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Prevalence and distribution of *Aggregatibacter actinomycetemcomitans* serotypes and the JP2 clone in a Greek population

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Abstract

Aim: To investigate the distribution of *Aggregatibacter actinomycetemcomitans* serotypes and the prevalence of the JP2 clone in subgingival samples of Greek subjects.

Materials and Methods: Two hundred and twenty eight subjects participated in the present study. Each contributed with one pooled subgingival sample from the mesiobuccal surface of the four first molars. Samples were analysed using polymerase chain reaction for five serotypes of *A. actinomycetemcomitans* and the JP2 clone, using primers and conditions described previously. Subjects were stratified according to periodontal status (untreated periodontitis, non-periodontitis and periodontitis patients receiving supportive treatment). Comparisons between and within groups were performed by applying non-parametric tests (Kruskall–Wallis, Pearson χ^2, z-test with Bonferroni's corrections and Kramer's V-test) at $p = 0.05$ level.

Results: *A. actinomycetemcomitans* was detected statistically more frequently in untreated patients (27.5%) compared with the other two groups (11.7% for non-periodontitis and 10% for periodontitis patients receiving supportive treatment). No statistical differences were observed concerning the distribution of serotypes among groups (z-test with Bonferroni's corrections $p > 0.05$). Serotype c was more predominant within the periodontally diseased groups (Kramer's V-test $p < 0.05$). The JP2 clone was not detected.

Conclusions: *A. actinomycetemcomitans* serotype b was not statistically correlated with periodontal disease in the investigated sample and the utility of microbiological testing before antimicrobial administration is emphasized.

Key words: *Aggregatibacter actinomycetemcomitans*; Greek; serotypes

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The aetiological relationship between *Aggregatibacter actinomycetemcomitans*, aggressive periodontitis and possibly other forms of periodontal disease has long been investigated and discussed in the literature (American Academy of Periodontology 1996). The leukotoxin expressed by this microorganism is considered a primary virulence factor enabling the evasion of host immune mechanisms, and therefore, attenuates its pathogenic potential (Brogan et al. 1994). However, it is known that the species is genetically heterogeneous and can be grouped into six serotypes (a–f), which may differ regarding virulence characteristics (Kilian et al. 2006),although it has been shown that several phylogenetically diverse strains of *A. actinomycetemcomitans* may have pathogenetic material (Kaplan et al. 2002).

Conflict of interest and source of funding statement

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Subjects are usually colonized by a single serotype, which can persist for life (Asikainen & Chen 1999, Saarela et al. 1999), and the frequency distribution of *A. actinomycetemcomitans* serotypes differs among various populations (Rylev & Kilian 2008). Data in the literature have shown that serotypes a–c can be isolated from North European and other Caucasian populations (Asikainen et al. 1991, Haubek et al. 1995), while there is evidence that serotype c is more prevalent in individuals of Asian origin, such as Chinese, Vietnamese, Thai and Korean subjects (Chung et al. 1989, Holtta et al. 1994, Mombelli et al. 1998, 1999, Dahlen et al. 2002).

Furthermore, the correlation of different serotypes with various periodontal conditions remains unclear. Data in the literature have consistently shown that serotype b is detected more frequently in localized aggressive periodontitis (LAgP) patients, mainly of African origin (Newman et al. 1976, Slots 1976, Newman & Socransky 1977, DiRienzo et al. 1994, Haubek et al. 1997), serotype a can be isolated from chronic periodontitis lesions in American subjects (Zambon et al. 1983a), while in Northern Europe the three serotypes are equally represented and serotype c may be more prevalent in periodontal health (Asikainen et al. 1991, Haubek et al. 1995). 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 LAgP in Korean subjects (Chung et al. 1989).

Although all strains have been shown to carry the complete leukotoxin gene operon, which consists of the ltxA, ltxB, ltxC and ltxD genes, significant differences in the level of leukotoxin expression have been demonstrated among them (Poulsen et al. 1994). The highly toxic strains demonstrate a 530-base deletion in the promoter region, which results in increased (10–times) leukotoxic activity (Brogan et al. 1994). Genetic studies have shown that *A. actinomycetemcomitans* isolates, which demonstrate high leukotoxic activity, all belong to the same clone (JP2) of serotype b (Haubek et al. 1997). Notably, this clone demonstrates racial tropism and can be isolated from subjects originating from – but not necessarily residing in – Africa (Haubek et al. 1996, 1997, 2001, 2007, 2009).

