A multicenter survey of antimicrobial susceptibility of Prevotella species as determined by Etest methodology

ESCMID Study Group for Anaerobic Infections (ESGAI)

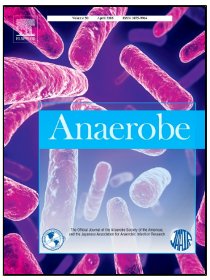
Published in:
Anaerobe

DOI:
10.1016/j.anaerobe.2018.05.005

Publication date:
2018

Document version:
Accepted manuscript

Accepted Manuscript



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PII:	S1075-9964(18)30087-8
DOI:	10.1016/j.anaerobe.2018.05.005
Reference:	YANAE 1886
To appear in:	*Anaerobe*
Received Date:	28 January 2018
Accepted Date:	16 May 2018

Please cite this article as: Nurver Ulger Toprak, Alida C.M. Veloo, Edit Urban, Ingrid Wybo, Ulrik S. Justesen, Helene Jean-Pierre, Trefor Morris, Oncu Akgul, Guven Kulekci, Guner Soyletir, Elisabeth Nagy, Vesna Tripkovic, Eva Leitner, Samo Jeverica, Suzana C. Stingue, Arne C. Rodloff, Joseph Paparaskevas, Wafaa Jamal, Vincent O. Rotimi, Hrisi B. Tokman, ESCMID Study Group for Anaerobic Infections (ESGAI), A multicenter survey of antimicrobial susceptibility of *Prevotella* species as determined by Etest methodology, *Anaerobe* (2018), doi: 10.1016/j.anaerobe.2018.05.005

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26 **Running Title: Antimicrobial susceptibility of *Prevotella* species**

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Highlights

- Twelve antimicrobials were tested on 508 *Prevotella* clinical isolates from 13 countries
- All isolates were susceptible to piperacillin/tazobactam, carbapenems, tigecycline and metronidazole
- High non-susceptibility rate was found to ampicillin, clindamycin, tetracycline and moxifloxacin
- A total of 49 (9.6%) *Prevotella* isolates were resistant to three or more antimicrobials.
- *P. bivia* was most prevalent (n=118), accounting for most resistant isolates.

Abstract

Knowledge about the antimicrobial susceptibility patterns of different *Prevotella* species is limited. The aim of this study was to determine the current antimicrobial susceptibility of clinical isolates of *Prevotella* species from different parts of Europe, Kuwait and Turkey. Activity of 12 antimicrobials against 508 *Prevotella* isolates, representing 19 species, were tested according to Etest methodology. EUCAST, CLSI and FDA guidelines were used for susceptibility interpretations. All *Prevotella* species were susceptible to piperacillin/tazobactam, imipenem, meropenem, tigecycline and metronidazole. Ampicillin/sulbactam and cefoxitin also showed good activity. Ampicillin, clindamycin, tetracycline and moxifloxacin were less active; 51.2%, 33.7 %, 36.8% and 18.3% of isolates were non-susceptible, respectively. A total of 49 (9.6%) isolates were resistant to three or more antimicrobials. *Prevotella bivia* was the most prevalent species (n=118) and accounted for most of the multidrug-resistant isolates. In conclusion, the level of non-susceptibility to antimicrobials, which may be used for treatment of infections involving *Prevotella* species, are a cause of concern. This data emphasizes the need for species level identification of clinical *Prevotella* isolates and periodic monitoring of their susceptibility to guide empirical treatment.

Keywords: *Prevotella*; antimicrobial susceptibility; Etest; multidrug-resistance; surveillance; multicenter study

Introduction

Prevotella species are obligate anaerobic Gram-negative bacteria and important constituents of the oral, upper respiratory, intestinal and female genital tract microbiota. These organisms may be involved in infections of the periodontal region and root canal, head and neck, lower respiratory tract, central nervous system, abdominal and female genital tract. Sometimes *Prevotella* species cause bacteremia, endocarditis and meningitis [1].

