Discussion

The changes in oral microflora have been related directly to aging and usually associated with major changes to habitat such as tooth eruption, hormonal changes and the presence of active disease in the young (19). In addition to the influence of ageing, we have studied epidemiologically the relation between the requirement for systemic care in the elderly and the isolation frequency of oral bacteria in residents of nursing homes. The isolation frequencies of *C. albicans*, some Enterobacteriaceae, Pseudomonadaceae and *S. aureus* (MSSA and MRSA) in plaque, and *C. albicans* and *X. maltophilia* in pharynx were significantly higher in the older adults requiring care than those not (mean age: 83.9 vs 71.0 years) (Table 3). The increased prevalence of these bacteria was associated with the requirement for care and also due to ageing. In short, the elderly in the nursing homes could not care for themselves and were in poor oral condition (3). Previous studies have suggested that the oral carriage of bacteria pneumonia: *K. pneumoniae*, Pseudomonas sp., Staphylococci etc., is low in healthy subjects, but higher in immunodeficient and myelosuppressed subjects (20, 21, 22), and in patients with severe periodontal disease (4, 15). The significantly higher prevalence of these bacteria in the older adults requiring care is of interest as these people may be at greater risk of developing systemic disease such as pneumonia and heart disease (23). The proportion of those with one underlying medical condition (bedridden state) accounted for 89% of all the older adults in the nursing homes (Table 1). It is possible that the individuals completely bedridden tend to suffer a deterioration of host defense and poor oral hygiene. Little is known about the relation between the isolation frequencies for bacteria on the tooth surface and the bedridden state in older adults. Therefore, we analyzed the relation and demonstrated that the frequency of Pseudomonas sp. and *S. marcescens* was significantly higher in bedridden than control (Not Bedridden) subjects (Table 4). It is suggested that being bedridden encouraged infection or accumulation of Pseudomonas sp. and *S. marcescens* in the plaque and is a risk factor for respirotory tract infection by aspiration. The bedridden subjects were also given to the progressive loss of protective reflexes, which is one of the causes of aspiration pneumonia in the elderly (24).

Mixtures of these bacteria including mutans streptococci cause biofilm

infections and show a higher level of attachment than a single species biofilm (11, 25). Our studies showed that a complex bacterial community was formed by numerous bacterial species in plaque and pharynx. Pseudomonas sp., *K. pneumoniae* and *S. marcescens* were shown to be more active in biofilm formation than either the pathogen, Escherichia coli (26, 27), or opportunistic pathogens in a wide range of human infections (28, 29). They also interact with each other in these biofilms. Parts of the biofilm including pathogens on the tooth can break off in the flow, and may colonize new surfaces and release planktonic bacteria into same organs. Moreover, the biofilm bacteria may invade into subgingival tissue and be carried in the blood stream. Our results demonstrated a significant association of multiple biofilm infection by Pseudomonas sp. including *P. aeroginosa* and *K. pneumoniae*, and/or *C. albicans* with heart disease. However, the small significant differences may well be spurious as a result of multiple cross-correlations with other pathogens inducing heart disease. During the growth of the biofilm on the teeth, the pathogenic strains are incorporated into the biofilm in association with oral bacteria. Taken together, they were likely to be key species for biofilm development on severe infection of the lower respiratory tract and various bodily sites such as heart through the blood stream. Interestingly, unlike the association in the plaque samples, the isolation frequency of *K. pneumoniae* and/or *C. albicans* in pharynx was significantly associated with hypertension. The isolation frequency of the organisms detected abundantly in the plaque samples was not identified among those in the pharynx except *C. albicans* (Table 3). They acquire different levels of attachment and development on the tooth surface and pharynx. Therefore, it is supposed that the other pathogens incorporated into tooth biofilms differ from those incorporated into pharynx biofilms. The infection and accumulation of these bacteria on the tooth surface did not correlate closely with the use of full dentures that are one of the effectors for microflora change in the oral cavity.

S. aureus is the most common pathogen in various infectious diseases (30). MRSA often causes nosocomical infections. The increasing number of cases of nosocomical infections caused by MRSA can indeed be serious for elderly and compromised hosts (31). Therefore, it is very important to survey appearance of MRSA in the oral cavity of elderly in the nursing homes. The frequency of MRSA in the plaque samples that was relatively low, was significantly higher in the elderly requiring care than those not.

