

Subgingival microbial profiles of Sudanese patients with aggressive periodontitis

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Background and Objective: Aggressive periodontitis (AgP) is prevalent and shows a rapid course in African individuals. Although a strong focus has been placed on *Aggregatibacter actinomycetemcomitans*, new methods support the existence of a complex subgingival microflora in AgP. The purpose of the present study was to map the subgingival microbiota as well as explore the presence of *A. actinomycetemcomitans* and the JP2 clone in a group of Sudanese individuals with AgP, using different analytical methods.

Material and Methods: A study population consisting of 19 patients with AgP was recruited from patients seeking treatment at University of Science and Technology (UST) in Khartoum. Fifteen healthy subjects were included as controls. Plaque samples were analyzed for 272 taxa using human oral microbe identification microarrays and for 26 periodontal taxa using DNA-DNA hybridization checkerboard. Conventional polymerase chain reaction (PCR) was applied for the detection of *A. actinomycetemcomitans* and the JP2 clone in plaque. Saliva from patients with AgP was analyzed using quantitative PCR (qPCR) for the detection of *A. actinomycetemcomitans*.

Results: *Eubacterium yurii* was detected more frequently in patients with AgP than in controls, and *E. nodatum* was found in patients with AgP only. *A. actinomycetemcomitans* was found in plaque samples of two (12%) patients by human oral microbe identification microarrays and in five (29%) patients with AgP by conventional PCR, as well as in six (32%) of the AgP saliva samples by qPCR. The JP2 clone was identified in only one patient.

Conclusion: The classical periodontal pathogens were not present in high amounts in AgP in the population studied here. Species of *Eubacterium*, which are not typically associated with AgP, were often detected in individuals with disease. Using laboratory methods with different sensitivities and detection levels allowed identification of variances in microbial communities. The findings reported in this study provide a basis for the further understanding of AgP.

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Aggressive periodontitis (AgP) represents the most severe, rapidly progressing type of periodontal disease (1,2). The prevalence of AgP varies among races and geographical loca-

tions, being low in young European Caucasians (about 0.1%) and higher in Hispanics, African-Americans and Africans (3). A prevalence of 0.3–7.6% has been reported in young age

cohorts in Africa (3,4). In some African countries, the incidence of AgP has been reported to be even higher, but likely, due to differences in recruitment, methods and criteria used for

diagnosis (5). Recently, 3.4% of Sudanese students, aged 13–19 years old, were reported to have AgP, with males being more affected (6).

AgP, as well as other forms of periodontal diseases, are associated with a complex microbial community, composition and population dynamics, making it difficult to identify specific causative agents. In association studies, following the criteria of Socransky (7) to determine putative etiologic agents of periodontal disease, a close association between *Aggregatibacter actinomycetemcomitans* and bone loss has been reported in the localized type of the disorder (LAgP). Other bacterial species associated with progression of LAgP have been identified as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Campylobacter gracilis*, *Eubacterium nodatum* and *Prevotella intermedia* (8). In generalized AgP (GAgP), bacterial species found in the plaque of affected patients may be more similar to those present in other types of periodontitis (9). Conflicting results for the association between colonization with *A. actinomycetemcomitans* and LAgP compared with GAgP have been reported for different populations (10). *A. actinomycetemcomitans* is detected in all forms of periodontal disease, as well as in healthy individuals (11,12). *A. actinomycetemcomitans*, especially the highly leukotoxic JP2 clone, was found to be associated with the initiation of destruction at diseased sites in a Moroccan population and in populations in northwest Africa with AgP (13,14).

Despite close association between *A. actinomycetemcomitans* and bone destruction, *A. actinomycetemcomitans* has not been detected in all diseased sites of AgP (15), and the bacterium has also been found in pockets without any signs of pathology in the same mouth (16). These studies highlight the complexity of periodontal disease pathogenesis, including variation in microbial composition and virulence of the strains, in combination with various host and environmental factors.