Due to the fastidious nature of anaerobes as well as the nature of appropriate collection, transportation and culture methods, only a few clinical laboratories carry out anaerobic culture and even less perform antimicrobial susceptibility testing. This means that antimicrobial treatment is frequently empirical and comprises an antimicrobial agent known to be effective against anaerobes. However, antimicrobial resistance among anaerobic bacteria has been increasing consistently and the susceptibility of anaerobes to antimicrobials has become less predictable [2].

A significant increase of resistance to some antimicrobials has been detected among *Prevotella* species as well [3, 4]. Resistance rates vary among isolates from different infection sources and between geographic locations. However, knowledge about the antimicrobial susceptibility patterns of different *Prevotella* species is limited, as most previous studies, the species of the tested *Prevotella* isolates were not differentiated or only a limited number of isolates representing different species were tested [5]. Recently, significant taxonomic changes have occurred in the *Prevotella* genus and several new species have been described and accepted as human pathogens [6]. These are some of the reasons why providing recent antimicrobial susceptibility data of these isolates is very important for effective empirical treatment.

As a part of the studies of the ESCMID Study Group on Anaerobic Infections (ESGAI), dealing with the antimicrobial resistance of Gram negative anaerobic bacteria [7-9], the aim of this study was to obtain susceptibility data for 12 antimicrobial agents against *Prevotella* isolates originating from human infections, collected in 11 European countries, Kuwait and Turkey. We also wanted to evaluate the resistance patterns of *Prevotella* isolates belonging to different species, isolated from various clinical samples and originating from different countries.

Materials and methods

Bacterial isolates collected for the studies

Prevotella spp. isolated from non-hospitalized or hospitalized patients from the following countries Austria, Belgium, Croatia, Denmark, France, Germany, Great Britain, Greece, Hungary, Kuwait, Netherlands, Slovenia, and Turkey were included in this study. Non-duplicate *Prevotella* isolates were collected between January 2014 and April 2016. Clinical data regarding basic patient information (age, gender), type of infection and sample type was recorded. The organisms were sent to Turkey, and the study was carried out at the Department of Clinical Microbiology, Marmara University School of Medicine, Istanbul. The study was approved by the Ethics Committee of Marmara University, No. 09.2013.0248. The collecting laboratories and the central laboratory both identified the isolates to the species level using conventional and modern diagnostic tests including Matrix Assisted Laser Desorption Time-of-Flight Mass Spectrometry (MALDI-TOF MS [VITEK MS, bioMerieux, France and Biotyper MS, Bruker, Germany]) and 16S rRNA gene sequencing. Following identification, the isolates were stored at -80°C in 10% skim milk until antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

Etest methodology (bioMerieux, France) was used in determining the minimum inhibitory concentrations (MICs) as previously described [10, 11]. The following antimicrobials recommended for anaerobes by the Clinical and Laboratory Standards Institute (CLSI) were tested: ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefoxitin, imipenem, meropenem, clindamycin, tetracycline, tigecycline, moxifloxacin and metronidazole [13]. Erythromycin, the first-choice antimicrobial for patients with penicillin allergy, was also included in the study [12]. The isolates were suspended in Brucella broth (BD Difco, USA) to match a density of 1 McFarland. The bacterial suspensions were swabbed on Brucella blood agar supplemented with vitamin K1 and haemin (Sigma-Aldrich, Germany), and the Etest strips were applied within 15 minutes. The plates, which the Etest strips had been applied were incubated in anaerobic boxes (GENbox, bioMerieux, France) at 37°C for 48 hours. *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were included as quality control strains for every assay.

