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JANDENILSON ALVES BRÍGIDO

PREVALÊNCIA E DISTRIBUIÇÃO DE SOROTIPOS DE *Aggregatibacter actinomycetemcomitans* ISOLADOS DE PACIENTES BRASILEIROS COM DOENÇA PERIODONTAL

FORTALEZA
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Dissertação de mestrado submetida ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para a obtenção do título de Mestre em Odontologia. Área de concentração: Clínica Odontológica

Orientadora: Profa. Dra. Nádia Accioly Pinto Nogueira

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RESUMO

Estudos indicam que indivíduos com lesões periodontais mais severas apresentam maior probabilidade de serem colonizados por *Aggregatibacter actinomycetemcomitans.* Essa espécie é geneticamente heterogênea e pode ser agrupada em seis sorotipos (a-f), que podem diferir quanto a suas características de virulência. As diferenças étnicas e populações geográficas podem influenciar na distribuição e prevalência desses sorotipos em relação ao tipo de doença periodontal. Os objetivos dessa dissertação, constituída por dois artigos, foram: revisar a literatura concernente aos sorotipos de *A. actinomycetemcomitans* em relação à condição periodontal e origem geográfica dos indivíduos (capítulo 1); e avaliar a prevalência e distribuição dos sorotipos de *A. actinomycetemcomitans* em pacientes brasileiros com periodontite crônica e agressiva, identificando a possível relação dos diferentes sorotipos de *A. actinomycetemcomitans* com a patologia periodontal (capítulo 2). No estudo 1, foi realizada uma revisão sistemática da literatura pertinente ao assunto e no estudo 2, amostras de biofilme bacteriano subgengival de 71 pacientes com periodontite agressiva ou crônica positivos para *A. actinomycetemcomitans* foram analisadas através da reação em cadeia da polimerase (PCR). A análise da literatura apresentada no estudo 1 mostrou que diferentes grupos étnicos são preferencialmente colonizados por diferentes sorotipos de *A. actinomycetemcomitans.* Os sorotipos a, b e c foram largamente encontrados e o sorotipo c foi o mais prevalente na maioria dos estudos. Os resultados do estudo 2 demonstraram que o sorotipo c foi encontrado com maior frequência e os sorotipos d-f não foram detectados. Foi verificado também que indivíduos com periodontite agressiva apresentaram maior prevalência de ambos os sorotipos b e c ($p<0.05$), e que em pacientes com periodontite crônica o sorotipo c foi significativamente mais prevalente ($p<0.05$). Em conclusão, os resultados desses estudos indicam que a relação entre os diferentes sorotipos e a condição periodontal permanece obscura (capítulo 1). O sorotipo c foi dominante entre pacientes brasileiros com doença periodontal e os indivíduos com periodontite agressiva foram associados com os sorotipos b e c (capítulo 2).

Palavras-Chave: Doença periodontal. Periodontite. Sorotipo. *Aggregatibacter actinomycetemcomitans.* Biologia molecular.

ABSTRACT

Studies suggest that subjects with severe periodontal lesions are more likely to colonize *Aggregatibacter actinomycetemcomitans*. This species is genetically heterogeneous and can be grouped into six serotypes (a-f), which may differ regarding virulence characteristics. Ethnic differences and geographic population can influence the distribution and prevalence of these serotypes regarding periodontal disease. The aims of this dissertation, comprised of two manuscripts, were to review the literature concerning *A. actinomycetemcomitans* serotypes regarding to periodontal status and geographic origin of individuals (chapter 1); and to investigate the prevalence and distribution of *A. actinomycetemcomitans* serotypes in Brazilian subjects with chronic and aggressive periodontitis, identifying possible relationship of the different *A. actinomycetemcomitans* serotypes with periodontal disease (chapter 2). In study 1 was performed a systematic review of the pertinent literature related to the issue and in study 2, subgingival plaque sample of 71 subjects with aggressive or chronic periodontitis positive to *A. actinomycetemcomitans* were analysed by polymerase chain reaction (PCR). The literature analysis presented in study 1 showed that different ethnic groups are preferentially colonized by different *A. actinomycetemcomitans* serotypes. Serotypes a, b and c were largely found, and serotype c was the most prevalent in the majority of studies. The results of study 2 demonstrated that serotype c was detected with the highest frequency and serotypes d-f were not detected. It was also observed that individuals with aggressive periodontitis showed higher prevalence of both serotypes b and c ($p<0.05$), and in chronic periodontitis subjects the serotype c was significantly more prevalent ($p<0.05$). In conclusion, the results of these studies suggest that the relationship between the different serotypes and periodontal conditions remains unclear (chapter 1). Serotype c was dominant among Brazilian subjects with periodontal disease and aggressive periodontitis subjects were associated both serotypes b and c (chapter 2).

Keywords: Periodontal disease. Periodontitis. Serotype. *Aggregatibacter actinomycetemcomitans. Molecular biology.*

SUMÁRIO

1 INTRODUÇÃO GERAL

A microbiota periodontal é uma comunidade complexa de micro-organismos, muitos dos quais ainda são difíceis de isolar em laboratório. Múltiplas espécies, que funcionam como patógenos em um sítio, podem estar presentes em menor número em sítios saudáveis. Isso significa que a presença de periodontopatógenos pode ser frequentemente detectada em pacientes saudáveis (CORTELLI *et al*., 2003).

Um pequeno grupo de patógenos é reconhecido devido à sua associação com a doença periodontal, por sua capacidade de implantar-se no sulco gengival, produzir substâncias tóxicas, invadir o tecido e possuir mecanismos que lhes permitem resistir aos fatores de defesa do hospedeiro, e o *Aggregatibacter actinomycetemcomitans* está incluído nesse grupo (SLOTS *et al*., 1984).

O *A. actinomycetemcomitans* é um bastonete Gram-negativo curto, não formador de esporo, imóvel, anaeróbio facultativo, sacarolítico, forma pequenas colônias convexas com um "centro estrelado" quando cultivadas em Agar sangue 5% (ZAMBON *et al*., 1996), desenvolve-se melhor em condições de anaerobiose, principalmente na temperatura de 37º C (SLOTS, 1982), e tem demonstrado a capacidade de invadir as células epiteliais da gengiva humana *in vitro*, células endoteliais vasculares humanas e células epiteliais bucais *in vivo*. Além disso, estudos têm mostrado que induz à morte celular por apoptose (ARAKAWA *et al*., 2000) e está intimamente relacionado ao homem, pois faz parte da microbiota oral indígena e, ocasionalmente, pode ser encontrado no cérebro e no sangue, causando abscessos e endocardites (ZAMBON *et al*., 1996).

Os anos iniciais da infância são um período crítico para a aquisição de determinadas bactérias, e o contato com familiares que apresentam periodontopatógenos é a principal fonte de contaminação para bebês e crianças, que quando periodontalmente saudáveis mostram ocorrência de *A. actinomycetemcomitans* de 0 a 26% (CONRADS *et al*., 1996, CHEN *et al*., 1997), enquanto nos indivíduos jovens acometidos com a doença periodontal, esta

ocorrência pode variar de 40% a 100% (LÓPEZ *et al.*, 1996; CLEREHUGH *et al.*, 1997).

A periodontite agressiva localizada (AAP, 1999) é a forma de doença periodontal mais associada à presença deste patógeno. Alguns estudos mostram que em indivíduos com esta periodontite, *A. actinomycetemcomitans* foi isolado de bolsas periodontais ativas numa proporção de 75 a 100% (ZAMBON *et al.*, 1983, 1996).

Indivíduos adultos com saúde periodontal parecem não apresentar níveis subgengivais detectáveis de *A. actinomycetemcomitans,* enquanto estudos realizados em indivíduos adultos com periodontite crônica demonstram uma ocorrência deste patógeno de 10% a 50% (LEE *et al.*, 1999; VIEIRA *et al.*, 2009; CORTELLI *et al.*, 2010). A periodontite crônica é a forma de doença periodontal destrutiva mais comum em adultos, embora possa acometer indivíduos jovens. A patologia, em geral, progride lentamente e apresenta relação com a presença de irritantes locais, o que se mostra compatível com a severidade da doença (AAP, 1999). A*. actinomycetemcomitans* pode ainda ser detectado em proporções variáveis em quadros de gengivite em que há exclusivamente o acometimento dos tecidos de proteção do elemento dentário (MÜLLER *et al.,* 1993; LIE *et al.*, 1995; AMANO *et al.*, 2001).

O *A. actinomycetemcomitans* possui vários mecanismos potenciais de virulência, tais como a presença da leucotoxina, fatores imunossupressores e lipopolissacarideos, ativação policlonal de células, inibição de fibroblastos, células endoteliais e de células epiteliais, além da capacidade de invadir os tecidos do hospedeiro. Esses fatores parecem contribuir para o início e a progressão da doença periodontal (SLOTS, 1982). Algumas cepas de *A. actinomycetemcomitans* apresentam uma deleção de 530 pb na região promotora do operon do gene da leucotoxina, produzindo assim maiores quantidades de leucotoxina (HAUBEK *et al.*, 1997).

O *A. actinomycetemcomitans* apresenta antígenos em sua superfície que irão determinar o seu sorotipo. Diferentes cepas dessa espécie apresentam sorotipos específicos, que podem variar de a-f (SAARELA *et al.*, 1992; KAPLAN *et al.*, 2001) e diferir quanto a características de virulência. Os antígenos determinantes dos sorotipos são polissacarídeos de superfície termoestáveis que aparentam ser

exclusivos da espécie (KILIAN *et al*., 2006). Sugeriu-se recentemente a inclusão de um novo sorotipo, designado como g (TAKADA *et al*., 2010).

Estudos iniciais investigaram a relação entre os sorotipos do *A. actinomycetemcomitans* e a condição periodontal dos indivíduos, demonstrando que as cepas do sorotipo b seriam mais frequentemente encontradas em periodontites agressivas. Já o sorotipo c seria frequentemente isolado em indivíduos que apresentam saúde periodontal (ASIKAINEN *et al*., 1991; HAUBEK *et al*., 1997). Com isso, sugere-se que o antígeno de superfície estaria relacionado à patogenicidade do micro-organismo, assim como com a patogênese da periodontite agressiva (YANG *et al*., 2005). Entretanto, há estudos relacionando a periodontite mais prevalentemente com sorotipo c (FINE *et al*., 2007; CHEN *et al*., 2010; SAKELLARI *et al*., 2011).

As diferenças étnicas e distribuição geográfica podem influenciar na distribuição e prevalência desses sorotipos em relação ao tipo de doença periodontal (KIM *et al*., 2009; ROMAN-TORRES *et al*., 2010). A maioria dos estudos defende a concepção de uma monoinfecção, ou seja, os pacientes tendem a serem colonizados por apenas um único sorotipo (ZAMBON *et al*., 1983), enquanto outros trabalhos afirmam que foram detectados pacientes com dois ou três sorotipos diferentes (CHUNG *et al*., 1989; ASIKAINEN *et al*., 1991; MOMBELLI *et al*., 1999).

O desenvolvimento de técnicas de biologia molecular, objetivando a detecção de espécies patogênicas, permitiu não apenas a aquisição do conhecimento da genética microbiana, mas também determinou a base para o desenvolvimento de métodos diagnósticos avançados (ROTIMI *et al*., 2010). Muitas espécies desconhecidas anteriormente foram detectadas e outras foram reclassificadas. Além disso, o emprego dessa metodologia permitiu o avanço na compreensão do papel de tais micro-organismos na etiopatogenia de doenças como periodontite, gengivite e cárie. A reação em cadeia da polimerase (PCR) mostrou-se uma técnica altamente sensível e eficaz na detecção de periodontopatógenos, mesmo quando estes estão presentes em pequenas quantidades (KOMIYA *et al*., 2010).

Estudos indicam que indivíduos com lesões periodontais mais severas apresentam maior probabilidade de serem colonizados por *A. actinomycetemcomitans.* Todavia, ainda há poucos estudos no Brasil que

relacionam antígenos sorotipos-específicos desse micro-organismo com a patologia periodontal (TINOCO *et al*., 1997; TEIXEIRA *et al*., 2006; ROMAN-TORRES *et al*., 2010; CORTELLI *et al*., 2012) e nenhum estudo na região norte-nordeste do Brasil.

A eliminação de *A. actinomycetemcomitans* de bolsas periodontais tem sido um alvo da terapia periodontal, e é correlacionada com resultados estáveis de tratamento (SAKELLARI *et al*., 2011). O teste microbiológico é importante para orientar as prescrições, detectando possíveis sorotipos envolvidos com a severidade da doença periodontal, pelo menos nos grupos com periodontite agressiva, em que a presença de *A. actinomycetemcomitans* é geralmente relacionada. Essas duas afirmativas fortalecem a relevância clínica deste estudo.

Assim, pela indisponibilidade de estudos brasileiros na região norte e nordeste e pela divergência da possível associação dos sorotipos de *A. actinomycetemcomitans* com a periodontite, justifica-se a realização do presente estudo do tipo transversal, no sentido de avaliar a distribuição dos sorotipos de *A. actinomycetemcomitans* em pacientes com doença periodontal e identificar possível relação dos diferentes sorotipos de *A. actinomycetemcomitans* com a condição clínica periodontal.

