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## Original article

Prevalence of antimicrobial resistance genes in *Bacteroides* spp. and *Prevotella* spp. Dutch clinical isolatesA.C.M. Veloo<sup>\*</sup>, W.H. Baas, F.J. Haan, J. Coco, J.W. Rossen

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## ABSTRACT

**Objectives:** The prevalence of resistance genes in two important anaerobic genera, *Bacteroides* and *Prevotella*, was assessed by applying PCR specifically directed to genes of interest.

**Methods:** A total of 101 *Bacteroides* spp. and 99 *Prevotella* spp. human clinical isolates were identified using MALDI-TOF MS. The presence of the resistance genes *cfxA*, *cepA*, *cfiA*, *tetQ*, *ermF* and *nim*, was assessed. Prevalence of resistance genes was compared with the phenotypic resistance against amoxicillin, clindamycin, meropenem and metronidazole.

**Results:** Even though the majority of the *Bacteroides* isolates (95.0%) showed resistance towards amoxicillin, only 52/101 of the isolates harboured one of the resistance genes, accounting for this resistance. Within the genus *Prevotella* the presence of *cfxA* (50/99) almost perfectly matched the amoxicillin resistance (48/99). No difference in prevalence of the *ermF* gene (16/101 and 9/99) and clindamycin resistance (16/101 and 10/99) was observed within *Bacteroides* and *Prevotella*, respectively. Two isolates of *Prevotella* were resistant to metronidazole. One harboured the *nim* gene. One metronidazole-susceptible isolate of *Bacteroides* harboured a *nim* gene. Within the *Bacteroides* and *Prevotella* genera, 6/101 strains and 5/99 isolates harboured three different resistance genes, respectively, among them *tetQ*. *TetQ* is often located on a conjugative transposon, increasing the chance of horizontal gene transfer between isolates.

**Conclusions:** An unknown mechanism in *Bacteroides non-fragilis* isolates causes resistance to  $\beta$ -lactam antibiotics. The fact that the prevalence of the *tetQ* gene among *Prevotella* is increasing and the existence of isolates harbouring three resistance genes are worrisome developments. **A.C.M. Veloo, Clin Microbiol Infect 2019;25:1156.e9–1156.e13**

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## Introduction

Members of the phylum Bacteroidetes are a major part of the human commensal oral and gut microbiota. Two anaerobic genera from this phylum, *Bacteroides* and *Prevotella*, are regularly isolated from human clinical specimens and are known to play an important role in mixed anaerobic infections. Members of the *Bacteroides* group are the most prevalent anaerobic bacteria in infections [1].

As among aerobic bacteria, the antibiotic resistance in anaerobic bacteria is increasing. Decades ago the resistance to clindamycin among the *Bacteroides* group isolates was 6% [2] compared with

21% in 2015 [3]. Resistance genes among anaerobic bacteria can be exchanged by horizontal gene transfer. Conjugative transposons (CTn) and/or plasmids harbour one or several resistance genes that can be transferred under conditions of, for example, low concentrations of antibiotic [4]. The most studied CTn in anaerobic bacteria is CTnDOT, encountered in several *Bacteroides* species. This CTn harbours a tetracycline resistance gene, *tetQ*, regularly accompanied by the *ermF* gene. The latter causes resistance to clindamycin. The conjugative transfer of CTnDOT is a complex series of events, triggered by exposing the bacterial cell to low concentrations of tetracycline. About 80% of *Bacteroides* strains are now resistant to tetracycline, as a consequence of its intensive use in the past [5].

The most important feature for a bacterium to protect itself against  $\beta$ -lactam antibiotics is the production of  $\beta$ -lactamases. Garcia *et al.* [6], described that in *Bacteroides* the presence of a *cfxA* gene is the most frequent indicator for  $\beta$ -lactamase production.

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Furthermore,  $\beta$ -lactamases can be produced by the *cepA* gene, which has only been found in *Bacteroides fragilis* strains [6,7]. The expression of either the *cfxA* or *cepA* gene results in high resistance to penicillins and cephalosporins. A worrisome development is the production of metallo- $\beta$ -lactamases by *Bacteroides* strains. This enzyme, encoded by the *cfiA* gene, cannot be inhibited by  $\beta$ -lactamase inhibitors. Similar to the *cepA* gene, the *cfiA* gene is strictly restricted to strains of *B. fragilis* [8].

