

---

# Detection of bacterial virulence genes associated with infective endocarditis in infected root canals

A. L. Bate<sup>1</sup>, J. K-C. Ma<sup>2</sup> & T. R. Pitt Ford<sup>1</sup>

<sup>1</sup>Department of Conservative Dentistry; and <sup>2</sup>Department of Oral Medicine and Pathology, GKT Dental Institute, King's College, University of London, London, UK

---

## Abstract

**Bate AL, Ma JK-C, Pitt Ford TR.** Detection of bacterial virulence genes associated with infective endocarditis in infected root canals. *International Endodontic Journal*, **33**, 194–203, 2000.

**Aim** The aim of this study was to examine whether bacteria associated with root canals possess genes that might predispose to bacterial colonization of the endocardium.

**Methodology** Oligonucleotides were designed from DNA sequences encoding the functional binding regions of streptococcal fibronectin-binding protein (FnBP) and staphylococcal fibrinogen-binding protein (FgBP). The specificity and cross-reactivity of the oligonucleotide primers were investigated; streptococcal primers were tested for recognition of FnBP genes in other strains of streptococci, and the staphylococcal primers for detection of FgBP from other staphylococci. Interspecies specificity of these primers was also investigated. In a pilot clinical study, the pulp chambers of 16 nonvital teeth without sinus tracts, were opened

aseptically. Root canal samples were collected, along with samples from the gingival sulcus and anterior nares. From these samples DNA was extracted, subjected to polymerase chain reaction (PCR) and analysed by agarose gel electrophoresis.

**Results** Using the streptococcal FnBP primers, PCR bands were amplified from eight root canal samples, eight gingival samples and three nasal samples. With the staphylococcal primers, PCR bands were amplified from seven root canals, 11 gingival and nine nasal samples. This study showed that PCR could be used to detect bacteria in root canals that possess genes with homology to functional regions of those encoding FnBP or FgBP.

**Conclusions** If bacteria in root canals possess FnBP or FgBP, they may have the potential to cause infective endocarditis.

**Keywords:** bacteria, genes, PCR, root canal, streptococci, staphylococci.

*Received 4 March 1999; accepted 22 June 1999*

---

## Introduction

Bacteraemia in patients with damaged heart valves can lead to infective endocarditis (Debelian *et al.* 1994). Antibiotics, however, are routinely used in such at-risk patients as a prophylactic measure in the dental surgery. Clinical procedures such as extractions and endodontic treatment may cause passage of microorganisms from the oral cavity to the bloodstream (Bender & Montgomery 1986, Debelian *et al.* 1995).

The anatomical proximity of the microflora of the gingivae and the root canal to the bloodstream potentially facilitates bacteraemia. Thus, even the most trivial procedure such as toothbrushing may cause a bacteraemia (Cawson 1981).

Infective endocarditis is the most common heart disease caused by oral microorganisms (Marsh & Martin 1992). It is a serious life-threatening condition with a 30% mortality rate, and can be difficult to diagnose and treat. Microorganisms associated with endocarditis can be divided into three groups (Topley 1990): 'viridans' streptococci (65%), *Staphylococcus aureus* (27%) and others (8%).

---

Correspondence: Professor T. R. Pitt Ford, GKT Dental Institute, Guy's Hospital, London SE1 9RT, England, UK.

Oral viridans streptococci are most commonly isolated in infective endocarditis. A relationship between the microflora of local apical periodontitis and the microorganisms of a bacteraemia has been shown (Otten *et al.* 1987), involving obligate as well as facultative anaerobes. However, a history of a dental procedure has been found in only 5–10% of cases of infective endocarditis (Cawson 1981). There is little evidence that the incidence of endocarditis has changed since the introduction of antibiotics (Cawson 1981) and as there are now many more risk factors, the evidence suggests that dental procedures may be relatively unimportant. However, a review of 53 episodes of infective endocarditis related to dental procedures (Martin *et al.* 1997) found that seven could have been due to endodontic treatment without antibiotic cover. Experimentally, infected vegetations were produced in three out of 20 rabbits, with intentionally damaged heart valves, that were later subjected to oral surgery (McGowan & Hardie 1974); it was claimed that the condition was probably induced by bacteraemia from the mouth. In a human study (Roberts *et al.* 1992), a peak in bacteraemia was detectable 30 s after dental procedures. Normally, the microorganisms are removed by the reticuloendothelial system over a period of a few minutes.

A critical step in any bacterial infection is adherence to host tissues, which leads to colonization. Two important bacterial components are fibronectin-binding protein (FnBP) and fibrinogen-binding protein (FgBP), that are found on the surface of staphylococci and streptococci, as well as other oral bacteria.

Fibronectin is a multifunctional dimeric glycoprotein found in high concentrations in extracellular matrix and body fluids. Its primary role is to serve as a substrate for adhesion of animal cells (Joh *et al.* 1994), but many parasitic bacteria have developed FnBPs that can mediate their adherence to host tissues. For both *S. aureus* (Kuypers & Proctor 1989) and *S. sanguis* (Lowrance *et al.* 1990), expression of a FnBP correlates with the ability to cause infective endocarditis in the rat model.

