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# Incidence and antimicrobial susceptibility of *Porphyromonas gingivalis* isolated from mixed endodontic infections

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## Abstract

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**Aim** To investigate the prevalence of *Porphyromonas gingivalis* in root canals of infected teeth with periapical abscesses and to investigate the antimicrobial susceptibility of this species to some frequently prescribed antibiotics.

**Methodology** Samples were obtained from 70 root canals of abscessed teeth. Microbial sampling, isolation and bacterial identification were accomplished using appropriate culture methods for anaerobic species. The antimicrobial susceptibility of the 20 strains of *P. gingivalis* isolated was determined by using the E-test. The antimicrobial agents tested were amoxicillin, amoxicillin + clavulanate, azythromycin, benzylpenicillin, cephaclor, clindamycin, erythromycin, metronidazole and tetracycline.

**Results** A total of 352 individual strains, belonging to 69 different species, were isolated. Eighty three percent of the strains were strict anaerobes and 47.5% of the isolated bacteria were Gram-negative. *Porphyro-*

*monas gingivalis* was found in 20 root canals and was most frequently found in symptomatic cases. Statistically, the presence of *P. gingivalis* was related to purulent exudates and pain on palpation (both  $P < 0.05$ ). All *P. gingivalis* strains were sensitive to amoxicillin, amoxicillin + clavulanate, cephaclor, clindamycin, benzylpenicillin, metronidazole and tetracycline. The lowest range of minimum inhibitory concentration (MIC) ( $0.026\text{--}0.125\ \mu\text{g mL}^{-1}$ ) was observed against amoxicillin + clavulanate and clindamycin. The lowest MIC 90 was observed against clindamycin ( $0.064\ \mu\text{g mL}^{-1}$ ). One strain was resistant to erythromycin and eight strains were resistant to azythromycin.

**Conclusion** *Porphyromonas gingivalis* pathogen is isolated with frequency from root canals of infected teeth with periapical abscesses. Amoxicillin, as well as amoxicillin–clavulanic acid and benzylpenicillin were effective against *P. gingivalis*.

**Keywords:** anaerobic bacteria, antibiotics, antimicrobial susceptibility, endodontic infection, *Porphyromonas gingivalis*.

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## Introduction

Odontogenic infections, such as periapical abscess, are generally polymicrobial and dominated by anaerobic

bacteria. Although several different microorganisms can colonize the root canal system, some specific species have been related to features of periradicular diseases. *Porphyromonas gingivalis*, for instance, has been associated with symptomatic periradicular lesions, including abscessed teeth (Sundqvist *et al.* 1989, Rocas *et al.* 2002).

*Porphyromonas gingivalis* is a nonfermentative black-pigmented Gram-negative, obligate anaerobic rod (Shah & Collins 1988), which has its primary

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ecological niche in the human oral cavity and is well known as an important periodontopathogen (Van Winkelhoff *et al.* 1988). However, this microorganism is frequently present in root canal infections and other odontogenic abscesses of patients not suffering from periodontal disease, which confirms its pathogenic role (Sundqvist *et al.* 1989). *Porphyromonas* possesses a large number of putative virulence determinants, which suggests it may be one of the most pathogenic species found in the oral cavity. These include fimbriae, haemagglutinin, capsule, lipopolysaccharide (LPS), outer membrane vesicles and powerful hydrolytic enzymes activities that can perturb host defence mechanisms as well as initiate tissue destruction (Mayrand & Holt 1988).

Although clinical isolates of *P. gingivalis* tend to be susceptible to most antimicrobial agents and generally do not produce  $\beta$ -lactamase, relatively little information is available on its *in vitro* antibiotic susceptibility. Furthermore, antibiotic resistance among anaerobes is continuously increasing which may be related to the selective pressure exerted by the use of antibiotics.

The determination of *in vitro* antimicrobial susceptibility can be important in certain situations, for example, to monitor patterns of susceptibility and resistance in the population and to aid in the selection of an appropriate antibiotic when indicated in endodontic treatment. The E-test has been evaluated for determining minimum inhibitory concentration (MIC) of various antimicrobial agents against anaerobic bacteria and shown to be appropriate (Bolmstrom 1993).

Hence, the purpose of this study was to investigate the prevalence of *P. gingivalis* in root canals of infected teeth with periapical abscesses and to investigate the antimicrobial susceptibility of this species to some antibiotics frequently prescribed by clinicians.

## Material and methods

### Patients and clinical examination

This study was based on 70 patients who attended the State University of Campinas – UNICAMP for emergency treatment because of infection of the root canal. The human Volunteers Research and Ethics Committee of the Dental School of Piracicaba approved the study, and all patients signed an informed consent.

