

# Prevalence of *Actinobacillus actinomycetemcomitans* in moderate and advanced periodontitis

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## ABSTRACT

*Actinobacillus actinomycetemcomitans* is a gram-negative, small, nonspore-forming, nonmotile, facultatively anaerobic rod. This bacterium has been strongly implicated in the etiology of localized juvenile periodontitis, but may also be isolated in different forms of periodontal disease like adult periodontitis. The aim of this study was to determine the prevalence of *Actinobacillus actinomycetemcomitans* in individuals diagnosed with moderate and advanced periodontitis, presenting to the general dental clinic at University of Taubaté, São Paulo. The study population comprised 87 individuals, thirty males and 57 females, 25 – 72 years old (mean age  $40.1 \pm 9.8$ ). Through periodontal probing depth (PD), was chosen two test teeth (including > PD) and two control teeth (including < PD) at the same subject. From these target teeth, subgingival bacterial plaque was obtained from all subjects by placing three paper point (by tooth) for 10 seconds into the sulci/pocket of four teeth. The points were transferred to tubes containing Ringer's solution and processed separately for each tooth. The results showed that in 17 subjects (19.3%) positive samples of *Actinobacillus actinomycetemcomitans* was detected. This pathogen was present in six and 11 individuals, respectively, with moderate and advanced periodontal lesions. Out of 348 teeth examined (174 test and 174 control), 16 test teeth and four control teeth were *Actinobacillus actinomycetemcomitans* positive. The results

observed in the present study showed a major prevalence of *Actinobacillus actinomycetemcomitans* in cases of advanced lesions of adult periodontitis.<sup>1</sup>

## UNITERMS

*Actinobacillus actinomycetemcomitans*; moderate periodontitis; advanced periodontitis.

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## RESUMO

*Actinobacillus actinomycetemcomitans* é um bastonete Gram negativo, pequeno, não formador de esporo, imóvel, anaeróbio facultativo. Esta bactéria tem sido fortemente associada na etiologia da periodontite juvenil localizada, podendo também ser isolada em outras formas de doença periodontal como a periodontite do adulto. O objetivo do presente estudo foi determinar a prevalência de *Actinobacillus actinomycetemcomitans* em indivíduos diagnosticados com periodontite moderada e avançada, que se apresentaram para tratamento clínico geral na clínica odontológica da Universidade de Taubaté,

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São Paulo. A população estudada compreendeu 87 indivíduos, trinta homens e 57 mulheres, entre 25 e 72 anos de idade (média de idade de  $40,1 \pm 9,8$ ). Através das medidas de profundidade de sondagem (PS), foram determinados dois dentes teste (>PS) e dois dentes controle (<PS) do mesmo indivíduo. A partir destes quatro dentes selecionados, amostras de placa bacteriana subgingival foram obtidas, de todos os indivíduos examinados, colocando-se três cones de papel esterilizados subgingivalmente (por dente) e mantidos em posição por 10 segundos. Os cones de papel foram então colocados em tubos contendo solução de Ringer e processados separadamente por dente. Os resultados mostraram que 17 (19,3%) dos indivíduos apresentaram amostras positivas de *Actinobacillus actinomycetemcomitans*. Esta bactéria foi distribuída entre seis e 11 indivíduos, respectivamente apresentando periodontite moderada e avançada. Dos 348 dentes examinados (174 dentes teste e 174 dentes controle), 16 dentes teste e quatro dentes controle apresentaram amostras positivas de *Actinobacillus actinomycetemcomitans*. Os resultados observados no presente estudo evidenciam uma maior prevalência de *Actinobacillus actinomycetemcomitans* nos indivíduos com lesões periodontais avançadas.

## UNITERMOS

*Actinobacillus actinomycetemcomitan*; periodontite moderada; periodontite avançada.

## INTRODUCTION

Many studies have showed that some oral pathogens are able to development gingivitis and periodontitis in human (Moore & Moore<sup>10</sup>, 1994; Haffajee & Socransky<sup>4</sup>, 1994). Despite the fact that many oral pathogens cannot be definitely ruled out as periodontal pathogens, a small number have been implicated as important in the etiology of periodontal infections. They can colonize gingivae, cheeks and tongue and, when teeth are present, bacteria colonize them both below and above the gingival margin. It is estimated that between 300 and 400 different species are capable of colonizing the mouth, and any individual may typically harbor 150 to 200 different species. These organisms live in harmony with the host, but in certain circumstances a select group of organisms have the potential to cause disease. Among the most often studied are *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Veillonella parvula*, *Treponema denticola*, *Eikenella corrodens*, *Fusobacterium nucleatum* e *Capnocytophaga rectus*.

