The Journal of Laryngology & Otology (2010), ***124***, *594–598.*

doi:10.1017/S0022215109992672

Review Article

Effect of vancomycin-coated tympanostomy tubes on methicillin-resistant *Staphylococcus aureus* biofilm formation: *in vitro* study

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Abstract

Background and objective: Bacterial biofilm formation has been implicated in the high incidence of persistent otorrhoea after tympanostomy tube insertion. It has been suggested that the tube material may be an important factor in the persistence of such otorrhoea. Development of methicillin-resistant *Staphylococcus aureus* otorrhoea after tympanostomy tube placement is a growing concern. We evaluated the effect of using vancomycin and chitosan coated tympanostomy tubes on the incidence of methicillin-resistant *Staphylococcus aureus* biofilm formation *in vitro*.

Materials and methods: Three sets each of vancomycin-coated silicone tubes ($n = 5$), commercial silver oxide coated silicone tubes ($n = 5$) and uncoated tympanostomy tubes (as controls; $n = 5$) were compared as regards resistance to methicillin-resistant *Staphylococcus aureus* biofilm formation after *in vitro* incubation.

Results: Scanning electron microscopy showed that the surfaces of the silver oxide coated tubes supported the formation of thick biofilms with crusts, comparable to the appearance of the uncoated tubes. In contrast, the surface of the vancomycin-coated tympanostomy tubes was virtually devoid of methicillin-resistant *Staphylococcus aureus* biofilm.

Conclusion: Vancomycin-coated tympanostomy tubes resist methicillin-resistant *Staphylococcus aureus* biofilm formation. Pending further study, such tubes show promise in assisting the control of methicillin-resistant *Staphylococcus aureus* biofilm formation.

Key words: Middle Ear; Ventilation Tubes; Otorrhea; Methicillin-Resistant *Staphylococcus Aureus*

Introduction

Tympanostomy tube insertion is the most commonly performed surgical procedure in children younger than 15 years. Purulent otorrhoea is the most common complication following tympanostomy tube insertion, occurring in 9 to 34 per cent of patients.[1–5] The causative pathogens in young children with acute otorrhoea are the same as those found in paediatric patients with acute otitis media and an intact tympanic membrane.[6]

There has been a steady increase in the number of cases of otorrhoea caused by methicillin-resistant *Staphylococcus aureus* (MRSA).[7] Recently, concern has been expressed over the increasing incidence of community-acquired MRSA infection presenting in paediatric patients with otitis media with otorrhoea.[8] An increased incidence of MRSA tympanostomy tube otorrhoea has also been observed.[9] Bacterial biofilm formation has been implicated in the high rate of persistent otorrhoea after tympanostomy tube insertion.[10–12] Once a staphylococcal biofilm has formed on an implanted medical device or damaged tissue, it is difficult to disrupt. Biofilm-infected implants must often be removed and replaced, placing the patient at increased risk of complications.[13] Current antimicrobial therapies for biofilms have proven largely unsuccessful.[14]

An ideal tympanostomy tube material would be well tolerated by the patient and resistant to bacterial adhesion. However, it has been suggested that currently used tube materials may be influential in the development of otorrhoea. Silicone is currently the most commonly used material for tympanostomy tubes. The use of fluoroplastic tympanostomy tubes has resulted in a significant reduction in otorrhoea incidence, compared with silicone tympanostomy tubes.[10] Silver ions inhibit the growth of staphylococcus, streptococcus, *Escherichia coli* and pseudomonas, and the incorporation of silver oxide into tympanostomy tubes appears to decrease the long-term

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Accepted for publication: 21 October 2009. First published online 8 January 2010.

incidence of otorrhoea.[15] The use of silver oxide confers to tympanostomy tubes the ability to prevent adherence and colonisation of selected bacteria, rather than affecting an already established infection.

