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Prevalence of antibiotic (β-lactams, tetracycline, metronidazole, erythromycin) resistance genes in periodontic infections

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Abstract

Objective: *Porphyromonas gingivalis* and *Prevotella intermedia* are thought to be pathogens in adult periodontitis. Antibiotherapy is usually needed in the treatment of periodontitis being often prescribed empirically. To allow prescription of a specific antibiotic treatment, identification of resistance genes should be performed. The aim of this study was the identification of the presence of *TetM*, *TetQ*, *TEM*, *cfxA*, *MefA*, *ErmB* and *Nim* resistance genes in previously identified *P. intermedia* and *P. gingivalis* isolated from samples collected from periodontal infections.

Method: PCR was used for the identification of *TetM*, *TetQ*, *TEM*, *cfxA*, *MefA*, *ErmB* and *Nim* resistance genes in strains isolated from samples collected from periodontal infections.

Results: It was seen that 8% of isolates had one of the tested tetracycline resistance genes. A total of 32% of β-lactamases resistance genes was observed in isolated strains. It was also observed that 2% of isolates had one of the analysed erythromycin resistance genes. None of the isolates showed the presence of the metronidazole resistance gene.

Conclusions: Most strains harboring β-lactamase resistance genes had been previously identified as *P. intermedia*. No tetracycline resistance gene and a very low percentage of β-lactamase resistance genes were observed in *P. gingivalis* strains.

Introduction

The oral cavity constitutes a special environment in which more than 700 commensal or resident bacterial species, may store and exchange their genetic material [1]. Two of the most common human diseases (caries and inflammatory periodontal disease) result from the accumulation of bacterial biofilms (plaques) on tooth surfaces. Oral health is the result of a balance between the resident flora and defence systems of the host. When this balance is disturbed, commensal and transient bacteria will be responsible for various local infections [1]. Black-pigmented, Gram negative oral anaerobes such as *Porphyromonas gingivalis* and *Prevotella intermedia* are thought to be pathogens in adult periodontitis [2,3]. These bacteria are frequently isolated from patients under periodontitis treatment. Odontogenic local infections require surgical treatment and, if required, a probabilistic antibiotherapy is needed that is effective on most recognized oral pathogens [4].

Treatment of this infection is primarily probabilistic, favoring β-lactam, macrolide–lincosamide–streptogramin and nitromidazole antibiotic families. β-Lactams (especially amoxicillin) are used as the first-line treatment against infections of the oral cavity, because of their suitable antimicrobial spectrum, bactericidal activity, low incidence of adverse effects, and cost-effectiveness [1,5]. Intensive or inadequate use of β-lactam antibiotics in medicine and dentistry favors the selection of bacteria that have acquired resistance to other antibiotics [1].

In most cases, antibiotic prescription is empirical and based on the clinical condition of the patient. As a result, treatment is often inappropriate and leads to the development of bacterial resistance and even multiple resistances [6]. β-Lactams (especially amoxicillin) are used as the first-line treatment against infections of the oral cavity, but, the intensive or inadequate use of β-lactam antibiotics in medicine and dentistry favors the selection of bacteria that have acquired resistance to other antibiotics. Antibiotic resistance genes gradually spread among other pathogenic bacterial species by horizontal gene transfer in resident or transient bacterial populations. So, antibiotic resistance has become a serious problem in nowadays medical and dental practice, and data from the literature suggest that antibiotic resistance in the periodontal microbiota has increased.

The main mechanism of resistance to β-lactam antibiotics in the oral cavity appears to be production of a β-lactamase. This enzyme is frequently detected in diseased periodontal sites and appears to be positively correlated with increased periodontal pocket depth.

For this reason, antibiotic resistances have been under extensive microbiological, biochemical and genetic investigations [4].

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β-Lactamases produced by oral Gram-negative bacilli belong to Ambler class A (CepA, CblA, CfxA, CSP-1 and TEM), class B (CfiA) or class D (FUS-1).

Reports from different countries show an increasing prevalence of patients with oral and subgingival β-lactamase producing bacteria. The use of antibiotics (defined as daily doses/100 000 inhabitants) was significantly higher in Mediterranean countries than in the rest of Europe [7,8]. However, in Portugal there is no data concerning antibiotic resistance of oral flora. Little is known about the variety of β-lactamases in periodontal isolates [9].