The selection of the patients was determined by the dental history as well as clinical and radiographic examination. The following features were noted for

each patient: age, gender, tooth and pulpal status, nature of pain, previous pain, tenderness to percussion, pain on palpation, mobility, presence of a sinus tract and its origin, presence of swelling of the periodontal tissues, depth of periodontal pocket, previous antibiotic therapy and internal condition of the root canal (e.g. presence of clear, haemorrhagic or purulent exudates). Patients with acute or chronic periapical abscess, who had not received antibiotic therapy in the last 6 months, excluding pathology associated to periodontal disease, were selected for this study.

### Specimen sampling

At the time of sampling, every effort was made to ensure aseptic surgical techniques. A rubber dam was used to isolate the tooth, which had the restorations and carious tissue completely removed previously. The tooth and surrounding field were then cleansed with 30% hydrogen peroxide and decontaminated with a 2.5% sodium hypochlorite solution for 30 s each, followed by neutralization of the solution with 5% sodium thiosulphate. In multirouted teeth the largest canal was sampled, in order to confine the microbial evaluation to a single ecological environment. Access preparations were made using sterile burs without water spray. After initial entry to the pulp space, the enlargement of the root canal was established with minimal instrumentation, where possible, and without the use of any irrigant. A sterile paper point was then introduced into the full length of the canal, based on diagnostic radiographs, and held in place for 60 s. If the root canal was dry, a small amount of sterile saline solution was introduced into the canal. Four sequential paper points were then placed to the same level and used to soak up the fluid of the canal. As previously, each paper point was retained in position for 60 s.

A sterilized prerduced transport fluid (RTF) (Syed & Loesche 1972) was used to transport the sample to the laboratory and immediately processed. All culture procedures were accomplished under an anaerobic atmosphere, consisting of 80% nitrogen, 10% hydrogen and 10% carbon dioxide, at 37 °C.

### Microbial isolation and identification

The isolation and identification of the microorganisms was performed using culture techniques for phenotypic characterization as described previously (Gomes *et al.* 1994, 2004, Jacinto *et al.* 2003). In summary, the samples were vortexed for 60 s and diluted with

Fastidious Anaerobe Broth (FAB; Lab M, Bury, UK) by 10-fold serial dilution to  $10^{-4}$  in the anaerobic chamber. A volume of 50  $\mu\text{L}$  of each dilution was spread onto 5% defibrinated sheep blood Fastidious Anaerobe Agar (FAA; Lab M) containing 5  $\text{mg mL}^{-1}$  of haemin (final concentration of 5  $\mu\text{g mL}^{-1}$ ) and 1  $\text{mg mL}^{-1}$  of vitamin K1 (final concentration of 1  $\mu\text{g mL}^{-1}$ ). Selective culture media were also used. The plates were incubated at 37 °C in an anaerobic atmosphere for up to 14 days. The same dilutions were plated in 5% sheep blood Brain Heart Infusion (BHI) agar (Oxoid, Basingstoke, UK), and incubated aerobically at 37 °C. All aerobic cultures were examined at 24–48 h, whereas anaerobic cultures were kept for at least 2 weeks but examined for growth every 3 days.

Preliminary characterization of microbial species was based on their growth in the anaerobic chamber, in the aerobic incubator and also in 5%  $\text{CO}_2$  incubator (IG 150  $\text{CO}_2$  incubator; Jouan SA, Saint-Herblain, France), colonial pigmentation and morphology, Gram-stain and catalase production.

Biochemical tests were used for the primary specification of individual isolates as follows: Rapid ID 32 A (BioMérieux SA, Marcy-l'Étoile, France) for strict anaerobic rods; API Staph (BioMérieux SA) for staphylococci and micrococci; Rapid ID 32 Strep (BioMérieux SA) for streptococci; Rapid ID NH System (Innovative Diagnostic Systems Inc.) for *Eikenella*, *Haemophilus*, *Neisseria* and *Actinobacillus*; and API C Aux (BioMérieux SA) for yeasts. Miniapi software (BioMérieux SA) was used to automatically read ID 32 tests and visually-read API range tests BioMérieux.

Additional tests were performed to ensure the identification of the black pigmented *Porphyromonas* and *Prevotella* sp., including fluorescence under long-wave (366 nm) UV light – Black-Ray lamp, model UVL-56 (UVP Inc., San Gabriel, CA, USA); lactose fermentation by the application of the fluorogenic substrate 4-methylumbelliferyl-b-galactoside (M-1633; Sigma Chemical Co., St Louis, MO, USA), and the determination of proteinase activity by use of the synthetic fluorogenic peptide (Alcoforado *et al.* 1987, Slots 1987).

