Detection of Six Periodontopathogens in the Subgingival Plaque of Patients with Chronic Periodontitis in Lagos, Nigeria

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ABSTRACT

Objective: This study was aimed at evaluating the occurrence of six periodontal pathogens in the subgingival plaque of patients with chronic periodontitis in Nigeria.

Methods: Forty-two subjects with chronic periodontitis participated in the cross-sectional study between June, 2015 and August, 2016. Subgingival plaque samples from one site of ≤3mm depth (gingivitis) and another site with the greatest depth of ≥4mm (periodontitis) respectively were collected from each subject. Culture and PCR assay using 16S rRNA species-specific primers, were performed. Fisher's exact test was used for statistical analysis.

Results: Majority (73.8%) of the subjects were positive for at least one of the organisms. Culture was positive for *P. gingivalis, F. nucleatum* and *P. intermedia* in 23.8%, 14.3% and 14.3% of the subjects respectively in sites of ≤3mm and at 33.3%, 23.8% and 33.3% respectively in sites of ≥4mm. PCR was able to detect all six periodontopathogens in sites of ≥4mm, with *A. actinomycetemcomitans, P. gingivalis F. nucleatum P. intermedia, T. forsythia* and *T. denticola* in 4.8%, 31.0%, 40.4%, 45.2%, 4.8% and 2.4% of the subjects respectively. The pathogen with the most frequent total occurrence in all sites was *P. intermedia* (54.8%). The difference between the occurrence of the organisms in the sites of periodontal probing depth of ≤3mm and ≥4mm was significant ($p<0.05$).

Conclusion: This study shows the distribution of subgingival periodontal pathogens in chronic periodontitis in a Nigerian population. Wider studies among the Nigerian population are required to assess differences in the pattern of distribution of these bacteria.

Keywords: Nigeria, patients, chronic periodontitis, PCR

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INTRODUCTION

Chronic periodontitis is the most prevalent type of periodontitis and is usually preceded by gingivitis. It characteristically involves a slow and gradual loss of periodontal attachment and alveolar bone.[1] The role of bacteria in the aetiology of periodontitis has been reported extensively.[2,3] The association between subgingival bacterial species and chronic periodontitis has been demonstrated by clinical studies worldwide.[4-7] From these studies it has become apparent that there are considerable differences in the composition of subgingival microbiota of populations from different countries. It has been advocated that these differences may be related to economic, environmental and genetic influences.[7] Evaluation of the subgingival bacterial diversity may therefore be significant in diagnosis, optimal treatment and prediction of further

periodontal destruction in periodontal disease. This suggests that the impact of investigations into the subgingival microbial organisms in a country may not only be pertinent to understanding their implication in the pathogenesis of periodontal disease, but also in predicting likely treatment outcomes especially in cases of non-response to non-surgical periodontal therapy and in addressing its association with some medical conditions.[8]

Culture is the usual microbiological method for the identification of microorganisms in biological specimens. Nevertheless, as some species are not readily cultured, the rate of recoverable bacteria from a culture specimen might be disparate with the proportion of the bacteria in a sample.[9] The exacting conditions required to cultivate anaerobic bacteria poses further challenges. Polymerase chain reaction (PCR) is a molecular technique which utilizes 16S rRNA gene-specific oligonucleotide primers to amplify specific DNA fragments of target microorganisms.[10] It has proved to be a versatile tool in the identification of periodontal pathogens in dental plaque samples.

While investigations into the microbiology of periodontal diseases have been on-going worldwide for decades, only a few studies have been conducted in West Africa.[11-13] In Nigeria, some studies have been reported which examined the detection of the subgingival microbial pathogens of periodontal diseases.[14-16]

The objective of this study was to assess the occurrence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Tannerella forsythia* and *Treponema denticola* in clinical subgingival plaque in a population of Nigerian patients with chronic periodontitis, utilizing both culture and PCR techniques.

