American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Association between Commensal Bacteria and Opportunistic Pathogens in the Dental Plaque of Elderly Individuals

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Received: 2024, 15, Apr
Accepted: 2025, 21, May
Published: 2025, 25, Jun

Annotation: The prevalence of systemic disease may rise in older adults with opportunistic mouth infections. Investigating the variations in oral bacterial ecology between independent elderly (community-dwelling residents) and dependent elderly (inpatients) was The rationale of this analysis was to find the incidence of α-hemolytic streptococci ($p < 0.001$) and Neisseria types ($p = 0.004$) was significantly lower in hospitalized individuals when compared with community dwellers after multiple confounders were adjusted. In contrast, they showed a higher detection frequency of Significant detection rates were observed for Pseudomonas aeruginosa ($p = 0.024$) MRSA ($p = 0.011$) and Actinomyces organisms ($p = 0.005$) among bacterial isolates.

Increased need for intensive care among hospitalized patients were significantly associated with MRSA ($p = 0.004$) and P. aeruginosa ($p = 0.018$), but inversely related to α-streptococci.

Patients on enteral nutrition by feeding tube were significantly less likely to be positive for streptococci ($p = 0.041$) and

significantly more likely to have Pseudomonas aeruginosa (p = 0.004) than those in whom feeding tubes were not necessary.

Similarly, α-streptococci were significantly less recovered from inpatients with history of previous antimicrobial therapy (p = 0.049) and MRSA more detectable (p = 0.007) that those without history.

After adjustment for age and sex, inpatients who were negative for α-streptococci were more commonly detected with P. aeruginosa (p = 0.006) and MRSA (p = 0.001), in comparison to those colonised with oral α-streptococci.

In conclusion, the detection of α-streptococci in the oral microbial flora was negatively correlated with MRSA and P. aeruginosa colonisation, and it may serve as an indicator of the oral microbial dysbiosis and a potential pathogenic colonisation.

Keywords: MRSA, Pseudomonas aeruginosa, α-streptococci, oral biofilm, elderly, prevalent microorganisms.

Introduction

Elderly people, particularly those who are bedridden, often exhibit low levels of oral hygiene, leading to the accumulation of dental biofilm hosting opportunistic microorganisms. The age-related decline in immune system efficiency may partly explain this phenomenon. As noted by Saltzman and Peterson [4], individuals over 70 experience changes in their oral bacterial communities, which can lead to opportunistic infections associated with weakened immunity. Moreover, research indicates that dental plaque from seniors contains opportunistic bacterial pathogens [5–9]. Since microbes residing within biofilms form sessile colonies and exhibit resistance to antibiotics, they can cause persistent and recurrent infections [10]. Many conditions, such as aspiration pneumonia and septicemia, are believed to be influenced by bacterial biofilms developing on teeth and denture surfaces [10]. Prior studies have investigated the relationship between Oral biofilm derived microorganisms from frail or institutionalized older adults individuals and their overall health. For instance, the prevalence of *Pseudomonas aeruginosa* was notably higher among elderly who relied on tube feeding compared to those who did not, according to research on functional dependence [6]. Additionally, Methicillin-resistant Staphylococcus aureus (MRSA) was detected within the mouths of immobile elderly individuals

presenting with reduced blood albumin concentrations [7]. Additionally, various harmful bacterial organisms were found at notably higher frequencies among aging patients suffering from cardiovascular conditions who required caregiving support, in contrast to their counterparts without cardiac diagnoses [8]. The typical oral microbiota consists of numerous bacterial species. Changes in the oral microbiome are impacted by immunodeficiency and antibiotic medication therapy [11,12]. It is also feasible to hypothesize that infections due to opportunistic oral flora could alter the equilibrium of the indigenous microbial flora. An assessment was performed in this research to investigate the correlation between the pathogenic species, which are found in the oral biofilm of elderly individuals, and the indigenous flora.

Participants: Participants were only Japanese participants older than 60 years. The study included 108 community-dwelling Japanese seniors in Niigata City The mean age of the participants was (64 male and 44 female) 74.5 years. who were classified as independent elderly. Additionally, 49 hospitalized elders (Among 20 male and 29 female subjects, mean age 75.1 years), all were institutionalized for more than 3 months in two different facilities in Chiba-city, Japan, and were classified as dependent elderly. Prior to the commencement of the research, residents living in the community, inpatients, and/or their relatives were informed of the study's purpose, purpose, and verbal consent was obtained. Chronic health conditions affected the hospitalized group, including one case of cancer, five cases of diabetes mellitus, three with heart disease, and twenty-one with cerebrovascular disease. The details concerning their daily care requirements, dietary habits, and history of antibiotic use are summarized in Table 1. The "level of care needed" describes the assistance required for daily activities.

