PCR detection of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* in dental plaque samples from Haitian adolescents

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Abstract

Streptococcus mutans and *Aggregatibacter actinomycetemcomitans* are oral pathogens associated with dental caries and periodontitis, respectively. The aim of this study was to determine the colonization of these two microorganisms in the dental plaque of a group of Haitian adolescents using two different polymerase chain reaction (PCR) methods, standard PCR, and quantitative real-time PCR (qPCR) assays. Fifty-four pooled supra-gingival plaque samples and 98 pooled sub-

gingival plaque samples were obtained from 104 12- to 19-year-old rural-dwelling Haitians. The total genomic DNA of bacteria was isolated from these samples, and all participants also received caries and periodontal examinations. Caries prevalence was 42.2%, and the mean decayed, missing, and filled surface (DMFS) was 2.67±5.3. More than half of the adolescents (53.3%) experienced periodontal pockets (Community Periodontal Index score :3). *S. mutans* was detected in 67.3% by qPCR and 38.8% by PCR of the supra-gingival plaque samples (p<0.01), and 36.6% by qPCR and 8.1% by PCR of the sub-gingival samples (p<0.01). *A. actinomycetemcomitans* was detected in 85.1% by qPCR and 44.0% by PCR of the sub-gingival samples (*p*< 0.01), but the prevalence was similar, 67.3% by qPCR and 59.2% by PCR, in the supra-gingival plaque samples. Neither age nor gender was significantly correlated to the bacterial colonization. The results demonstrated a moderate-to-high prevalence of *S. mutans* and *A. actinomycetemcomitans* in the Haitian adolescent population, and qPCR is more sensitive than standard PCR in field conditions. These findings suggest that qPCR should be considered for field oral epidemiologic studies and may be necessary in investigations having major logistic challenges.

Keywords

Haitian adolescents; PCR; qPCR; Dental caries; Periodontitis; *Streptococcus mutans*; *Aggregatibacter actinomycetemcomitans*

Introduction

The microbial population in dental plaque is known to be highly complex. A number of microorganisms are significantly associated with the development of human dental caries and periodontitis. Specifically, *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* are two oral pathogenic bacteria which have been extensively studied for their positive associations with dental caries [1 ,2] and periodontal diseases [3–5], respectively. Traditionally, determination of these two microorganisms in the oral cavity has been based on bacterial cultivation methods that are impractical for field epidemiological studies. In recent years, molecular approaches, such as standard polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) with species-specific primers, have been developed, commonly used, and demonstrated a high degree of efficiency and accuracy in the detection of target micro- organisms and the evaluation of bacterial colonization in saliva and dental plaque samples [6–9].

Although numerous studies have qualitatively and quantitatively described the microbiology of caries and periodontitis in various populations [10, 11], studies on the colonization of *S. mutans* and *A. actinomycetemcomitans* in Haitian adolescents have not been conducted, mainly due to the lack of comprehensive microbiology laboratories for oral bacterial cultivation and quantification in Haiti, a situation applicable to other developing countries. In the present study, we aimed to qualitatively and quantitatively determine, using both standard PCR and qPCR assays, the colonization of *S. mutans* and *A. actinomycetemcomitans* in the dental plaque of a group of Haitian adolescents. Meanwhile, we sought to determine the feasibility of utilizing PCR techniques for an epidemiological

field study, especially in remote rural areas. The information generated from the study will provide baseline data and validated microbiological approaches for future studies in Haiti and elsewhere.

Methods

Study population

The present study was pa11 of a larger investigation to determine whether early childhood (<60 months of age) protein-energy malnutrition (ECPEM) is related to oral diseases and conditions in adolescents [12, 13]. The original study included a total of I ,058 Haitian adolescents in the rural Jeremie area of Haiti [12]. All subjects were selected from a list of children maintained by the Haitian Health Foundation on whom anthropometric data were collected during 1988–1996. For this study, 104 adolescents aged 12–19 years old who were in the original ECPEM study and from two road-accessible villages, Fr. Torbeck and Carretour-Prince, were re-examined. The subjects had a range of ECPEM levels from malnourished to normaL Informed consent was obtained from each parent and assent from adolescents. The study proposal was approved by the New York University School of Medicine Institutional Review Board. Field data collection was conducted between May and August 2005.

