Beta-lactamic Resistance Profiles in *Porphyromonas*, *Prevotella*, and *Parvimonas* Species Isolated from Acute Endodontic Infections

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Abstract

Introduction: Susceptibility to beta-lactamic agents has changed among anaerobic isolates from acute endodontic infections. The aim of the present study was to determine the prevalence of the *cfxA*/*cfxA2* gene in *Prevotella* spp., *Porphyromonas* spp., and *Parviomonas micra* strains and show its phenotypic expression. **Methods:** Root canal samples from teeth with acute endodontic infections were collected and *Porphyromonas*, *Prevotella*, and *Parvimonas micra* strains were isolated and microbiologically identified with conventional culture techniques. The susceptibility of the isolates was determined by the minimum inhibitory concentration of benzylpenicillin, amoxicillin, and amoxicillin + clavulanate using the E-test method (AB BIODISK, Solna, Sweden). The presence of the *cfxA*/*cfxA2* gene was determined through primer-specific polymerase chain reaction. The nitrocefin test was used to determine the expression of the lactamase enzyme. **Results:** *Prevotella disiens*, *Prevotella oralis*, *Porphyromonas gingivalis*, and *P. micra* strains were susceptible to benzylpenicillin, amoxicillin, and amoxicillin + clavulanate. The *cfxA*/*cfxA2* gene was detected in 2 of 29 isolates (6.9%). Simultaneous detection of the *cfxA*/*cfxA2* gene and lactamase production was observed for 1 *Prevotella buccalis* strain. The gene was in 1 *P. micra* strain but was not expressed. Three strains were positive for lactamase production, but the *cfxA*/*cfxA2* gene was not detected through polymerase chain reaction. **Conclusions:** There is a low prevalence of the *cfxA*/*cfxA2* gene and its expression in *Porphyromonas* spp., *Prevotella* spp., and *P. micra* strains isolated from acute endodontic infections. Genetic and phenotypic screening must be performed simultaneously to best describe additional mechanisms involved in lactamic resistance for strict anaerobes. *(J Endod 2014;40:339–344)*

Key Words

Anaerobic bacteria, antimicrobial agents, E-test, lactamases, nitrocefin, polymerase chain reaction, susceptibility

Acute endodontic infections harbor heterogeneous, virulent, and unique multispecies microbial communities that are able to promote acute inflammation (1). Strict anaerobes comprise the most frequent species isolated/detected inside the root canal (RC) system of symptomatic infections (2, 3), and species belonging to the genus *Porphyromonas*, *Prevotella*, and *Parvimonas* are frequently found (4–7).

The treatment for acute endodontic infections depends on local microbial reduction that can be achieved by RC debridement and drainage of the apical exudates. In specific clinical situations, their appropriate management requires the administration of antimicrobial agents. Currently, beta-lactam antibiotics are useful therapeutic agents to combat these infections (8). Because of their effectiveness, low cost, and good compatibility, their patient benefits are unquestionable (8).

The resistance to many commonly available antimicrobial drugs has already been reported (9). Changes in the susceptibility patterns of anaerobic isolates over time have been observed, especially among gram-negative bacilli (9). Beta-lactamase production is the most important resistance mechanism in gram-negative bacteria (10). This group of enzymes can open the beta-lactam ring of beta-lactam antibiotics, resulting in inactivation of the drug (10). Beta-lactamase production is encoded by chromosomal genes. The *cfxA* and *cblA* genes were initially described for *Bacteroides vulgatus* and *Bacteroides uniformis* (11, 12). The *cepA*, *cfxA*, and *cblA* genes encode enzymes that can also act as penicilinases that are inhibited by the clavulanic acid. Broad-spectrum beta lactamases are encoded by *cfiA*. Despite the presence of a complex immune system in certain prokaryotic cells that might regulate the ingress of foreign genetic elements (13), the resistance genes can be carried by transposons that enable their spread through microbial strains.

Although beta-lactamase production and concomitant resistance to some beta-lactams is the rule in the *Bacteroides fragilis* group, both phenomena have been increasingly encountered in the *Prevotella* and *Fusobacterium* species (14). The

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increase in isolation of strains that harbor the beta-lactamases gene is associated with the fast genetic interaction intra- and interspecies (15). Recently, the *cfxA2* gene was cloned and sequenced in a *Prevotella intermedia* strain. The *P. intermedia cfxA2* gene shared 98% identity with *cfxA*, the structural gene of a β-lactamase previously described in *B. vulgatus* (16).