Another possible mechanism in the pathogenesis of infective endocarditis is the direct aggregation of platelets by bacteria; this can be mediated by the FgBP of *S. aureus* (Kehoe 1994). For example, in the rat model for infective endocarditis, an *S. aureus* mutant deficient in FgBP produced 50% less disease than the nonmutant strain (Moreillon *et al.* 1995). *Staphylococcus aureus* also forms macroscopic clumps when mixed with plasma due to binding of fibrinogen to a clumping

factor (FgBP) on the bacterial cell surface (Foster & McDevitt 1994). The same clumping factor can also promote attachment of bacteria to fibrinogen-coated surfaces *in vitro*, and fibrinogen binding is the most important determinant of adherence of *S. aureus* to newly implanted biomaterials.

The genes encoding FnBP in *Streptococcus pyogenes* (Talay *et al.* 1992), *Streptococcus dysgalactiae* (Lindgren *et al.* 1993) and *Staphylococcus aureus* (McDevitt *et al.* 1995) have been cloned and sequenced. The amino acid sequences all share characteristic conserved features, and the fibronectin binding site is composed of 37–48 residue motifs that are repeated 3–5 times and located upstream of a proline-rich putative cell wall spanning domain (Joh *et al.* 1994). The repeated sequence motifs result in multivalent binding and a high affinity of interaction for the N-terminal region of fibronectin.

The *S. aureus* gene for FgBP (clumping factor *clfA*) has been cloned and characterized by McDevitt *et al.* (1994); it comprises 896 residues. Significant amino acid sequence similarity with the FnBPs occurs in the N- and C-terminal sequences associated with secretion and wall-localization; in addition, some similarity occurs in region A, which is thought to include the ligand-binding domain.

Bacterial cell-surface proteins can be detected by a number of techniques; these range from immunological assays using specific antisera or monoclonal antibodies to functional assays examining ligand binding. Alternatively, molecular biological techniques can be used, such as the polymerase chain reaction (PCR). This approach has the advantage of being relatively easy to perform, but more importantly, of being significantly more sensitive. The technique of PCR is a highly specific way of amplifying and detecting segments of DNA from a solution containing as little as one copy of the target sequence. The test solution is incubated with DNA primers, and DNA is replicated repeatedly in the presence of the enzyme, DNA polymerase. The presence or absence of an amplified fragment of DNA is determined by agarose electrophoresis and staining of DNA with ethidium bromide.

The aim of this study was to examine by the use of PCR whether bacteria associated with infected root canals possess genes that might predispose to bacterial colonization of the endocardium.

## Materials and methods

Bacterial DNA from pure cultures and also from clinical samples was first purified and then subjected to

PCR. A preliminary investigation was performed to test the specificity of the two sets of synthetic oligonucleotide primers (binding of primers within one genus, e.g. streptococci only) and the cross-reactivity of the primers (binding of primers in other groups of bacteria). A pilot study of clinical samples was then conducted.

### Designing oligonucleotides

Synthetic oligonucleotides were designed, based on sequences of two FnBP genes from *Streptococcus dysgalactiae* (Lindgren et al. 1993) and one from *Streptococcus pyogenes* (Talay et al. 1992), as well as FgBP from *Staphylococcus aureus* (McDevitt et al. 1995). The primers were from the conserved functional regions encoding adhesive domains of streptococcal FnBP and staphylococcal FgBP. The streptococcal primers were 5' – TTTACWRAAGAYACTCAA, corresponding to residues [3044–3061] of *S. dysgalactiae* FnBP gene and [1450–1467] of *S. pyogenes* FnBP and 3' – CY-GTTGCAGGAA, corresponding to residues [3513–3524] of *S. dysgalactiae* FnBP gene and [1937–1949] of *S. pyogenes* FnBP gene. The staphylococcal primers were 5' – ACTCATATGTTGACCATGCCCGCTTATATT, corresponding to residues [1298–1318] and 3' – AGGGTCGACTTACTCATCAGGTTGTTTCAGG corresponding to residues [1934–1954] of *S. aureus* FgBP gene.

### Investigation of cross-reactivity of primers

The interspecies cross-reactivity of the streptococcal primers was tested by PCR of DNA extracted from: *Streptococcus sanguis* (two clinical samples), *S. vestibularis* (AS2), *S. gordonii* (7865), *S. oralis* (7864), *S. salivarius* (11389), *S. milleri* (MILL. 154). The specificity was analysed using DNA from human cells (Sigma, Poole, UK), *Candida albicans*, *Staphylococcus aureus* and five other anaerobic oral bacteria, namely: *Veillonella parvula* (NCTC 11463), *Fusobacterium nucleatum* (NCTC 10562), *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611) and *Actinomyces viscosus* (NCTC 10951).

A similar investigation was carried out to study the cross-reactivity of the staphylococcal primers. These were tested by PCR of DNA extracted from: coagulase positive *S. aureus* (1671), *S. saprophyticus* (1672), *S. xylophilus* (1673), *S. simulans* (1674), *S. epidermidis* (1675), *S. haemolyticus* (1676), and two coagulase negative staphylococci (1179 and 2755). Specificity

was analysed using DNA from human cells, *Candida albicans*, *Streptococcus sanguis* and five other anaerobic oral bacteria, as above.

