was isolate-to-isolate variation in MICs within each of the groups tested, the MIC ranges recorded were narrow (0·25–2, 0·5–2 and 0·25–1 for MRSA, MSSA and CoNS isolates, respectively). Similarly, although isolate-to-isolate variation in MBCs within each of the groups tested was apparent, the MBC ranges recorded were also narrow (2–8, 2–8 and 0·5–4 for MRSA, MSSA and CoNS isolates, respectively). The isolates we tested were not, therefore, as susceptible to TTO as others have described previously using a similar method, where $MIC_{90}$ values of 0·25 % were reported (Carson *et al.*, 1995; Chan & Loudon, 1998; Elsom & Hide, 1999; Nelson, 1997). Similarly, the $MBC_{90}$ value of 4 % was higher than the value of 0·5 % reported in these earlier studies. However, our results were similar to those reported by Banes-Marshall *et al.* (2001), who reported an MIC range of 2–4 and an MBC of 4 for MRSA isolates. Statistical analysis of the results using the Kruskal–Wallis test revealed significant differences in both the MIC and the MBC results between the groups of isolates. Further analysis of the results, using the Mann–Whitney and Bonferonni tests, revealed that the MRSA and MSSA isolates were significantly less susceptible than the CoNS isolates to both the bacteriostatic and bactericidal properties of TTO as measured by MIC and MBC, respectively. However, there was no significant difference in TTO susceptibility between MRSA and MSSA isolates. This finding is in contrast to the results of the study by Banes-Marshall *et al.* (2001), who reported that MSSA were more susceptible to TTO than MRSA. However, in comparison with our study in which the susceptibility of 30 MRSA and 25 MSSA isolates was determined, Banes-Marshall *et al.* (2001) only compared the susceptibility of six MRSA and ten MSSA isolates and performed no statistical analysis to establish whether the differences highlighted were significant.

The bactericidal activity of TTO against the three groups of isolates was also determined using a time–kill assay, as this method, unlike an MIC/MBC assay, allows determination of how quickly an agent acts on an organism. A TTO concentration of 5 % was chosen for the time–kill assay to reflect the TTO concentration found in many commercially available products. Time–kill data are shown in Table 2, with the antimicrobial effect of 5 % TTO reported as the mean $\log_{10}$ reduction in viable count for all isolates in each group in comparison with the control. A significant decrease in mean viable count of MRSA, MSSA and CoNS isolates was seen at each time interval in comparison with the control. At each time point, CoNS isolates demonstrated the smallest reduction in viable count, followed by MRSA isolates, with MSSA isolates demonstrating the greatest reduction in viable count. However, statistical analysis of the results revealed that there was no significant difference in the

**Table 1.** MICs and MBCs of TTO against MRSA, MSSA and CoNS isolates

| Bacteria ($n$) | MIC (%, v/v) | | | MBC (%, v/v) | | |
|---|---|---|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ | Range | $MBC_{50}$ | $MBC_{90}$ |
| MRSA (30) | 0·25–2 | 0·5 | 2 | 2–8 | 4 | 4 |
| MSSA (25) | 0·5–2 | 1 | 2 | 2–8 | 4 | 4 |
| CoNS (28) | 0·25–1 | 0·5 | 1 | 0·5–4 | 2 | 4 |

**Table 2.** $Log_{10}$ reduction in viable count of MRSA, MSSA and CoNS isolates in suspension following challenge with 5 % TTO

Values are the $\log_{10}$ reduction in c.f.u. $ml^{-1}$ after treatment [mean ($\pm$SD)] at the time points indicated.