Monitoring the usage of antimicrobials and investigating the patterns of drug resistance are essential approaches in determining the causes of antimicrobial resistance. The minimum inhibitory concentration (MIC) for each drug to each bacterial isolate can be assessed using the E-test (AB BIODISK, Solna, Sweden) according to the guidelines and breakpoints suggested by the Clinical and Laboratory Standards Institute (CLSI) (17). The E-test is a simple and reliable method that needs viable bacterial cells in pure culture, allowing for testing the antimicrobial susceptibility of the isolates. It is based on the diffusion of a continuous exponential gradient of antimicrobials from a plastic strip (17). The presence of the *cfxA/cfxA2* gene, which encodes the production of lactamases, can be assessed through polymerase chain reaction (PCR) (18–20). However, it is important not only to describe the presence of the resistance gene but also to determine if the bacteria are functionally resistant to antibiotics. Nitrocefin can be used to determine lactamase production, representing gene activity. The nitrocefin test comprises a chromogenic test in which a very rapid color change takes place as the beta-lactam ring of nitrocefin is hydrolyzed by a beta-lactamase.

Antibiotic resistance genes have been identified in microbial samples collected from RCs with primary and persistent endodontic infections (18). According to Rôças and Siqueira (19), an unexpectedly large proportion of RC isolates, including as-yet-uncharacterized strains, can carry antibiotic resistance genes. However, it is important to determine if these genes are expressed and can exert their pathogenicity in isolates from acute endodontic infections. The aim of the present study was to detect the presence of the *cfxA/cfxA2* gene through molecular methods and to observe its expression through the MIC and degradation of a lactamase substrate.

Materials and Methods

Clinical isolates from acute endodontic infections belonging to the species *Prevotella intermedia/nigrescens, Prevotella buccae, Prevotella oralis, Prevotella disiens, Parvimonas micra, Porphyromonas endodontalis*, and *Porphyromonas gingivalis* strains were selected for the present study. The sampling methods were previously described by Montagner et al (21). Culturing methods for anaerobic isolation and the susceptibility test were performed according to Gomes et al (9). The protocols for the detection of the cfxA/cfxA2 gene were described by Fosse et al (20) and Giraud-Morin et al (22).

Patient Selection and Clinical Examination

The present study was approved by the Ethics Committee in Research of Piracicaba Dental School (State University of Campinas, Piracicaba, São Paulo, Brazil) and the Ethics Committee in Research of the Federal University of Rio Grande do Sul (Porto Alegre, RS, Brazil). Informed consent was obtained from all subjects. Twenty patients presenting to the emergency clinic with spontaneous pain and pulp necrosis determined by vitality tests participated in the study. Subjects with systemic disease and those who had used antibiotics in the last 3 months were excluded from the study. The impossibility of tooth isolation, the presence of dental caries or coronal destruction that allowed the communication between the RC and the oral cavity, previous endodontic manipulation, poor access to the apical region for sampling (as determined by previous radiographic examination), and the presence of marginal periodontitis were also exclusion factors.

Sample Collection from RCs

All clinical procedures were performed under local anesthesia with 2% lidocaine and epinephrine (1:100,000). Briefly, all dental caries and restorations were removed without pulp exposure. The tooth was individually isolated from the oral cavity with a previously disinfected rubber dam. Disinfection of the rubber dam and tooth was performed using 30% hydrogen peroxide and then 2.5% sodium hypochlorite. The solution was inactivated with 5% sodium thiosulfate to avoid interference with the sampling procedure. The pulp chambers were exposed using sterile diamond burs under manual irrigation with sterile saline. Root canals were classified as wet or dry based on the presence of exudate. Samples were collected by inserting sterile paper points to the radiographic working length. All samples were immediately transferred to 1 mL Viability Medium Göteborg Agar III transport medium. In multirooted teeth, the largest canal or the one with periapical radiolucency was chosen.