However, the frequency did not correlate with bedridden status and incidence of systemic diseases such as heart disease. These results might be indicated that the surveillance was important about the horizontal transmission of MRSA rather than harmful biofilm formation in elderly health.

Dental plaque is defined as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin (32). In the elderly requiring care, biochemical gradients develop that allow for the co-existence of a number of species including opportunistic organisms that form a bacterial community during the process of colonization. Opportunistic organisms are those that rarely if ever cause disease in immunocompetent people but can cause serious synergistic diseases in the elderly with underlying systemic problems. To identify high-risk individuals, the surveillance of potential indicators such as Pseudomonas sp. and *K. pneumoniae* guiding harmful oral biofilms may be a necessary condition for health care in elder adults requiring systemic care. Furthermore, these biofilms should be removed by improving oral hygiene, the use of chemical agents, and a professional such as a dental hygienist etc.. However, one should first remove the dental biofilm and disrupt microbial adhesion and biofilm formation, otherwise chemotherapeutic and antimicrobial agents can not function effectively.

Therefore, attention to oral hygiene including professional care may diminish the risk of systemic disease. In addition to oral hygiene, identification of high-risk patients, staff education in the nursing homes, hand washing and use of disposable glove were also proposed on the diminishing. These findings may be usually beneficial for continued health of elderly people.

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References

1. Streele JG, Sheiham A, Marcenes W, Walls AWG, National diet and nutrition survey: people aged 65 year and over. Vol 2: Report of the oral health survey. London: Stationery Office, 1998.

2. Simons D, Kidd EA, Beighton D. Oral health of elderly occupants in residential homes. Lancet 1999; 353: 1761.

3. Yoneyama T, Yoshida M, Matsui T, Sasaki H, and the oral care working group. Oral care and pneumonia, Lancet 1999; 354: 515.

4. Scannapieco FA, Mylotte JM. Relationship between periodontal disease and bacterial pneumonia. J. Periodontol. 1996; 67: 1114-22.

5. McEldowney S, Fletcher M. Adhesion of bacteria from mixed cell suspension to solid surfaces. Arch. Microbiol. 1987; 148: 57-62.

6 Loesche WJ, Syed SA, Laughon BE, Stoll J. The bacteriology of acute necrotizing ulcerative gingivitis. J. Periodontal. 1982; 53: 223-230.

7. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbial Rev. 1986; 50: 353-380.

8. Moore WE, Holdeman LV, Cato EP, Smibert RM, Burmeister JA, Ranney RR. Bacteriology of moderate (chronic) periodontitis in mature adult humans. Infect. Immun. 1983; 42: 540-515.

9. Bowden GH. Microbiology of root surface caries in humans. J. Dent. Res. 1990; 69: 1205-1210.

10. Gibbons RJ. Role of adhesion in microbial colonization of host tissues. a contribution of oral microbiology. J. Dent. Res. 1996; 75: 866-870.

11. Costerton JW, Stewart PS, Greeberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999; 284: 1318-22.

12. Millward TA, Wilson M. The effect of chlorhexidine on *Streptococcus sanguis* biofilms. Microbios 1989; 58: 155-164.

13 Wilson M. Susceptibility of oral bacterial biofilms to antimicrobial agents. J. Med. Microbiol. 1996; 44: 79-87.

14. Slots J, Rams TE, Listgarten MA. Yeasts, enteric rods and pseudomonads in the subgingival flora of severe adult periodontitis. Oral Microbiol. Immunol. 1988; 3: 47-52.

15. Slots J, Felk D, Rams TE. Prevalence and antimicrobiol susceptibility of Enterobacteriaceae, Pseudomonadaceae and Actinetobacter in human periodontitis. Oral Microbiol Immunol. 1990; 5: 149-54.

16. Kornman KS, Age, supragingival plaque and steroid hormones as ecological determinants of the sub-gingival flora. In: Genco RJ, Mergenhagen SE (eds) Host-parasite interactions inperiodontal disease. Wachington DC, American Society for Microbiology. 1982: 132-38.

17. Murray RGE, Brenner DJ, Bryant MR, Holt JG, Krieg NR, Moulder JW, Pfennig N, Sneath PHA, Steley JT, Williams ST. 1989. Bergey's manual of systematic bacteriology Vol 1 - Vol 4.