| Bacteria ($n$) | 1 h | 2 h | 3 h |
|---|---|---|---|
| MRSA (10) | 2·54 ($\pm$1·56) | 3·69 ($\pm$2·33) | 4·59 ($\pm$2·54) |
| MSSA (10) | 3·59 ($\pm$2·34) | 5·27 ($\pm$2·54) | 6·78 ($\pm$2·84) |
| CoNS (11) | 2·70 ($\pm$2·76) | 3·57 ($\pm$3·14) | 3·96 ($\pm$3·17) |

mean reduction in viable count between the isolate groups with the exception of CoNS and MSSA at 3 h, where there was a significantly lower mean reduction in the viable count for CoNS isolates. These results suggest that TTO at a concentration of 5 % does not display any particular antimicrobial specificity in relation to the different *Staphylococcus* species tested. Our results contrast with those reported in a small study by May *et al.* (2000), who also compared the bactericidal activity of TTO against two MRSA and two MSSA isolates using time–kill assays and found that the MRSA isolates were killed more slowly than the MSSA isolates. However, our results for TTO do concur with those reported previously for chlorhexidine by Cookson *et al.* (1991), who found that MRSA isolates were as susceptible as MSSA isolates to the bactericidal action of chlorhexidine. Similarly, Haley *et al.* (1985) demonstrated that the bactericidal activity of both chlorhexidine and povidone-iodine against MRSA and MSSA isolates was similar.

A number of reports have suggested that resident skin bacteria grow as microcolonies encased in glycocalyx (Edwards & Harding, 2004; Marples, 1994) and may exhibit the biofilm property of decreased antimicrobial susceptibility. However, most studies investigating the bactericidal properties of TTO, and indeed other biocides, have been carried out using suspension tests and therefore are not truly representative of the situation *in vivo*. In an attempt to provide a better simulation of conditions *in vivo* on the skin, we also compared the bactericidal activity of TTO against the clinical skin isolates growing in biofilms. Similar to the time–kill assays, a TTO concentration of 5 % was used to

**Table 3.** Effect of treatment with 5 % TTO on biofilm-grown MRSA, MSSA and CoNS isolates

Values are numbers of adherent bacteria (c.f.u. $cm^{-2}$) following treatment [mean ($\pm$SD)].

| Isolate | 5 % TTO | Control |
|---|---|---|
| **MRSA ($n=12$)** | | |
| 180 | 0 | $1{\cdot}99\times10^{4}$ ($\pm0{\cdot}99\times10^{4}$) |
| 181 | 0 | $3{\cdot}97\times10^{3}$ ($\pm3{\cdot}36\times10^{3}$) |
| 182 | 0 | $1{\cdot}80\times10^{2}$ ($\pm1{\cdot}72\times10^{2}$) |
| 183 | 0 | $2{\cdot}84\times10^{4}$ ($\pm1{\cdot}41\times10^{4}$) |
| 184 | 0 | $2{\cdot}74\times10^{4}$ ($\pm2{\cdot}71\times10^{4}$) |
| 185 | 0 | $4{\cdot}85\times10^{5}$ ($\pm3{\cdot}71\times10^{5}$) |
| 186 | 0 | $2{\cdot}81\times10^{3}$ ($\pm2{\cdot}25\times10^{3}$) |
| 190 | 0 | $2{\cdot}39\times10^{3}$ ($\pm1{\cdot}35\times10^{3}$) |
| 193 | 0 | $5{\cdot}31\times10^{3}$ ($\pm2{\cdot}34\times10^{3}$) |
| 195 | 0 | $8{\cdot}54\times10^{3}$ ($\pm8{\cdot}71\times10^{3}$) |
| 197 | 0 | $6{\cdot}41\times10^{3}$ ($\pm6{\cdot}64\times10^{3}$) |
| 208 | 0 | $3{\cdot}22\times10^{3}$ ($\pm1{\cdot}18\times10^{3}$) |
| **MSSA ($n=10$)** | | |
| AH1 | 0 | $2{\cdot}52\times10^{6}$ ($\pm0{\cdot}87\times10^{6}$) |
| AH2 | 0 | $1{\cdot}14\times10^{5}$ ($\pm1{\cdot}36\times10^{5}$) |
| AH3 | 0 | $7{\cdot}17\times10^{5}$ ($\pm10{\cdot}1\times10^{5}$) |
| AH17 | 0 | $1{\cdot}92\times10^{3}$ ($\pm3{\cdot}24\times10^{3}$) |
| AH19 | 0 | $1{\cdot}78\times10^{5}$ ($\pm1{\cdot}02\times10^{5}$) |
| AH21 | 0 | $1{\cdot}49\times10^{5}$ ($\pm1{\cdot}32\times10^{5}$) |
| AH22 | 0 | $4{\cdot}23\times10^{5}$ ($\pm7{\cdot}20\times10^{5}$) |
| AH23 | 0 | $2{\cdot}60\times10^{5}$ ($\pm2{\cdot}67\times10^{5}$) |
| AH24 | 0 | $2{\cdot}88\times10^{4}$ ($\pm2{\cdot}19\times10^{4}$) |
| AH25 | 0 | $2{\cdot}20\times10^{5}$ ($\pm1{\cdot}73\times10^{5}$) |
| **CoNS ($n=9$)** | | |
| *S. epidermidis* S10 | 0 | $4{\cdot}49\times10^{4}$ ($\pm3{\cdot}20\times10^{4}$) |
| *S. epidermidis* S11 | $9{\cdot}33\times10^{4}$ ($\pm5{\cdot}58\times10^{4}$) | $2{\cdot}01\times10^{6}$ ($\pm0{\cdot}41\times10^{6}$) |
| *S. epidermidis* S14 | $4{\cdot}03\times10^{3}$ ($\pm4{\cdot}19\times10^{3}$) | $2{\cdot}27\times10^{6}$ ($\pm2{\cdot}03\times10^{6}$) |
| *S. epidermidis* S16 | 0 | $3{\cdot}79\times10^{3}$ ($\pm3{\cdot}55\times10^{3}$) |
| *S. epidermidis* S17 | 0 | $1{\cdot}26\times10^{4}$ ($\pm0{\cdot}96\times10^{4}$) |
| *S. capitis* S12 | $6{\cdot}28\times10^{1}$ ($\pm3{\cdot}15\times10^{1}$) | $1{\cdot}05\times10^{6}$ ($\pm0{\cdot}50\times10^{6}$) |
| *S. capitis* S15 | 0 | $6{\cdot}89\times10^{3}$ ($\pm8{\cdot}26\times10^{3}$) |
| *S. lugdunensis* S20 | 0 | $2{\cdot}47\times10^{6}$ ($\pm0{\cdot}79\times10^{6}$) |
| *S. warneri* S18 | $1{\cdot}99\times10^{2}$ ($\pm1{\cdot}27\times10^{2}$) | $4{\cdot}61\times10^{2}$ ($\pm3{\cdot}53\times10^{2}$) |