Materials and Methods

Table 1. General health status of inpatients (n = 49) included in the study

	No. (%)
Degree of care necessary for daily activities	
None	9 (18.4)
Partial	29 (59.2)
Complete	11 (22.4)
Mode of nutritional intake	
Oral feeding	45 (91.8)
Tube feeding	4 (8.2)
History of antibiotic use	
None	39 (79.6)
Current or previous	10 (20.4)

required for everyday tasks including eating, washing, moving, and excreting. During the three months of the trial, no long-term antibiotic treatment was given, No one in thehospital developed any major infectious diseases. The rate of natural teeth preservation was 81.5%, including the rate of inpatient 61.2%, and that of the community-dwelling 90.7%.

For microbiological evaluation, dental plaque was harvested from the upper molars or the same sites of upper dentures. Swab sampling was carried out by swabbing tooth surfaces five times to obtain biofilm samples. These swabs were inserted into a transport medium containing Samples in 0.4% w/v agar 0.15% w/v thioglycolate PBS were then transferred to the microbiology lab for further analysis.

Both aerobic and anaerobic growth conditions were used for the bacterial species' identification and growth (see below). Each species and genus had a detection limit of 103 CFU⁄mL. According to current understanding, Non-pathogenic anaerobes and indigenous oral flora detected at the genus level. Each specimen was rapidly cultured onto Drigalski agar plates (Nippon Becton Dickinson Co., Tokyo, Japan) under aerobic conditions, as well as onto chocolate agar and OPA staphylococcus-selective media. The culture plates were then incubated at 37°C for 24--48 h in 5% (v/v) CO 2 enriched air. A few typical bacterial colonies from each medium were stained by Gram's method and studied. Colony identification was initially determined by colony morphology, enzyme reactions, namely oxidase and catalase reactions, and hemolysis.

[13]. To facilitate species identification, bacterial colonies—such as those occurring frequently in the majority of the participants—were resuspended in 1 ml of 0.5% (w/v) saline with slight stirring to give a homogeneous suspension. Identification of the Pseudomonas strains was carried in the VITEK system (bioMérieux, Tokyo, Japan). with Haemophilus influenzae were estimated by using a Haemophilus ID4 culture medium (manufactured by Nippon Becton Dickinson). Methicillin-sensitive staphylococcus aureus and MRSA were differentiated using PS latex agglutination on MRSA-specific agar plates (Nippon Becton Dickinson, Tokyo) and rabbit plasma coagulation. Brucella HK blood agar (Kyokuto Seiyaku, Tokyo, Japan) was used for anaerobic incubation were immediately coated with each sample, and the Gas Pack system was used to incubate them anaerobically for 48–72 hours.

Identification was done by the RapID ANA system (Innovative Diagnostic Systems, Norcross, GA, USA).

used to identify representative colonies from each plate after they had been analyzed using Gram's stain. Before being inoculated onto media containing the following, In some experiments the colonies of microorganism were diluted in solution of 0.6% (w/v) potassium chloride (KCl), 0.05% (w/v) calcium chloride ($CaCl_2$), and 0.16 mM NaOH. The biochemical screening substrates were: (1) 0.4% (w/v) urea; (2) 0.1% (w/v) p-nitrophenyl-β-D-disaccharide; (3) 0.1% (w/v) p-nitrophenyl-α-L-arabinoside; (4) 0.1% (w/v) p-nitrophenyl-β-D-galactoside; (5) 0.08% (w/v) p-nitrophenyl-β-D-glucoside; (7) 0.08% (w/v) p-nitrophenyl-α-D-galactoside; (8) 0.08% (w/v) p-nitrophenyl-α-L-fucoside; (9) 0.1% (w/v) p-nitrophenyl-N-acetyl-β-D-glucosaminide; and (10) 0.1% (w/v) p-nitrophenyl phosphate.