The MIC results were interpreted according to the breakpoints described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14]. Breakpoints recommended by CLSI were applied for cefoxitin, tetracycline and moxifloxacin since there are no EUCAST breakpoints for these antimicrobials [13]. In the case of tigecycline we used the breakpoint suggested by the U.S. Food and Drug Administration (FDA) [15]. Some anaerobes are a good target for therapy with erythromycin as well, however clinical breakpoints are unavailable in both EUCAST and CLSI guidelines, so this antimicrobial agent was only tested to collect data on the erythromycin MIC distribution among *Prevotella* species for further studies [16].

We used antimicrobial consumption data from the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) at ECDC in order to compare bacterial non-susceptibility rates and antimicrobial consumption in 11 European countries [17]. The results were analyzed with Pearson's correlation using R Software (The R Foundation for Statistical Computing platform), specifically R commander package (R, version 3.5.0). A p value ≤ 0.05 was considered statistically significant [18].

Results

Clinical isolates of *Prevotella* spp.

The number of *Prevotella* isolates submitted per country varied between 14 and 79, most isolates (15.6%) were collected in Turkey. All 508 *Prevotella* isolates included in this study were identified at the species level using 16S rRNA gene sequencing. Altogether 19 different species were identified (most *P. bivia* (n=118), *P. buccae* (n=68)). Some rare species such as "*P. conceptionensis", P. veroralis* and *P. corporis* were also present among the isolates. See Table 1 for distribution of *Prevotella* species by country included in the study.

Patient age range was 1 to 91 years (mean age, 43; median age, 45 years). Specimens were collected from various clinics. The majority of patients (30.1%) were treated at surgical departments, followed by dentistry (17.3%) and departments of internal medical (14.2%). The proportion of patients from gynecology and urology departments was 5.9%. The strains were mainly isolated from abscesses (37.4%) and wounds (18.1%). Other major categories of clinical samples were from intraoral infections (17.4 %), biopsy (7.7%) and sterile body fluids (7.7%).

Antimicrobial susceptibility testing results

The MIC ranges, MIC_{50}, MIC_{90}, the proportions of susceptible, non-susceptible and resistant isolates of 508 *Prevotella* spp. to 12 different antimicrobials is presented in Table 2. All *Prevotella* isolates were susceptible to piperacillin/tazobactam, imipenem, meropenem, tigecycline and metronidazole. Ampicillin/sulbactam and cefoxitin also showed good activity. However, for both antimicrobials intermediate resistance was encountered in 0.4% of the *Prevotella* isolates. Non-susceptibility to ampicillin, clindamycin, tetracycline and moxifloxacin (resistant isolates and those with intermediate resistance taken together) was detected at different rates; 51.2%, 33.7% 36.8% and 18.3%, respectively (Table 2).

Ampicillin non-susceptible *Prevotella* isolates were isolated from pulmonary samples (76.9 %), abscesses (61.1%), bone biopsies (58%) and wounds (56.5%). The highest prevalence of ampicillin non-susceptible strains was detected among isolates from orthopedic clinics (83.3%), followed by dermatology (75%), medical departments (66.6%) and dentistry (26.1%) (data not shown).

In case of clindamycin, all non-susceptible isolates were in the resistant category. Most resistant isolates were obtained from orthopedic (66.6%) and dermatology clinics (50%). These isolates were associated with wounds (47.8 %) and diabetic foot infections (40%). Seven of the 12 *Prevotella* strains isolated from bone biopsy samples were highly resistant (MIC >256 mg/L) to clindamycin.

Tetracycline non-susceptibility was found in 36.8% of the isolates, and in surgical site infections (47.8%), abscesses (47.3%), bone and wound infections (41%). Most non-susceptible organisms were obtained from the dermatology (75%), diabetic (50%) and orthopedic (50%) departments.

Of all isolates, 18.3% were non-susceptible to moxifloxacin. Most of the moxifloxacin non-susceptible *Prevotella* spp. were isolated from pleural effusion (83.3%).

