J Anesth (2003) 17:84–91

Journal of Anesthesia

© JSA 2003

Reduction of potential respiratory pathogens by oral hygienic treatment in patients undergoing endotracheal anesthesia

Minori Okuda[1], Yuzuru Kaneko[1], Tatsuya Ichinohe[1], Kazuyuki Ishihara[2], and Katsuji Okuda[2]

[1]Oral Health Science Center and Department of Dental Anesthesiology, Tokyo Dental College, 1-2-2 Masago, Chiba 261-8502, Japan
[2]Oral Health Science Center and Department of Microbiology, Tokyo Dental College, Chiba, Japan

Abstract

Purpose. This study was conducted to evaluate the usefulness of mechanical and chemical prophylactic oral cleansing treatments for reducing potential respiratory pathogens existing in the oral cavity.

Methods. Thirty-two patients scheduled to undergo oral and maxillofacial surgery that required endotracheal anesthesia were randomly allocated to one of the two groups, the oral cleansing group ($n = 16$) or the noncleansing group ($n = 16$). Culture and polymerase chain reaction (PCR) methods were used to detect and enumerate pathogens. Oral cleansing was carried out with an electric toothbrush capable of automatically supplying and aspirating povidone-iodine solution before surgery, followed by rinsing twice a day after surgery. Cephazolin ($3\,g \cdot day^{-1}$) was given to all patients for 5 days after surgery.

Results. The PCR detection rates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Porphyromonas gingivalis* in gargle samples before treatment were 87.5%, 68.8%, 53.1%, and 40.6%, respectively. Oral cleansing reduced the detection rates and numbers of methicillin-sensitive Staphylococcus species, *S. pneumoniae*, and *H. influenzae*. In contrast, there was no significant reduction of methicillin-resistant Staphylococcus species, *S. pneumoniae, H. influenzae*, or *P. aeruginosa* in subjects who underwent systemic cephazolin administration without oral cleansing.

Conclusion. The combination of mechanical and chemical oral cleansing resulted in a significant reduction of potential respiratory pathogens in the oral cavity.

Key words Respiratory pathogens · Ventilator-associated pneumonia · Oral prophylactic cleansing

Introduction

The prevention of ventilator-associated pneumonia in patients receiving endotracheal anesthesia is a major challenge, because this complication is frequent and, once present, carries a high morbidity and mortality, even with adequate treatment [1,2]. Infectious respiratory diseases such as pneumonia resulting from salivary bacterial aspiration during mechanical ventilation are common [3–6]. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, Staphylococcus species, and *Candida albicans* are found in samples from the human oral cavity as well as in nasopharyngeal secretions, and all of these bacteria have the potential to cause infectious respiratory diseases [7–10].

It is well known that various kinds of microorganisms form biofilms in which organisms are intimately associated with each other and the solid substratum through binding and inclusion within an ex-polymer matrix; such systems are widely distributed in nature and disease [11–15]. In the oral cavity, multispecies biofilms form well-ordered structures on dental plaque, the tongue, and other oral soft-tissue surfaces [16]. These biofilms may serve as reservoirs for respiratory pathogens, especially in higher-risk patients with poor oral hygiene [4,17–19]. Such biofilms are resistant to antibiotic and chemical attack [20–23]. Povidone-iodine (polyvinylpyrrolidone) is an effective broad-spectrum disinfectant that is widely used in medicine for topical applications [24,25].

The present study evaluated the effect of mechanical and chemical oral prophylactic cleansing using povidone-iodine solution on the reduction of the

Address correspondence to: M. Okuda
Received: September 5, 2002 / Accepted: January 15, 2003

biofilm-forming respiratory pathogens in patients receiving endotracheal anesthesia.

Materials and methods

A total of 32 patients classified as American Society of Anesthesiolosists Physical Status I or II (mean age and SD, 28.9 + 8.4 years; range, 19–55 years) at Chiba Hospital, Tokyo Dental College, who were scheduled to undergo oral surgery requiring endotracheal intubation, were enrolled after written informed consent had been obtained. None of the subjects had been given any antibiotic during the 3 weeks prior to the study. No finding of infectious disease, such as sinusitis or respiratory infections, was shown in the hemanalysis or the chest X-ray photograph. The patients underwent total intravenous anesthesia with propofol and fentanil. Nasotracheal intubation was performed on all patients. The Blue Line (Portex, Hythe Kent, UK) with a cuff was used. Five percent povidone-iodine solution (Isodine, Meiji, Tokyo) at an iodine concentration of $5\,mg \cdot ml^{-1}$ was used for preoperative decontamination of the oral cavity. After the operation, extubation was performed immediately, and the intubation time was not prolonged. All patients received nutritional support by nasogastric tube for 3 days after the operation and were not given antacids or H_2-blockers during the investigation period. All patients were given cephazolin ($3\,g \cdot day^{-1}$) starting 12 h before the induction of anesthesia until 5 days after surgery. The surgical procedures mainly consisted of orthognathic surgery; procedures involving infectious diseases were excluded. We enrolled 32 subjects and randomly assigned them to two groups: the cleansing group (n = 16) and the non-cleansing group (n = 16).

