

A microbiological study of Papillon-Lefèvre syndrome in two patients

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Abstract

Aim—To analyse the microflora of subgingival plaque from patients with Papillon-Lefèvre syndrome (PLS), which is a very rare disease characterised by palmar-plantar hyperkeratosis with precocious periodontal destruction.

Methods—Bacterial isolates were identified using a combination of commercial identification kits, traditional laboratory tests, and gas liquid chromatography. Some isolates were also subjected to partial 16S rDNA sequencing. Plaque samples were also assayed for the presence of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* in a quantitative enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies.

Results—The culture results showed that most isolates were capnophilic and facultatively anaerobic species—mainly *Capnocytophaga* spp and *Streptococcus* spp. The latter included *S constellatus*, *S oralis*, and *S sanguis*. Other facultative bacteria belonged to the genera *gemella*, *kingella*, *leuconostoc*, and *stomatococcus*. The aerobic bacteria isolated were species of *neisseria* and *bacillus*. Anaerobic species included *Prevotella intermedia*, *P melaninogenica*, and *P nigrescens*, as well as *Peptostreptococcus* spp. ELISA detected *P gingivalis* in one patient in all sites sampled, whereas *A actinomycetemcomitans* was detected in only one site from the other patient. *Prevotella intermedia* was present in low numbers.

Conclusions—Patients with PLS have a very complex subgingival flora including recognised periodontal pathogens. However, no particular periodontopathogen is invariably associated with PLS.

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Keywords: Papillon-Lefèvre syndrome; periodontopathogens

Papillon-Lefèvre syndrome (PLS) was first described in 1924 by Papillon and Lefevre.¹ It is a rare autosomal recessive disease,² with an incidence of 1-4 cases/million people,³ and with consanguinity between parents seen in one third of cases.⁴ The disease is characterised by palmar-plantar hyperkeratosis, a thickening of the skin on the palms of the hands and the soles of the feet.⁵ In addition, precocious periodontal destruction of both the deciduous and permanent teeth is seen.⁶ The gingiva become red and inflamed and may be ulcerated⁵ and a form of rapid, severe periodontitis occurs.⁴ Alveolar bone is resorbed and deep periodontal

pockets form leading to increased tooth mobility.⁶ Usually, both deciduous teeth⁷ and permanent teeth⁶ are lost prematurely.

PLS is a systemic disease with immune system defects,⁸ which might result in the proliferation of certain periodontopathogens. Microbiological studies of the oral microflora of patients with PLS have shown that the predominant organisms in the periodontal sites are Gram negative anaerobic rods,⁹ including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella loescheii*, *Bacteroides gracilis*,¹⁰ and *Fusobacterium nucleatum*.^{10 11} *Eikenella corrodens*, capnocytophaga,¹¹ veillonella, anaerobic streptococci,¹² and spirochaetes have also been reported.^{13 14} Although the presence of cultivatable *Actinobacillus actinomycetemcomitans* and raised serum antibody titres to *A actinomycetemcomitans* have been found,^{10 12 13 15 16} this is not always the case.^{11 17}

The aims of our study were to identify the subgingival plaque microflora of two patients with PLS and to assess the amounts of the periodontopathic bacteria, *A actinomycetemcomitans*, *P gingivalis*, and *P intermedia* using a quantitative enzyme linked immunosorbent assay (ELISA).

Methods

PLAQUE SAMPLES

Two patients with PLS attended the University of Manchester Dental Hospital. Patient 1 had only six teeth remaining and subgingival plaque samples were taken from each tooth. A further five subgingival plaque samples were also collected from the molars and canine teeth of patient 2. All samples were collected with a curette and (1) placed into reduced transport fluid for rapid transportation to Manchester Royal Infirmary for initial bacterial cultivation; (2) resuspended in phosphate buffered saline (PBS) containing 0.01% thiomersal, frozen, and sent to the University of Queensland, St Lucia, Australia for analysis with specific monoclonal antibodies by means of ELISA.

