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# OCCURRENCE OF *ACTINOBACILLUS ACTINOMYCETEMCOMITANS* IN PATIENTS WITH CHRONIC PERIODONTITIS, AGGRESSIVE PERIODONTITIS, HEALTHY SUBJECTS AND CHILDREN WITH GINGIVITIS IN TWO CITIES OF THE STATE OF SÃO PAULO, BRAZIL

## *OCORRÊNCIA DE* ACTINOBACILLUS ACTINOMYCETEMCOMITANS *EM PACIENTES COM PERIODONTITE CRÔNICA, PERIODONTITE AGRESSIVA, PESSOAS SAUDÁVEIS E CRIANÇAS COM GENGIVITE EM DUAS CIDADES DO ESTADO DE SÃO PAULO, BRASIL*

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## ABSTRACT

The aim of this study was to determine the frequency of isolation of *Actinobacillus actinomycetemcomitans* (*Aa*) in 100 patients with chronic periodontitis, 14 patients with aggressive periodontitis, 142 pre-school children with gingivitis and 134 periodontally healthy subjects. Samples of subgingival plaque were taken using sterilized paper points introduced into periodontal pockets or gingival crevice for 60 seconds and inoculated on TSBV agar, which was incubated under anaerobiosis at 37ºC, for 4 days. Microbial identification was performed through biochemical methods and morphocellular and morphocolonial analysis. *Aa* was detected in 40.3% of healthy subjects, 68% of patients with chronic periodontitis, 92.86% of patients with aggressive periodontitis and 40.14% of children with gingivitis. The rate of recovery of *Aa* in the tested human groups proved to be higher than previously reported and in agreement with participation of this facultative anaerobe as a member of native microbiota of the periodontium and its relation with aggressive and chronic periodontitis in Brazil.
**Uniterms:** Periodontitis; Microbiology; Infection; Bacteria; Gingivitis.

## RESUMO

Avaliou-se a ocorrência de *Actinobacillus actinmycetemcomitans* (*Aa*) em pacientes 100 pacientes com periodontite crônica, 14 com doença periodontal agressiva, 142 crianças com gengivite em idade pré-escolar e 134 indivíduos adultos saudáveis. Amostras de placa subgengival foram coletadas usando cones de papel estéreis introduzidos nas bolsas periodontais ou no sulco gengival por 60 segundos e inoculadas em ágar TSBV, que foram incubadas em anaerobiose a 37ºC, por 4 dias. A identificação microbiana foi realizada através de análises bioquímicas, morfocelulares e morfocoloniais. *Aa* foi detectado em 40,3% de indivíduos saudáveis, 68% de pacientes com periodontite crônica, 92,86% de pacientes com periodontite agressiva e 40,14% das crianças com gengivite. A taxa de ocorrência de *Aa* nos grupos testados provou ser mais alta do que a previamente descrita na literatura e que esse microrganismo é membro freqüente da microbiota de indivíduos adultos periodontalmente sadios e de crianças com idade pré-escolar com gengivite além de sua relação com a periodontite crônica e agressiva no Brasil.
**Unitermos:** Periodontite; Microbiologia; Infecção; Bactéria; Gengivite.

## INTRODUCTION

Periodontal diseases are one of the causes of early loss of teeth and represent a worldwide socioeconomic and public health problem. There is a close association between certain bacterial species and their susceptible hosts[14]. However, the role of each microbial species in the development of these pathologies must be better understood. *Actinobacillus actinomycetemcomitans* has been implicated in the pathogenesis of aggressive periodontitis, especially aggressive periodontitis[1, 21], as well as chronic periodontitis[3, 5, 20].

There have been controversies about the role of *A. actinomycetemcomitans* in the etiology of different modalities of periodontopathies around the world[2, 8], since its distribution does not seem to be homogeneous[10] and the available data on the occurrence of this amphibiontic rod in Brazilian population, which shows some peculiar cultural, dietary and ethnic characteristics, are very scarce[18].

Therefore, the aim of this study was to evaluate the distribution of *Aa* in patients with chronic periodontitis, aggressive periodontitis, healthy subjects and children with gingivitis.

## MATERIAL AND METHODS

### Sample collection

One hundred patients with chronic periodontitis (PCP), ranging in age from 33 to 59 years (average 40), showing at least 4 periodontal pockets of depth equal to or exceeding 5mm, gingival bleeding and radiographic evidences of bone loss; 134 healthy subjects (HS), ranging in age from 16-29 years (mean 20), with no evidence of attachment loss and gingival inflammation; 14 patients with localized aggressive periodontitis (PAP), 13-20 years and 142 preschool children with marginal gingivitis (CG), ranging in age from 1-6 years, participated in this study from 1992 to 2000.