Clinical examination

Each examination included a dental examination and dental plaque sample collection. Dental caries examinations were completed by four study examiners and were scored for DMFS, decayed (D), missing due to caries (M), and filled (F) surface (S), using World Health Organization (WHO) diagnostic criteria. Two trained and calibrated examiners performed all periodontal examinations and plaque sampling. Periodontal pockets were measured using a pressure-sensitive periodontal probe (Vivadent, Lichtenstein), and disease was classified using the WHO Community Periodontal Index (CPI).

Bacterial sample collection and transportation

From the 104 subjects, a total of 152 viable pooled dental plaque samples were randomly collected: 54 were supragingival plaque samples defmed as *supra-gingival group,* and 98 were sub-gingival plaque samples defmed as *sub-gingival group*. For the supra-gingival plaque sample collection, all teeth present in the mouth were sampled using a modified plaque sample collection method for field studies [14,15]. Briefly, a sterile toothpick was rubbed across all upper and lower teeth buccaVlabial gingival margin swfaces. Tn order to maximize plaque sample collection, a second toothpick was placed into each interproximal tooth swface. After carefully removing supra-gingival plaque, sterile periodontal curettes were used to collect sub-gingival plaque from all available gingival sulci and periodontal pockets [16]. The toothpicks and the curettes were immediately immersed in separate DNase- and RNase-free 2-ml pre-labeled Eppendorf tubes containing 500 μl lysis buffer (Epicentre, Madison, WI, USA).

All specimen vials were packed in coolers with cold packs supplemented with ice. The plaque samples were transfered from the field sites to a field office with a 4°C refrigerator

within a maximum of 8 h. Flights were scheduled so that the plaque samples were transferred from the field office in Haiti to the microbiology laboratory at the NYU College of Dentistry within a maximum of 24 h packed in coolers with ice refreshed after 8 h if necessary, and then stored immediately in a −80°C freezer.

PCR evaluation methods of bacterial colonization

Total bacterial genomic DNA was extracted from each plaque sample using the Epicentre method (Epicentre, Madison, WI, USA) as described previously [17, 18]. Briefly, a MasterPure DNA purification kit was used for bacterial cells lysis and total DNA precipitation. The final quantity and quality of the DNA was evaluated using DU-7400 UV-VIS spectrophotometer at $OD_{260}/0D_{280}$. A standard concentration of 10 ng/μl was prepared for each individual sample for all PCR assays.

Initially, a standard PCR was performed using a standardized protocol and GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The *S. mutans* species-specific primer set generated a 479-bp amplicon: forward, 5'-TCG CGA AAA AGA TAA ACA AAC A-3', and reverse, 5'-GCC CCT TCA CAG TTG GTT AG-3' [19]. The PCR reaction mixture for *S. mutans* contained a total volume of 25 μl, which consisted of 1 Ox buffer, 2.5 mM/ea dNTPs, 10 pmol/μl primers, 5 U/μl Taq, 50 mm $MgCI_2$, and 10 ng/μl DNA template. The positive control for S. *mutans* was UA159 (ATCC700610). The standard PCR conditions were performed at 95°C for 5 min for initial denaturing, followed by 35 cycles at 95°C for 15 s, 56°C for 30 s, 72°C for 1 min, and a 72°C extension for 7 min. The minimum detectable level was found to be 16 fg or 7 copies/μl for the S. *mutans* genomic DNA.

The species-specific primers for *A. actinomycetemcomitans* were selected based on published literature. The primer set generated a 194-bp amplicon: forward, 5'-ATT GGG GTT TAG CCC TGG T-3', and reverse, 5'-GGC ACA AAC CCA TCT CTG A-3' [20]. The PCR reaction mixture for *A. actinomycetemcomitans* contained a total vo lume of 25 μl, which consisted of 10× buffer, 2.5 mM/ea dNTPs, lO pmol/μl primers, 5 U /μl Taq, 50 mm $MgCI_2$, and 10 ng/μl DNA template. The positive control for *A. actinomycetemcomitans* was ATCC29522. The standard PCR conditions were performed at 95°C for 4 min for initial denaturing, followed by 40 cycles at 95°C for 15 s, 58°C for 20 s, 72°C for 40 s, and a 72°C extension for 4 min. The minimum detectable level was found to be 10 fg or 4 copies/μl for the *A. actinomycetemcomitans* genomic DNA.