ENZYME LINKED IMMUNOSORBENT ASSAY

Plaque samples were thawed, six to eight 1 mm glass beads added to each vial, and the bacteria dispersed by vortexing and then sonicated for five seconds. Samples were diluted in an equal volume of 0.1 M carbonate buffer pH 9.6. A 100 µl volume of each sample was pipetted into triplicate wells of a 96 well Maxisorp microtitre plate (Nunc, Roskilde, Denmark). A known concentration of bacterial cells of either *A actinomycetemcomitans* Y4, *P gingivalis* FDC-381, or *P intermedia* ATCC 25611 (ranging from 9 to 150 × 10⁴ cells/ml) in carbonate buffer were assayed on each plate with the

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plaque samples. Microtitre plates were incubated overnight at 4°C and washed three times with PBS/Tween 20 (0.05%; PBS-T). Non-specific binding was blocked with PBS-T containing 1% fetal calf serum (Commonwealth Serum Laboratory, Melbourne, Australia) and the plates were incubated for one hour at room temperature (RT). After washing (×3) with PBS-T, diluted horseradish peroxidase labelled monoclonal antibodies specific to either *A actinomycetemcomitans*, *P gingivalis*, or *P intermedia* were added to all the coated wells and incubated for two hours at RT. After further washing (×3) with PBS-T, colour development was achieved by adding 150 µl of 2.5 mM σ-tolidine (Kodak Eastman, Rochester, New York, USA) in 100 mM phosphate citrate buffer (pH 3.5) containing 0.025 mM EDTA and activated by 3% H₂O₂. The colour development was stopped after 10 minutes by the addition of 50 µl of 1 M HCl. The plates were read in a Bio-Rad microplate reader model 3550 (Bio-Rad Laboratories, Hercules, California, USA) at 450 nm and 655 nm.

DETECTION OF SPIROCHAETES

Smears were prepared from the plaque samples, air dried, and stained with a modified Gram stain, where the counter stain was 20% carbol fuchsin, applied for five minutes. Slides were viewed under a Zeiss (Jena, Germany), Axioplan light microscope at ×1000 magnification.

CULTIVATION OF PLAQUE SAMPLES

Plaque samples were plated on to Columbia blood agar plates supplemented with 5% defibrinated horse blood (Oxoid, Basingstoke, UK) and fastidious anaerobe agar (FAA; Lab M, Bury, UK) plates supplemented with 5% defibrinated horse blood (Oxoid). Columbia agar plates were incubated in an aerobic atmosphere for three days at 37°C. The latter were incubated in a Compact M anaerobic cabinet (Don Whitley Scientific, Shipley, UK) in an atmosphere of 10% hydrogen, 10% carbon dioxide, and 80% nitrogen for three to five days at 37°C. Selected isolated colonies were subcultured.

IDENTIFICATION OF ISOLATES

Plaque contains many species; thus, we selected the predominant organisms for further study. All isolates were Gram stained and tested for catalase, oxidase activity, aerobic growth, and anaerobic growth. In some cases, other tests were used such as Hugh and Leifson's oxidation fermentation test, nitrate reduction, extracellular polysaccharide production on TYC agar, and spore production. Isolates were identified using a range of commercial identification kits, namely: API 20 NE; Rapid ID 32 Strep; API Coryne; Rapid ID 32A (BioMérieux, Basingstoke, Hampshire, UK), and the Rapid ANA II system (Prolab, Liverpool, UK). Microcodes generated using API 20 NE, ID 32 Strep, API Coryne, or ID 32A were analysed by BioMérieux. Microcodes generated using Rapid ANA II were analysed by Prolab. Anaerobe identification also made

use of schemes in the *Wadsworth anaerobic bacteriology manual* and *Virginia Polytechnic Institute anaerobe laboratory manual*.^{18 19} Bacterial culture supernatants from Gram positive bacteria were analysed for the presence of non-volatile acids.¹⁸ For gas chromatographic analysis of end products, established methods were followed,¹⁸ except that supernatant fluid from 48 hour gas liquid chromatography (GLC) broth (Lab M) cultures was used. Samples (2 µl) were analysed on a column of 6% Carbowax 20M TPA on Chromosorb W AWDMCS (Phase Separations, Queensferry, UK), whether ether extracts of volatile end products or chloroform extracts of methylated non-volatile acids. The injector and detector temperatures of the chromatograph (AI, Cambridge, UK) were both 150°C. The initial temperature of the column was 65°C, which was held for 30 seconds before being increased linearly to 115°C over two minutes, and held for six minutes and 20 seconds. The equilibration time between samples was three minutes.