The influence of silver oxide on MRSA is ill-defined. There is reason to believe that silver oxide may exert antibacterial effects against MRSA, since antibiotic coatings are effective against biofilm formation. For example, we have observed that tympanostomy tubes coated with a mixture of chitosan, piperacillin and tazobactam display effective resistance to the formation of ciprofloxacin-resistant pseudomonas biofilms.[16]

Vancomycin is not an aminoglycoside antibiotic but is often mistaken for one due to the 'mycin' suffix. In experimental animal models using large single vancomycin doses, there was no convincing evidence of ototoxicity from vancomycin.[17] In fact, one report of a patient who inadvertently received six 185-mg doses of vancomycin described no resulting auditory damage.[18] Topical vancomycin has been found to be effective in patients with MRSA otorrhoea refractory to conventional antibiotic treatment; in addition, none of these patients showed any adverse effects during topical vancomycin treatment, and there were no statistically significant differences in mean bone conduction hearing thresholds as a result of treatment.[7]

In the current study, we evaluated the efficacy of vancomycin-coated tympanostomy tubes in preventing MRSA biofilm formation *in vitro*.

Materials and methods

Vancomycin coating

To create the vancomycin coating, we used 90–95 per cent deacetylated, water-soluble chitosan (molecular weight <10 kDa; Ecobio, Gwangju, South Korea). Chitosan solutions of 5 per cent by weight and pH 4.5 were prepared by dissolution of chitosan in 0.2 M acetic acid. The mixture was stirred at 50°C for 2 hours to obtain a homogeneous polymer solution. The reaction mixture was then filtered through a fine cloth to remove air bubbles trapped in the viscous liquid. Vancomycin powder and a Paparella type one tympanostomy tube with an inner diameter of 1.14 mm (Medtronic Xomed, Jacksonville, Florida, USA) were added to the prepared chitosan solution to create a chitosan and vancomycin coated tympanostomy tube. The tube was rapidly transferred to a −20°C freezer for 8 hours to solidify the solvent and induce solid–liquid phase separation. The tympanostomy tubes were then transferred to a freeze-dryer and were lyophilised for five days to completely remove the solvent.

In vitro *testing*

We evaluated three sets each of vancomycin-coated, commercial silicone tubes ($n = 5$; Medtronic Xomed), commercial silver oxide coated silicone tubes with an inner diameter of 1.14 mm ($n = 5$; Activent Silic, Medtronic Xomed), and Paparella type one uncoated tympanostomy tubes with an inner diameter of 1.14 mm (as controls; $n = 5$; Medtronic Xomed).

Twenty clinical MRSA isolates were obtained from patients with otorrhoea due to chronic suppurative otitis media treated at the Chonnam National University Hospital, Gwangju City, South Korea, from March 2006 to February 2007.

Each isolate was grown to logarithmic phase in tryptone soy broth at 37°C for 24 hours, harvested by centrifugation, resuspended in a volume of tryptone soy broth and monitored spectrophotometrically to yield approximately 10^9 colony-forming units per millilitre. Before each experiment, several colonies were used to inoculate 5 ml tryptone soy broth at 37°C for 24 hours in ambient air. Subsequently, 100 μl of this culture was used to inoculate 50 ml of medium, from which biofilms were grown on tympanostomy tubes introduced by suspension from a stainless steel hook. After 72 hours' incubation at 37°C with continuous shaking at 60 rpm, the