To collect samples for microbial studies, the patients gargled with 5-ml samples of distilled phosphate-buffered saline (PBS, pH 7.4) for 20 s on the day before surgery. For mechanical and chemical oral cleansing, we used an Electronic Toothbrush System (Dento-Erac 910, Ozkqa, Lion, Tokyo, Japan) capable of automatically supplying and aspirating solution. The cleansing, including brushing the teeth; scrubbing the periodontium, buccal mucosa, and tongue; and rinsing, was carried out 24 h before surgery using 200 ml of 0.5% povidone-iodine solution (Isodine-Gargle, Meiji, Tokyo, Japan) at an iodine concentration of $0.5\,mg \cdot ml^{-1}$. To avoid contamination of the gargle sample by povidione-iodine solution, all procedures were completed with a thorough rinse with tap water. Thirty minutes later, we examined the effect of cleansing on the total number of colony-forming-units (CFUs) of anaerobic organisms and *C. albicans* cells. All patients in the oral cleansing group performed oral rinsing with 50 ml of 0.5% povidone-iodine solution twice a day after surgery [24]. Seven days after surgery, between 9:00 and 10:00 A.M. and before oral rinsing with povidone-iodine solution, gargle samples were obtained for microbial examination.

After obtaining the gargle samples, we carried out the microbial analysis as a non-double-blind study. The samples were diluted with PBS in a gradient of 1:10 steps down to $1:10^{-5}$, and 100 μl of each dilution was inoculated onto duplicate trypticase soy agar plates (Becton Dickinson, Cockeysville, MD, USA) supplemented with hemin ($5\,\mu g \cdot ml^{-1}$), menadione ($0.5\,\mu g \cdot ml^{-1}$), and 10% defibrinated horse blood. These inoculated plates were incubated at 37°C for 1 week in an anaerobic chamber containing 10% CO_2, 10% H_2, and 80% N_2. The total CFUs of anaerobic bacteria in each sample were counted.

For detection of Staphylococcus species, a selective medium (No. 110 Medium, Difco Laboratories, Detroit, MI, USA) was used. For detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE), a selective medium containing $16\,g \cdot l^{-1}$ of phenol-red broth base (Difco), $10\,\mu g \cdot ml^{-1}$ of polymyxicin-B sulfate (Pfizer, Tokyo, Japan), and $6\,\mu g \cdot ml^{-1}$ of oxacillin sodium salt (Stacillin, Banyu Pharmaceutical, Tokyo, Japan) was used [26]. Colonies isolated from this selective medium were confirmed to harbor the *mec*A gene by polymerase chain reaction (PCR) [27]; their biochemical characteristics were examined, and then they were identified as MRSA and MRSE as described previously [28]. For detection of *P. aeruginosa* by examination of culture, we used a modified selective medium, Cetrimide agar (Nippon Pharmaceutical, Tokyo, Japan) as reported by Fonseca et al. [29] and Campbell et al. [30]. For detection of *C. albicans*, Candida GE agar (Nippon Pharmaceutical) was used. After examination of the biochemical characteristics of the colonies on selective medium, we identified *C. albicans* as described previously [28].

PCR was used to detect *S. pneumoniae* [31], *H. influenzae* [32], *P. aeruginosa* [33], *Porphyromonas gingivalis* [34], *Actinobacillus actinomycetemcomitans* [34], and *Chlamydia pneumoniae* [35]. The primers used in these reactions are listed in Table 1 and have been described in previous studies [31–35]. The reagents used in this experiment were purchased from Wako Pure Chemical Industries, Tokyo, Japan. Each 2-ml aliquot sample of gargled fluid was centrifuged at $10\,000 \times g$ for 15 min. The precipitate was resuspended by vortex mixing in 200 μl of 10 mM Tris-HCl buffer (pH 7.8) containing 5 mM EDTA, 0.5% sodium dodecylsulfate (SDS), and 80 μg of proteinase K and lysed by incubation at 56°C for 120 min. This was followed by phenol extraction and precipitation of DNA by ethanol. The DNA