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 help in the selection of the appropriate antibiotic in dentistry treatment [10]. Although antibiotic sensitivity can be determined from standard cultural microbiological analysis, this generally takes several days due to the slow growth of fastidious anaerobic bacteria. Since infection can spread rapidly and cause severe complications such as sepsis and obstruction of the airway, such a delay can prove problematic and undesirable. The introduction of PCR-based techniques has resulted in the development of tests that can detect specific pathogens and genes directly and rapidly from clinical samples. Indeed, conventional PCR has already become an important tool in clinical diagnostic and research laboratories [5].

The main goal of this study was to identify the presence of antibiotic resistance genes in strains isolated from periodontal infections. These strains had been previously identified as *P. intermedia* and *P. gingivalis*. Analyzed genes were *TetM* and *TetQ* genes that confer resistance to tetracycline, *cfxA* and *TEM* genes giving resistance to β-lactamases, *nim* gene responsible for metronidazole resistance and *ermB* and *mefA* genes involved in erythromycin resistance. These correspond to the most frequently prescribed antibiotics for periodontitis treatment.

Methods

This study was based on 50 adult patients with ages ranging from 35 to 75 years old, having a diagnosis of periodontitis and that did not receive antimicrobial therapy in the previous 30 days. The sampling was done in a clinical of oral medicine in a Portuguese University School.

Isolation and identification of bacterial strains had been previously performed [11].

For the identification of the tetracycline resistance genes, DNA (5 μL) was amplified in a reaction mixture containing 10 μL of 5x PCR buffer, 3 μL of $MgCl_2$ 25mM, 1 μL of dNTP mixture 10 mM, 2,5 μL of each primer 10 μM and 0,25 μL Taq polymerase in a total volume of 50 μL [12].

Primers used for identification of the *TetM* gene were 5'-GACACGCCAGGACATATGG-3' and 5'-TGCTTTCCTCTTGTTCGAG-3' [12]. PCR was performed according with Koukos, *et al.* [12] as followed: 5 minutes at 94ºC; 37 cycles of 30 seconds at 94ºC, 1 minute at 55ºC and 90 seconds at 72ºC; final extension for 10 minutes at 72ºC.

Primers used for identification of the *TetQ* gene were 5'-GGCTTCTACGACATCTATTA-3' and 5'-CATCAACATTTATCTCTCTG-3' [12]. PCR was performed according with Koukos, *et al.* [12] as followed: 5 minutes at 94ºC; 37 cycles of 30 seconds at 94ºC, 1 minute at 50ºC and 160 seconds at 72ºC; final extension for 10 minutes at 72ºC.

For identification of the *TEM* gene, DNA (5 μL) was amplified in a reaction mixture containing 10 μL of 5x PCR buffer, 4 μL of $MgCl_2$ 25mM, 2 μL of dNTP mixture 10 mM, 5 μL of each primer 10 μM and 0,25 μL Taq polymerase in a total volume of 50 μL [12]. Primers used for identification of the *TEM* gene were 5'-AGATCAGTTGGGTGCACGAG-3' and 5'-CAGTGCTGCAATGATACCGC-3' [12]. PCR was performed according with Koukos, *et al.* [12] as followed: 5 minutes at 94ºC; 35 cycles of 1 minute at 94ºC, 1 minute at 62ºC and 1 minute at 72ºC; final extension for 10 minutes at 72ºC.

For identification of the *cfxA* gene, DNA (5 μL) was amplified in a reaction mixture containing 10 μL of 5x PCR buffer, 4 μL of dNTP mixture 10 mM, 5 μL of each primer 10 μM and 0,25 μL Taq polymerase in a total volume of 50 μL. Primers used for identification of the *cfxA* gene were 5'-GCAAGTGCAGTTTAAGATT-3' and 5'-GCTTTAGTTTGCATTTTCATC-3' [9]. PCR was performed according with Handal, *et al.* [9] as followed: 5 minutes at 94ºC; 25 cycles of 1 minute at 94ºC, 1 minute at 58ºC and 30 seconds at 72ºC; final extension for 10 minutes at 72ºC.