It has been shown that the JP2 clone can be endemic in North African populations and strongly related to the particularly high prevalence and progression of aggressive periodontitis in Moroccan schoolchildren (Haubek et al. 2001, 2004, 2008). This specific clone was not detected in subjects of different origins such as Asian (Mombelli et al. 1999, Contreras et al. 2000) or North European (Haubek et al. 1995) individuals, while a clone with an insertion sequence was correlated to periodontal disease in Japanese subjects (He et al. 1999). Limited data exist regarding the distribution of various *A. actinomycetemcomitans* serotypes in Greek subjects. Data from clinical studies in chronic and aggressive periodontitis subjects, using whole genomic probes and the ''checkerboard'' DNA–DNA hybridization technique, have reported prevalence rates for *A. actinomycetemcomitans* serotype b ranging between 14% and 80% (Sakellari et al. 2003, Xajigeorgiou et al. 2006, Ioannou et al. 2009). However, the prevalence and distribution of other serotypes or the highly leukotoxic clone JP2 remain unknown.

The aim of the present study was, therefore, to investigate the prevalence and distribution of different *A. actinomycetemcomitans* serotypes and the JP2 clone in Greek subjects.

Materials and Methods

Subject sample

Seventy-seven non-periodontitis and 151 periodontally diseased subjects participated in the present study. The periodontally diseased subjects were either un-treated or patients treated previously and currently at supportive periodontal treatment (SPT). All participants were patients of the Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School, Aristotle University, Thessaloniki, Greece or personnel of the Dental School. Demographic data for participants are presented in Table 1.

Criteria for inclusion

All subjects were systemically healthy or not taking medication known to affect periodontal tissues. Subjects reporting antibiotic intake during the previous 6 months, pregnant or lactating women were excluded from the present study. The selection of participants fulfilling the above criteria was performed by one of the investigators (D. S.) in chronological order of their check-in the Clinic of the Department. All subjects agreed to cooperate and participate to the investigation.

Subjects were stratified according to their periodontal status, as follows: subjects were considered as non-periodontitis cases when they displayed no probing depth or probing attachment level >3 mm and no radiographic signs of bone loss.

Subjects were considered as periodontitis cases (chronic or aggressive) according to the analytical criteria of the American Academy of Periodontology (Armitage 1999). Subjects in the supportive treatment phase (SPT) had been diagnosed previously and treated for generalized chronic or aggressive periodontitis according to criteria described by the American Academy of Periodontology (Armitage 1999), and displayed fewer than five pockets <5 mm. Subjects had been undergoing SPT for at least 3 years.

Care was taken to include age-matched individuals across the three groups.

All subjects signed an informed consent, and the study was conducted according to the protocol outlined by the Research Committee, Aristotle University of Thessaloniki, Greece, and approved by the Ethical Committee of the School of Dentistry.

Clinical recordings and sampling

Clinical recordings from periodontitis patients were performed by a calibrated examiner (II) using the FP32 Florida probe (Florida Probe Corporation, Gainesville, FL, USA). Assessments from the remaining individuals were performed by another examiner (D. S.), using a manual Williams probe (POW, Hu-Friedy, Chicago, IL, USA) The examiner has reproducible assessments (Pearson's test, $r = 0.971$) as determined in 10% of her weekly registrations.

Parameters assessed included probing depth, recession and bleeding at six sites of all teeth present in the dentition. Bleeding was scored dichotomously as presence or absence upon completion of probing (Ainamo & Bay, 1975).

All participants from the three groups contributed with one pooled subgingival sample. This sample was taken from the mesial sites of first molars using a sterile Gracey curette after isolation with cotton rolls, air-drying and careful removing of supragingival plaque. When the first molars were not present, the sample

was taken from the mesial sites of the second molars.

All samples were immediately placed in 200 μl of TE buffer (Tris HCL 10 mM, EDTA 1 mM, pH 7.5) and stored at −20°C, until assayed.

Sampling always preceeded clinical recordings.

Analysis

All samples were analysed with the polymerase chain reaction (PCR), using primers and conditions described in the literature. All samples were analysed by the same operator (A. K.) who was unaware of the clinical condition (coded samples).