For the primary test, incubation was place for 4–6 hours at 37C in an environment with 5% v⁄vinN2. The secondary test involved adding "INNOVA C=O indole was applied to reaction mixture (10) and 0.01% (w/v) 3-phenylmethylaminoacrolein, 0.1% (v/v) hydrochloric acid and 1.0% (v/v) acetic acid were introduced into reaction mixtures (3) to (9)."Analysis of statistics The chi-square test was used to examine the connections Links were investigated between the functional status of each person and the detected bacterial species, such as comparisons of pathogenic organisms and harmless species that normally colonize the body. To further elucidate the factors contributing to the presence of individual species of bacteria, the logistic regression tests and multivariate linear regression model were used

The predictors included in the analysis were sex, age group (categorized as 60–79 or 80 years and older), level of physical autonomy (independent versus dependent), and oral condition (presence or absence of natural teeth). The outcome variables consisted of whether each bacterial species was identified (presence versus absence). Logistic regression analysis and multiple regression analysis were used to correct for age and gender in the Differences in odds of detection of pathogenic bacterial strains were compared between the two α-streptococcus categories. All statistical values were considered significant at the level of $p < 0.05$. All data were analyzed using SPSS for Windows version 10.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 2 explains the identification frequencies against individual bacterial genus. "Patients did

have significantly lower rates of detection of commensal organisms (e.g., α-streptococci, Neisseria) when the specimens were cultured aerobically than community dwellers. Compared to 4–12% of inpatients, 0–6% of community members had pathogenic bacterial species. Inpatients had much greater The frequencies of Pseudomonas aeruginosa, Klebsiella pneumoniae, and MRSA were calculated—than community residents.

Table 2. Number (%) of patients from whom each bacterial species was isolated[a]

	Community residents (n = 108)	Inpatients (n = 49)	p value[b]
(1) Aerobic growth conditions			
Commensal bacteria			
α-Streptococci	106 (98.1)	37 (75.5)	0.000
Neisseria spp.	72 (66.7)	21 (42.9)	0.004
Pathogenic bacteria			
Pseudomonas aeruginosa	1 (0.9)	6 (12.2)	0.004
Klebsiella pneumoniae	3 (2.8)	6 (12.2))	0.027
Klebsiella oxytoca	4 (3.7)	2 (4.1)	0.609
Enterococcus cloacae	6 (5.6)	3 (6.1)	0.573
Stenotrophomonas maltophilia	0 (0)	2 (4.1)	0.097
Streptococcus agalactiae	1 (0.9)	2 (4.1)	0.230
MRSA	0 (0)	3 (6.1)	0.007
(2) Anaerobic growth conditions			
Capnocytophaga spp.	79 (80.6)	22 (73.3)	0.269
Actinomyces spp.	5 (5.1)	7 (23.3)	0.007
Prevotella melaninogenicus	70 (71.4)	20 (66.7)	0.387
Prevotella corporis	28 (28.6)	5 (16.7)	0.142
Bacteroides capillosus	0 (0)	1 (3.3)	0.234
Bacteroides fragilis	1 (1.0)	1 (3.3)	0.415
Fusobacterium nucleatum	21 (21.4)	8 (26.7)	0.355

dents. About 70–80% of both inpatients and community members had Capnocytophaga spp. and Prevotella melaninogenicus in anaerobic conditions. Compared to community residents, inpatients had a four-fold higher detection rate for Actinomyces spp. Seldom were Bacteroides species found in either category. About 20% of the participants have Fusobacterium nucleatum. The findings of multiple Univariate analysis using regression and logistic regression were used to evaluate potential risk factors associated with the detection of pathogenic bacteria are displayed in Table 3. The subjects' functional state was taken into consideration while calculating the ORs and 95% CIs.

Compared to community members, inpatients had reduced odds of detecting Neisseria and a-streptococci. After adjusting When adjusting for sex and age, inpatients had higher probabilities of detecting P. aeruginosa ($p = 0.024$), MRSA ($p = 0.011$), and Actinomyces spp. ($p = 0.002$) than community residents. Regarding the other detected bacterial species, no statistically significant difference was found (data not shown). Table 4 displays Isolated frequencies of α-streptococci, Neisseria, P. aeruginosa, K. pneumoniae, MRSA, and Actinomyces spp. were assessed. in inpatients based on their overall health state. In participants with poor overall health status (as determined by the amount of care needed, dietary considerations, and antibiotic administration

A greater detection rate for harmful bacterial species and Reduced incidence of non-pathogenic oral bacteria were observed. The presence of MRSA and P. aeruginosa was substantially associated with a requirement for care. Compared to inpatients who received nutrition orally, Those patients who receive nourishment through a feeding-tube had significantly lower detection rates of the α-streptococci but higher frequencies of Pseudomonas aeruginosa. A higher rate of MRSA was isolated from inpatients with previous antibiotic exposure.