18. Ellen RP, Gibbons RJ. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces; prerequisite for virulence. Infect. Immun. 1972; 5: 826-830.
19. van Winkelhoff AJ, van Steenbergen TJ, de Graaff J, The role of black-pigmented bacteroides in human oral infections. J. Clin. Periodontol. 1988; 15: 145-55.

20. Minah GE, Rednor JL, Peterson DE, Overholser CD, Depaola LG, Suzuki JB. Oral succession of gram-negative bacilli in myelosuppressed cancer patients. J. Clin. Microbiol. 1986; 24: 210-13.

21. Bergmann OJ, Kilian M, Ellegaard J. Potentially pathogenic microorganisms in the oral cavity during febrile episodes in immunocompromised patients with haematologic malignancies. Scand. J. Infect. Dis. 1989; 21: 43 - 51.

22. Costerton JW, Lewandowski Z. Microbiol biofilms. Annu Rev Microbiol 1995; 49: 711-45.

23. Sedgley CM, Samaranayake LP. The oral prevalence of aerobic and facultatively anaerobic gram-negative rods and yeasts in Hong Kong Chinese. Arch Oral Biol, 1994; 39: 459-66.

24. Sasaki H, Sekizawa K, Yanai M, Arai H, Yamaya M, Ohrui T. New strategies for aspiration pneumonia. Int. Med. 1997; 36: 851-55.

25. Jones K, Bradshaw SB. Synergism in biofilm formation between *Salmonella enteritidis* and a nitrogen-fixing strain of *Klebsiella pneumoniae*. J. Applied Microbiol. 1997; 82: 663 - 8.

26. Skillman LC, Sutherland IW, Jones MV, Goulsbra A. Green flourescent protein as a novel species-specific marker in enteric dual-species biofilms. Microbiology 1998; 144: 2095-101.

27. Brown LR, Mackler BF, Levy BM, Wright TE, Handler SF, Moylan JS, Perkins DH, Keene HJ. Comparison of the plaque microflora in immunodeficient and immunocompetent dental patients. J Dent Res 1979; 58: 2344-52.

28. Bodey GP, Bolivar R, Fainstein V, Jadeja L. Infections caused by *Pseudomonas aeruginosa*. Rev. Infect. Dis. 1983; 5: 279 - 313.

29. Young LS, Martin WJ, Meyer RD, Weinstein RJ, Anderson ET. Gram-negative rod bacteremia: microbiologic, immunologic, and therapeutic considerations. Ann Inter Med. 1977; 86: 456-71.

30. Sheagren JN. *Staphylococcus aureus* the persistant pathogen (second of two parts). New Enbl. J. Med. 1984; 310: 1437-1443.

31. Lingnau W, Allerberger F. 1994. Control of an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) by hygenic measures in a general intensive care unit. Infection 1994; 22(2 Suppl.): 135-139.

32. Marsh PD, Bradshow DJ. Dental plaque as a biofilm. J. Industr. Microbiol. 1995; 15: 169-175.

Table 1 Subject details

Population of older adults	n	Age	Male(%) : Female(%)
Group: not requiring care	464	72.0 ± 0.3	247(53%) : 217(47%)
Group: requiring care	291	83.9 ± 7.5	61(21%) : 230(79%)
Bedridden status: Not	29	81.8 ± 6.6	4(14%) : 25(86%)
Slightly	88	84.0 ± 6.9	21(24%) : 67(76%)
Moderately	94	83.3 ± 8.7	19(20%) : 75(80%)
Completely	67	85.2 ± 8.0	17(25%) : 50(75%)

Age is given as a mean ± SD, all other data are no. (%).
Bedridden status: terms were described in Materials and Methods

Table 2 Lists of bacteria and fungi detected in the plaque and pharynx samples

Bacteria and fungi	
Coagulase(-)staphylococcus	*Haemophilus influenzae*
Staphylococcus aureus (MSSA, MRSA)	*Haemophilus parainfluenzae*
Streptococcus pneumoniae	*Klebsiella oxytoca*
Streptococcus anginosus	*Klebsiella pnuemoniae*
β-Streptococcus (type A)	*Moraxella catarrhalis*
β-Streptococcus (type B)	*Morganerlla morganii*
β-Streptococcus (type C)	*Proteus mirabilis*
β-Streptococcus (type D)	Pseudomonas sp.
Enterococcus faecalis	*Pseudomonas aeruginosa*
Enterococcus faecium	*Pseudomonas cepacia*
Citrobacter freundii	*Serratia marcescens*
Enterobacter aerogenes	*Xanthomonas maltophilia*
Enterobacter cloacae	*Candida albicans*
Flavobacterium meningosepticum	*Candida glabrata*
	Candida tropicalis

