

## Laboratory and Epidemiology Communications

# Controlling Opportunistic Pathogens in the Oral Cavity of Preschool Children by the Use of 3DS

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Dental caries and periodontal disease are two major diseases that affect the oral cavity. *Streptococcus mutans* and *Lactobacillus* are known as major pathogens for dental caries, while *Porphyromonas gingivalis* and *Actinobacillus actinomycetem-comitans* have been shown to be involved with periodontal disease. Other types of bacterial infection also have clinical significance in aged groups (1) predisposing them to serious systemic infection (2). *Staphylococcus aureus* may cause pneumonia in older veterans (3).

Though data on the prevalence of oral bacteria in the elderly (4-6) are available, such data regarding young people are few. In the present study, we focused on opportunistic pathogens in the oral cavities of preschool children.

Deciduous teeth are replaced by permanent teeth generally at the age of 5 to 16. During this period, immunological activities against pathogens as well as mineralization on tooth surfaces develop. Formation of biofilm by pathogenic microbes on surfaces in the oral cavity and changes in microflora may take place and cause systemic diseases (7) such as endocarditis (8) and rheumatic diseases (9).

In this study, 645 children during medical check-ups prior to entrance to elementary school were surveyed for their salivary levels of *S. mutans* and *Lactobacillus*. Informed consent was obtained from the parents prior to the survey. Salivary levels greater than 10<sup>5</sup> CFU/ml saliva for *S. mutans* and greater than 10<sup>3</sup> CFU/ml saliva for *Lactobacillus* were considered to indicate a high risk for dental caries (10). Forty-seven of the subjects fulfilling these criteria were then randomly selected for treatment with the Dental Drug Delivery System (3DS) to eliminate the opportunistic bacteria (11, 12).

The 3DS method consists of individual trays, called drug retainers, that fit onto teeth and deliver small amounts of anti-bacterial drugs. After the oral biofilm was removed by physical treatment, known as Professional Mechanical Tooth Cleaning (PMTc), a commercially available 10% povidone iodine gel (ISODINE GEL: Meijiseika Co. Ltd., Tokyo) was applied to the dentition using a drug retainer for 3 min. Any gel remaining on the tooth surfaces or in the inter-dental spaces was removed

by rinsing with water and hand flossing. The subjects were then advised not to eat or drink for 2 h after the treatment. Following 3DS, all subjects were provided with a new toothbrush to prevent re-infection by *S. mutans*, and were also given instructions regarding the application of a commercially available 0.4% povidone iodine (Isogin nodo flesh: Meijiseika) by drug retainer for 5 min after tooth brushing at night. This home care was continued for 1 month.

Dental plaque samples were obtained at a private dental office following the home care period. The plaque samples were placed in transport fluid (0.4% agar, 0.15% thioglycollate/phosphate buffered saline) and taken to Bio Medical Laboratory (Tokyo) for analysis. There, each sample was poured directly onto chocolate agar, blood agar, OPA staphylococcus, and drigalski agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo) using a stick. The plates were incubated in an atmosphere of 5% CO<sub>2</sub> in H<sub>2</sub> at 37°C for 24-48 h. Representative microbial colonies from each plate were gram stained and isolated by identification of their characteristic appearance, as well as by their hemolytic, catalytic, and oxidase reactions (13). Those species found in a majority of the subjects were suspended in 1 ml of 0.5% saline, gently shaken, and tested using microbial detection kits. The following bacteria and fungi were identified in the detection plates: *S. aureus* (methicillin sensitive [MSSA] and resistant [MRSA]) by PS latex, rabbit plasma, and MRSA screening plates (Nippon Becton Dickinson); *Pseudomonas* sp. by VITEK (BioMérieux Vitek Japan [BVJ], Tokyo);  $\beta$ -hemolytic streptococcus by a Seroidenstrepto kit (Eiken Chemical Co., Ltd., Tokyo), API strepto (BVJ), and VITEK; *S. pneumoniae* by a Streptococcus identification disk (Nippon Becton Dickinson); *Haemophilus influenzae* by a Haemophilus ID4 plate (Nippon Becton Dickinson); *Serratia marcescens* by VITEK; and *Candida* sp. by a Candida check (Iatron Laboratories Inc., Tokyo). The levels of detection for each organism were determined according to the manufacturer's instructions.

As shown in Table 1, among the 47 subjects investigated, 21 (44.7%) had species of bacteria that were not designated as a part of normal flora (14). The detection rates of these bacteria were higher in higher grade children (data not shown). Three species, *Haemophilus* sp., *Enterobacter* sp., and *Candida*

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Table 1. Opportunistic pathogens detected before and after 3DS treatment with 10% povidone iodine gel

	Before treatment		After treatment	
	n	%	n	%
<i>Haemophilus</i> sp.	7	14.9	0	0.0
<i>Enterobacter</i> sp.	6	12.8	0	0.0
<i>Candida</i> sp.	4	8.5	0	0.0
<i>Serratia</i> sp.	1	2.1	0	0.0
<i>S. aureus</i> (MSSA)	2	4.3	4	8.5
<i>S. pyogenes</i>	3	6.4	2	4.3
<i>Flavobacterium</i> sp.	1	2.1	1	2.1
<i>Klebsiella</i> sp.	1	2.1	1	2.1
<i>Pseudomonas</i> sp.	1	2.1	4	8.5
<i>P. aeruginosa</i>	1	2.1	1	2.1
<i>Acinetobacter</i> sp.	0	0.0	1	2.1
<i>M(B). catarrhalis</i>	0	0.0	1	2.1

sp., that showed high frequencies in plaque samples were eliminated by the 3DS treatment, though other species was not affected by the treatment.

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