2 PROPOSIÇÃO

Os objetivos deste estudo foram:

- Revisar os estudos que avaliaram a prevalência e distribuição dos sorotipos de *Aggregatibacter actinomycetemcomitans* em relação à condição periodontal e origem geográfica dos indivíduos.

- Avaliar a prevalência e distribuição dos sorotipos de *Aggregatibacter actinomycetemcomitans* em pacientes brasileiros com periodontite crônica e agressiva e identificar possível relação dos diferentes sorotipos desse micro-organismo com a patologia periodontal.

3 CAPÍTULOS

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, e permite a inserção de artigos científicos de autoria ou co-autoria do candidato (Anexo A). Por se tratar de pesquisa envolvendo seres humanos, ou parte deles, o projeto de pesquisa foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovado (Anexo B). Assim sendo, esta dissertação é composta de dois capítulos contendo artigos que serão submetidos para publicação em revistas científicas, conforme descrito abaixo:

Capítulo 1:
"Serotypes of *Aggregatibacter actinomycetemcomitans* in relation to periodontal status and geographic origin of individuals - a systematic review." Brígido JA, Silveira VRS, Rego RO, Nogueira NAP. Este artigo será submetido à publicação no periódico **European Journal of Clinical Microbiology & Infections Diseases.**

Capítulo 2:
"Prevalence and distribution of Aggregatibacter actinomycetemcomitans serotypes in periodontal disease Brazilian subjects." Brígido JA, Silveira VRS, Rego RO, Nogueira NAP. Este artigo será submetido à publicação no periódico **Molecular Oral Microbiology.**

3.1 CAPÍTULO 1

Serotypes of *Aggregatibacter actinomycetemcomitans* in relation to periodontal status and geographic origin of individuals - a systematic review

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Abstract

Purpose: Several studies have focused on the relationship among serotype distribution, ethnical status and geographic populations, and periodontal conditions. Studies that have investigated the prevalence and the distribution of *A. actinomycetemcomitans* serotypes and the relation between the different serotypes of the bacterium and periodontal status were reviewed. **Methods:** A systematic literature search for publications regarding the distribution of *A. actinomycetemcomitans* serotypes in subgingival samples of periodontitis patients and periodontally healthy subjects by employing polymerase chain reaction (PCR) was conducted. **Results:** From the 85 studies identified in the first analysis, only 14 met all inclusion and exclusion criteria. Clinical isolates from diverse geographic populations with different periodontal conditions were evaluated. Serotypes a, b and c were largely found, and serotype c was the most prevalent. They were isolated from various periodontal conditions, including aggressive periodontitis. **Conclusions:** The available literature suggests that serotypes a, b, and c are globally dominant, serotypes d and e are rare, and the prevalence of the most recently identified serotype f is still unknown. It is widely accepted that distribution patterns of *A. actinomycetemcomitans* vary among subjects of different ethnicity and geographic regions. The correlation of different serotypes with various periodontal conditions remains unclear.

Keywords: *Aggregatibacter actinomycetemcomitans;* Serotypes; Periodontal disease; Prevalence

Introduction

Periodontitis is a collective term for inflammatory conditions affecting supporting tissues of the teeth induced by microbial deposits [1]. Progressive loss of tooth attachment in periodontis may eventually culminate in loss of affected teeth. As a consequence, periodontal disease is one of the most important concerns for dentists, patients and the public dental healthcare system.

Epidemiological studies have shown that periodontal disease occurs predominantly in a slowly progressing form, chronic periodontitis, which in the majority of patients involves a limited number of teeth and rarely interferes with tooth function before adulthood [2]. Periodontitis also occurs in a severe and rapidly progressing form, denoted aggressive periodontitis, which most often starts at an early age [2,3].

Clinical and microbiological studies have identified only a few bacterial species associated with periodontal disease in adults [4]. *Aggregatibacter actinomycetemcomitans* is a Gram-negative, nonmotile, facultative anaerobic cocobacillus bacterium that colonizes the human oral cavity, associated with the etiology of aggressive periodontitis [5- 7], and can also be detected in the oral cavity of chronic periodontitis patients and periodontally healthy subjects [8, 9]. This microorganism produces a variety of virulence factors, such as lipopolysaccharide, leukotoxin and cytolethal distending toxin (CDT) [10]. The natural population of *A. actinomycetemcomitans* comprises distinct clonal lineages that exhibit little recombination between clones [11, 12].

A. actinomycetemcomitans can be grouped into six serotypes (a-f) based on the polysaccharide antigen on the cell surface [13]. Numerous studies have examined the relationship of *A. actinomycetemcomitans* serotype, ethnical status and geographic populations, and periodontal disease status, but with conflicting results [14-17]. Subjects are

usually colonized by a single serotype, which can persist for life [18], and the frequency distribution of *A. actinomycetemcomitans* serotypes differs among various populations [19]. There are no epidemiological studies on the distribution of *A. actinomycetemcomitans* serotypes, but the available literature suggests that serotypes a, b, and c occur much more frequently among oral isolates than serotypes d, e, and f [13, 20-22]. Differences in serotype distribution have been shown among African, Asian, Europeans, and North and South American populations [21-25].

The objective of the present study was to review the studies that have investigated the prevalence and the distribution of *A. actinomycetemcomitans* serotypes in subgingival samples of periodontitis patients and periodontally healthy subjects by employing polymerase chain reaction (PCR) and to examine the possible association between periodontal conditions and serotypes.

Materials and methods

Data sources and search strategy

The electronic database PubMed was searched systematically for studies published between January 2002 and July 2012. No language restrictions were applied. Both Mesh and Major terms were used in the search and Boolean operators (OR, AND) were used to combine the searches. The bibliographies of all potentially relevant studies and review articles were also searched. The search terms included "serotypes" AND "*Aggregatibacter actinomycetemcomitans*" OR "A*ctinobacillus actinomycetemcomitans*" AND "periodontal disease" OR "periodontitis". The search was carried out twice by two different people.

Study selection

Eligibility criteria applied to all studies retrieved by the search were established. Duplicate records or double-published studies and articles published before 2002 were excluded. No limitations were placed on the geographical location. Studies involving the distribution of *A. actinomycetemcomitans* serotypes in subgingival samples of periodontitis patients and periodontally healthy subjects by employing PCR were eligible for inclusion in this review. All abstracts were reviewed in order to identify any studies of interest. Two reviewers independently assessed the full-text articles for eligibility. Only studies which met all the eligibility criteria were finally included. Relevant data were abstracted from all studies meeting the eligibility criteria. The following data were extracted from each study: (1) the first author and year of publication; (2) the country where the study was conducted; (3) searched serotypes; (4) Aim of study; (5) Principal findings and (6) possible association between periodontal conditions and serotypes.

Results

Eighty-five potentially articles were identified, of which 66 were excluded based on their titles and abstracts. The full text of each of the 19 remaining papers was reviewed, and seven were excluded because they did not match the inclusion criteria for this review. The remaining 12 studies were included in the systematic review, nine cross-sectional studies and three longitudinal studies.

The study selection is presented in Table 1. The publication dates ranged from 2003 to 2012. The study sample sizes ranged from 49 to 486 individuals and the number of

participants positive for *A. actinomycetemcomitans* ranged from 13 to 204 individuals. Participants' ages ranged from 4 to 82 years. Definition of periodontal disease varied greatly between the studies. Although majority of the studies defined periodontitis based on probing pocket depth (PPD) and/or clinical attachment level (CAL) measurements, their definitions varied in terms of the threshold for the extent and severity of these criteria. Various researches included a control group of periodontally healthy participants. Eight studies evaluated serotypes a-f and four studies examined serotypes a-e.

Clinical isolates from diverse geographic populations with different periodontal conditions were evaluated. The samples were obtained of the subjects from Japan, Brazil, United States, Indonesia, Sweden, Germany, Korea, Greece and Thailand.

Table 2 shows the prevalence and distribution of *A. actinomycetemcomitans* serotypes and the relationship with periodontal status of the studies included in the systematic review. Serotypes a, b and c were largely found, and serotype c was the most prevalent. These serotypes were isolated from various periodontal conditions, including aggressive periodontitis. Serotypes d, e, and f were either not detected or were relatively infrequent.

Discussion

There is convincing evidence of differences in serotype distribution related to geography and/or ethnic group. Available data indicate that the geographic distribution of serotypes is not uniform [6, 32, 33]. The distribution pattern of *A. actinomycetemcomitans* serotypes varies greatly depending on the periodontal status of the allocated population and the country where the study takes place [22, 26, 27, 32, 33].

It has been suggested that patients are usually infected by only one serotype and colonization is stable over time [28, 30], however occasional individuals are colonized with two or three serotypes [31, 33, 35]. Most authors reported frequencies of multiple-serotype infection up to 20% [22, 27, 31]. There have been a few exceptions, as in a Japanese study population where two or three serotypes of *A. actinomycetemcomitans* were detected in a percentage as high as 33% of the sites tested positive [26].

In general, the serotypes a–c occur much more frequently among oral isolates than serotypes d–f. The *A. actinomycetemcomitans* serotype presence in African-American students appears to be equally distributed among serotypes a, b, and c, whereas, Hispanic students show a strong association with serotype c [6]. Serotypes a, b and c are equally dominant and collectively comprise 95% or more of all *A. actinomycetemcomitans* strains in Greece [33]. In Brazilian subjects, serotypes a, b and c were largely found (98%), and serotype c was the most prevalent. Serotypes d, e, and f were either not detected or relatively rare [22, 31, 35]. The distribution pattern of *A. actinomycetemcomitans* serotypes in the subgingival plaque of subjects residing in the United States showed that serotype c is the dominant serotype, followed by serotypes a and b, and serotypes d, e, and f were either not detected or relatively rare [32].

The studies showed that Asian populations were commonly colonized with *A. actinomycetemcomitans* serotype c, but were occasionally infected with serotype b [26, 27, 30, 34]. Two studies have examined the serotype distribution patterns of *A. actinomycetemcomitans* in a Japanese population. In both studies serotypes c was the dominant serotype, while serotype b was relatively rare [26, 27]. For 86 *A. actinomycetemcomitans* strains in Thai adults with varying degrees of periodontal disease severity the serotype c was the dominant serotype, followed by serotypes a (33%) and b (7%) [34], whereas in Korean patients, the serotype distribution was different, the serotypes

detected most frequently were c (61.9%) and d (19.0%) [30]. The differences between the results from these Asian populations shows that serotype distribution patterns may be affected by geographic variations, even between subjects of the same race/ethnicity.

In contrast, serotype b was frequently observed in Caucasian populations [30]. In German patients, the serotypes detected most frequently were b (33.3%), c (25.0%), and a (20.8%) [30].

The serotype distribution pattern of *A. actinomycetemcomitans* within a local population may change over time, as was documented in Indonesian subjects with periodontitis between 1994 and 2002. In 1994, the predominant serotype was b (53.7%), whereas a and c occurred in 17.1% and 14.6% of the subjects, respectively. In 2002, a reduction in serotypes a (7.5%) and b (30.2%) occurred. Serotypes c and e increased in prevalence from 14.6% to 35.8% and 2.4% to 9.4%, respectively [28].

Serotypes d-f were rarely detected in most populations worldwide [32, 33, 35]. However, a high prevalence of serotype e (19–47%) was noted in Indonesian [28] and Japanese [26] individuals.

The application of molecular techniques has allowed a more detailed discrimination among different serotypes of *A. actinomycetemcomitans* and therefore the investigation of potential differences between populations of various origins as well as periodontal conditions. It has been suggested that some *A. actinomycetemcomitans* serotypes are more closely associated with periodontal disease than others.

In the United States, serotype c was the dominant serotype among *A. actinomycetemcomitans* from subjects with periodontitis [32], and in addition to the JP2 serotype b phenotype, there are other strains that are equally associated with disease initiation [6]. In Japanese patients, *A. actinomycetemcomitans* serotype c was predominantly identified in the gingival tissues of localized aggressive periodontitis patients, while the prevalence of

serotype b was rather low [27], and the distribution of *A. actinomycetemcomitans* serotypes was influenced by the presence of *Porphyromonas gingivalis* [26].

In Indonesian subjects was observed that the mean increase in probing pocket depth between 1994 and 2002 was significantly greater in subjects culture positive in 2002 in comparison to subjects without detectable *A. actinomycetemcomitans* in 2002 [28]. This confirms that subgingival presence of *A. actinomycetemcomitans*, but not a specific serotype is associated with a higher degree of inflammation [6].

In Brazil, an association between serotype b and healthy periodontium and between serotype c and chronic periodontitis was observed [22], differing from other data, which associated serotype b strains with patients with aggressive periodontitis [35]. Aggressive periodontitis subjects were not exclusively associated with *A. actinomycetemcomitans* serotype b [31, 35]. In general, isolates from healthy subjects belonged to serotypes a or c [35]. Serotype c was the most prevalent serotype among Brazilian *A. actinomycetemcomitans*, and they were isolated from various periodontal conditions, including aggressive periodontitis [35].

In a Greek population, *A. actinomycetemcomitans* was more prevalent in untreated periodontitis subjects, but no clear predominance of a specific *A. actinomycetemcomitans* serotype and absence of the JP2 clone were observed [33]. In Sweden, the findings indicate that periodontitis affecting the primary dentition does not necessarily indicate the presence of periodontal attachment loss in the permanent dentition [29].