MICs of erythromycin ranged between < 0.016 and > 256 mg/L, with a MIC_{50} of 2 mg/L and a MIC_{90} of >256 mg/L. The distribution of erythromycin MIC's was also evaluated because erythromycin is an alternative for penicillin (in case of penicillin allergy) in various types of infections. There were more isolates with a high MIC (≥4 mg/L) for erythromycin among ampicillin non-susceptible *Prevotella* spp. compared with all the *Prevotella* isolates (Figure 1).

Only 26.6% of *Prevotella* isolates were susceptible to all antimicrobials, the remaining isolates were non-susceptible to at least one of the tested antimicrobial agents. Non-susceptibility for one antimicrobial, frequently ampicillin, was observed for 28.7% of the isolates (not shown). With the exception of moxifloxacin, the highest non-susceptibility rates against antimicrobials were observed among the *P. bivia* isolates. The proportions of non-susceptibility among these isolates to ampicillin, clindamycin, tetracycline and moxifloxacin were 70.3%, 48.3%, 63.4% and 50.4%, respectively. The second highest proportion of moxifloxacin non-susceptible isolates was found in the *P. nanciensis* isolates (76.9%). See Table 3 for non-susceptibility rates of ampicillin, clindamycin, tetracycline and moxifloxacin in different *Prevotella* species.

A total of 49 isolates were resistant to three or more antimicrobials. At the species level, multidrug-resistant isolates were mostly found among *P. bivia* isolates. While only 5.9 % of the *P. bivia* isolates proved to be susceptible to all tested antimicrobials, 27.1% of the *P. bivia* isolates showed resistance to three or more antimicrobials. See Table 4 for distribution of multidrug-resistant *Prevotella* isolates encountered in the different countries.

The prevalence of *Prevotella* species non-susceptible to ampicillin, clindamycin, tetracycline and moxifloxacin varied considerably between countries (Table 5). The highest non-susceptibility rate was detected among isolates from Kuwait. These isolates were non-susceptible to ampicillin (92.9%), clindamycin (64.3%), tetracycline (57.1%) and moxifloxacin (43.1%). However, this country submitted the lowest number of strains, namely 14 isolates. Among the isolates collected from Germany, the prevalence of isolates non-susceptible to ampicillin, clindamycin and moxifloxacin was found to be low compared to the other countries, 5.9%, 14.7% and 8.8%, respectively (Table 5). The percentage of multidrug-resistant isolates was also the highest among the isolates from Kuwait (28.6%). No multidrug-resistance was found among the German isolates (Table 4).

We found that in the 11 countries, there was a statistically significant correlation between the antimicrobial non-susceptibility rate for ampicillin (a β-lactam agent) ($p=0.02$) and the antimicrobial consumption data provided by the European Antimicrobial Consumption Network Observation (ESAC-Net) at ECDC [17]. There was no such correlation found between non-susceptibility and antimicrobial consumption for clindamycin, tetracycline and moxifloxacin in these countries. When the study results were evaluated by country, the highest rate of clindamycin resistance among *Prevotella* isolates (55.6%) was found in Greece, the country with the highest use of macrolide, lincosamine, streptogramine (MLS) agents. A similar result was observed between the highest consumption of quinolones in Hungary and the highest rate of non-susceptibility to moxifloxacin (31.9%) amongst the countries with data available. Very low use of penicillins in Germany was also reflected by the lowest number of strains showing non-susceptibility to ampicillin (5.9%). On the other hand, in The Netherlands the consumption of penicillins was also very low compared to other countries, but the *Prevotella* isolates collected for this study showed a high amount of ampicillin non-susceptible isolates (44.4%) (Table 5).