Before the introduction of the culture-independent methods, the bacterial species frequently detected and

associated with periodontally diseased sites were proteolytic gram-negative species. In more recent studies, periodontal disease has also been associated with large number of gram-positive bacteria (17,18). This draws attention to the importance of the quality of methods used for the outcome and limitation of bacterial identification in samples from the periodontal pocket. Reports on the prevalence of periodontal bacteria at a population level reveal substantial variation in estimates with regard to sampling strategy, systems for bacterial identification and ethnicity of the population (19). As it was observed that AgP was a serious problem for young individuals, leading to loss of permanent teeth at a young age, one of the goals for this study was to map the microbial profiles of Sudanese individuals with AgP and to compare these with the profiles of healthy controls using the culture independent techniques such as 16S rRNA gene amplification and subsequent human oral microbe identification microarray (HOMIM). The results were also compared with the DNA-DNA hybridization checkerboard assay outcome. Further, we examined the presence of *A. actinomycetemcomitans* in subgingival plaque and saliva samples using conventional polymerase chain reaction (PCR) and quantitative PCR (qPCR).

Materials and methods

A flow chart of the study populations and methods is shown in Fig. 1.

Study populations

Nineteen AgP subjects (15 females and four males) were recruited from subjects seeking treatment at the University of Science and Technology (UST), Khartoum, Sudan, from December 2008 to July 2009. Of these, 10 were classified as GAgP and nine as LAgP (1). Nineteen systemically and periodontally healthy employees or students at UST were recruited as healthy controls. Both patients and healthy controls originated from Khartoum.

To be included in the study as a patient, bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level ≥ 5 mm all had to be present at least at one central incisor and one first molar. Diagnosis of AgP was confirmed by analysis of radiographs (horizontal bitewings and periapical radiographs). Medical history was recorded for each individual, for participants and for controls, and subjects were excluded from the study if they had received antibiotic or periodontal treatment within 3 months before the examination, were pregnant or had any systemic disease that could affect the progression of periodontal disease, or were smokers.

To be included in the study as a healthy control, PPD ≤ 3 mm (excluding third molars) and no attachment loss were required.

Ethical approvals for this study were obtained from the Research Ethical Committee at UST, Sudan and the Regional Committee for Medical Research Ethics (REK) Western Norway, Norway (REK 177.04, Biobank 08/1325-3). Signed informed consent was obtained from all subjects before their enrolment.

Clinical examination

All study procedures, including clinical measurements and sample collection, were conducted in a single clinical visit. The periodontal clinical registrations in this cross-sectional study were performed at six sites per tooth for all teeth excluding third molars. The plaque index used in this study was the Turesky modification of the original index of Quigley and Hein (20,21). Gingival index was measured according to the modified gingival index (22). Gingival recession (GR) was registered in millimeters and with either (+) or (–) according to the position of the gingival margin to the cemento-enamel junction. The PPD was measured in millimeters from the gingival margin to the bottom of the periodontal pocket. Clinical attachment level was calculated according to the formula [clinical attachment level = PPD – GR]. Both GR and PPD were measured by a