Table 3 Isolation frequency of microbial infection in plaque and pharynx samples from older adults

Bacteria	Group: requiring care		Group: not requiring care	
	Plaque n=291	Pharynx n=215	Plaque n=464	Pharynx n=453
Candida albicans	107 (37%)*	83 (39%)*	135 (30%)	101 (27%)
Enterobacter cloacae	45 (16%)**	17 (8%)	26 (6%)	27 (6%)
Pseudomonas sp.	41 (14%)**	7 (3%)**	27 (6%)	35 (8%)
Klebsiella pneumoniae	30 (10%)	35 (16%)**	32 (7%)	40 (9%)
Xanthomonas maltophilia	23 (8%)**	5 (2%)	8 (2%)	17 (4%)
Klebsiella oxytoca	21 (7%)**	3 (1%)	6 (1%)	13 (3%)
Staphylococcus aureus (MSSA)	16 (5%)**	7 (2%)	8 (2%)	20 (4%)
Coagulase negative staphylococci: CNS	15 (5%)	5 (2%)	15 (3%)	7 (2%)
Serratia marcescens	12 (4%)	4 (2%)	5 (1%)	5 (1%)
Psudomonas aeruginosa	9 (3%)*	16 (5%)	4 (<1%)	4 (<1%)
β-Streptococcus (type B)	9 (3%)*	4 (2%)	2 (<1%)	2 (<1%)
Actinetobacter calcoaceticus	8 (3%)**	6 (3%)	41 (9%)	30 (7%)
Candida parapsilosis	7 (2%)	3 (4%)	23 (5%)	17 (4%)
Staphylococcus aureus (MRSA)	7 (2%)*	4 (2%)	2 (<1%)	2 (<1%)

All data are no. (%).

*: $p<0.05$ (χ^2 test with continuing correction, Plaque and pharynx samples in group: requiring care vs group: not requiring care)

**: $p<0.01$ (χ^2 test with continuing correction, Plaque and pharynx samples in group: requiring care vs group: not requiring care)

Table 4 Correlation between bacteria in dental plaque and bedridden degree in the elderly

Bacteria	Bedridden degree			
	Not (n = 29)	Slightly (n = 88)	Moderately (n = 94)	Completely (n = 67)
Candida albicans	8 (28%)	30 (34%)	34 (36%)	30 (45%)
Enterobacter cloacae	7(24%)	7 (8%)*	14 (15%)	9 (13%)
Pseudomonas sp.	0 (0%)	15 (17%)**	14 (15%)**	6 (9%)
Klebsiella pneumoniae	5 (17%)	10 (11%)	4 (4%)	9 (13%)
Xanthomonas maltophilia	5 (17%)	7 (8%)	5 (5%)	1 (2%)
Klebsiella oxytoca	3 (10%)	4 (5%)	5 (5%)	10 (15%)
Staphylococcus aureus (MSSA)	1 (3%)	5 (6%)	2 (2%)	3 (4%)
Coagulase negative staphylococci: CNS	2 (7%)	2 (2%)	3 (3%)	6 (9%)
Serratia marcescens	0 (0%)	2 (2%)	1 (1%)	7 (10%)*
Psudomonas aeruginosa	2 (7%)	3 (3%)	2 (2%)	2 (3%)
β-Streptococcus (type B)	0 (0%)	0 (0%)	4 (4%)	5 (8%)
Acinetobacter calcoaceticua	0 (0%)	3 (3%)	3 (3%)	1 (2%)
Candida parapsilosis	6 (21%)	3 (3%)**	1 (1%)**	0 (0%)**
Staphylococcus aureus (MRSA)	1 (3%)	1 (1%)	2 (2%)	3 (4%)

All data are no (%). Bedridden status: terms were described in Materials and Methods.
*: $p<0.05$, (χ^2 test with continuing correction, Not vs Slightly, Moderately and Completely)
**: $p<0.01$, (χ^2 test with continuing correction, Not vs Slightly, Moderately and Completely)

Table 1

Isolation frequency of microbial infection in Dental plaque, Saliva and tonsil samples from older adults.