Table 1. List of PCR primers used in this study

Species	Nucleotide sequence 5′→3′	No. of bases amplified
Streptococcus pneumoniae		
SP I	AGGATAAGGAACTGCG	
SP II	CTTATTTTCTGACCTTTCA	247
Haemophilus influenzae		
F 1	AACTTTTGGCGGTTACTCTG	
R 2	CTAACACTGCACGACGGTTT	351
Pseudomonas aeruginosa		
PAL 1	ATGGAAATGCTGAAATTCGGC	
PAL 2	CTTCTTCAGCTCGACGCGACG	504
Porphyromonas gingivalis		
PG I	ATAATGGAGAACAGCAGGAA	
PG II	TCTTGCCAACCAGTTCCATTGC	131
Actinobacillus actinomycetemcomitans		
Aa I	CAGCAAGCTGCACAGTTTGCAAA	
Aa II	CATTAGTTAATGCCGGGCCGTCT	238
Chlamydia pneumoniae		
Cpn A	TGACAACTGTAGAAATACAGC	
Cpn F	GGTTGAGTCAACGACTTAAGG	446
Staphylococcus aureus		
aureusF	AATCTTTGTCGGTACACGATATTCTTCACG	
aureusR	CGTAATGAGATTTCAGTAGATAATACAACA	422
Methicillin-resistant *Staphylococcus aureus*		
MSAF	AAAATCGATGGTAAAGGTTGGC	
MSAR	AGTTCTGCAGTACCGGATTTGC	533

was then dissolved in 20 µl of distilled water. PCR was performed using a GeneAmp PCR System 9700 (Perkin-Elmer, Foster City, CA, USA). The reaction mixture (50 µl) contained 1 µl of DNA template, 50 *p*M of primers, 1.25 U of Taq DNA polymerase (Takara, Shuzo, Shiga, Japan), 5 µl of 10× reaction buffer, and 200 µM of the four deoxynucleotide triphosphates supplied with Taq polymerase. For *S. pneumoniae*, *H. influenzae*, and *P. aeruginosa,* the total number of PCR cycles, denaturation, annealing, and extension followed the recommendations in our previous reports [28].

Five microliters of each of the amplified products were analyzed by electrophoresis in 2% agarose gel in 1 × TBE buffer (90 mM Tris-borate, 2 mM EDTA; pH 8.3) at 100 V for 40 min. A low-DNA Mass Ladder (Life Technologies, Gaithersburg, MD, USA) was used as the molecular size standard. The gel was stained with ethidium bromide (0.5 µg·ml^{-1}) and photographed under ultraviolet illumination with Polaroid film (Polaroid, St. Albans, UK). The optical density of the various bands was quantified with Kodak Digital Science 1D Image Analysis Software (Eastman Kodak, Rochester, NY, USA).

The differences in the rates of detection in cultures before and after surgery were evaluated by the chi-square test. One-way analysis of variance was used to compare the numbers of CFUs before and after oral cleansing and 7 days postoperatively.

Results

There were no significant differences in age or sex between the cleansing and the noncleansing groups. The detection ratios of microorganisms examined by PCR and culture methods before the cleansing treatment are summarized in Fig. 1. We found a high prevalence (87.5%) of *S. pneumoniae* in gargle samples from 32 patients undergoing surgery under endotracheal anesthesia. The DNA sequences of the amplified *S. pneumoniae* spacer regions of the 16S and 23S rRNA genes were confirmed to be identical to those of this species. The PCR detection rates of *H. influenzae*, *P. aeruginosa*, and *P. gingivalis* were 68.8%, 53.1%, and 40.6%, respectively. Neither *A. actinomycetemcomitans* nor *C. pneumoniae* was detected in any of the gargle samples by PCR.

The culture detection rates of *S. aureus*, MRSA, *S. epidermidis*, and MRSE in gargle samples from 32 patients before surgery were 34.4%, 9.4%, 56.3%, and 15.6%, respectively. We confirmed that the identified MRSA and MRSE possessed the *mec*A gene. The detection rates of *P. aeruginosa* and *C. albicans* by the culture method were 15.6% and 28.1%, respectively. The detection rates of *P. aeruginosa* by the culture method were lower than those by PCR ($P = 0.068$), but the differences were not significant.