Laboratory Processing

In the anaerobic chamber, the RC samples were serially diluted 10 times in tubes containing Fastidious Anaerobe Broth (Laboratory M, Bury, UK). A 50-μL sample of each serial dilution as well as of the undiluted sample were plated onto fastidious anaerobe agar (Laboratory M) with 5% defibrinated sheep blood containing 1 mL/L of hemin and 1 mL/L of vitamin K1. Bacterial plates were incubated at 37°C under anaerobic conditions (10% CO_2, 10% H_2, and 80% N_2) for up to 14 days. Samples were also plated onto brain-heart infusion agar with 5% sheep blood (Oxoid, Basingstoke, UK) to allow growth of aerobic and facultative anaerobic microorganisms. Brain-heart infusion plates were aerobically incubated at 37°C for 2 days.

From each bacterial plate, representative colonies of each morphologic type were cultured. Pure cultures were initially characterized according to their gaseous requirements, Gram stain characteristic, and ability to produce catalysis. Strict anaerobes were then selected for further biochemical identification by using the Rapid ID 32 A kit (bioMérieux, Marcy-l'Etoile, France).

The isolates belonging to the species *P. intermedia/nigrescens* (n = 5), *P. buccae* (n = 5), *P. oralis* (n = 3), *P. disiens* (n = 2), *P. micra* (n = 9), *P. endodontalis* (n = 1), and *P. gingivalis* (n = 1) were further analyzed.

Antimicrobial Susceptibility Test

The selected strains' susceptibility was determined by the MIC of benzylpenicillin (PG), amoxicillin (AC), and amoxicillin + clavulanate (XL) using the E-test method. Colonies were suspended in Brucella broth (Oxoid; Basingstoke, Hampshire, UK) to achieve a density corresponding to McFarland turbidity standard 1.0. 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 mg/mL of hemin and 1 mg/mL of vitamin K. The E-test strip was applied separately to the center of the plate with the high-MIC end toward the edge of the plate. The plates were then immediately incubated in an anaerobic chamber (Don Whitley Scientific, Bradford, England) for 24–48 hours. After growth, 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 an interpretative scale (AB BIODISK). An MIC less than or equal to the breakpoints recommended by the CLSI (17) were considered susceptible; those above the breakpoints were considered resistant. The reference values are shown in Table 1. Isolates were

TABLE 1. MIC Reference Values for Anaerobic Strains

Antimicrobial agent	MIC (μg/mL)		
	Susceptible	Intermediary	Resistant
Benzylpenicillin	≤0.5	1	≥2
Amoxicillin	≤4	8	≥16
Amoxicillin + clavulanate	≤2	4	≥8

MIC, minimum inhibitory concentration.

considered susceptible if the MIC for each antibiotic was the following: PG <0.5 mg/L, AC <4 mg/L, and XL <2 mg/L.

Detection of the *cfxA*/*cfxA2* Gene

Bacterial isolated cells belonging to each strain were suspended in 200 μL of ultrapure water free of DNAase and RNAase. One hundred microliters was used to isolate DNA with a Qiagen DNA MiniAmp Kit (Qiagen, Valencia, CA) using the tissue protocol according to the manufacturer's instruction. The DNA was eluted in 100 μL of Tris-HCl + EDTA.

PCR was accomplished in a volume of 50 μL for each sample as follows: 5 μL of PCR reaction buffer (10× Reaction Buffer; Invitrogen, São Paulo, SP, Brazil), 1 μL of a mixture of deoxyribonucleotides of phosphate (Invitrogen), 2.50 μL of a solution of magnesium chloride (MgCl2, Invitrogen), 1.5 μL of a solution of 100 mmol/L of Forward Primer (Invitrogen), 1.5 μL of a solution of 100 mmol/L of reverse primer (Invitrogen), 37.25 μL of ultrapure water free of DNAase and RNAase, 0.25 μL of Taq DNA Polymerase (Platinum Taq, Invitrogen), and 1.0 μL of DNA extracted from the isolated strains.