Although there were no appreciable differences, The lowest rate of this organism was among hospitalized subjects who were completely dependent or were on enteral feeding. There was no correlation for any clinical parameter in the inpatient group and detection of Klebsiella pneumoniae or Actinomyces spp. The presence of pathogenic bacteria (Pseudomonas aeruginosa and MRSA) and normal commensal species (α- streptococci and Neisseria) was also investigated

Table 3. Multivariate analysis of potential risk-factors for detection of bacteria in the study population[a]

	α-Streptococci	*Neisseria*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	MRSA	*Actinomyces*
Gender						
Female vs. male	1.572 (0.440–5.620)	1.432 (0.724–2.831)	2.294 (0.395–13.330)	0.506 (0.104–2.470)	0.020	1.031 (0.351–3.023)
Age group (years)						
80+ vs. 60–79	2.241 (0.437–11.488)	1.375 (0.400–4.724)	0.718 (0.099–5.200)	10.569 (1.273–87.725)	– 0.033	0.826 (0.183–3.730)
Level of care						
Dependent vs. independent	0.053[b] (0.010–0.274)	0.331[c] (0.141–0.774)	16.304[d] (1.799–155.574)	3.081 (0. 481–19.728)	0.226[d]	5.066[c] (1.527–16.811)
Dental state						
Dentures vs. own teeth	0.553 (0.133–2.292)	0.777 (0.307–1.962)	0.732 (0.107–4.999)	0.102 (0.009–1.161)	– 0.014	0.879 (0.237–3.265)

[a]For bacterial species except MRSA, ORs and 95% CIs derived from multiple logistic regression analysis including all variables in the models are shown. Statistical analysis for MRSA detection was limited to linear regression analysis, as MRSA was not detected in independent subjects; thus correlation coefficients are presented.
[b]$p < 0.001$; [c]$p < 0.01$; [d]$p < 0.05$.

Table 4. Detection of bacteria according to general health status of inpatients

	α-Streptococci	*Neisseria*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	MRSA	*Actinomyces*
Degree of care necessary for daily activities						
None (n = 9)	9 (100)	4 (44.4)	0 (0)[a]	1 (11.1)	0 (0)[b]	1 (11.1)
Partial (n = 29)	22 (75.9)	15 (51.7)	2 (6.9)	4 (13.8)	0 (0)	8 (27.6)
Complete (n = 11)	6 (54.5)	2 (18.2)	4 (36.4)	1 (9.1)	3 (27.2)	2 (18.2)
Mode of nutritional intake						
Oral feeding (n = 45)	36 (80.0)[a]	21 (46.7)	3 (6.7)[b]	6 (13.3)	2 (4.4)	10 (22.2)
Tube feeding (n = 4)	1 (25.0)	0 (0)	3 (75.0)	0 (0)	1 (25.0)	1 (25.0)
History of antibiotic use						
None (n = 39)	32 (82.1)	17 (43.6)	4 (10.2)	6 (15.4)	0 (0)[b]	8 (20.5)
Current or previous (n = 10)	5 (50)	4 (40)	2 (10.0)	0 (0)	3 (30.0)	3 (30.0)

[a]$p < 0.05$; [b]$p < 0.01$
MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 5. Relationship between the detection of pathogenic bacterial species and the detection of non-pathogenic bacterial species among inpatients

	α-Streptococci		*Neisseria*	
	No. of patients (%)	p value	No. of patients (%)	p value
Pseudomonas aeruginosa		0.002		0.175
Detected (n = 6)	1 (16.7)		1 (16.7)	
Not detected (n = 43)	36 (83.7)		20 (46.5)	
MRSA		0.012		0.178
Detected (n = 3)	0 (0)		0 (0)	
Not detected (n = 46)	37 (80.4)		21 (45.7)	

MRSA, methicillin-resistant *Staphylococcus aureus*.

inpatients who are gated in (Table 5). Compared to inpatients without P. aeruginosa, Incidence of detection of α-streptococci was substantially lower in P. aeruginosa patients. Additionally, compared to inpatients without MRSAThere was a statistically significantly lower prevalence of α-streptococci in MRSA positive patients who had been hospitalized. There was no significant correlation between the presence of pathogenic bacterial species and the presence of Neisseria. After adjusting for age and gender, individuals without α-streptococci were more likely to test

positive for Pseudomonas aeruginosa or MRSA in comparison with those positive for α-streptococci. (Table 6).