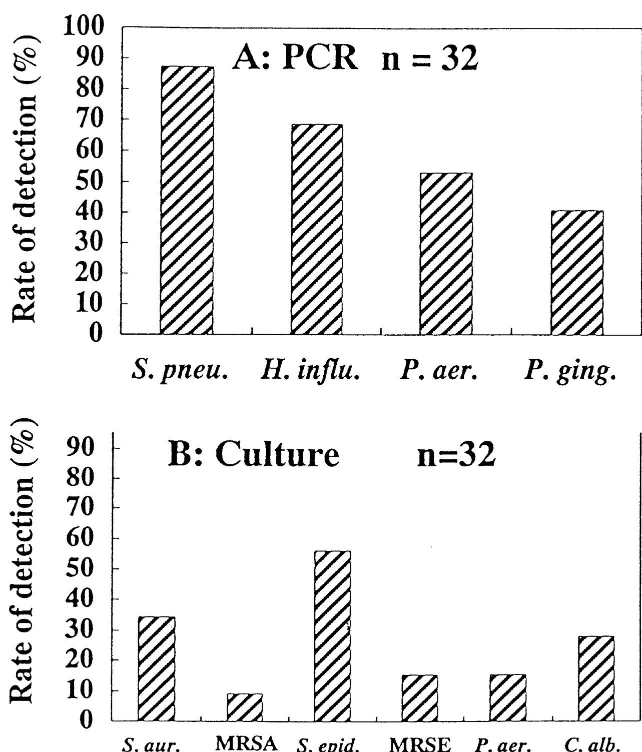


Fig. 1. Detection rates of (**A**) *Streptococcus pneumoniae* (*S. pneu.*), *Haemophilus influenzae* (*H. influ.*), *Pseudomonas aeruginosa* (*P. aer.*), and *Porphyromonas gingivalis* (*P. ging.*) by the PCR method and of (**B**) *Staphylococcus aureus* (*S. aur.*), methicillin-resistant *S. aureus* (*MRSA*), *S. epidermidis* (*S. epid.*), methicillin-resistant *S. epidermidis* (*MRSE*), and *Candida albicans* (*C. alb.*) examined by culture methods in gargle samples obtained from 32 patients before mechanical and chemical prophylactic oral cleansing. Neither *Actinobacillus actinomycetemcomitans* nor *Chlamydia pneumoniae* was detected by PCR

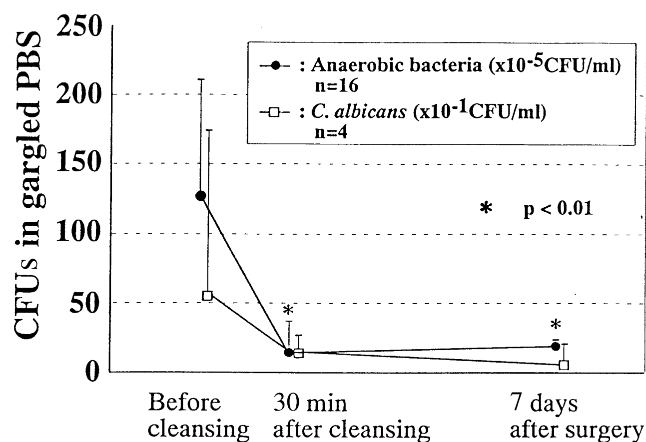


Fig. 2. Effects of mechanical and chemical prophylactic oral cleansing with povidone-iodine solution on viable cell numbers of oral anaerobic bacteria and *C. albicans* in gargle samples obtained from 16 patients. Mean number of colony-forming units (CFUs) with standard deviations examined before the cleansing treatment, 30 min after cleansing, and 7 days after surgery. * $P < 0.01$ vs. before cleansing. PBS, phosphate-buffered saline

The means and standard deviations of the number of anaerobic bacterial CFUs grown on blood agar plates and CFUs of *C. albicans* on Candida GE medium obtained before cleansing, 30 min, after cleansing, and 7 days after surgery from 16 patients are shown in Fig. 2. The average number of CFUs in the gargle samples obtained after oral cleansing using povidone-iodine solution were significantly reduced 30 min after cleansing and 7 days after surgery ($P < 0.01$). We detected *C. albicans* cells in only 4 samples from the 16 patients. Reduction in the mean number of *C. albicans* was also noted 30 min after cleansing ($P = 0.108$) and 7 days after surgery ($P = 0.068$), but these reductions were not significant.

The oral cleansing effects on microorganisms examined by PCR and culture methods are summarized in Fig. 3. We compared pre- and posttreatment findings among 16 subjects in a nonrandomized study. It was found by the PCR method that the combination of mechanical and chemical oral prophylactic cleansing resulted in significant reductions in the detection rates of *S. pneumoniae* ($P < 0.05$) and *H. influenzae* ($P < 0.05$), but not of *P. aeruginosa*. Culturing demonstrated that the treatment including cephazolin administration reduced both methicillin-sensitive *S. aureus* ($P < 0.01$) and *S. epidermidis* ($P < 0.05$), but not MRSA, MRSE, *P. aeruginosa*, or *C. albicans*. However, no reduction in *S. pneumoniae*, *H. influenzae,* or *P. aeruginosa* was found by the PCR method in the group taking cephazolin for 5 days without oral prophylactic cleansing (Fig. 4). Cephazolin administration without the oral cleansing treatment significantly reduced methicillin-sensitive *S. epidermidis* ($P < 0.01$). However, we unexpectedly found increases in the detection rates of *P. aeruginosa* ($P = 0.068$) and *C. albicans* ($P < 0.05$) in the group without oral cleansing.