reflect the concentration found in commercially available products. Biofilms formed by MSSA and MRSA isolates were completely eradicated following exposure to 5 % TTO for 1 h. In contrast, of the biofilms formed by the nine CoNS isolates tested, only five were completely killed, although a reduction in viable count was apparent for the other four isolates (Table 3). As the number of adherent bacteria in the biofilms formed by CoNS isolates was similar to the number in biofilms formed by both MRSA and MSSA isolates, this difference in biofilm susceptibility cannot be attributed to a difference in the size of the biofilm populations. To date, several studies have investigated the possible antimicrobial mechanism of action of TTO (Carson & Riley, 1995; Carson *et al.*, 2002; Cox *et al.*, 1998, 2000), with the consensus being that the antibacterial activity of TTO is related to disruption of hydrophobic structures within phospholipid bilayers of the bacterial cell. Whether this mechanism is replicated in killing of bacteria growing in a biofilm is yet to be investigated and it is possible that differences in biofilm formation by CoNS and *S. aureus* may be responsible for the resistance of some biofilm-grown CoNS to killing by TTO. Further studies using an animal model of skin colonization would be useful for *in vivo* confirmation of the differences in biofilm susceptibility that are apparent *in vitro* between MSSA and MRSA isolates and some CoNS isolates.

In conclusion, the results of this study are promising in that they show that TTO at a concentration of 5 %, which has previously been shown to be well tolerated and non-irritant to the skin (Carson & Riley, 2001; Carson *et al.*, 1998), could be used as an alternative agent for MRSA decolonization, as it is effective in eradicating biofilm-grown MRSA. A number of clinical studies have already compared the efficacy of TTO and standard decolonization regimens for the eradication of MRSA carriage and demonstrated no significant difference (Caelli *et al.*, 2000; Dryden *et al.*, 2004). Furthermore, a recent study has also shown that hand-cleansing formulations containing 5 % TTO and 10 % alcohol or a solution of 5 % TTO in water are more effective than soft soap in removing bacteria from the surface of the hand in both an *in vivo* hand-washing test and an *ex vivo* model (Messager *et al.*, 2005). However, prior to the widespread use of TTO for MRSA decolonization, further evidence of efficacy in clinical trials is required, as are investigations to determine whether the organic debris encountered on the skin and nasal mucosa affect the ability of TTO to kill bacteria growing in biofilms.