Microorganisms	Dental plaque n=125 No. (%)	Saliva n=125 No. (%)	Pharynx 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)
Ekenella corrodons	1 (1)	0 (0)	0 (0)
Kebsiella pneumoniae	9 (7)	29 (23)	13 (11)
Kebsiella oxytoca	4 (3)	6 (5)	6 (5)
Kebsiella 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 flourescens	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 agalactae	0 (0)	4 (3)	4 (3)
α streptococcus	106 (85)	111 (88)	101 (81)
β streptococcus G	0 (0)	1 (2)	0 (0)
β streptococcus non group A	0 (0)	1 (2)	0 (0)
γ streptococcus	66 (53)	74 (58)	77 (61)
Neisseria sp.	85 (68)	78 (63)	67 (54)
Acintobacter sp.	3 (3)	5 (4)	1 (1)
Acintobacter calcoaceticus	3 (3)	10 (8)	5 (4)
Acintobacterlwoffii	1 (1)	2 (2)	2 (2)
Alcaligenes xylosoxydans	1 (1)	2 (2)	1 (1)
Alcaligenes faecalis	0 (0)	1 (1)	0 (0)
Serrata marcescens	3 (3)	4 (3)	4 (3)
Serrata liqifaciens	4 (3)	6 (5)	6 (5)
Leclericia adecarboxylata	1 (1)	2 (2)	0 (0)
H. 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 (12	1 (1)
Xanthomonas maltophilia	5 (4)	5 (4)	4 (3)

Table 2 Correlation of isolation frequency of between Dental plaque, Saliva and tonsil samples.

	Dental plaque	saliva	Tonsil
Dental plaque	.....	79.8±26.7%	78±27.3%
Saliva	63.7±27.4%	.....	64.5±26.7%
Tonsil	71.8±27.2%	75.9±27.5%	.....

特別養護老人ホーム等施設内高齢者の口腔バイオフィルム内細菌群と全身疾患との関係

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要旨：感染に対する抵抗力の低下や自己による口腔清掃力の低下がみられる要介護高齢者は，口腔に多くの細菌が蓄積しそれが気道や血流に入る事により全身疾患の発症へ関与する可能性が考えられている．そこで特別養護施設内の要介護高齢者の口腔内細菌の検出を試み，細菌感染の有無と全身疾患の関連について検討を行った．特別養護老人ホーム 329 名（平均年令 82 才）を対象に，歯垢，咽頭粘膜上および舌の試料を採取し，その微生物の有無を培養法にて同定した．それぞれの微生物が検出された人数の全体における割合を算出した．歯垢内，咽頭，舌において，*Candida albicans*, *Enterobacter cloacae*, Pseudomonas sp., *Klebsiella pneumoniae*, *Xanthomonas maltophilia*, *Staphylococcus aureus* (MSSA, MRSA), *Serratia marcescens*, *Pseudomonas aeroginosa* が高率に検出された．また Pseudomonas sp. と *S. marcescens* は，寝たきりの程度に依存して，歯垢内で高率に検出された．要介護者の歯垢内細菌群と全身疾患との関連を検討した結果，*C. albicans* と *K. pneumoniae* や Pseudomonas sp. のどちらかが検出された要介護者は，検出されない群より有意に心疾患を有していた．要介護高齢者の口腔は肺炎に関わるような多種の細菌感染を受け，また *C. albicans* と *K. pneumoniae* や Pseudomonas sp. の歯垢内感染と心臓病の発症との相関関係が認められた。よって要介護高齢者は、口腔からそれらの菌を指標菌として、除去する口腔ケアが必要であると示唆された．

