

Anaerobic, non-sporulating, Gram-positive bacilli bacteraemia characterized by 16S rRNA gene sequencing

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Owing to the difficulties in identifying anaerobic, non-sporulating, Gram-positive bacilli in clinical microbiology laboratories, the epidemiology and clinical spectrum of disease of many of these bacteria have been poorly understood. The application of 16S rRNA gene sequencing in characterizing bacteraemia due to anaerobic, non-sporulating Gram-positive bacilli during a 4-year period is described. The first case of *Olsenella uli* bacteraemia, in a patient with acute cholangitis, is also reported. Among 165 blood culture isolates of anaerobic, Gram-positive bacilli, 75 were identified as *Propionibacterium acnes* by phenotypic tests and 21 as members of other anaerobic, non-sporulating Gram-positive bacilli by 16S rRNA gene sequencing. Of these 96 isolates, 16 (17%) were associated with cases of clinically significant bacteraemia, among which 10 (63%) were caused by *Eggerthella*, four (25%) by *Lactobacillus* and one (6%) by each of *Eubacterium tenue* and *O. uli*. Five of the 10 *Eggerthella* isolates were *Eggerthella lenta*, whereas the other five belonged to two novel *Eggerthella* species, with *Eggerthella hongkongensis* being almost as prevalent as *Eggerthella lenta*. Underlying disease in the gastrointestinal tract, isolation of *Eggerthella* and *Lactobacillus*, and monomicrobial bacteraemia were associated with clinically significant bacteraemia, whereas isolation of *P. acnes* and polymicrobial bacteraemia were associated with pseudobacteraemia. Most patients with clinically significant bacteraemia had underlying diseases, with diseases in the gastrointestinal tract being most common. The overall mortality rate was 31%. Immunocompromised patients with clinically significant bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli other than *P. acnes* should be treated with appropriate antibiotics. The unexpected frequency of isolation of *Eggerthella* from blood cultures and its association with clinically significant disease suggest that this genus is probably of high pathogenicity. Further studies to look for specific virulence factors are warranted.

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INTRODUCTION

Medically important anaerobic, non-sporulating, Gram-positive bacilli include members of the genera *Actinomyces*, *Bifidobacterium*, *Eggerthella*, *Eubacterium*, *Lactobacillus* and *Propionibacterium*. Identification of this group of bacteria in clinical microbiology laboratories is difficult. Analysis of cell wall peptidoglycans and metabolic end products by GC-MS, which is important for accurate identification to the genus level, requires special equipment and expertise, and is usually not available in clinical laboratories. Moreover, these organisms are often unrecognized in clinical specimens because of their complex transport and growth requirements, and frequent co-existence of less fastidious, aerobic bacteria in mixed infections. As a result, the epidemiology, clinical spectrum of disease and pathogenic potential of the various genera are not fully understood.

Comparison of the gene sequences of bacterial species has shown that the 16S rRNA gene is highly conserved within a species and among species of the same genus. Thus it can be used as the new standard for classification and identification of bacteria (Relman *et al.*, 1990, 1992). Recently, we have reported the application of this technique for identifying species of this group of bacteria, including a strain of *Lactobacillus salivarius* isolated from a patient with cholecystitis (Woo *et al.*, 2002a) and a strain of *Actinomyces odontolyticus* from a patient with pelvic inflammatory disease (Woo *et al.*, 2002b), and for the discovery of a novel *Actinomyces* species (Woo *et al.*, 2003) and two novel *Eggerthella* species (Lau *et al.*, 2004). In this article, using 16S rRNA gene sequencing as the standard of bacterial identification, we define the epidemiology, clinical disease spectrum and outcome of patients with bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli during a 4-year period.