*Actinobacillus actinomycetemcomitans* is a gram-negative, small, nonspore-forming, nonmotile, facultatively anaerobic rod. It produces several biologically active substances such as leukotoxin, inhibition of neutrophil functions, endotoxin-mediated complement activation, polyclonal cell activation that individually or collectively could be involved in the production of disease. This specie was first recognized as a possible periodontal pathogen by its increased frequency of detection and higher numbers in lesions of localized juvenile periodontitis (Slots<sup>16</sup>, 1976; Newman & Socransky<sup>12</sup>, 1977; Mandell & Socransky<sup>7</sup>, 1981; Zambon et al<sup>29</sup>. 1983) compared with low number in plaque samples from other clinical conditions including health, gingivitis and periodontitis. Although its role is less clear, *Actinobacillus actinomycetemcomitans* has also been implicated in adult forms of destructive periodontal disease. The species has been isolated from adult periodontitis lesions but less frequently and in lower numbers than from lesions in localized juvenile periodontitis individuals (Rodenburg et al<sup>14</sup>. 1990; Slots et al.<sup>19</sup> 1990).

The aim of the present study was to determine the prevalence of *Actinobacillus actinomycetemcomitans* in individuals diagnosed with moderate and advanced periodontitis, presenting to the general dental clinic at University of Taubaté, São Paulo.

## MATERIALS AND METHODS

The subjects examined in the present study were obtained through the Graduate Clinic of the Department of Periodontics at University of Taubaté, São Paulo. All subjects were informed about the study protocol and signed an informed consent that was previously approved by the Council of Ethics Research of University of Taubaté. The study population comprised 87 individuals, thirty males and 57 females, 25 – 72 years old (mean age  $40,1 \pm 9,8$ ) and had been diagnosed from moderate to advanced periodontal lesions (Brown et al.<sup>2</sup>, 1989).

All the subjects met the following inclusion criteria: a) no periodontal therapy in the previous six months; b) no local or systemic antibiotic therapy in the previous six months and; c) no systemic disease. Clinical parameters in-

cluded assessments of gingival index/ GI (Løe & Silness<sup>5</sup>, 1963) and plaque index / PII (Silness & Løe<sup>15</sup>, 1964) and periodontal probing depth (PD).

A total of 348 teeth, four teeth by subject, were sampled for bacterial analysis, two control teeth (> PD), and two test teeth (< PD). The sampling area was isolated with cotton rolls, carefully cleaned supragingivally with sterile cotton pellets, and then air-dried. Three sterile paper point (Johnson and Johnson # 30) were inserted to the bottom of the pocket and allowed to absorb subgingival bacteria for 10 seconds (Renvert et al.<sup>13</sup>, 1997). The paper points were then transferred to a vial containing Ringer's solution. All samples were processed in the laboratory within 2 hours. Appropriate dilutions were plated on Tryptic soy-Serum-Bacitracin-Vancomycin (TSBV) agar plates (Slots<sup>18</sup>, 1982). The TSBV plates were incubated in a CO<sub>2</sub> incubator, 5% CO<sub>2</sub> in air, at 37°C (Van Steenberg et al.<sup>25</sup>, 1986). After five days, the plates were examined for presence of *Actinobacillus actinomycetemcomitans*. The criteria for identification were colony morphology, the

presence of a star-like inner structure and catalase production (Slots<sup>17</sup>, 1982).

Immediately after the clinical and microbiological examination all subjects received periodontal treatment at the Graduate Clinic of the Department of Periodontics at University of Taubaté. The statistical analysis was performed by Chi-square and analysis of variance test (ANOVA), and the significance level with  $p < 0.05$ .

## RESULTS

The study population comprised 87 individuals, thirty males and 57 females, between 25-72 years old (mean age  $40.1 \pm 9.8$ ) presenting to the general dental clinic proceedings. This population received diagnosis of moderate periodontitis (PD from 5 to 6mm) that included 35 (40.2%) individuals and advanced periodontitis (PD  $\geq 7$ mm), with 52 (59.8%) individuals. These diagnosis were established by periodontal probing depth, including 6 sites/tooth. Figure 1 shows the distribution of 87 individuals divided in moderate and advanced periodontitis.