Discussion

In the present study antimicrobial susceptibility patterns of 508 *Prevotella* isolates collected from clinical specimens in 11 European countries, Kuwait and Turkish expert laboratories between 2014 and 2016 were tested. We wanted to use a simple MIC method, which can be used in routine clinical microbiology laboratory work. For that reason, we chose the Etest, which is a simple, rapid and reliable method. Our results display three important issues in the antimicrobial susceptibility of *Prevotella* isolates today. First, there were considerable non-susceptibility rates to ampicillin, clindamycin, tetracycline and moxifloxacin among the tested isolates. Second, 9.6% of the isolates were multidrug-resistant. Third, among the tested 19 *Prevotella* species, *P. bivia* was the most prevalent and most resistant species found, when compared to other *Prevotella* species.

The first Europe-wide study about the antimicrobial susceptibility of *Prevotella* spp., together with other Gram-negative anaerobes, was carried out in 1995-1996. A total of 488 isolates were studied. These isolates represented nine *Prevotella* species, *P. bivia* being most prevalent, from 13 European countries. Although there were differences between the species, the highest resistance rates were in *P. bivia*, *Prevotella* species were highly resistant to penicillin and tetracycline [19]. The most recent representative surveillance studies have indicated high rates of penicillin resistance among *Prevotella* species [4, 5]. In the present study, a total of 51.2% of isolates were found to be non-susceptible to ampicillin, though susceptibility profiles do vary by *Prevotella* species, source of isolation and geographic origin. In previous studies, some authors observed that two thirds of *Prevotella* isolates were penicillin resistant, and some researchers reported resistance rates of > 90% in certain areas [20-22]. In *Prevotella,* resistance to the β-lactams is mainly due to β-lactamase production. In

288 average laboratories, β-lactamase production is mostly determined by using a nitrocefin [1-3].
289 β-lactamase activity was not evaluated in this study because pigmented *Prevotella* species
290 gave indistinguishable results. However, we presumed that the resistance to ampicillin was
291 caused by β-lactamase production as, except for the two intermediate-resistant *Prevotella*
292 species, combinations of β-lactams with β-lactamase inhibitors had good antimicrobial
293 activity against the ampicillin resistant isolates. As mentioned previously, combinations of β-
294 lactams with β-lactamase inhibitors had excellent activity against other anaerobes as well, so
295 they are good candidates for effective empiric treatment of pure or mixed *Prevotella*
296 infections [16].

297 Several studies have reported an increase in clindamycin resistance among *Prevotella*
298 species. The clindamycin resistance rate varies depending on time period, country, the
299 population studied and the source of the specimen. In Belgium, the clindamycin non-
300 susceptibility rate increased continuously from 8% in 1987 to 31% in 2011-12, while in
301 Bulgaria resistance to clindamycin remained stable (13%, 2007-2009) [23, 3]. Resistance
302 rates in other countries have been reported to be, between 0% and 33% [4, 24-26]. In the
303 present study, a total of 33.7% of isolates were resistant to clindamycin. Resistance rates of
304 isolates from Greece (55.6%) and Kuwait (64.3 %) had increased compared to previous
305 studies, 31% in 2006-2007 and 28.8% in 2002-2007 [27,21]. However, the differences may be
306 due to the testing of different *Prevotella* species or real development of resistance in the last
307 decade.

308 Data on *Prevotella* species susceptibility to tetracycline are limited [27, 28].
309 Tetracycline resistance rate in *P. intermedia* isolated from Spanish patients with periodontitis
310 was approximately 30% [24]. However, the resistance rate was higher (36.7%) in patients
311 who had received treatment compared to those who had not (19.1%). Conversely, the non-
312 susceptibility of *Prevotella* spp. has decreased 2.5-fold from 1987 to 2005 in Belgium and

2.1-fold in Bulgaria over six years because of a decrease in tetracycline consumption [3, 29]. The present study shows the variability of tetracycline non-susceptibility patterns among *Prevotella* isolates originating from different countries ranging from 24% to 59%. Non-susceptibility to tetracycline was associated with national antimicrobial consumption and possibly with previous antimicrobial treatments.