METHODS

Patients and microbiological methods. The bacterial strains used in this study were isolates from blood cultures of patients hospitalized at the Queen Mary Hospital in Hong Kong during a 4-year period (January 1998 to December 2001). Clinical data were collected prospectively as described in our previous publication (Luk *et al.*, 1998). Clinical specimens were collected and handled according to standard protocols (Murray *et al.*, 1999). The BACTEC 9240 blood culture system (Becton Dickinson) was used. All anaerobic Gram-positive bacilli isolated from blood cultures were identified to the species level. Isolates were identified as *Clostridium perfringens* by the presence of double zone haemolysis on blood agar, the ability to produce lecithinase on egg yolk-glucose agar and by the Vitek system (ANI) (bioMérieux Vitek). Isolates of *Propionibacterium acnes* were identified by the ability to produce catalase and indole, and by the Vitek system (ANI). All isolates other than *C. perfringens* and *P. acnes* were subjected to 16S rRNA gene sequencing. All isolates finally identified as species belonging to genera of anaerobic, non-sporulating, Gram-positive bacilli were included in this study. Each isolate was categorized as clinically significant or a contaminant (pseudobacteraemia) by clinical and laboratory criteria as described in a previous study (Weinstein *et al.*, 1997). The criteria included the patients' clinical presentation, physical examination findings, body temperature at the time of the blood culture, leukocyte and differential cell counts, imaging or operative findings, histopathological results, number of positive blood cultures out of the total number performed, and response to treatment. The same isolate recovered from the same patient was counted only once.

Extraction of bacterial DNA for 16S rRNA gene sequencing. Bacterial DNA extraction was modified from our previously published protocol (Lau *et al.*, 2003; Woo *et al.*, 2002c). Briefly, 80 µl NaOH (0.05 M) was added to 20 µl of a loopful of bacterial cells suspended in 100 µl distilled water and the mixture was incubated at 60 °C for 45 min, followed by addition of 6 µl Tris/HCl (pH 7.0), achieving a final pH of 8.0. The resultant mixture was diluted ×100 and 5 µl of the diluted extract was used for PCR.

PCR, gel electrophoresis and 16S rRNA gene sequencing. PCR amplification and DNA sequencing of the 16S rRNA genes were performed according to our previous publications (Woo *et al.*, 2002a, b; Yuen *et al.*, 2001). Briefly, DNase-I-treated distilled water and PCR master mix [which contained deoxynucleoside triphosphates (dNTPs), PCR buffer and *Taq* polymerase] were used in all PCR reactions by adding 1 U DNase I (Pharmacia) to 40 µl distilled water or PCR master mix, and incubating the mixture at 25 °C for 15 min and subsequently at 95 °C for 10 min to inactivate the DNase I. The bacterial DNA extracts and control were amplified with 0.5 µM primers [LPW81 (5'-TGGCGAACGGGTGAGTAA-3') and LPW58 (5'-AGGCCCGGGAA CGTATTACAC-3'), corresponding to nucleotide positions 91–1350 of the 16S rRNA gene of *Eggerthella lenta*, GenBank accession no. AB011817] (Gibco-BRL). The PCR mixture (50 µl) contained bacterial DNA, PCR buffer (10 mM Tris/HCl, pH 8.3; 50 mM KCl; 2 mM MgCl₂; and 0.01 % gelatin), 200 µM of each dNTP and 1.0 U *Taq* polymerase (Boehringer Mannheim). The mixtures were amplified in 40 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 10 min, in an automated 0.5 ml GeneAmp PCR system 9700 (Applied Biosystems). DNase-I-treated distilled water was used as the negative control. Ten microlitres of each amplified product was electrophoresed in a 1.0 % (w/v) agarose gel with a molecular size marker (lambda DNA *Ava*II digest; Boehringer Mannheim) in parallel. Electrophoresis in Tris/borate/EDTA buffer was performed at 100 V for 1.5 h. The gel was stained with ethidium bromide (0.5 µg ml⁻¹) for 15 min, rinsed and photographed under UV light illumination.

The PCR products were gel-purified using the QIAquick PCR purification kit (QIAGEN). Both strands of the PCR products were sequenced

twice with an ABI 377 automated sequencer according to the manufacturer's instructions (Perkin-Elmer), using the PCR primers LPW58 and LPW81. The 16S rRNA gene sequences of novel *Eggerthella* species were further determined by PCR and sequencing as described in our previous publication (Lau *et al.*, 2004). The sequences of the PCR products were compared with known 16S rRNA gene sequences in GenBank (<http://www.ncbi.nlm.nih.gov>) by multiple sequence alignment using the CLUSTAL W program (Thompson *et al.*, 1994).

Statistical analysis. A comparison of characteristics was made between patients with clinically significant bacteraemia and those with pseudobacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli. The chi-square test was used for categorical variables and the Student's *t*-test for age. *P* < 0.05 was regarded as statistically significant.