The studies have varied widely in periodontal disease diagnosis and status, sampling protocols, study design and microbial detection methods and serotype analysis techniques, hindering comparison of the studies.

The elimination of *A. actinomycetemcomitans* from periodontal pockets has long been considered a target of periodontal therapy, and has been correlated with stable outcomes of treatment. If *A. actinomycetemcomitans* continues to be highly associated with disease development, its detection may be used as a risk marker for disease progression.

The findings from the studies reviewed indicate that different ethnic groups are preferentially colonized by different *A. actinomycetemcomitans* serotypes and the relationship between different *A. actinomycetemcomitans* serotypes and periodontal conditions remains unclear.

References

1. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366:1809–1820

2. Armitage GC (2004) Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 34:9–21

3. Armitage GC (1999) Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1–6

4. Haffajee AD, Socransky SS (1994) Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 5:78–111

5. Henderson B, Wilson M, Sharp L, Ward JM (2002) Actinobacillus actinomycetemcomitans. J Med Microbiol 51:1013–1020

6. Fine DH, Markowitz K, Furgang D *et al* (2007) Aggregatibacter actinomycetemcomitans and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. J Clin Microbiol 45:3859–3869

7. Faveri M, Figueiredo LC, Duarte PM, Mestnik MJ, Mayer MP, Feres M (2009) Microbiological profile of untreated subjects with localized aggressive periodontitis. J Clin Periodontol 36:739–749

8. Fives-Taylor PM, Meyer DH, Mintz KP, Brissette C (1999) Virulence factors of Actinobacillus actinomycetemcomitans. Periodontol 2000 20:136–167

9. Torrungruang K, Bandhaya P, Likittanasombat K, Grittayaphong C (2009) Relationship between the presence of certain bacterial pathogens and periodontal status of urban Thai adults. J Periodontol 80: 122–129

10. Curtis MA, Slaney JM, Aduse-Opoku J (2005) Critical pathways in microbial virulence. J Clin Periodontol 32:(Suppl 6) 28–38

11. Kaplan JB, Schreiner HC, Furgang D, Fine DH (2002) Population structure and genetic diversity of Actinobacillus actinomycetemcomitans strains isolated from localized juvenile periodontitis patients. J Clin Microbiol 40:1181–1187

12. Kilian M, Frandsen EV, Haubek D, Poulsen K (2006) The etiology of periodontal disease revisited by population genetic analysis. Periodontol 2000 42: 158–179

13. Kaplan JB, Perry MB, MacLean LL, Furgang D, Wilson ME, Fine DH (2001) Structural and genetic analyses of O polysaccharide from Actinobacillus actinomycetemcomitans serotype f. Infect Immun 69:5375–5384

14. Zambon JJ, Slots J, Genco RJ (1983) Serology of oral Actinobacillus actinomycetemcomitans and serotype distribution in human periodontal disease. Infect Immun 41:19–27

15. Saarela M, Asikainen S, Alaluusua S, Pyhala L, Lai CH, Jousimies-Somer H (1992) Frequency and stability of mono- or poly-infection by Actinobacillus actinomycetemcomitans serotypes a, b, c, d or e. Oral Microbiol Immunol 7:277–9

16. Asikainen S, Chen C, Slots J (1995) Actinobacillus actinomycetemcomitans genotypes in relation to serotypes and periodontal status. Oral Microbiol Immunol 10:65–68

17. Tsuzukibashi O, Takada K, Saito M et al (2008) A novel selective medium for isolation of Aggregatibacter (Actinobacillus) actinomycetemcomitans. J Periodontal Res 43: 544–548

18. Asikainen S, Chen C (1999) Oral ecology and person-to-person transmission of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. Periodontol 2000 20: 65–81

19. Rylev M, Kilian M (2008) Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol 35:346–361

20. Dahlén G, Widar F, Teanpaisan R, Papapanou PN, Baelum V, Fejerskov O (2002) *Actinobacillus actinomycetemcomitans* in a rural adult population in southern Thailand. Oral Microbiol Immunol 17:137–142

21. Yang HW, Huang YF, Chan Y, Chou MY (2005) Relationship of Actinobacillus actinomycetemcomitans serotypes to periodontal condition: prevalence and proportions in subgingival plaque. Eur J Oral Sci 113:28–33

22. Teixeira RE, Mendes EN, Roque de Carvalho MA, Nicoli JR, Farias Lde M, Magalhães PP (2006) Actinobacillus actinomycetemcomitans serotype-specific genotypes and periodontal status in Brazilian subjects. Can J Microbiol 52:182–188

23. Asikainen S, Lai CH, Alaluusua S, Slots J (1991) Distribution of Actinobacillus actinomycetemcomitans serotypes in periodontal health and disease. Oral Microbiol Immunol 6:115–118

24. Haubek D, DiRienzo JM, Tinoco EM et al (1997) Racial tropism of a highly toxic clone of Actinobacillus actinomycetemcomitans associated with juvenile periodontitis. J Clin Microbiol 35: 3037–4302

25. Tinoco EM, Lyngstadaas SP, Preus HR, Gjermo P (1997) Attachment loss and serum antibody levels against autologous and reference strains of Actinobacillus actinomycetemcomitans in untreated localized juvenile periodontitis patients. J Clin Periodontol 24: 937–944

26. Yoshida Y, Suzuki N, Nakano Y, Shibuya K, Ogawa Y, Koga T (2003) Distribution of Actinobacillus actinomycetemcomitans serotypes and Porphyromonas gingivalis in Japanese adults. Oral Microbiol Immunol 18:135–139

27. Thiha K, Takeuchi Y, Umeda M, Huang Y, Ohnishi M, Ishikawa I (2007) Identification of periodontopathic bacteria in gingival tissue of Japanese periodontitis patients. Oral Microbiol Immunol 22:201–207

28. Van Der Reijden WA, Bosch-Tijhof CJ, Van Der Velden U, Van Winkelhoff AJ (2008) Java project on periodontal diseases: serotype distribution of *Aggregatibacter actinomycetemcomitans* and serotype dynamics over an 8-year period. J Clin Periodontol 35: 487–492

29. Höglund Åberg C, Sjödin B, Lakio L, Pussinen PJ, Johansson A, Claesson R (2009) Presence of *Aggregatibacter actinomycetemcomitans* in young individuals: a 16-year clinical and microbiological follow-up study. J Clin Periodontol 36: 815–822

30. Kim TS, Frank P, Eickholz P, Eick S, Kim CK (2009) Serotypes of Aggregatibacter actinomycetemcomitans in patients with different ethnic backgrounds. J Periodontol 80:2020–2027

31. Roman-Torres CV, Aquino DR, Cortelli SC *et al* (2010) Prevalence and distribution of serotype-specific genotypes of Aggregatibacter actinomycetemcomitans in chronic periodontitis Brazilian subjects. Arch Oral Biol 55:242–248

32. Chen C, Wang T, Chen W (2010) Occurrence of Aggregatibacter actinomycetemcomitans serotypes in subgingival plaque from United States subjects. Mol Oral Microbiol 25:207–214

33. Sakellari DA, Katsikari A, Slini T, Ioannidis I, Konstantinidis A, Arsenakis M (2011) Prevalence and distribution of Aggregatibacter actinomycetemcomitans serotypes and the JP2 clone in a Greek population. J Clin Periodontol 38:108–114

34. Bandhaya P, Saraithong P, Likittanasombat K, Hengprasith B, Torrungruang K (2012) *Aggregatibacter actinomycetemcomitans* serotypes, the JP2 clone and cytolethal distending toxin genes in a Thai population. J Clin Periodontol 39: 519–525

35. Cortelli JR, Aquino DR, Cortelli SC, Roman-Torres CVG, Franco GCN, Gomez RS, Batista LHB, Costa FO (2012) Aggregatibacter actinomycetemcomitans serotypes infections and periodontal conditions: a two-way assessment. Eur J Clin Microbiol Infect Dis 31:1311–1318

Table 1 Description of the studies included in the systematic review

Study	Searched serotypes / Type of study	Aim of study	Participants number(s), Aa-positive individuals, Age	Periodontal status/ Clinical Parameters examined
Yoshida et al., 2003 [26]	a-e Cross-sectional study	To examine the frequency of mono- or poly-infection by Aa serotypes and the relationship between the detection of Pg and the distribution of Aa serotypes.	Aa was detected in 64 (19.5%) of 328 subjects (190 males aged 25–64 years and 138 females aged 22–59 years).	Minimal periodontal disease or periodontally healthy/ PPD.
Teixeira et al. , 2006 [22]	a-f Cross-sectional study	To evaluate the distribution of Aa serotypes in subjects with and without periodontitis and whether there is an association between serotype and periodontal status.	Aa strains isolated from subgingival specimens of 49 Brazilian subjects (from 4 to 58 years).	Healthy periodontium; AgP; CP (AAP, 1999 [3]) / PPD; attachment loss; bleeding or exudation on deep sites; radiographic evidence of alveolar bone loss.
Thiha et al., 2007 [27]	a-e Cross-sectional study	To identify periodontopathic bacteria in diseased gingival tissue of periodontitis patients. The distribution of Aa serotypes in tissue samples was also examined.	56 subjects consisting of 32 CP (mean age 55.13 ± 7.46), 16 GAgP (mean age 35.07 ± 8.23) and 8 LAgP (mean age 31.29 ± 5.56). Prevalence of Aa was higher in the LAgP (63%) group.	CP; GAgP; LAgP (AAP, 1999 [3]) / PPD; CAL; BOP.
Fine et al., 2007 [6]	a-e Longitudinal Cohort Study	To study the prevalence of localized aggressive periodontitis (LAP), the prevalence of Aa carriage, the relationship of Aa carriage to disease initiation.	A cohort of 96 students was established that included a test group of 38 Aa-positive students and 58 healthy Aa-negative controls (from 11 to 17 years – initial).	Healthy; LAP / One 4- or 5-mm pocket; At least two 5-mm pockets; At least one 6-mm pocket with 2 mm of attachment loss.
Van der Reijden et al., 2008 [28]	a-f Longitudinal Cohort Study	To investigate the serotype distribution and stability of Aa over an 8-year period in untreated Indonesian subjects.	From the total number of 158 patients in 1994, 65 (41.1%) were positive for Aa (mean age 29.4 years). In 2002, 53 (49.5%) subjects out of a total of 107 subjects were Aa positive (mean age 38.2 years).	Untreated periodontal disease / PI; bleeding index; PPD; CAL.

Table 1 (continued)

Study	Searched serotypes / Type of study	Aim of study	Participants number(s), Aa-positive individuals , Age	Periodontal status/ Clinical Parameters examined
Hoglund A°berg et al., 2009 [29]	a-f Longitudinal study	To look for clinical signs of periodontal disease in young adults who exhibited bone loss and detectable numbers of Aa in their primary dentition.	13 subjects who all exhibited bone loss and were colonized by Aa 16 years ago (aged 7–9 years). Aa was recovered from six of these subjects (aged 23–25 years).	Detection of bone loss and Aa in primary dentition / PPD; BOP; ABL, alveolar bone loss; PI.
Kim et al., 2009 [30]	a-f Cross-sectional study	Compared serotypes of Aa in two groups of periodontal patients with different ethnic backgrounds.	194 samples of subgingival plaque from periodontal patients (98 Koreans and 96 Germans) were analyzed (ages ranged between 27 and 63 years). 45 (23.2%) tested positive for Aa.	Generalized severe periodontitis (≥ 30% sites with CAL > 4 mm, more than two teeth with >50% periodontal bone loss in relation to the total root length) / PI; GBI; PPD; CAL.
Roman-Torres et al., 2010 [31]	a-f Cross-sectional study	To elucidate the prevalence of Aa and the distribution of Aa serotypes in Brazilian subjects with CP.	Out of 486 subjects examined, Aa was isolated in 85 (17.5%) individuals (mean age 33.41 ± 9.76 years).	CP (slight [1–2mm CAL], moderate [3–4mm CAL] or severe [>5 mm CAL]) / PPD; CAL; GI; PI.
C. Chen et al., 2010 [32]	a-f Cross-sectional study	Examined the distribution pattern of Aa serotypes in the subgingival plaque of subjects residing in the United States.	Aa was examined in 256 subgingival plaque samples from 161 subjects. A total of 82 distinct Aa strains were identified (from 11 to 32 years).	No periodontitis; LAP (with at least two teeth, either central incisors and/or first molars, CAL ≥ 2 mm, and with fewer than other four teeth with AL); GAgP (with at least two teeth with ≥ 2 mm of AL in each of the four quadrants) / PPD; Attachment loss (AL).
Sakellari et al., 2011 [33]	a-e Cross-sectional study	To investigate the distribution of Aa serotypes and the prevalence of the JP2 clone in subgingival samples of Greek subjects.	228 subjects examined for Aa. 40 subjects were positive for Aa (from 29 to 82 years).	Non-periodontitis; Untreated periodontitis Periodontitis patients receiving supportive treatment (CP or AgP - AAP, 1999 [3]) / PPD; BOP.

