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Isolation of Opportunistic Pathogens in Dental Plaque, Saliva and Tonsil Samples from Elderly

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The oral cavity is a potential reservoir of pathogenic microorganisms (1-3). In the elderly, oral hygiene is poor (4) and dental biofilm containing opportunistic pathogens forms on tooth surfaces (4-6). Oral pathogens are risk factors for bacterial pneumonia as they may be aspirated into the respiratory tract (6,7). Pathogenic bacteria in the biofilm in the dental plaque might also be released to colonize new surfaces, e.g., the tonsils, pharynx, and respiratory tract. To test these possibilities, we studied the isolation frequencies of opportunistic bacteria on tooth and tonsil surfaces, and in saliva in the elderly.

One hundred twenty-five elderly people (mean age: 73.0 ± 0.3 years old, 66 males and 59 females) from Niigata City in Japan participated in this study, which was conducted in July, 2000. None of the subjects was hospitalized or institutionalized, and all came to the examination center either by themselves or accompanied by their family. They were in good general health and did not require special assistance for their daily activities. Informed consent was obtained from each subject prior to the study. Ethical clearance for the study's methods used was obtained from the Ethics Committee of the Faculty of Dentistry, Niigata University.

Supragingival plaque was collected from the upper right second premolar and first molar teeth by swabbing back and forth with a cotton swab (SEEDSWAB No. 1: Eiken Chemical Co., Ltd. [Eiken], Tokyo). In the case of subjects using full dentures, samples were collected from the same regions of the dentures after removal from the oral cavity. From subjects not having any of the above-mentioned teeth, samples were obtained from the opposite teeth. The subjects were asked to chew paraffin gum for 3 min to stimulate secretion of saliva, which was collected into ice-chilled sterile bottles using a cotton swab. Samples from the tonsils were also collected using a cotton swab. All specimens were transferred into 1 ml of reduced transport fluid medium (0.4% agar, 0.15% thioglycollate/phosphate buffered saline) in sterile bottles on ice and processed within one night of collection. The entire samples in tubes were inoculated onto chocolate agar, blood agar, OPA staphylococcus, and drigalski agar plates (Nippon Becton Dickinson Co., Ltd., Tokyo) with an aid of a stick. The plates were incubated in an atmosphere of 5% CO_2 in H_2 at 37°C for 24-48 h. Representative microbial colonies from each plate were gram stained and identified in terms of their characteristic appearance, hemolytic, catalytic reaction, and oxidase reaction (8). Colonies of microbes suspected to be those responsible for pneumonia and which were found in a majority of the subjects were suspended in 1 ml of 0.5% saline and tested by using diagnosis kits indicated below (9). We identified *Staphylococcus aureus*, both methicillin sensitive (MSSA) and resistant (MRSA) strains, by using PS latex, rabbit plasma, and MRSA screening plates (Nippon Becton

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Dickinson); *Pseudomonas* sp. by using VITEK (BioMérieux Vitek Japan [BVJ], Tokyo); β-hemolytic streptococcus by using a Seroidenstrepto kit (Eiken), API strepto (BVJ), and VITEK; *Streptococcus pneumoniae* by using a Streptococcus identification disk (Nippon Becton Dickinson); *Haemophilus influenzae* by using a Haemophilus ID4 plate (Nippon Becton Dickinson); *Serratia marcescens* by using VITEK; and *Candida* sp. by using Candida check (Iatron Laboratories Inc., Tokyo).

The isolation frequencies of microorganisms from retrieved from dental plaque, saliva, and tonsils, as determined using the manufacturers' instruction, are shown in Table 1. *C. albicans*, *Enterobacteriaceae*, *Pseudomonas* sp., *S. aureus*, *Xanthomonas maltophilia*, *Klebsiella pneumoniae*, *S. marcescens*, coagulase-negative staphylococci (CNS) and *K. oxytoca* were most frequently isolated from these specimens. The higher prevalence of these microorganisms in elderly subjects indicates that this age population is at greater risk of developing systemic diseases such as pneumonia and heart disease (6). Near 80% of the opportunistic pathogens isolated from dental plaque were found in saliva or tonsils, while 63-64% of the microbes present in saliva were present in dental plaques or tonsils (Table 2). This may indicate that these organisms might be released from tooth surfaces into saliva

Table 1. Isolation frequency of microbial pathogens in dental plaque, saliva, and tonsil samples from older adults

