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Detection and genetic characterization of β-lactamases in *Prevotella intermedia* and *Prevotella nigrescens* isolated from oral cavity infections and peritonsillar abscesses

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ABSTRACT

A prospective analysis on β-lactam resistance mechanisms and β-lactamase prevalence was conducted on *Prevotella intermedia* and *Prevotella nigrescens* recovered from patients with chronic periodontitis and peritonsillar abscesses. Both phenotypic and genotypic methods were performed to characterize the β-lactamases, their coding genes and their genetic contexts. Overall, β-lactamase production was observed in 64% (16/25) *P. intermedia* and 23.8% (5/21) *P. nigrescens* ($p < 0.01$). Besides higher β-lactamase production rates were observed in *P. intermedia* (8/16) than in *P. nigrescens* (2/16) recovered from chronic periodontitis, almost all isolates from peritonsillar abscesses were producers (8/9 and 3/3, respectively). *cfxA*, but not *cepA* and *cblA*, was detected in those isolates, which were previously categorized as β-lactamase producers. CfxA producing isolates displayed higher β-lactam MICs than non-producers in both species. The most frequent allele was *cfxA2*, followed by *cfxA3* and a new allelic variant named *cfxA6*. The analysis of the downstream flanking region in the three *cfxA* variants revealed the association with *mobA* of Tn*4555*, suggesting their localization in a mobilizable element. β-lactam resistance and *cfxA* carriage prevalence seems to be not only related to the bacterial species but also to the infection site.
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1. Introduction

Pigmented *Prevotella* spp. belong to the normal oral microbiota, however, they can also be pathogenic and be prevalent in odontogenic infections as well as in head and neck infections such as peritonsillar abscesses [1,2]. Mechanical treatment in periodontal disease or drainage in suppurative processes are usually complemented with β-lactam antibiotic treatment, despite increasing β-lactam resistance [3–6].

β-Lactamase production is the most important β-lactam resistance mechanism in anaerobic gram-negative bacteria. Aside from the *Bacteroides fragilis* group, resistance and β-lactamase production is more frequent in *Prevotella* spp. than in any other gram-negative anaerobe. However, differing β-lactamase-production rates have been reported, depending on species, sample origin or analyzed populations [7–13].

It is difficult to assess β-lactamase prevalence and identity in *Prevotella intermedia* and *Prevotella nigrescens* isolated from oral infections [14–17], as reports are scarce, and, even more, most of them were conducted before modern genetic and proteomic approaches led to accurate identification within this genus. However, a high prevalence of β-lactamase-producing strains has been reported in *P. intermedia sensu lato* and *P. melaninogenica* isolated from odontogenic infections [18,19]. The corresponding enzymes, as well as those initially reported for the *B. fragilis* group, were initially referred as cephalosporinases, inhibited by suicide inhibitors, with common characteristics of functional group 2e [20]. Nevertheless, differences in substrate profile, kinetic parameters and pIs were reported [21–23]. Among them, CfxA2 was initially characterized by Madinier et al. in a periodontal strain of *P. intermedia*. Its coding gene, *cfxA2*, shares 99% identity with *cfxA*, first described in *Bacteroides vulgatus* [24].

The purpose of this study was to analyze the prevalence of β-lactamase-producing isolates in *P. intermedia* and *P. nigrescens* recovered from subgingival plaques and peritonsillar abscesses and to characterize β-lactamase coding genes, their genetic contexts and their association with mobile elements.

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2. Materials and methods

2.1. Bacterial isolates

A prospective study was conducted on *P. intermedia and P. nigrescens* recovered from patients with chronic periodontitis and peritonsillar abscesses during 2000–2004. Patients with periodontitis had not received antibiotic treatment in the previous three months while patients with peritonsillar abscesses had not received antibiotics in the previous week before sampling.