Key words: Oral biofilm, Systemic disease, Plaque, Bacteria

はじめに

口腔は様々な細菌によって感染を受け，歯表面で歯垢のような粘着性の高いバイオフィルムが形成される事により，さらに多くの病原性細菌を蓄積しやすい環境となる[1]．近年高齢者において，口腔内でバイオフィルム感染症が成立すると細菌の誤嚥により容易に不顕性感染が起こり，肺炎のような感染症が起こる事が社会的な問題となっている[2]．すなわち口腔内にいる時は感染症の原因とならないような口腔微生物が歯面および口腔内表層に付着し表面をフイルム状に被覆し，強固な薬剤抵抗性，感染性を有して蓄積されて行くと，誤って肺の方へ侵入しはじめて病原性を発揮するという疾患が起こる．高齢者は，老化や様々な全身疾患を有する事から感染に対する防御力が低下し，口腔感染への感受性は高まっているものと考えられる．感染に対する抵抗力の低下に加え自己による口腔清掃力の低下がみられる要介護高齢者は，口腔に多くの細菌が蓄積しそれが気道や血流に入る事により全身疾患の発症に関与する可能性が考えられている[2,3,4]．そこで特別養護施設内の要介護高齢者の口腔内細菌の検出を試み，細菌の感染の有無と全身疾患の関連について検討を行った．

材料と方法

1. 対象者

65才から105才までの介護の必要な特別養護老人ホーム329名（平均年令82才）で，男性が67名，女性が262名を対象にした(Table 1). 要介護者のうち，生活自立者が10%，準寝たきり者が30%，寝たきり者（座位可能）が32%，寝たきり者（座位不可）が23%，その他が5%であった.

2. 菌検出試料の採取および検出方法

歯垢試料は，対象者の左側上顎臼歯部5, 6, 7番（第2小臼歯・第1大臼歯・第2大臼歯）相当部，頬側歯頚部の歯垢をシードスワブ1号の滅菌キャップ付綿棒で数回（5往復）擦過し，更に綿棒の綿球を180度回転し5往復擦過後，キャリブレア・チューブに投入する. 咽頭粘膜上および舌上試料も歯垢と同様にシードスワブ1号にて擦過後試料を擦過後，キャリブレア・チューブに投入する. その微生物の有無は培養法にて同定した. 培養法は，試料の入った溶液をスパイラルシステムを用いて血液寒天培地へ植菌し，48時間炭酸ガス培養後コロニーを採取し，それぞれの菌に対する選択培地にさらに植菌し，培養後菌の同定を行った[5]. 口腔微生物のリストをTable 1に示す. それぞれの微生物が検出された人数の全体における割合を算出した. 有意差検定は，1および5％危険率でχ自乗検定を行った.

3. 全身疾患の既往暦

対象者の全身疾患の既往暦は，脳血管疾患，心疾患，高血圧，糖尿病，肝臓疾患，腎臓疾患，パーキンソン病，悪性腫瘍，整形外科疾患などが記載されたチェックシートに，対象者が内科医によって診断された疾患を記録することにより判定された.

結果

要介護者の歯垢内, 咽頭, 舌において, それぞれ *C. albicans*, 38%, 38%, 40 % ; *E. cloacae*, 16%, 11%, 13% ; Pseudomonas sp., 12%, 3%, 17% ; *K. pneumaniae*, 9%, 14%, 13%; *X. maltophilia* , 8%, 3%, 4%; *S. aureus* (MSSA, 5%, 2%, 5% ; MRSA, 2%, 2%, 4%); *S. marcescens*, 4%, 1%, 9%; *P. aeroginosa*, 4%, 6%, 7%; *Streptococcus anginosus*, 1%, 1%, 1%; *Haemophilus influemzae*, 1%, 1%, 0%が検出された (Table 3). また歯垢内にPseudomonas sp. が検出された人の割合は, 寝たきりの程度が Slightly (準寝たきり) で 17%, Moderately [寝たきり(座位可能)]で 15% とNot (生活自立) 0 % に比べ有意に ($p < 0.01$) 高い事が明かとなった (Table 4). また *S. marcescens* 歯垢内感染者も, Completely [寝たきり(座位不可)] で 10% と寝たきりの程度の低い高齢者よりも有意に ($p < 0.05$) 高率である事が明かとなった. よって Pseudomonas sp. と *S. marcescens* の歯垢内感染は、寝たきりの程度に依存していた. 要介護者の歯垢内および咽頭内細菌の検出と全身疾患との関連を検討した結果, *C. albicans* と *K. pneumoniae* のどちらかが歯垢内から検出された被験者の心疾患を有する割合 (24%) は, どちらも検出されない群 (13%) に比べ有意に ($p < 0.05$) 高い事が明かとなった (Table 5). またそれらの菌が咽頭から検出された高齢者の高血圧を有する割合 (52 %) は, どちらも検出されない群 (36%) に比べ有意に ($p < 0.01$) 高い事も明かとなった. *C. albicans* や Pseudomonas sp. が単独あるいは混合で歯垢内で検出された被験者は, 検出されない群より高率に心疾患を有していたが, 有意差は認められなかった (Fig. 1). しかし, *C. albicans* や Pseudomonas sp. のどちらかが検出された被験者は, 検出されない群より有意に ($p < 0.05$) 心疾患を有していた(Fig. 1).