A limited number of studies have been performed on the prevalence of antibiotic-resistance genes in *Bacteroides* strains isolated from human clinical specimens [7,9,10]. Most studies focus on the prevalence of the *cfiA* gene in *B. fragilis* [8,11]. To our knowledge, studies focusing on the prevalence of antibiotic-resistance genes in the genus *Prevotella* are scarce.

In this study, we determined the prevalence of resistance genes in *Bacteroides* and *Prevotella* isolates obtained from human clinical specimens, at the University Medical Centre, Groningen, the Netherlands. Besides the most prevalent resistance genes against antibiotics used nowadays, we also determined the prevalence of the *tetQ* gene. In this study the prevalence of *cfxA*, *tetQ*, *ermF* and *nim* genes in *Prevotella* and, additionally, of the *cepA* and *cfiA* genes in *Bacteroides* clinical isolates, was assessed.

## Material and methods

### Bacterial strains

A total of 101 *Bacteroides* and 99 *Prevotella* isolates, isolated from a variety of human clinical specimens, were included in this study. All *Bacteroides* isolates and most *Prevotella* isolates ( $n = 77$ ) were collected, consecutively, in 2016; some *Prevotella* isolates were collected in 2015 ( $n = 11$ ) and 2017 ( $n = 11$ ) to obtain a similar number of isolates as for the *Bacteroides* group. Isolates were revived from the  $-80^{\circ}\text{C}$  freezer, subcultured on Brucella blood agar (Mediaproducs, Groningen, the Netherlands), supplemented with haemin (5 mg/L) and vitamin K (1 mg/L), and incubated at  $37^{\circ}\text{C}$  for 48 hours in an anaerobic jar (Mart Microbiology, Drachten, the Netherlands) or anaerobic cabinet (Don Whitley, Bingley, UK). In both cases the anaerobic environment was created from the same gas mixture (80%  $\text{N}_2$ , 10%  $\text{CO}_2$ , 10%  $\text{H}_2$ ). An anaerobic indicator (Oxoid, Badhoevedorp, the Netherlands) was included. Isolates were identified using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany), as described previously [12]. Obtained log scores were interpreted as advised by the manufacturer, e.g. a log score  $\geq 2$  was recorded as an identification with high confidence, log score  $\geq 1.7$  and  $< 2$  as an identification with low confidence and a log score  $< 1.7$  as no reliable identification. Since MALDI-TOF MS is unable to differentiate between *Bacteroides ovatus* and *Bacteroides xylanisolvens* or between *Bacteroides vulgatus* and *Bacteroides dorei* [13], these species were listed as *B. ovatus/xylanisolvens* and *B. vulgatus/dorei*, respectively. No patient consent or approval from the ethics committee was required as isolates were obtained during routine microbiological diagnostics and upon admission patients can indicate if they do not want leftover material/isolates being used for research and/or improvement of diagnostic procedures.

### Antibiotic susceptibility testing

For each strain, the antibiotic susceptibility profile for amoxicillin, clindamycin, metronidazole and meropenem was determined using an Etest (Biomérieux, Marcy-l'Étoile, France). Briefly, Brucella blood agar was confluent inoculated with a bacterial suspension of 1 McFarland prepared in saline. After applying an Etest strip, plates were incubated in an anaerobic environment, as

mentioned above, at  $37^{\circ}\text{C}$ . After 48 hours of incubation, the MIC value was determined as advised by the manufacturer. The susceptibility testing was part of the diagnostic procedure in our laboratory. Resistance was determined according to the EUCAST guidelines (v6.0).

### Prevalence of resistance genes

Isolates belonging to the genus *Bacteroides* were tested for the presence of *cfxA*, *cepA*, *cfiA*, *tetQ*, *ermF* and *nim* antibiotic-resistance genes and isolates belonging to the genus *Prevotella* were tested for the presence of *cfxA*, *tetQ*, *ermF* and *nim* genes using targeted PCR. As a positive control, a *Bacteroides* strain and a *Prevotella* strain were used in which antibiotic-resistance genes were known to be present, as assessed by whole genome sequencing. An overview of the primers used in the PCR is shown in the Supplementary material (Table S1).