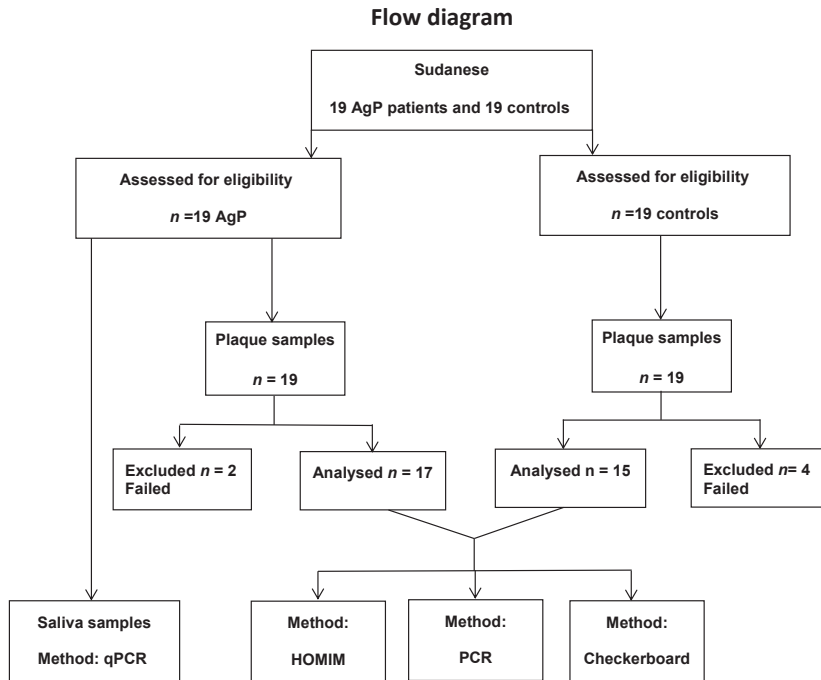


Fig. 1. Flow chart of participants and methods used in this study. AgP, aggressive periodontitis; HOMIM, human oral microbe identification microarrays; qPCR, quantitative polymerase chain reaction.

color-coded Williams' periodontal probe, and BOP was recorded as 1 if present or 0 if not present (23). All clinical registrations were conducted by the same examiner (H.Z.E.) who had been trained and calibrated against a gold standard examiner (M.M.). The inter- and intra-examiner reproducibility of PPD was assessed by the intraclass correlation coefficient (ICC) (24).

Subgingival plaque sampling

Subgingival plaque samples were collected from all enrolled subjects. Before collecting subgingival plaque, supragingival plaque was first removed by sterile gauze and sites were isolated with a sterile cotton roll. For each individual, plaque samples were collected subgingivally at the mesiobuccal sites of one posterior tooth in each quadrant, either from the first molar, second molar or the second premolar in this priority, depending on whether the teeth were present or not. The plaque was sampled from all quadrants using a sterile Gracey curette scraped against the tooth subgingivally from the bot-

tom of the pocket. In healthy controls, the samples were collected by placing the curette into the gingival sulcus with 1–3 mm depth. The collected plaque was diluted in 200 μ L Tris buffer before the addition of 200 μ L fresh prepared NaOH solution (consisting of 0.1 g NaOH in 5 mL distilled water) to a final concentration of 0.25 M. The samples were transferred to a -80°C freezer and stored until needed.

Bacterial DNA isolation

DNA from plaque and saliva to be used for either HOMIM, checkerboard or qPCR analyses was isolated in accordance with the DNA isolation protocol from MasterPure™ DNA purification kit (Epicentre® Biotechnologies, Madison, WI, USA). The amount and purity of DNA was measured on a NanoDrop ND 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and stored at -80°C . For conventional PCR, plaque samples within each individual were pooled and 20 μ L of each pooled plaque sample was subjected to boiling for 5 min to release DNA.

Human oral microbe identification microarrays

Frozen subgingival plaque DNA samples were transferred to the Forsyth Institute, Cambridge, MA, USA and analyzed using HOMIM (25). In this method, 16S rDNA reverse-capture oligonucleotide probes, 18–20 bases in length, were printed on aldehyde-coated glass slides (25,26). In total, 422 unique probes were used to investigate overall 272 species (e.g. there were multiple probes for some target species). 16S rRNA genes were PCR amplified from plaque-derived DNA of each subject, labelled in another nested PCR and hybridized to the probes on the aldehyde-coated glass slides (18). The detection limit for the HOMIM assay is about 10,000 bacterial cells. A detailed description of the methods used can be found elsewhere (18,25). Spot intensities of the array of score of 1–5 were regarded as positive (present).

DNA-DNA hybridization checkerboard

DNA from subgingival plaque of patients and controls was hybridized to DNA from 24 bacterial species by DNA-DNA hybridization checkerboard technique. The procedures of Haffajee *et al.* (27) and Socransky *et al.* (28) were followed with minor modifications. Signals were evaluated and converted to absolute counts by comparison with the standards (10^5 and 10^6 number of cells) on the same membrane. Failure to detect signals was recorded as zero. Only species present above the detection level ($> 100,000$ cells) were examined for statistical significance.

