in vivo *21*: 1027-1030 (2007)

Antibacterial Effect of Tea-tree Oil on Methicillin-resistant *Staphylococcus aureus* Biofilm Formation of the Tympanostomy Tube: An *In Vitro* Study

HAEKYUN PARK[1], CHUL-HO JANG[2,3], YONG BUM CHO[2] and CHEOL-HEE CHOI[3]

[1]*College of Natural Science, Chosun University, Gwangju;*
[2]*Department of Otolaryngology, Chonnam National University Medical School, Gwangju;*
[3]*Research Center for Resistant Cells, Chosun Medical School, Gwangju, South Korea*

Abstract. *The antibacterial effects of tea-tree oil against the formation of methicillin-resistant Staphylococcus aureus (MRSA) biofilm on the surface of the tympanostomy tubes was evaluated. Materials and Methods: Silicone tympanostomy tubes were pretreated with normal saline for 12 hours, the control group (n=4), with 100% tea-tree oil, experimental group A (n=3), or with 50% tea-tree oil, experimental group B (n=3). All the tubes were incubated in a MRSA solution for 2 days and then processed for evaluation using scanning electron microscopy. Results: The development of the biofilm mode of growth of MRSA was observed in the saline-treated control group. In contrast, only focal biofilms were present on the tube surface in experimental group A and considerable reduction of biofilm with destruction of the MRSA cells was shown in experimental group B. Conclusion: From these results, the antimicrobial effect of tea-tree oil against biofilm formation on tympanostomy tubes in vitro has been verified.*

Acute tympanostomy tube otorrhea is a common problem (1-3) that mainly occurs with upper respiratory infection (4). In children with tympanostomy tubes, acute otitis media can be diagnosed in the presence of acute otorrhea and acute symptoms. The causative pathogens in young children with acute otorrhea are the same as those found in acute otitis media with an intact tympanic membrane (5).

Bacterial biofilm is a polysaccharide formation believed to be an important mediator of infection at the site of implanted materials (6, 7). The organisms within this polysaccharide matrix, or glycocalyx slime layer, are relatively resistant to antibiotics and can become a source of persistent and relapsing infection, often necessitating the removal of the implanted material (8). Bacterial biofilm formation has been implicated in the high rate of persistent otorrhea after tympanostomy tube insertion (9, 10). It has been shown that coating medical implants with antimicrobials may effectively prevent the initial adherence of staphylococcal biofilms to the implants (11, 12).

There has been a steady increase in the number of cases of methicillin-resistant *Staphylococcus aureus* (MRSA) otorrhea (13); this is a growing concern in particular the increasing incidence of MRSA infections in pediatric otitis media with otorrhea (14, 15). Once a Staphylococcal biofilm has formed on an implanted medical device or damaged tissue, it is difficult to disrupt. A biofilm-infected implant must often be removed and replaced, placing the patient at increased risk of complications due to these additional procedures (11). Current antimicrobial therapies for biofilms have largely proven unsuccessful (16).

Tea-tree oil is an essential oil produced by steam distillation from leaves of the indigenous Australian plant, *Melaleuca alternifolia* or tea-tree. Tea-tree oil is known to possess antimicrobial activity, and *in vitro* activity against *Staphylococcus aureus*, MRSA, *S. epidermidis*, and *Proteus mirabilis* has been documented (17-19). A recent paper by Anderson and Fennessy (20) concluded that there was compelling *in vitro* evidence of the effectiveness of tea-tree oil against MRSA. Sherry *et al.* (21) have described the clinical efficacy of tea-tree oil against MRSA postoperative wound infections. To our knowledge, there have been no studies into how tea-tree oil might influence the development of bacterial biofilms on the tympanostomy tube.

In this study, the antibacterial effects of tea-tree oil against the formation of MRSA biofilm on the surface of the tympanostomy tubes were evaluated.

Correspondence to: Chul Ho Jang, MD, Deparment of Otolaryngology, Chonnam National University Hospital, Hak-dong 8, Gwangju, 501-757, South Korea. Tel: +82 62 2206774, e-mail: chulsavio@hanmail.net

Key Words: MRSA, tympanostomy tube, biofilm, tea-tree oil.