As the standard PCR cannot provide quantitative measurement of the two bacterial colonizations, we further performed quantitative real-time PCR to determine the amounts of *S. mutans* and *A. actinomycetemcom itans* DNA in the plaque samples, using DNA Engine Opticon™ 2 System (MJ Research, San Francisco, CA, USA) and the SYBR Green T fluorescent dye detection kit (Qiagen, Valencia, CA, USA). The same sets of primers used for standard PCR were used for qPCR. Tn order to validate the qPCR assay, standard curves were first obtained by externally quantified standards of UA159 for *S. mutans* and ATCC29522 for *A. actinomycetemcomitans*. The amount of DNA template for the standard curve was tenfold serial dilutions from 10^7 to 10 fg/μl. The minimum detectable level by using qPCR was 10 fg or 4 copies/μl of genomic DNA for both *S. mutans* and *A. actinomycetemcomitans* ATCC type strains, respectively (Fig. 1).

For detecting *S. mutans* in the plaque samples, the qPCR reaction mixture contained a total volume of 25 μl, which consisted of SYBR master Mix 2X (12.5 μl; QuantiTect SYBR Green PCR Kits, Qiagen), 10 pmool/μl of each primer (2 μl), and 10 ng/μl DNA template (10.5 μl). The standard curve mixture contained a total volume of 25 μl, which consisted of SYBR master Mix 2X (12.5 μl), 10 pmol/μl of each primer (2 μl), 3d H_2O (9.5 μl), and UA159 genomic DNA from 10 ng/μl to 10 fg/μl (1 μl). The reagents and reaction conditions used in the plaque samples mixtures were the same as those used for the standard curve to ensure reaction consistency. The qPCR conditions were performed at 95°C for 15 min, followed by 44 cycles at 95°C for 15 min, 56°C for 30 s, and 72°C for 30 s. For detecting *A. actinomycetemcomitans*, the qPCR reaction mixture of the plaque sample DNA and the standard strain DNA used were the same as for the qPCR for S. *mutans*. The qPCR conditions were performed at 95°C for 15 min, followed by 44 cycles at 94°C for 15 s, 58°C for 20 s, and 72°C for 40 s. Each plate was read after each cycle and then incubated at 72°C for 4 min. The melting curves were performed from 60°C to 90°C and read every 0.5°C for l s, and fmally, the plate was incubated at 4°C.

All output data were analyzed using Opticon Monitor™ software (BioRad, Hercules, CA, USA). The qPCR results demonstrated that both standard curves, representative of typical experiments, were linear generated from a plot of C_t against log concentration for the known standard DNA for *S. mutans* ($r^2 = 0.998$) and *A. actinomycetemcomitans* ($r^2 = 0.999$; Fig. 2), both melting curves for *S. mutans* at 78°C and *A. actinomycetemcomitans* at 83°C demonstrated homogeneous products without primer-dimers (data not shown), and all the amplifications of clinical samples were linear generated within the standard range (Fig. 2).

All qPCR for the standards and the plaque samples were performed in duplicate to eliminate variation between tubes with the same templates. The final analysis was based on the mean of the two reactions, and the specificity of all PCR and q PCR products of clinical samples was confirmed for correct molecular size by electrophoresis in a 1.5% agarose gel. The gel was then stained with ethidium bromide (l ug/ml) for 15 min and washed with water for 5 min. The gels were photographed and recorded with Alphalmager 3300 imaging system (Alpha lnnotech Corp., San Leandro, CA, USA).

Statistical analysis

The PCR and qPCR results were organized and analyzed using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). Descriptive statistics including prevalence, mean, range, standard deviation, and variance were examined. Nonparametric Mann- Whitney *U* test was used to examine the significance of the distribution among continuous variables. The Pearson Chi-square test was used to examine the significance of the distribution among categorical variables. McNemar's test was used to examine the significance of the association between matched paired categorical variables. The *p* value of the SEgnificance probability less than or equal to 0.05 was ,considered statistically significant.