In order to ensure reproducibility of the results, all samples were analysed twice and 10% of patients were sampled twice and analysed under different codes.

Firstly, the PCR was performed for the detection of *A. actinomycetemcomitans* (Goncharoff et al. 1993). Positive samples were then further analysed by PCR in order to identify the serotype of *A. actinomycetemcomitans* (Suzuki et al. 2001). Finally, the template DNA from serotype-b-positive subgingival plaque samples was used in a PCR assay to identify members of the JP2 clone among *A. actinomycetemcomitans* strains (Poulsen et al. 2003).

The final volume of the reaction mixture for each PCR assay was 25 μl consisting of 2.5 μl of DNA sample, 0.25 mM of each deoxynucleotide triphosphate, 2.5 μl of 10 × Standard *Taq* Reaction Buffer (670 mM Tris-HCL, pH 8.8, 166 mM $(NH_4)_2$ SO_4, 0.1% Tween 20), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1.25 U *Taq* DNA Polymerase (HyTest Ltd., Turku, Finland) and distilled water. A Peltier Thermal Cycler (PTC-100, Peltier Thermal Cycler, MJ Research, Waltham, MA, USA) was used for PCR.

PCR for *A. actinomycetemcomitans*

The LKT2 and LKT3 primers specific to the *lktA* gene of *A. actinomycetemcomitans* were used for identifying *A. actinomycetemcomitans*. The sequences of the primers were 5′-CTA GGT ATT GCG AAA CAA TTT G-3′ and 5′-CCT GAA ATT AAG CTG GTA ATC-3′ as described by Goncharoff et al. (1993). The thermocycling programme was as follows: an initial denaturation step for 2 min at 95°C and 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final elongation step at 72°C for 5 min. Samples produced a 262 bp DNA fragment after amplification with primers LKT2 and LKT3.

Serotyping

For serotyping, we used five pair of primers from specific DNA sequences for each serotype as described by Suzuki et al. (2001). The sequences of the primers were: 5′-GCA ATG ATG TAT TGT CTT CTT TTG GA-3′ CTT CAG TTG AAT GGG GAT TGA CTA AAA C-3′ for serotype a, 5′-CGG AAA TGG AAT GCT TGC-3′ and 5′-CTG AGG AAG CCT AGC AAT-3′ for serotype b, 5′-AAT GAC TGC TGT CGG AGT-3′ 5′-CGC TGA AGG TAA TGT CAG-3′ for serotype c, 5′-TTA CCA GGT GTC TAG TCG GA-3′ and 5′-GGC TCC TGA CAA CAT TGG AT-3′ for serotype d, and 5′-CGT AAG CAG AAG AAT AGT AAA CGT-3′ 5′-AAT AAC GAT GGC ACA TCA GAC TTT-3′ for serotype e. PCR parameters included an initial denaturation step for 2 min at 95°C and 30 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min, followed by a final elongation step at 72°C for 5 min. The resulting amplification products produced, respectively: single bands of 428 bp for serotype a, 298 bp for serotype b, 559 bp for serotype c, 690 bp for serotype d and 211 bp for serotype e.

JP2 clone

Primers ltx3 (5′-GCC GAC ACC AAA GAC AAA GTC T-3′) and ltx4 (5′-GCC CAT AAC CAA GCC ACA TAC-3′) designed by Poulsen et al. (2003) span the Δ530 deletion present in the JP2 clone. The thermocycling programme was as follows: an initial denaturation step for 5 min at 94°C and 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min, followed by a final elongation step at 72°C for 8 min. The amplified fragment size was 686 bp.

For each set of samples analysed by PCR, both a negative and a positive control were used. Amplification products for the identification of *A. actinomycetemcomitans* and serotyping were loaded into 2% (w/vol) and those for the JP2 clone into 1% (w/vol) agarose gels by electrophoresis, stained with ethidium bromide, exposed under UV light and photographed. A 100 bp DNA ladder (New England Biolabs Inc., Ipswich, MA, USA) was also loaded on agarose gels as a molecular weight standard.

Statistical Analysis

The statistical analysis of the data was carried out with the statistical packages SPSS, (17.0 version), Statmate® and Instat® (Graphpad Inc., San Diego, CA USA).