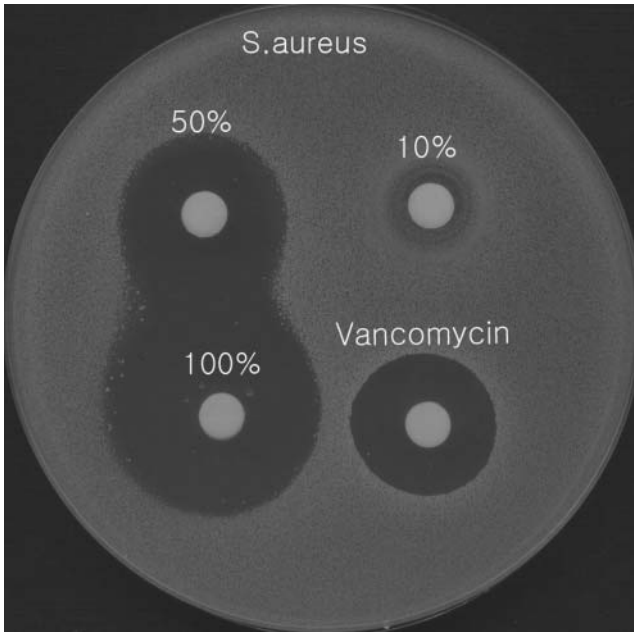

Figure 1. *Zone of inhibition of MRSA tested against individual teat tree oils and vancomycin placed in direct contact.*

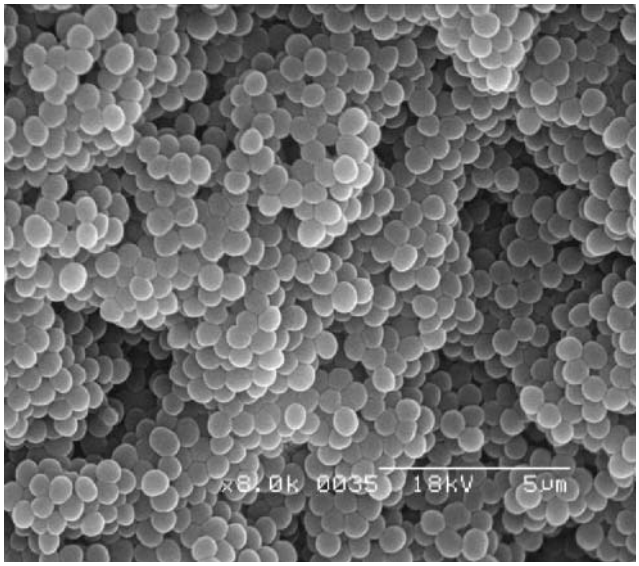

Figure 2. *Thick biofilms with colonies of MRSA in the saline-treated control group.*

Materials and Methods

Clinical MRSA (n=20) bacteria samples were obtained from patients at the Chonnam National University Hospital in Gwangju city, South Korea (March 2006 through May 2007). Bacterial cultures were obtained from otorrhea in chronic suppurative otitis media patients. All patients were initially treated with ciprofloxacin otic drops but the otorrhea failed to resolve. Ear fluid for culture was collected from the external auditory canal using a swab. Clinical samples were processed and identified with standard cultures. Susceptibilities to various antibiotics were determined by modified Kirby-Bauer disk diffusion methods according to the Clinical Laboratory Standards Institute (22). In all cases, MRSA was the only organism grown. Susceptibility of MRSA was to few antibiotics. Vancomycin was the most effective antibiotics against MRSA. Therefore, vancomycin (SamjinPharm. Co, Seoul, South Korea) was tested in comparison to three individual tea-tree oil concentrations (100%, 50%, 10% in tween). The biofilm susceptibility of the MRSA was tested using the modified microplate Alamar blue assay (23).

Medtronic Xomed (Jacksonville, FL, USA) silicone tympanostomy tube were prepared for 12 hours with 100% tea-tree oil, group A (n=3), with 50% tea-tree oil diluted with tween, group B (n=3), or with normal saline, control (n=4). The MRSA was grown to a logarithmic phase in trypticase soy broth (TSB) at 37˚C for 24 hours. The bacteria were harvested by centrifugation, and then resuspended in TSB and evaluated with a spectrophotometer to yield approximately 10^9 colony-forming units per milliliter. Flasks containing TSB were inoculated with MRSA using a sterile wire loop. The pretreated tympanostomy tubes were incubated in biofilm susceptible MRSA solution for 2 days. All the tympanostomy tubes were immersed in fresh 2% glutaraldehyde overnight. They were prepared by critical point drying and gold sputter coating. All the prepared specimens were investigated for MRSA biofilm formation on the surface of the tube using scanning electron microscopy (SEM). Each specimen was examined for 30 minutes.

Results

All the bacterial strains showed some susceptibility to each concentration of tea-tree oil when tested in direct contact using the disc diffusion method. The size of the zone of inhibition varied depending upon the concentration with 100% tea-tree oil giving the largest zones of inhibition. The MRSA appeared to be similarly susceptible to 50% tea-tree oil and vancomycin (Figure 1).