The intragenic primers 5′-CGTAGTTTTGAGTATAGCTTT-3′ and 5′-GATGTTGCCTATATATGTC-3′ were used to amplify an 802–base pair (bp) conserved region within the *cfxA* and *cfxA2* genes (20, 22). The steps of the PCR cycle included an initial denaturation step (94°C, 10 minutes) and 25 cycles of denaturation (94°C, 1 minute), annealing (58°C, 1 minute), and extension (72°C, 1.5 minutes) followed by a final extension (72°C, 10 minutes). Aliquots of extracted DNA from the strain *B. vulgatus* CLA 341, the wild-type strain from which *cfxA* was initially cloned and sequenced, and aliquots of ultrapure water were used as positive and negative controls, respectively.

The presence of specific amplicons in each sample for the sequences studied was verified by gel electrophoresis on 1% agarose (Invitrogen) in diluted 10× Trisborate EDTA (Invitrogen, São Paulo, SP, Brazil) buffer (pH = 8.0) and stained with ethidium bromide (5 mg/mL, Invitrogen). For each gel, a molecular weight marker of 100 bp was added (DNA Ladder, Invitrogen). The conditions for electrophoresis were 120 V for 45 minutes. After this period, the gels stained with ethidium bromide were visualized under an ultraviolet light transilluminator. Photographic documentation was obtained using VDS Image Master software (Pharmacia Biotech, Cambridge, England). The capture of the images was performed using LISCAP Image Capture (Pharmacia Biotech). Positive reactions were determined by the presence of bands that had the appropriate length (≈802 bp).

Beta-lactamase Production

The production of beta-lactamases was determined by the chromogenic cephalosporin method using reconstituted lyophilized nitrocefin (Glaxo Research, Oxoid, Hampshire, UK). The total content of lyophilized nitrocefin was dissolved in the solution provided by the manufacturer. An isolated bacterial colony from the surface of the agar plate was applied to a nitrocefin solution drop positioned in a 20-μL plastic tube. The plastic tube was incubated at 37°C. A pink reaction developing within 30 minutes indicated beta-lactamase production.

Data Analysis

The MIC was determined, and each strain was classified as susceptible or resistant for each antibiotic. The frequency of *cfxA*/*cfxA2* strains was determined according to the presence of a specific band at the length of 802 bp. Strains were classified as positive or negative for beta-lactamase production.

Results

Bacterial identification, MIC for each antimicrobial agent, PCR results for detection of the *cfxA*/*cfxA2* gene, and beta-lactamase production are summarized in Table 2. Susceptibility profiles revealed that all the *P. disiens*, *P. oralis*, *P. gingivalis*, and *P. micra* strains were susceptible to PG, AC, and XL.

The *cfxA*/*cfxA2* gene was detected in 2 of 29 isolates (6.9%). One strain of *Prevotella buccalis* (Pb6) harbored the *cfxA*/*cfxA2* gene and showed a positive result for lactamase production. However, 1 strain of *P. micra* (Pm4) had the beta-lactamic resistance gene but was not positive for lactamase production and showed a low MIC for all the tested antibiotics. The strains Pb1, Po3, and Pm3 were positive for lactamase production, but the *cfxA*/*cfxA2* gene was not detected through PCR. The strains Pb3, Pb8, Pi/n2, and Pe1 showed a high MIC for the tested antibiotics; however, no positive results were observed for the detection of the gene and the degradation of nitrocefin.

Discussion

Although it is known that resistance to antimicrobial agents changes over time (9) and that resistance genes have been detected in samples from endodontic origin and RC isolates (18, 19), there is little evidence that correlates the presence of resistance genes and their expression in endodontic infections. Using a culture-based approach and molecular methods allowed comparisons to be made between the susceptibility profiles and the presence of a selected resistance gene in strict anaerobes isolated from symptomatic endodontic infections.

Even though molecular methods have been determined to be the best method for describing and depicting the composition of microbial communities associated with endodontic diseases, culture methods enable the study of bacterial physiology and pathogenicity, including the antimicrobial susceptibility profile of clinical isolates (23). In the present study, anaerobic techniques for microbial recovery, cultivation, isolation, and characterization were used to obtain pure cultures of *Porphyromonas*, *Prevotella*, and *Parvimonas* species isolated from acute RC infections. It should be emphasized that the sampling procedures adopted in the present study recovered planktonic strains of the selected genus suspended in the main RC or attached to the dentin walls. It is known that some bacteria can colonize dentin tubules or RC irregularities, contributing to the disease process (1). Environmental challenges may contribute to genetic differentiation between strains such as the presence or absence of specific resistance genes, as observed for *Enterococcus faecalis* strains isolated from different human sources (24). Furthermore, other species that participate in acute primary endodontic infections may carry and express genes associated with microbial resistance to antibiotics.