For PCR, DNA was obtained by suspending bacterial colonies in DNase/RNase-free water. The PCR mastermix consisted of 100  $\mu\text{L}$  HotStart-Taq mastermix (100 U/mL DNA polymerase, 400  $\mu\text{M}$  of each dNTP; Qiagen, Hilden, Germany), 4  $\mu\text{L}$  of each of the primers (10  $\mu\text{M}$ ; Eurogentec, Luik, Belgium) and 84  $\mu\text{L}$  DNase/RNase-free water. For each PCR 24  $\mu\text{L}$  PCR mastermix and 1  $\mu\text{L}$  DNA suspension were used, yielding an end concentration of 5  $\mu\text{M}$  per primer. The PCR reactions were run in a T100™ Thermal cycler (Bio-Rad, Hercules, CA, USA), using the conditions presented in the Supplementary material (Table S1). Strains harbouring the *cfiA* gene were also checked for the presence of an insertion sequence (IS) element upstream of the gene according to the method described by Soki et al. [8].

## Results

### Bacterial strains

The most prevalent species within the *Bacteroides* group were *B. fragilis* ( $n = 38$ ), *Bacteroides thetaiotaomicron* ( $n = 21$ ), *B. ovatus/xylanisolvens* ( $n = 11$ ) and *B. vulgatus/dorei* ( $n = 11$ ). Among the genus *Prevotella* the most prevalent species were *Prevotella melaninogenica* ( $n = 21$ ), *Prevotella bivia* ( $n = 17$ ) and *Prevotella buccae* ( $n = 13$ ) (Tables 1 and 2).

### Antibiotic susceptibility and prevalence of resistance genes

Only 2 of the 38 tested *B. fragilis* isolates (5.3%) were meropenem resistant, and the *cfiA* gene was present in six of the strains (15.8%; Table 1). In one isolate an IS-element, upstream of the *cfiA* gene, was present. Of the six isolates harbouring the *cfiA* gene, two showed complete resistance to meropenem and four were intermediate resistant (data not shown). The isolate harbouring both the *cfiA* gene and the IS-element showed complete resistance to meropenem (MIC  $> 32$  mg/ $\mu\text{L}$ ).

Most of the *Bacteroides* isolates were resistant to amoxicillin. Among the 38 *B. fragilis* isolates, 34 were resistant to amoxicillin (89.5%), and the *cepA* and *cfiA* genes (with and without IS-element) were present in 31 and 6 isolates (81.6% and 15.8%), respectively. At least one of these two genes was found in 37 of the tested isolates (97.4%). The *cfxA* gene was found in 14 of the 63 tested non-*fragilis* species of *Bacteroides*. However, 48 isolates of *B. non-fragilis* were resistant to amoxicillin, but did not harbour a *cfxA* gene.

Resistance to clindamycin differed among the *Bacteroides* species. In most cases the prevalence of the *ermF* gene was similar to the number of isolates showing resistance to clindamycin.

**Table 1**  
The number of resistant isolates and the percentage resistance against different antibiotics and the prevalence of corresponding resistance genes in the different species of *Bacteroides*

Species (n)	Resistant strains, n (%)				Prevalence of antibiotic-resistance genes, n (%)						
	Amoxicillin	Meropenem	Clindamycin	Metronidazole	<i>cfxA</i>	<i>cepA</i>	<i>cfiA</i>	IS-element	<i>tetQ</i>	<i>ermF</i>	<i>nim</i>
Breakpoint (mg/L)	R>2	R>8	R>4	R>4							
<i>B. cellulosilyticus</i> (n = 2)	2 (100)	0	1 (50.0)	0	0	0	0	na <sup>b</sup>	2 (100)	0	0
<i>B. clarus</i> (n = 2)	2 (100)	0	0	0	1 (50.0)	0	0	na	2 (100)	0	0
<i>B. fragilis</i> (n = 38)	34 (89.5)	2 (5.3)	2 (5.3)	0	1 (2.6)	31 (81.6)	6 (15.8)	1 (2.6)	23 (60.5)	5 (13.2)	0
<i>B. nordii</i> (n = 2)	2 (100)	0	0	0	0	0	0	na	0	0	0
<i>B. ovatus/xylanisolvans</i> (n = 11)	11 (100)	0	6 (54.5)	0	3 (27.3)	0	0	na	6 (54.5)	4 (36.4)	0
<i>B. salyersiae</i> (n = 4)	4 (100)	0	0	0	0	0	0	na	1 (25.0)	0	0
<i>B. stercoris</i> (n = 2)	2 (100)	0	1 (50.0)	0	1 (50.0)	0	0	na	2 (100)	1 (50.0)	0
<i>B. thetaotaomicron</i> (n = 21)	21 (100)	0	3 (14.3)	0	4 (19.0)	0	0	na	10 (47.6)	2 (9.5)	0
<i>B. uniformis</i> (n = 4)	4 (100)	0	1 (25.0)	0	0	0	0	na	3 (75.0)	1 (25.0)	0
<i>B. vulgatus/dorei</i> (n = 11)	11 (100)	0	2 (18.2)	0	4 (36.4)	0	0	na	8 (72.7)	3 (27.3)	1 (9.1)
<i>Bacteroides</i> spp. (n = 4) <sup>a</sup>	3 (75.0)	0	0	0	1 (25.0)	0	0	na	2 (50.0)	0	0
Total, n (%)	96 (95.0)	2 (2.0)	16 (15.8)	0	15 (14.9)	31 (30.7)	6 (5.9)	1 (1.0)	59 (58.4)	16 (15.8)	1 (1.0)