For identification of the *nim* gene, DNA (5 μL) was amplified in a reaction mixture containing 10 μL of 5x PCR buffer, 3 μL of $MgCl_2$ 25mM, 1 μL of dNTP mixture 10 mM, 5 μL of each primer 10 μM and 0,25 μL Taq polymerase in a total volume of 50 μL [12]. Primers used for identification of the *nim* gene were 5'-ATGTTCAGAGAAATGCGGCGTAAGCG-3' and 5'-GCTTCCTTGCCTGTCATGTGCTC-3' [12]. PCR was performed according with Koukos, *et al.* [12] as followed: 5 minutes at 94ºC; 35 cycles of 30 seconds at 94ºC, 1 minute at 62ºC and 1 minute at 72ºC; final extension for 10 minutes at 72ºC.

For identification of the *ermB* and *mefA* genes, DNA (1 μL) was amplified in a reaction mixture containing 4 μL of 5x PCR buffer, 0,4 μL of $MgCl_2$ 25mM, 0,8 μL of dNTP mixture 10 mM, 1 μL of each primer 10 μM and 0,25 μL Taq polymerase in a total volume of 20 μL [13]. Primers used for identification of the *ermB* gene were 5'-CGTACCTTGGATATTCACCG-3' and 5'-GTAAACAGTTGACGATATTC-3' [13]. Primers used for identification of the *mefA* gene were 5'-CCCAGCTTAGGTATACGTAC-3' and 5'-CTGTATGGAGCTACCTGTCTGG-3' [13]. PCR was performed according with Ubukata, Iwata & Sunakawa [14] as followed: 5 minutes at 94ºC; 30 cycles of 20 seconds at 94ºC, 20 seconds at 52ºC and 15 seconds at 72ºC; final extension for 10 minutes at 72ºC.

PCR products were analyzed by electrophoresis on a 1% agarose gel.

Results and Discussion

Concerning all tested antibiotic resistance genes, it was observed a total of 42% of antibiotic resistance genes in strains isolated from periodontal infections (Table 1). These results agree with most studies of other countries that show high levels of antibiotic resistance among anaerobes [12,15-18].

Analysis of β-lactamase resistance genes showed that 32% of total isolates harboured one of the analysed genes. From these, 2% corresponded to the presence of the *cfxA* gene. These were identified as *P. intermedia*. The remaining 30% were strains harbouring the *TEM* gene. Most strains having the *TEM* gene were identified as *Prevotella intermedia* (20%) while 8% belonged to unidentified black-pigmented species and 2% were *P. gingivalis* (Table 1).

Resistance genes identified in this study are in agreement with most published studies that report a high level of β-lactamase resistance in *P. intermedia* [16,17], or lower prevalences of resistance [4,19]. However,

Table 1. Results obtained in the identification of antibiotic resistance genes in strains previously isolated from periodontal infections

Antibiotic Resistance Genes								
		Tetracycline		β-Lactamases		Erythromycin		Metronidazole
Strain	% identified by PCR[11]	*Tet Q*	*Tet M*	*CfxA*	*TEM*	*ErmB*	*MefA*	*Nim*
P. gingivalis	20%	0%	0%	0%	2%	0%	0%	0%
P. intermedia	44%	4%	2%	2%	20%	2%	0%	0%
Other black-pigmented	36%	0%	2%	0%	8%	0%	0%	0%
Total isolates	100%	4%	4%	2%	30%	2%	0%	0%

other studies also observe β-lactamase resistance in *Porphyromonas* sp. [20,21], that was not detected in this study.

In agreement with this work, studies report high prevalence of the *TEM* gene (48-71%) in periodontitis samples [22,23].

It has been reported that *cfxA* and *cfxA2* occur in 100% of β-lactamase-positive *Prevotella* strains from American and Norwegian patients with periodontal disease [24]. French investigators have also demonstrated a 100% prevalence of the resistance genes in β-lactamase-positive *Prevotella* strains [5]. Some antibiotics, such as amoxicillin-clavulanate, cefmetazole, clindamycin and metronidazole, have been demonstrated to be effective for treatment of β-lactamase-positive *Prevotella* infections.