Table 1 (continued)

Study	Searched serotypes / Type of study	Aim of study	Participants number(s), Aa-positive individuals , Age	Periodontal status / Clinical Parameters examined
Bandhaya et al., 2012 [34]	a-f Cross-sectional study	To examine Aa serotypes, the ltx promoter and the presence of cdtABC genes in a group of Thai adults.	Subgingival plaque samples from 453 subjects were analysed for Aa. Eighty six subjects (19%) were positive for Aa (aged 38–59 years).	Mean PD; % sites with PD ≥ 5 mm; Mean CAL; % sites with CAL ≥ 4 mm / PPD; recession; CAL.
Cortelli et al., 2012 [35]	a-f Cross-sectional study	Investigated a large population of individuals positive for Aa and performed a two way analysis assessing the relation between the different serotypes of the bacterium and periodontal conditions.	204 individuals (mean age 33.54 ± 11.11) positive for Aa.	Non-periodontitis; AgP (PPD > 5 mm and CAL ≥ 4 mm at first molars and incisors); Mild CP (PPD > 3 mm in at least four teeth and mean periodontal CAL > 3 mm); Moderate/severe CP (PPD > 3 mm in at least four teeth and mean periodontal CAL ≥ 5 mm) / PPD;CAL; GI; PI.

Aa, *Aggregatibacter actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*; AgP, Aggressive Periodontitis; CP, chronic periodontitis; LAgP, localized aggressive periodontitis; GAgP, generalized aggressive periodontitis; PPD, probing pocket depth; CAL, clinical attachment level; BOP, bleeding on probing; PI, plaque index; GI, gingival index; GBI, gingival bleeding index.

Table 2 Prevalence and distribution of *A. actinomycetemcomitans* serotypes and association with periodontal status

Study / Location	Occurrence of Aa serotypes	Periodontal conditions and serotypes
Yoshida et al., 2003 [26] Japan	Aa serotype c was detected more frequently in sites that were positive for both Pg and Aa (76.9%) than in sites that were Pg-negative and Aa-positive (33.9%). The numbers of sites in which two different serotypes and three different serotypes were detected were 18 (25.0%) and 7 (9.3%), respectively.	The distribution of Aa serotypes was influenced by the presence of Pg. The findings suggest that the characteristics of serotype c may differ from those of the other serotypes.
Teixeira et al., 2006 [22] Brazil	Serotypes b and c were observed in similar frequencies, and no subject harboured d, e, or f serotype strains.	An association between serotype b and healthy periodontium and between serotype c and CP was observed.
Thiha et al., 2007 [27] Japan	Aa serotype c was detected in 50% of LAP patients.	Aa serotype c was predominantly identified in the gingival tissues of Japanese LAP patients, while the prevalence of serotype b was rather low.
Fine et al., 2007 [6] United States	Aa serotype presence in African-American students appears to be equally distributed among serotypes a, b, and c, whereas, Hispanic students show a strong association with serotype c.	The detection of Aa in periodontally healthy children can serve as a risk marker for initiation of LAP.
Van der Reijden et al., 2008 [28] Indonesia	In 1994, the predominant serotype was b (53.7%), whereas a and c occurred in 17.1% and 14.6% of the subjects, respectively. In 2002, a reduction in serotypes a (7.5%) and b (30.2%) occurred. Serotypes c and e increased in prevalence from 14.6% to 35.8% and 2.4% to 9.4%, respectively.	Subgingival presence of Aa, but not a specific serotype is associated with a higher degree of inflammation. Aa serotypes distribution in Indonesian young adults shifts from predominantly serotype b to a more equal prevalence of serotypes b and c.
Hoglund A°berg et al., 2009 [29] Sweden	Serotypes a–c and e, but not d or f, were found from the fourteen 7–9-year-old subjects at the baseline examination. Among the strains isolated from the six Aa-positive young adults, serotypes a–c, and f were identified.	The presence of Aa and early bone loss in the primary dentition does not necessarily predispose the individual to periodontal attachment loss in the permanent dentition.
Kim et al., 2009 [30] Germany, Korea	In German patients, the serotypes detected most frequently were b (33.3%), c (25.0%), and a (20.8%), whereas in Korean patients, the serotype distribution was different, with serotypes c (61.9%) and d (19.0%).	Even if the percentage of patients who tested positive for Aa was identical in patients with GAgP and severe CP and different ethnic backgrounds, the distribution of Aa serotypes may exhibit marked differences.

Table 2 (continued)

Study / Location	Occurrence of Aa serotypes	Periodontal conditions and serotypes
Roman-Torres et al., 2010 [31] Brazil	Out of 85 positive samples, 68 were infected by at least 1 serotype, 7 by mixed, and 10 were non-serotyped. Serotypes d and f were not detected. Serotype c showed the highest prevalence (52.9%), followed by serotype a (31.8%).	The prevalence of serotype c in severe periodontitis was significantly greater than that of serotypes a and b.
C. Chen et al., 2010 [32] United States	The serotype distribution pattern of the strains was 21 (25.6%) serotype a, 12 (14.6%) b, 41 (50%) c, 6 (7.3%) e, 1 (1.2%) f, and 1 (1.2%) non-typeable.	Serotype c is the dominant serotype among Aa from subjects with periodontitis in the United States.
Sakellari et al., 2011 [33] Greece	No statistical differences were observed concerning the distribution of serotypes among groups. Serotype c was more predominant within the periodontally diseased groups.	Aa serotype b was not statistically correlated with periodontal disease in the investigated sample and the utility of microbiological testing before antimicrobial administration is emphasized.
Bandhaya et al., 2012 [34] Thailand	Serotype c was the most prevalent (57%), followed by serotypes a (33%) and b (7%).	No significant relationship between serotypes and the extent or severity of periodontal disease.
Cortelli et al., 2012 [35] Brazil	Serotypes a, b and c were largely found (98%), and serotype c was the most prevalent. Serotypes d, e, and f were either not detected or relatively rare.	Serotype c was the most prevalent in both diseased and healthy subjects. AgP subjects were not exclusively associated with Aa serotype b.

Aa, *Aggregatibacter actinomycetemcomitans*; Pg, *Porphyromonas gingivalis*; CP, chronic periodontitis; AgP, Aggressive Periodontitis; LAP, localized aggressive periodontitis; GAgP, generalized aggressive periodontitis.

3.2 CAPÍTULO 2

Title: Prevalence and distribution of *Aggregatibacter actinomycetemcomitans* serotypes in periodontal disease Brazilian subjects

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Running title: Serotypes of *A. actinomycetemcomitans*

Keywords: *Aggregatibacter actinomycetemcomitans*; Serotypes; Periodontal disease; Prevalence

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Summary

Aggregatibacter actinomycetemcomitans is usually isolated from the oral cavity where it is associated with active periodontitis and can be divided into 6 serotypes (a-f). This study investigated the occurrence of *A. actinomycetemcomitans* serotypes in Brazilian subjects (n=71) with chronic (n=35) and aggressive periodontitis (n=36), assessing possible relationship of the different *A. actinomycetemcomitans* serotypes and periodontal disease. All patients received clinical examinations that included periodontal pocket depth, clinical attachment loss, plaque, and gingival indexes. Subgingival plaque sample of subjects with aggressive or chronic periodontitis were analysed by Polymerase Chain Reaction (PCR). The results demonstrated that serotype c was the most prevalent and serotypes d-f were not detected. It was also observed that individuals with aggressive periodontitis showed higher prevalence of both serotypes b and c ($p<0.05$), and in chronic periodontitis subjects the serotype c was significantly more prevalent ($p<0.05$). In conclusion, serotype c was dominant among Brazilian subjects with periodontal disease and aggressive periodontitis subjects were related both serotypes b and c.

Introduction

Aggregatibacter actinomycetemcomitans is a facultative anaerobic, Gram-negative coccobacillus bacteria that has been associated with the etiology of periodontal diseases, especially in aggressive periodontitis (Kaplan *et al*. 2001;Fine *et al*., 2007; Cortelli *et al*., 2008) and also with chronic periodontitis (Cortelli *et al*., 2005).

A. actinomycetemcomitans strains can be grouped into six serotypes (a-f) based on the composition of the polysaccharides present on the surface of the organism, which function as immunodominant antigens (Kaplan *et al*., 2002; Kilian *et al*., 2006). The global serotype distribution is not homogeneous and the association between serotype and periodontal status

(periodontal health, aggressive or chronic periodontitis) may be depending on the geographical location and/or ethnical status of the study population (Haubek *et al*., 1997; Celenligil & Ebersole, 1998; Kaplan *et al*., 2001; Fine *et al*., 2007).

Data on the prevalence of *A. actinomycetemcomitans* serotypes in populations from geographically distant regions showed that serotypes a–c occur much more frequently among oral isolates than serotypes d–f (Tinoco *et al*., 1997; Yoshida *et al*., 2003; Teixeira *et al*., 2006; Chen *et al*., 2010; Cortelli *et al*., 2012). In several studies the numbers of strains with these serotypes have been too small to allow any firm conclusions as to particular associations between strains of these serotypes and periodontal disease and geographical origin of carriers (Tinoco *et al*., 1997; Haubek *et al*., 2001; Teixeira *et al*., 2006).

Data in the literature have consistently shown that serotype b strains have been more frequently associated with periodontitis, especially with the aggressive form of the disease, mainly of African origin, and serotype c isolates with periodontal health (Dirienzo *et al*., 1994; Haubek *et al*., 1997; Lakio *et al*., 2002; Yang *et al*., 2004, 2005). In contrast, in Japanese subjects periodontal disease appears to be associated mainly with serotype c (Thiha *et al*., 2007), while it has been reported that serotype b may not be associated with aggressive periodontitis in Korean subjects (Chung *et al*., 1989).

Most studies defend the concept of monoinfection putting forward that one subject tends to be colonized by one unique serotype (Zambon *et al*., 1983a) while other studies detected subjects with two or three serotypes (Chung *et al*., 1989; Asikainen *et al*., 1991; Saarela *et al*., 1992; Mombelli *et al*., 1999).

Although several studies have examined *A. actinomycetemcomitans* serotype distribution among various populations, the relationship between a specific serotype and periodontal disease remains unclear (Rylev & Kilian, 2008).

Furthermore, observations reported for one geographic region or ethnicity have not always been confirmed in other places, which makes available data inconclusive, and no single study, up to now, was conducted in the north-northeast of Brazil. Therefore, the present study aimed to establish the prevalence and distribution of *A. actinomycetemcomitans* serotype-specific antigens in Brazilian subjects diagnosed with aggressive or chronic periodontitis.

Materials and methods

Subject Sample

Participants included in the present cross-sectional study were selected among those who sought care at the Periodontology Clinic, Faculty of Pharmacy, Dentistry and Nursing, at the Federal University of Ceará, Brazil. All subjects were screened from September 2011 to August 2012.

Patients with aggressive periodontitis were classified according to the clinical criteria suggested by the American Academy of Periodontology (AAP, 1999):

- Localized aggressive periodontitis - interproximal attachment loss on at least two permanent teeth, involving no more than two teeth other than incisors and first molars; and
- Generalized aggressive periodontitis - generalized interproximal attachment loss affecting three permanent teeth other than the first molars and incisors.

The disease affects individuals systemically healthy and with rapid attachment loss and bone destruction.

Patients with chronic periodontitis were also classified according to the criteria of the AAP (1999, 2000a, 2000b):

- Slight to moderate destruction is characterized by the presence of periodontal sites with probing depth of up to 6 mm and clinical attachment loss of up to 4 mm;
- Advanced destruction is characterized by the presence of probing depth greater than 6 mm with clinical attachment loss greater than 4 mm.

Exclusion criteria were as follows: periodontal treatment within the previous six months, antibiotics therapy within the previous three months, systemic alterations that could have interfered with the periodontal conditions, and pregnancy or women currently breast-feeding

The research protocol has been approved by the Ethics Committee, Federal University of Ceará, Brazil (183/11). All participants or guardians were informed about the purpose of the study and signed a written consent form.

Clinical Measurements

Clinical measurements were made on all totally erupted permanent teeth, except the third molars, using a periodontal probe PCP-UNC 15 (Trinity, São Paulo, Brazil). The following parameters were evaluated: plaque index (PI) (Ainamo & Bay, 1975), gingival index (GI) (Ainamo & Bay, 1975), probing depth (PD), and clinical attachment level (CAL). The clinical parameters of PD and CAL were examined at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). A single examiner evaluated all the clinical periodontal parameters. Measurement reproducibility was calculated by using intraclass correlation coefficient (ICC) for PD and CAL. The agreement between replicate measurements was high (ICC > 0.80).

Microbiological sampling

The supragingival plaque sample of 151 subjects with aggressive or chronic periodontitis was removed with curettes and sterile cotton pellets, and the area was isolated with sterile cotton rolls. Subgingival plaque sample was collected from the proximal tooth site with greater attachment loss and increased probing depth (four sites per patient) by means of two sterile paper points (Dentsply, Rio de Janeiro, Brazil). All samples were immersed in microtubes containing 1 mL of sterile Ringer's solution and stored at - 80 °C until processing.

Microbiological evaluation

The presence of *A. actinomycetemcomitans* was determined by Polymerase Chain Reaction (PCR), as described below. The subgingival samples in the microtube were dispersed using a Vortex and centrifuged (3 min at 12,000 rpm). From the cellular bacteria pellet, genomic DNA was extracted using a commercial DNA purification Kit (InstaGene1, Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. Aliquots of 20 μL of the supernatant obtained were added to 30 μL of a reaction mixture containing PCR buffer with 25 μM (Promega Corporation, USA), 25 μM $MgCl_2$ (Promega Corporation, USA), 0.2 μM of dNTP Mix (Promega Corporation, USA), 1.25 U Taq polymerase (Promega Corporation, USA) and corresponding volume 100 ng of each primer (Invitrogen, São Paulo, SP, Brazil), resulting in 50 μL of final volume. Negative and positive controls were included in each reaction.