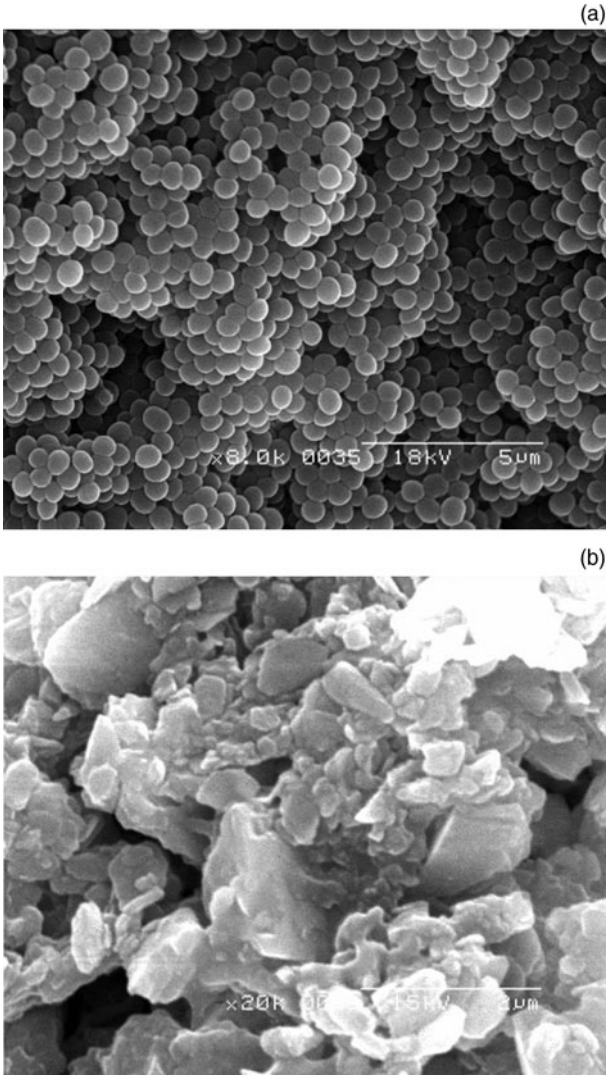

FIG. 1

Scanning electron micrograph of the surface of an untreated silicone tympanostomy tube. Diffuse methicillin-resistant *Staphylococcus aureus* biofilm formation is evident at (a) lower and (b) higher magnification.

https://doi.org/10.1017/S0022215109992672 Published online by Cambridge University Press

inoculated tryptone soy broth was refreshed with sterile tryptone soy broth.

After a total incubation period of six days, the tympanostomy tubes were collected, dipped once in sterile 0.9 per cent NaCl and then gently dried by a minimal touch with a soft tissue, to wash off planktonic micro-organisms as well as any remaining tryptone soy broth. All tympanostomy tubes were immersed in fresh 2 per cent glutaraldehyde overnight. The tubes were then cut longitudinally into two segments for scanning electron microscopy.

The tubes were prepared for microscopy by critical point drying and gold sputter coating. All prepared specimens were investigated, for 30 minutes each, for MRSA biofilm formation on the surface of the tube.

Results

The surface of the uncoated control tympanostomy tubes showed diffuse MRSA biofilm formation on both the outer and inner surfaces (Figure 1).

The silver oxide coated tubes showed no resistance to MRSA; their surfaces showed thick biofilms with crusts (Figure 2).

In contrast, the surface of the vancomycin-coated tympanostomy tubes was devoid of biofilm (Figure 3).

All of the tubes in each group showed similar findings.

Discussion

Methicillin-resistant *S aureus* otorrhoea is a growing concern. Intravenously administered vancomycin hydrochloride is the drug of choice in treating MRSA-infected patients. As an alternative approach, we evaluated the resistance of vancomycin-coated tympanostomy tubes to MRSA biofilm formation *in vitro*. Tubes coated with vancomycin were less prone to MRSA biofilm formation, compared with silver oxide coated tympanostomy