Moxifloxacin is recommended to adult patients for the treatment of various types infections, caused by *Prevotella* species and a broad range of other anaerobes and aerobes [2]. In earlier studies, clinical isolates were fully susceptible to moxifloxacin and over time, resistance rates have increased up to 36% [30, 30, 4, 31]. There is very little comparable data on moxifloxacin resistance from European countries. Two multicenter surveys conducted in Belgium showed that *Prevotella* non-susceptibility to moxifloxacin showed highly similar rates in 2004 (24%) and 2011–12 (23%) [29, 23]. Papaparaskevas et al. [32] found that 42% of the 141 *Prevotella* spp. tested were non-susceptible to moxifloxacin. This resistance was particularly frequent among *P. oralis* and *P. bivia* isolates, for which moxifloxacin resistance rates were 62% and 71%, respectively. In our study, 18.3% of all tested *Prevotella* isolates were non-susceptible to moxifloxacin. The rates of non-susceptibility to moxifloxacin in Belgian and Greek isolates in this study were lower than the rates from the previous Belgian and Greek studies, 15.6% and 11.2% respectively. The differences in non-susceptibility rates may be due to differences in the number of isolates, the kind of *Prevotella* species, and the origin of the strains that were tested. In our study, non-susceptibility to moxifloxacin is shown to vary considerably between countries, ranging from 6.6% to 43.1%. This suggests that moxifloxacin should be used cautiously in the empirical treatment of anaerobic infections involving *Prevotella* spp.

In this study, the most prevalent species, *P. bivia*, was more resistant to antimicrobials than the other *Prevotella* species. It is also noteworthy that the majority of multidrug-resistant

organisms were also *P. bivia* strains. As member of the human microbiota, *P. bivia* may represent a reservoir for resistance genes, and may contribute to resistance gene transmission [1]. This data makes highlights the need to study the pathogenesis and resistance mechanisms of *P. bivia* in the future.

The variation in resistance rates between isolates originating from different countries is closely related to use of antimicrobials [33]. National antimicrobial consumption data can offer a partial explanation for resistance due to antimicrobial exposure and selection [34]. We know that patients with low compliance and self-medication may also contribute to resistance. Moreover, antimicrobial resistance can differ from one city to another or even between hospitals within the same city. In this study data representing each country was presented. Although the study did not cover all types of *Prevotella* species, this survey represents the most comprehensive report on the susceptibility patterns of the most prevalent *Prevotella* species. Recent antimicrobial susceptibility patterns of these isolates can help develop strategies to prevent antimicrobial resistance in *Prevotella* species.

In conclusion, ampicillin-sulbactam, piperacillin/tazobactam, cefoxitin, metronidazole and tigecycline display high in vitro activity against *Prevotella* spp. and they all remain good candidates for empiric therapy. Imipenem and meropenem were also found to be very active, but the usage of carbapenems should be reserved for serious mixed infections, potentially accompanied by other resistant organisms. The high rates of resistance to ampicillin, clindamycin, tetracycline and moxifloxacin indicate that these antimicrobials should not be used for empirical treatment of infections caused by *Prevotella* species without prior antimicrobial susceptibility testing. In addition, because of multidrug-resistance in certain *Prevotella* species such as *P. bivia*, we recommended routine identification at the species level and antimicrobial susceptibility testing of isolates for the confirmation of the appropriate antimicrobial therapy.

Acknowledgements

This study was supported by research grants from the ESCMID for ESCMID Study Group on Anaerobic Infections (ESGAI). We thank Mike Cox for providing us transport medium.

We also thank to Sedef Glover for editing the manuscript.

Transparency Declaration

The authors declare that they have no conflicts of interest.

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504 Figure 1. Erythromycin MIC distribution among *Prevotella* species.