MATERIALS AND METHODS

Study design and patient selection

This was a cross-sectional analytic study conducted in the Periodontology clinic, at the Lagos University Teaching Hospital, Lagos, Nigeria among patients with chronic periodontitis. The clinical status of chronic periodontitis was determined according to American Academy of Periodontology and the Centre for Disease Prevention and Control (AAP-CDC) Joint Group where the patient had at least ≥2 interproximal sites with ≥3mm clinical attachment loss (CAL) and ≥2 interproximal sites with ≥4mm pocket depth (not on the same tooth) or 1 site with ≥5mm pocket depth with radiographic evidence of bone loss.[17]

A convenience sampling of 42 consecutive patients who presented at the clinic and were diagnosed with chronic periodontitis between June, 2015 and August, 2016 was done and these patients gave their consent to participate in the study.

The inclusion criteria were; systemically healthy patients, aged ≥20 years with at least 12 teeth present excluding the third molars. The exclusion criteria were; pregnancy or nursing, history of smoking, periodontal therapy in the last six months, systemic or topical oral antibiotics use in last three months, as well as use of anti-inflammatory, anti-convulsant, immunosuppressant or calcium-channel blocker medications in the last three months.

Clinical examination

The examination and subgingival plaque sample collection were performed by a single calibrated investigator. A periodontal examination was conducted at the first visit using a dental mouth mirror and a UNC-15 periodontal probe. The periodontal probing depth (PPD) was taken 3 times in 6 sites (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual) of all teeth present and the mean recorded. The clinical attachment loss (CAL) was measured and all findings recorded accordingly.

Sample collection

Subgingival plaque samples were collected on a second visit. Two sites of probing depths ≤3mm (inflammation with no CAL) and ≥4mm (with bleeding on probing (BOP) and CAL, with or without gingival recession), were selected in each subject. The sites selected were on the first tooth with at least 3 contiguous sites with PPD at ≤3mm or ≥4mm respectively. The upper right quadrant was the starting point, working clockwise to upper left quadrant until the required site was obtained. The selected sites were isolated with cotton rolls and the supragingival plaque removed from the teeth with a cotton pellet. Two sterile paper points (ISO number 30 UnoDent, England) were inserted into the depth of each site for 30 seconds. Four subgingival plaque samples were obtained from each subject for both culture and PCR totaling 168 samples. The paper points were immediately placed in an anaerobic Dental Transport Medium (Anaerobe Systems, USA) and the specimens were transported within 2 hours of collection to the Anaerobe Unit, Molecular Biology Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos.

Anaerobic culture

One sample from each of gingivitis and periodontitis sites were processed and cultured for the identification of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythia* and *Treponema denticola* according to the method outlined by Nwaokorie et al.[14] Each sample was vortexed for 30 seconds and thereafter streaked on fastidious anaerobic agar (FAA) (Lab M) plate supplemented with 5µg/ml haemin, 1µg/ml vitamin K and 5% of sheep blood. Incubation in an anaerobic jar (Merck KGaA, Germany) was at 37°C for 7 days using 90% nitrite and 10% carbon dioxide generated by sachets of gas generating kit (Merck, Germany) in accordance with the manufacturer's instructions.

The growth on each plate was examined macroscopically on day 7 to assess colony morphology. To obtain pure cultures for subsequent biochemical tests, each discrete colony was sub-cultured on FAA. After 48 hours of incubation, the isolates were Gram-stained and examined under a microscope for cell morphology.

Biochemical tests

Biochemical tests using API 20A (bioMérieux SA, Mercy-l'Etoile, France) were performed according to the manufacturer's instructions. Indole reaction was performed on both API 20A and indole/nitrate reagent (Anaerobe Systems). Oxidase, catalase and urease (Anaerobic Systems) enzymatic activities were carried out. The identified bacterial isolates were stored in 10% skimmed milk at -80°C.