Conventional polymerase chain reaction

Primers used for detection of the *LktA* gene of *A. actinomycetemcomitans* were constructed as described (29); LKT2 (5'-CTA-GGT-ATT-GCG-AAA-CAA-TTT-G-3') and LKT3 (5'-CCT-GAA-ATT-AAG-CTG-GTA-ATC-3'), producing a 262 bp amplicon. The primers

spanning the 530 bp deletion in the promoter region of the highly leukotoxic JP2 clone Ltx3 (5'-GCC-GAC-ACC-AAA-GAC-AAA-GTC-T-3') and Ltx4 (5'-GCC-CAT-AAC-CAA-GCC-ACA-TAC-3') were constructed as previously described (30). Strains of *A. actinomycetemcomitans* (CCUG 37399) and the JP2 clone (CCUG 56172) were used as positive controls for PCR.

Quantitative polymerase chain reaction assay

DNA from saliva samples of the patients was investigated for presence of *A. actinomycetemcomitans* by qPCR on 96-well thermal cycle plates in ABI StepOne Plus equipment (Applied Biosystem Inc., Foster City, CA, USA). Samples were run in duplicates. Each TaqMan PCR assay was performed in a 20 µL volume containing 10 µL TaqMan Fast Master Mix (Applied Biosystem), 1 µL of the primer/probe mix addressing the infectious agent *A. actinomycetemcomitans* (AF359451.1) and Actin Beta (ACTB, endogenous control) from Ingene Advanced Kit (PrimerDesign™ Ltd, Millbrook Technology Campus, Southampton, UK), 1 µL internal extraction control, 3 µL of RNase/DNase free water and 5 µL of the purified DNA. The qPCR cycling program included 20 s at 95°C for hot start, then 40 cycles each consisting of 95°C for 1 s and 60°C for 20 s. The data were analyzed by STEPONE-PLUS™ Software v2.1 using the standard curve method. The results obtained from this technique were compared with standard curves, which were constructed for specific detection of *A. actinomycetemcomitans*. The detection limit is about 100 cells.

Data analysis

The statistical analysis was performed by the PREDICTIVE ANALYTICS Software (PASW) v18. Descriptive statistics were used to calculate the mean and standard deviation for the clinical parameters of the AgP group. Differences between AgP and controls, and between the GAgP and LAgP, were

computed and examined for significance using Mann–Whitney test. $p < 0.05$ was considered significant.

Analysis was performed on the absolute intensity of HOMIM data (frequency of scores from 0 to 5) as well as binary data (presence/absence). The prevalence of each taxon was computed for each subject and averaged within groups. Mann–Whitney test was used to identify statistically significant differences between groups. The Benjamini–Hochberg procedure, which allows calculation of the false discovery rate, was used to correct for multiple testing. An adjusted $p < 0.05$ was considered significant.

Results

Study population

The mean age for the study population consisting of patients with AgP and healthy controls was $23.74 \pm .84$ years and 24.40 ± 3.54 years, respectively. The age of participants with AgP ranged from 13 to 30 years old. The GAgP population (age = 26.7 ± 6.2 year) was older than the LAgP population (age = 20.4 ± 5.4 years). The gender distribution was similar for LAgP and GAgP, 77% and 78%, respectively, were females. The participants with GAgP had a mean periodontal pocket depth of 3.7 ± 0.6 mm while this was 3.0 ± 0.8 mm in LAgP, and the mean clinical attachment level was 4.0 ± 2.2 mm in GAgP compared with 2.9 ± 2.3 mm in LAgP (Table 1). The proportion of deep pockets (≥ 6 mm) in each individual ranged between 0 and 32% and the propor-

tion of sites with BOP ranged between 17% and 100% in all patients with AgP (Table S1).

Inter- and intra-examiner reliability

Measurements of PPD at posterior teeth in nine individuals were used for intra- and inter-examiner calibration. The agreements between the repeated measurements of the examiner H.Z.E. and the reference standard M.M. ranged between 0.73 and 0.93, indicating very good agreement (24), except for one variable (the PPD of the mesio-buccal site of the first premolar) where ICC was equal to 0.06, and the only reason for that was the small variation between the measured values. The intra-examiner ICC ranged between 0.61 and 1.00, which was also good except for one variable that was equal to 0.52, which was acceptable.

Human oral microbe identification microarray data analysis

DNA extracted from subgingival plaque of patients and controls was analyzed by HOMIM. In total, 272 oral bacterial species were investigated.