First, a PCR with specific primers for the 16S ribosomal DNA (16S rDNA) was performed to confirm the presence of bacterial DNA. Then, the samples were evaluated by PCR with specific primers for the presence of *A. actinomycetemcomitans*. For the *A. actinomycetemcomitans* positive samples, serotyping was carried out using primers specific

for each serotype (a–f). DNA sequences of the primers used in the present study are also described in Table 1.

The amplification (Biocycler, Biosystems, Curitiba, PR, Brazil) of 16S rDNA and *A. actinomycetemcomitans* (16S ribosomal DNA) was performed with an initial cycle at 94 °C for ten minutes followed by 30 cycles at 96 °C for 30 s, 55 °C for 30 s and 72 ° C for 30 s, with final extension at 72 °C for ten minutes (Haraszthy et al., 2000). PCR primers for serotypes a-e were used as previously described by Suzuki et al. (2001), whereas those for serotype f were used according to Kaplan et al. (2001).

The amplification products were analyzed by electrophoresis separated on a 1.5% agarose gel, stained with SYBR Safe1 (Invitrogen, Carlsbad, CA, USA) and photographed (Canon Powershot A640, Canon, USA) under ultraviolet light (LTA/LTB GE, Loccus Biotecnologia - São Paulo, SP, Brazil). A 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as the molecular weight marker. Positive controls (serotype a: ATCC 29523; serotype b: Y4; serotype c: NCTC 9710; serotype d: IDH 781; serotype e: IDH 1705; and serotype f: CU 1000) and negative controls (purified PCR-grade water instead of the DNA template) were included for the PCR reactions. No amplification was observed in the negative controls.

Statistical analysis

The frequency of *A. actinomycetemcomitans* serotypes in subjects with chronic and aggressive periodontitis was analysed using the chi-square test. The values of PPD, CAL, PI and GI were analysed by Student's t-test. Significance of differences was established at 5% ($p<0.05$). All tests were performed using GraphPad Prism 6 software (GraphPad Software Inc. San-Diego, CA, USA).

Results

Demographic and periodontal characteristics of *A. actinomycetemcomitans*-positive subjects are presented in Table 2. The study included a total of 71 periodontally diseased subjects positive for *A. actinomycetemcomitas* consisting of 35 chronic periodontitis (mean age 41,8 ± 9,8) and 36 aggressive periodontitis (mean age 28,8 ± 5,6) patients. A statistically significant difference between the ages of two groups was observed. The aggressive periodontitis subjects presented signs of more severe periodontal destruction, as observed for the significantly higher mean of PD in comparison to chronic periodontitis group ($p<0.05$).

The expected bands of the sizes listed in Table 1 were observed (data not showed). The resulting amplification products produced respective single bands of 428 bp (serotype a), 298 bp (serotype b), 559 bp (serotype c), which corresponded to the size of the amplification band when the genomic DNA sample of individuals included in this study and positive controls were used. For the serotypes d-f, no bands were amplified when the genomic DNA sample of subjects with aggressive or chronic periodontitis was used.

Out of 151 subjects with periodontal disease, *A. actinomycetemcomitans* was isolated from 71 (47%) individuals. The serotype-specific antigens used in *A. actinomycetemcomitans* analysis showed that out of 71 positive samples, 60 individuals were positive for a single serotype, while 7 individuals were not positive for any serotype tested. Multiple serotypes were simultaneously found in a few samples. Two serotypes (serotype b and c) were identified in three samples from the chronic periodontitis group. Two serotypes (serotype a and b) were detected in one sample from the aggressive periodontitis group. Serotype c was detected with the highest prevalence and serotypes d-f were not detected (Table 3).

The data were interpreted considering periodontal status as the primary variable. Therefore, the allocated population was assessed as to relate their periodontal conditions with

single infections by serotype-specific strains a, b or c. Out of the 35 chronic periodontitis subjects, 29 were infected with serotypes a (5), b (7) or c (17), 3 by mixed infection, and 3 were non-serotyped. Out of the 36 aggressive periodontitis individuals, 31 were infected with serotypes a (4), b (14) or c (13), 1 by mixed infection, and 4 were non-serotyped (Table 3). Individuals diagnosed with chronic periodontitis were more commonly infected with serotype c ($p<0.05$); and aggressive periodontitis subjects showed high prevalence of serotypes b and c ($p<0.05$) (Fig. 1).

Discussion

A. actinomycetemcomitans has long been implicated in periodontal disease (Socransky & Haffajee, 2008). The frequency distribution of A. *actinomycetemcomitans* serotypes (a–f) varies among populations from geographically distinct areas and the specific serotypes may be associated with periodontal status (Rylev & Kilian, 2008; Chen *et al*., 2010; Sakellari *et al*., 2011).

We observed that serotype c was the most prevalent, followed by serotypes b and a (Table 3). These findings are in line with previous observations that Brazilian populations were commonly colonized with *A. actinomycetemcomitans* serotypes a-c (Teixeira *et al*., 2006; Roman-Torres *et al*., 2010; Cortelli *et al*., 2012). In a Thai population, serotype c was the most prevalent, followed by serotypes a and b (Bandhaya *et al*., 2012). *A. actinomycetemcomitans* serotype presence in African-American students appears to be equally distributed among serotypes a, b, and c, whereas, Hispanic students show a strong association with serotype c (Fine *et al*., 2007). In contrast, serotype b was frequently observed in Caucasian populations (Zambon *et al*., 1983b; Asikainen *et al*., 1991; Kim *et al*., 2009).

In this study, no subject harboured d, e, or f serotype strains. Serotypes d-f are usually either not detected or relatively rare (Teixeira *et al*., 2006; Chen *et al*., 2010; Sakellari *et al*., 2011; Bandhaya *et al*., 2012). However, a high prevalence of serotype e was noted in Japanese (Yamamoto *et al*., 1997; Yoshida *et al*., 2003) and Indonesian (Van der Reijden *et al*., 2008) individuals.

Most subjects harbored only a single serotype (Table 3). Data in the literature have shown that single serotype infections are the most commonly found (Saarela *et al*., 1992; Asikainen *et al*., 1995; Yang *et al*., 2004). Occasionally, individuals are colonized with two or three serotypes (Rylev & Kilian, 2008). Previous studies demonstrated that infection with multiple serotypes is possible. This was especially evident in a Japanese population where two or three serotypes of *A. actinomycetemcomitans* were detected in 33% of the sites that tested positive (Yoshida *et al*., 2003) and in Indonesian individuals, where were observed a relatively high prevalence of subjects (17%) with multiple serotypes (Van der Reijden *et al*., 2008). The high carriage rate of multiple serotypes may be partly explained by the high prevalence of serotype e because more than 50% of subjects carrying multiple serotypes were infected by this serotype. In the present study, a low percentage of individuals testing positive for *A. actinomycetemcomitans* were found to be colonized by two serotypes simultaneously (5.63%).

In our study, a low percentage of samples *A. actinomycetemcomitans* could not be serotyped (9.86%). The nonserotypeable isolates may express altered antigenicity or suggest the existence of new serotypes (Asikainen *et al*., 1995). These strains may belong to the recently identified serotype g (Takada et al. 2010), or a yet unrecognized serotype.

Several studies suggest that different *A. actinomycetemcomitans* serotypes are associated with periodontal health, periodontitis, and non-oral infections (Asikainen *et al*., 1995; Haubek *et al*., 1997; Kaplan *et al*., 2001). Among isolates of *A. actinomycetemcomitans*

from Finland, Sweden, and Denmark, the serotypes a through c are usually represented by almost equal proportions (Saarela *et al*., 1992; Haubek *et al*., 1997; Lakio *et al*., 2002). In contrast, several studies showed a clear predominance of serotype c in Japanese (Yoshida *et al*., 2003; Thiha *et al*., 2007), Chinese (Mombelli *et al*., 1998, 1999; Lakio *et al*., 2002), Brazilian (Teixeira *et al*., 2006; Roman-Torres *et al*., 2010) and from Turkish (Dogan *et al*., 2003) patients with periodontal disease.

Studies from the United States have shown that serotype b was more often isolated from patients with aggressive periodontitis (Zambon *et al*., 1983b, Yang *et al*., 2004). In Finland subjects, serotype b was predominant in periodontitis patients and serotype c was frequently isolated in periodontally healthy individuals (Asikainen *et al*., 1991). However, studies from Asia, including Thailand (Bandhaya *et al*., 2012) and Indonesia (Van der Reijden *et al*., 2008) showed no significant relationship between serotypes and the extent or severity of periodontal disease. Similarly, a study in United States school-children consisting primarily of African-Americans and Hispanics, showed that the presence of *A. actinomycetemcomitans*, but not a specific serotype, was related to the initiation of aggressive periodontitis (Fine *et al*., 2007).

To date, no study was conducted in the north-northeast of Brazil to investigate the presence and distribution of *A. actinomycetemcomitans* serotypes. Other studies were conducted in southeastern Brazil. The first study described that serotype c was more frequently found in both aggressive periodontitis and chronic periodontitis than serotype b (Tinoco *et al*., 1997). Then, an association between serotype b and healthy periodontium and between serotype c and chronic periodontitis was observed (Teixeira *et al*., 2006). Then, Roman-Torres *et al*. (2010) conducted a study describing that the prevalence of serotype c in severe periodontitis was significantly greater than that of serotypes a and b. Recently, Cortelli *et al*. (2012) examined a large population of individuals positive for *A.*

actinomycetemcomitans and investigated the relation between the different serotypes of the bacterium and periodontal conditions and demonstrated that serotype c was the most prevalent in both diseased and healthy subjects.

These Brazilian studies as well as our findings showed a higher prevalence of serotype c in patients diagnosed with aggressive and chronic periodontitis. This finding agrees with data from a Japanese study (Thiha *et al*., 2007) and similar findings were also verified in subjects residing in the United States (Chen *et al*., 2010).

Although several authors have associated the diagnosis of aggressive periodontitis subjects with serotype b (Dirienzo *et al*., 1994; Haubek *et al*., 1996; Yang *et al*., 2005), we found both serotypes b and c to be equally statistically more prevalent than the other serotypes investigated. This similar finding was also observed in a Turkish population (Celenligil & Ebersole, 1998). More information is required to make conclusions as to the potential importance of ethnicity for these differences.

In conclusion, data from the present study have shown that serotype c was highly prevalent in periodontal disease subjects. Also, aggressive periodontitis subjects were related both serotypes b and c. Serotypes d, e and f were not found in our population.

References

A.A.P. (1999) International Workshop for a Classification of Periodontal Diseases and Conditions. American Academy of Periodontology. Ann Periodontol **4:** 53-54.

A.A.P. (2000a) Parameter on chronic periodontitis with advanced loss of periodontal support. American Academy of Periodontology. J Periodontol **71:** 856-858.

A.A.P. (2000b) Parameter on chronic periodontitis with slight to moderate loss of periodontal support. American Academy of Periodontology. J Periodontol **71**: 853-855.

Ainamo, J. and Bay, I. (1975) Problems and proposals for recording gingivitis and plaque. Int Dent J **25:** 229-235.

Ashimoto, A., Chen, C., Bakker, I. and Slots, J. (1996) Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol **11:** 266-73.

Asikainen, S., Lai, C.H., Alaluusua, S. and Slots, J. (1991) Distribution of Actinobacillus actinomycetemcomitans serotypes in periodontal health and disease. Oral Microbiol Immunol **6:** 115-8.

Asikainen, S., Chen, C. and Slots, J. (1995) Actinobacillus actinomycetemcomitans genotypes in relation to serotypes and periodontal status. Oral Microbiol Immunol **10:** 65–68.

Bandhaya, P., Saraithong, P., Likittanasombat, K., Hengprasith, B. and Torrungruang, K. (2012) Aggregatibacter actinomycetemcomitans serotypes, the JP2 clone and cytolethal distending toxin genes in a Thai population. J Clin Periodontol **39:** 519–525.

Celenligil, H. and Ebersole, J.L. (1998) Analysis of serum antibody responses to periodontopathogens in early-onset periodontitis patients from different geographical locations. J Clin Periodontol **25:** 994-1002.

Chen, C., Wang, T. and Chen, W. (2010) Occurrence of Aggregatibacter actinomycetemcomitans serotypes in subgingival plaque from United States subjects. Mol Oral Microbiol **25:** 207–214.

Chung, H. J., Chung, C. P., Son, S. H. and Nisengard, R. J. (1989) Actinobacillus actinomycetemcomitans serotypes and leukotoxicity in Korean localized juvenile periodontitis. Journal of Periodontology **60:** 506–511.

Cortelli, J.R., Cortelli, S.C., Jordan, S., Haraszthy, V.I. and Zambon, J.J. (2005) Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol **32:** 860-866.

Cortelli, J.R., Aquino, D.R., Cortelli, S.C. et al (2008) Etiological analysis of initial colonization of periodontal pathogens in oral cavity. J Clin Microbiol **46:** 1322-1329.