DNA extraction

Each sample for PCR assay was incubated in Brain Heart infusion (BHI) broth (5ml) for 48 hours and the bacterial cells were harvested by centrifugation at 14,000 x g for 10 minutes. The cells were washed three times in 1ml of ultra-pure water by centrifuging at 12,000 x g for 5 minutes before the chromosomal DNA was extracted using Fungal/Bacterial DNA MiniPrep™ 50 Preps, Model D6005.

DNA amplification

Amplification of the individual DNA was performed using previously published 16S rRNA species-specific forward and reverse primer pairs (Table 1). A final volume of 25µl, with a reaction mix containing 16.5µl of sterile water, 5µl of Mastermix (containing 1 x PCR buffer, 2.5mMol $MgCl_2$, 0.2mMol dNTP and 0.5µl *Taq* DNA polymerase), 0.5µl of each primer and 3µl of extracted DNA, was used to initiate the amplification reaction. The amplification reactions were carried out in a Thermocycler Gene Amp PCR system 9700. Amplification was programmed to run following cycling conditions of initial denaturing at 94°C for 5 minutes; 35 cycles of denaturing at 94°C for 1-minute, annealing temperature was set as listed on Table 1 for designated primers for 2 minutes, extension at 72°C for 2 minutes, followed by final extension at 72°C for 10mins. The reference strains American Type Culture Collection (ATCC) were used as positive controls and PCR mix without DNA templates were included as negative controls.

Agarose gel electrophoresis

The amplification products were analysed with agarose gel electrophoresis by adding them to a mixture of 1.5% agarose gel in 40mMol triacetate, 2mM EDTA (TAE) buffer (pH 8.3) stained with 3µl ethidium bromide. The electrophoresis was performed at 70V for 2.5 hours. The resultant bands were viewed through a UV transilluminator (CLINIX 1500 Japan). The microorganisms were identified by the presence or absence of a band corresponding with the expected band size (Figure 1). A 3µl DNA ladder of 1kbp (Promega, USA) was used as molecular weight marker.

Data analysis

Data were entered into Excel Spreadsheet and exported to SPSS (IBM Inc., Chicago, USA version 17.0) for analysis. Quantitative variables were expressed as means and standard deviation. Frequency distribution tables were generated for nominal and ordinal variables. The association between the occurrence of the six microorganisms and the PPD at the ≤3mm and ≥4mm sites were analysed using Fisher's exact test. Significant values were determined at p-value < 0.05.

Ethical approval

Ethical clearance was obtained from the Lagos University Teaching Hospital Health Research Ethics Committee (ADM/DCST/HREC/1944) prior to commencement of the study. A written informed consent was obtained from the participants after an explanation of the purpose of the study.

RESULTS

There were 25 (59.5%) males and 17 (40.5%) females with age range from 22 to 85 years old with a mean of 56.69 years (SD 13.40). One hundred and sixty-eight subgingival samples from 84 separate sites (84 samples from sites ≤3mm deep and 84 samples from sites ≥4mm deep) were collected from the subjects. The anterior teeth yielded 73.8% of the samples collected from gingivitis sites (≤3mm). All the samples from sites of periodontitis were collected

from varying PPD of ≥4mm with the deepest PPD at 14mm. Thirty (71.4%) of the samples from periodontitis sites were obtained from posterior teeth, of which 18 (60%) were from the second molars. Thirty-one (73.8%) of the subjects were positive for at least one of the microorganisms. The characteristics of the subjects are outlined in Table 2.

Detection of bacteria by culture

Culture was able to isolate *P. gingivalis*, *F. nucleatum* and *P. intermedia* in samples obtained from sites of gingivitis (≤3mm) and from sites of periodontitis (≥4mm). *A. actinomycetemcomitans*, *T. forsythia* and *T. denticola* were not detectable by culture in either site. The prevalence of the 6 bacteria is shown in Figure 2.