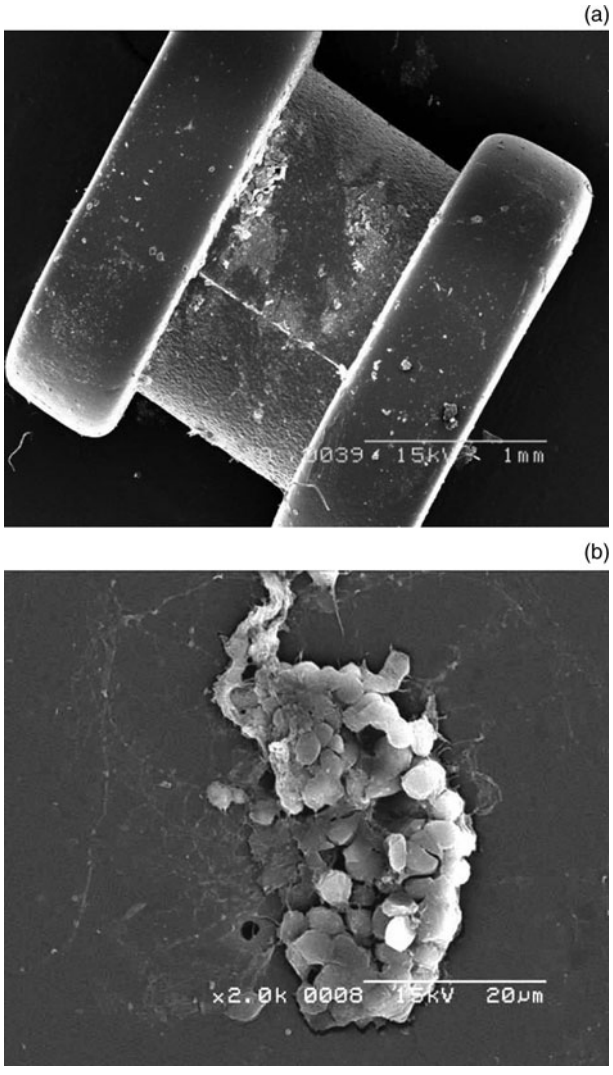

FIG. 2

Scanning electron micrograph of the surface of a silver oxide coated silicone tympanostomy tube. Methicillin-resistant *Staphylococcus aureus* biofilm formation is evident at (a) lower and (b) higher magnification.

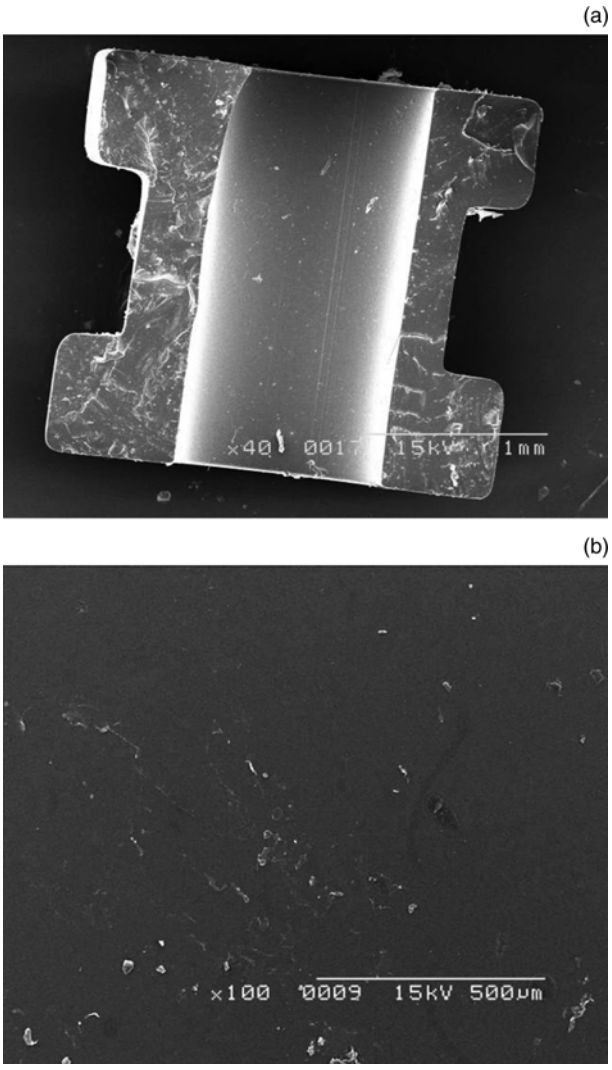

FIG. 3

Scanning electron micrograph of the surface of a vancomycin-coated silicone tympanostomy tube. Almost complete absence of methicillin-resistant *Staphylococcus aureus* biofilm is evident at (a) lower and (b) higher magnification.