The densities of amplified DNA bands of *S. pneumoniae* in samples obtained before prophylactic oral cleansing were compared with those obtained 7 days after the cleansing treatment (Fig. 5). The net intensity ranged from 0 to 87719 in the samples examined. As shown in Fig. 5, prophylactic oral cleansing resulted in either an intensity of 0 or a decreased net intensity of *S. pneumoniae* PCR bands in gargle samples obtained from 16 patients 7 days after surgery. However, there was no significant reduction of the intensity in 11 of 16 patients who had not undergone mechanical and chemical oral cleansing treatment. There was no diagnosis of postoperative infectious disease, including respiratory infection, in any of the 32 patients.

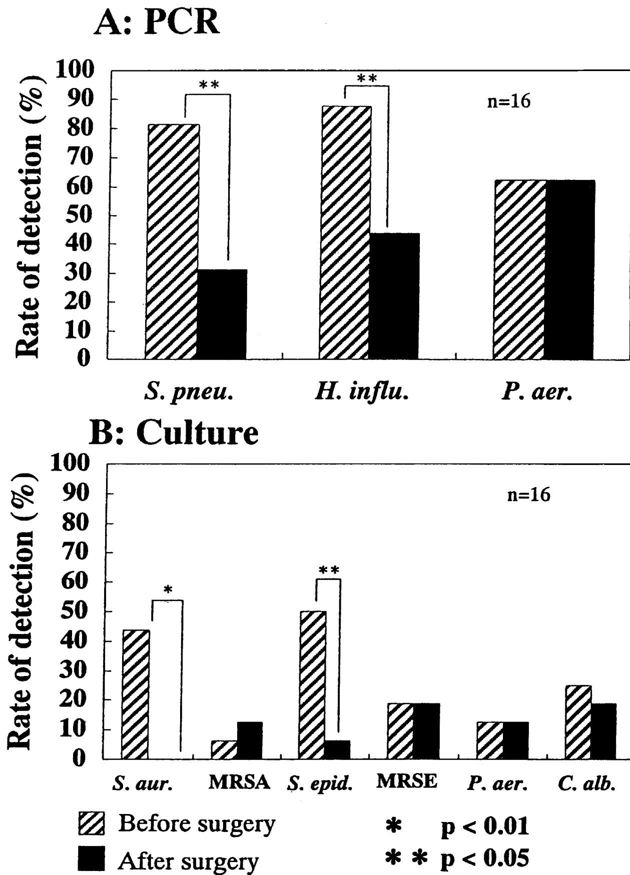


Fig. 3. Comparison of detection rates between gargle samples obtained from 16 patients before mechanical and chemical prophylactic oral cleansing and those obtained 7 days after the cleansing treatment. **A** *S. pneumoniae* (*S. pneu.*), *H. influenzae* (*H. influ.*), *P. aeruginosa* (*P. aer.*), and *P. gingivalis* (*P. ging.*) were examined by the PCR method. **B** *S. aureus* (*S. aur.*), *MRSA*, *S. epidermidis* (*S. epid.*), *MRSE*, and *C. albicans* (*C. alb.*) were examined by culture methods

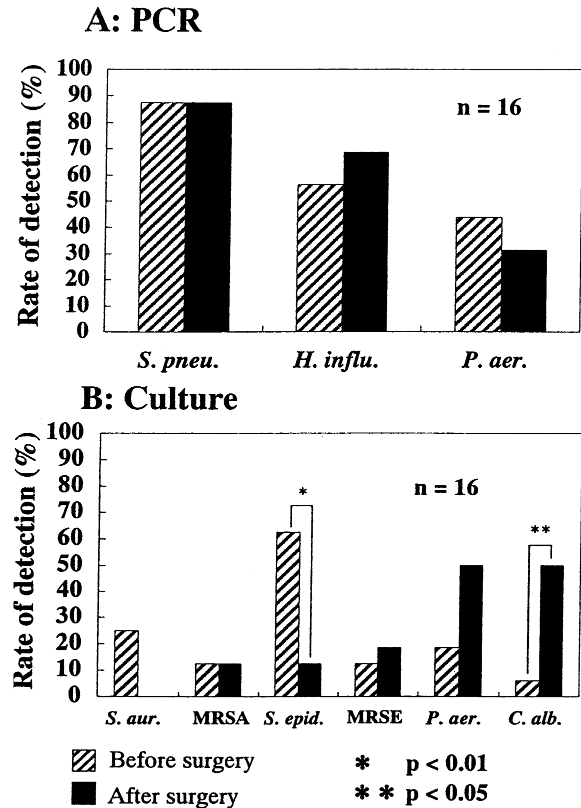
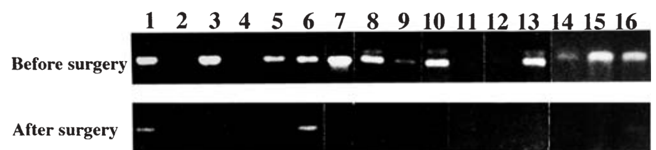