<sup>a</sup> *Bacteroides* spp. includes *B. caccae*, *B. coagulans*, *B. intestinalis* and *B. massiliensis*.

<sup>b</sup> Not applicable.

A *nim* gene was present in one of the *B. vulgatus/dorei* isolates, showing no resistance to metronidazole. All other tested *Bacteroides* isolates were susceptible to metronidazole and no *nim* gene was present.

Of all tested *Bacteroides* isolates, 59 (58.4%) harboured the *tetQ* gene, among these were all isolates of *Bacteroides cellulosilyticus*, *Bacteroides clarus* and *Bacteroides stercoris*.

None of the 99 tested *Prevotella* isolates showed resistance to meropenem. The prevalence of resistance against the other tested antibiotics differed depending on the *Prevotella* species (Table 2). The prevalence of the *cfxA* gene corresponded with the percentage of resistance against amoxicillin in six of the tested species whereas in the other species a difference in phenotypic resistance and prevalence of the resistance gene was observed.

In general, the prevalence of the *ermF* gene corresponded with the percentage of clindamycin-resistant isolates; nine isolates harboured the *ermF* gene and ten were phenotypically resistant.

Metronidazole resistance was observed for one isolate of *P. melaninogenica* (MIC >256 mg/L) and one isolate of *P. bivia* (MIC 6 mg/L). The *nim* gene was detected in the metronidazole-resistant *P. bivia* isolate. None of the other tested *Prevotella* isolates harboured this gene.

Of the 99 tested *Prevotella* isolates, 30 harboured the *tetQ* gene. Its prevalence was highest in *P. bivia* and *P. bergensis*, i.e. 12/17 (70.6%) and 2/3 (66.7%), respectively.

Several *Bacteroides* isolates harboured more than two antibiotic-resistance genes (Table 3; see Supplementary material, Table S2). Two *B. fragilis* isolates harboured the *cepA*, *tetQ* and *ermF* genes. In addition, two *B. ovatus/xylanisolvans*, one *B. stercoris* and one *B. vulgatus/dorei* isolate harboured the *cfxA*, *tetQ* and *ermF* genes. Four *Prevotella* strains harboured three resistance genes (Table 3; see Supplementary material Table S3). One *P. bergensis*, one *Prevotella disiens* and one *P. melaninogenica* isolate harboured the *cfxA*, *tetQ* and *ermF* genes. In addition, one isolate of *P. bivia* harboured the *cfxA*, *tetQ* and *nim* genes.

## Discussion

In this study, we describe the prevalence of antibiotic-resistance genes in human clinical isolates of *Bacteroides* and *Prevotella* species, isolated in the Netherlands. *Bacteroides fragilis* is the most prevalent anaerobic species isolated from human clinical specimens and also the one studied most extensively. Within this species we encountered a prevalence for the *cfiA* and *cepA* genes of,

**Table 2**  
The number of resistant isolates and the percentage of resistance against different antibiotics and the prevalence of the corresponding resistance genes in *Prevotella* isolates