RESULTS

Identification of anaerobic, non-sporulating, Gram-positive bacilli

A total of 165 anaerobic Gram-positive bacilli were isolated from the blood cultures during the 4-year study period. Of the 165 isolates, 51 were identified as *C. perfringens* and 75 as *P. acnes* by conventional phenotypic tests. The remaining 39 isolates were subjected to 16S rRNA gene sequencing. PCR of the 16S rRNA genes of these isolates showed bands at about 1200 bp. Sequencing of the 16S rRNA genes revealed that 21 had 16S rRNA genes with 92.7–100 % nucleotide identity with those of known species belonging to genera of anaerobic, non-sporulating, Gram-positive bacilli, indicating that they were anaerobic, non-sporulating, Gram-positive bacilli (Table 1). The remaining 17 isolates were identified as *Clostridium* species and one isolate as a novel bacterial genus and species and will not be discussed in the present report. The 96 blood culture isolates of anaerobic, non-sporulating, Gram-positive bacilli (75 of *P. acnes* and 21 of other species) were recovered from 96 different patients. Out of these 96 isolates, 16 (17 %) were associated with clinically significant bacteraemia, while the remaining 80 (83 %) were associated with pseudobacteraemia due to contamination. Ten of the 16 cases (63 %) of clinically significant bacteraemia were caused by *Eggerthella* spp., four (25 %) by *Lactobacillus* spp. and one (6 %) by each of *Eubacterium tenue* and *Olsenella uli* (Table 2). Five of the 10 *Eggerthella* isolates belonged to two novel *Eggerthella* spp., which have been described recently (Lau *et al.*, 2004). All the 75 isolates of *P. acnes*, three isolates of *Lactobacillus* spp. and one each of *Eubacterium tenue* and *Bifidobacterium pseudocatenulatum* were associated with pseudobacteraemia.

Patient characteristics

The characteristics of the 16 patients with clinically significant bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli are tabulated and summarized in Tables 2 and 3. The clinical details of patient 15 with *Lactobacillus* cholecystitis have been described previously (Woo *et al.*, 2002a). The incidence of anaerobic, non-sporulating, Gram-positive bacilli bacteraemia was similar throughout the 4-year study period and there was no obvious seasonal

Table 1. Identification of 21 blood culture isolates of anaerobic, non-sporulating, Gram-positive bacilli other than *P. acnes* by 16S rRNA gene sequencing

Isolate no.	Identification by 16S rRNA gene sequencing	Closest GenBank match (accession no.)	Nucleotide identity to closest match (%)
1	<i>Bifidobacterium pseudocatenulatum</i>	<i>Bifidobacterium pseudocatenulatum</i> (D86187)	99.8
2	<i>Eggerthella lenta</i>	<i>Eggerthella lenta</i> (AF292375)	99.5
3	<i>Eggerthella lenta</i>	<i>Eggerthella lenta</i> (AF292375)	100.0
4	<i>Eggerthella lenta</i>	<i>Eggerthella lenta</i> (AF292375)	98.7
5	<i>Eggerthella lenta</i>	<i>Eggerthella lenta</i> (AF292375)	99.4
6	<i>Eggerthella lenta</i>	<i>Eggerthella lenta</i> (AF292375)	99.8
7	<i>Eggerthella hongkongensis</i>	<i>Eggerthella lenta</i> (AF292375)	92.7
8	<i>Eggerthella hongkongensis</i>	<i>Eggerthella lenta</i> (AF292375)	92.7
9	<i>Eggerthella hongkongensis</i>	<i>Eggerthella lenta</i> (AF292375)	92.7
10	<i>Eggerthella hongkongensis</i>	<i>Eggerthella lenta</i> (AF292375)	92.7
11	<i>Eggerthella sinensis</i>	<i>Eggerthella lenta</i> (AF292375)	96.3
12	<i>Eubacterium tenue</i>	<i>Eubacterium tenue</i> (M59118)	99.8
13	<i>Eubacterium tenue</i>	<i>Eubacterium tenue</i> (M59118)	99.2
14	<i>Lactobacillus casei</i>	<i>Lactobacillus casei</i> (AF385770)	100.0
15	<i>Lactobacillus casei</i>	<i>Lactobacillus casei</i> (AF385770)	100.0
16	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus rhamnosus</i> (AF243146)	99.8
17	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus rhamnosus</i> (AF243146)	99.8
18	<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus rhamnosus</i> (AF243146)	99.7
19	<i>Lactobacillus salivarius</i>	<i>Lactobacillus salivarius</i> (AF420311)	100.0
20	<i>Lactobacillus salivarius</i>	<i>Lactobacillus salivarius</i> (AF420311)	99.9
21	<i>Olsenella uli</i>	<i>Olsenella uli</i> (AF292373)	100.0