Fig. 4. Comparison of detection rates between gargle samples obtained from 16 patients who had not received any chemical oral cleansing treatment before surgery and those obtained from the same group 7 days after surgery. **A** *S. pneumoniae* (*S. pneu.*), *H. influenzae* (*H. influ.*), *P. aeruginosa* (*P. aer.*), and *P. gingivalis* (*P. ging.*) were examined by the PCR method. **B** *S. aureus* (*S. aur.*), *MRSA*, *S. epidermidis* (*S. epid.*), *MRSE*, and *C. albicans* (*C. alb.*) were examined by culture methods

A: Mechanical and chemical cleansing group



B: Non-cleansing group

Fig. 5. Comparison of densities of amplified *S. pneumoniae* DNA bands between the gargle samples obtained before and after surgery in the mechanical and chemical prophylactic oral cleansing group (**A**) and in the noncleansing group (**B**)

Discussion

Pneumonia that develops within 48 h in critically ill patients under mechanical ventilation is thought to be associated with the ventilation process [3,7,8,36]. It has been reported that poor oral hygiene and severe periodontal disease increase the colonization of oral bacteria in the oropharynx and lung [17,18,28]. In the present study, we found high detection rates of *S. pneumoniae*, *H. influenzae*, and *P. aeruginosa* by the PCR method in gargle samples from patients undergoing oral and maxillofacial surgery that required endotracheal intubation. In our previous study, we found a high prevalence of *S. pneumoniae* in gargle samples from healthy young and elderly persons [28]. To confirm the presence of *S. pneumoniae* in these gargle samples, we examined them by an immunofluorescence method. Fluorescent cells stained with antiserum against *S.*

pneumoniae (Biogenesis, Poole, UK) were found only in strongly PCR-positive gargle samples (data not shown).

More than 500 bacterial species are found in the human oral cavity [37]. *S. pneumoniae* is a member of the *Streptococcus mitis* group that is predominant in the human oral cavity. Haemophilus species are also endogenous in the human oral cavity. We used the PCR method, which can detect low cell numbers of both species in gargle samples. In this study, we first tried to detect and enumerate *S. pneumoniae* and *H. influenzae* by culture methods. Although we isolated many colonies, we could not identify most isolates as *S. pneumoniae* or *H. influenzae*. It is possible that various oral bacterial species interact competitively with each other in an attempt to survive and prevent other bacterial growth [38,39]. These antagonistic actions make it difficult to detect and enumerate *S. pneumoniae* and *H. influenzae* species by culture methods.

We detected Staphylococcus species, including MRSA and MRSE, *C. albicans,* and *P. aeruginosa* by culture methods. These microorganisms are known to form biofilms and to be resistant to many antibiotics [11–13,23,40,41]. Oral microorganisms grown in biofilms have also been shown to be more resistant to various antibacterial agents than their planktonic counterparts [16,23,42]. We did not find any significant reductions in *S. pneumoniae*, *H. influenzae*, *P. aeruginosa*, MRSA, or MRSE in gargle samples obtained from surgical patients administered systemic cephazolin for 5 days without undergoing mechanical and chemical oral prophylactic cleansing with povidone-iodine solution. Unexpectedly, the detection rates of *P. aeruginosa* and *C. albicans* after cephazolin administration were higher than those before surgery. These results indicated that systemic cephazolin administration for 5 days was not effective in elimination of either microorganism and might have induced superinfection by *P. aeruginosa* and *C. albicans* in some patients.

Previous clinical trials have examined the possibility of eliminating nosocomial respiratory pathogens by topical antimicrobial prophylaxis rather than systemic antibiotic administration. Pugins et al. [2] demonstrated, by a randomized, placebo-controlled, double-blind clinical trial, that topical oropharyngeal antibiotic application of polymyxin B, neomycin sulfate, and vancomycin hydrochloride produced a significant reduction in aerobic gram-negative bacterial colonization and ventilator-associated pneumonia. Rodriguez-Roldan et al. [43] also showed that nosocomial pneumonia, which is a frequent complication in critically ill patients on mechanical ventilation, could be prevented by local application of nonabsorbable antibiotics containing tobramycin, amphotericin B, and polymyxin E to the oropharynx. In addition, DeRiso et al. [44] investigated whether a chlorhexidine oral rinse would be useful in preventing pneumonia in patients scheduled for heart surgery. The results showed a 65% reduction in pneumonia, which included a significant reduction in respiratory infections caused by bacteria. The authors concluded that the use of chlorhexidine might have fewer side effects than broad-spectrum antibiotic administration in selective decontamination for the prevention of pneumonia. Bergmans et al. [45] showed the significance of oral decontamination in the prevention of nosocomial infections in critically ill patients and of ventilator-associated pneumonia. In the present study, we found that mechanical and chemical prophylactic oral cleansing using povidone-iodine effectively reduced the numbers of anaerobic bacteria. This oral hygienic treatment also resulted in the elimination or reduction of *S. pneumoniae* and *H. influenzae*. The detection rates of *H. influenzae* before cleansing with mechanical and chemical oral hygienic treatment were slightly higher than those in the noncleansing group, but the difference was not significant by the chi-square test. However, further analysis in the larger series is essential to completely eliminate sampling bias.