Streptococcus clusters II and III were detected in all subjects. There were significantly different proportions of bacterial species in participants with AgP compared with controls, as shown in Fig. 2. The most prevalent bacterial species detected in participants with AgP by HOMIM was *Eubacterium yurii*. *Campylobacter* and *Leptotrichia* species were more prevalent in controls than in participants with AgP

Table 1. Clinical parameters of patients with AgP

Clinical parameters	GAgP* (n = 10)	LAgP* (n = 9)
Probing pocket depth [†]	3.7 ± 0.6	3.0 ± 0.8
Clinical attachment level [†]	4.0 ± 2.2	2.9 ± 2.3
Bleeding on probing [‡]	0.8 ± 0.4	0.6 ± 0.5
Gingival index	2.7 ± 0.7	2.6 ± 0.7
Plaque index	4.1 ± 1.3	4.0 ± 2.0

AgP, aggressive periodontitis; GAgP, generalized aggressive periodontitis; LAgP, localized aggressive periodontitis. Mann–Whitney test was used to test for difference between LAgP and GAgP.

*Mean ± SD.

[†]Measured in millimeters.

[‡]Bleeding on probing for all sites of all patients.

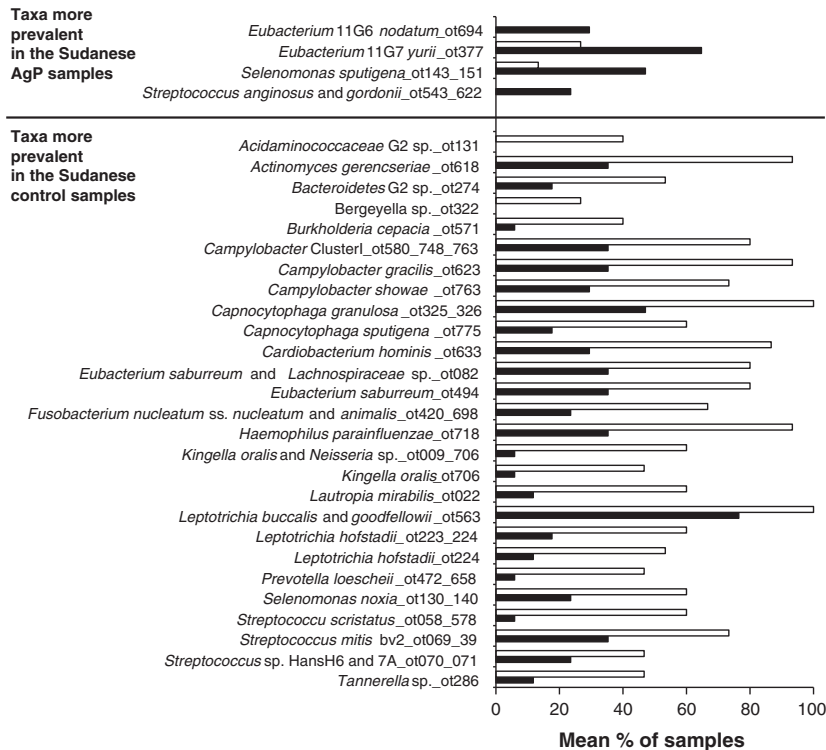


Fig. 2. Bacterial taxa significantly different in Sudanese subjects with AgP compared with controls when analyzed by HOMIM. The x-axis indicates the proportion of individuals in the cohorts that tested positive by HOMIM for the respective bacteria listed on the left in the figure ($p < 0.05$; Mann–Whitney test). Black bar, patients with AgP; white bar, controls; AgP, aggressive periodontitis; HOMIM, human oral microbe identification microarrays.

(Fig. 2). Using HOMIM, there was an absence of *P. gingivalis* in the AgP group, while it was present only in two controls. *A. actinomycetemcomitans* was detected only in two patients with AgP and was totally absent in controls. *E. nodatum* was also totally absent from the control group, but significantly present in AgP ($p < 0.05$).

Parvimonas micra, *Solobacterium moorei* and *Shuttleworthia satelles* were the only bacterial taxa that differed between participants with LAgP and GAgP, being more frequently detected in GAgP (data not shown).