Results

The mean age of the pat1tctpants was 14.9 years (SD = 1.7 years, median=l4.7; range, 12.2 to 18.7 years), and 54.6% of the adolescents were male. The caries prevalence was 42.2%, 48.1% for males and 33.3% for females (p= 0.37), and the DMFS averaged 2.67 (SD =5.3; Table 1). The distribution of DMFS scores was skewed, the Kurtosis value was 7.5, and the median values were 0 for both males and females. In terms of the gingival-periodontal complex, we found that more than half of the adolescents (53.3%), 62.8% for males and 43.6% for females (p= 0.09), exhibited gingival or periodontal pockets (CPI score 3 or **4;** Table 1). The distribution of periodontal pocket depth was also skewed, the Kurtosis value was 5.8, and the median values were 1.5 and 0 for males and females, respectively. Therefore, all statistical analyses were conducted by using nonparametric statistical tests. The mean values were presented in Table 1 sole for clinical relevance. Though male Haitian adolescents had more dental caries and higher CPI scores, the differences were not statistically significant. Additionally, age was not significantly related to either caries status or CPT score (Table 1).

Our results showed that the prevalence of S. *mutans* was statistically significantly higher in supra-gingival samples compared to that in sub-gingival samples using either the standard PCR ($p<0.001$) or qPCR (p<0.001) method. Using the qPCR method, *S. mutans* was detected in 67.3% of the total supra-gingival samples and 38.8% of the total supra-gingival samples by using standard PCR ($p< 0.00$ 1; Table 2). For the sub-gingival plaque samples, S. *mutans* was found in 36.6% and 8.1% of the samples using the qPCR and the standard PCR, respectively ($p<0.001$; Table 2). Hence, the prevalence of bacteria detected by qPCR was significantly higher than the prevalence determined by standard PCR (McNemar's test ($p<0.001$)).

The results also showed that *A. actinomycetemcomitans* was detected in 44.0% of the total sub-gingival samples using standard PCR, 85.1% by using the qPCR (McNemar's test, $p<0.001$ Table 2). When examining only the supra-gingival plaque samples, the percentage of *A. actinomycetemcomitans-positjve* was 59.2% and 67.3% using the standard PCR and qPCR, respectively (McNemar's test, $p = 0.219$). When comparing a single PCR assay for the detection of *A. actinomycetemcomitans* in different samples types (supra- vs. sub-gingival), we observed using standard PCR resulted in a prevalence that was significantly higher in the supra-gingival samples (59.2%) compared to that in the sub-gingival samples (44.0%; Pearson Chi-square test, $p =0.031$). However, in the samples analyzed using qPCR group, the prevalence in the sub-gingival samples was higher (85.1 %) compared to that in the suprd-gingival samples (67.3%; Pearson Chi-square test, $p=0.055$; Table 2).

There were no statistically significant relationships found between the prevalence of colonization and gender, age, ECPEM levels, caries status, and CPI score (data not shown).

Discussion

Information on the oral health status of Haitian adolescents is lacking. Tn the 1999 Oral Health National Survey of Haiti [21], Psoter et al. reported a mean DMFS of 2.53 and a

caries prevalence of 46% in 12- to 15-year-old urban and rural Haitian adolescents. The present study sampled two rural villages and found a mean DMFS of 2.67 and a caries prevalence of 42.2%, which was similar to the national data, suggesting that the caries status found in this study population may be comparable to that generally found in rural Haiti.

To date, there are no published reports on oral bacterial colonization in this population. As part of a major epidemiological study on ECPEM and its relationship to oral diseases and conditions [12, 13,22], the present study further assessed the colonization of *S. mutans*, known as a key cariogenic bacteria associated with caries development, and *A. actinomycetemcomitans*, known as a key periodontal pathogen associated with periodontal disease, in a subsample of the Haitian subjects. We found a moderate level of dental caries and periodontal disease and also detected a moderate-to-high prevalence of *S. mutans* and *A. actinomycetemcomitans* among these adolescents. Neither age nor gender was significantly related to the colonization of these two microorganisms. Although a trend indicated a positive relationship between bacterial colonization and caries and periodontitis, this present study was not statistically powered to test for those correlations. As the adolescent population under consideration was recruited from only two rural vi llages, our microbiologic findings may limit the generalizability of these findings, although life-style conditions are generally similar throughout rural Haiti. Importantly, however, as the present study employed validated molecu lar techniques, these findings can be used as baseline data for future microbiology and epidemiologic studies in the Haitian as well as other populations.