The development of the biofilm mode of growth of MRSA was observed in the control (saline pretreated tympanostomy tubes) group. Thick colonies of MRSA were evident on most of the tube surfaces with no intervening spaces (Figure 2). On the other hand, a marked change in the appearance of the biofilm with considerable reduction in the density of adherent bacteria and biofilm structures was shown in experimental group A (Figure 3). Experimental group B (Figure 4) showed destruction of the surface MRSA and partial reduction of the biofilm compared to experimental group A.

Discussion

The resistance to antibiotics of bacteria growing in biofilms remains an incompletely understood process and is an area of active research. The antibiotic resistance of bacteria in established biofilms may be due to a number of factors,

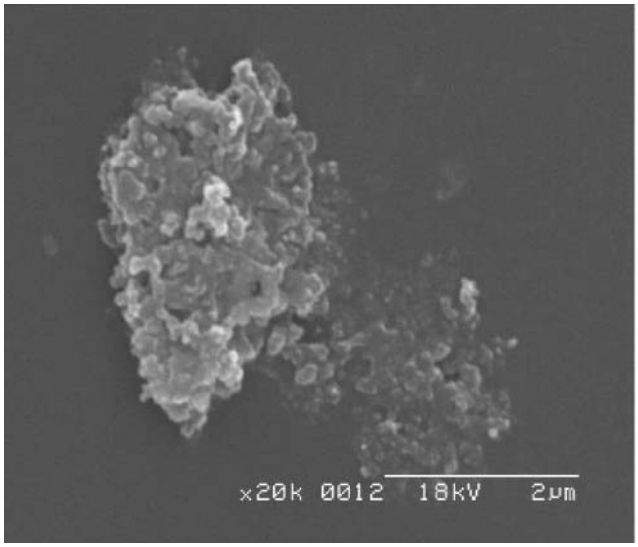

Figure 3. *Considerable reduced focal appearance of biofilm on tube surface in group A (100% tea-tree oil).*

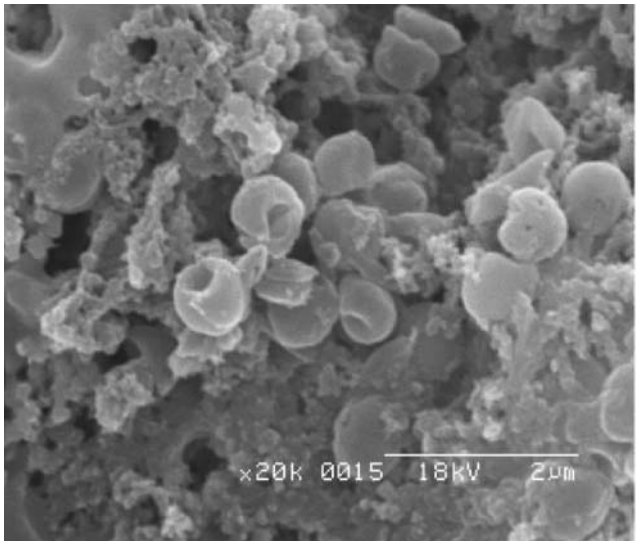

Figure 4. *Surface destruction of the MRSA with intervening spaces is shown in the group B (50% tea-tree oil).*

including the multilayer structure of biofilms (24). Recently, in an *in vitro* study, Biedlingmaier *et al.* (25) showed the development of bacterial biofilms on tympanostomy tubes composed of silicone, fluoroplastic and silver oxide-impregnated silicone. Jang *et al.* (26) reported biofilm formation on the tympanostomy tubes with ciprofloxacin-resistant *Pseudomonas* otorrhea.

In the present study, SEM confirmed that tea-tree oil eradicated the biofilm of MRSA. A diverse range of essential oils are known to possess antimicrobial activity and *in vitro* activity of oils such as tea-tree oil against MRSA has been documented (20). The exact mechanism of tea-tree oil action against MRSA biofilms remains unclear.

The reduced bacterial adherence to the tympanostomy tube caused by tea-tree oil may be explained by the alteration of adherence factors present on the bacterial cell surface. Tea-tree oil can be used topically for the MRSA otorrhea (17). However, if tea-tree oil is to be used in pediatric MRSA otorrhea with tympanostomy tubes, its ototoxicity should be assessed.

Conclusion

From these results, the antimicrobial effect of tea-tree oil against biofilm formation on the tympanostomy tubes *in vitro* was verified. However, further *in vivo* studies are necessary.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (R13-2003-009).