Species (n)	Resistant strains (n [%])				Prevalence of antibiotic-resistance genes, n (%)			
	Amoxicillin	Meropenem	Clindamycin	Metronidazole	<i>cfxA</i>	<i>tetQ</i>	<i>ermF</i>	<i>nim</i>
Breakpoint (mg/L)	R>2	R>8	R>4	R>4				
<i>P. baroniae</i> (n = 2)	1 (50.0)	0	0	0	1 (50.0)	0	0	0
<i>P. bergensis</i> (n = 3)	2 (66.7)	0	2 (66.7)	0	2 (66.7)	2 (66.7)	2 (66.7)	0
<i>P. bivia</i> (n = 17)	9 (52.9)	0	2 (11.8)	1 (5.9)	12 (70.6)	12 (70.6)	1 (5.9)	1 (5.9)
<i>P. buccae</i> (n = 13)	5 (38.5)	0	0	0	3 (23.1)	2 (15.4)	1 (7.7)	0
<i>P. buccalis</i> (n = 3)	0	0	0	0	1 (33.3)	1 (33.3)	0	0
<i>P. copri</i> (n = 2)	1 (50.0)	0	1 (50.0)	0	0	1 (50.0)	0	0
<i>P. denticola</i> (n = 7)	4 (57.1)	0	0	0	4 (57.1)	0	0	0
<i>P. disiens</i> (n = 4)	1 (25.0)	0	2 (50.0)	0	3 (75.0)	2 (50.0)	2 (50.0)	0
<i>P. histicola</i> (n = 2)	1 (50.0)	0	0	0	1 (50.0)	1 (50.0)	0	0
<i>P. intermedia</i> (n = 4)	1 (25.0)	0	0	0	0	1 (25.0)	0	0
<i>P. jejuni</i> (n = 2)	2 (100)	0	0	0	1 (50.0)	1 (50.0)	0	0
<i>P. melaninogenica</i> (n = 21)	14 (66.7)	0	1 (4.8)	1 (4.8)	14 (66.7)	2 (9.5)	1 (4.8)	0
<i>P. nigrescens</i> (n = 4)	3 (75.0)	0	1 (25.0)	0	0	0	1 (25.0)	0
<i>P. oris</i> (n = 2)	2 (100)	0	0	0	2 (100)	1 (50.0)	0	0
<i>P. timonensis</i> (n = 6)	1 (16.7)	0	1 (16.7)	0	4 (66.7)	2 (33.3)	1 (16.7)	0
<i>Prevotella</i> spp. (n = 7) <sup>a</sup>	1 (14.3)	0	0	0	2 (28.6)	2 (28.6)	0	0
Total, n (%)	48 (48.5)	0	10 (10.1)	2 (2.0)	50 (50.5)	30 (30.3)	9 (9.1)	1 (1.0)

<sup>a</sup> *Prevotella* spp. includes two *Prevotella* spp. isolates, and one of *P. oralis*, *P. loescheii*, *P. massiliensis*, *P. dentalis* and *P. oulorum* isolate.

**Table 3**  
Distribution of antibiotic-resistance genes in *Bacteroides* and *Prevotella* species, harbouring three different antibiotic-resistance genes

Species (n)	<i>cfxA</i>	<i>cepA</i>	<i>cfiA</i>	IS	<i>tetQ</i>	<i>ermF</i>	<i>nim</i>
<i>Bacteroides fragilis</i> (n = 1)	—	—	+	+	+	—	—
<i>Bacteroides fragilis</i> (n = 2)	—	+	—	na <sup>a</sup>	+	+	—
<i>Bacteroides ovatus/xylanisolvens</i> (n = 2)	+	—	—	na	+	+	—
<i>Bacteroides stercoris</i> (n = 1)	+	—	—	na	+	+	—
<i>Bacteroides vulgatus/dorei</i> (n = 1)	+	—	—	na	+	+	—
<i>Prevotella bergensis</i> (n = 1)	+	na	na	na	+	+	—
<i>Prevotella bivia</i> (n = 1)	+	na	na	na	+	—	+
<i>Prevotella disiens</i> (n = 1)	+	na	na	na	+	+	—
<i>Prevotella melaninogenica</i> (n = 1)	+	na	na	na	+	+	—

<sup>a</sup> Not applicable.

