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ORIGINAL ARTICLE

Identification of *Capnocytophaga* species from oral cavity of healthy individuals and patients with chronic periodontitis using phenotypic tests

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Abstract

Background: The role of *Capnocytophaga* species in oral health and disease is not well studied, and there are no reports from India about their prevalence in the oral cavity. Few attempts have been made to identify all seven cultivable species of *Capnocytophaga* from gingival pocket. The aim of this study was to detect the prevalence of *Capnocytophaga* species in healthy individuals, gingivitis, and periodontitis using phenotypic tests.

Materials and Methods: A total of 150 adult subjects between the age ranges of 20–55 years were included in the study comprised of 50 each of subjects with gingivitis, periodontitis, and healthy individuals. Subgingival plaque was collected and cultured on blood agar, TBBP, and Dentaid media. Species identification was done by performing biochemical tests and hydrolysis tests.

Results: Among 150 samples, 28 (18.67%) yielded *Capnocytophaga* species. The prevalence of *Capnocytophaga* species was statistically analyzed using Chi-square test, Mann–Whitney U-test, and Fisher's exact test. The prevalence was higher in healthy individuals (30%), compared to gingivitis (14%) and periodontitis (12%). The prevalence of *Capnocytophaga ochracea, Capnocytophaga gingivalis,* and *Capnocytophaga granulosa* was more in healthy individuals than in gingivitis and periodontitis.

Conclusion: We conclude that *Capnocytophaga* is more frequent in healthy human mouth than in diseased individuals. There is a need to further study both sub- and supra-gingival plaques for the presence of this organism.

Introduction

A number of studies have shown that periodontitis is a microbial infection caused by various bacteria residing in the gingival pocket. These include mainly Gram-negative anaerobic bacilli with varying nutritional and environmental requirements. Some researchers have also recognized the importance of facultative anaerobic bacteria such as *Capnocytophaga* in the etiology of periodontal diseases.[1]

The organisms belonging to the genus *Capnocytophaga* are known to be members of the oral microbial flora both in health and disease. The genus was established by Leadbetter in 1979 and then consisted of only three species isolated from dental plaque.[2] Two new species were added by later investigators in 1994.[3] According to the most recent reports, this genus includes six well-characterized species and 13 unnamed/unassigned phylotypes.[4]

Capnocytophaga species are frequently isolated from patients with various infections such as septicemia, osteomyelitis, abscesses, and keratitis from adults and children, more so from individuals with immunocompromised status.[5-8] In the oral cavity, they are isolated from both healthy individuals and patients with localized aggressive periodontitis, advancing adult periodontitis, periodontal abscesses, and diabetics with chronic periodontitis with varying frequency.[2] However, the role of these organisms in oral health and disease is not well studied and there are no reports from India about their prevalence in the oral cavity of periodontally diseased or healthy individuals. In addition, there are no reports available where attempts have been made to identify all seven cultivable species of *Capnocytophaga* from subgingival pocket. Keeping this in mind, the present study was aimed to investigate the prevalence of *Capnocytophaga* species from the gingival pockets of healthy individuals and patients suffering from periodontitis with different grades of severity.

A battery of phenotypic tests was used for the identification and differentiation of species.

Materials and Methods

The present study was performed in the Department of Microbiology, Maratha Mandal Dental College, Belagavi, Karnataka. The study was initiated after obtaining approval from the ethical committee of the institution. A total of 150 adult subjects between the age ranges of 20–55 years belonging to both the genders were included after obtaining informed consent from each individual. They were categorized into three groups. Among them, 50 were apparently healthy individuals (Group I), 50 with gingivitis (Group II), and 50 with periodontitis (Group III).

Inclusion criteria for subjects in healthy group were absence of any clinical sign of gingival inflammation, probing depth ≤3 mm, and no clinical attachment loss. Inclusion criteria for patients with gingivitis were generalized presence of clinical signs of gingival inflammation, probing depth ≤3 mm, and no clinical attachment loss. Criteria for inclusion of patients with periodontitis were generalized presence of clinical signs of gingival inflammation, generalized probing depth ≥5 mm, and generalized clinical attachment loss of ≥3 mm. Exclusion criteria for all three groups were patients with any systemic disease, smokers, pregnant or lactating women, cervical or subgingival caries or restorations, and periodontal or antimicrobial therapy within 3 months before sampling.