## ACKNOWLEDGEMENTS

A. B. was funded by a European Social Fund Grant. The supply of bacterial isolates by Dr Sheila Patrick, Department of Microbiology and Immunobiology, School of Medicine, Queen's University Belfast, UK, is gratefully acknowledged.

## REFERENCES

**Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997).** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

**Andrews, J. M. (2001).** Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* **48**, 5–16.

**Banes-Marshall, L., Cawley, P. & Phillips, C. A. (2001).** *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against bacterial and *Candida* spp. isolates from clinical specimens. *Br J Biomed Sci* **58**, 139–145.

**Boyce, J. M. (2001).** MRSA patients: proven methods to treat colonization and infection. *J Hosp Infect* **48** (Suppl. A), S9–S14.

**Caelli, M., Porteous, J., Carson, C. F., Heller, R. & Riley, T. V. (2000).** Tea tree oil as an alternative topical decolonization agent for methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* **46**, 236–237.

**Carson, C. F. & Riley, T. V. (1995).** Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J Appl Bacteriol* **78**, 264–269.

**Carson, C. F. & Riley, T. V. (2001).** Safety, efficacy and provenance of tea tree (*Melaleuca alternifolia*) oil. *Contact Dermatitis* **45**, 65–67.

**Carson, C. F., Cookson, B. D., Farrelly, H. D. & Riley, T. V. (1995).** Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia*. *J Antimicrob Chemother* **35**, 421–424.

**Carson, C. F., Riley, T. V. & Cookson, B. D. (1998).** Efficacy and safety of tea tree oil as a topical antimicrobial agent. *J Hosp Infect* **40**, 175–178.

**Carson, C. F., Mee, B. J. & Riley, T. V. (2002).** 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. *Antimicrob Agents Chemother* **46**, 1914–1920.

**Carson, C. F., Hammer, K. A. & Riley, T. V. (2006).** *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clin Microbiol Rev* **19**, 50–62.

**Chan, C. H. & Loudon, K. W. (1998).** Activity of tea tree oil on methicillin-resistant *Staphylococcus aureus* (MRSA). *J Hosp Infect* **39**, 244–245.

**Colsky, A. S., Kirsner, R. S. & Kerdel, F. A. (1998).** Analysis of antibiotic susceptibilities of skin wound flora in hospitalized dermatology patients. The crisis of antibiotic resistance has come to the surface. *Arch Dermatol* **134**, 1006–1009.

**Cookson, B. D., Bolton, M. C. & Platt, J. H. (1991).** Chlorhexidine resistance in methicillin-resistant *Staphylococcus aureus* or just an elevated MIC? An in vitro and in vivo assessment. *Antimicrob Agents Chemother* **35**, 1997–2002.

**Cox, S. D., Gustafson, J. E., Mann, C. M., Markham, J. L., Liew, Y. C., Hartland, R. P., Bell, H. C., Warmington, J. R. & Wyllie, S. G. (1998).** Tea tree oil causes $K^+$ leakage and inhibits respiration in *Escherichia coli*. *Lett Appl Microbiol* **26**, 355–358.

**Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R. & Wyllie, S. G. (2000).** The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol* **88**, 170–175.

**Diekema, D. J., Dodgson, K. J., Sigurdardottir, B. & Pfaller, M. A. (2004).** Rapid detection of antimicrobial-resistant organism carriage: an unmet clinical need. *J Clin Microbiol* **42**, 2879–2883.

**Dryden, M. S., Dailly, S. & Crouch, M. (2004).** A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. *J Hosp Infect* **56**, 283–286.