Cortelli, J.R., Aquino, D.R., Cortelli, S.C., Roman-Torres, C.V.G., Franco, G.C.N., Gomez, R.S., Batista, L.H.B., Costa and F.O. (2012) Aggregatibacter actinomycetemcomitans serotypes infections and periodontal conditions: a two-way assessment. Eur J Clin Microbiol Infect Dis **31:** 1311–1318.

Dirienzo, J. M., Slots, J., Sixou, M., Sol, M. A., Harmon, R. and McKay, T. L. (1994) Specific genetic variants of Actinobacillus actinomycetemcomitans correlate with disease and health in a regional population of families with localized juvenile periodontitis. Infect Immun **62:** 3058–3065.

Dogan, B., Antinheimo, J., Cetiner, D. et al. (2003) Subgingival microflora in Turkish patients with periodontitis. J Periodontol **74:** 803-814.

Fine, D.H., Markowitz, K., Furgang, D., Fairlie, K., Ferrandiz, J., Nasri, C., McKiernan, M. and Gunsolley, J. (2007) Aggregatibacter actinomycetemcomitans and its relationship to

initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. J Clin Microbiol **45:** 3859-3869.

Haraszthy, V.I., Hariharan, G., Tinoco, E.M. et al. (2000) Evidence for the role of highly leukotoxic Actinobacillus actinomycetemcomitans in the pathogenesis of localized juvenile and other forms of early-onset periodontitis. J Periodontol **71:** 912-922.

Haubek, D., Poulsen, K., Westergaard, J., Dahlen, G. and Kilian, M. (1996) Highly toxic clone of Actinobacillus actinomycetemcomitans in geographically widespread cases of juvenile periodontitis in adolescents of African origin. J Clin Microbiol **34:** 1576–1578.

Haubek, D., Dirienzo, J.M., Tinoco, E.M. et al. (1997) Racial tropism of a highly toxic clone of Actinobacillus actinomycetemcomitans associated with juvenile periodontitis. J Clin Microbiol **35:** 3037-3042.

Haubek, D., Ennibi, O.K., Poulsen, K., Poulsen, S., Benzarti, N. and Kilian, M. (2001) Early-onset periodontitis in Morocco is associated with the highly leukotoxic clone of Actinobacillus actinomycetemcomitans. J Dent Res **80:** 1580-1583.

Kaplan, J.B., Perry, M.B., MacLean, L.L., Furgang, D., Wilson, M.E. and Fine, D.H. (2001) Structural and genetic analyses of O polysaccharide from Actinobacillus actinomycetemcomitans serotype f. Infect Immun **69:** 5375-5384.

Kaplan, J.B., Schreiner, H.C., Furgang, D. and Fine, D.H. (2002) Population structure and genetic diversity of Actinobacillus actinomycetemcomitans strains isolated from localized juvenile periodontitis patients. J Clin Microbiol **40:** 1181-1187.

Kilian, M., Frandsen, E.V., Haubek, D. and Poulsen, K. (2006) The etiology of periodontal disease revisited by population genetic analysis. Periodontol 2000 **42:** 158-179.

Kim, T.S., Frank, P., Eickholz, P., Eick, S. and Kim, C.K. (2009) Serotypes of Aggregatibacter actinomycetemcomitans in patients with different ethnic backgrounds. J Periodontol **80:** 2020–2027

Lakio, L., Kuula, H., Dogan, B., and Asikainen, S. (2002) *Actinobacillus actinomycetemcomitans* proportion of subgingival bacterial flora in relation to its clonal type. Eur J Oral Sci **110**: 212–217.

Mombelli, A., Gmu¨r, R., Frey, J. et al. (1998) Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in young Chinese adults. Oral Microbiol Immunol **13:** 231-237.

Mombelli, A., Gmur, R., Lang, N.P., Corbert, E. and Frey, J. (1999) Actinobacillus actinomycetemcomitans in Chinese adults. Serotype distribution and analysis of the leukotoxin gene promoter locus. J Clin Periodontol **26:** 505–510.

Roman-Torres, C.V., Aquino, D.R., Cortelli, S.C. et al (2010) Prevalence and distribution of serotype-specific genotypes of Aggregatibacter actinomycetemcomitans in chronic periodontitis Brazilian subjects. Arch Oral Biol **55:** 242–248.

Rylev, M. and Kilian, M. (2008) Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol **35:** 346-361.

Saarela, M., Asikainen, S., Alaluusua, S., Pyhalea, L., Lai, C.H. and Jousimies Somer, H. (1992) Frequency and stability of mono- or poly-infection by Actinobacillus actinomycetemcomitans serotypes a, b, c, d or e. Oral Microbiol Immunol **7:** 277–279.

Sakellari, D., Katsikari, A., Slini, T., Ioannidis, I., Konstantinidis, A. and Arsenakis, M. (2011) Prevalence and distribution of Aggregatibacter actinomycetemcomitans serotypes and the JP2 clone in a Greek population. J Clin Periodontol **38:** 108–114.

Socransky, S. S., and Haffajee, A. D. (2008) Periodontal infections. In Lindhe, J., Lang, N. P. & Karring, T. (eds). Clinical Periodontology and Implant Dentistry, pp. 207–267. Oxford, Blackwell Munksgaard.

Suzuki, N., Nakano, Y., Yoshida, Y., Ikeda, D. and Koga, T. (2001) Identification of Actinobacillus actinomycetemcomitans serotypes by multiplex PCR. J Clin Microbiol **39:** 2002–2005.

Takada, K., Saito, M., Tsuzukibashi, O., Kawashima, Y., Ishida, S. and Hirasawa, M. (2010) Characterization of a new serotype g isolate of Aggregatibacter actinomycetemcomitans. Molecular Oral Microbiology **25:** 200–206.

Teixeira, R.E., Mendes, E.N., Roque, D.E., *et al*. (2006) Actinobacillus actinomycetemcomitans serotype-specific genotypes and periodontal status in Brazilian subjects. Can J Microbiol **52:** 182–188.

Thiha, K., Takeuchi, Y., Umeda, M., Huang, Y., Ohnishi, M. & Ishikawa, I. (2007) Identification of periodontopathic bacteria in gingival tissue of Japanese periodontitis patients. Oral Microbiol Immun **22:** 201–207.

Tinoco, E.M, Stevens, R.H., Haubek, D., Lai, C.H., Balachandran, S. and Preus, H.R. (1997) Relationship of serotype, leucotoxin gene type and lysogeny in Actinobacillus actinomycetemcomitans to periodontal disease status. Eur J Oral Sci **105:** 9–14.

Van der Reijden, W.A., Bosch-Tijhof, C.J., Van der Velden, U. and Van Winkelhoff, A.J. (2008) Java project on periodontal diseases: serotype distribution of Aggregatibacter actinomycetemcomitans and serotype dynamics over an 8-year period. J Clin Periodontol **35:** 487-492.

Wilson, K.H., Blitchington, R.B. and Greene, R.C. (1990) Amplification of bacterial 16s ribosomal DNA with polymerase chain reaction. J Clin Microbiol **28:** 1942-1946.

Yamamoto, M., Nishihara, T., Koseki, T., He, T., Yamato, K., Zhang, Y. Z., Nakashima, K., Oda, S. and Ishikawa, I. (1997) Prevalence of Actinobacillus actinomycetemcomitans serotypes in Japanese patients with periodontitis. J Periodontal Res **32:** 676–681.

Yang, H.W., Asikainen, S., Dogan, B., Suda, R. and Lai, C.H. (2004) Relationship of Actinobacillus actinomycetemcomitans serotype b to aggressive periodontitis: frequency in pure cultured isolates. J Periodontol **75:** 592–599.

Yang, H.W., Huang, Y.F., Chan, Y. and Chou, M.Y. (2005) Relationship of Actinobacillus actinomycetemcomitans serotypes to periodontal condition: prevalence and proportions in subgingival plaque. Eur J Oral Sci **113:** 28–33.

Yoshida, Y., Suzuki, N., Nakano, Y., Shibuya, K., Ogawa, Y. and Koga, T. (2003) Distribution of Actinobacillus actinomycetemcomitans serotypes and Porphyromonas gingivalis in Japanese adults. Oral Microbiol Immunol **18:** 135–139.

Zambon, J.J., Christersson, L.A. and Slots, J. (1983a) Actinobacillus actinomycetemcomitans in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. J Periodontol **54:** 707–711.

Zambon, J.J., Slots, J. and Genco, R.J. (1983b) Serology of oral Actinobacillus actinomycetemcomitans and serotype distribution in human periodontal disease. Infect Immun **41:** 19–27.

Table 1 Primers used for the determination of A. actinomycetemcomitans-positive samples and for the serotyping (a-f) procedure.

Primers	Sequence 5`- 3` [a]	PCR product (bp)	References
16S rDNA	GGACTAYAGGGTATCTAAT AGAGTTTGATCMTGG	789	Wilson *et al*. 1990
A. actinomycetemcomitans	AAACCCATCTCTGAGTTCTTCTTC ATGCCAACTTGACGTTAAAT	637	Ashimoto *et al*. 1996
Serotype a	GCAATGATGTATTGTCTTCTTTTGGA CTTCAGTTGAATGGGGATTGACTAAAAC	428	Suzuki et al. 2001
Serotype b	CGGAAATGGAATGCTTGC CTGAGGAAGCCTAGCAAT	298	Suzuki et al. 2001
Serotype c	AATGACTGCTGTCGGAGT CGCTGAAGGTAATGTCAG	559	Suzuki et al. 2001
Serotype d	TTACCAGGTGTCTAGTCGGA GGCTCCTGACAACATTGGAT	690	Suzuki et al. 2001
Serotype e	CGTAAGCAGAAGAATAGTAAACGT AATAACGATGGCACATCAGACTTT	211	Suzuki et al. 2001
Serotype f	ARAAYTTYTCWTCGGGAATG CCTTTATCAATCCAGACAGC	232	Kaplan et al. 2001

[a] R = A ou G; Y= C ou T; W= A ou T

Table 2 Individuals positive for *A. actinomycetemcomitans* distributed according to periodontal conditions and clinical parameters.

Demographic and Clinical parameters	CP	AgP
Total (n [%])	35 (49,30)	36 (50,70)
Male (n [%])	18 (51,43)	13 (36,11)
Female (n [%])	17 (48,57)	23 (63,89)
Mean age ± SD (years)	41,8 ± 9,8*	28,8 ± 5,6
Number of teeth (mean ± SD)	22,9 ± 3,8	25,6 ± 2,1
PD FM (mean ± SD)	3,1 ± 0,72	3,6 ± 0,90*
CAL FM (mean ± SD)	3,8 ± 1,07	4,1 ± 1,20
PI FM (mean ± SD)	32,2 ± 12,8	33,3 ± 17,4
GI FM (mean ± SD)	16,2 ± 9,9	13,5 ± 12,0
PD SS (mean ± SD)	7,6 ± 2,2	8,3 ± 2,3*
CAL SS (mean ± SD)	8,7 ± 2,7	8,8 ± 2,8

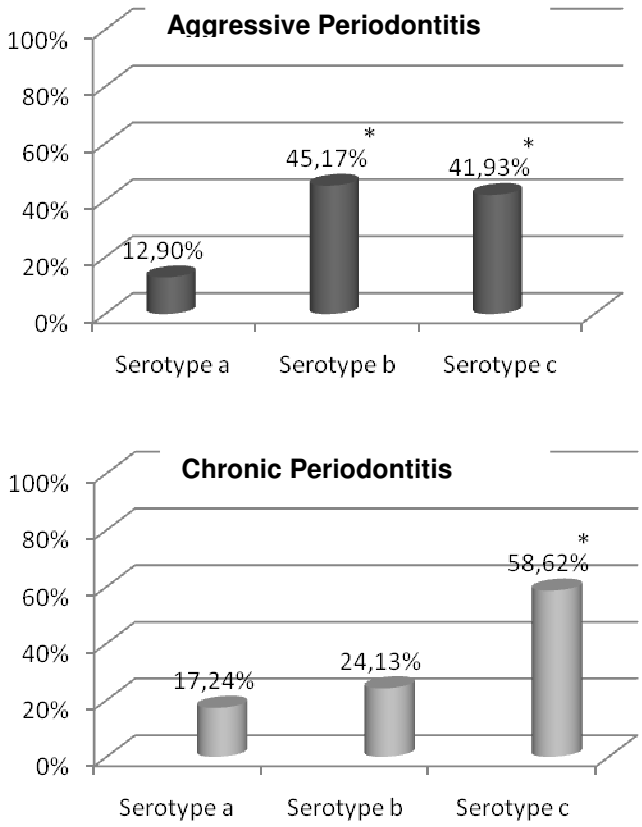
* Statistically significant difference between the groups ($p<0.05$), Student's t-test
CP- Chronic periodontitis; AgP - Aggressive periodontitis
PD – periodontal pocket depth (mm); CAL – clinical attachment level (mm);
GI – Gingival Index (%); PI – Plaque Index (%)
FM - Full mouth; SS – Sampled sites for microbial analysis
SD - Standard deviation

Table 3 Individuals positive for *A. actinomycetemcomitas* distributed according to periodontal condition and serotype infection.