In conclusion, mechanical and chemical oral cleansing using povidone-iodine appears to have been effective in eliminating these microorganisms in oral biofilms such as dental plaque. All the patients in the oral cleansing group carried out oral rinsing with povidone-iodine twice a day after surgery. It is possible that continuous oral cleansing with povidone-iodine solution could effectively eliminate these pathogens from the oral cavity. In fact, we and other groups have shown that continuous professional mechanical cleansing did reduce the cell numbers of potential respiratory pathogens [28,45–47]. Based on these findings, we emphasize that mechanical and chemical prophylactic cleansing of the oral cavity with povidone-iodine is an effective protocol for reducing potential respiratory pathogens in patients undergoing endotracheal anesthesia.

Acknowledgments. We thank Dr. B.L. Pierce for assistance with the English of this manuscript. This study was supported in part by a grant from the Oral Health Center, Tokyo Dental College.

References

1. Andrews CP, Coalson JJ, Smith JD, Johanson WG Jr (1981) Diagnosis of nosocomial bacterial pneumonia in acute, diffuse lung injury. Chest 80:254–258
2. Pugin J, Auckenthaler R, Lew DP, Suter PM (1991) Oropharyngeal decontamination decreases incidence of ventilator-associated pneumonia. A randomized, placebo-controlled, double-blind clinical trial. JAMA 265:2704–2710