https://doi.org/10.1017/S0022215109992672 Published online by Cambridge University Press

tubes. Vancomycin was applied along with chitosan, a linear polysaccharide derived from crustacean shells and fungi cell walls, which has many reported medical applications including use as an implant coating.[16,19–21] The advantages of chitosan include biocompatibility, drug delivery and reported bacteriostatic properties.[22,23]

Reduction in biofilm contamination could substantially reduce the incidence of otorrhoea. Chole and Hubbell have reported a decreased incidence of otorrhoea with the use of silver oxide coated silicone tympanostomy tubes, compared with plain silicone tympanostomy tubes.[15] However, silver oxide coated tubes are also susceptible to biofilm formation.[23,24] In the present study, MRSA biofilm formation occurred on silver-treated tympanostomy tubes, indicating resistance of MRSA to silver oxide. Ion-bombarded silicone tympanostomy tubes and phosphorylcholine-coated tubes have been reported to resist biofilm formation.[25,26] However, to date, these types of tubes have not been reported to resist MRSA biofilms.

- **Bacterial biofilm formation has been implicated in the high rate of persistent discharge occurring after tympanostomy tube insertion**
- **The tympanostomy tube material may be an important factor in the development of aural discharge**
- **This *in vitro* study found that vancomycin-coated tympanostomy tubes resisted methicillin-resistant *Staphylococcus aureus* biofilm formation**
- **Further studies are required to establish the possible clinical value of this finding in human patients**

Cağavi *et al.* studied microbial colonisation of vancomycin-coated catheters and silicone elastomer catheters.[27] Their results showed us that a coated catheter more important than the topical application of vancomycin to the shunt catheters (Regular vancomycin coated catheters are superior to silicone catheters that were topically immersed using vancomycin. They prevent bacterial colonization in some respect.)

Conclusion

This study compared vancomycin-coated tympanostomy tubes with silver oxide coated tubes and uncoated, control tubes as regards resistance to MRSA biofilm formation *in vitro*. The vancomycin coating inhibited MRSA biofilm formation. Further studies are necessary to investigate this potential *in vivo*.

Acknowledgement

This work was supported by a National Research Foundation grant funded by the Korean government Ministry of Education, Science and Technology (MEST) through the Research Center for Resistant Cells (R13-2003-009).