One of the major objectives of this study was to conduct a field ep idemiological study with a microbiological component in rural areas of Haiti. Traditionally, laboratory-based bacterial cultivation is used as the "gold standard" to determine the presence, absence, and levels of bacterial colonization. Qualitative and quantitative findings from enriched or species-specific selective culture media are then analyzed for associations with oral disease. Although bacteriological methods have been the method of choice for the evaluation of bacterial colonization for decades, PCR with *S. mutans-specific* and *A. actinomycetemcomitans*-specific primers has become a more commonly used and important technique for more rapid and reliable detection of these two oral pathogens in the saliva and dental plaque. PCR-based assay is particularly useful and cost-effective when bacterial pure culture is not required or a lab-based operation is not available. In this study, we first obtained the prevalence of *S. mutans* or *A. actinomycetemcomitans* rapidly by using standard PCR assays with limited expenses. However, it has been recognized that the presence of *S. mutans* or *A. actinomycetemcomitans* in the saliva and dental plaque may not be necessarily correlated with the development and severity of dental caries or periodontal disease, respectively [1 ,23). Consequently, we took a second approach to conduct qPCR to determine the colonization level of *S. mutans* and *A. actinomycetemcomitans* in dental plaque. Although significant differences in the DNA levels were not found, all results consistently demonstrated higher detection rate by qPCR compared to that by standard PCR.

Using the standard PCR method, we found that the prevalence of *S. mutans* was 38.8% in the supra-gingival samples and 8.1% in the sub-gingival samples. When qPCR assay was performed, the prevalence of S. *mutans* increased from 38.8% to 67.3% in the supra-gingival

plaque sample of Haitian adolescents. Overall, this prevalence is lower than the 90% prevalence reported by Linossier (culture method) in 1989 for 9- to 13-year-old Chilean children, but higher than the prevalence reported in Colombian and Venezuelan children (culture method) [24–26]. The observed difference in prevalence could be possibly attributable to the different methods used. Meanwhile, the low prevalence of *S. mutans* in the sub-gingival samples was not unexpected, since the presence of S. *mutans* in subgingival samples is associated with root caries and the prevalence of root caries was likely low in the study population.

Based on previously published cross-sectional studies, the prevalence of *A. actinomycetemcomitans* varies from 20% to 75%, depending on the population and stage of periodontitis [11 ,23]. Tinoco et al., for instance, reported that *A. actinomycetemcomitans* was detected in 80% of young patients with localized juvenile periodontitis, but also found in 43.9% of all siblings who did not have the disease and 35.3% of their parents [27]. Cortelli et at. reported an *A. actinomycetemcomitans* prevalence of 41.6% among subjects with chronic periodontitis and 72% among subjects with aggressive periodontitis [28]. In the present study, the prevalence of *A. actinomycetemcomitans* detected by qPCR in Haitian adolescents is similar to the prevalence reported among American adolescents and young adults [29], but higher than that reported from some other countries using different methods [30,31]. As the association between *A. actinomycetemcomitans* and aggressive fonns of periodontitis is well established [5,32,33], the high prevalence of *A. actinomycetemcomitans* may serve as a risk indicator for future initiation of aggressive periodontitis in rural Haitian adolescents.

Interestingly, we fotmd that PCR-positive detection rate for *A. actinomycetemcomitans* was higher in the supragingival plaque (59.2%) than in the sub-gingival plaque samples (44.0%), but 85. 1% in sub-gingival plaque samples and 67.3% in supra-gingival samples by using qPCR. We speculate that the difference found between the supra- and sub-gingival samples may be associated with diminished salivary gland antimicrobial function resulting of early childhood malnutrition (ECPEM) [13]. The differences observed between the PCR-based methods could also attribute to the fact that the colonization level of *A. actinomycetemcomitans* is higher and concentrated at subgingival level than in the saliva, but collecting sufficient amount of sub-gingival plaque samples for bacterial DNA isolation and consequently PCR experiment is often challenging when there is little periodontal pocketing. As qPCR is more sensitive and less bacterial DNA samples are required for pe1forming the experiment, the qPCR results, therefore, are likely to be accurate for reporting Uevels of *A. actinomycetemcomitans* colonization in both supra- and sub-gingival samples.