### Pilot clinical study

The samples were taken from 16 adult patients who required root canal treatment and presented with symptomatic teeth with necrotic pulps. Care was taken to select only teeth with no coronal leakage around restoration margins, and which were without sinus tracts. An aseptic technique was employed throughout. For each sample, a freshly sterilized bottle of paper points or cotton pledgets was used; sterile tweezers were employed for every step to ensure no microbial contamination. A sterile handpiece was also used. A 'sterile' and 'nonsterile' tray system was used.

**Gingival sample** Before any presterilization procedures were carried out in the mouth, samples were taken from the gingival area adjacent to the tooth by placing three sterile paper points in the gingival sulcus of mandibular premolars for 5 s.

**Root canal sample** The root canal procedures were carried out according to established methods (Möller 1966, Dahlén & Möller 1992). A rubber dam was applied and the operative field cleansed by repeated application of 35% hydrogen peroxide, until bubbling ceased when fresh hydrogen peroxide was applied. Subsequently, 2.5% iodine tincture was applied for 1 min to sterilize the field. This was inactivated using 5% sodium thiosulphate solution. A sterility test was then performed; the operative field was wiped with a cotton pellet, which was transferred to a bottle of Todd-Hewitt medium. Sterile burs were used to prepare an access cavity to the pulp chamber. At this stage a thin layer of dentine was left over the pulp chamber. The field and cavity was sterilized again and a second sterility test performed. A new sterile bur was used to complete the access cavity through the final layer of dentine. The pulp chamber was irrigated with Todd-Hewitt medium, which was agitated with a file to the apical part of the canal. The fluid in the root canal was soaked up with five paper points until all sampling fluid from the root canal was absorbed.

**Nasal sample** The anterior nares were sampled near the opening of the lacrimal duct using a nasal swab, which was placed into Todd-Hewitt medium.

All points or swabs used for sampling were immediately transferred into 5 mL of Todd-Hewitt medium and incubated overnight at 37°C.

### Extraction of DNA from bacterial samples

DNA was extracted as described by Bollet *et al.* (1991). A 1.5-mL aliquot of each culture was pelleted in a microcentrifuge, washed twice with 1 mL TE (10 m mol L<sup>-1</sup> Tris pH 8, 10 m mol L<sup>-1</sup> EDTA) and resuspended in 100 µL TE. Next, 50 µL of 10% SDS were added and the solution was incubated for 30 min at 65°C. The lysate was centrifuged and the supernatants removed. The microtubes were then placed in a microwave oven and heated for three 1-min cycles (750 W). The pellets were dissolved in 200 µL TE and shaken with an equal volume of chloroform-isoamyl alcohol-phenol (24 : 1 : 25) for 15 min. The aqueous phase was recovered after centrifuging and precipitated in 2.2 Vol. absolute ethanol and 0.1 Vol. 3 mol L<sup>-1</sup> sodium acetate (pH 5.2). This was incubated at -20°C for 1 h, pelleted by centrifuging for 20 min at 4°C, then washed with ethanol (70%). The DNA pellets were resuspended in 20 µL of distilled water.

### Polymerase chain reaction

For each DNA sample, two PCR reactions were performed using oligonucleotides specific for streptococcal FnBP or staphylococcal FgBP. In each, 1 µL of DNA was mixed with 50 µM deoxynucleotide triphosphate (dNTP) mixture, 0.4 µM oligonucleotides, Taq polymerase buffer in a total volume of 49.5 µL. The reactions were heated at 94°C for 3 min and the PCR commenced with 2.5 units of Taq DNA polymerase (Boehringer Mannheim, Lewes, UK) using a hot-start procedure. The reaction conditions were 30 cycles of: 94°C for 30 s (denaturation), 48°C for 1 min (annealing) and 72°C for 2 min (polymerization) in a Perkin Elmer DNA thermal cycler 480. To confirm the PCR reaction conditions, positive control reactions contained staphylococcal DNA or streptococcal DNA with their respective oligonucleotide primers. Negative control reactions contained human DNA (Sigma) with either the streptococcal or staphylococcal oligonucleotide primers. Analysis of 10 µL of each sample was carried out by 1% agarose gel electrophoresis. The PCR products were stained with ethidium bromide and viewed under ultraviolet light.

### Cloning and sequencing of PCR products

The PCR-amplified DNA bands were purified following agarose gel electrophoresis and extracted using the QIAQuick gel extraction system (Qiagen Ltd, Crawley,

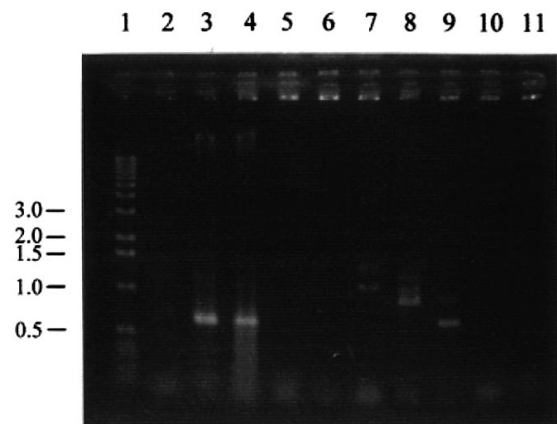
UK); DNA sequencing was performed using the respective 5' primers (Sanger *et al.* 1992).