Specific identification of *Porphyromonas* sp. using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) (Waters Corporation, Manchester, UK). MALDI-TOF-mass spectral analysis of strains was carried out as described previously (Keys *et al.* 2004). Reference strains of *Porphyromonas* spp. from the National Collection of Type Cultures (*Porphyromonas gingivalis* NCTC 11894,

*Porphyromonas endodontalis* NCTC 13058 and *Porphyromonas asaccharolytica* NCTC 9337) were grown at 37 °C on Fastidious Anaerobe Agar (FAA) plated and incubated in anaerobic conditions. Small amounts of growth from the surface of several single colonies on an agar plate was removed with plastic loops and spread evenly over the surface of the wells of the target plate. Twelve replicates were analysed per sample. One microlitre matrix solution containing acetonitrile, water and methanol (1 : 1 : 1), 0.01 M 18-crown-6 ether, 0.1% formic acid (v/v) saturated with *a*-cyano-4-hydroxy-cinnamic acid at a concentration of 14.0  $\text{mg mL}^{-1}$ . This was then added over the sample wells and allowed to air dry. Matrix solutions were sonicated in a sonic bath (Ultrawave Ltd, Cardiff, UK) for 10 min prior to application. Lock mass wells were spotted with 1  $\mu\text{L}$  of a 1 : 1 mixture of  $\alpha$ -cyano and peptide mixture. The peptide mixture contained bradykinin, angiotensin I, glu-fibrinopeptide B, rennin, ACTH (18–39 clip), all at 1  $\text{pmol } \mu\text{L}^{-1}$ , bovine insulin 2  $\text{pmol } \mu\text{L}^{-1}$  and ubiquitin 10  $\text{pmol } \mu\text{L}^{-1}$ . All reagents unless otherwise stated were from Sigma, Gillingham, UK. Mass spectral analysis and comparative analysis were as described previously (Keys *et al.* 2004).

#### Antimicrobial susceptibility test

Susceptibility of the *P. gingivalis* strains isolated was determined by the MIC of amoxicillin, amoxicillin + clavulanate, azythromycin, benzylpenicillin, cephaclo, clindamycin, erythromycin, metronidazole and tetracycline using the E-test system (AB BIODISK, Solna, Sweden) according to the user's manual. Colonies were suspended in Brucella broth to achieve a density corresponding to 1.0 McFarland turbidity standard. A cotton wool swab soaked in the inoculum was used to inoculate the surface of plates containing 5% defibrinated sheep blood Brucella agar enriched with 5  $\text{mg mL}^{-1}$  of haemin and 1  $\text{mg mL}^{-1}$  of vitamin K1. The E-test strip was applied separately to the centre of the plate with the high MIC end towards the edge of the plate. The plates were then immediately incubated in an anaerobic chamber (Don Whitley Scientific, Shipley, UK) for 24–48 h. After growth had occurred, an ellipse of inhibition was seen around the strip. At the point of intersection of the ellipse with the strip, the MIC was read from the interpretative scale provide (AB BIODISK, Solna, Sweden). The MICs of the antibiotics that inhibited 50% and 90% of the isolates were calculated and expressed as MIC50 and MIC 90. MIC less than or equal to the breakpoints recommended by

the National Committee for Clinical Laboratory Standards (NCCLS) were considered susceptible; those above the breakpoints were considered resistant.

## Results

This study evaluated 70 root canals from different patients (range 10–64 years old) with acute or chronic periapical abscesses. Twenty patients had no pain at the moment of sampling and seven were asymptomatic. Signs and symptoms observed were spontaneous pain ( $n = 50$ ), tenderness to percussion ( $n = 49$ ), pain on palpation ( $n = 41$ ), swelling ( $n = 33$ ), exudate ( $n = 40$ ), presence of endodontic sinus tract ( $n = 9$ ). Radiographically, it was possible to observe periapical radiolucent areas in all cases selected for sampling.

A total of 352 individual strains, belonging to 69 different species, were isolated from the 70 cases, and cultivable bacteria were found in all of them. A maximum of nine species per root canal was found.