Differences in the prevalence of *A. actinomycetemcomitans* and investigated serotypes were sought among non-periodontitis, periodontitis and subjects undergoing SPT, by applying the Pearson χ^2 and the *z*-test for proportions adjusted with Bonferroni's corrections. Differences regarding the distribution of serotypes within each group were examined by applying the χ^2 and Kramer's *V*-test. The experiment was set to provide at least a 80% probability of detecting changes of 25% with a significance level (α) of 0.05 (two-tailed).

For clinical parameters, indicators of descriptive statistics were used, such as mean and standard deviation for each group, with the patient as the observational unit. The same indicators were calculated for the subset of sampled sites. Differences in clinical parameters and age were sought by applying the Kruskall–Wallis test between groups, with a significance level of 0.05.

Results

Demographic and clinical data for participants and sampled sites are presented in Table 1. No differences were detected concerning age between the three groups (Kruskall–Wallis test $p > 0.05$), while the three groups demonstrated statistically significant differences regarding clinical parameters examined in the whole dentition and the subset of sampled sites (Kruskall–Wallis test $p < 0.05$).

Results regarding the prevalence of *A. actinomycetemcomitans* and serotypes according to clinical condition are presented in Table 2. Periodontitis patients were statistically more frequently colonized by *A. actinomycetemcomitans* than the other two groups (Pearson's χ^2 test and *z*-test for proportions with Bonferroni's corrections, $p < 0.05$), while no differences were observed among the three groups

Table 1. Demographic and clinical characteristics of the subject sample

Diagnosis	Total	Male	Female	Age range	Mean age ± SD	Sampled sites					
						probing depth (mean ± SD)	recession (mean ± SD)	bleeding on probing (mean ± SD)	probing depth (mean ± SD)	recession (mean ± SD)	bleeding on probing (mean ± SD)
Non-periodontitis	77	36	41	30–73	47.3 ± 10.5	**1.76 ± 0.22**	**0.09 ± 0.13**	**0.14 ± 0.11**	**1.95 ± 0.81**	**0.10 ± 0.4**	**0.19 ± 0.39**
Periodontitis	91	46	45	29–82	51.5 ± 11.2	**2.96 ± 0.77**	**0.9 ± 0.66**	**0.53 ± 0.39**	**3.27 ± 1.73**	**0.84 ± 1.3**	**0.58 ± 0.49**
Periodontitis at SPT	60	28	32	34–74	49.3 ± 8.3	**1.34 ± 0.87**	**1.84 ± 0.92**	**0.29 ± 0.21**	**1 ± 1.27**	**2.86 ± 1.59**	**0.38 ± 0.48**

No differences were observed between groups concerning mean age (Kruskall–Wallis test $p > 0.05$).
Differences in clinical parameters among groups are indicated by bold lettering (Kruskall–Wallis test $p < 0.05$).
SD, standard deviation; SPT, supportive periodontal treatment.

Table 2. Prevalence and distribution of *Aggregatibacter actinomycetemcomitans* serotypes, combinations of serotypes and the JP2 clone

Diagnosis	$N = 228$	Positive (%)	a (%)	b (%)	c (%)	d (%)	e (%)	Non-serotypable (%)	a+b (%)	b+c (%)	b+e (%)	c+e (%)	JP2 clone
Non-periodontitis	77	9 (11.7)	3 (3.9)	2 (2.6)	2 (2.6)	ND	ND	1 (1.3)	ND	ND	ND	1 (1.3)	ND
Untreated periodontitis	91	**25(27.5)**	6 (6.6)	2 (2.2)	9 (9.9)	ND	1 (1.1)	2 (2.2)	1 (1.1)	3 (3.3)	1 (1.1)	ND	ND
Periodontitis at SPT	60	6 (10.0)	1 (1.7)	ND	4 (6.7)	ND	ND	1 (1.7)	ND	ND	ND	ND	ND

Bold lettering indicates statistical differences of prevalence of *Aggregatibacter actinomycetemcomitans* (z-test with Bonferroni's corrections, $p < 0.05$).
No statistical differences were detected for the distribution of serotypes (z-test with Bonferroni's corrections, $p < 0.05$).
ND, not detected; SPT, supportive periodontal treatment.

concerning the distribution of the serotypes (Pearson's χ^2 test and z-test for proportions with Bonferroni's corrections, $p > 0.05$). Serotype d was not detected in any of the samples, while non-serotypable *A. actinomycetemcomitans* was found in four subjects (Table 2). Six subjects were found to be colonized by two serotypes of *A. actinomycetemcomitans* simultaneously. The highly leukotoxic JP2 clone was not detected in any of the investigated samples testing positive for serotype b (Table 2). Differences in the patterns of distribution of the five serotypes within each group are presented in Table 3. Serotype c was more predominant in the untreated periodontitis and SPT groups, but no differences were observed in the non-periodontitis group (Kramer's V-test $p < 0.05$).