From each individual, subgingival plaque was collected with curette, after stripping away the supragingival plaque. The material was immediately transferred to reduced transport fluid (RTF) and brought to the laboratory for processing. The RTF received was vortexed to break the plaque samples and release the organisms in broth. It was then cultured on Blood agar, Dentaid media, and Trypticase soya agar with bacitracin and polymyxin (TBBP), which is a selective medium for *Capnocytophaga*. The plates were prepared as per the guidelines of original authors.[9,10] The plates were then incubated in a jar with 5–10% CO_2 for 72 h. The plates were then removed from the jar and examined for colony characters typical of *Capnocytophaga*. Characteristic gliding motility on blood agar with or without hemolysis and/or yellow-orange or beige-colored flat thin colonies on selective media was used to provisionally identify the species of *Capnocytophaga.* Gram staining of suspected colony was performed to see for the presence of Gram-negative fusiform bacilli; simultaneously, catalase and oxidase tests were also performed [Figures 1-3]. Provisionally identified *Capnocytophaga* colonies were further subjected to phenotypic identification by performing biochemical tests that included fermentation of glucose, lactose, sucrose, maltose, mannose, fructose, amygdalin, cellobiose, salicin, mannitol, sorbitol, melibiose, inulin, and raffinose. In addition, nitrate reduction test and hydrolysis of aesculin, urea, starch, and gelatin were also performed. The procedures used by earlier investigators were followed for carrying out the biochemical reactions.[11,12]

Differentiation among seven species was done by adopting the criteria used by Frandsen *et al.*[12] The species investigated include *Capnocytophaga ochracea, Capnocytophaga granulosa, Capnocytophaga haemolytica, Capnocytophaga gingivitis, Capnocytophaga sputigena, Capnocytophaga leadbetteri,* and *Capnocytophaga genospecies AHN 8471.*

Results

Subgingival plaque samples from 150 individuals with an equal distribution of healthy, gingivitis, and periodontitis group were analyzed by culture to detect the presence of various *Capnocytophaga s*pecies. Among the participants, 65 (43.3%) were males and 85 (56.6%) were females. A total of 28 (18.67%) samples yielded *Capnocytophaga* species. Of seven species, only five could be detected and *C. sputigena* and *C. haemolytica* were not seen in any of the subjects from all three groups [Table1].

Analysis of the data showed that the prevalence of *Capnocytophaga gingivalis, C. ochracea,* and *C. granulosa* was more in healthy individuals than in gingivitis and periodontitis. They were also found to be in much higher frequency in all study subjects than the other two species detected. Among them, the intergroup differences were significant for *C. ochracea* and *C. gingivalis* [Figure 4]. On the other hand, *C. leadbetteri* and *C. genospecies AHN 8471* were found only in patients with gingivitis [Figure 4]. When the prevalence of *Capnocytophaga* species among different groups was studied, it could be seen that these organisms were present in 30% of healthy individuals in contrast to 14% of patients

Table 1: Descriptive statistics with frequencies and averages (*n*=150) of study volunteers

Frequencies	Frequency (%)
Group	
Healthy	50 (33.33)
Gingivitis	50 (33.33)
Chronic periodontitis	50 (33.33)
Gender	
Male	65 (43.33)
Female	85 (56.66)
Prevalence of microorganisms	
C. gingivalis	8 (5.33)
C. ochracea	10 (6.66)
C. granulosa	7 (4.66)
C. sputigena	0 (0)
C. haemolytica	0 (0)
C. genospecies (AHN8471)	1 (0.66)
C. leadbetteri (AHN8855)	2 (1.33)

C. gingivalis: Capnocytophaga gingivalis, C. ochracea: Capnocytophaga ochracea, C. granulosa: Capnocytophaga granulosa, C. sputigena: Capnocytophaga sputigena, C. haemolytica: Capnocytophaga haemolytica, C. genospecies: Capnocytophaga genospecies, C. leadbetteri: Capnocytophaga leadbetteri