## Results

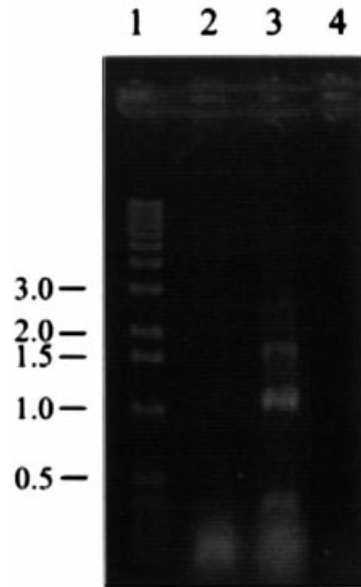
### Staphylococcal FgBP PCR

The PCR of DNA from *S. aureus* using the staphylococcal FgBP primers yielded a single product of the expected size, approximately 700 bp (Fig. 1, lane 3). Homology to the DNA sequence of FgBP was confirmed by sequence analysis. There was no recognition of human DNA (Fig. 1, lane 2) nor of DNA from *Candida albicans* (Fig. 2) or *S. sanguis* (not shown). Of the other staphylococcal species tested, only *S. haemolyticus* and another *S. aureus* isolate produced an amplified product of the expected size (Fig. 1, lanes 4 and 9) although a distinct DNA fragment was also clearly amplified from *S. epidermidis* (Fig. 1, lane 8). Poorly defined bands were also observed from *S. simulans* but these were much larger DNA fragments than expected (Fig. 1, lane 7). A DNA fragment was also amplified from one of the coagulase negative staphylococci (1179), although this was slightly smaller, approximately 400 bp, than the expected size (Fig. 1, lane 11).

In a PCR using five other oral bacterial species, DNA products of approximately the correct size were amplified from *P. intermedia* (Fig. 3, lane 4) and *P.*



**Figure 1** Conservation of fibrinogen binding protein in coagulase positive and negative staphylococci. Agarose gel electrophoresis of PCR products generated using staphylococcal template DNA and fibrinogen binding protein primers. Lanes: 1) 1 Kbp ladder; 2) Human DNA; 3) Staphylococcal positive control; 4) Coagulase positive *S. aureus*; 5) *S. saprophyticus*; 6) *S. xylosum*; 7) *S. simulans*; 8) *S. epidermidis*; 9) *S. haemolyticus*; 10) Coagulase negative staphylococcus 2755; 11) Coagulase negative staphylococcus 1179.



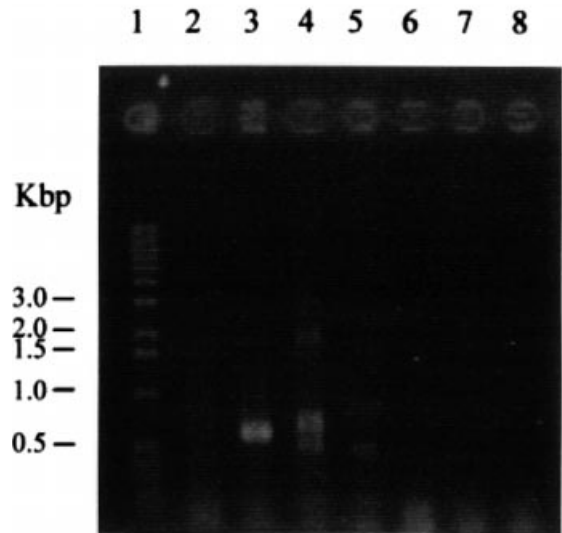
**Figure 2** Cross-reactivity between staphylococcal and streptococcal oligonucleotide primers with DNA from *Candida albicans* in PCR. Lanes: 1) 1 Kbp ladder; 2) *C. albicans* DNA PCR with staphylococcal primers; 3) *C. albicans* DNA PCR with candida-specific primers; 4) *C. albicans* DNA PCR with streptococcal primers.

*gingivalis* (Fig. 3, lane 5). No PCR products were detected from *F. nucleatum*, *A. naeslundii* or *V. parvula*.

#### Streptococcal FnBP PCR

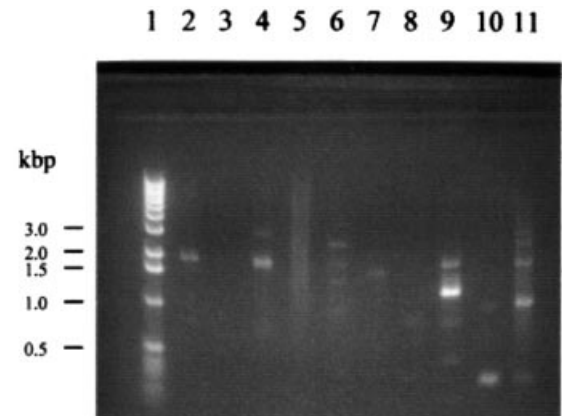
A faint PCR product of approximately expected size, 600 bp, was amplified using the streptococcal FnBP primers and *S. sanguis* as the source for template DNA (Fig. 4, lane 2). However, a much more intense band of approximately 1.9 Kbp was also consistently amplified. Sequence analysis of this band showed a region of homology with fibronectin-binding proteins of *S. pyogenes* and *S. dysgalactiae*. The streptococcal primers did not, however, anneal to any human DNA sequences (Fig. 4, lane 3), candidal (Fig. 2) or staphylococcal DNA (not shown). Of five further oral streptococcal species tested, PCR products of 1.9 Kbp were also amplified from two of three isolates of *S. sanguis*, *S. vestibularis*, *S. oralis* and *S. milleri*. In addition, DNA products of different sizes were amplified from the other two species: *S. gordonii* – 350 and 800 bp; and *S. salivarius* – 350 and 1000 bp.