When satisfactory identification could not be achieved, the partial sequence of the 16S rRNA gene was determined and compared with library data for known organisms.

DNA EXTRACTION: GRAM NEGATIVE BACTERIA

Bacterial isolates were grown to purity on FAA. Isolated colonies were subcultured into fastidious anaerobe broth and DNA was extracted from a 72 hour culture. Briefly, 5 ml of each bacterial culture was centrifuged at 3000 ×g for 10 minutes and the cells resuspended in sucrose/Tris/EDTA (STE). Lysozyme (Sigma, Poole, Dorset, UK) was added to give a final concentration of 2 mg/ml and incubated at 37°C for 30 minutes. Proteinase K (0.3 mg/ml) and sodium dodecyl sulphate (SDS; 1% final concentration) were added and incubated at 55°C for one hour, to induce cell lysis. An equal volume of phenol/chloroform/isoamyl alcohol (25/24/1, vol/vol/vol) was added to each suspension and vortexed. After centrifugation at 16 000 ×g for 10 minutes, 24 µl 5 M NaCl and 600 µl propan-2-ol were added. Gentle mixing and incubation at 0°C for 10 minutes were followed by centrifugation at 16 000 ×g for 10 minutes. The pellet was resuspended in water and one volume of ammonium acetate was added (final concentration of 2.5 M). The suspension was then cooled at 0°C for one hour then centrifuged at 16 000 ×g for 20 minutes. The supernatant was removed and the DNA was reprecipitated by the addition of 600 µl of chilled ethanol (-85°C). The DNA was pelleted by centrifugation at 16 000 ×g for 10 minutes and then washed in 500 µl 70% chilled ethanol (-85°C). The ethanol was removed and the DNA was resuspended in 100 µl Tris/EDTA (TE) and stored at 4°C.

DNA EXTRACTION: GRAM POSITIVE BACTERIA

DNA was extracted from all Gram positive clinical isolates using the Puregene DNA isolation kit (Flowgen Instruments, Staffordshire, UK), according to the manufacturer's instructions.

MEASUREMENT OF DNA

Samples (5 µl) of DNA were stained with ethidium bromide (1 µg ml⁻¹ in 1 × trisphosphate EDTA (TPE)) after electrophoresis in an 0.8% agarose gel and then visualised by ultraviolet light (λ = 254 nm) and photographed on Polaroid 667 film. The amount of DNA was determined by visual comparison with known amounts of uncut λ DNA (Sigma; 40–400 ng µl⁻¹).