Porphyromonas species possess pathogenic properties, including fimbriae, proteinases, exopolysaccharides, and hemin-binding proteins (25). The genus *Prevotella* comprises species such as *P. intermedia*/*nigrescens*, *Prevotella melaninogenica*, *Prevotella corporis*, and

TABLE 2. Susceptibility Profile, Presence of the *cfxA/cfxA2* Gene, and Nitrocefin Test of Strains Isolated from Root Canals in Acute Endodontic Infections

		Benzylpenicillin		Amoxicillin		Amox + Clavulanate		*cfxA/cfxA2*	Nitrocefin
Species	Strain	MIC	Profile	MIC	Profile	MIC	Profile	Gene	Test
P. buccae	Pb1	0.19	S	3	S	0.5	S	–	+
	Pb2	0.08	S	0.94	S	0.94	S	–	–
	Pb3	1	R	0.19	S	0.23	S	–	–
	Pb4	0.023	S	0.016	S	0.125	S	–	–
	Pb5	0.047	S	0.016	S	0.032	S	–	–
	Pb6	0.094	S	0.125	S	0.125	S	+	+
	Pb7	0.032	S	0.032	S	0.064	S	–	–
	Pb8	48	R	4	R	3	R	–	–
P. disiens	Pd1	0.008	S	0.016	S	0.016	S	–	–
	Pd2	0.016	S	0.016	S	0.016	S	–	–
P. oralis	Po1	0.016	S	0.016	S	0.016	S	–	–
	Po2	0.002	S	0.016	S	0.016	S	–	–
	Po3	0.016	S	0.016	S	0.016	S	–	+
P. int./nigr.	Pi/n1	0.016	S	0.016	S	0.016	S	–	–
	Pi/n2	256	R	1.5	S	0.25	S	–	–
	Pi/n3	0.016	S	0.016	S	0.023	S	–	–
	Pi/n4	0.002	S	0.016	S	0.016	S	–	–
	Pi/n5	0.016	S	0.016	S	0.016	S	–	–
P. endodontalis	Pe1	24	R	6	R	4	R	–	–
P. gingivalis	Pg1	0.016	S	0.016	S	0.016	S	–	–
P. micra	Pm1	0.002	S	0.016	S	0.032	S	–	–
	Pm2	0.016	S	0.016	S	0.023	S	–	–
	Pm3	0.064	S	0.016	S	0.023	S	–	+
	Pm4	0.016	S	0.64	S	0.094	S	+	–
	Pm5	0.016	S	0.016	S	0.032	S	–	–
	Pm6	0.002	S	0.016	S	0.016	S	–	–
	Pm7	0.006	S	0.016	S	0.023	S	–	–
	Pm8	0.016	S	0.032	S	0.016	S	–	–
	Pm9	0.016	S	0.016	S	0.016	S	–	–

I, intermediate; Pb (n), *Prevotella buccae* (number of the strain); Pd (n), *Prevotella disiens* (number of the strain); Pe (n), *Porphyromonas endodontalis* (number of the strain); Pg (n), *Porphyromonas gingivalis* (number of the strain); *P. int/nigr* (n), *Prevotella intermedia/nigrescens* (number of the strain); Pm (n), *Parvimonas micra* (number of the strain); Po (n), *Prevotella oralis* (number of the strain); R, resistant; S, susceptible.

Prevotella loescheii that are able to produce black-pigmented colonies on blood agar. Nonpigmented *Prevotella* species include *P. buccae*, *P. disiens*, and *P. oralis*. These species are often present in association with other organisms in acute endodontic infections (4, 7). *P. micra* has been frequently isolated/detected in primary endodontic infections (4, 7), and a positive association was observed between *P. micra* and the presence of spontaneous pain (2). Therefore, it is important to describe and understand the pathogenicity components of these groups of anaerobic microorganisms that are strongly related to acute endodontic disease.