Table 1: Control bacterial strains and sequences of the species-specific oligonucleotides used in the PCR

Bacteria	Primer pair (5'- 3')	Base pair	PCR annealing temperature	Reference
A. actinomycetemcomitans ATCC 33384	AAA CCC ATC TCT GAG TTC TTC TTC ATG CCA ACT TGA CGT TAA AT	557	50° C	18
P. gingivalis ATCC 33277	AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404	60° C	19
F. nucleatum ATCC 25586	AGA GTT TGA TCC TGG CTC AG GTC ATC GTG CAC ACA GAA TTG CTG	360	60° C	20
P. intermedia ATCC 2564	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	259	64° C	19
T. forsythia ATCC 43037	TAC AGG GGA ATA AAA TGA GAT ACG ACG TCA TCC CCA CCT TCC TC	745	59° C	20
T. denticola ATCC 35405	TAA TAC CGA ATG TGC TCA TTT ACA T TCA AAG AAG CAT TCC CTC TTC TTC TTA	316	60° C	20

Table 2: Demographic characteristics and clinical periodontal parameters of subjects (N = 42)

Demographics and clinical parameters	Mean (SD)
Age (years)	56.69 (13.40)
Gender (M/F)	25/17
PPD_{all} (mm)	3.12 (0.70)
PPD_{dx} (mm)	5.35 (0.98)
CAL (mm)	3.79 (1.86)
Sites with CP	39.48 (24.09)
BOP sites	37.33 (26.66)

Key: SD = Standard deviation, PPDall = periodontal probing depth of all sites, PPDx = periodontal probing depth of periodontitis sites, CAL = clinical attachment loss, CP = chronic periodontitis, BOP = bleeding on probing

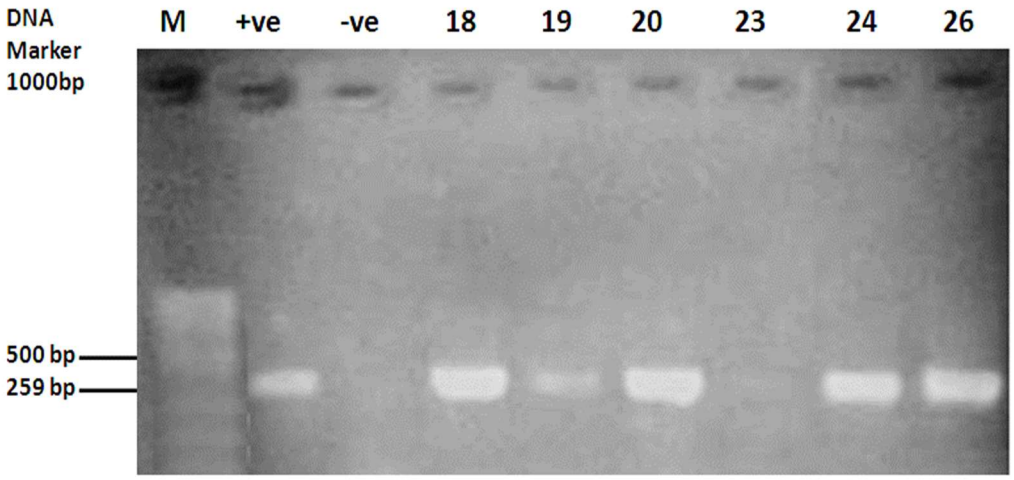


Figure 1: Agarose gel containing representative amplicon with specific primers. Lane M: 1kbp DNA ladder, Lane -ve: negative control, Lane +ve: positive control *P. intermedia* ATCC 2564, Lanes 18, 19, 20, 24 and 26 show positive amplification for *P. intermedia* species.

Detection of bacteria by PCR

PCR analysis of samples from the gingivitis sites detected *P. gingivalis*, *F. nucleatum* and *P. intermedia* respectively. All six periodontopathogens were detected by PCR in samples obtained from sites of periodontitis. *P. intermedia* was the most prevalent organism in 19 (45.2%) of the periodontitis sites while *F. nucleatum* was most prevalent at 12 (28.6%) of gingivitis sites (Figure 2). Both culture and PCR demonstrated statistically significant association ($p<0.05$) between the occurrence of *P. gingivalis, F. nucleatum* and *P. intermedia* and the sites of gingivitis (≤3mm) and sites of periodontitis (≥4mm) as shown in Table 3. Detection of the presence of any one of the bacteria in the subjects by a combination of both culture and PCR technique demonstrated *P. intermedia* with the highest occurrence at 23 (54.8%) sites and *T. denticola* at 1 (2.4%) site with the least occurrence (Table 4).