β-Lactamases vary considerably from one organism to another. Some are chromosomally-encoded while others are plasmid-mediated; some are constitutive while others require induction [16]. The presence of resistance genes on plasmids and transposable elements allows resistance to be transferred even between genetically distantly related organisms [9].

Plasmid-specified β-lactamases are present in many Gram-negative bacteria. The most common of these is the TEM-type enzyme originally isolated from ampicillin-resistant *Escherichia coli*.

Concerning tetracycline resistance genes, it was observed that 8% of isolated strains had one of the analysed tetracycline resistance genes. The *TetQ* gene was detected in 4% of total isolates. The *TetQ* harbouring strains were all identified as *P. intermedia*. The *TetM* gene was also detected in 4% of total isolates, corresponding 1% of these to *P. intermedia* strains. The remaining 3% belonged to other black-pigmented unidentified strains. None of these tetracycline resistance genes was present in *P. gingivalis* strains (Table 1).

Ioannidis, *et al.* [22] also observed high levels of *tetQ* (70-80%) and *tetM* (76-82%) genes in samples collected from periodontal infections.

Moreover, unlike our data, another study [12] on implants showed that the most abundant genes were the tetracycline resistance genes. Collins, *et al.* [18] also detected seven tetracycline resistance genes in bacterial isolates from chronic periodontitis, including the *tetQ* gene present in 72% of tested patients.

Isolated strains were also tested for the presence of the erythromycin resistance genes *ermB* and *mefA*. Only 2% of total isolates showed the presence of the *ermB* gene and none harboured the *mefA* gene (Table 1).

The *nim gene*, responsible for metronidazole resistance, was not detected in any of the isolates. Although, in accordance with this work, some studies did not detect the *nim* gene in collected samples [22,23], Xie, *et al.* [25] reported the presence of this gene in strains isolated from periodontal abscesses.

β-Lactam resistance has been associated with resistance to tetracycline (*Tet* genes) and to erythromycin (*erm* genes) [26]. A recent study of oral anaerobes from patients with periodontitis identified a high prevalence (97%) of *CfxA* β-lactamase production by aminopenicillin-resistant *Prevotella* in subgingival plaque [9].

In the same way, it was observed in this work that strains with tetracycline resistance genes (*TetQ* or *TetM*) also harboured the *TEM* gene. Moreover, the *ermB* gene was detected in strains that also had the *TEM* and the *TetM* genes. These results may indicate a combined transfer of antibiotic resistance genes. However, due to the sampling size, these results should be confirmed with a bigger collection of sample isolates from periodontal infections.

Divergence in bacterial frequencies observed in this study and in other reports might be explained by geographical differences and divergences in sampling [4,17] as well as sample size. This study should be enlarged to the analysis of a bigger number of patients carrying periodontal infections.

Due to the increasing prevalence of antibiotic resistance among bacterial strains isolated from periodontal infections, the empirical prescription of these antibiotics as a therapy strategy for periodontitis should be avoided.

This study contributes to the knowledge on subgingival microbiota and its resistance genes present in periodontal infections. Knowing the prevalence of resistance genes can have impact on their clinical prescription and might raise awareness to the appropriate use of antibiotics.

Conclusion

Our results showed that the genes coding for β -lactamases were the most prevalent resistance genes found in periodontal infections, with high prevalence of the *TEM* gene. Most resistance genes were found in strains previously identified as *P. intermedia*. This strain together with *P. gingivalis* are commonly isolated from periodontal infections being considered the most abundant putative black pigmented species [2,4,9,11,18]. When considering isolates from patients with periodontal disease, *Prevotella* sp. has been the most prevalent anaerobe isolate [16,25]. The presence of β -lactamases resistance genes in these strains is probably correlated with the fact that the β-lactams antibiotics are used as the first-line treatment in oral medicine.

As expected, oral bacterial species also carry in addition other resistance genes, such as tetracycline and erythromycin antibiotic resistance genes, probably due to the diversity of mechanisms of transfer of genetic material.

These results support the idea that the prescription of an antibiotic in oral medicine must be a carefully act based in clinical data, and not an empirical attitude, particularly in oral infections such as periodontitis.

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Conflicts of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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