Antibiotic therapy should be reserved for patients who have systemic signs and symptoms associated with an endodontic infection, patients with progressive infections, or patients who are immunocompromised (26). In the present study, the *Porphyromonas*, *Prevotella*, and *Parvimonas* isolates were tested against PG, AC, and XL. Lactamics are the first choice of antimicrobial drugs to treat acute orofacial infections. In the 1950s, the entire beta-lactam family of antibiotics consisted of 2 compounds with a limited spectrum of activity: penicillin G and penicillin V. The identification of broad-spectrum penicillin occurred in 1961 with the synthesis of ampicillin and, later in 1970, with amoxicillin (27). Amoxicillin is very closely related to ampicillin with the same spectrum of activity and potency but is much better absorbed when given orally (15, 27). However, in the 1965s, the frequency of clinical isolates producing enzymes that were able to inactivate penicillins increased (15). In 1972, a potent inhibitor of lactamic enzymes with low antibacterial activity was identified and named clavulanic acid (15). The beta-lactam ring of clavulanic acid binds irreversibly to the bacterial beta-lactamase, preventing it from inactivating beta-lactam antibiotics (10, 15).

In the present study, resistance to lactamics was not frequently detected among *Prevotella* isolates. Only 3 of 18 *Prevotella* strains were resistant to PG, 1 of 18 was resistant to AC, and 1 of 18 was also resistant to XL. *Prevotella* species have shown resistance to PG through lactamase production (28, 29). Kuryiama et al (30) showed that both pigmented and nonpigmented *Prevotella* isolates had low susceptibility values to PG. The *P. micra* isolates were not resistant to PG, AC, and XL. Kuryiama et al (29) reported that *P. micra* strains isolated from orofacial infections were resistant to PG. However, other lactamic agents not frequently prescribed in endodontics such as cefmetazole, flomoxef, and imipenem were effective against lactamase-positive or lactamase-negative isolates. *P. gingivalis* (Pg1) was susceptible to PG, AC, and XL. A high MIC for all tested antimicrobial agents was observed for *P. endodontalis* (Pe1). *Porphyromonas* strains isolated from orofacial infections were highly susceptible to PG with low MIC 50 and MIC 90 values (30). According to Warnke et al (31), anaerobic strains isolated from odontogenic abscesses were more susceptible to broad-spectrum lactamic agents (moxifloxacin, amoxicillin with clavulanic acid, and doxycycline) than penicillin G.

The addition of clavulanic acid reduced the MIC values for AC in strains Pb1, Pi/Pn2, Pm4, and Pm8. In the present study, these strains had not expressed lactamase production as detected by using the nitrocefin test. Koeth et al (32) reported that any bacterial isolates that might produce beta-lactamase would not be expected to have similar MIC data for AC and XL. The hydrolytic activity of beta-lactamases can be

overcome by inhibiting components such as clavulanic acid, tazobactam, and sulbactam. These components resemble penicillin and have a high affinity for beta-lactamases. They are slowly or poorly hydrolyzed by enzymes, occupy the active site for long periods, and act like "suicide inhibitors" (10). Therefore, the combination between a beta-lactamase inhibitor and a partner beta-lactam antibiotic has been shown to be effective, especially for AC, because the former was protected by the latter from enzymatic hydrolysis.

The genes bla_{TEM-1} and *cfxA/cfxA2* are responsible to encode lactamase production for many gram-negative bacteria (33). The prevalence of *cfxA/cfxA2* was assessed in the present study because Fosse et al (20) reported that 97% of aminopenicillin-resistant *Prevotella* isolates from subgingival plaque in patients with periodontitis harbored this gene (33). In the present study, the *cfxA/cfxA2* gene was detected in 2 isolates. Rôças and Siqueira (19) detected the presence of the *cfxA/cfxA2* in only 1 *Prevotella* strain isolated from RCs of patients presenting apical periodontitis. Jungermann et al (18) reported that the prevalence of *cfxA/cfxA2* producing strains is low, but the positive strain also showed resistance to amoxicillin. However, when the total bacterial load was assessed for the target gene, it was detected in 11% of the preoperative samples from RCs with primary endodontic infections. Therefore, the *cfxA/cfxA2* gene was not highly prevalent in intraradicular endodontic infections.