Table 5 shows the evaluation of the presence of the six microorganisms in single or co-occurrence with one or more of the other bacteria at the different sites using culture and PCR. Culture demonstrated a more frequent co-occurrence of *P. gingivalis, P. intermedia* and *F. nucleatum* (14.3%) in the periodontitis sites. *F. nucleatum* and *P. intermedia* (7.1%) was the only combination of bacteria detected in gingivitis sites by PCR. Both the gingivitis and periodontitis sites were devoid of the co-occurrence of the red complex group of *P. gingivalis*, *T. forsythia* and *T. denticola*.

Table 3: Differences in the occurrence of 6 periodontopathogens in gingivitis and periodontitis sites by culture and PCR. (N=42)

Bacteria	Culture		*P*-value	PCR		*P*-value
	≤3mm n (%)	≥4mm n (%)		≤3mm n (%)	≥4mm n (%)	
A. actinomycetemcomitans	-	-	-	-	2 (4.8)	-
P. gingivalis	10 (23.8)	14 (33.3)	0.001	7 (16.7)	13 (31.0)	0.021
F. nucleatum	6 (14.3)	10 (23.8)	0.002	12 (28.6)	17 (40.4)	0.001
P. intermedia	6 (14.3)	14 (33.3)	0.011	9 (21.4)	19 (45.2)	0.033
T. forsythia	-	-	-	-	2 (4.8)	-
T. denticola	-	-	-	-	1 (2.4)	-

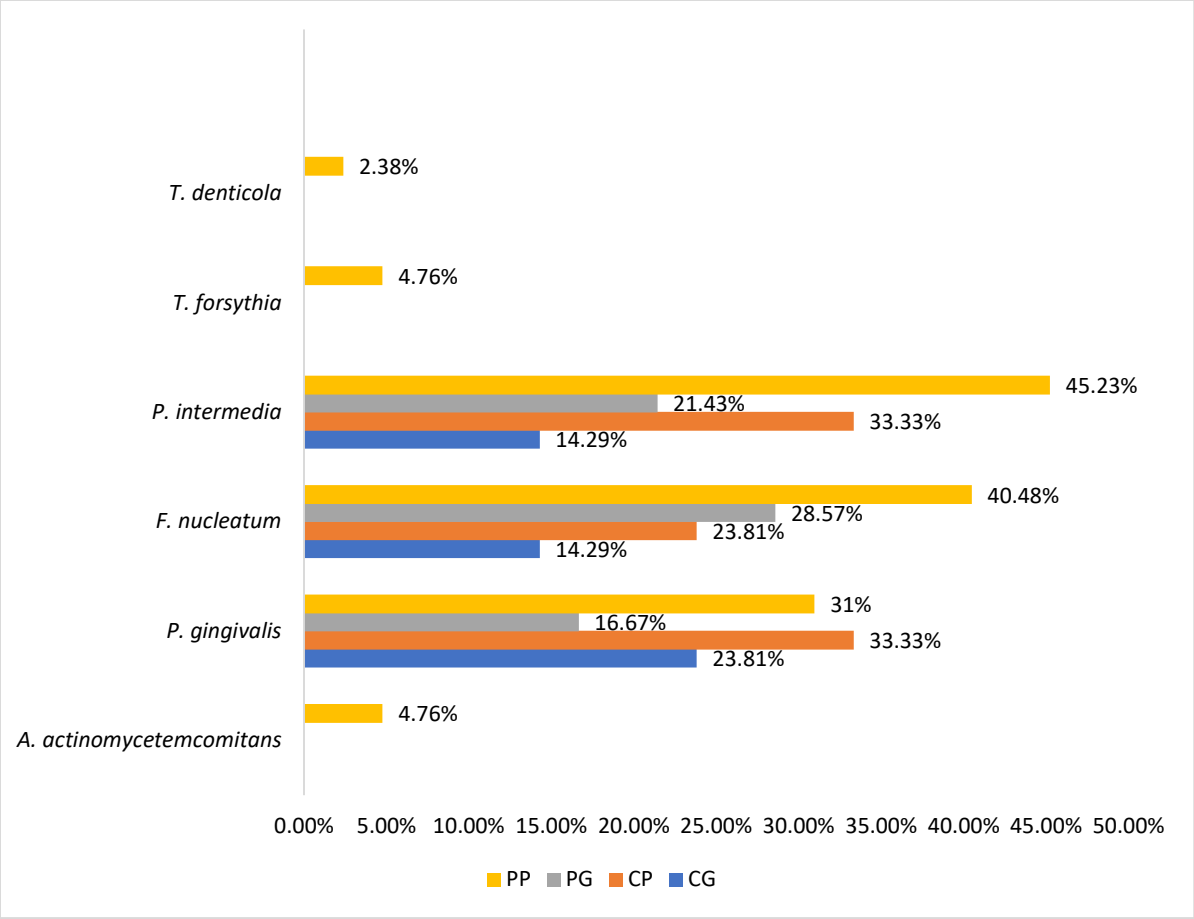
Table 4: Total occurrence of six periodontopathogens using culture and PCR (N=42)

