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Reproducibility of Oral Bacterial Isolation in the Elderly

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Aspiration pneumonia is a major cause of death among elderly persons (1) because dysphagia and decreased cough reflex are often associated with aging. Oral bacterial flora is an important factor of the occurrence of aspiration pneumonia (2). Oral bacterial flora is composed of both aerobic and anaerobic bacteria species due to the complex structure of the oral cavity. Data regarding pathogenic aerobic bacteria in the oral cavity of the elderly (3) and the association of periodontal disease-causing anaerobic bacteria with pneumonia (4) have been reported. This report focuses on the detection rates of oral anaerobic bacteria measured at two different times in elderly subjects.

Thirty-three elderly people (73.4 ± 5.6 year-old in average), 12 males and 21 females, from Saitama Prefecture were enrolled in the present study which was conducted in July and August, 2001.

Samples were taken from dental plaque on the upper molar teeth or upper molar portions of dentures. The plaque samples were placed in transport fluid (0.4% agar, 0.15% thioglycollate/ phosphate buffered saline) and sent to Bio Medical Laboratory (Tokyo) for analysis. For the detection and identification of aerobic bacteria species, each sample was poured directly onto chocolate 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_2 in H_2 gas at 37°C for 24-48 h. Representative microbial colonies from each plate were examined for appearance, gram stain, hemolytic, catalytic, and oxidase reaction characters

Table 1. Detection rate of microbial pathogens in dental plaque from the elderly

(1) aerobic microorganisms

	No. positives at 1st exam (% total) (I)	No. positives at 2nd exam among positives at 1st exam (II)	II/I (%)
Candida sp.	14 (42.4)	7	7/14 (50.0)
Haemophilus parainfluenzae	13 (39.4)	4	4/13 (30.8)
Acinetobacter calcoaceticus	10 (30.3)	4	4/10 (40.0)
Corynebacterium sp.	7 (21.2)	2	2/7 (28.6)
Bacillus sp.	6 (18.2)	2	2/6 (33.3)
Enterobacter cloacae	4 (12.1)	0	0/4 (0)
Coagulase (-) *Staphylococcus*	4 (12.1)	1	1/4 (25.0)
Klebsiella pneumoniae	4 (12.1)	1	1/4 (25.0)

(2) anaerobic bacteria species

	No. positives at 1st exam (% total) (I)	No. positives at 2nd exam among positives at 1st exam (II)	II/I (%)
Capnocytophaga sp.	23 (69.7)	23	23/23 (100)
Prevotella melaninogenica	22 (66.7)	12	12/22 (54.5)
Prevotella oris	10 (30.3)	9	9/10 (90.0)
Fusobacterium nucleatum	10 (30.3)	9	9/10 (90.0)
Prevotella intermedia	7 (21.2)	7	7/7 (100)
Fusobacterium necrophorum	5 (15.2)	3	3/5 (60.0)
Prevotella denticola	3 (9.1)	3	3/3 (100)
Fusobacterium sp.	3 (9.1)	0	0/3 (0)

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(5). The bacteria identified were methicillin-sensitive (MSSA) and -resistant *Staphylococcus aureus* (MRSA), (detected by PS latex, rabbit plasma, and MRSA screening plates [Nippon Becton Dickinson]), *Pseudomonas* sp. (detected by VITEK [BioMerieux Vitek Japan, Tokyo]), *Haemophilus influenzae* (*H. influenzae*) (detected by a Haemophilus ID4 plate (Nippon Becton Dickinson), and *Candida* sp. (detected by Candida check [Intron Laboratories, Inc., Tokyo]). For detection and identification of anaerobic bacteria, each sample was poured directory onto an HK agar plate and incubated for 48-72 h under anaerobic conditions using a gas pack system. Representative microbial colonies from each plate were gram stained and isolated using a RapID ANAII system (AMCO, Tokyo).

The detection rates of microorganisms from dental plaque are shown in Table 1. In the first examination, *Candida* sp. and *H. parainfluenzae* were most frequently detected aerobic microorganisms. Of the anaerobic species, *Capnocytophaga* sp. and *Prevotella melaninogenica*, were isolated from more than two-thirds of the subjects. The detection rate of *P. oris* and *Fusobacterium nucleatum* was 30%. Most of the subjects enrolled in this study had at least one of the four major pathogenic anaerobes, *Capnocytophaga* sp., *P. melaninogenica*, *P. oris*, and *F. nucleatum*.

A second examination was performed a month later. Less than half of the subjects retained the same aerobic species detected in the first examination, while more than half retained anaerobic species (except *Fusobacterium* sp.) detected in the first examination. Anaerobes appeared more stable in the elderly persons' oral flora than did aerobes probably because of the biofilm in the oral cavity which provides anaerobes with an optimal environment.

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