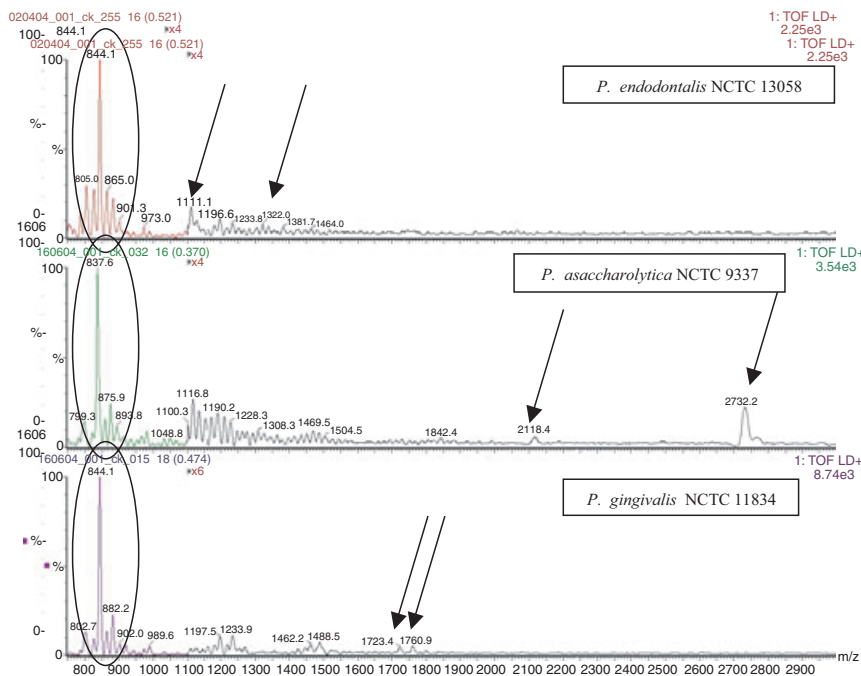
Eighty-three percent of the strains (293/352) were strict anaerobes and could be found in 68 of 70 root canals. Gram-negative strains were found in 67 of 70 root canals composing 47.5% of the isolated bacteria. Specific attention was given to the identification of *P. gingivalis*. Here isolates were further characterized

using MALDI-TOF – mass spectrometry (MALDI-TOF-MS). Significant mass ions at 803 (+2 daltons) and 837.6–844.1 daltons characterized members of the genera *Porphyromonas*. However, unique signal mass ion for each species was apparent. *P. gingivalis* had 1723.4 and 1760.9 daltons, *P. endodontalis* had 1111.1 and 1322 daltons while *P. assacharolytica* possessed distinguishing mass ions at 2118.4 and 2732.2 daltons (Fig. 1).

*Porphyromonas gingivalis* was found in 20 root canals, all of them with mixed infections dominated by obligate anaerobes (93.5%) and Gram-negative bacteria (62%). In the root canal infections where *P. gingivalis* was isolated, four different species were found on average. The species most commonly associated with *P. gingivalis* was *Fusobacterium necrophorum* ( $n = 11$ ). *Porphyromonas gingivalis* was most frequently found in symptomatic cases. Statistically, the presence of *P. gingivalis* was related to purulent exudates and pain on palpation (both  $P < 0.05$ ).

Other black-pigmented rods were found associated to *P. gingivalis* such as *P. endodontalis*, *P. intermedia*, *P. denticola*, *P. loeschei* and *P. corporis*. All species associated with *P. gingivalis* are shown on Table 1.

All *P. gingivalis* strains were sensitive to amoxicillin, amoxicillin + clavulanate, cephaclor, clindamycin,



**Figure 1** Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry mass spectral profiles of *Porphyromonas gingivalis*, *Porphyromonas endodontalis* and *Porphyromonas asacharolytica*.

**Table 1** Bacterial species found in 20 infected root canals where *Porphyromonas gingivalis* was isolated

Bacterial species	No. of strains
<i>Fusobacterium nucleatum</i>	11
<i>Eubacterium limosum</i>	4
<i>Prevotella oralis</i>	4
<i>Gemella morbilorum</i>	4
<i>Micromonas micros</i>	3
<i>Anaerococcus prevotii</i>	3
<i>Veillonella</i> spp.	3
<i>Prevotella loescheii</i>	2
<i>Clostridium butyricum</i>	2
<i>Porphyromonas endodontalis</i>	1
<i>Prevotella intermedia</i>	1
<i>Prevotella buccae</i>	1
<i>Prevotella corporis</i>	1
<i>Bacteroides fragilis</i>	1
<i>Bacteroides ureolyticus</i>	1
<i>Peptostreptococcus anaerobius</i>	1
<i>Eubacterium limosum</i>	1
<i>Fusobacterium necrophorum</i>	1
<i>Actinomyces viscosus</i>	1
<i>Clostridium sporogens</i>	1
<i>Propionibacterium acnes</i>	1
<i>Streptococcus sanguis</i>	1
<i>Streptococcus anginosus</i>	1
<i>Streptococcus constellatus</i>	1

benzylpenicillin, metronidazole and tetracycline. The lowest range of MIC (0.026–0.125 µg mL<sup>-1</sup>) was observed against amoxicillin + clavulanate and clindamycin. The lowest MIC 90 was observed against clindamycin (0.064 µg mL<sup>-1</sup>). One strain was resistant to erythromycin, and eight strains were resistant to azythromycin (Table 2).