PARTIAL 16S rRNA GENE SEQUENCE ANALYSIS

Bacterial DNA was amplified in a Crocodile III thermal cycler (Appligene, County Durham, UK). The reaction mix (total volume, 50 µl) consisted of 2 µl of DNA, Taq polymerase (1.5 units), 0.2 mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), MgCl₂ (1.5 mM), 1 × buffer IV, and 0.3 µM of both primers: RE-TPU1 (5'-AGA GTTTGATCMTGGCTCAG) and RE-RTU3 (5'-GWATTACCGCGCKGCG)²⁰ (Oligonucleotide Synthesising Service, School of Biological Sciences, University of Manchester, UK). The reaction mix was covered with an equal volume (20 µl) of mineral oil to prevent evaporation. Negative controls contained all components of the reaction mix except the bacterial template DNA. The thermal cycling profile was as follows: one cycle of four minutes at 94°C, one minute at 55°C, one minute at 72°C; 29 cycles of one minute at 94°C, one minute at 57°C, one minute at 72°C; one cycle of one minute at 94°C, one minute at 57°C, and five minutes at 72°C. Polymerase chain reaction (PCR) products (3 µl) were resolved in 1.0% agarose gels, stained with ethidium bromide, and visualised under ultraviolet light (λ = 254 nm). A λ PstI digest was used as a size marker (270 ng/µl). Gels were photographed on Polaroid 665 and 667 films and the presence of the correct size fragment confirmed. QIAquick PCR purification kit (Qiagen, Crawley, Sussex, UK) was used to clean the PCR product. The quantity of the product was estimated by visual comparison in a 0.8% agarose gel with a known amount of λ DNA. Concentrations of PCR products (30–90 ng) were amplified further in a Perkin Elmer (Warrington, UK) model 2400 thermal cycler. Two reaction mixes were set up for each PCR product, one with each primer. The reaction mix had a total volume of 20 µl, which consisted of 8 µl ABI PRISM™ terminator cycle sequence ready reaction mix (Perkin Elmer) or 4 µl ABI PRISM™ BigDye™ terminator cycle sequence ready reaction mix (Perkin Elmer) and either RE-RTU3 or RE-TPU1 (0.15 µM). The PCR product was added and the volume made up with water. The thermal cycling profile was as follows: 25 cycles of 10 seconds at 96°C, five seconds at 50°C, and four minutes at 60°C. The cycle sequencing product was purified by ethanol precipitation. The automated sequence analysis was performed by Oswals DNA Service, University of Southampton. Sequences were manipulated using the Genetics Computer Group (GCG) package version 8.0 (Wisconsin, USA).

Sequences resulting from amplification with primer RE-RTU3 were reverse transcribed and changed to the complement. Results from each primer were then aligned using GAP function and checked twice against each other to produce a consensus sequence. The consensus sequence was submitted for a FASTA search to determine the most likely relation.

Results

A total of 108 pure cultures of predominant organisms was obtained for identification and comprised the genera and species listed in tables 1–3. The bacterial species isolated (including those determined by ELISA) were separated into aerobic, anaerobic, facultative, and capnophilic species and 17 genera were found to be present. The facultative microorganisms identified consisted mainly of species of streptococcus, including *Streptococcus anginosus*, *S bovis*, *S constellatus*, *S mitis*, and *S sanguis*. Species of capnocytophaga and gemella were also identified. The obligately anaerobic flora consisted mostly of prevotella species, namely: *Prevotella intermedia*, *P nigrescens*, *P melaninogenica*, and “unidentified” prevotella (formerly “PINLO”). In addition, *Peptostreptococcus anaerobius*, *Pstr micros* and species of mobiluncus were identified. Table 1 gives a comprehensive list of species cultured for each patient. *Gemella* spp, *Neisseria* spp,

Table 1 Complete list of bacterial species isolated from two patients with Papillon-Lefèvre syndrome, displaying association with patient

	Patient 1	Patient 2
Obligate aerobes		
<i>Neisseria</i> sp	2	0
<i>N cinerea</i>	1	1
<i>N elongata</i>	0	1
<i>N flavescens</i>	0	2
<i>Neisseria</i> sp possibly <i>N subflava</i>	1	0
Obligate anaerobes		
<i>Eubacterium</i> sp	0	1
<i>Porphyromonas gingivalis</i>	1	0
<i>Prevotella</i> sp possibly <i>P oralis</i>	1	0
<i>P intermedia</i>	1	1
<i>P melaninogenica</i>	0	2
<i>P nigrescens</i>	0	2
<i>Peptostreptococcus</i> sp	1	0
<i>Pstr anaerobius</i>	0	1
<i>Pstr micros</i>	1	0
Facultative		
<i>Actinobacillus actinomycetemcomitans</i>	0	1
<i>Actinomyces</i> sp	1	0
<i>A israelii</i>	1	0
<i>Bacillus cereus</i>	1	0
<i>Gemella haemolysans</i>	1	0
<i>G morbillorum</i>	1	1
<i>Kingella denitrificans</i>	1	0
<i>Leuconostoc</i> sp	0	1
<i>L mesenteroides</i>	0	1
<i>Mobiluncus</i> sp	1	0
<i>Streptococcus</i> sp (to genus only)	1	1
<i>S anginosus</i>	0	2
<i>S bovis</i> II	1	0
<i>S constellatus</i>	3	0
<i>S gordonii</i>	0	1
<i>S intermedius</i>	0	1
<i>S milleri</i> group	1	1
<i>S mitis</i>	2	1
<i>S oralis</i>	1	0
<i>S salivarius</i> subspecies <i>P salivarius</i>	1	1
<i>S sanguis</i>	3	1
<i>Stomatococcus mucilaginosus</i>	1	0
<i>Suttonella indologenes</i>	1	0
Capnophilic		
<i>Capnocytophaga</i> sp	1	0