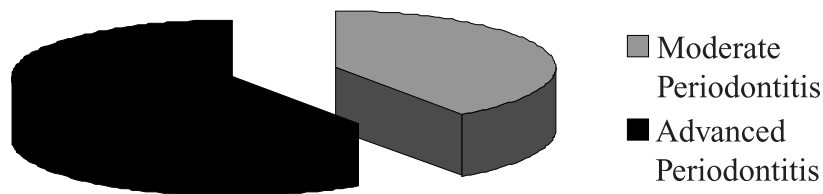


FIGURE 1 – Distribution of individuals with moderate (35 individuals) and advanced (52 individuals) periodontitis.

Seventeen out of 87 individuals examined (mean age  $36.4 \pm 6.51$ ) demonstrated positive samples of *Actinobacillus actinomycetemcomitans*, 6 (mean age  $37.8 \pm 5.9$ ) moderate and 11 ( $35.5 \pm 6.9$ ) advanced periodontitis. Table 1 showed the association of *Actinobacillus actinomycetemcomi-*

*tans* in test (> PD) and control (< PD) periodontal sites in moderate and advanced periodontitis. The Chi-square test applied showed high association between periodontal pocket depth and presence of *Actinobacillus actinomycetemcomitans* ( $\chi^2 = 16.97$  e  $p \leq 0.001$ ).

**Table 1 - Description of 17 individuals diagnosed by moderate and advanced periodontitis, presence of *Actinobacillus actinomycetemcomitans* in test and control teeth**

Patient Number	Test teeth		Control teeth	
	PD (mm)	A.a	PD (mm)	A.a
<u>09</u>	7	+	3	-
12	6	+	3	-
<b>15</b>	<b>9</b>	<b>+</b>	<b>3</b>	-
<b>26</b>	<b>12</b>	<b>+</b>	<b>4</b>	-
<b>29</b>	<b>8</b>	<b>+</b>	<b>3</b>	-
<b>38</b>	<b>8</b>	<b>+</b>	<b>3</b>	-
46	5	+	2	-
<b>57</b>	<b>9</b>	<b>+</b>	<b>3</b>	-
<b>64</b>	<b>7</b>	<b>+</b>	<b>3</b>	-
<b>66</b>	<b>9</b>	<b>+</b>	<b>4</b>	-
<b>71</b>	<b>7</b>	<b>+</b>	<b>3</b>	<b>+</b>
<b>74</b>	<b>7</b>	<b>+</b>	<b>3</b>	-
80	6	-	3	+
<b>81</b>	<b>7</b>	<b>+</b>	<b>3</b>	-
83	6	+	3	+
85	5	+	2	+
86	5	+	3	-
Total				
A. <i>actinomycetemcomitans</i> positive	16	4		

$\chi^2 = 16,97$  e  $p < 0,001$

PD : periodontal probing depth

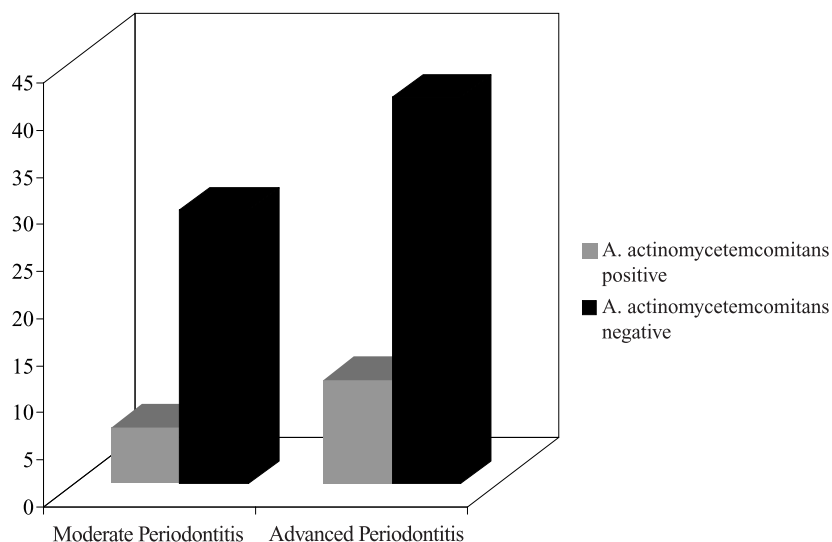
mm : milimeters

A.a : *Actinobacillus actinomycetemcomitans*

= : individuals with advanced periodontitis

The total number of individuals with moderate (6) and advanced (11) periodontitis related to presence of *Actinobacillus actinomycetemcomitans* is present in Figure 2. This Figure showed yet that in

29 individuals (moderate periodontitis) and 41 individuals (advanced periodontitis) weren't detected positive samples of *Actinobacillus actinomycetemcomitans*.