**Discussion**

Current evidence indicates that endodontic infections are directly associated with bacterial invasion into the

dentine, root canal system and periradicular tissues. Treatment is largely dependent on the chemo-mechanical removal of infected pulp and dentine debris to prevent re-infection. Failure rates because of contamination are variable (11–20%) (Smith *et al.* 1993, Field *et al.* 2004, Kojima *et al.* 2004). It is evident that an understanding of the infecting microflora is essential for elucidating the pathogenic mechanisms involved in disease development. Consequently over the years, the composition of the flora has been studied (Van Winkelhoff *et al.* 1985, Sundqvist *et al.* 1989, Gomes *et al.* 1994, Rocas *et al.* 2002) and accumulating evidence indicates that some taxa such as *Porphyromonas* spp., which produce a plethora of virulence factors, may be significant contributors to the development of endodontic infections. Van Winkelhoff *et al.* (1985) found *Porphyromonas endodontalis* in 53% and *Porphyromonas gingivalis* in 12% of periapical abscesses with endodontal origin. In this study, all root canals presented mixed infections dominated by anaerobic bacteria, with a high incidence of *Porphyromonas* spp., especially *P. gingivalis*. In common with other members of the genus, *P. gingivalis* is nonfermentative and cannot be delineated by conventional biochemical tests, which are based upon carbohydrate fermentation tests. Here, its identity was confirmed by its unique signal mass ions revealed by MALDI-TOF mass spectrometry (Fig. 1). Figure 1 shows a comparison of the mass spectral profiles of these closely related species.

Although Haapasalo *et al.* (1986) did not find a relationship between endodontic symptoms and the presence of black pigmented *Bacteroides*, they isolated the asaccharolytic oral species *P. gingivalis* and *P. endodontalis* only in acute infections. *P. gingivalis* may be involved in the induction of acute periapical lesions and sometimes long-standing fistulae associated

Antimicrobial agent	MIC (µg mL <sup>-1</sup> )			Percentage of susceptibility	Number of resistant strains
	50%	90%	MIC range		
Amoxicillin	0.016	0.125	0.016–1.0	100	0
Amoxicillin + clavunate	0.016	0.064	0.016–0.125	100	0
Azythromycin	0.25	2.0	0.016–12.0	60	8
Benzylpenicillin	0.023	0.125	0.016–1.0	100	0
Cephaclor	0.064	0.125	0.016–0.19	100	0
Clindamycin	0.016	0.047	0.016–0.125	100	0
Erytromycin	0.19	1.5	0.016–4.0	95	1
Metronidazole <sup>a</sup>	0.125	0.75	0.016–1.5	100	0
Tetracycline	0.032	0.75	0.016–2.0	100	0

<sup>a</sup>Antimicrobial susceptibility of 18 *Porphyromonas gingivalis* strains (the results of two strains against metronidazole were not included as they could not be verified).

**Table 2** Antimicrobial susceptibility of 20 *Porphyromonas gingivalis* strains isolated from 70 root canals

with periapical infections refractory to conventional therapy (Tronstad *et al.* 1987). Rocas *et al.* (2002) found *P. gingivalis* in 30% of teeth with primary root canal infections associated with symptoms and Gomes *et al.* (2004) found associations between *Porphyromonas* spp. and tenderness to percussion, swelling and wet canals. Sundqvist *et al.* (1989) examined teeth with asymptomatic periapical destruction and teeth with acute periapical inflammation. *Porphyromonas gingivalis* spp. were recovered from all teeth with acute exacerbation but not from any tooth free of pain. In the present study, *P. gingivalis* was most frequently found in symptomatic cases, being statistically related to purulent exudates and pain on palpation.

In the root canal infections where *P. gingivalis* was isolated, four different species were found on average. The activities of neighbouring populations act as external factors affecting the dynamics of growth and the physiological behaviour of that population, resulting in negative pressure or beneficial relationships (Gharbia & Shah 1993, Gomes *et al.* 1994). Gharbia *et al.* (1989) have shown that the proteolytic degradation by *P. gingivalis* is enhanced by the addition of peptidase-producing species such as *Fusobacterium nucleatum*. Furthermore, *F. nucleatum* preferentially incorporates peptides rich in glutamate, when compared with the higher affinity of *P. gingivalis* for aspartate may contribute to the coexistence and interaction of both species (Gharbia & Shah 1991). Such data supports the results of the present research, where *F. nucleatum* was the bacterium most frequently found associated with *P. gingivalis*. Close statistical association between *F. nucleatum* and *P. gingivalis* isolated from root canal infections has also been reported (Jung *et al.* 2000).

*Porphyromonas gingivalis* is one of the most pathogenic species among the group of black-pigmented Gram-negative anaerobes (Sundqvist 1993). The morphology of the cell surface of *P. gingivalis* has been examined in a number of studies. The capsule and LPS are located external to a typical Gram-negative outer membrane, and have antigenic, haemagglutinating and adherence activities (Okuda *et al.* 1991). It has been shown that *P. gingivalis* is the only black pigmented bacteria that possesses cell-bound collagenolytic enzymes, contributing to the high pathogenic potential of this species (Sundqvist *et al.* 1987). Bone destruction in the periapical area is a result of antigenic stimulation from the canal. LPS-free, surface-associated material extracted from *P. gingivalis*, i.e. capsular material, S-layer, slime, fibrils etc. can inhibit bone

formation as well as stimulate bone resorption (Wilson *et al.* 1993).