The numbers refer to the total number of sites with the isolate.

Table 2 Predominant species isolated from patient 1 according to site sampled

Site A (upper right deciduous canine)	
<i>Actinomyces</i> sp	<i>Peptostreptococcus</i> sp
<i>Bacillus cereus</i>	<i>Prevotella</i> sp
<i>Capnocytophaga</i> sp	<i>Streptococcus constellatus</i>
<i>Kingella denitrificans</i>	<i>S mitis</i>
<i>Neisseria cinerea</i>	<i>S sanguis</i>
<i>Neisseria</i> sp	<i>Streptococcus</i> sp
Site B (lower left deciduous canine)	
<i>Actinomyces israelii</i>	<i>S milleri</i> group
<i>Mobiluncus</i> sp	<i>S oralis</i>
<i>Neisseria</i> sp	<i>S sanguis</i>
<i>Streptococcus constellatus</i>	
Site C (upper left deciduous 1st molar)	
<i>Streptococcus bovis</i> II	<i>Stomatococcus mucilaginosus</i>
<i>S constellatus</i>	<i>Neisseria subflava</i>
<i>S sanguis</i>	
Site D (lower left deciduous 1st molar)	
<i>Gemella haemolysans</i>	<i>Peptostreptococcus micros</i>
<i>G morbillorum</i>	<i>Streptococcus sanguis</i>
<i>Prevotella intermedia</i>	
Site E (lower right deciduous canine)	
<i>Suttonella indologenes</i>	<i>Capnocytophaga</i> sp
<i>Actinomyces meyeri</i>	
Site F (lower right deciduous 1st molar)	
<i>Streptococcus mitis</i>	<i>S salivarius</i> subspecies <i>salivarius</i>

Table 3 Predominant species isolated from patient 2 separated according to site of sample

Site A (upper right permanent 1st molar)	
<i>Eubacterium</i> sp	<i>P nigrescens</i>
<i>Leuconostoc</i> sp	<i>Peptostreptococcus anaerobius</i>
<i>Neisseria cinerea</i>	<i>S anginosus</i>
<i>N elongata</i>	<i>S intermedius</i>
<i>N flavescens</i>	<i>S salivarius</i> subspecies <i>salivarius</i>
<i>Prevotella melaninogenica</i>	<i>Actinomyces meyeri</i>
<i>Streptococcus sanguis</i>	
Site B (upper right permanent canine)	
<i>P melaninogenica</i>	<i>S milleri</i> group
<i>P nigrescens</i>	<i>S mitis</i>
<i>Streptococcus gordonii</i>	<i>Streptococcus</i> sp
Site C (lower right permanent canine)	
<i>Leuconostoc mesenteroides</i>	
Site D (lower left canine)	
<i>Peptostreptococcus micros</i>	
Site E (lower left first molar)	
<i>Actinomyces meyeri</i>	

Peptostreptococcus spp, *P intermedia*, and *Streptococcus* spp were isolated from both patients. *Actinomyces* spp, *Bacillus* spp, *Capnocytophaga* spp, *Kingella* spp, *Mobiluncus* spp, *P gingivalis*, and *Stomatococcus* spp were isolated only from patient 1, whereas *A actinomycetemcomitans*, *Eubacterium* spp, *Leuconostoc* spp, *P nigrescens*, and *P melaninogenica* were isolated only from patient 2. Tables 2 and 3 show the correlation between the species identified and the site sampled in the patients.