Checkerboard results from analysis of the population

The mean counts of 24 bacterial species (27 strains) in subgingival plaque were calculated for the study population according to the results of the checkerboard assay (Fig. 3). *Actino-*

myces naeslundii was found in high amounts in patients with AgP; however, there was no significant difference compared to controls. *Actinomyces viscosus* II, *Campylobacter concisus*, *Escherichia coli*, *Veillonella parvula*, *Filifactor alocis* and *Fusobacterium nucleatum* ssp. *vincentii* were present in significantly lower amounts in patients with AgP compared with healthy controls, only *Streptococcus sanguinis* was found in an increased number in AgP ($p < 0.05$).

Conventional polymerase chain reaction results

PCR was carried out on pooled plaque samples from the 17 patients with AgP and the 15 controls with primers for *A. actinomycetemcomitans*. Using PCR, *A. actinomycetemcomitans* was detected in five of the plaque samples (29%) from patients with AgP and was totally absent in controls. In

addition to the primers for *A. actinomycetemcomitans*, the JP2 clone was tested in the AgP samples and it was found in only one of those five *A. actinomycetemcomitans*-positive patients (2%), a patient diagnosed with GAgP (Fig. 4).

Quantitative polymerase chain reaction results

The presence of *A. actinomycetemcomitans* was further investigated in the saliva of the 19 AgP patients using qPCR with probes for *A. actinomycetemcomitans*. In this analysis, *A. actinomycetemcomitans* was detected in six saliva samples (32%) of the 19 patients tested.

Discussion

In this study, the main aim was to define and compare the microbial composition of subgingival plaque of Sudanese patients with AgP and healthy controls using current microbiological techniques.

By use of HOMIM, *E. yurii* was found significantly more prevalent in patients with AgP compared to healthy controls. In a study carried out in China, *Eubacterium* species were found in high amounts in patients with AgP (31). Moreover, in a group of patients with refractory periodontitis, *E. nodatum* was among the putative periodontal pathogens that were found in higher frequency in patients with refractory periodontitis in comparison with periodontal healthy individuals (18). Interestingly, *E. nodatum* was totally absent from the controls.

The microbiological differences between GAgP and LAgP were not great in our study, only few species were detected significantly different using HOMIM. This finding was supported by Pocolos *et al.* (32). They investigated 15 bacterial species, including the classical periodontal pathogens. In their study, patients with LAgP and GAgP tended to exhibit no differences in harboring any of the tested species.