2.1.1. Bacterial identification

Bacteria were grown on Brucella agar plates supplemented with hemin (5 μg/ml), vitamin K (1 μg/ml) and 5% sheep blood (SBBA), and incubated at 37 °C during 48 h in jars under an anaerobic atmosphere generated by commercial pouches (Anaero Pack, Mitsubishi Gas Chemical Company, inc). The isolates were identified based on their phenotypic and biochemical characteristics [25] and stored at −70 °C in glycerol broth and subcultured onto SBBA for further testing.

Genomic DNA was purified from 48 h cultures on SBBA, in an anaerobic atmosphere, using a Genomic DNA Extraction Kit (BIONEER, AccuPrep®, USA).

16S rRNA amplification was conducted as previously described [26] (Table 1), amplicons were purified and sequenced in both strands. Nucleotide sequences were compared with databases using NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/).

2.2. Antimicrobial susceptibility tests and β-lactamase screening assays

2.2.1. β-lactam susceptibility tests

Minimal inhibitory concentrations (MICs) of penicillin, ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, ceftriaxone, ceftazidime and cefoxitin were determined by the agar dilution test according to the Clinical and Laboratory Standard Institute (CLSI) recommendations [27]. Briefly, SBBA were inoculated with approximately 10^5 CFU/spot using a Steers multipoint replicator, and incubated at 37 °C during 48 h in jars under an anaerobic atmosphere. *B. fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used as controls.

2.2.2. β-Lactamase screening assays

Chromogenic detection was performed using 10 μl of nitrocefin (500 mg/l) and 10 μl of a bacterial suspension containing approximately 10^8 CFU/ml [28]. Bacterial colonies were scraped off from SBBA plates and suspended in saline solution obtaining a turbidity comparable to that the 0.5 McFarland standard. Variation of the nitrocefin color from yellow to red in less than 10 min was interpreted as positive.

Phenotypic screening for extended spectrum β-lactamases (ESBL) was performed by double disk diffusion synergy tests using BSSA and inoculated by streaking using an inoculum of approximately 5×10^8 CFU/ml. Disks containing ceftazidime (30 μg) were placed 2 cm from disks containing amoxicillin/clavulanic acid (20:10 μg) and phenylboronic acid (300 μg) as selective inhibitors of class A and C enzymes, respectively, whereas disks containing imipenem (10 μg) were placed 2 cm from disks containing Ethylenediaminetetraacetic acid (EDTA)/sodium mercaptoacetic acid (SMA) (372/900 μg) as selective inhibitors of class B enzymes. *Bona fide* CepA producing *B. fragilis*, CfiA-15 producing *B fragilis* and CMY-2 producing *E. coli* were used as controls. Plates were incubated at 37 °C as previously described. An enhancement in the inhibition zone of ceftazidime and imipenem close to an inhibitor containing disk was considered as an indicator of possible class specific β-lactamase production. All disks were obtained from Britania S.A., Buenos Aires, Argentina.

A preliminary hydrolytic antibiotic profile was determined on crude extracts obtained from bacterial cultures grown under anaerobic conditions during 48 h, in Brain Heart Infusion broth (Britania S.A., Buenos Aires, Argentina) supplemented with hemin (5 μg/ml) and vitamin K (1 μg/ml). β-lactamase activity was revealed using an agar-iodometric method containing ampicillin (500 μg/ml), ampicillin-sulbactam (500 μg/ml), ampicillin-clavulanate (500 μg/ml), cephalotin (1000 μg/ml), ceftazidime (1000 μg/ml) or ceftriaxone (1000 μg/ml) as substrates [29].

2.3. Genotypic characterization of β-lactamases

2.3.1. Detection of β-lactamase coding genes

PCR amplification of *cfxA*, *cepA* and *cblA* were performed in all isolates using total DNA and primers already described by Fosse et al. [16] (Table 1). *cfxA* amplicons (802 bp) were sequenced on both strands and compared with databases as mentioned previously.

2.3.2. Analysis of cfxA flanking regions

The genetic structures surrounding the different *cfxA* alleles were studied in five representative isolates: *P. intermedia* AP29 (*cfxA2*), *P. nigrescens* AP30 (*cfxA2*), *P. intermedia* AP32 (*cfxA3*), *P. intermedia* AP34 (*cfxA3*) and *P. intermedia* AP51 (*cfxA6*).