References

1 Mandel EM, Casselbrant ML and Kurs-Lasky M: Acute otorrhea: bacteriology of a common complication of tympanostomy tubes. Ann Otol Rhinol Laryngol *103*: 713-718, 1994.
2 Ah-Tye C, Paradise JL and Colborn DK: Otorrhea in young children after tympanostomy-tube placement for persistent middle-ear effusion: prevelance, incidence, and duration. Pediatrics *107*: 1251-1258, 2001.
3 Debruyne F and Degroote M: One-year follow-up after tympanostomy tube insertion for recurrent acute otitis media. ORL J Otorhinolaryngol Relat Spec *55*: 226-229, 1993.
4 Salata JA and Derkay CS: Water precautions in children with tympanostomy tubes. Arch Otolaryngol Head Neck Surg 122: 276-280, 1996.
5 Bluestone CD and Klein JO: Otitis media in Infants and Children. 3rd ed. Philadelphia, PA: WB Saunders. pp. 1-15, 2001.
6 Post JC: Direct evidence of bacterial biofilms in otitis media. Laryngoscope *111(12)*: 2083-2094, 2001.
7 Dohar JE, Kenna MA and Wadowsky RM: *In vitro* susceptibility of aural isolates of *Pseudomonas aeruginosa* to commonly used ototopical antibiotics. Am J Otol *17*: 207-209, 1996.
8 Dougherty SH: Pathobiology of infection on prosthetic devices. Rev Infect Dis *10*: 1102-1117, 1988.
9 Gander S: Bacterial biofilms: resistance to antimicrobial agents. J Antimicrob Chemother *37*: 1047-1050, 1996.
10 Karlan MS, Mufson RA, Grizzard MB, Cassisi NJ, Singleton GT, Buscemi P and Goldberg EP: Myringotomy tube materials: bacterial adhesion and infection. Otolaryngol Head Neck Surg *88*: 783-795, 1980.
11 Raad I, Darouiche R, Hachem R, Abi-Said D, Safar H, Darnule T, Mansouri M and Morck D: Antimicrobial durability and rare ultrastructural colonization of indwelling central catheters coated with minocycline and rifampin. Crit Care Med *26*: 219-224, 1998.

12 Schierholz J, Steinhauser H, Rump A, Berkels R and Pulverer G: Controlled release of antibiotics from biochemical polyurethanes: morphological and structural features. Biomaterials *18*: 839-844, 1997.
13 Jang CH, Song CH and Wang PC: Topical vancomycin for chronic suppurative otitis media with methicillin-resistant *Staphylococcus aureus* otorrhoea. J Laryngol Otol *118*: 645-647, 2004.
14 AI-Shawwa BA and Wegner D: Trimethoprim-sulfamethoxazole plus topical antibiotics as therapy for acute otitis media with otorrhea caused by community-acquired methicillin-resistant *Staphylococcus aureus* in children. Arch Otolaryngol Head Neck Surg *131*: 782-784, 2005.
15 Coticchia JM and Dohar JE: Methicillin-resistant *Staphylococcus aureus* otorrhea after tympanostomy tube placement. Arch Otolaryngol Head Neck Surg *131*: 868-873, 2005.
16 Donlan RM and Costerton JW: Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev *15*: 167-193, 2002.
17 Farnan TB, McCallum J, Awa A, Khan AD and Hall SJ: Tea tree oil: *in vitro* efficacy in otitis externa. J Laryngol Otol *119*: 198-201, 2005.
18 Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR and Wyllie SG: The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J Appl Microbiol *88*: 170-175, 2000.
19 Carson CF and Riley TV: Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia.* J Appl Bacteriol *78*: 264-269, 1995.
20 Anderson JN and Fennessy PA: Can tea tree *(Melaleuca alternifolia)* oil prevent MRSA? Med J Aust *173*: 489, 2000.
21 Sherry E, Boeck H and Warnke PH: Percutaneous treatment of chronic MRSA osteomyelitis with a novel plant derived antiseptic. BMC Surg *1*: 1, 2001.
22 National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. M7–A5. Wayne, Pa: National Committee for Clinical Laboratory Standards, 2000.
23 Wei GX, Campagna AN and Bobek LA: Effect of MUC7 peptides on the growth of bacteria and on Streptococcus mutans biofilm. J Antimicrob Chemother *57*: 1100-1109, 2006.
24 Rachid S, Ohlsen K, Wallner U, Hacker J, Hecker M and Ziebuhr W: Alternative transcription factor sigma(B) is involved in regulation of biofilm expression in a *Staphylococcus aureus* mucosal isolate. J Bacteriol *182*: 6824-6826, 2000.
25 Biedlingmaier JF, Samaranayake R and Whelan P: Resistance to biofilm formation on otologic implant materials. Otolaryngol Head Neck Surg *118*: 444-451, 1998.
26 Jang CH, Cho YB and Choi CH: Structural features of tympanostomy tube biofilm formation in ciprofloxacin-resistant *Pseudomonas* otorrhea. Int J Pediatr Otorhinolaryngol *71*: 591-595, 2007.

Received July 7, 2007
Revised October 15, 2007
Accepted October 22, 2007