All patients were assisted at the clinics of the Dental School of São Paulo-USP, Araçatuba Dental School-UNESP and private dental offices. None of the subjects had received antibiotics or periodontal therapy in the 6 months prior to acceptance to the study.

Samples were taken by the same examiner using sterilized paper points, which were introduced into the periodontal pocket or gingival crevice for 60s and then transferred, under $CO_2$ flux, to tubes containing 2.0mL of Ringer-PRAS. In the periodontal patients the samples were collected from deepest sites showing clinically evidenced inflammation, while the samples from healthy subjects were taken from mesio-buccal and mesio-lingual sites of maxillary and mandibular first molars.

### Microbial isolation and identification

The samples were submitted to serial dilutions in Ringer-PRAS and plated on TSBV agar[17], incubated at 37°C, under anaerobiosis (90% $N_2$, 10% $CO_2$), for 4 days. After incubation, suggestive colonies of *A. actinomycetemcomitans* were inoculated on trypticase soy agar, enriched with yeast extract (0.5%) and supplemented with sheep blood (5%). The isolated bacteria were identified by means of their morphocellular, morphocolonial and biochemical-physiological characteristics[17].

## RESULTS

The prevalence of *Actinobacillus actinomycetemcomitans* in different groups is presented in Table 1. It was verified that this pleomorphic rod was frequently isolated (40.14% in children with gingivitis). The data were submitted to statistical analysis and performed by means of chi-square and Fisher tests ($\alpha \leq 0.05$) and the difference between groups is presented in Table 2.

## DISCUSSION

*Actinobacillus actinomycetemcomitans* is a Gram-negative rod frequently involved in human periodontitis, especially in the most aggressive forms[1,18,21]. However, some studies have been arguing the universality of its relevance in the pathogenesis of periodontitis[2,4,8].

The data presented in table 1 evidenced that *Aa* was isolated from 40.3% of healthy subjects, which is in accordance with Papapanou, et al.[16] (1997), who evaluated the occurrence of this pathogen in Chinese living in rural areas. However, literature has presented a smaller frequency of isolation, varying from 6% to 27% in healthy subjects[13,19,20,21].

Similar phenomenon was also observed in preschool

**TABLE 1-** Prevalence (%) of *Aa* in the studied groups

| Group (n) | n (%) of positive subjects and patients |
|---|---|
| HS (134) | 54 (40.3) |
| PCP (100) | 68 (68.0) |
| PAP (14) | 13 (92.86) |
| CG (142) | 57 (40.14) |

**TABLE 2**- Difference between studied groups about the presence of *Aa*

| Groups | Difference (significance) |
|---|---|
| HS x PCP | Significant ($\alpha$=0.01) |
| HS x PAP | Significant ($\alpha$=0.01) |
| HS x CG | Not significant ($\alpha$>0.05) |
| PCP x PAP | Significant ($\alpha$=0.05) |
| PCP x CG | Significant ($\alpha$=0.01) |
| PAP x CG | Significant ($\alpha$=0.01) |

children, where 40.14% of them proved to be colonized by this pathogen. This value is much higher than previously reported[15, 20]. Könönen, et al.[7] (1994) and Kamma, et al.[6] (2000) were not able to detect this pathogen in children under 10 years old. Perhaps the presence of gingival inflammation in these children could collaborate with the colonization of gingival sulcus by *Aa*, hence these bacteria seem to be frequently related to initial periodontal inflammation in patients with minimal attachment loss[11].

The results presented on table 1 evidenced the close association between *Aa* and aggressive periodontitis, as observed in literature[1,18,21]. However, studies in Chile[9], North Ireland[12] and China[4], did not find any relation with these aggressive early-onset periodontitis and occurrence of *Aa*.

The data presented in table 2 demonstrate that it did not only have statistical differences when we compare the groups with gingival health and children with gingivitis, what it means that the studied microorganisms are found in similar percentages in the two groups.

The association between *A. actinomycetemcomitans* and chronic periodontitis seems to be less evident than observed in relation to aggressive forms of periodontitis and the frequency of isolation of this rod varies from 20% to 75%[13,15,21]. The results of our study confirmed this association, and showed that this Gram-negative rod was isolated from 68% of patients with chronic periodontitis, but its participation in the microbiota was very reduced, always smaller than others pathogens like *Prevotella intermedia*, *Porphyromonas gingivalis* and particularly *Fusobacterium nucleatum* (unpublished data).

## CONCLUSIONS

The present study showed a close association between *Aa* and aggressive periodontitis in two cities of the state of São Paulo, Brazil, and higher frequency of isolation of this facultative anaerobe in healthy subjects and children with gingivitis. Although *Aa* has been isolated in high percentage in chronic periodontitis patients, the number of microorganisms, in these patients, was extremely low.

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