Furthermore, the different findings of *S. mutans* and *A. actinomycetemcomitans* colonization between the standard PCR and qPCR were statistically significant, which may be due to the difference in the detection technique designed for each method. Standard PCR method detects PCR amplification at the end-point of PCR reaction. The use of agarose gels tor the detection presents low sensitivity and low resolution. Ethidium bromide for staining is not quantitative and cannot delineate differences less than tenfold among samples. In addition, these results are based on size discrimination, wh ich is not precise. Quantitative real-time

PCR, on the other hand, invented in the mid- 1990s [6], combines standard PCR with the use of fluorescent markers to measure the amount of PCR-amplified bacterial DNA during the exponential amplification phase. The technique can be used to determine the amount of sta1ting material, as little as a twofold change can be detected. Thus, qPCR provides much precise, accurate, and reliable results compared to standard PCR [6,34]. Since its introduction, qPCR has been used increasingly in the evaluation of oral microbial infection and colonization (35–41]. The results of our study further support the contention that qPCR is more sensitive than standard PCR and should be considered as a preferred method to detect and quantify dental pathogens in clinical and field epidemiologic studies, at least under the difficult conditions of storage and transpottation as reported here.

In summary, the findings (1) demonstrate a moderate-to-high prevalence of S. *mutans* and *A. actinomycetemcomitans* in a Haitian adolescent population and (2) suggest that qPCR is more sensitive than standard PCR in difficult field conditions. Thus, qPCR should be considered for field oral epidemiologic studies and may be necessary in investigations having major logistic challenges.

Acknowledgment

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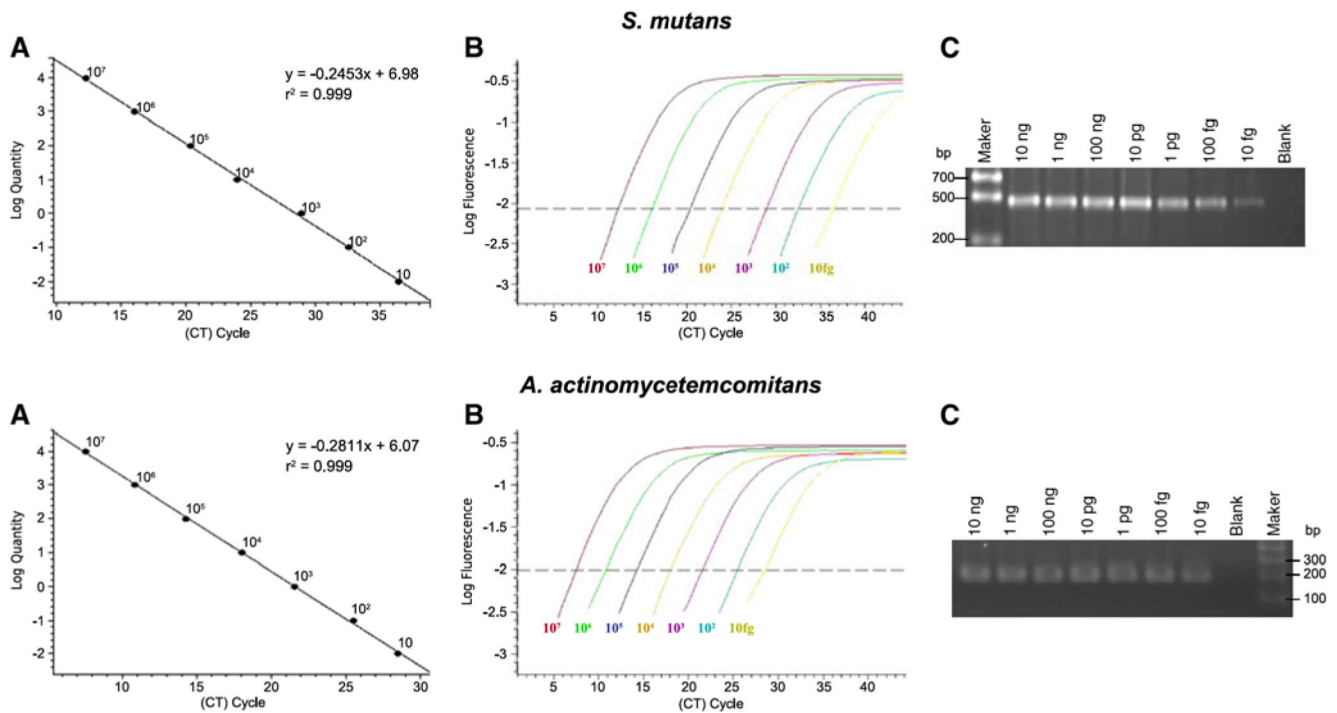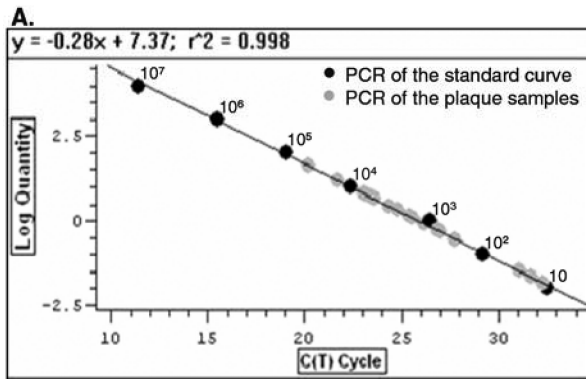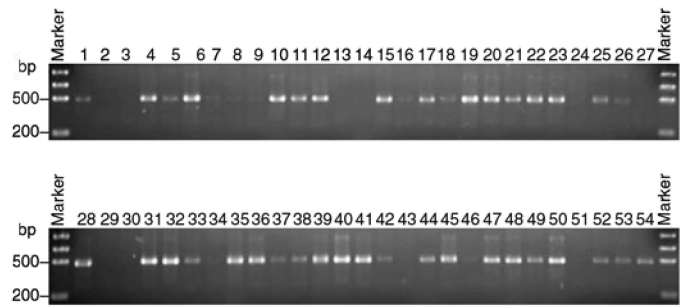
Fig. 1.