Table 6. Multivariate analysis of potential factors for detection of pathogenic bacteria among inpatients

(1) *Pseudomonas aeruginosa*

	OR (95% CI)	p value
Gender		
Female vs. male	1.3 (0.1–11.2)	0.823
Age group		
80+ vs. 60–79 years	0.6 (0.1–11.2)	0.608
α-Streptococci detection		
Not detected vs. detected	26.3 (2.6–267.2)	0.006

(2) Methicillin-resistant *Staphylococcus aureus* (MRSA)[a]

	β	*t*	p value
Gender	0.056	0.002	0.690
Age group	− 0.170	− 1.215	0.231
α-Streptococci detection	0.461	3.489	0.001

[a]Statistical analysis of MRSA detection was limited to linear regression analysis, as MRSA was not detected in subjects who had a detectable level of α-streptococci in the oral cavity.

Discussion

Compared to community residents, The case detection of α-streptococci was markedly reduced among hospitalised patients, and MRSA was more prevalent and P. aeruginosa. Compared to inpatients lacking a-streptococci, inpatients with a-streptococci had decreased Frequency of P. aeruginosa and methicillin-resistant Staphylococcus aureus Additionally, only 19 out of 37 inpatients with a-streptococci produced harmful bacteria under aerobic culture conditions, whereas all 12 ⁄ 12 inpatients without a-streptococci did. Almost everyone has streptococci in their oral cavity, which are As a key family of commensal microbes in the oral environment, they form the bulk of the bacterial community found in dental plaque [14]. According to reports, certain a-streptococci generate H2O2 and bacteriocins.

, which can temporarily stop Development of microflora in infants not previously exposed to oral factors [15–18]. Consequently, the normal oral bacterial ecology may be maintained in part by this group of commensal bacteria. The fact that patients in the hospital not harbouring α-streptococci had, in general, a higher number of treatments with antibiotics indicates that broad-spectrum antimicrobial drugs inhibited the growth of α-streptococci.

A-streptococci are dominant species in healthy people and may outgrow opportunistic bacterial species like P

MRSA and Pseudomonas aeruginosa were substantially more frequently identified in the mouth of hospitalized patients as compared to community controls. The likelihood of finding these pathogens was higher in patients in critical care, tube-fed patient and previously treated with antimicrobials usage than in other inpatients. There is a chance that participants who needed more intensive care or were tube-fed may have had worse immune function. According to earlier reports, inpatients who receive tube feedings had noticeably greater oral cavity P. aeruginosa detection rates than those who do not.

[6], and this result validates the current study's findings. Compared to other bacterial species, P. aeruginosa is better at producing biofilms [10,19,20] and does so with ease on plastic tubes [21,22]. This could lead to a higher rate of P. aeruginosa colonization in inpatients who are tube-fed. Every inpatient who tested positive for MRSA had previously used antibiotics. Since

antibiotic treatment reduced other bacterial species, it is believed that MRSA could proliferate easily in these patients. Despite earlier studies of a higher prevalence of opportunistic infections in older people's oral cavities

[23–25], It is yet unclear what mechanisms are causing these behaviors. The current study's findings imply that a rise On the finding of opportunist bacterial forms in the buccal cavity is linked to a decline in commensal bacterial species. A number of disorders in other organs may be preceded by harmful bacterial species colonizing the mouth cavity. Aspiration pneumonia has been linked to oral bacteria [3,26], and by Gosney et al. [27], a patient was found to present with oral colonization by P. aeruginosa experienced septicemia brought on by the same strain of the bacterium.

The danger of microorganisms entering the bloodstream is increased when periodontal disease causes gingival bleeding. Elderly people who are bedridden run the danger of developing systemic disease due to Infectious bacteria of the oral cavity. Accordingly, much of the elderly's general care should be dental hygiene to prevent the colonization of harmful germs.

Conclusion

According to the current investigation, inpatients who did not have a-streptococci were more likely than those who did to have opportunistic microorganisms detected in their dental plaque. It is easy to get oral samples, and the results of the current study's bacterial inspection using culture methods were available in less than a week. It has been proposed that Virulent oral bacteria colonization and there is an inverse association with the proliferation of a-streptococci. Not much research has been done on the relationship between astreptococci and health. The current work offers fresh insights into the possible function of commensal bacterial species in averting pathogenic bacterial infection.

An examination The relation between the oral α-streptococci and of pathogenic bacterial species need to be investigated for the improvement of data accuracy.

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