In the present study, microorganisms included *Haemophilus parainflu-*

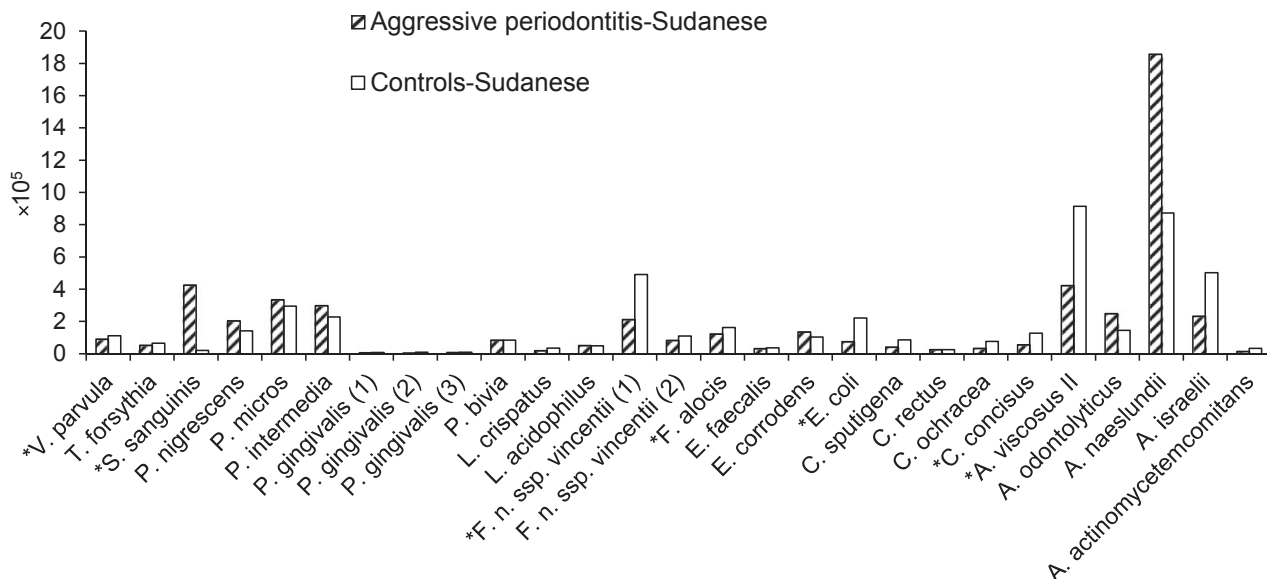


Fig. 3. Distribution of subgingival bacterial taxa measured by checkerboard. Profiles of bacterial DNA probe counts (mean) ($\times 10^5$ cells) of Sudanese patients with AgP and controls. The bacterial taxa are listed on the x-axis in alphabetical order. *Significantly different ($p < 0.05$; Mann-Whitney test). Statistical significance was calculated only for bacteria present above the detection limit ($> 100,000$ cells). *P. gingivalis* 1, 2 and 3 represent strains 33, 277, 381 and A7436, respectively, and *F. nucleatum* ssp. *vincentii* 1 and 2 represent strains 49, 256 and 364, respectively.

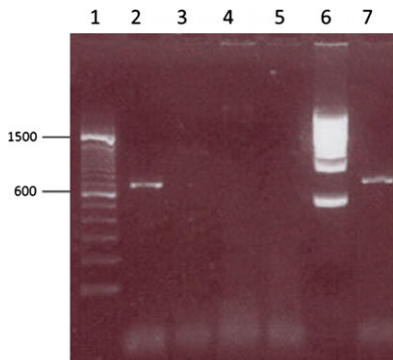


Fig. 4. Detection of the JP2 clone of *A. actinomycetemcomitans* in subgingival plaque samples and positive controls by polymerase chain reaction using the Ltx3 and the Ltx4 primer pairs. Lane 1: DNA marker (100 bp ladder; Invitrogen, Carlsbad, CA, USA); lane 2: JP2-positive control; lanes 3–4: JP2-negative plaque samples; lane 5: JP2- and *A. actinomycetemcomitans*-negative plaque sample; lane 6: 1 kb DNA marker starting at 0.5 kb (New England Biolabs, Hitchin, Hertfordshire, UK); lane 7: JP2-positive sample.

enzae, *Cardiobacterium hominis*, *Lautropia mirabilis*, *Actinomyces gengenceriae*, *Capnocytophaga granulosa* and *Campylobacter gracilis* were significantly more common in the con-

trol group compared with subjects with AgP. These findings are in line with previous reports (9,18). Notably, *Tannerella* ssp. were found in healthy conditions, which was also reported by others (33). Some bacterial species were present in plaque samples of more than 50% of both AgP and control groups, which may indicate that they belong to the normal microflora in subgingival plaque of the study population (18).

Our findings showed that the classical periodontal pathogens were not frequently detected in the subjects with AgP; this is in contrast to some previous reports (34), but in line with others (8,31). Here, plaque from all individuals was examined by both the HOMIM and the checkerboard techniques, as well as PCR for a subset of individuals with AgP. *A. actinomycetemcomitans* was detected in two patients with AgP while *P. gingivalis* was not detected in subgingival plaque of patients with AgP using HOMIM. This outcome is similar to a finding obtained by a Chinese study previously referred to, where *A. actinomycetemcomitans* was not detected in patients with “juvenile periodonti-

tis” (31). However, it is in contrast to what was reported by Mombelli *et al.* (35), where many serotypes of *A. actinomycetemcomitans* were regularly detected in the clinical samples. In a study by Favari *et al.* (8), the proportions of *A. actinomycetemcomitans* were elevated in shallow and intermediate-sized pockets of subjects with LAgP, but not in deep sites, suggesting an association between *A. actinomycetemcomitans* and the onset of LAgP. A negative relationship between *A. actinomycetemcomitans* and deep pockets has been demonstrated by Hamlet *et al.* (36).