variation. The median age was 62 (range 27–87). Ten patients (63 %) were over 60. The male:female ratio was 1:1. Only one patient did not have underlying disease. The major underlying diseases included diseases in the gastrointestinal tract in eight (50 %), malignancy in seven (44 %), hepatobiliary tract diseases in five (31 %), immobilization and/or bed sores in four (25 %), diabetes mellitus in three (19 %) and cerebrovascular accident in two (13 %). No definite source of the bacteraemia was identified (primary bacteraemia) in six patients (38 %), whereas four (25 %) had infections in the gastrointestinal tract, three (19 %) had hepatobiliary tract infections, two (13 %) had infected bed sores and one (6 %) had genital tract infections. Twelve (75 %) and four (25 %) had community- and hospital-acquired bacteraemia, respectively. Eight patients (50 %) had anaerobic, non-sporulating, Gram-positive bacilli as the only bacteria recovered in their blood cultures, whereas in the other eight (50 %), other pathogens were recovered concomitantly, with the *Bacteroides fragilis* group isolated in three cases, and *Prevotella intermedia*, *Morganella morganii*, *Bacteroides splanchnicus*, *Arcanobacterium haemolyticum*, *Peptostreptococcus* spp., *Streptococcus constellatus*, *Escherichia coli* and *Candida glabrata* each in one. Overall five patients (31 %) died.

The characteristics of patients with clinically significant bacteraemia and those with pseudobacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli are compared and summarized in Table 3. Underlying gastrointestinal tract disease, isolation of *Eggerthella* and *Lactobacillus*

species, and monomicrobial bacteraemia were associated with clinically significant bacteraemia ($P = 0.003$, $P < 0.0005$, $P = 0.014$ and $P = 0.002$, respectively), whereas isolation of *P. acnes* and polymicrobial bacteraemia were associated with pseudobacteraemia ($P < 0.0005$ and $P = 0.002$, respectively).

DISCUSSION

In this study, we defined the epidemiology, clinical spectrum and outcome of bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli with the aid of 16S rRNA gene sequencing. Almost all patients with clinically significant bacteraemia had underlying diseases, with gastrointestinal tract disease and malignancy being the most common. Most patients (75 %) had community-acquired bacteraemia. Among the 10 patients with documented foci of infection, the gastrointestinal and hepatobiliary tracts were the major portal of entry. The four patients with hospital-acquired infection had primary bacteraemia of which the source was unidentified. The most common genus associated with clinically significant bacteraemia was *Eggerthella* (63 %), followed by *Lactobacillus* (25 %).

From the present report, *Eggerthella* is always of clinical significance when isolated from blood cultures. In contrast to the rare reports of *Eggerthella* bacteraemia in the literature, the present study suggests that this genus is an important cause of clinically significant bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli, and that the novel

Table 2. Characteristics of the 16 patients with clinically significant bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli

For all patients, the anaerobic, non-sporulating, Gram-positive bacilli were isolated once from their blood cultures.