The region downstream of the *cfxA* gene was accessed by PCR

Table 1
Primers and PCR conditions used in this study.

Gene	Primer name	Primer sequence (5′–3′)	Ref. no.	PCR conditions	Cycles
Bacterial identification					
16S rRNA	27 F	AGAGTTTGATCMTGGCTCAG	[26]	94 °C 45 s, 50 °C 45 s, 72 °C 1 min 30 s	34
	1492 R	GYTACCTTGTTACGACTT			
Detection of β-lactamase coding genes					
bla_{CfxA}	CfxA F	GCTTTAGTTTGCATTTTCATC	[16]	94 °C 1 min, 58 °C 1 min, 72 °C 90 s	25
	CfxA R	GCAAGTGCAGTTTAAGATT			
$bla_{CepA/CblA}$	CepA-F	CAAAGYGACAAYAATGCCTGCG	[16]	94 °C 1 min, 58 °C 1 min, 72 °C 90 s	25
	CepA-R	TSACGAAGRCGGCWAT			
Analysis of *cfxA* flanking regions					
mobA	MobA F	GGCGTTTCTCCCCTGAGAAC	This study	94 °C 1 min, 58 °C 1 min, 72 °C 90 s	25
	MobA R	TCATGTCAAGTTCCTGCCTG			
mobA- bla_{CfxA}	MobA-CfxA F	ACAGGTGAAATCAGAAATACGC	This study	94 °C 1 min, 58 °C 1 min, 72 °C 90 s	25
	MobA-CfxA R	GCAGCTCACAATGATGTTGC			
cfxA upstream region	CfxA-up F	GCCGACAAAGGTACATAACT	This study	94 °C 1 min, 58 °C 1 min, 72 °C 90 s	25
	CfxA-up R	ACAGTCTTGAATGATGGGCG			

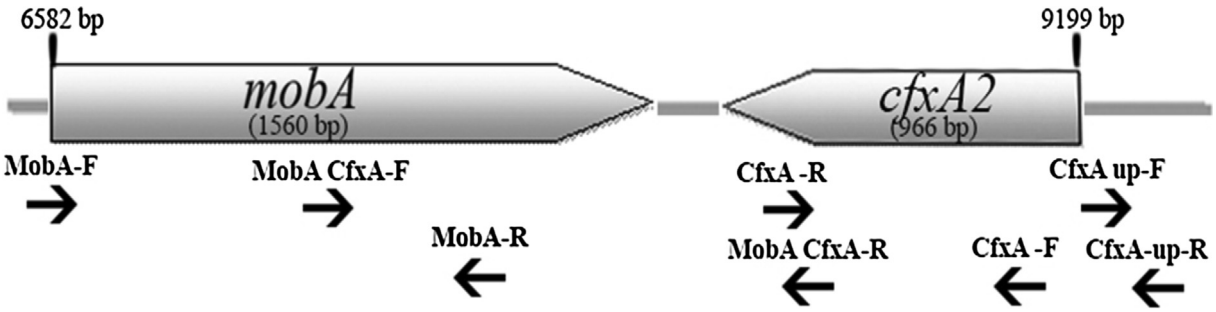

Fig. 1. Description of the PCR strategy to achieve the genetic context of *cfxA*. *mobA* and *cfxA2* genes and their corresponding transcription orientations are indicated by gray arrows. Primers are indicated by black arrows. 6582 bp and 9199 bp correspond to the *mobA* and *cfxA2* origin in the sequence used as reference (access number U75371.3).

mapping using primers designed on transposon Tn*4555* of *B. fragilis* (access number U75371.3) (Table 1) (Fig. 1). A modified thermal asymmetric interlaced- PCR (TAIL-PCR) approach was used to study the *cfxA* upstream region [30]. Purified amplicons were sequenced on both strands as previously mentioned and analyzed using the VECTOR NTI 11.0 software program.