The therapeutic approach to endodontic infections is similar to treatment of anaerobic infection in general. Surgery plays a key role; debridement of necrotic tissue and drainage of collections of pus are critical. However, in some cases such as swelling, surface erythema, trismus, fever, lymphadenopathy or if surgical treatment cannot be performed because of the general condition of the patient, antimicrobial therapy should be considered with careful selection of the antibiotic to be prescribed.

The establishment of drug susceptibility patterns in pathogenic bacteria is an essential step for developing a rational antimicrobial guideline. In dentistry, when the use of antimicrobial drugs is necessary, the choice of the antibiotic is based on susceptibility pattern research drawn from the international literature.

It has been established that anaerobic bacteria can cause a number of serious human infections, and that they are becoming increasingly resistant to many traditionally anti-anaerobic antibiotics currently in use (Tamaka-bandon *et al.* 1995). Indeed, over recent years, resistance to different antimicrobial agents has been frequently described worldwide and the susceptibility patterns are becoming less predictable (Bianchini *et al.* 1997). These facts emphasize the need to study frequently the susceptibility of anaerobic bacteria.

The epsilometer method (E-test) is a commercial test based on the principle of a diffusion antimicrobial gradient in agar medium as a mean of determining the MIC. It is a plastic strip coated with an antibiotic gradient on one side and an interpretative scale on the other. Although acceptable correlation with the standard techniques has been reported (Citron *et al.* 1991, Croco *et al.* 1995), some discrepancies have also been described (Jorgensen & Ferraro 1998). This test differs from other commercial methods in the flexibility of drug selection. However, the expense is one of the main limitations of this test. Nachnani *et al.* (1992), using the standard agar dilution method as a control, validated the E-test method for antimicrobial susceptibility testing of freshly isolated periodontopathic microorganisms, including *P. gingivalis*, with standard strains from the American Type Culture Collection (ATCC). They found high percentage of agreement and high sensitivity for all antibiotics for both groups of bacteria, documenting the potential feasibility of using the E-test as a quick and useful method for testing antimicrobial susceptibility for oral pathogens. Pajukanta *et al.* (1994) also showed high agreement between

reference-agar dilution method and the E-test, when testing *P. gingivalis* against benzylpenicillin, ampicillin, cefaclor, erythromycin clindamycin, metronidazole etc. and as well as the drugs that were assayed in the present study.

A significant level of resistance to clindamycin, cephalosporins, penicillins and  $\beta$ -lactam antibiotics has been reported among black-pigmented bacteria. However, in this work almost all strains of *P. gingivalis* isolated from infected root canals of teeth with periapical abscesses were susceptible to the assayed drugs. In addition, one strain was resistant to erythromycin and eight strains were resistant to azythromycin. Resistance of *P. gingivalis* to erythromycin (44%) was previously reported by Chan & Chan (2003).

Resistance to metronidazole has not being reported in the literature. In the present study two strains of *P. gingivalis* were resistant to metronidazole. However, such data are not shown in the results because the experiment could not be repeated as these strains were no longer viable and false positive results might have occurred. The reason for the discrepancy usually is too much oxygen present during the incubation, which is the most usual mechanism for false resistant results with metronidazole.

The high sensitivity of *P. gingivalis* to drugs was in accordance with the results found by Pajukanta *et al.* (1993, 1994), Kleinfelder *et al.* (1999, 2000), and Chan & Chan (2003). Strains of *Porphyromonas* spp. were susceptible to  $\beta$ -lactam antibiotics as well as to clindamycin and metronidazole in the study of Behara-Miellet *et al.* (2003). The results of Pajukanta *et al.* (1993) indicated that  $\beta$ -lactamase production is currently not a problem amongst clinical isolates of *P. gingivalis*.

Amoxicillin, a penicillin derivative with a broader spectrum, has been indicated as the first drug of choice in oral infections for patients without allergies. However, its broad spectrum is more than that required for endodontic needs, and its use in a healthy individual may contribute to the global antibiotic resistance problem (Yingling *et al.* 2002).

Clindamycin is the first choice for patients with an allergy to penicillin, but erythromycin can also be chosen. In this study the MIC<sub>90</sub> for clindamycin was 0.047 while for erythromycin was 1.5  $\mu\text{g mL}^{-1}$ .

Azithromycin is a new generation semisynthetic macrolide derivative of erythromycin that has been modified to create a broader spectrum of antibacterial activity and improved tissue penetration. Nevertheless, in this study, 40% of strains were resistant to azithromycin. As the NCCLS has not yet set down the

breakpoint of this agent against anaerobic bacteria, the present study considered the crevicular fluid concentration for the antibiotic (0.5  $\mu\text{g mL}^{-1}$ ) as a breakpoint according to Carrasco *et al.* (1999). This may explain the high percentage of resistance to this antibiotic compared to the others for which the NCCLS breakpoints were used.