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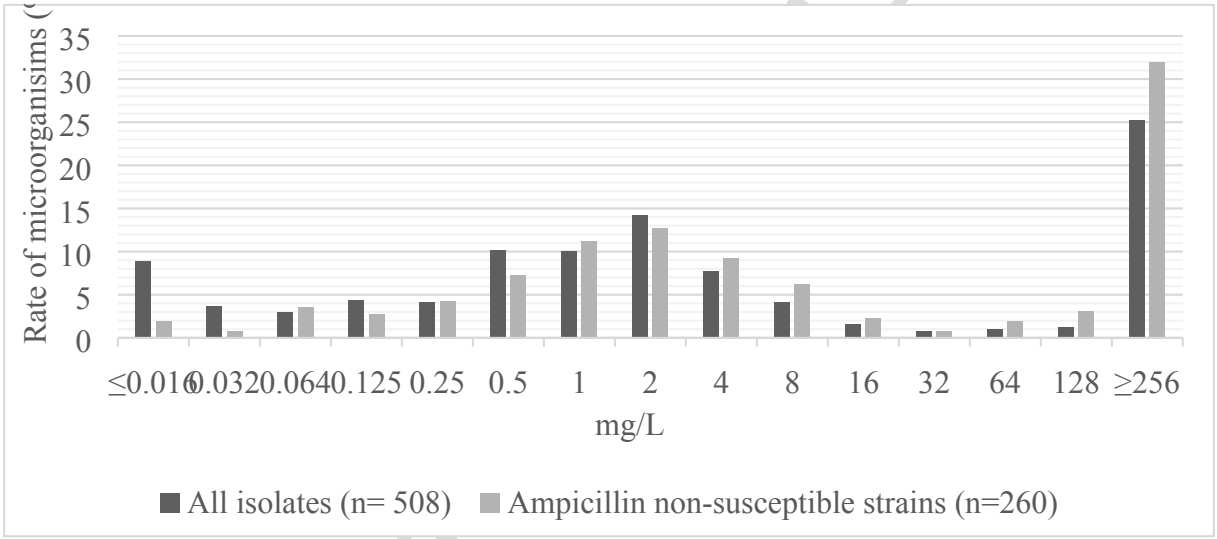

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508 Table 1. Distribution of *Prevotella* species submitted by the different countries

Prevotella spp. (n)	Countries* (no of isolates)												
	AT	BE	HR	DK	FR	DE	GB	GR	HU	KW	NL	SI	TR
P. baroniae (13)		1	1		2		1		5		3		
P. bergensis (13)		2		5	3						1	2	
P. bivia (118)	7	10	16	4	9	7	7	6	23	12	8		9
P. buccae (68)	5	7	2	8	5	5	4	2	6		9	3	12
P. buccalis (4)		2					1					1	
"P. conceptionensis"(1)									1				
P. corporis (5)		1					1	1			2		
P. denticola (57)	4	3		3	9	5		1	7		9	5	11
P. disiens (31)	4	3	7	1	1	2		2		1	2	4	4
P. histicola (11)		1				6			1		2		1
P. intermedia (21)	1	1				1	8	2				1	7
P. melaninogenica (44)	1	2	5	8	3	2		2	2		3	6	10
P. nanceiensis (13)	1	1		7	1							3	
P. nigrescens (62)	3	3		1	3	1	22	1	2	1	4	1	20
P. oralis (7)					3	1					1	1	1
P. oris (16)	2		2	3	3			1			1	1	3
P. salivae (11)		1		5		3	1					1	
P. timonensis (9)	1	6			2								
P. veroralis (4)		1			1	1							1
Total: 508	29	45	33	45	45	34	45	18	47	14	45	29	79

509 *AT, Austria; BE, Belgium; HR, Croatia; DK, Denmark; FR, France; DE, Germany; GB,
510 Great Britain; GR, Greece; HU, Hungary; KW, Kuwait; NL, the Netherlands; SI, Slovenia;
511 TR, Turkey.