Microorganisms	Dental plaque $n = 125$ No. (%)	Saliva $n = 125$ No. (%)	Tonsils $n = 125$ No. (%)
Enterobacter cloacae	13 (11)	23 (19)	20 (16)
Enterobacter aerogenes	2 (2)	3 (3)	6 (5)
Enterobacter sazakii	0 (0)	2 (2)	0 (0)
Enterobacter sp.	5 (4)	7 (6)	7 (6)
Enterobacter agglomerans	4 (3)	7 (6)	4 (3)
Enterococcus faecalis	4 (3)	5 (4)	9 (8)
Enterococcus sp.	0 (0)	2 (2)	1 (1)
Escherichia coli	1 (1)	4 (3)	2 (2)
Eikenella corrodens	1 (1)	0 (0)	0 (0)
Klebsiella pneumoniae	9 (7)	29 (23)	13 (11)
Klebsiella oxytoca	4 (3)	6 (5)	6 (5)
Klebsiella ozaenae	0 (0)	0 (0)	1 (1)
Kluyvera sp.	1 (1)	3 (2)	1 (1)
Candida albicans	18 (14)	21 (17)	1 (1)
Candida parapsilosis	0 (0)	1 (1)	1 (1)
Candida tropicalis	0 (0)	1 (1)	0 (0)
Corynebacterium sp.	7 (6)	1 (1)	2 (2)
Citrobacter freundii	3 (3)	3 (3)	1 (1)
Comamonas acidovoran	3 (3)	1 (1)	1 (1)
Pseudomonas sp.	5 (4)	3 (3)	5 (4)
Pseudomonas fluorescens	3 (3)	6 (5)	5 (4)
Pseudomonas putida	1 (1)	3 (3)	5 (4)
Pseudomonas aeruginosa	1 (1)	1 (1)	0 (0)
Pseudomonas cepacia	1 (1)	0 (0)	0 (0)
Flavobacterium sp.	0 (0)	1 (1)	0 (0)
Flavobacterium indolgens	10 (8)	6 (5)	5 (4)
Flavobacterium meningosepticum	0 (0)	0 (0)	1 (1)
Staphylococcus aureus (MSSA)	3 (3)	5 (4)	13 (11)
Staphylococcus aureus (MRSA)	5 (4)	7 (6)	12 (10)
Staphylococcus aureus (CNS)	6 (5)	3 (3)	2 (2)
Streptococcus agalactiae	0 (0)	4 (3)	4 (3)
α-hemolytic streptococcus	106 (85)	111 (88)	101 (81)
β-hemolytic streptococcus	0 (0)	1 (2)	0 (0)
β-hemolytic streptococcus non group A	0 (0)	1 (2)	0 (0)
γ-hemolytic streptococcus	66 (53)	74 (58)	77 (61)
Neisseria sp.	85 (68)	78 (63)	67 (54)
Acinetobacter sp.	3 (3)	5 (4)	1 (1)
Acinetobacter calcoaceticus	3 (3)	10 (8)	5 (4)
Acinetobacter lwoffii	1 (1)	2 (2)	2 (2)
Alcaligenes xylosoxydans	1 (1)	2 (2)	1 (1)
Alcaligenes faecalis	0 (0)	1 (1)	0 (0)
Serratia marcescens	3 (3)	4 (3)	4 (3)
Serratia liquefaciens	4 (3)	6 (5)	6 (5)
Leclericia adecarboxylata	1 (1)	2 (2)	0 (0)
Haemophilus parainfluenzae	2 (2)	4 (3)	2 (2)
Edwardsiella sp.	0 (0)	1 (1)	0 (0)
Morxella sp.	0 (0)	1 (1)	0 (0)
Branhamella catarrhalis	0 (0)	1 (1)	1 (1)
Xanthomonas maltophilia	5 (4)	5 (4)	4 (3)

Table 2. Agreement rates of opportunistic pathogens isolated from dental plaque, saliva, and tonsil samples

	Dental plaque	Saliva	Tonsils
Dental plaque	–	79.8 ± 26.7*	78.0 ± 27.3
Saliva	63.7 ± 27.4	–	64.5 ± 26.7
Tonsil	71.8 ± 27.2	75.9 ± 27.5	–

Agreement rate: Number of opportunistic pathogens which was identical in both saliva and dental plaque, divided by the number of opportunistic pathogens isolated from dental plaque (saliva/dental plaque).
*: $P < 0.05$, saliva/dental plaque vs dental plaque/saliva.

and then colonize on oral cavity surfaces such as the tonsils. Professional oral hygiene to remove pathogens from dental biofilm may significantly reduce the risk of systemic diseases in elderly people.

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