2.4. Statistical analysis

The rates of *cfxA* in different species of *Prevotella* and isolates source were analyzed for statistical significance by the Fisher's exact test (p-values <0.01 were considered significant). Geometric means of the MICs were compared by the non-parametric test of Kruskal–Wallis using the Statistix 8 *software* program for Windows (p-values <0.05 were considered significant).

3. Results

3.1. Bacterial isolates

A total of 46 successive isolates phenotypically identified as *P. intermedia/nigrescens* were included. Based on the 16S rRNA gene sequence analysis, 25 isolates could be accurately identified as *P. intermedia* and 21 as *P. nigrescens*. Thirty-four isolates were recovered from subgingival plaque samples collected from 30 patients (ages 35–60 years), 16 of which corresponded to *P. intermedia* and the remaining 18 to *P. nigrescens*. Twelve isolates were recovered from peritonsillar abscesses from 32 patients (aged 12–46 years), 9 of which were *P. intermedia* and the remaining ones *P. nigrescens*. Seventy eight percent (78%) of the patients with peritonsillar abscesses had a history of repeated tonsillitis and 37% of them suffered recurrent peritonsillar abscesses.

3.2. Antimicrobial susceptibility tests and β-lactamase screening assays

From a total of 46 pigmented *Prevotella* spp. isolates included in this study, 21 (45.7%) rendered a positive nitrocefin reaction. β-lactamase producer rates were higher in isolates from peritonsillar abscess (91.7%) than in those from subgingival plaque (29.4%) ($p < 0.01$) (Table 2).

Production of β-lactamases was observed in 16/25 (64%) *P. intermedia* and in 5/21 (23.8%) *P. nigrescens* isolates ($p < 0.01$). Moreover, differences in the proportion of β-lactamase-producing isolates was observed between species in those isolates recovered from subgingival plaque ($p = 0.023$). Conversely, despite the low number of isolates recovered from abscesses, no difference was found in the rate of β-lactamase-producing isolates between species ($p > 0.01$) since almost all of them were nitrocefin-positive (Table 2).

β-lactamase- producing isolates displayed higher MICs than non-producing ones for both species (summarized in Table 3). Not a single producer was susceptible to penicillin, and only two producing *P. intermedia* isolates were categorized as susceptible to ampicillin according to the current CLSI breakpoints. Activity of ampicillin was restored in the presence of sulbactam. Even if piperacillin and cefoxitin MICs were higher ($p < 0.05$) with respect to non β-lactamase-producing isolates, they would still be under the current resistance breakpoints.

Synergy was observed between clavulanic acid and the oxyiminocephalosporin containing disks in all β-lactamase producing isolates, suggesting the presence of an extended spectrum class A β-lactamase. None of the isolates displayed any synergy with EDTA or phenylboronic acid (however the latter has not been previously tested in anaerobic bacteria).

Crude extracts of β-lactamase-producing isolates were able to hydrolyze ampicillin, cephalotin, ceftazidime and ceftriaxone. The hydrolytic activity against ampicillin was partially reduced by sulbactam and clavulanate.

3.3. Genotypic characterization of β-lactamases

Genotypic detection of *cfxA* was only positive in those isolates previously categorized as β-lactamase producers. The nucleotide sequence of 15/21 *cfxA* amplicons corresponded to *cfxA2* (11 *P. intermedia* and 4 *P. nigrescens*), while four amplicons corresponded

Table 2
Distribution of *P. intermedia* and *P. nigrescens* isolates by sample and β-lactamase production.

Species	Subgingival plaque no. (%)		Peritonsilar abscesses no. (%)		Total	β-lac + no. (%)
	Total	β-lac +	Total	β-lac +		
P. intermedia	16	8 (50.0)[3;5]	9	8[4;5]	25	16 (64.0)[2]
P. nigrescens	18	2 (11.1)[3;6]	3	3[4;6]	21	5 (23.8)[2]
Total	34	10 (29.4)[1]	12	11(91.7)[1]	46	21 (45.7)

β-lac +: β-lactamase-producing isolates. 1 and 2: $p < 0.01$; 3: $p = 0.023$; 4, 5: $p > 0.01$; 6: $p = 0.01$.