References

1 Barfoed C, Rosborg J. Secretory otitis media. *Arch Otolaryngol* 1980;**106**:553–6
2 Balknay TH, Barkin RM, Suzuki BH, Watson WJ. A prospective study of infection following tympanostomy and tube insertion. *Am J Otol* 1983;**4**:288–91
3 Baker RS, Chole RA. A randomized clinical trial of topical gentamicin after tympanostomy tube placement. *Arch Otolaryngol Head Neck Surg* 1988;**114**:755–7
4 Giebink SG, Daly K, Buran DJ, Satz M, Ayre T. Predictors for postoperative otorrhea following tympanostomy tube insertion. *Arch Otolaryngol Head Neck Surg* 1992;**118**: 491–4
5 Younis RT, Lazar RH, Long TE. Ventilation tubes and prophylactic antibiotic eardrops. *Otolaryngol Head Neck Surg* 1992;**106**:193–5
6 Bluestone CD, Klein JO. *Otitis Media in Infants and Children*, Chapter 8, 3rd edn. Philadelphia: WB Saunders, 2001; 1–15
7 Jang CH, Song CH, Wang PC. Topical vancomycin for chronic suppurative otitis media with methicillin-resistant *Staphylococcus aureus* otorrhoea. *J Laryngol Otol* 2004; **118**:645–7
8 AI-Shawwa BA, 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* 2005;**131**:782–4
9 Coticchia JM, Dohar JE. Methicillin-resistant *Staphylococcus aureus* otorrhea after tympanostomy tube placement. *Arch Otolaryngol Head Neck Surg* 2005;**131**:868–73
10 Karlan MS, Mufson RA, Grizzard MB, Buscemi PA, Hench L, Goldberg EP. Myringotomy tube materials: bacterial adhesion and infection. *Otolaryngol Head Neck Surg* 1980;**88**:783–95
11 Jang CH, Cho YB, Choi CH. Structural features of tympanostomy tube biofilm formation in ciprofloxacin-resistant *Pseudomonas* otorrhea. *Int J Pediatr Otorhinolaryngol* 2007;**71**:591–5
12 Johnson JR, Roberts PL, Olsen RJ, Moyer KA, Stamin WE. Prevention of catheter-associated urinary tract infection with a silver oxide-coated urinary catheter. Clinical and microbilogic correlates. *J Infect Dis* 1990;**162**: 1145–50
13 Raad I, Darouiche R, Hachem R, Abi-Said D, Safar H, Darnule T *et al*. Antimicrobial durability and rare ultrastructural colonization of indwelling central catheters coated with minocycline and rifampin. *Crit Care Med* 1998;**26**:219–24
14 Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002;**15**:167–93
15 Chole RA, Hubbell RN. Antimicrobial activity of silastic tympanostomy tubes impregnated with silver oxide. *Arch Otolaryngol Head Neck Surg* 1995;**121**:562–5
16 Jang CH, Park H, Cho YB, Choi CH, Park IY. The use of piperacillin-tazobactam coated tympanostomy tubes against ciprofloxacin-resistant *Pseudomonas* biofilm formation: an *in vitro* study. *Int J Pediatr Otorhinolaryngol* 2009;**73**:295–9
17 Brummett RE. Ototoxicity of vancomycin and analogues. *Otol Clin* 1993;**26**:821–8
18 Mellor JA, Kingdom J, Cafferkey M, Keane C. Vancomycin ototoxicity: a prospective study. *J Antimicrob Chemother* 1985;**15**:773–80
19 Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D. Antibiotic loaded chitosan bar: an in vitro, in vivo study of a possible treatment for osteomyelitis. *Clin Orthop Relat Res* 1999;**366**:239–47
20 Yuan XB, Yuan YB, Jiang W, Liu J, Tian EJ, Shun HM *et al*. Preparation of rapamycin-loaded chitosan/PLA

nanoparticles for immunosuppression in corneal transplantation. *Int J Pharm* 2008;**349**:241–8
21 Bumgardner JD, Wiser R, Gerard PD, Bergin P, Chesnutt B, Marin M *et al*. Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants. *J Biomater Sci Polym Ed* 2003;**14**:423–38
22 Campbell AA, Song L, Li XS, Nelson BJ, Bottoni C, Brooks DE *et al*. Development, characterization, and antimicrobial efficacy of hydroxyapatite-chlorhexidine coatings produced by surface-induced mineralization. *J Biomed Mater Res* 2000;**53**:400–7
23 Hamilton V, Yuan Y, Rigney DA, Chesnutt BM, Puckett AD, Ong JL *et al*. Bone cell attachment and growth on well-characterized chitosan films. *Polymer International* 2007;**56**:641–7
24 Biedlingmaier JF, Samaranayake R, Whelan P. Resistance to biofilm formation on otologic implant materials. *Otolaryngol Head Neck Surg* 1998;**118**:444–51
25 Saidi IS, Biedlingmaier JF, Whelan P. In vivo resistance to bacterial biofilm formation on tympanostomy tubes as a function of tube material. *Otolaryngol Head Neck Surg* 1999;**120**:621–7
26 Licameli G, Johnston P, Luz J, Daley J, Kenna M. Phosphorylcholine-coated antibiotic tympanostomy tubes: are post-tube placement complications reduced? *Int J Pediatr Otorhinolaryngol* 2008;**72**:1323–8
27 Cağavi F, Akalan N, Celik H, Gür D, Güçiz B. Effect of hydrophilic coating on microorganism colonization in silicone tubing. *Acta Neurochir (Wien)* 2004;**146**:603–10

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Dr C H Jang takes responsibility for the integrity of the content of the paper.
Competing interests: None declared