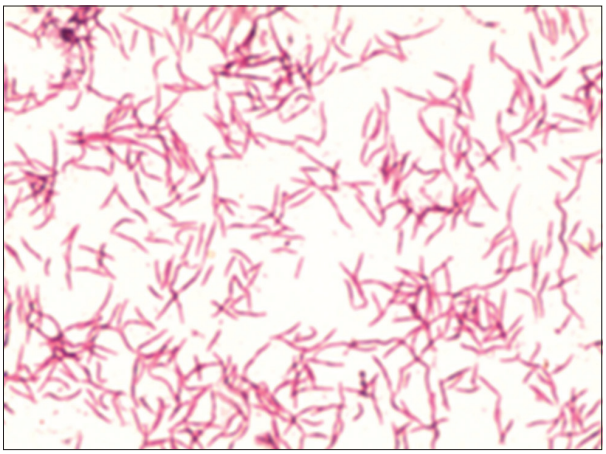

Figure 1: Gram-negative fusiform bacilli of *Capnocytophaga* (Gram staining)

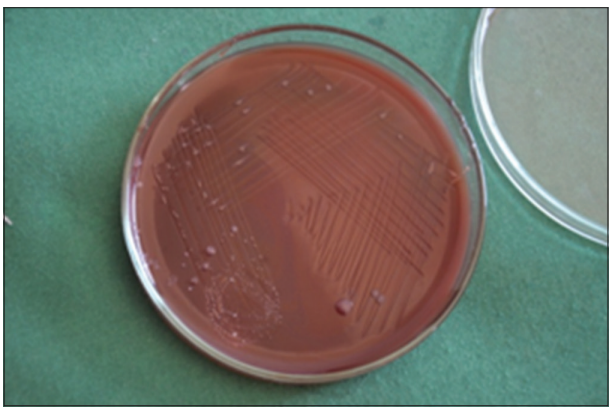

Figure 2: *Capnocytophaga* colonies on TBBP

with gingivitis and 12% of patients with periodontitis [Table 2]. This difference was statistically significant.

We also compared median colony-forming units of *Capnocytophaga* among different groups. The colony counts were significantly higher in periodontitis patients than in healthy individuals and periodontitis than in gingivitis group. On the other hand, these differences were not significant between healthy and gingivitis groups [Tables 3-5].

Discussion

Organisms belonging to the Genus *Capnocytophaga* are Gram-negative, fusiform, facultatively anaerobic bacteria that prefer an atmosphere with 5–10% CO_2 for their growth.[13] They are considered to be a significant component of human oral bacterial flora. They have been isolated from periodontally healthy and diseased sites in the oral cavity; however, the reports on their role in periodontal disease are conflicting.[14] This could be due to differences in the isolation rate of these bacteria from healthy and diseased sites, heterogeneity of the organism, and variability in the production of different virulence factors from individual bacteria.[12]

Figure 3: Gliding motility of *Capnocytophaga* on blood agar

Table 2: Prevalence of *Capnocytophaga* species-culture

Groups	Culture		Total	Fisher's exact test
	Negative	Positive		
Periodontitis	44 (88)	6 (12)	50 (100.0	<0.0405, significant
Healthy	35 (70)	15 (30)	50 (100.0)	
Gingivitis	43 (86)	7 (14)	50 (100.0)	
Total	122 (81.33)	28 (18.67)	150 (100.0)	

Interpretation: The healthy group had significantly prevalence (30%) of *Capnocytophaga* compared to chronic periodontitis (12%) and gingivitis (14%). The difference was statistically significant when compared by Fisher's exact test (P<0.05)

In the present study, an attempt was made to investigate the prevalence of all seven cultivable species of *Capnocytophaga,* and the overall prevalence was found out to be 18.67%. We could identify only five species, and the presence of *C. haemolytica* and *C. sputigena* could not be detected in any of the samples from either healthy or diseased subjects. In earlier studies using culture methods, the isolation frequency of *Capnocytophaga* species has been reported to range from 20 to 67% in samples from gingival pockets.[1,15] However, in most of these studies, only selected species of *Capnocytophaga* were targeted. These bacteria are known to be found in higher proportions in children and adolescents.[16,17] This could be the probable reason for slightly lower detection rate than expected in our subjects since the entire study population consisted of adults.