考察

要介護高齢者の歯垢は肺炎に関わるような *C. albicans*, Pseudomonas sp., *K. pneumaniae*, *X. maltophilia* , *S. aureus* (MSSA; MRSA), *S. marcescens*; *P. aeroginosa* の菌群などの感染を受けている (Table 3)。これらの菌は歯表面のバイオフィルム内に蓄積されて, そのバイオフィルムの一部がはがれ, 要介護高齢者において特に誤嚥などを起こした場合は肺へ侵入して行くことになる. その結果, 肺炎を誘発する危険性が高まって行く.

Pseudomonas sp. は寝たきりの程度に依存して歯垢内にて検出された (Table 4). 重度な寝たきりの要介護高齢者は, 体力の低下から菌への抵抗力が低下し, また自力による口腔清掃もおろそかになりがちであることから Pseudomonas sp. の感染を受けやすくなった可能性が考えられる. Pseudomonas sp. は強くバイオフィルムを形成する菌でもあることから, 歯表面に従来のバイオフィルムに加えさらに強固なバイオフィルムを形成することが推測される. また*C. albicans* とともに *K. pneumoniae* や Pseudomonas sp. の歯垢内感染と心臓病との間に相関関係がある(Table 5, Fig. 1) ことからも、強くバイオフィルムを形成する *K. pneumoniae* や Pseudomonas sp. が, 全身疾患に関わる病原性細菌を歯表面で蓄積させる環境に導いている可能性も考えられる[6, 7, 8]. よって要介護高齢者は, 口腔から *K. pneumoniae* や Pseudomonas sp. のような菌を指標として除菌する口腔ケアが必要である. これらの口腔ケアが要介護高齢者の健康増進に役立つことが示唆された.

文献

1) Costerton JW, Stewart PS, Greeberg EP : Bacterial biofilms: a common cause of persistent infections. Science 284: 1318-22, 1999.

2) Yoneyama T, Yoshida M, Matsui T, Sasaki H, and the oral care working group : Oral care and pneumonia, Lancet 354: 515, 1999.

3) Streele JG, Sheiham A, Marcenes W, Walls AWG : National diet and nutrition survey: people aged 65 year and over. Vol 2: Report of the oral health survey. London: Stationery Office, 1998.

4) Simons D, Kidd EA, Beighton D : Oral health of elderly occupants in residential homes. Lancet 353: 1761, 1999.

5) Murray RGE, Brenner DJ, Bryant MR, Holt JG, Krieg NR, Moulder JW, Pfennig N, Sneath PHA, Steley JT, Williams ST : Bergey's manual of systematic bacteriology Vol 1 - Vol 4. 1989.

6) Bentley DW : Bacterial pneumonia in the elderly : clinical features, diagnosis, etiology, and treatment. Gerontology 30: 297-307, 1984.

7) Rosenthal S, Tager IB : Prevalence of gram-negative tods in the normal pharyngeal flora. Ann. Intern Med. 83: 355-57, 1975.

8) Bartlett, JG, O'Keefe P, Tally FP, Louie TJ, Gorbach SL : Bacteriology of hospital-acquired pneumoniae. Arch Intern Med. 146: 868-71, 1986.

Figure legend

Fig. 1 Correlation between only *C. albicans*, Pseudomonas sp. including *P. aeroginosa*, *C. albicans* and/or Pseudomonas sp. and heart disease. Negative group (□): Neither *C. albicans* nor Pseudomonas sp. was isolated in plaque samples. Positive group (■): *C. albicans* or Pseudomonas sp. was isolated in plaque samples. Two groups were studied for the correlation to heart disease. $\chi 2$ test < 0.05 with continuing correction , negative group vs positive group.