Bacteria	Frequency	Percentage (%)
A. actinomycetemcomitans	2	4.8
P. gingivalis	20	47.6
P. intermedia	23	54.8
F. nucleatum	22	52.4
T. forsythia	2	4.8
T. denticola	1	2.4

Table 5: Evaluation of co-occurrence of six periodontopathogens at sites of ≤3mm and ≥4mm

Co-occurrence of Bacteria	Culture (N = 42)		PCR (N = 42)	
	≤3mm	≥4mm	≤3mm	≥4mm
	n (%)	n (%)	n (%)	n (%)
PG	4 (9.5)	2 (4.8)	7 (16.7)	1 (2.4)
FN	4 (9.5)	3 (7.1)	9 (21.4)	5 (11.9)
PI	-	1 (2.4)	6 (14.3)	4 (9.5)
PG + PI	4 (9.5)	6 (14.3)	-	6 (14.3)
FN + TF	-	-	-	1 (2.4)
PI + FN	-	1 (2.4)	3 (7.1)	3 (7.1)
PG + FN	-	-	-	1 (2.4)
PG + PI + FN	2 (4.8)	6 (14.3)	-	4 (9.5)
AA + PI + FN	-	-	-	1 (2.4)
AA + FN + TF	-	-	-	1 (2.4)
PG + PI + FN + TD	-	-	-	1 (2.4)

Key: AA = *A. actinomycetemcomitans,* PG = *P. gingivalis*, FN = *F. nucleatum*, PI = *P. intermedia*, TF = *T. forsythia*, TD = *T. denticola*



Key: PP = PCR of periodontitis sites, PG = PCR of gingivitis sites, CP = Culture of periodontitis sites, CG = Culture of gingivitis sites

Figure 2: Prevalence of six periodontopathogens at gingivitis and periodontitis sites using culture and PCR (N = 42).

DISCUSSION

In this study, anaerobic culture was able to detect *P. gingivalis, F. nucleatum* and *P. intermedia* species in sites with gingivitis (≤3mm) and with periodontitis (≥4mm). The detection of one or more of these organisms in sites of gingivitis and periodontitis using culture has been demonstrated by various studies and is reportedly due to their frequent abundance in the subgingival plaque.[16,21] *T. forsythia* and *T. denticola* were not isolated by culture in this study. This may be due to their fastidious nature and stringent needs for special nutritional requirements. They have commonly been implicated in the aetiology of chronic periodontitis and recognized as part of the red complex of subgingival plaque.[5,22,23] Kotsilkov et al.[24] reported a similar finding in their study in Bulgaria.