Additionally to the detection of the *cfxA/cfxA2* gene, the functional resistance of the strains was assessed through phenotypic resistance tests (ie, E-test and nitrocefin test). High values for MIC, detection of the *cfxA/cfxA2* gene, and a positive response to the nitrocefin test was only observed for Pb6. Pm4 harbor the cfxA/cfxA2 gene. However the MIC values were lower than the breakpoints suggested by the CLSI (AC = 0.64 μg/mL, XL = 0.094 μg/mL). Gatignol et al (34) observed that beta-lactamase–negative *P. intermedia* strains had a range of 0.016–0.094 μg/mL MIC values for AC and XL, whereas a wide range of 0.125–96 μg/mL MICs was observed for the beta-lactamase–positive strains. Therefore, authors also reported that the threshold MIC separating the beta-lactamase–producing strains from the others was 0.125 μg/mL. This breakpoint is lower than the described by the CLSI as the value for bacterial classification. Pm4 also had a negative response to the nitrocefin test. It can be speculated that Pm4 has the potential to become a resistant strain depending on the regulation of gene expression in the cells. On the other hand, Pb6 harbored the *cfxA/cfxA2* gene, and it was expressed as observed for the positive result to the nitrocefin test. However, the MIC values for PG, AC, and XL were lower than the CLSI breakpoints that determine resistance to the antimicrobial agent. The Pb6 behavior confirms the hypothesis that bacteria presenting active *cfxA/cfxA2* genes might show MIC values lower than the CLSI breakpoints. The circumstances under which a particular gene is up-regulated or down-regulated might provide important clues about gene function, which can influence the disease process (18). It must also be emphasized that CLSI breakpoints were determined not only by the range in which specific microbial resistance mechanisms are likely to occur but are also based on the drug concentration usually achieved with normal dosage schedules and on the clinical efficacy of the agent against the isolate has been reliably shown in treatment studies (17).

Pb1, Po3, and Pm3 had a positive response to the degradation of nitrocefin substrate. However, the *cfxA/cfxA2* gene was not detected through PCR. Another behavior was observed for Pe1 and Pb8 strains. Even presenting high values for MIC for all the tested agents, the *cfxA/cfxA2* gene was not detected or expressed for Pe1 and Pb8. Lactamic resistance might be associated with other chromosomal genes families that also encode lactamases production such as TEM, SHV, OXA, ampC, and cepA/CblA (33). Jungermann et al (16) and Rôças and Siqueira (17) also reported a high frequency of the *blaTEM* gene in RCs associated with endodontic infections through PCR. Babic et al (10) described other mechanisms rather than lactamase production that are associated with this resistance profile for gram-negative bacteria. Altered penicillin-binding proteins that exhibit low affinity for beta-lactam antibiotics or lack/diminished expression of outer membrane proteins that restrict the entry of certain beta-lactams into the periplasmic space are alternative mechanisms of resistance associated with the strains. Pe1 and Pb8 showed high MIC values for PG and low MIC values for AC and XL when compared with PG. These data confirm that the antimicrobial spectrum for PG is more limited than the ones shown by AC (15). However, low MIC values for PG compared with AC were observed for Pb2, Pb6, Pd1, Po2, Pi/Pn4, and Pm1. These results could be associated with difficulties in specifying the point where the inhibition halo intersected the E-test strip because the MIC values for both agents were in close proximity on the scale. Therefore, the low MIC variation has not influenced the susceptibility profile of the mentioned strains. The results also suggested that the addition of clavulanic acid has not significantly decreased the MIC value for AC, especially for Pe1 and Pb8. Therefore, other mechanisms than lactamase production might be associated with resistance to lactamics for Pe1 and Pb8.

In conclusion, there is a low prevalence of the gene *cfxA/cfxA2* in *Porphyromonas* spp., *Prevotella* spp., and *P. micra* strains isolated from acute endodontic infections and when present may or may not be expressed. Besides, other mechanisms rather than the expression of the investigated gene might lead to resistance against beta-lactamic antibiotics. Genetic and phenotypic screening must be performed simultaneously to best describe additional mechanisms involved in lactamic resistance for strict anaerobes. This combined approach also allows for understanding and discussing the mechanisms involved in lactamic resistance for strict anaerobes.

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The authors deny any conflicts of interest related to this study.

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