	CP, N (%)	AgP, N (%)	Total, N (%)
Single infection	29 (82,86)	31 (86,11)	60 (84,51)
a	5 (14,29)	4 (11,11)	9 (12,68)
b	7 (20,00)	14 (38,89)	21 (29,58)
c	17 (48,57)	13 (36,11)	30 (42,25)
d	0 (0,00)	0 (0,00)	0 (0,00)
e	0 (0,00)	0 (0,00)	0 (0,00)
f	0 (0,00)	0 (0,00)	0 (0,00)
Mixed infection	3 (8,57)	1 (2,78)	4 (5,63)
a + b	0 (0,00)	1 (2,78)	1 (1,41)
b + c	3 (8,57)	0 (0,00)	3 (4,23)
Non-serotyped	3 (8,57)	4 (11,11)	7 (9,86)
Total	35 (49,30)	36 (50,70)	71 (100,00)

CP- Chronic periodontitis; AgP - Aggressive periodontitis

Fig. 1 Periodontal conditions related to *A. actinomycetemcomitans* serotype (a, b and c) infections.



*Statistically significant difference ($p<0.05$), chi-square test

4 CONCLUSÃO GERAL

- A relação entre os diferentes sorotipos e a condição periodontal permanece obscura (capítulo 1).

- O sorotipo c foi dominante entre pacientes brasileiros com doença periodontal e os indivíduos com periodontite agressiva foram associados com ambos os sorotipos b e c (capítulo 2).

REFERÊNCIAS

AMANO, A. Relationship of periodontophatic bacteria with early-onset periodontitis in Down's syndrome. **J. Periodontol**., v. 72, n.2, p. 368-73, 2001.

ARAKAWA, S.; NAKAJIMA, T.; ISHIKURA, H.; ICHINOSE, S.; ISHIKAWA, I.; TSUCHIDA, N. Novel apoptosis-inducing activity in *Bactreroides forsythus*: a comparative study with three serotypes of *Actinobacillus actinomycetemcomitans*. **Infect Immun**, v.68, p. 4611-4615, 2000.

AMERICAN ACADEMY OF PERIODONTOLOGY. International Workshop for a Classification of Periodontal Diseases and Conditions. **Annals of Periodontol,** v.4, n. 1, p. 53-54, 1999.

ASIKAINEN, S. ; LAI, C. H. ; ALALUUSUA, S. ; SLOTS, J. Distribution of *Actinobacillus actinomycetemcomitans* serotypes in periodontal health and disease. **Oral Microbiol Immunol**, v.6, n.2, p. 115-118, 1991.

CHEN, C. Oral food consumption and subgingival microorganisms: sub-gingival microbiota of gastrostomy tube-fed children and healthy children and health controls. **J Periodontol,** v. 68, n. 12, p.1163-8, 1997.

CHEN, C.; WANG, T.; CHEN, W. Occurrence of *Aggregatibacter actinomycetemcomitans* serotypes in subgingival plaque from United States subjects. **Molecular Oral Microbiol**, v.25, p. 207–214, 2010.

CHUNG, H. J.; CHUNG, C. P. *Actinobacillus actinomycetemcomitans* serotypes and leukotoxicity in Korean localized juvenile periodontitis. **J Periodontol**, v.60, n.9, p. 506-511, 1989.

CLEREHUGH *et al.* The detection of *A. actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* using an ELISA in an adolescent population with early periodontitis. **J. Clin. Periodontol**, v. 24, n. 1, p. 57-64, 1997.

CONRADS, G. *et al.* PCR reation and dot-blot hybridization to monitor the distribuition of oral pathogens within plaque samples of periodontally healthy individuals. **J. Periodontol**, v. 67, n. 10, p. 994-03, 1996.

CORTELLI, S.C. Detecção de cepas de *Actinobacillus actinomycetemcomitans* de máxima e mínima leucotoxicidade em pacientes com doença periodontal. **Pesq. Odontol. Bras.,** v.17, n.2, p.183-188, 2003.

CORTELLI, J. R.; ROMAN-TORRES, C. V. G.; AQUINO, D. R.; FRANCO, G. C. N.; COSTA, F. O.; CORTELLI, S. C. Occurrence of *Aggregatibacter actinomycetemcomitans* in Brazilians with chronic periodontitis. **Braz Oral Res**., v. 24, n. 2, p. 217-223, 2010.

CORTELLI, J. R.; AQUINO, D. R.; CORTELLI, S. C.; ROMAN-TORRES C.V.G.; FRANCO, G.C.N.; GOMEZ, R. S.; BATISTA, L. H. B.; COSTA, F. O. Aggregatibacter actinomycetemcomitans serotypes infections and periodontal conditions: a two-way assessment. **Eur J Clin Microbiol Infect Dis.,** v. 31, p. 1311-1318, 2012.

FINE, D. H.; MARKOWITZ, K.; FURGANG, D.; FAIRLIE, K.; FERRANDIZ, J.; NASRI C. *Aggregatibacter actinomycetemcomitans* and its relationship to initiation of localized aggressive periodontitis: longitudinal cohort study of initially healthy adolescents. **J Clin Microbiol**., v. 45, n. 12, p. 3859-3869, 2007.

HAUBEK, D.; DIRIENZO, J. M.; TINOCO, E. M. B.; WESTERGAARD, J.; LOPEZ, N. J.; CHUNG, C. P.; POULSEN, K.; KILIAN, M. Racial tropism of a highly toxic clone of *Actinobacillus actinomycetemcomitans* associated with juvenile periodontitis. **J Clin Microbiol.,** v. 35, p. 3037-3042, 1997.

KAPLAN, J. B.; PERRY, M. B.; MACLEAN, L. L.; FURGANG, D.; WILSON, M. E.; FINE, D.H. Structural and genetic analyses of O polysaccharide from *Actinobacillus actinomycetemcomitans* serotype f. **Infect Immun**., v. 69, n. 9, p. 5375-5384, 2001.

KILIAN, M.; FRANDSEN, E. V.; HAUBEK, D. & POULSEN, K. The etiology of periodontal disease revisited by population genetic analysis. **Periodontology,** v. 42, p.158–179, 2006.

KIM, T. S.; FRANK, P.; EICKHOLZ, P.; EICK, S.; KIM, C. K. Serotypes of *Aggregatibacter actinomycetemcomitans* in Patients with different ethnic backgrounds**. J Periodonto**l, v. 80, p. 2020-2027, 2009.

KOMIYA ITO, A.; ISHIHARA, K.; TOMITA, S.; KATO, T.; YAMADA, S. Investigation subgingival profile of periodontopathic bacteria using polymerase chain reaction. **Bull Tokyo Dent Coll**, v.51, n. 3, p. 139-144, 2010.

LEE, K. H. Microbiota of sucessful osseointegrated dental implants. **J. Periodontol**., v. 70, n. 2, p. 131-8,1999.

LIE, M. A. et al. Oral microbiota in subjects with a weak or strong response in experimental gingivitis. **J. Clin Periodontol**, v. 22, n. 7, p. 642-5, 1995.

LÓPEZ, N. J. *et al.* Occurrence of *A. actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* in juvenile periodontitis. **J. Clin Periodontol**, v. 23, n. 2 . p. 101-5, 1996.

MOMBELLI, A.; GMUR, R. *Actinobacillus a*. in Chinese adults. Serotype distribution and analysis of the leukotoxin gene promoter locus. **J Clin Periodontol**, v. 26, n. 8, p. 505-510, 1999.

MÜLLER, H. P. et al. *A. actinomycetemcomitans* recovery from extracrevicular locations of the mouth. **Oral Microbiol Immunol**, v. 8, n. 5, p. 334-48, 1993.

ROMAN-TORRES, C. V. G.; AQUINO, D. R.; CORTELLI, S. C.; FRANCO, G. C. N.; SANTOS, J. G.; CORRAINI, P.; HOLZHAUSEN, M.; GOMEZ, R. S.; CORTELLI, J. R. Prevalence and distribution of serotype-specific genotypes of *Aggregatibacter actinomycetemcomitans* in chronic periodontitis Brazilian subjects. **Archives of oral biology**,v. 55, p. 242-248, 2010.

ROTIMI, V. O.; SALAKO, N. A.; DIVIA, M.; ASFOUR, L.; KONONEN E. Prevalence of periodontal bacteria in saliva of Kuwaiti children at different age groups. **J Infect Public Health**, v. 3, p. 76-82, 2010.

SAARELA, M. Frequency and stability of mono- or poly-infection by *Actinobacillus actinomycetemcomitans* serotypes a, b, c, d or e. **Oral Microbiol Immunol**, v.7, p. 277-279, 1992.

SAKELLARI, D.; KATSIKARI, A.; SLINI, T.; IOANNIDIS, I.; KONSTANTINIDIS, A.; ARSENAKIS, M. Prevalence and distribution of *Aggregatibacter actinomycetemcomitans* serotypes and the JP2 clone in a Greek population. **J Clin Periodontol**, v. 38, p. 108–114, 2011.

SLOTS, J.; ZAMBON, J. J.; ROSLING, B. G.; REYNOLDS, H. S.; CHRISTERSSON, L. A.; GENCO, R. J. *Actinobacillus actinomycetemcomitans* in human periodontal disease. Association, serology, leukotoxicity, and treatment. **J Periodontal Res**., v. 17, n. 5, p. 447-448, 1982.

SLOTS, J.; GENCO, R. J. Black pigmented Bacteroides species, Capnocytophaga species, and *Actinobacillus actinomycetemcomitans* in human periodontal disease:

virulence factors in colonization, survival, and tissue destruction. **J. Dent. Res**., v.63, p. 412-421, 1984.

TAKADA, K.; SAITO, M.; TSUZUKIBASHI, O.; KAWASHIMA, Y.; ISHIDA, S.; HIRASAWA, M. Characterization of a new serotype g isolate of *Aggregatibacter actinomycetemcomitans* **Molecular Oral Microbiol**., v. 25, p. 200-206, 2010.

TEIXEIRA, R. E.; MENDES, E. N.; CARVALHO, M. A. R.; NICOLI, J. R.; FARIAS, L. M.; MAGALHÃES, P. P. *Actinobacillus actinomycetemcomitans* serotype-specific genotypes and periodontal status in Brazilian subjects. **Can J Microbiol**, v. 52, p. 182-188, 2006.

TINOCO, E. M.; STEVENS, R. H.; HAUBEK D.; LAI, C. H. BALACHANDRAN, S.; PREUS, H. R. Relationship of serotype, leucotoxin gene type and lysogeny in *Actinobacillus actinomycetemcomitans* to periodontal disease status. **Eur J Oral Sci**., v.105, p. 09-14, 1997.

VIEIRA, E. M. M.; RASLAN, S. A.; WAHASUGUI, T. C.; AVILA-CAMPOS, M. J.; MARVULLE, V.; GAETTI-JARDIM JÚNIOR, E. Occurrence of *Aggregatibacter actinomycetemcomitans* in Brazilian indians from Umutina Reservation, Mato Grosso, Brazil. **J Appl Oral Sci.,** v. 17, n. 5, p. 440-445, 2009.

YANG, H-W.; HUANG Y-F.; CHAN Y.; CHOU M-Y. Relationship of *Actinobacillus actinomycetemcomitans* serotypes to periodontal condition: prevalence and proportions in subgingival plaque. **Eur J Oral Sci**., v.113, p. 28–33, 2005.

ZAMBON, J.J.; CHRISTERSSON, L. A.; SLOTS, J. *Actinobacillus actinomycetemcomitans* in human periodontal disease. Prevalence in patient groups and distribution of biotypes and serotypes within families. **J Periodontol**., v. 54, p. 707-711, 1983.

ZAMBON, J. J. Periodontal diseases: microbial factors. **Ann Periodontol**., v. 1, n. 1, p. 879-925, 1996.

APÊNDICE A – Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Você está sendo convidado por Jandenilson Alves Brígido (pesquisador responsável) a participar como voluntário de uma pesquisa. Você não deve participar contra a sua vontade. Leia atentamente as informações abaixo e faça qualquer pergunta que desejar, para que todos os procedimentos desta pesquisa sejam esclarecidos.

1- Fui esclarecido(a) que esta pesquisa tem como objetivo analisar a presença de bactérias que causam doença em gengiva e osso. Para tanto, serei submetido(a) a um exame clínico odontológico para verificar se possuo essa doença e para coletar uma quantidade de placa bacteriana subgengival de algumas áreas dentárias, com cones de papel absorvente. Procedimentos que podem causar certo desconforto, mas não prejudiciais à minha saúde.

2- Fui esclarecido(a) que a realização da pesquisa não implica em risco algum para os participantes, pois o exame clínico a que serei submetido(a) é um exame odontológico realizado com instrumentos devidamente esterilizados.

3- Apresenta como benefícios a detecção de doença periodontal e o encaminhamento para tratamento, se for detectada doença, para a clínica de Periodontia do Curso de Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem.

4- Estou ciente de que serei esclarecido(a) durante todo o decorrer da pesquisa sobre quaisquer dúvidas relacionadas a esta e que possuo plena liberdade para desistir da referida pesquisa, retirando o meu consentimento a qualquer momento, sem sofrer nenhuma penalização ou prejuízo no atendimento clínico.

5- Estou ciente de que os dados e resultados obtidos na pesquisa serão utilizados para fins didáticos e de divulgação em revistas científicas brasileiras ou estrangeiras, entre os profissionais estudiosos no assunto; porém será garantido o sigilo de identidade, assegurando a privacidade. Os dados e o material coletado serão utilizados somente para esta pesquisa.