Conventional PCR has been shown to be able to detect the presence of these six periodontopathogens.[5,6,13,25,26] The detection of *P. gingivalis, F. nucleatum* and *P. intermedia* in this study were greater in sites of periodontitis (≥4mm) than in sites (≤3mm). PCR was however, unable to detect certain *P. gingivalis* organisms detected by culture. This could be due to the presence of *Porphyromonas spp* with phenotypic features similar to *P. gingivalis,* which the species-specific primer used in this study seemed to have been able to discern. The ability of PCR to discriminate between periodontal bacteria with significant phenotypic similarity has been reported by Avila-Campos and Velásquez-Meléndez.[26] It is possible that the oligonucleotide primer set used in the present study may not be specific to the strains that might be present in this environment. This is most probably due to a difference in the DNA sequence of the *P. gingivalis* strains than that targeted by the oligonucleotide primer set.

The greater percentage of the subjects (73.8%) were positive for the presence of at least one of the

periodontopathogens investigated in this study. A higher occurrence of *P. gingivalis, F. nucleatum* and *P. intermedia* was demonstrated in the periodontitis sites by both culture and PCR than what was obtained by culture and PCR in gingivitis sites. This finding contrasts with the findings by Egwari et al[15] and Anyiam et al.[16] who both reported lower detection levels in sites of periodontitis than this study. Sites of gingivitis were not included in their investigation. Other studies have however reported similar findings between gingivitis and periodontitis sites using either or both culture and PCR techniques.[5,6,27] Nevertheless, the detection levels for some of these studies, differed from the present study. In a study in Congo, a higher prevalence was reported.[13] In another population of south Indians, Mahalakshmi et al,[25] with the aid of PCR only, demonstrated greater detection of *P. gingivalis* in periodontitis sites and lower figure in gingivitis sites than in the present study. In a study similar to this study, Avila-Campos and Velásquez-Meléndez[26] in São Paulo, Brazil, demonstrated higher detection figures than this study for these three bacteria (*P. gingivalis, P. intermedia* and *F. nucleatum*). Among another population of Brazilians, Farias et al[5] reported higher occurrence for *P. gingivalis* in both periodontitis and gingivitis sites with conventional PCR only, than was obtained with this study. Gatto et al's[6] study among Italians utilized multiplex PCR and had higher occurrence in periodontitis sites for the three bacteria than observed in this study, however, their study did not include gingivitis sites. Ready et al.[27] in the UK, had reported higher levels than this study for *P. gingivalis*, *A. actinomycetemcomitans* and *T. forsythia* in periodontitis sites of their subjects. In their study in the Netherlands, van Winkelhoff et al[21] using culture reported a lower occurrence for *P. gingivalis* (10.6%) in gingivitis sites than in the present study while its occurrence in periodontitis sites was greater (59.9%) than in this study. Their findings on *A. actinomycetemcomitans, F. nucleatum, P. intermedia* and *T. forsythia* were considerably higher than this study. A greater detection of *F. nucleatum* and lower values for *P. gingivalis* and *P. intermedia* in periodontitis sites using multiplex PCR among a Ugandan population was reported by Mboowa et al.[28] Low frequencies of *A. actinomycetemcomitans, T. forsythia and T. denticola* were detected at the periodontitis sites by PCR alone in this study. The low detection of *A. actinomycetemcomitans* in adults in this study corroborate with findings reported in other studies.[4,5,11,29] Other studies of chronic periodontitis have reported higher figures. Avila-Campos and Velásquez-Meléndez[26] reported greater detection for *A. actinomycetemcomitans, T. forsythia and T. denticola* than in this study. Gatto et al.[6] reported *A. actinomycetemcomitans* with the least occurrence among the organisms in their subjects but at levels higher than this study. However, a high occurrence of *A. actinomycetemcomitans* in some populations with chronic periodontitis, especially in Asia have been reported in certain studies.[7] Conversely, Egwari et al.[15] were unable to demonstrate *A. actinomycetemcomitans* in their adult subjects but did in the adolescents. Kalala-Kazadi et al.[13] in their study among adolescent and adult Congolese patients, reported non-detection of *A. actinomycetemcomitans.* A high occurrence of *A. actinomycetemcomitans* has been demonstrated in periodontitis sites of children and adolescents with aggressive periodontitis in West Africa.[12]