Table 3
β-lactam activity and β-lactamase production in *P. intermedia* (25) and *P. nigrescens* (21) isolates recovered from oral and oropharyngeal infections.

Antimicrobial agent	Species	bla_{CfxA}	MIC (μg/ml)			GM[1]	n (%) I + R
			Range	MIC_{50}	MIC_{90}		
Penicillin	*P. intermedia*	bla_{CfxA} + (16)	1–16	4	16	4.00	16(100)
		bla_{CfxA} − (9)	≤0.06–≤0.06	≤0.06	≤0.06	0.06	0
	P. nigrescens	bla_{CfxA} + (5)	2–32	16	16	12.1	5(100)
		bla_{CfxA} − (16)	≤0.06–≤0.06	≤0.06	≤0.06	0.06	0
Ampicillin	*P. intermedia*	bla_{CfxA} + (16)	0.25–>32	1	>32	2.71	16(87.5)
		bla_{CfxA} − (9)	≤0.06–≤0.06	≤0.06	≤0.06	0.06	0
	P. nigrescens	bla_{CfxA} + (5)	1–>32	16	>32	12.1	5(100)
		bla_{CfxA} − (16)	≤0.06–≤0.06	≤0.06	≤0.06	0.06	0
Ampicillin/sulbactam	*P. intermedia*	bla_{CfxA} + (16)	≤0.06–2	0.25	2	0.37	0
		bla_{CfxA} − (9)	≤0.06-≤0.06	≤0.06	≤0.06	0.06	0
	P. nigrescens	bla_{CfxA} + (5)	0.25–4	1	2	1.0	0
		bla_{CfxA} − (16)	≤0.06–≤0.06	≤0.06	≤0.06	0.06	0
Piperacillin	*P. intermedia*	bla_{CfxA} + (16)	1–32	2	32	3.51	0
		bla_{CfxA} − (9)	≤0.06–1	0.25	0.5	0.21	0
	P. nigrescens	bla_{CfxA} + (5)	2–32	32	32	13.9	0
		bla_{CfxA} − (16)	0.125–0.5	0.5	0.5	0.35	0
Piperacillin/tazobactam	*P. intermedia*	bla_{CfxA} + (16)	0.25–2	0.5	2	0.77	0
		bla_{CfxA} − (9)	≤0.06–1	0.125	0.125	0.14	0
	P. nigrescens	bla_{CfxA} + (5)	1–4	2	2	1.74	0
		bla_{CfxA} − (16)	0.125–0.5	0.25	0.5	0.27	0
Antimicrobial agent	Species	bla_{CfxA}	MIC (μg/ml)			GM[1]	n (%) I + R
			Range	MIC_{50}	MIC_{90}		
Ceftriaxone	*P. intermedia*	bla_{CfxA} + (16)	0.125–32	4	16	2.95	1(6.25)
		bla_{CfxA} − (9)	≤0.06–1	≤0.06	0.5	0.17	0
	P. nigrescens	bla_{CfxA} + (5)	1–32	32	32	12.1	3 (60)
		bla_{CfxA} − (16)	≤0.06–0.5	0.125	0.5	0.20	0
Ceftazidime	*P. intermedia*	bla_{CfxA} + (16)	0.125–64	2	32	4.00	4 (25.0)
		bla_{CfxA} − (9)	≤0.06–1	≤0.06	0.5	0.13	0
	P. nigrescens	bla_{CfxA} + (5)	4–32	16	32	13.9	2(40.0)
		bla_{CfxA} − (16)	≤0.06–0.5	0.125	0.5	0.20	0
Cefoxitin	*P. intermedia*	bla_{CfxA} + (16)	0.25–4	0.5	4	0.65	0
		bla_{CfxA} - (9)	≤0.125–0.25	≤0.125	≤0.125	0.14	0
	P. nigrescens	bla_{CfxA} + (5)	0.5–4	2	4	1.74	0
		bla_{CfxA} − (16)	≤0.125–≤0.125	≤0.125	≤0.125	0.13	0