When tested in a PCR using five other representative species of oral bacteria, three species yielded amplified DNA products (Fig. 5). Only *P. gingivalis* DNA gave a

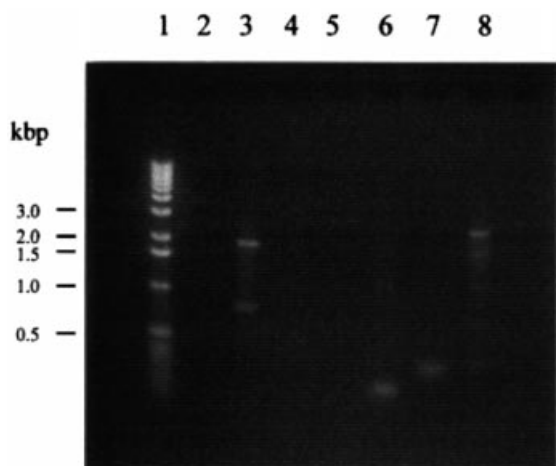


**Figure 3** Conservation of staphylococcal fibrinogen binding protein in five oral bacterial species. Electrophoresis of PCR products generated using staphylococcal FgBP primers. Lanes: 1) 1 Kbp ladder; 2) Human negative control; 3) Staphylococcal positive control; 4) *P. intermedia*; 5) *P. gingivalis*; 6) *F. nucleatum*; 7) *A. naeslundii*; 8) *V. parvula*.

PCR product of a similar size to the streptococci (1.9 Kbp; Fig. 5, lane 8), whereas the bands derived from *P. intermedia* and *F. nucleatum* were less than 500 bp. There was no PCR product associated with *A. naeslundii* nor *V. parvula*.



**Figure 4** Conservation of fibronectin binding protein in oral streptococci. Electrophoresis of PCR products generated using streptococcal template DNA and streptococcal fibronectin binding protein primers. Lanes: 1) 1 Kbp ladder; 2) Streptococcal positive control; 3) Human DNA; 4) *S. sanguis*; 5) *S. sanguis*; 6) *S. sanguis*; 7) *S. vestibularis*; 8) *S. gordonii*; 9) *S. oralis*; 10) *S. salivarius*; 11) *S. milleri*.



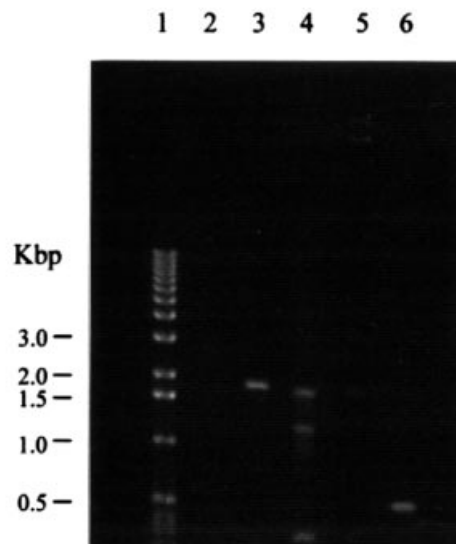
**Figure 5** Conservation of streptococcal fibronectin binding protein in five oral bacterial species. Electrophoresis of PCR products generated using streptococcal FnBP primers. Lanes: 1) 1 Kbp ladder; 2) Human DNA; 3) Streptococcal positive control; 4) *A. naeslundii*; 5) *V. parvula*; 6) *P. intermedia*; 7) *F. nucleatum*; 8) *P. gingivalis*.

#### Detection of streptococcal FnBP and staphylococcal FgBP in clinical samples

In all cases the field sterility samples showed no evidence of bacterial growth after incubation, confirming that there was no contamination on the tooth before the root canal was sampled.

The PCR results from the clinical samples were scored positive or negative. A positive result indicated amplification of a DNA product of the approximate expected molecular size. Other samples were marked negative because either no PCR product was present, or bands of widely divergent molecular size were amplified. A representative result is shown in Fig. 6 using the streptococcal primers, where the gingival and root canal samples generated bands of approximately the expected size and were considered positive (Fig. 6, lanes 4 and 5). A 400-bp fragment was amplified from the nasal sample and for the purposes of this study was considered negative (Fig. 6, lane 6).

The distribution of positive and negative tests for the sites from each patient is summarized in Table 1. For streptococcal FnBP, PCR products of the correct size were identified in eight root canal samples, eight gingival samples and three nasal samples. Of the eight patients with positive findings in their root canal samples the majority also had positive gingival samples, and in only two cases were the gingival and nasal samples negative. Five of the 16 patients had negative results at all three sites.