Several studies have reported detection of *T. forsythia* and *T. denticola* at levels far greater than what was obtained in this study.[5,7,18] Utilizing PCR, Kalala-Kazadi et al.[13] reported higher levels in periodontitis. Gatto et al.[6] utilizing real-time PCR, had reported higher values for *T. forsythia* and *T. denticola* in chronic periodontitis. Mahalakshmi et al.[25] also demonstrated higher occurrence of *T. forsythia* and *T. denticola* in periodontitis sites.

The differences in the frequencies of detection of *P. gingivalis, F. nucleatum* and *P. intermedia* between the periodontitis sites and the gingivitis sites with either culture or PCR, agrees with findings in other studies, which have reported the positive relationship between these three anaerobic bacteria and chronic periodontitis, where they are frequently detected in sites of periodontitis.[4-7,22,23,29]

The more prevalent and equal levels of co-occurrence of *P. gingivalis* plus *P. intermedia* and *P. gingivalis* plus *P. intermedia* as well as *F. nucleatum* than any other combination of the bacteria in periodontitis sites differs from studies by Farias et al[5] and Mahalakshmi et al,[25] which did not report either of these combinations of bacteria in periodontitis sites. The occurrence of *A. actinomycetemcomitans*, *T. forsythia* and *T. denticola* in combination with other bacteria in periodontitis sites is similar to their reports. The red complex combination was not detected in this study unlike in their studies. Certain complexes of periodontal bacteria have been associated with disease severity, of which the red complex of *P. gingivalis*, *T. forsythia* and *T. denticola* is most associated with severe periodontitis.[2]

The occurrence of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, F. nucleatum, T. forsythia*

and *T. denticola* in gingivitis and periodontitis sites among a Nigerian population with chronic periodontitis in this study was generally lower than other populations.[7] Wide-ranging differences have been reported on the subgingival microbial composition in chronic periodontitis among different geographical and ethnic populations.[4-7,25] Due to the variety of techniques that have been utilized, including microbiological cultivation methods and different molecular techniques as well as the differences in environment, study designs, sample sizes, sample selection and subgingival plaque sampling methods, findings are to be interpreted with caution.[7]

Factors such as genetic background, diet, culture, healthcare practices, socioeconomic status, oral hygiene procedures and access to dental care have been attributed to be likely causes for the differences in the subgingival plaque microbial composition they observed among their subjects.[4,7] A high prevalence of antibiotic self-medication among Nigerians,[30] the homogeneity of the sample population, possible existence of wild strains of periodontal pathogens, geographical location, environmental and genetic influences are to be considered in evaluating the occurrence of these periodontopathogens in this environment. This study was conducted in Lagos, Nigeria, a cosmopolitan city of which many of the ethnic groups in Nigeria may be found but 73.8% of the sample population was composed of Yoruba, the predominant ethnic group in this part of the country. The small sample size was the major limitation of this study. Future studies to assess the periodontopathogenic profile of periodontitis, utilizing larger sample sizes and cutting across the different parts of the country are needed.

CONCLUSION

This study shows the distribution of subgingival periodontal pathogens in chronic periodontitis in an adult Nigerian population. Wider studies among the Nigerian population are required to assess differences in the pattern of distribution of these and other organisms, and their role in the pathogenesis of periodontitis. The identification of local wild strains of *P. gingivalis* would require DNA sequencing of the culture isolates obtained and using them to design specific primers.

Source of Support
Nil.

Conflict of Interest
None declared.

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