Table 3. Distribution of *Aggregatibacter actinomycetemcomitans* serotypes within the three groups

Diagnosis	$N = 228$	a	b	c	d	e	Non-serotypable
Non-periodontitis	77	3	2	3	ND	1	1
Untreated periodontitis	91	7	7	**12**	ND	2	2
Periodontitis at SPT	60	1	ND	**4**	ND	ND	1

Bold lettering indicates statistical differences of prevalence of serotype c within each group (Kramer's V-test $p < 0.05$).
No statistical differences were detected for the distribution of other serotypes.
ND, not detected; SPT, supportive periodontal treatment.

Discussion

A. actinomycetemcomitans has long been implicated in periodontal disease. Existing data in the literature strongly support its role as an aetiological factor in LAgP (localized juvenile), while the relationship with other forms of periodontal disease or periodontal health, remains unclear (Socransky & Haffajee 2008).

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.

Data concerning the prevalence of *A. actinomycetemcomitans* serotypes in periodontally diseased and healthy individuals of Greek origin are limited. Serotype b was found in 35 out of 40 subjects with generalized aggressive periodontitis in a randomized clinical trial examining the efficacy of various systemic antibiotics by applying ''checkerboard'' DNA–DNA hybridization (Xajigeorgiou et al. 2006), while in 120 sites from another group of 10 aggressive periodontitis patients the same serotype was recovered in $< 14\%$ of sites (Sakellari et al. 2003). By applying the same technique, serotype b was detected in 70% of 40 chronic periodontitis subjects in a randomized clinical trial regarding the efficacy of instrumentation (Ioannou et al. 2009). In studies using cultural techniques, *A. actinomycetemcomitans* was detected in 14% of 85 severe lesions from 10 young Greek adults with Rapidly Progressive periodontitis (Kamma et al. 1995), while a study using both cultures and immunofluoresence showed *A. actinomycetemcomitans* as detectable in 25% of 264 sites from early-onset periodontitis patients (Kamma et al. 2004). Data from the present study showed that *A. actinomycetemcomitans* was more prevalent in untreated periodontitis subjects (Table 2). Periodontally healthy indivi-

duals were also colonized by *A. actinomycetemcomitans* and the frequency of detection in the present study is similar to those reported for Caucasian populations, usually of the order of <20% (Rylev & Kilian 2008). However, a predominance of serotype b was not observed in the present study and no statistical differences were observed concerning the distribution of *A. actinomycetemcomitans* serotypes among the three groups (Table 2). Serotype c was numerically more prevalent in the untreated periodontitis group, compared with the other two groups, although differences did not reach statistically significant levels. Regarding the rare serotypes, in the present study, serotype d was not detected in any of the investigated samples, something in agreement with findings from other populations such as Brazilian (Teixeira et al. 2006) and serotype e was detected at a low frequency (<5%). Data from European populations have also shown that serotypes d and e can rarely be recovered from the oral cavity, in contrast to Japanese subjects where serotype e was detected in 23% of the sample (Yamamoto et al. 1997, Dogan et al. 1999a, b). These discrepancies might be due to genetic or host factors but the explanation remains unclear.

Although no differences were observed concerning the distribution of serotypes among the three groups, when examining the distribution of serotypes within each group, serotype c displayed a predominance in the untreated periodontally diseased and the SPT groups, while no differences were observed within the non-periodontally diseased group, indicating a possible pathogenetic association of this serotype in Greek subjects which requires further investigation (Table 3). In contrast to the present findings, relevant data from the United States and European countries have shown that serotype b can be correlated with LAgP (Newman & Socransky 1977, Zambon et al. 1983b, Asikainen et al. 1991, Gmur et al. 1993), while serotype c has also been isolated from non-infected individuals and correlated to periodontal health, at least in Scandinavian individuals (Asikainen et al. 1991) but in some cases was found in periodontally diseased subjects (Kaplan et al. 2002, Fine et al. 2007).