**Figure 6** Analysis of a representative clinical sample. Electrophoresis of PCR products amplified using streptococcal fibronectin binding protein primers. Lanes: 1) 1 Kbp ladder; 2) human DNA; 3) Streptococcal DNA; 4) DNA extracted from gingival sample; 5) DNA extracted from root canal sample; 6) DNA extracted from nasal sample.

Seven of the 16 patients had positive root canal samples when tested with the staphylococcal FgBP. Correct size PCR products were also detected in 11 of the gingival samples and nine nasal samples. In this group, all of the seven positive root canal samples were associated with positive samples elsewhere, indeed in four patients all three sites were positive. However, gingival and nasal carriage only occurred concurrently in five of the 16 patients. There was only one patient with negative results from all three sites.

Taken together, 10 out of 16 patients had root canal samples that were positive with either virulence factor, and five had samples that were positive for both.

#### Discussion

There is a paucity of studies on the potential risk of infective endocarditis posed by endodontic treatment (Bender & Montgomery 1986, Debelian *et al.* 1995), although it is logical to assume that during instrumentation of an infected root canal, microorganisms could be pushed through the apex and into the bloodstream. However, there have been no studies which demonstrate a link between microorganisms of the root canal and cardiac disease. In this study, the virulence factors, which determine the pathogenic potential of bacteria, were in-

Patient samples	Streptococcal primers			Staphylococcal primers		
	Root canal	Gingival	Nasal	Root canal	Gingival	Nasal
1	-	+	-	-	-	+
2	+	-	-	-	-	+
3	+	-	-	-	+	-
4	-	-	-	-	-	-
5	+	+	-	+	+	-
6	-	+	+	-	-	+
7	-	-	-	+	+	-
8	+	+	-	+	+	+
9	-	-	-	+	+	+
10	-	-	-	-	+	-
11	-	-	-	-	+	-
12	+	+	-	+	+	-
13	+	-	+	-	+	+
14	+	+	-	+	+	+
15	-	+	-	-	-	+
16	+	+	+	+	+	+

**Table 1** The presence (+) or absence (-) of PCR products from clinical samples

investigated, rather than the bacterial flora of the infected tooth. The rationale for this approach is that although certain bacterial species can commonly be found, not all strains are necessarily pathogenic. Similarly, key virulence determinants tend to be highly conserved amongst bacterial species, such as the SA I/II adhesion proteins of oral streptococci (Ma *et al.* 1991), so that potential uncharacterized pathogens might be recognized.

Previous studies have shown the range of bacteria commonly isolated from infected root canals; the most common being viridans streptococci, enterococci and lactobacilli (Möller 1966, Sundqvist 1994). Facultative bacteria such as streptococci may make up a significant part of the more coronal flora, when the pulp has been exposed by caries; whilst obligately anaerobic Gram-positive cocci and Gram-negative rods are found in deeper aspects.

In contrast to the range of organisms found in infected root canals, 95% of cases of infective endocarditis are caused by streptococci and staphylococci (Topley 1990). For this reason, the presence of streptococcal FnBP and staphylococcal FgBP genes were examined in this study as they both have an important role in the bacterial colonization of host tissues, and thus in pathological mechanisms of infective endocarditis. It was reasoned that if sufficiently specific oligonucleotide primers could be designed, their detection of bacteria possessing functional, conserved regions of these genes might be achieved by PCR.

The oligonucleotide primers for both streptococcal FnBP and staphylococcal FgBP were designed from regions of the genes that encoded the adhesion

determinant of the proteins. Only a few sequences have been determined, for two streptococcal and one staphylococcal species. In order to provide broad specificity for the two streptococcal FnBP genes (Fnba and FnbB) some degeneracy was incorporated into the primers, and one was rather short. However, the specificity studies demonstrated that there is broad reactivity with other oral streptococci (that are known to bind to fibronectin) but not to human DNA, candidal DNA, or staphylococcal FnBP. The predominant PCR band of 1.9 Kbp was somewhat larger than predicted; however, as DNA sequencing has demonstrated that this band was related to FnBP, it was considered that the 1.9 Kbp band was the correct size for oral  $\alpha$ -haemolytic streptococci. Somewhat surprisingly, the strain of *P. gingivalis* tested also resulted in a PCR product of approximately that size; FnBP has not previously been described in *P. gingivalis*, but this will be investigated further.

The staphylococcal FgBP primers identified homologous sequences in the coagulase positive as well as one of the two coagulase negative staphylococcal species. This may be important, as coagulase negative staphylococci are increasingly being recognized as extremely virulent causes of infective endocarditis (Vandenesch *et al.* 1993). As with the streptococcal FnBP primers, a PCR band was also amplified in *P. gingivalis* using the staphylococcal FgBP primers as well as from *P. intermedia*. The finding that *P. gingivalis* may possess both virulence factors is interesting, as this is an obligate anaerobe which is

difficult to grow and may not be readily identified by culture microbiological testing of patients with infective endocarditis.

The PCR bands obtained from *P. gingivalis* with streptococcal FnBP primers and with staphylococcal FgBP primers, and from *P. intermedia* with staphylococcal FgBP primers will be cloned and sequenced in a later study.