GM: geometric mean; I: intermediate susceptibility, R: resistance. Penicillin and Ampicillin breakpoint: I: 1 μg/ml- R: ≥ 2 μg/ml; Ampicillin/sulbactam: I: 16/8 μg/ml - R: ≥32/16 μg/ml; Piperacillin: I: 64 μg/ml − R: ≥128 μg/ml; Piperacillin/tazobactam: I: 64/4 μg/ml − R: ≥128/4 μg/ml: Ceftriaxone, Ceftazidime and Cefoxitin breakpoint: I: 32 μg/ml; R: ≥64 μg/ml 1- Significances between beta-lactamase positive and negative strains for the examined antibiotics were $p < 0.05$, except for ampicillin and ampicillin-sulbactam for *P. nigrescens*.

to *cfxA3* (3 *P. intermedia* and 1 *P. nigresces*) and the remaining 2 *cfxA* amplicons corresponded to a new allelic variant, named *cfxA6* (2 *P. intermedia*) (acc. number FN376426.1). *cfxA6* displays 99% identity with *cfxA* (acc. number U38243.1) [31], and *cfxA2*, *cfxA3* and *cfxA5* from *Parabacteroides distasonis* (acc. number AY769934). *cfxA6* differs from *cfxA2* in three substitutions translated in one aminoacid substitution, I125V. No association was observed between allelic variants, species and susceptibility patterns.

No positive amplifications for *cepA* and *cblA* could be obtained for any isolate.

3.3.1. Analysis of cfxA flanking regions

Sequences downstream to *cfxA2 in P. intermedia* AP29 and *P. nigrescens* AP30 and *cfxA3* in *P. intermedia* AP32 and AP34 display 99% identity with the *mobA* sequence of Tn*4555* described in *B. fragilis* (acc. number U75371) [32], *B. vulgatus* (acc. number U382431) [31] and *Capnocytophaga ochracea* (acc. Number AY860640 and AY839943) [33] (Fig. 1). The downstresam region of *cfxA6* analyzed *in P. intermedia* AP51 displays 95% identity with *mobA of C. ochracea* (acc. number AY860640 and AY839943) [33], *B. fragilis* (acc. number U75371) [32], and *B. vulgatus* (acc. number U382431) [31]. The presence of promoters upstream of *cfxA* could be detected by the Tail PCR approach only in *cfxA2* (*P. intermedia* AP 29, *P. nigrescens* AP 30) and *cfxA3* (*P. intermedia* AP 32 and AP34) according to Handal et al. [33], but not in *cfxA6* in *P. intermedia* AP51. A ~1000 bp IS (acc. NumberAY860640), described in *C. ochracea* isolates in which *cfxA2* and *cfxA3* are plasmid-encoded beta-lactamases, is absent.

4. Discussion

High β-lactam resistance rates due to β-lactamase production were observed among the studied isolates. The prevalence of β-lactamase-producing pigmented *Prevotella* spp. among subgingival plaque isolates was similar to that observed in isolates recovered from odontogenic infections [9,18,34]. On the other hand, β-lactamase production in isolates recovered from peritonsillar abscesses was significantly higher than in those isolates from patients with periodontitis (91.7% vs. 29.4%) ($p < 0.01$), what may be probably related to selection of resistant strains due to previous (and repeated) antibiotic administration courses for recurrent infections [8,35]. These results are similar to those observed in previous resistance surveillance studies conducted on non-odontogenic body site infections. Almost 95% penicillin resistance was observed among bloodstream infection isolates recovered in Taiwan [36], and 71% in isolates recovered from abdominal, soft tissue, respiratory tract infections and bacteremia in Greece [37]. Furthermore, β-lactamase production was observed in 70% *Prevotella* spp. isolates recovered from different clinical specimens [3] and 83% *Prevotella* spp. recovered from intra-abdominal, obstetric-

gynecologic and body fluid specimens isolates [10].

cfxA carriage (and resistance) was more frequent in *P. intermedia* than in *P. nigrescens* among subgingival plaque isolates, while no differences were observed in the highly resistant peritonsillar abscess isolates. These results suggest than *cfxA* carriage is not only related to the bacterial species but also to the infection type, being more common in suppurative non-oral processes.