Data in the literature have shown that although subjects′ oral cavities are usually colonized by a single clone, up to 15% can carry two different clonal types (Yang et al. 2004). In the present study, a low percentage of individuals testing positive for *A. actinomycetemcomitans* were found to be colonized by two serotypes simultaneously (Table 2). In addition, in a low percentage of samples *A. actinomycetemcomitans* could not be serotyped.

The highly leukotoxic JP2 clone was not detected in any of the samples tested positive for serotype b, and these data are in agreement with previous data from North European subjects (Haubek et al. 1995), Caucasians residing in the US (Contreras et al. 2000) or Asian (Mombelli et al. 1999). Therefore, although Greece is a Mediterranean country, the current data suggest that it is outside the habitat of the leukotoxic clone endemic in the Southern areas of the Mediterranean basin.

It has been suggested that *A. actinomycetemcomitans* can persist for life in infected individuals, even after periodontal treatment (Saarela et al. 1991, Asikainen & Chen 1999, Ehmke et al. 1999). This is the reason periodontal patients undergoing supportive treatment were also included in the present study. According to these findings, 10% of these subjects receiving supportive treatment for at least 3 years were colonized by *A. actinomycetemcomitans*. These patients displayed satisfactory clinical conditions (Table 1) but unfortunately there are no microbiological data from their baseline condition. From the epidemiological point, serotype b was not detected in any of the investigated samples from this group of subjects and serotype c was again the most prevalent.

A caveat regarding the present study concerns the absence of substantial number of aggressive periodontitis cases enabling a clearer colonization profile to be distinguished for this group of patients. Clearly, aggressive periodontitis cases in Greece merit further investigation regarding the distribution of *A. actinomycetemcomitans* serotypes. A further limitation concerns the choice of the four molars as representative subgingival sampling sites. Although molars have been used as sampling sites in several studies, the issue of correctly obtaining representative bacterial samples from the oral cavity remains controversial and data indicate that there may be differences in the distribution patterns of subgingival pathogens within the same individual (Asikainen & Chen 1999).

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. The combination of metronidazole plus amoxicillin has been shown to successfully suppress or eliminate *A. actinomycetemcomitans* from infected individuals (van Winkelhoff et al. 1992, Pavicic et al. 1994, Berglundh et al. 1998, Guerrero et al. 2005, Xajigeorgiou et al. 2006) and is favoured and prescribed by an increasing number of dentists in Greece (unpublished data), particularly as these antimicrobials, administered together, are widely thought to eliminate *A. actinomycetemcomitans* in periodontitis patients. Data from the current study do not support this indiscriminate prescription practice, at least in chronic periodontitis patients, due the relatively low prevalence of this species and especially serotype b in the periodontally diseased groups. The present findings also support the utility of microbial testing in guiding antimicrobial prescription, at least in subject groups such as aggressive periodontitis cases, where the presence of *A. actinomycetemcomitans* is suspected. Few data exist in the literature regarding differences in antimicrobial susceptibility between different serotypes of *A. actinomycetemcomitans*. In general it has been shown that no major differences exist between serotypes, except in the case of metronidazole, while all serotypes are susceptible to the tetracyclines, certain cephalosporins and quinolonic antibiotics (Pajukanta et al. 1992, Paju et al. 2000).

In conclusion, data from the present study have shown that *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 was observed. We intend to expand our investigation by examining the specific distribution of *A. actinomycetemcomitans* serotypes in aggressive periodontitis cases.

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Clinical Relevance

Scientific rationale for the study: The distribution of various *A. actinomycetemcomitans* serotypes and the highly leukotoxic JP2 clone appears to be racially correlated but limited data exist regarding Greek subjects.

Principal findings: *A. actinomycetemcomitans* was detected more frequently in untreated periodontitis subjects, but no statistical differences were observed in the distribution of serotypes among groups. Serotype c was more predominant within the periodontally diseased groups (Kramer's V-test $p < 0.05$). The JP2 clone was not detected.

Practical implications: The prevalence of *A. actinomycetemcomitans* and the distribution of *A. actinomycetemcomitans* serotypes vary according to ethnic group.