Culture provided qualitative data only. However, monoclonal antibodies provided quantitative data and detected *P gingivalis* in low numbers in all six sites sampled in patient 1; *A actinomycetemcomitans* was detected in one site in low numbers in patient 2. *Prevotella intermedia* was detected in low numbers from both patients—in one of six sites from patient 1 and three of six sites from patient 2.

The occurrence of spirochaetes in the plaque was confirmed by microscopy.

Discussion

A range of bacteria was found in the subgingival plaque of both these patients with PLS. In addition, the periodontopathic bacteria *P gingivalis*, *P intermedia*, and *A actinomycetemcomitans* were detected, albeit in low numbers.

“Control” sites were not studied because in the case of severe PLS there are no healthy control sites.

Aerobes identified were species of bacillus and neisseria. The Gram positive, spore former *Bacillus cereus* is not considered part of the normal oral flora, although it has been reported in plaque samples.²⁰ With no reports of an association with periodontal disease and because of its universal occurrence and the formation of spores, the possibility that this is a contaminant cannot be ruled out. *Neisseria* spp—*N sicca/subflava*¹⁰ and *N pharyngis*²¹—have been reported previously in other patients with PLS. In our study, the identification of isolates as either species of neisseria or prevotella was second only to identification as streptococcus.

Five genera of obligately anaerobic bacteria were identified—eubacterium, peptostreptococcus, porphyromonas, prevotella, and treponema. Species of eubacterium have been isolated from subgingival plaque²² and are associated with oral health, gingivitis,²³ and periodontal disease in humans,^{24 25} although little evidence is available for an association with PLS. One study¹³ has failed to isolate eubacterium from plaque samples despite the use of selective media, although low numbers were cultured from mouth rinse samples, suggesting that eubacterium was present in the oral cavity of patients with PLS. Unidentified *Peptostreptococcus* spp have been isolated from the subgingival plaque of patients with periodontal disease²⁵ and *Pstr micros* has been associated with gingivitis.²³ *Peptostreptococcus micros*¹⁰ and unspiciated peptococcaceae¹³ have also been identified previously from patients with PLS.

Porphyromonas gingivalis is an accepted periodontal pathogen,^{26 27} which was detected by monoclonal antibodies in one of the patients. The use of monoclonal antibodies to *P gingivalis* in a previous study¹⁰ detected this species in one of the two patients. It has also been isolated previously from patients with PLS using culture methods.^{10 13}

Prevotella intermedia, *P melaninogenica*, *P nigrescens*, and *P oralis* were identified in our present study. *Prevotella oralis*, *P oris*, *P loeschii*,^{10 13} *P intermedia*,^{10 13} and other unspiciated black pigmented anaerobes^{9 13 17} have been associated previously with PLS. In earlier studies, *P nigrescens* was probably misidentified as *P intermedia*. *Prevotella melaninogenica* has not been isolated previously from PLS samples but it occurs in subgingival plaque²⁸ and has been associated with gingivitis²⁶ and periodontal disease²⁹. *Prevotella intermedia* was detected both by culture and ELISA in our study, methods by which it has previously been detected in PLS.¹⁰

Unspiciated spirochaetes were seen within gingival smears during our study and have been reported previously in patients with PLS.^{13 14}

Actinobacillus actinomycetemcomitans is an accepted periodontopathogen,^{23 27 30} which has been associated particularly with prepubertal periodontitis³¹ and localised juvenile periodontitis.³² It has been associated with PLS on many occasions^{10 12 13 15 16} and is considered an important pathogen for the periodontal component

of PLS.³² We detected this organism using monoclonal antibodies, but only in one sample from patient 2. Therefore, the presence of *A actinomycetemcomitans* might not be necessary for PLS to progress. This argument could also apply to *P gingivalis*, which was only detected in patient 1.