Patient no.	Isolate no.	Sex*/age	Underlying disease	Diagnosis	Blood culture isolate	CA/HA†	Other organisms recovered in blood culture	Outcome
1	2	F/69	Carcinoma of lung, intestinal obstruction	Primary bacteraemia	<i>Eggerthella lenta</i>	HA	<i>Prevotella intermedia</i>	Cured
2	3	M/74	Alcoholic cirrhosis, gallstones, gastrointestinal bleeding	Primary bacteraemia	<i>Eggerthella lenta</i>	CA	None	Died
3	4	F/75	None	Pelvic inflammatory disease	<i>Eggerthella lenta</i>	CA	<i>Morganella morganii</i>	Cured
4	5	F/87	Diabetes mellitus, hypertension, cerebrovascular accident	Infected bed sore	<i>Eggerthella lenta</i>	CA	<i>Bacteroides splanchnicus</i> , <i>Arcanobacterium haemolyticum</i>	Died
5	6	F/84	Cerebrovascular accident, hypercholesterolaemia	Infected bed sore	<i>Eggerthella lenta</i>	CA	None	Cured
6	7	M/30	Alcoholic cirrhosis with ascites, epilepsy	Perianal abscess	<i>Eggerthella hongkongensis</i>	CA	None	Died
7	8	M/72	Carcinoma of rectum, diabetes mellitus	Infected rectal tumour	<i>Eggerthella hongkongensis</i>	CA	<i>Bacteroides fragilis</i> , <i>Peptostreptococcus</i> spp.	Cured
8	9	M/64	Recurrent pyogenic cholangitis	Liver abscess	<i>Eggerthella hongkongensis</i>	CA	<i>Streptococcus constellatus</i>	Cured
9	10	F/27	Cerebral palsy	Acute appendicitis	<i>Eggerthella hongkongensis</i>	CA	<i>Escherichia coli</i> , <i>Bacteroides fragilis</i>	Cured
10	11	F/59	Carcinoma of cervix, irradiation proctitis	Acute proctitis	<i>Eggerthella sinensis</i>	CA	<i>Bacteroides fragilis</i>	Cured
11	13	F/85	Sick sinus syndrome, carcinoma of rectum, intestinal obstruction	Primary bacteraemia	<i>Eubacterium tenue</i>	CA	None	Cured
12	14	F/41	Chronic myeloid leukaemia	Primary bacteraemia	<i>Lactobacillus casei</i>	HA	None	Died
13	16	M/50	Diabetes mellitus, liver cirrhosis, gastrointestinal bleeding, pulmonary tuberculosis	Primary bacteraemia	<i>Lactobacillus rhamnosus</i>	HA	None	Cured
14	17	M/68	Carcinoma of oesophagus, chronic obstructive pulmonary disease, ischaemic heart disease	Primary bacteraemia	<i>Lactobacillus rhamnosus</i>	HA	<i>Candida glabrata</i>	Died
15	20	M/70	Parkinsonism, chronic obstructive pulmonary disease	Acute cholecystitis	<i>Lactobacillus salivarius</i>	CA	None	Cured
16	21	M/43	Cholangiocarcinoma, choledochal cyst, history of liver abscess	Acute cholangitis	<i>Olsenella uli</i>	CA	None	Cured

*M, Male; F, female.

†CA, Community acquired; HA, hospital acquired.

Table 3. Comparison of characteristics of patients with clinically significant bacteraemia and those with pseudobacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli

Characteristics	No. of patients (%)		P value
	Clinically significant bacteraemia (n = 16)	Pseudobacteraemia (n = 80)	
Sex			
Male	8 (50)	52 (65)	NS
Female	8 (50)	28 (35)	
Age (mean ± SD)	62 ± 19	52 ± 31	NS
Underlying diseases			
Gastrointestinal tract disease	8 (50)	11 (14)	0.003
Malignancy	7 (44)	22 (28)	NS
Hepatobiliary tract disease	5 (31)	12 (15)	NS
Immobilization and/or bed sore	4 (25)	19 (24)	NS
Diabetes mellitus	3 (19)	5 (6)	NS
Cerebrovascular accident	2 (13)	20 (25)	NS
None	1 (6)	6 (8)	NS
Blood culture isolate			
<i>Bifidobacterium</i> spp.	0 (0)	1 (1)	NS
<i>Eggerthella</i> spp.	10 (63)	0 (0)	< 0.0005
<i>Eubacterium</i> spp.	1 (6)	1 (1)	NS
<i>Lactobacillus</i> spp.	4 (25)	3 (4)	0.014
<i>Olsenella</i> spp.	1 (6)	0 (0)	NS
<i>Propionibacterium</i> spp.	0 (0)	75 (94)	< 0.0005
Community/hospital acquired			
Community	12 (75)	55 (69)	NS
Hospital	4 (25)	25 (31)	
Mono/polymicrobial bacteraemia			
Monomicrobial	8 (50)	10 (13)	0.002
Polymicrobial	8 (50)	70 (88)	