In the clinical part of the study, the gingival and nasal samples served partly as a control, as streptococci are always found in dental plaque, whereas it has been estimated that 30% of the population carry *S. aureus* in the anterior nares (Topley 1990). It was surprising initially to find that only eight out of 16 of the patients had streptococcal FnBP in gingival samples, but this may be explained in that not all strains of a species are pathogenic, therefore some may not possess the FnBP gene, or its functional regions. Indeed, this might explain why infective endocarditis is not more common in high-risk patients despite the multitude of opportunities for bacteraemia through everyday activities such as chewing. Another possibility lies in the microbiological sampling technique; paper point gingival sampling is simple, but may leave more firmly attached organisms on the tooth.

Of the gingival samples, 11 out of 16 were also PCR positive using the staphylococcal primers, even though staphylococcal carriage in the mouth is uncommon. This result may reflect the conserved nature of this region of the gene, as demonstrated by the results found in *P. gingivalis* and *P. intermedia*. In other words, the positive PCR results may indicate the presence of this or homologous genes in other microorganisms as well as staphylococci. Of the nasal samples, 9 out of 16 gave PCR bands with the staphylococcal primers, this being slightly higher than expected if the primers were detecting *S. aureus* alone. In contrast, only three out of 16 nasal samples were PCR positive with the streptococcal primers, a result which probably reflects the specificity of these primers and the fact that they do not cross-react with staphylococcal FnBP.

The virulence genes were detected from the root canal samples of 10 out of 16 patients, and in five patients both genes were present. Although no statistical significance can be drawn from this pilot study, it can be suggested that the results from the root canals correlate with those from gingival samples ( $n = 12$ ) rather than nasal samples ( $n = 6$ ). At this stage, only PCR bands of approximately the expected molecular size were regarded as positive; these results may therefore be an underestimate of the true

situation. There are many reasons why PCR bands of different sizes might have been generated. The most obvious is nonspecific random priming during the PCR, but given that DNA was not amplified using human, candidal or some bacterial DNA, this would seem improbable. The heterogeneity of amplified bands might represent different organization of homologous genes in different bacterial species. The binding epitopes of FnBP and FgBP are encoded by motifs that are repeated in both cases. Thus, more or less repeats would result in larger or smaller PCR bands. Alternatively, although the binding sites in these proteins are located toward the C terminus in streptococci and staphylococci, this may not be the case in all bacterial species. Indeed, we have already shown an FnBP gene in *S. sanguis* which differs from those in *S. dysgalactiae*, from which the PCR primers were designed. An important aspect of the continuing investigations is to confirm that all the PCR bands generated derive from FnBP and FgBP homologues, particularly in the case of *P. gingivalis* and *P. intermedia*.

Although PCR is a highly sensitive technique, the chances of success were maximized in this pilot study by growing bacterial samples prior to DNA extraction, particularly as the main interest was in streptococcal and staphylococcal pathogens. This, of course, favours certain bacterial species, but PCR should still be sensitive enough to detect DNA from bacteria that did not multiply during the incubation period. In the next stage of this study, it will be important to investigate the use of PCR with DNA extracted immediately from a larger number of clinical samples.

The results suggest that the majority of infected root canals contain bacteria that may have the potential to cause infective endocarditis. Other factors must also be considered. The actual number of microorganisms introduced into the bloodstream will probably be extremely important, and that depends upon the size of the apical foramen, degree of infection of the root canal and method of root canal treatment. The proportion of microorganisms that survive in the bloodstream is significant and dictated in part by the health of the patient and the host response. Moreover, FnBP and FgBP are unlikely to be the only virulence factors involved. These aspects of the pathogenic process need further investigation. In a small clinical study it was interesting to note that the same strain of bacteria found in the root canal was detected in the blood after root canal overinstrumentation (Debelian et al. 1992).

A further aspect of root canal treatment that could have a significant effect on the numbers of bacteria



inoculated into the bloodstream is the placement of a rubber dam clamp. It has been suggested that patients rinse with chlorhexidine gluconate mouthrinse beforehand to reduce the bacteria present (Bender & Montgomery 1986). The present study demonstrated that 14 out of 16 patients were PCR positive for one or both virulence factors in gingival plaque.

If the root canal is considered a potential source for endocarditis-causing microorganisms, then it may be advisable to provide antibiotic cover to patients who are at risk of contracting infective endocarditis when carrying out endodontic treatment; this, however, is not currently recommended (British Society for Antimicrobial Chemotherapy 1990).

### Acknowledgements

The authors thank Charles Kelly for designing the staphylococcal primers. Julian Naglick kindly provided the candidal DNA samples. Craig van Dolleweerd and Justine Younson are thanked for their technical support, and William Wade and Rob Whiley for supplying the anaerobes. The authors acknowledge a grant from the European Society of Endodontology that helped to support the project.