Although the present results show a high susceptibility pattern of the *P. gingivalis* strains, some resistance was observed. Patterns of antibiotic resistance can change over the years, making susceptibility testing necessary and to enhance accurate selection of initial antibiotic therapy, as an adjuvant to endodontic treatment. As resistance cannot be prevented, attempt must be made to modulate its progress by reducing antibiotic use and restricting prophylaxis to where it is of proven value.

## Conclusion

The E-test method allowed easy and relevant susceptibility testing against individual isolates of *P. gingivalis* strains in the clinical microbiology laboratory. The *P. gingivalis* pathogen was isolated with frequency from root canals of infected teeth with periapical abscesses. Therefore, attempts to monitor its susceptibility pattern are paramount in avoiding selection of resistant strains. Amoxicillin, which is the first choice penicillin, as well as amoxicillin-clavulanic acid and benzylpenicillin were effective against *P. gingivalis*.

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## References

- Alcoforado GAP, MacKay TL, Slots J (1987) Rapid method for detection of lactose fermentation oral microorganisms. *Oral Microbiology and Immunology* **2**, 35–8.
- Behara-Miellet J, Calvet L, Mory F *et al.* (2003) Antibiotic resistance among anaerobic Gram-negative bacilli: lessons from a French multicentric survey. *Anaerobe* **9**, 105–11.
- Bianchini H, Fernandez Canjila L, Bantar C, Smayevsky J (1997) Trends in antimicrobial resistance of the *Bacteroides*