Quantitative real-time polymerase chain reaction (qPCR) validation. **a** Standard curves for *Streptococcus mutans* (UAI59) and *Aggregatibacter actinomycetemcomitans* (ATCC29522) generated from a plot of threshold cycle (C,) values against log value for the tenfold serial dilutions of known concentrations (10^7–10 fg/μl). **b** The amplification plot obtained using SYBR Green I assay for *S. mutans* and *A. actinomycetemcomitans*. **c** The confirmations of the qPCR amplification detected on 1.5% agarose gels. As expected, the sizes of S. *mutans* and *A. actinomycetemcomiwns* amplicons are 479 and 194 bp, respectively
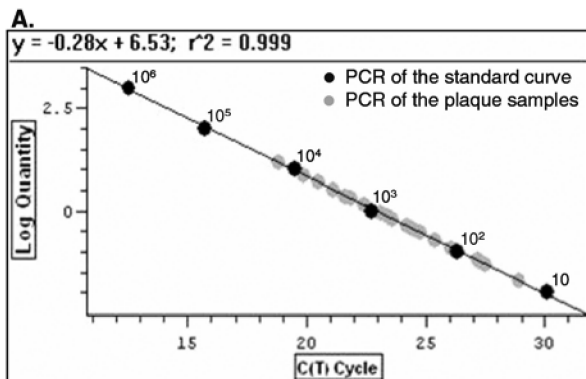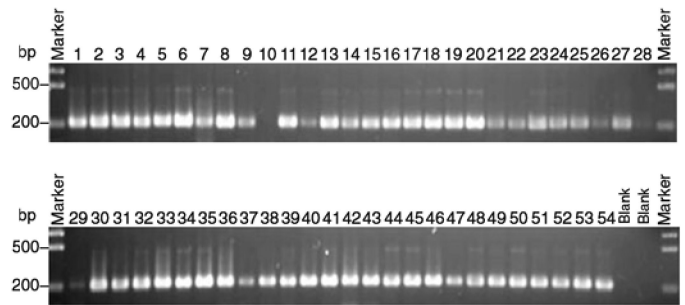
Fig. 2.