**FIGURE 2** - Distribution of individuals with moderate and advanced periodontitis by presence (six and 11 individuals) and absence (29 and 41 individuals) of *Actinobacillus actinomycetemcomitans*.

In the present study was correlated the mean of PII and GI with mean of PD from subjects with moderate and advanced periodontitis. The results showed no correlation between PII and GI and PD in all individuals examined.

## DISCUSSION

*Actinobacillus actinomycetemcomitans* is a facultatively anaerobic rod that produces several biologically active substances which can cause tissue destruction and impair host immune responses. Destructive periodontal disease in children is frequently associated with *Actinobacillus actinomycetemcomitans*. This bacteria is found in high numbers in deep pocket in localized juvenile periodontitis patients. Studies have isolated *Actinobacillus actinomycetemcomitans* from 75-100% in these patients (DiRienzo<sup>3</sup>, 1994; López et al<sup>6</sup>, 1996; Tinoco et al<sup>23</sup>, 1997). Mombelli et al<sup>9</sup>, (1994) related that *Actinobacillus actinomycetemcomitans* can be pathogenic in adult periodontitis according to the same criteria which established its importance in localized juvenile periodontitis and the elimination of this microorganism from periodontal pockets is associated with post-treatment increases in clinical attachment and decreases in clinical probing depth. The distribution of *Actinobacillus*

*actinomycetemcomitans* in oral sites is similar to the other species of periodontal pathogens such as *Porphyromonas gingivalis*. The purpose of this clinical and microbiological study was to evaluate the presence of *Actinobacillus actinomycetemcomitans* in 87 adults (mean age  $40.1 \pm 9.8$ ) with moderate and advanced periodontal lesions. The observed data showed positive samples of this bacteria in 17 (19.5%) individuals (mean age  $36.4 \pm 6.51$ ). The data agree with previous studies that showed the close percentage of affected periodontitis individuals. Muller et al<sup>11</sup>, (1993) recovery 28% of subjects with *Actinobacillus actinomycetemcomitans* and Van der Weijden et al.<sup>24</sup>, (1994) detect 33% of positive samples in 27 patients examined.

Several authors have showed the high presence of *Actinobacillus actinomycetemcomitans* and advanced periodontal lesions associated with angular alveolar bone defects (Slots et al.<sup>20</sup> 1980; Wolff et al.<sup>27</sup>1993; Melvin et al.<sup>8</sup> 1994; Zambon<sup>28</sup>, 1994; Tanner<sup>22</sup>, 1996; Ali et al.<sup>1</sup> 1996; Von Troil-Lindén et al.<sup>26</sup> 1996; Söder et al.<sup>21</sup> 1999). In our study out of 348 teeth examined (174 test and 174 control), 16 test teeth and four control teeth were *Actinobacillus actinomycetemcomitans* positive. These data confirm that this microorganism harbor periodontal lesions in advanced periodontitis subjects.



In the present study, positive samples of *Actinobacillus actinomycetemcomitans* were observed in 11 (64.7%) individuals with advanced periodontal lesions (PD from 7 to 12mm) and only 6 (35.5%) individuals diagnosed with moderate periodontal lesions (PD from 5 to 6mm) showed positive samples of this pathogen ( $\chi^2 = 16,97$  e  $p \leq 0,001$ ).

Our data also showed that 29.4% of individuals with positive samples of *Actinobacillus actinomycetemcomitans* had received periodontal therapy before previously 6 months to start this study. These data suggest that this pathogen could be able to invade gingival connective tissue and the degree of tissue invasion is related to the number of subgingival *Actinobacillus actinomycetemcomitans*.

The higher the number of subgingival pathogen, the greater the extent of tissue invasion (Zambon<sup>28</sup>, 1994). It is likely to be much more difficult to mechanically debride many deep periodontal pockets containing large numbers of *Actinobacillus actinomycetemcomitans*.

## CONCLUSION

Summarizing, in the present clinical and microbiological study was observed a prevalence of 19.3% of *Actinobacillus actinomycetemcomitans* in individuals that had received a diagnosis of periodontitis. This pathogen was present in 6 individuals with moderate periodontal lesions and 11 individuals with advanced periodontal lesions.

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