**Edwards, R. & Harding, K. G. (2004).** Bacteria and wound healing. *Curr Opin Infect Dis* **17**, 91–96.

**Elsom, G. K. F. & Hide, D. (1999).** Susceptibility of methicillin-resistant *Staphylococcus aureus* to tea tree oil and mupirocin. *J Antimicrob Chemother* **43**, 427–428.

**European Committee for Standardization (1997).** *Chemical disinfectants and antiseptics*. Basic Bactericidal Activity: Test Methods and Requirements (phase 1). European Standard EN 1040. Brussels: European Committee for Standardization.

**Gustafson, J. E., Liew, Y. C., Chew, S., Markham, J., Bell, H. C., Wyllie, S. G. & Warmington, J. R. (1998).** Effects of tea tree oil on *Escherichia coli*. *Lett Appl Microbiol* **26**, 194–198.

**Haley, C. E., Marling-Cason, M., Smith, J. W., Luby, J. P. & Mackowiak, P. A. (1985).** Bactericidal activity of antiseptics against methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **21**, 991–992.

**Hammer, K. A., Carson, C. F. & Riley, T. V. (1996).** Susceptibility of transient and commensal skin flora to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Am J Infect Control* **24**, 186–189.

**Hammer, K. A., Dry, L., Johnson, M., Michalak, E. M., Carson, C. F. & Riley, T. V. (2003).** Susceptibility of oral bacteria to *Melaleuca alternifolia* (tea tree) oil *in vitro*. *Oral Microbiol Immunol* **18**, 389–392.

**Higaki, S., Kitagawa, T., Kagoura, M., Morohashi, M. & Yamagishi, T. (2000).** Predominant *Staphylococcus aureus* isolated from various skin diseases. *J Int Med Res* **28**, 187–190.

**Johnson, A. P., Pearson, A. & Duckworth, G. (2005).** Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemother* **56**, 455–462.

**Kloos, W. E. & Musselwhite, M. S. (1975).** Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl Microbiol* **30**, 381–385.

**Leeming, J. P., Holland, K. T. & Cunliffe, W. J. (1984).** The microbial ecology of pilosebaceous units isolated from human skin. *J Gen Microbiol* **130**, 803–807.

**LiPuma, J. J., Dulaney, B. J., McMenamin, J. D., Whitby, P. W., Stull, T. L., Coenye, T. & Vandamme, P. (1999).** Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. *J Clin Microbiol* **37**, 3167–3170.

**Marples, R. R. (1994).** The normal flora of the skin is a biofilm. In *Bacterial Biofilms and Their Control in Medicine and Industry*, pp. 127–129. Edited by J. Wimpenny, W. Nichols, D. Stickler & H. Lappin-Scott. Cardiff: Bioline.

**May, J., Chan, C. H., King, A., Williams, L. & French, G. L. (2000).** Time–kill studies of tea tree oils on clinical isolates. *J Antimicrob Chemother* **45**, 639–643.

**Messager, S., Hammer, K. A., Carson, C. F. & Riley, T. V. (2005).** Effectiveness of hand-cleansing formulations containing tea tree oil assessed ex vivo on human skin and in vivo with volunteers using European standard EN 1499. *J Hosp Infect* **59**, 220–228.

**Mulligan, M. E., Murray-Leisure, K. A., Ribner, B. S., Standiford, H. C., John, J. F., Korvick, J. A., Kauffman, C. A. & Yu, V. L. (1993).** Methicillin-resistant *Staphylococcus aureus*: a consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *Am J Med* **94**, 313–328.

**Nelson, R. R. S. (1997).** In-vitro activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. *J Antimicrob Chemother* **40**, 305–306.

**Shapiro, S., Meier, A. & Guggenheim, B. (1994).** The antimicrobial activity of essential oils and essential oil components towards oral bacteria. *Oral Microbiol Immunol* **9**, 202–208.

**Strommenger, B., Kettlitz, C., Werner, G. & Witte, W. (2003).** Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J Clin Microbiol* **41**, 4089–4094.

**Valencia, I. C., Kirsner, R. S. & Kerdel, F. A. (2004).** Microbiologic evaluation of skin wounds: alarming trend toward antibiotic resistance in an inpatient dermatology service during a 10-year period. *J Am Acad Dermatol* **50**, 845–849.