respectively, 15.8% and 81.6%. Together they are responsible for the observed resistance against amoxicillin (89.5%) in the isolates of *B. fragilis*. Tran *et al.* [7], observed a prevalence of 90.4% of the *cepA* gene in *B. fragilis* and no *cfxA* gene, while Eitel *et al.* [9] reported, for a European study, prevalences of 78.9% and 14.8%, respectively. In this study we report a prevalences of 81.6% and 2.6%, respectively. None of the strains harboured both genes. Gutacker *et al.* [14] showed that *B. fragilis* strains belong to two genetic groups, based on the presence of *cepA* and *cfiA*: subdivision I, strains harbouring the *cepA* gene, and subdivision II, strains harbouring the *cfiA* gene. In this study, 15.8% of the *B. fragilis* strains belonged to subdivision II and 81.6% to subdivision I. It is noteworthy that in isolates of other *Bacteroides* species the prevalence of the *cfxA* gene was lower than the observed resistance for amoxicillin. This indicates that within non-*fragilis* isolates another antibiotic-resistance gene or mechanism must be present, which is responsible for resistance against  $\beta$ -lactam antibiotics, as reported by Tran *et al.* [7]. The fact that these strains were shown to produce  $\beta$ -lactamase using a cefinase disc (data not shown), supports this hypothesis.

We report prevalences of 15.8% and 9.1% of the *ermF* gene within the *Bacteroides* group and *Prevotella* species, respectively. Boente *et al.* [15] reported a similar percentage within *B. fragilis* strains, which were isolated in a clinical setting, whereas Tran *et al.* [7] reported 28.6%. Within the *B. fragilis* strains used in this study a prevalence of 12.8% was observed. Remarkably, the prevalence of the *ermF* gene was much higher within the *B. ovatus/xylanisolvens* and *B. vulgatus/dorei* species—36.4% and 27.3%, respectively.

The *tetQ* gene is known to be located on a CTn and can be associated with the *ermF* gene on the same CTn [16]. Exposing strains harbouring this kind of CTn to low concentrations of tetracycline stimulates the transfer of this CTn. This process also triggers the excision of other mobile elements out of the genome. In this case not only transfer of the CTn harbouring the *tetQ* and/or *ermF* gene takes place, but also the transfer of other mobile elements harbouring antibiotic-resistance genes [17]. In this study, the prevalences of the *tetQ* gene in *Bacteroides* and *Prevotella* were 58.4% and 30.3%, respectively. Often not only the *tetQ* gene is present, but also other antibiotic-resistance genes, *cfxA* for example. The *cfxA* gene is known to be located on a transposon, Tn4555 [18], as is the *nimK* gene in *P. bivia*, described in a previous study [19].

Generally, it is accepted that *nim* genes play a role in metronidazole resistance, even though the exact resistance mechanism for this antibiotic remains unknown. In this study we encountered a metronidazole-susceptible *B. vulgatus/dorei* strain harbouring a *nim* gene, using the described set of primers. Gal and Brazier [20], reported that silent *nim* genes can become activated when strains harbouring them are exposed to metronidazole for a prolonged period of time. A new *nim* gene, *nimJ*, was found in two multidrug-resistant *B. fragilis* strains [21]. This gene was not detected using the universal *nim* primers, as we did in this study. Therefore, we cannot exclude that more *nim* genes are present in our set of isolates.

Sherrard *et al.* [22], determined the prevalence of *cfxA*, *tetQ* and *ermF* in *Prevotella* strains isolated from individuals with cystic fibrosis and those without. A prevalence of 45% for the *cfxA* gene was observed, which is similar to the prevalence observed in this study, i.e. 50.5%. Furthermore, 4% of the tested strains harboured all three antibiotic-resistance genes. We found a similar number of isolates harbouring three antibiotic-resistance genes, randomly divided among the different species. Arzese *et al.* [23] reported a prevalence of 20% of the *tetQ* gene in *Prevotella* strains, isolated from clinical specimens and healthy individuals, and a prevalence of 8.3% for the *ermF* genes in the same collection of strains. As in this study, the presence of an *ermF* gene did not always correspond with the phenotypic susceptibility for clindamycin of isolates. In this study we were confronted with a higher prevalence of the *tetQ* gene (30.3%) in *Prevotella* isolates solely isolated from human clinical specimens. As the study by Arzese *et al.* [23] was performed on strains isolated in 1995–1997, we hypothesize that the prevalence of the *tetQ* gene (and hereby also the prevalence of CTn) among *Prevotella* strains is increasing.

Unfortunately, not all species are represented by a sufficient number of strains. Also, the role of efflux pumps in unexplained resistance remains uncertain.

The increase in prevalence and the presence of multiple resistance genes in one isolate warrants further research on this topic, for example whole genome sequencing of these isolates, especially since more and more multidrug-resistant anaerobes are reported.

## Transparency declaration

All authors have declared that they have no conflicts of interest. No external funding was received to perform this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2019.02.017>.

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