Low levels of *P. gingivalis* have been previously reported in periodontal disease (37). Recently, *P. gingivalis* and *A. actinomycetemcomitans* were only sporadically identified in saliva of a cohort of Danish patients with chronic periodontitis (38). Whole saliva or a periodontal pocket sample alone does not seem sufficient to determine the presence of *A. actinomycetemcomitans* and the detection of *A. actinomycetemcomitans* may require analysis of both whole saliva and periodontal pocket samples (39). As *A. actinomycetemcomitans* has

been reported as a key pathogen in AgP, we chose to analyze saliva to confirm the presence or absence of *A. actinomycetemcomitans* in patients with AgP.

Nibali and co-workers (40) in their study on differences in microbiologic profiles of patients with chronic periodontitis or AgP concluded that detection of known periodontopathogenic bacteria is not sufficient to discriminate between different forms of periodontitis. This concept was supported by Riep *et al.* (12). The absence of *A. actinomycetemcomitans* from all controls by HOMIM, checkerboard and conventional PCR in the present study confirms what was reported before in a study by Dahlén *et al.* (41), indicating the importance of disease activity and longitudinal examination. The conventional PCR and qPCR picked up more *A. actinomycetemcomitans*-positive individuals than HOMIM and checkerboard.

The laboratory method used for bacterial analysis is an important factor that may have an impact on the bacterial species detected. Different microbiological techniques were utilized in the present study in an attempt to overcome limitations in the use of a single method. The new techniques in molecular biology are superior to cultivation methods in many aspects, being more sensitive and making detection of dead in addition to live bacteria possible. Moreover, high throughput technology, such as HOMIM, provided simultaneous processing of a large number of samples (25,42). However, the non-cultivation methods have also been reported as a potential cause of bias (43). A major shortcoming of the well-known checkerboard DNA-DNA hybridization technology is the probability of cross-reaction between closely related species (42). Furthermore, the specificity of PCR primers and the high numbers of cycles used may aggravate the results of different detection techniques (44–46). The use of different analytical methods with different sensitivity and detection levels may explain the discrepancies of *A. actino-*

mycetemcomitans results in the present study (47).

A critical point in collecting subgingival plaque is to avoid contamination with supragingival plaque. A great effort was performed in cleaning the tooth before the curette was inserted into the pocket.

From the clinical examination and X-rays of patients with AgP it was probable that the disease had rapidly developed from the LAgP to the GAgP type of AgP because it had become so serious at an early age, and judged on the distribution of tooth groups that were affected, which was consistent with tissue destruction having started as localized periodontitis.

The notion of the later extension of LAgP to GAgP rather than considering the two forms of AgP as two different entities was raised in a recent review by Könönen and Müller (11). The plaque index was high among the participants and there was a tendency for tooth loss at a young age, suggesting a more rapid progression of the disease without treatment. Diet and oral hygiene may influence the differences in bacterial composition observed but, undoubtedly, an adequate immune response is essential for the outcome, which is well documented (48).

A limitation of this investigation is the relatively small number of patients. It was difficult to find a large group of cases with LAgP among the Sudanese seeking treatment at UST, mainly for two reasons. Antibiotics are often purchased without prescription in Sudan and frequent use of antibiotics is common. Patients with AgP fulfilling the inclusion criteria of not having used antibiotics within the last 3 months were not easy to find.

Another point that could be commented on is a possible difference between the study group and the control group regarding socio-economic status. Whereas the patients were recruited from subjects seeking treatment at UST, the healthy controls were employees or students at UST. Although both patients and controls come from Khartoum, it does not necessarily imply that they belong to a similar socio-economic group.

Conclusion

As determined by HOMIM, *E. yurii* was the most prevalent species in patients with AgP. In addition, *E. nodatum* was prevalent in patients with AgP and totally absent from controls. Though efforts have been made to profile the subgingival plaque in the studied population using different methods, the classical pathogens were not frequently detected. New technology such as next generation sequencing in combination with immunological and longitudinal studies of a statistically significant number of patients with AgP may be a future approach to expand our knowledge of periodontal disease pathogenesis.

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Conflict of interest

The authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Clinical data of Sudanese aggressive periodontitis patients.

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