species *Eggerthella hongkongensis* may be as prevalent as the previously sole species, *E. lenta* (Lau *et al.*, 2004). Further studies should be carried out to investigate the presence of specific virulence factors in this bacterial genus. As for *Lactobacillus* bacteraemia, previous reports have identified immunosuppression and prolonged hospitalization as predisposing factors (Husni *et al.*, 1997; Salminen *et al.*, 2004). This is in line with the present study, where all four patients with clinically significant *Lactobacillus* bacteraemia had severe underlying diseases and three acquired the infection in hospital. Although *Lactobacillus* appeared to be associated with clinically significant bacteraemia in the present study, which is likely the result of the huge number of cases of *Propionibacterium* pseudobacteraemia, three cases of pseudobacteraemia were identified of undetermined clinical significance. Nevertheless, previous studies have suggested that most lactobacilli in blood cultures are of clinical significance and treatment with antimicrobials effective *in vitro* was associated with lower mortality (Husni *et al.*, 1997; Salminen *et al.*, 2004).

Bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli other than *P. acnes* should be managed cautiously. Although no studies have been conducted to evaluate the

effect of antimicrobial therapy on the clinical outcome except for *Lactobacillus* bacteraemia (Salminen *et al.*, 2004), the clinical significance of each case should be evaluated individually. The attributable mortality of clinically significant bacteraemia in this study was high (31%) and all the five patients who died had severe underlying diseases. Therefore, when bacteraemia due to anaerobic, non-sporulating, Gram-positive bacilli occurs in immunocompromised patients, appropriate antibiotic treatment should be initiated and the source of the bacteraemia identified.

The present study also uncovered the first case of *O. uli* bacteraemia in humans. *O. uli* was first described in 1991 by Olsen after its isolation from human gingival crevices and periodontal pockets (Olsen *et al.*, 1991). It was formerly known as *Lactobacillus uli* but was reclassified under the new genus *Olsenella* in 2001 on the basis of phenotypic characteristics and 16S rRNA sequence analysis (Dewhirst *et al.*, 2001). Apart from its isolation from the oral cavity of patients with periodontitis, the bacterium has not been reported to cause other infections (Dewhirst *et al.*, 2001; Downes *et al.*, 2001). The present patient (patient 16) with *O. uli* bacteraemia was admitted for acute cholangitis because of a blocked percutaneous biliary drain which had been inserted

for malignant biliary obstruction. *O. uli* was isolated from his blood culture taken on admission. The patient was treated with cefuroxime and revision of the biliary drain. Since he did not have dental problems, it is likely that the biliary tract was the source of the bacterium, which had ascended from the gastrointestinal tract. The application of 16S rRNA gene sequencing to clinically significant isolates of anaerobic Gram-positive bacilli may help further identify more cases of *O. uli* infections and define its epidemiology and pathogenicity.

Identification of anaerobic, non-sporulating, Gram-positive bacilli is difficult, as many of them are slow-growing, fastidious and biochemically inert. By 16S rRNA gene analysis, revisions have been made in their classification, and new genera and species introduced (Kageyama & Benno, 2000; Kageyama *et al.*, 1999; Lau *et al.*, 2004; Nakazawa *et al.*, 1999; Wade *et al.*, 1999). Moreover, such application has allowed unambiguous identification of these bacteria. In one study, 105 *Eubacterium*-like isolates from odontogenic infections, infections associated with dental implants or saliva from healthy subjects were subjected to 16S rRNA gene analysis (Downes *et al.*, 2001). Ninety-one of the isolates were identified as belonging to 10 previously described genera while the remaining 14 did not correspond to existing species. This illustrates the diversity of organisms provisionally identified as *Eubacterium* and the potential of 16S rRNA gene analysis in the discovery of previously mis-identified, novel bacteria. Recently, we have also reported the use of this technique for identifying and defining the clinical significance of a strain of *L. salivarius* (Woo *et al.*, 2002a) and a strain of *A. odontolyticus* (Woo *et al.*, 2002b), and for the discovery of a novel *Actinomyces* species (Woo *et al.*, 2003) and two novel *Eggerthella* species (Lau *et al.*, 2004). Others have reported the application of a simpler approach, amplified 16S rDNA restriction analysis, to identify clinical isolates of putative *Actinomyces* spp. (Hall *et al.*, 2001). They found that only 70% of the isolates analysed belonged to recognized *Actinomyces* species while others were either novel species or members of other genera. Further application of similar techniques in identifying clinically important isolates of anaerobic, non-sporulating, Gram-positive bacilli will better define the epidemiology, clinical significance and pathogenic potential of the various genera.

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