No differences have been previously reported in the frequency of β-lactamase production between species and sample source [38,39]. Moreover, it has been reported that β-lactamase production was more frequent in *P. nigrescens* than in *P. intermedia* [40].

CfxA was originally reported in *B. vulgatus*, and later in others species of the *B. fragilis* group [41,42], as able to hydrolyze cefuroxime and cefotaxime more efficiently than penicillins. CfxA was also reported as being responsible for cefoxitin resistance in *Bacteroides* spp.. However as this enzyme displayed low hydrolytic rates on this cephamycin [31], other resistance mechanisms such as alterations in penicillin-binding proteins, impermeability and also efflux pump overexpression have been described to confer high level resistance to this agent [43–45]. Even if a more detailed kinetic characterization is pending, our preliminary results suggest a typical class A ESBL profile, easily inhibited by suicide inhibitors such as sulbactam or clavulanic acid.

At first, CfxA2 was described in *P. intermedia* (acc. number AF118110) [24] and was also reported for *B. ovatus* (acc. number AM940016) [46]. This enzyme was the most frequent variant detected in the pigmented species included in our study, as previously described in *Prevotella* spp. recovered from odontogenic infections, [14–17]. It has been suggested that the K272E substitution in CfxA2 with respect to CfxA increases affinity for cefazolin about 10-fold without modifying the overall catalytic properties on cefoxitin [24]. Several *cfxA2*-like genes have been described in species of *Prevotella*, preserving this characteristic substitution [15].

A few isolates of *P. intermedia* and *P. nigrescens* included in this study presented *cfxA3*, which has never been previously found in these species. This variant was initially described in *Capnocytophaga* spp (acc. Number AF472622 and AY860640) [47], usually found in the oropharyngeal tract and occasionally associated to oral and severe extra-oral infections. The aminoacid sequence of CfxA3 differs from CfxA2 in Y239D, preserving the characteristic substitution, K272E. The Y239D substitution is not supposed to affect the hydrolytic activity of the enzyme [47,48].

The new variant, CfxA6, described in this study, conserved the K272E substitution but differed from CfxA2 in I257V. No differences were observed in the resistance profile of CfxA6-producing isolates with respect to other CfxA producers.

mobA with high level identity to Tn*4555* [24], a non-autonomous conjugative transposon that is potentially involved in the horizontal transfer of β-lactamase genes in *Bacteroides*, *Prevotella* and *Capnocytophaga*, [24,32,41,49] was detected downstream *cfxA*. In contrast, the other β-lactamase coding genes described in *B. fragilis* such as the endogenous cephalosporinase encoded by *cepA* and the metallo-β-lactamase that is encoded by *cfiA* seem to be only vertically transferred, leading to a sharp division between *cfiA*- and *cepA*-positive *B. fragilis* strains [46,50].

The regions upstream of *cfxA2* and *cfxA3* were identical; a 1000 bp deletion upstream of *cfxA* gene with respect to the flanking regions in most *C. ochracea* and *B. fragilis* was observed [33]; Handal et al. already proposed that the *mobA-cfxA* region could represent the minimum DNA sequence responsible for the mobility of the *cfxA* gene in *Bacteroidaceae*. In addition, as the promoter region lies within this fragment in Tn*4555*, it may be important for differences in overall resistance levels.

5. Conclusions

A high prevalence of *cfxA* was observed in the pigmented species of *Prevotella* recovered from the odontogenic and non-odontogenic infections included in this study. Its prevalence seems to be related both to bacterial species and to the infection site characteristics.

The occurrence of ESBL-coding genes in a transposable element in *P. intermedia* and *P. nigrescens* isolates suggest that the oral microbiome seems to be the reservoir of antimicrobial resistance genes that could be transferred between related species and even different non-related genus.

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