### References

- Bender IB, Montgomery S (1986) Nonsurgical endodontic procedures for the patient at risk for infective endocarditis and other systemic disorders. *Journal of Endodontics* **12**, 400–7.
- Bollet C, de Gevaudan MJ, Lamballerie X, Zandotti C, de Micco P (1991) A simple method for the isolation of chromosomal DNA from Gram-positive or acid-fast bacteria. *Nucleic Acids Research* **19**, 1955.
- British Society for Antimicrobial Chemotherapy (1990) Antibiotic prophylaxis of infective endocarditis. *Lancet* **335**, 88–9.
- Cawson RA (1981) Infective endocarditis as a complication of dental treatment. *British Dental Journal* **151**, 409–14.
- Dahlén G, Möller AJR (1992) Microbiology of endodontic infections. In: Slots J, Taubman MA, eds. *Contemporary Oral Microbiology and Immunology*. St Louis, MO, USA: Mosby-Year Book, 444–75.
- Debelian GJ, Olsen I, Tronstad L (1992) Profiling of *Propionibacterium acnes* recovered from root canals and blood during and after endodontic treatment. *Endodontics and Dental Traumatology* **8**, 248–54.
- Debelian GJ, Olsen I, Tronstad L (1994) Systemic diseases caused by oral microorganisms. *Endodontics and Dental Traumatology* **10**, 57–65.
- Debelian GJ, Olsen I, Tronstad L (1995) Bacteraemia in conjunction with endodontic therapy. *Endodontics and Dental Traumatology* **11**, 142–9.
- Foster TJ, McDevitt D (1994) Surface-associated proteins of *Staphylococcus aureus*: their possible roles in virulence. *FEMS Microbiology Letters* **118**, 199–205.
- Joh HJ, House-Pompeo K, Patti JM, Gurusiddappa S, Höök M (1994) Fibronectin receptors from Gram-positive bacteria: comparison of active sites. *Biochemistry* **33**, 6086–92.
- Kehoe MA (1994) Cell wall associated proteins in Gram positive bacteria. In: Ghuysen JM, Hakenbeck R, eds. *Bacterial Cell Wall – New Comprehensive Biochemistry*, 27. New York: Elsevier, 217–61.
- Kuypers JM, Proctor RA (1989) Reduced adherence to traumatized rat heart valves by a low-fibronectin-binding mutant of *Staphylococcus aureus*. *Infection and Immunity* **57**, 2306–12.
- Lindgren PE, McGavin MJ, Signäs C et al. (1993) Two different genes coding for fibronectin-binding proteins from *Streptococcus dysgalactiae*. The complete nucleotide sequences and characterization of the binding domains. *European Journal of Biochemistry* **214**, 819–27.
- Lowrance JH, Baddour LM, Simpson WA (1990) The role of fibronectin binding in the rat model of experimental endocarditis caused by *Streptococcus sanguis*. *Journal of Clinical Investigation* **86**, 7–13.
- McDevitt D, Francois P, Vaudaux P, Foster TJ (1994) Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Molecular Microbiology* **11**, 237–48.
- McDevitt D, Francois P, Vaudaux P, Foster TJ (1995) Identification of the ligand-binding domain of the surface-located fibrinogen receptor (clumping factor) of *Staphylococcus aureus*. *Molecular Microbiology* **16**, 895–907.
- McGowan DA, Hardie JM (1974) Production of bacterial endocarditis in prepared rabbits by oral manipulation. *British Dental Journal* **137**, 129–31.
- Ma JK, Kelly CG, Munro G, Whiley RA, Lehner T (1991) Conservation of the gene encoding streptococcal Antigen I/II in oral streptococci. *Infection and Immunity* **59**, 2686–94.
- Marsh P, Martin M (1992) The role of oral bacteria in other infections. In: *Oral Microbiology*, 3rd edn. London: Chapman & Hall, 207–11.
- Martin MV, Butterworth ML, Longman LP (1997) Infective endocarditis and the dental practitioner: a review of 53 cases involving litigation. *British Dental Journal* **182**, 465–8.
- Möller ÅJR (1966) Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. (Thesis) Göteborg, Sweden: University of Göteborg, 1–380.
- Moreillon P, Entenza JM, Francioli P et al. (1995) Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. *Infection and Immunity* **63**, 4738–43.
- Otten JE, Pelz K, Christmann G (1987) Anaerobic bacteraemia following tooth extraction and removal of osteosynthesis plates. *Journal of Oral and Maxillofacial Surgery* **45**, 477–80.

- Roberts GJ, Gardner P, Simmons NA (1992) Optimum sampling time for detection of dental bacteraemia in children. *International Journal of Cardiology* **35**, 311–5.
- Sanger F, Nicklen S, Coulson AR (1992) DNA sequencing with chain-terminating inhibitors, 1977. *Biotechnology* **24**, 104–8.
- Sundqvist G (1994) Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral Surgery, Oral Medicine and Oral Pathology* **78**, 522–30.
- Talay SR, Valentin-Weigand P, Jerlstrom PG, Timmis KN, Chhatwal GS (1992) Fibronectin-binding protein of *Streptococcus pyogenes*: sequence of the binding domain involved in adherence of streptococci to epithelial cells. *Infection and Immunity* **60**, 3837–44.
- Topley WWC (1990) Bacteraemia, septicaemia and endocarditis. In: Smith GR, Easmon SF, eds. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, Vol. 3. *Bacterial Diseases*, 8th edn. London: Arnold, 264–86.
- Vandenesch F, Etienne J, Reverdy ME, Eykyn SJ (1993) Endocarditis due to *Staphylococcus lugdunensis*: report of 11 cases and review. *Clinical Infectious Diseases* **17**, 871–6.