Quantitative real-time polymerase chain reaction (qPCR) for plaque samples. **a** The linear standard curve was generated based on the log concentration of known DNA samples of *Streptococcus mutans* (UA 159) and *Aggregatibacter actinomycetemcomitans* (ATCC 29522) *(black circle)* and the plaque samples *(gray circle)*. **b** The qPCR final results were confirmed on a 1.5% agarose gel

Table 1

Demographic characteristics and clinical examination results of the study population

Study group	Demography				Clinical examinations			
	Gender[a]		Age (years)[a]		Prevalence (%)[b]		Mean±SD[c]	
		%	Median	Mean±SD	Subject with caries	Subject with CPI 3	DMFS	Pocket depth (mm)
Supra-gingival								
N=54	All	100	14.7	14.9±1.7	42.2		2.67±5.3	
	Male	57.1	15.0	15.0±1.8	48.1		2.59±4.6	
	Female	42.9	14.5	14.8±1.6	33.3		2.78±6.3	
Sub-gingival								
N=98	All	100	14.7	150± 1.6		53.3		2.33±3.4
	Male	56.0	15.0	15.0±1.7		62.8		3.00±4.0
	Female	44.0	14.6	14.9±1.5		43.6		1.49±2.1

[a]Pearson Chi-square test and nonparametric Mann-Whitney *U* test. No statistically significant differences in the gender and the age distributions were found between the two study groups

[b]Pearson Chi-square test. No statistically significant differences in the prevalence of caries and periodontal pockets were found between the males and the females

[c]Nonparametrie Mann-Whitney test for statistical testing the distributions of DMFS score and the depth of periodontal pockets. No statistically significant differences were found between the males and the Females. Because the distributions of DMFS score and the depth of periodontal pockets were skewed, the mean values presented in this table are for clinical relevance, not for statistical analysis

Table 2

Comparison of standard polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) in detection of *Streptococcus nutans* and *Aggregatibacter actinomycetemcomitans* in dental plaque of a Haitian adolescent population

Study group	Gender	PCR[a]			qPCR[b]	
			% positive for *S. mutans* or *A. actinomycetemcomitans*		% positive for *S. mutans* or *A. actinomycetemcomitans*	
		%	*S. mutants* [c]	*A. actinomycetemcomitans* [d]	*S. mutans* [c]	*A. actinomycetemcomitans* [d]
Supra-gingival						
N=54	All	100	38.8	59.2	67.3	67.3
	Male	57.1	42.9	67.9	67.9	75.0
	Female	42.9	33.3	47.6	66.7	57.1
Sub-gingival						
N=98	All	100	8.1	44.0	36.6	85.1
	Male	56.0	7.0	43.1	39.0	83.7
	Female	44.0	9.7	45.0	33.3	87.1

[a]Pearson Chi-square test showed no differences in the prevalence of *S. mutans* and *A. actinomycetemcomitans* detected by *PCR* between the males and the females. However, the prevalence were statistically significant higher in supra-gingival samples compared to that in sub-gingival samples for cither *S. mutans* ($p < 0.001$) or *A. actinomycetemcomitans* ($p=0.031$)

[b]Pearson Chi-squarc test showed no differences in the prevalence of *S. mutans* and *A. actinomycetemcomitans* detected by *qPCR* between the males and the females. However, the prevalence was statistically significant higher in supra-gingival samples compared to that in sub-gingival samples for *S. mutans* ($p<0.001$), but marginally higher for *A. actinomycetemcomitans* (p 0.055)

[c]The prevalence of *S. mutans* was significantly higher when detected by *qPCR* compared to the results detected by standard *PCR* for cither supra-gingival samples (McNcmar's test, $p<0.00l$) or sub-gingival samples (McNcmar's test, $p<0.001$)

[d]The prevalence of *A. actinomycetemcomitans* was significantly higher when detected by *qPCR* compared to the results detected by standard *PCR* for sub-gingival samples (McNcmar's test, $p<0.001$), but not for supra-gingival samples (McNcmar's test, $p=0.219$)