We identified three actinomyces species that have been associated with PLS previously.¹⁰ *Actinomyces* spp form part of the resident oral microflora of humans³³ and are therefore isolated from the healthy oral cavity and associated with gingivitis.²³

Gemella morbillorum has been isolated previously from patients with PLS and is also associated with a healthy mouth,²³ whereas no association with periodontal disease or PLS has been reported for *G haemolysans*. The likelihood of gemella causing similar infections to those caused by viridans streptococci has been noted,³⁴ so that the isolation of this microorganism from patients with PLS is not surprising.

Kingella species include known upper respiratory tract commensals³⁵; however, there are no reports of the isolation of members of this genus from patients with PLS and only limited associations with periodontal disease. The primary habitat of *K oralis* is human dental plaque and it been isolated from supragingival and subgingival plaque taken both from healthy oral cavities and patients with periodontitis.³⁶

The genus leuconostoc contains species similar to streptococci and often identified initially as *S sanguis* type II as well as *S salivarius* and *S pneumoniae*, when using automated identification systems.³⁷ Leuconostoc has not been previously identified in PLS samples. However, this genus has been reported to be a cause of bacteraemia in patients already critically ill with acute leukaemia, renal failure, and human immunodeficiency virus infection.³⁷ It has been suggested that the incorrect identification of leuconostoc as viridans streptococci means that the pathogenic associations of this genus have been underestimated.³⁷ During our study, both viridans streptococci (*S constellatus*, *S mitis*, *S oralis*, and *S sanguis*) and *S salivarius* were identified using Rapid ID 32 Strep; however, additional testing would be required to check that none of these isolates was a species of leuconostoc.³⁷ Of the ten streptococcal species identified here, *S bovis*, *S mitis*, “*S milleri* group”, and *S sanguis*¹⁰ have been associated with PLS previously; other studies^{12 13} have detected streptococci but not identified them further. Previously, *S oralis* has been associated with gingivitis.²³

One isolate was identified as *Stomatococcus mucilaginosus*, an organism found within the oral microflora,³⁸ although it has not been previously associated with the periodontal flora in PLS. The variable catalase reaction can delay identification and cause confusion with staphylococcal and streptococcal species, which has led to the underestimation of its prevalence.

Mobiluncus has been isolated from extra-vaginal sources³⁹ other than the mouth. This organism may have been misidentified as

actinomyces because of a positive Gram reaction and a similar GLC profile.

Members of the genus capnocytophaga have been frequently associated with gingivitis,²³ periodontal disease,²⁷ and juvenile periodontitis.²⁹ Species of this organism have been reported in patients with PLS.^{10 11} *Capnocytophaga ochracea*¹⁰ has been isolated, but most studies have identified isolates only to the genus level. Paradoxically, capnocytophaga has also been found to be associated negatively with periodontal disease.⁴⁰

Species not isolated here but reported in other studies^{10 11 13} include *Fusobacterium* spp, *Eikenella corrodens*, veillonella, and *Bacillus gracilis*. The recognised periodontal pathogens *P gingivalis* and *A actinomycetemcomitans* were found in low numbers in our patients with PLS. It is possible that *A actinomycetemcomitans* acts together with human herpesviruses in the development of the syndrome,⁴¹ but our present study did not include virological examination. Furthermore, a “massive occurrence of *A actinomycetemcomitans*” has been noted in periodontal pockets in PLS, although the same species was present also in the mouths of siblings and a parent without PLS.⁴² Similarly, in a group of 12 Saudi-Arabian adolescents with PLS, there was no PLS specific profile of the subgingival infection because the bacterial composition resembled that characterising deep pockets in adult patients with periodontitis.⁴³ It is possible that recognised periodontal pathogens might be involved in PLS. However, the severity of periodontitis in patients with PLS cannot be explained simply by the presence of any of the bacteria found in this study, even the recognised periodontopathogens. These patients are known to have a higher risk of developing disease when compared with other individuals, suggesting that host factors determine the individual’s disease susceptibility. The inherited components of PLS induce both immune and epithelial defects.⁴¹ We conclude that PLS occurs in genetically susceptible individuals whose periodontal disease is associated with periodontal pathogens but the species of periodontopathogen is not of overriding importance.

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