3. Estes RJ, Meduri GU (1995) The pathogenesis of ventilation-associated pneumonia: I. Mechanisms of bacterial transcolonization and airway inoculation. Intensive Care Med 21:365–383
4. Scannapieco FA, Stewart EM, Mylotte JM (1992) Colonization of dental plaque by respiratory pathogens in medical intensive care patients. Crit Care Med 20:740–745
5. Marik PE (2001) Aspiration pneumonitis and aspiration pneumonia. N Engl J Med 344:665–671
6. Levine SA, Niederman MS (1991) The impact of tracheal intubation on host defenses and risks for nosocomial pneumonia. Clin Chest Med 12:523–543
7. Van Uffelen R, Rommes JH, Van Saene HKF (1987) Preventing lower airway colonization and infection in mechanically ventilated patients. Crit Care Med 15:99–102
8. Allaouchiche B, Jaumain H, Chassard D, Bouletreau P (1999) Gram stain of bronchoalveolar lavage fluid in the elderly diagnosis of ventilator-associated pneumonia. Br J Anaesth 83:845–849
9. Mimoz O, Karim A, Mazoit JX, Edouard A, Leprince S, Nordmann P (2000) Gram staining of protected pulmonary specimens in the elderly diagnosis of ventilator-associated pneumonia. Br J Anaesth 85:735–739
10. Finegold SM (1999) Aspiration pneumonia. Rev Infect Dis 13: S737–742
11. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ (1987) Bacterial biofilms in nature and disease. Annu Rev Microbiol 41:435–464
12. Costerton JW, Lewandowski Z, DeBeer D, Caldwell D, Korber D, James G (1994) Biofilms, the customized microniche. J Bacteriol 176:2137–2142
13. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284: 1318–1322
14. Whitehead NA, Bernard AML, Slater H, Simpson NJL, Salmond GPC (2001) Quorum-sensing in gram-negative bacteria. FEMS Microb Rev 25:364–404
15. Miller MB, Bassler B (2001) Quorum-sensing in bacteria. Annu Rev Microbiol 55:165–199
16. Kolenbrander PE (2000) Oral microbial communities: biofilms, interactions, and genetic systems. Annu Rev Microbiol 54:413–437
17. Scannapieco FA, Papandonatos GD, Dunford RG (1998) Associations between oral conditions and respiratory disease in a national sample survey population. Ann Periodontol 3:251–256
18. Russell SL, Boylan RJ, Kaslick RS (1999) Respiratory pathogen colonization of dental plaque of institutionalized elders. SCD Special Care in Dentistry 19:128–134
19. Gilbert P, Das J, Foley I (1997) Biofilm susceptibility to antimicrobials. Adv Dent Res 11:160–167
20. Kobayashi H (2001) Airway biofilm disease. Int J Antimicrob Agents 17:351–356
21. Budhani PK, Struthers JK (1998) Interactions of *Streptococcus pneumoniae* and *Moraxella catarrahalis*: investigation of the indirect pathogenic role of beta-lactamase-producing moraxellae by use of a continuous-culture biofilm system. Antimicrob Agents Chemother 42:2521–2526
22. Xu KD, McFeters GA, Stewart PS (2000) Biofilm resistance to antimicrobial agents. Microbiology 146:547–549
23. Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34–39
24. Addy M, Griffiths C, Isaac R (1997) The effect of povidone-iodine on plaque and salivary bacteria. A double-blind crossover trial. J Periodontol 48:730–732
25. Okuda K, Adachi M, Iijima K (1998) The efficacy of antimicrobial mouth rinses in oral health care. Bull Tokyo Dent Coll 39:7–14
26. Van Enk R, Tompson KD (1992) Use of primary isolation medium for recovery of methicillin–resistant Staphylococcus. J Clin Microbiol 30:504–505
27. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S (1996) Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 29:2240–2244
28. Abe S, Ishihara K, Okuda K (2001) Prevalence of potential respiratory pathogens in the mouth of elderly patients and effects of professional oral care. Arch Gerontol Geriatr 32:45–55
29. Fonseca K, MacDougall J, Pitt T (1986) Inhibition of *Pseudomonas aeruginosa* from cystic fibrosis by selective media. J Clin Pathol 39:220–222
30. Campbell ME, Farmer SW, Speert DP (1988) New selective medium for *Pseudomonas aeruginosa* with phenanthroline and 9-chloro-9 [4-(diethylamino)Phenyl]-9, 10-dihydro-10-phenylacridine hydrochloride (C-390). J Clin Microbiol 26:1910–1912
31. Saruta K, Matsunaga T, Hoshina S, Kono M, Kitahara S, Kanemoto S, Sakai O, Machida K (1995) Rapid identification of *Streptococcus pneumoniae* by PCR amplification of ribosomal DNA spacer region. FEMS Microbiol Lett 132:165–171
32. Ueyama T, Kurono Y, Shirabe K, Takeshita M, Mogi G (1995) High incidence of *Haemophilus influenzae* in nasopharyngeal secretions and middle ear effusions as detected by PCR. J Clin Microbiol 33:1835–1838
33. De Vos D, Lim A Jr, Pirnay JP, Struelens M, Vandenvelde C, Duinslaeger L, Vandelkelen A, Cornelis P (1997) Direct detection and identification of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes. *oprI* and *oprL*. J Clin Microbiol 35:1295–1299
34. Watanabe K, Frommel TO (1996) *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans,* and *Treponema denticola* detection in oral plaque samples using the polymerase chain reaction. J Clin Periodontol 23:212–219
35. Jantos CA, Roggendorf R, Wuppermann FN, Hegemann JH (1998) Rapid detection of *Chlamydia pneumoniae* by PCR-enzyme immunoassay. J Clin Microbiol 36:1890–1894
36. Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE, Moore JE, Kerr JR, Curran MD, Hogg G, Webb CH, McCarthy GJ, Milligan KR (1999) Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med 25:1072–1076
37. Moore WEC, Moore HLV (1994) The bacteria of periodontal diseases. Periodontology 2000 5:66–77
38. Takazoe I, Nakamura T, Okuda K (1984) Colonization of subgingival area by *Bacteroides gingivalis*. J Dent Res 63:422–427
39. Ishihara K, Miura T, Kimizuka R, Ebihara Y, Mizuno Y, Okuda K (1997) Oral bacteria inhibit the *Helicobacter pylori* growth. FEMS Microbiol Lett 159:355–361
40. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA (2001) Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol 183:5385–5394
41. Wilson M (1996) Susceptibility of oral bacterial biofilms to antimicrobial agents. J Med Microbiol 44:79–87
42. Roberts MC (1998) Antibiotic resistance in oral/respiratory bacteria. Crit Rev Oral Biol Med 9:522–540
43. Rodriguez-Roldan JM, Altuna-Cuesta A, Lopez A, Carrillo A, Garcia J, Leon J, Martinez-Pelles AJ (1990) Prevention of nosocomial lung infection in ventilated patients: use of an antimicrobial pharyngeal nonabsorbable paste. Crit Care Med 18: 1239–1242
44. DeRiso AJ 2nd, Ladowski JS, Dillon TA, Justice JW, Peterson AC (1996) Chlorhexidine gluconate 0.12% oral rinse reduces the incidence of total nosocomial respiratory infection and non-prophylactic systemic antibiotic use in patients undergoing heart surgery. Chest 18:1556–1561
45. Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van der Geest S, van Tiel FH, de Leeuw PW, Stobberingh EE (2001) Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. Am J Respir Crit Care Med 16:382–388

46. Fourrier F, Cau-Pottier E, Boutingny H (2000) Effect of dental plaque antiseptic decontamination on bacterial colonization and nosocomial infections in critically ill patients. Intensive Care Med 26:1239–1247
47. Adachi M, Ishihara K, Abe S, Okuda K, Ishikawa T (2002) Effect of professional oral health care on elderly living in nursing homes. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics 94: 191–195