We found the prevalence of *Capnocytophaga* species to be significantly higher in samples from healthy group as compared to those of gingivitis and periodontitis group. Similar findings have been reported by several other investigators.[18,19] In contrast to this, some workers have reported a higher frequency of *Capnocytophaga* species in patients with gingivitis and periodontitis than in healthy individuals.[14,16,19,20] When the prevalence of individual species was considered, it could be seen that *C. ochracea* (6.66%) followed by *C. gingivalis* (5.33%) and *C. granulosa* (4.66%) was detected in higher proportions

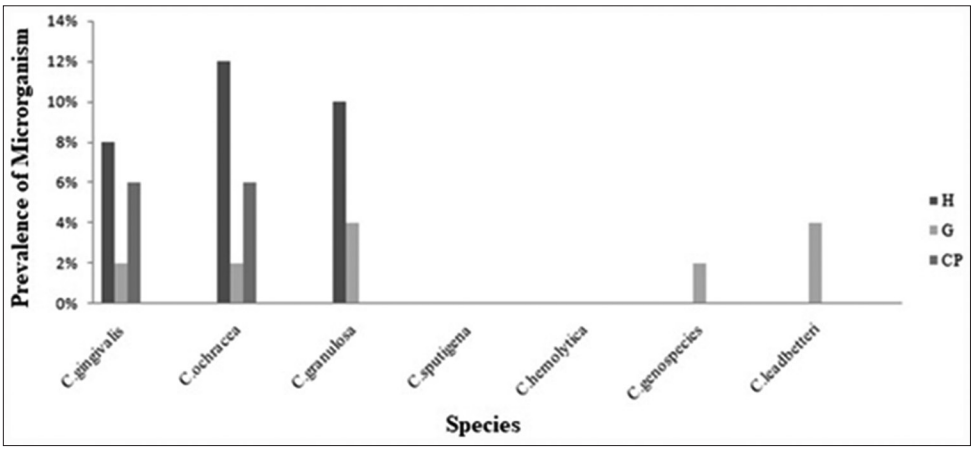

Figure 4: Prevalence of *Capnocytophaga* individual species in healthy, gingivitis, and periodontitis

Table 3: Comparison of median CFU between healthy and CP

Group	*n*	Mean±SD	Min	Max	Percentiles			Mann–Whitney test (*P*-value and significance)
					25th	50th	75th	
CP	50	54833±33313	15000	98000	24750	52000	87500	*P*<0.0.0363 Significant
Healthy	50	20438±17610	2000	63000	6500	16000	30750	

Interpretation: Chronic periodontitis group had significantly higher median CFUs compared to healthy group. CFUs: Colony-forming units, SD: Standard deviation, CP: Cerebral palsy

Table 4: Comparison of median CFU between healthy and gingivitis

Group	*n*	Mean±SD	Min	Max	Percentiles			Mann–Whitney test (*P*-value and significance)
					25th	50th	75th	
Gingivitis	50	24714±17036	8000	48000	12000	14000	46000	*P*<0.0.2413 not significant
Healthy	50	20438±17610	2000	63000	6500	16000	30750	

Interpretation: Gingivitis group had not significantly CFUs compared to healthy group. CFUs: Colony-forming units, SD: Standard deviation

Table 5: Comparison of median CFU between CP and gingivitis

Group	*n*	Mean±SD	Min	Max	Percentiles			Mann–Whitney test (*P*-value and significance)
					25th	50th	75th	
CP	50	54833±33313	15000	98000	24750	52000	87500	*P*<0.0.0367 Significant
Gingivitis	50	24714±17036	8000	48000	12000	14000	46000	

Interpretation: CP group had significantly CFUs compared to gingivitis group. CFUs: Colony-forming units, SD: Standard deviation, CP: Cerebral palsy

in our study. There appears to be a general consensus among investigators about higher occurrence of these three species in the oral cavity.[12,21] A few other workers also have found low prevalence of *C. leadbetteri* and *C. genospecies AHN 8471*, a finding similar to our study.[12] We could not isolate either *C. sputigena* or *C. haemolytica* from the participants in our study. There appears to be considerable variation in the detection rate of these two species, especially that of *C. sputigena* from several studies. A few workers have shown a high frequency of isolation, especially in children, whereas others have shown a low prevalence rate or complete failure to detect them. This wide variation in the prevalence rates could be due to geographical variation and methods of sampling.[12]

Correct identification of *Capnocytophaga* species is of paramount importance to determine their significance in oral health and disease. When phenotypic tests are being applied for differentiation of species, ambiguous results may give rise to problems. To avoid this, we have used a large panel of biochemical tests in our study. The outcome of our study reconfirmed that *Capnocytophaga* is more frequent in healthy human mouth than in diseased individuals. However, we feel that there is a need to perform studies with a larger sample size, more sensitive techniques such as polymerase chain reaction, and sampling from both subgingival and supragingival plaques to get a proper assessment of the role of these bacteria in periodontal diseases.

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