- fragilis* group: a 20-year study at a medical center in Buenos Aires. *Clinical Infectious Disease* **25**(Suppl. 2), S268–9.
- Bolmstrom A (1993) Susceptibility testing of anaerobes with E-test. *Clinical Infectious Disease* **16**(Suppl. 4), 367–70.
- Carrasco E, Martinez M, Calbacho M, Wilckens M (1999) In vitro activity of amoxicillin, tetracyclines, azithromycin, ofloxacin and metronidazole against *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* strains. *Anaerobe* **5**, 443–5.
- Chan Y, Chan CH (2003) Antibiotic resistance of pathogenic bacteria from odontogenic infections in Taiwan. *Journal of Microbiology and Immunology Infection* **36**, 105–10.
- Citron DM, Ostovari MI, Katlsson A, Goldstein EJC (1991) Evaluation of the E-test for susceptibility testing of anaerobic bacteria. *Journal of Clinical Microbiology* **29**, 2197–203.
- Croco JL, Erwin ME, Jennings JM, Putnam LR, Jones RN (1995) Evaluation of the E-test to determinations of antimicrobial spectrum and potency against anaerobes associated with bacterial vaginosis and peritonitis. *Clinical Infectious Disease* **20** (Suppl. 2) S339–41.
- Field JW, Gutmann JL, Solomon ES, Rakusin H (2004) A clinical radiographic retrospective assessment of the success rate of single-visit root canal treatment. *International Endodontic Journal* **37**, 70–82.
- Gharbia SE, Shah HN (1991) Utilization of aspartate, glutamate and their corresponding peptides by *Fusobacterium nucleatum* subspecies and *Porphyromonas gingivalis*. *Current Microbiology* **22**, 159–63.
- Gharbia SE, Shah HN (1993) Interactions between black-pigmented Gram-negative anaerobes and other species which may be important in disease development. *FEMS Immunology and Medical Microbiology* **6**, 173–7.
- Gharbia SE, Shah HN, Welsh SG (1989) The influence of peptides on the uptake of amino acids in *Fusobacterium*: predicted interactions with *Porphyromonas gingivalis*. *Current Microbiology* **19**, 231–5.
- Gomes BPFA, Lilley JD, Drucker DB (1994) Associations of endodontic symptoms and signs with particular combinations of specific bacteria. *International Endodontic Journal* **27**, 291–8.
- Gomes BP, Pinheiro ET, Gade-Neto CR et al. (2004) Microbiological examination of infected dental root canals. *Oral Microbiology and Immunology* **19**, 71–6.
- Haapasalo M, Ranta H, Ranta K, Shah H (1986) Black-Pigmented *Bacteroides* spp. in human apical periodontitis. *Infection and Immunity* **53**, 149–53.
- Jacinto RC, Gomes BPFA, Ferraz CC, Zaia AA, Souza-Filho FJ (2003) Microbiological analysis of infected root canals from symptomatic and asymptomatic teeth with periapical periodontitis and the antimicrobial susceptibility of some isolated anaerobic bacteria. *Oral Microbiology and Immunology* **18**, 285–92.
- Jorgensen JH, Ferraro MJ (1998) Antimicrobial susceptibility testing: general principles and contemporary practices. *Clinical Infectious Disease* **26**, 973–80.
- Jung Y, Choi B, Kum K et al. (2000) Molecular epidemiology and association of putative pathogens in root canal infection. *Journal of Endodontics* **26**, 599–604.
- Keys CJ, Diane JD, Sutton H et al. (2004) Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infections diseases. *Infection, Genetics and Evolution* **4**, 221–42.
- Kleinfelder JW, Muller RF, Lange DE (1999) Antibiotic susceptibility of putative periodontal pathogens in advanced periodontitis patients. *Journal of Clinical Periodontology* **26**, 347–51.
- Kleinfelder JW, Muller RF, Lange DE (2000) Bacterial susceptibility to amoxicillin and potassium clavulanate in advanced periodontitis patients not responding to mechanical therapy. *Journal of Clinical Periodontology* **27**, 846–53.
- Kojima K, Inamoto K, Nagamatsu K et al. (2004) Success rate of endodontic treatment of teeth with vital and nonvital pulps. A meta-analysis. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **97**, 95–9.
- Mayrand D, Holt SC (1988) Biology of asaccharolytic black pigmented species. *Microbiology Review* **52**, 134–52.
- Nachnani S, Scuteri A, Newman MG, Avanesian AB, Lomeli SL (1992) E-test: A new technique for antimicrobial susceptibility testing for periodontal microorganisms. *Journal of Periodontology* **63**, 576–83.
- Okuda K, Kato T, Ishihara K, Naito Y (1991) Adherence to experimental pellicle of rough-type lipopolysaccharides from subgingival plaque bacteria. *Oral Microbiology and Immunology* **6**, 241–5.
- Pajukanta R, Asikainen S, Forsblom B, Saarela M, Jousimies-Somer H (1993)  $\beta$ -lactamase production and in vitro antimicrobial susceptibility of *Porphyromonas gingivalis*. *FEMS Immunology and Medical Microbiology* **6**, 241–4.
- Pajukanta R, Asikainen S, Forsblom B, Piekola M, Jousimies-Somer H (1994) Evaluation of the E-test for antimicrobial susceptibility testing of *Porphyromonas gingivalis*. *Oral Microbiology and Immunology* **9**, 123–5.
- Rocas IN, Siqueira JF Jr, Andrade AFB, Uzeda M (2002) Identification of selected putative oral pathogens in primary root canal infections associated with symptoms. *Anaerobe* **8**, 200–208.
- Shah HN, Collins MD (1988) Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus *Porphyromonas*. *International Journal of Systemic Bacteriology* **3**, 128–31.
- Slots J (1987) Detection of colonies of *Bacteroides gingivalis* by a rapid fluorescence assay for trypsin-like activity. *Oral Microbiology and Immunology* **2**, 139–41.
- Smith CS, Setchell DJ, Harty FJ (1993) Factors influencing the success of conventional root canal therapy: a five-year retrospective study. *International Endodontic Journal* **26**, 321–33.
- Sundqvist G (1993) Pathogenicity and virulence of black-pigmented Gram-negative anaerobes. *FEMS Immunology and Medical Microbiology* **6**, 125–38.



- Sundqvist G, Carlsson J, Hanstrom L (1987) Collagenolytic activity of black-pigmented *Bacteroides* species. *Journal of Periodontal Research* **22**, 300–6.
- Sundqvist G, Johansson E, Sjogren U (1989) Prevalence of black-pigmented bacteroides species in root canal infections. *Journal of Endodontics* **15**, 13–9.
- Syed AS, Loesche WJ (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–44.
- Tamaka-bandon K, Kato N, Watanabe K, Ueno K (1995) Antibiotic-susceptibility profiles of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* in Japan from 1990 to 1992. *Clinical Infectious Disease* **20** (Suppl. 2), 352–5.
- Tronstad L, Barnett F, Riso K, Slots J (1987) Extraradicular endodontic infections. *Endodontics and Dental Traumatology* **3**, 86–90.
- Van Winkelhoff AJ, Carlee AW, de Graaff J (1985) *Bacteroides endodontalis* and other black-pigmented *Bacteroides* species in odontogenic abscesses. *Infection and Immunity* **49**, 494–7.
- Van Winkelhoff AJ, Van Steenberghe TJM, De Graaff J (1988) The role of black pigmented *Bacteroides* in human oral infections. *Journal of Clinical Periodontology* **15**, 145–55.
- Wilson M, Meghji S, Barber P, Henderson B (1993) Biological activities of surface-associated material from *P. gingivalis*. *FEMS Immunology and Medical Microbiology* **6**, 147–56.
- Yingling NM, Byrne E, Hartwell G (2002) Antibiotic use by members of the American Association of Endodontists in the year 2000: Report of a National Survey. *Journal of Endodontics* **28**, 396–404.

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