6- Estou ciente de que a participação na pesquisa não acarretará em nenhum gasto, a não ser aqueles de deslocamento até a Faculdade, uma vez que todo material utilizado será fornecido pelo pesquisador e que também não receberei nenhum pagamento por participar da pesquisa.

Endereço e telefone do responsável pela pesquisa:

Nome: Jandenilson Alves Brígido **Instituição:** Universidade Federal do Ceará **Endereço:** FFOE/UFC – Rua Monsenhor Furtado s/n **Telefones:** (85) 3217-7406/8805-0314

ATENÇÃO: Se você tiver alguma consideração ou dúvida sobre sua participação na pesquisa entre em contato com o Comitê de Ética em Pesquisa da UFC – Rua Coronel Nunes de Melo, 1127 Rodolfo Teófilo fone: 3366-8344

O abaixo assinado _____brasileiro(a), nascido(a) em ___/___/___, portador do RG nº _____ residente à _____, na cidade de _____ responsável por _____ brasileiro(a), nascido(a) em ___/____/___, portador do RG nº_____, na cidade de _____, concorda com a sua participação voluntária ou do menor acima, na pesquisa intitulada "**Prevalência e Distribuição de Sorotipos de *Aggregatibacter actinomycetemcomitans* Isolados de Pacientes Brasileiros com Doença Periodontal"** e declara que é de livre e espontânea vontade que está participando como voluntário da pesquisa. Eu declaro que li cuidadosamente esse Termo de Consentimento Livre e Esclarecido e que, após sua leitura tive a oportunidade de fazer perguntas sobre o seu conteúdo, como também sobre a pesquisa, e recebi explicações que responderam por completo minhas dúvidas. E declaro ainda estar recebendo uma cópia assinada deste termo.

Fortaleza, ____ de _____ de _____.

Assinatura do voluntário (ou responsável):

Assinatura do voluntário menor de 18 anos:

Assinatura do pesquisador:

Assinatura da testemunha:

Assinatura do profissional que aplicou o TCLE:

APÊNDICE B – Ficha de Exame Clínico

UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM

Número: _____

Nome:_____

Cor: _____ Sexo: M □ F

Data de nascimento: _____ Idade: _____Profissão: _____

Residência:_____

Cidade:_____Fone:_____

Estado Civil: _____ Data do exame: _____

Responsável:_____

HISTÓRICO GERAL

Está em tratamento médico? □ SIM □ NÃO Motivo:_____

Doenças sistêmicas _____

Usa ou usou medicamentos recentemente? □ SIM □ NÃO

_____

Sensibilidade a medicamentos? □ SIM □ NÃO

_____

Gravidez / lactação: _____

Fuma? □ SIM □ NÃO □ ex-fumante

_____

OBSERVAÇÕES:

HISTÓRICO BUCAL

Já fez tratamento periodontal: □ SIM □ NÃO

Quando: _____

Como escova os dentes? _____

Frequência: _____

Tipo de escova: _____

Tipo de dentifrício: _____

Usa fio dental? □ SIM □ NÃO

Freqüência: _____

Meios auxiliares de higiene oral?

_____

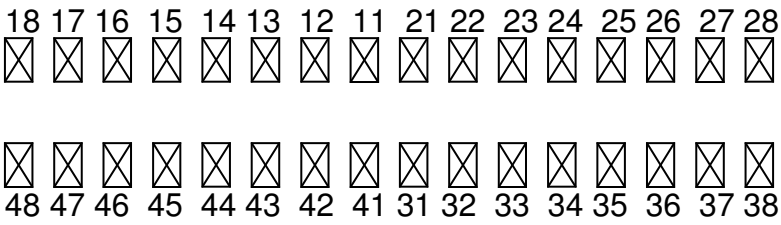
Antisséptico bucal? □ SIM □ NÃO

_____

DIAGNÓSTICO:

_____

OBSERVAÇÕES:



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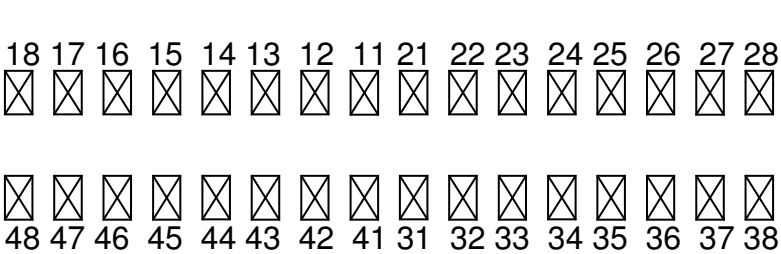

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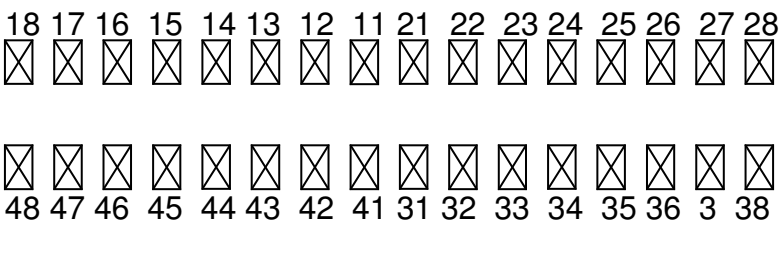

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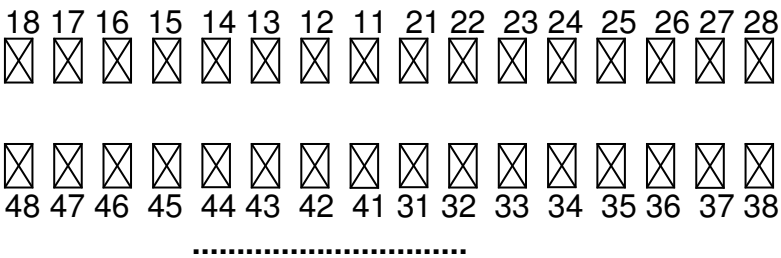

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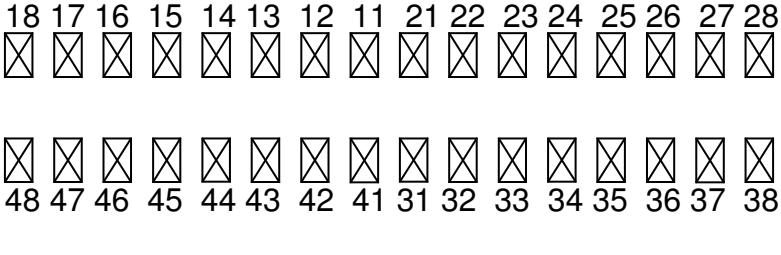

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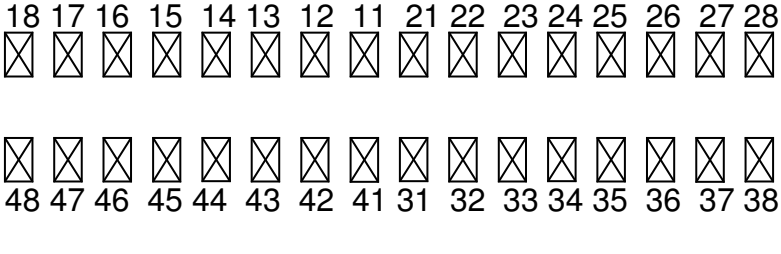

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APÊNDICE C – Eletroforese em gel de agarose (Sorotipos a, b e c)

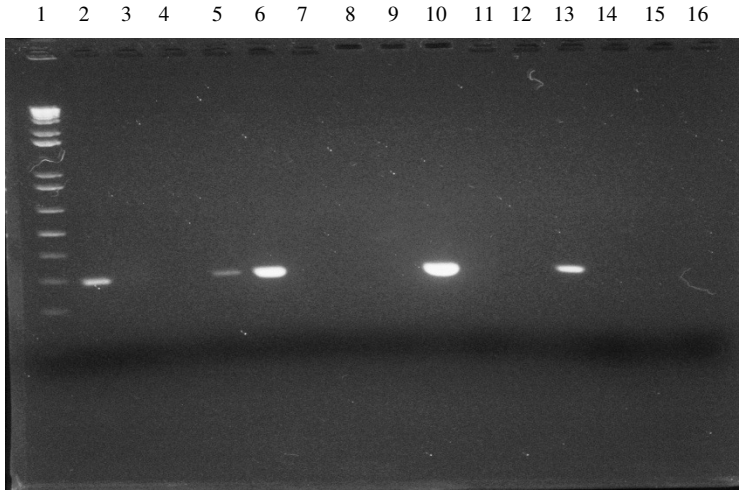

Figura 1 – Linha 1: marcador de peso molecular (100 pb); Linha 2: controle positivo (sorotipo b); Linhas 5, 6, 10 e 13: amostras amplificadas sorotipo b; Linha 16: controle negativo (água)

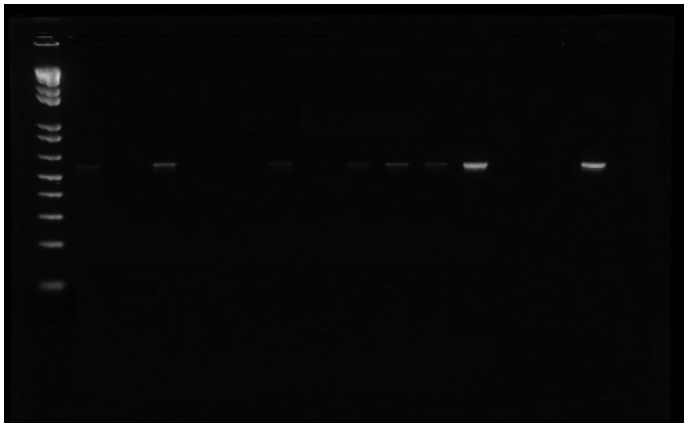

Figura 2 – Linha 1: marcador de peso molecular (100 pb); Linha 2: controle positivo (sorotipo c); Linhas 4, 7, 9, 10, 11, 12 e 15: amostras amplificadas sorotipo c; Linha 16: controle negativo (água)

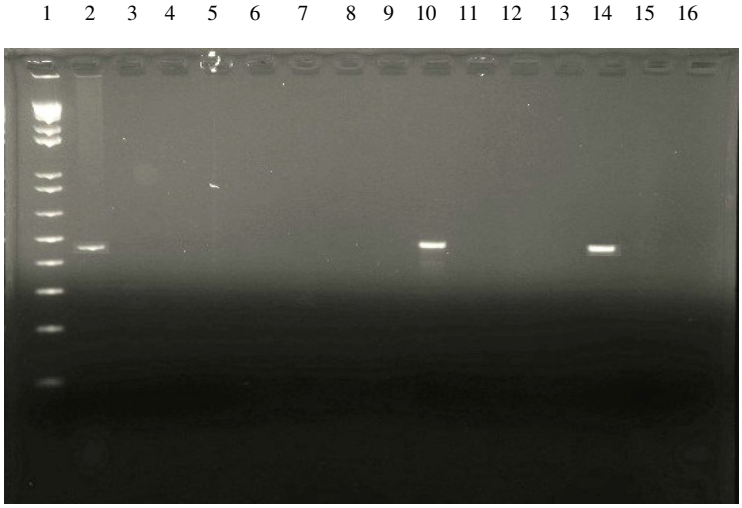

Figura 3 – Linha 1: marcador de peso molecular (100 pb); Linha 2: controle positivo (sorotipo a); Linhas 10 e 14: amostras amplificadas sorotipo a; Linha 16: controle negativo (água)

ANEXO A

DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas sujeitas a arbitragem, que constam da minha Dissertação de Mestrado, intitulada "**PREVALÊNCIA E DISTRIBUIÇÃO DE SOROTIPOS DE *Aggregatibacter actinomycetemcomitans* ISOLADOS DE PACIENTES BRASILEIROS COM DOENÇA PERODONTAL"** não infringem os dispositivos da Lei n.o 9.610/98, nem o direito autoral de qualquer editora.

Fortaleza, 28 de novembro de 2012

Autor

Jandenilson Alves Brígido

Orientadora

Nádia Accioly Pinto Nogueira

ANEXO B

Universidade Federal do Ceará
Comitê de Ética em Pesquisa

Of. Nº 211/11 Fortaleza, 21 de setembro de 2011
Protocolo COMEPE nº 183/ 11
Pesquisador responsável: Jandenilson Alves Brígido
Título do Projeto: "Prevalência e distribuição de sorotipos de *Aggregatibacter actinomycetemcomitans* isolados de pacientes brasileiros com doença perodontal"

Levamos ao conhecimento de V.Sª. que o Comitê de Ética em Pesquisa da Universidade Federal do Ceará – COMEPE, dentro das normas que regulamentam a pesquisa em seres humanos, do Conselho Nacional de Saúde – Ministério da Saúde, Resolução nº 196 de 10 de outubro de 1996 e complementares, aprovou o protocolo e o TCLE do projeto supracitado na reunião do dia 15 de setembro de 2011.

Outrossim, informamos, que o pesquisador deverá se comprometer a enviar o relatório final do referido projeto.

Atenciosamente,

Dr. Fernando A. Frota Mota
Coordenador do Comitê
de Ética em Pesquisa
COMEPE/UFC