512 Table 2. MIC range, MIC_{50}, MIC_{90} and percentage of susceptible and non-susceptible *Prevotella* isolates tested

Antimicrobial agents	Breakpoints[a] (mg/L)			MIC (mg/L)			Percentage (%)		
	S	I	R	Range	50%	90%	Susceptible	Non-susceptible[e]	Resistant
Ampicillin	≤0,5		>2	<0.016->256	1	>256	48.8	51.2	42.1
Ampicillin/sulbactam	≤4		>8	<0.016-8	0.125	2	99.6	0.4[f]	0
Piperacillin/tazobactam	≤8		>16	<0.016-4	<0.016	0.064	100	0	0
Cefoxitin	≤16	32	≥64	<0.016-32	0.5	2	99.6	0.4[f]	0
Imipenem	≤2		>8	<0.002-1	0.064	0.125	100	0	0
Meropenem	≤2		>8	<0.002-0.5	0.016	0.064	100	0	0
Clindamycin	≤4		>4	<0.016->256	0.016	>256	66.3	33.7	33.7
Erythromycin[d]	NA		NA	<0.016->256	2	>256	-	-	-
Tetracycline[b]	≤4	8	≥16	<0.016->256	1	32	61.6	36.8	17.7
Tigecycline[c]	≤4	8	≥16	<0.016-4	0.064	0.32	100	0	0
Moxifloxacin[b]	≤2	4	≥8	<0.002->32	0.032	8	81.7	18.3	9.4
Metronidazole	≤4		>4	<0.016-4	0.064	0.5	100	0	0

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514 [a] MIC breakpoints recommended by the EUCAST, [b]by the CLSI, and [c]by FDA,

[d]NA indicates that clinical breakpoints are unavailable in both EUCAST and CLSI guidelines

[e]Intermediate and resistant categories together.

[f] only intermediate resistant strains were found

535 Table 3. MIC range, MIC_{50}, MIC_{90} and percentage of susceptible and non-susceptible isolates belonging to different *Prevotella* species for four
536 different antibiotics

Organism (n) and antimicrobials	MIC (mg/L)			Percentage (%)	
	Range	50%	90%	Susceptible	Non-susceptible
***P. baroniae* (13)**					
Ampicillin	<0.016- >256	0.064	>256	53.9	46.1
Clindamycin	<0.016- >256	<0.016	>256	69.3	30.7
Tetracycline	0.023-16	1	8	84.6	15.4
Moxifloxacin	0.032->32	0.25	1	92.3	7.7
***P. bergensis* (13)**					
Ampicillin	<0.016- >256	1.5	16	46.2	53.8
Clindamycin	<0.016- >256	0.032	>256	69.2	30.8
Tetracycline	<0.016-16	1	16	53.8	46.2
Moxifloxacin	0.002->32	0.25	2	92.3	7.7
***P. bivia* (118)**					
Ampicillin	<0.016- >256	16	128	29.7	70.3
Clindamycin	<0.016- >256	0.25	>256	51.7	48.3
Tetracycline	<0.016- >256	16	64	36.6	63.4
Moxifloxacin	0.032->32	2	>32	49.6	50.4

***P. buccae* (68)**					
Ampicillin	<0.016- >256	0.064	>256	58.8	41.2
Clindamycin	<0.016- >256	<0.016	>256	60.3	39.7
Tetracycline	<0.016- 64	1	16	67.6	32.4
Moxifloxacin	0.016->32	0.25	0.5	98.5	1.5
***P. denticola* (57)**					
Ampicillin	<0.016- >256	0.5	>256	49.1	50.9
Clindamycin	<0.016- >256	0.016	>256	61.4	38.6
Tetracycline	0.016-16	0.25	16	71.4	28.6
Moxifloxacin	0.032->32	0.25	0.5	98.2	1.8
***P. disiens* (31)**					
Ampicillin	<0.016- >256	0.032	32	64.5	35.5
Clindamycin	<0.016- >256	<0.016	>256	67.8	32.3
Tetracycline	0.064-32	8	16	38.7	61.3
Moxifloxacin	<0.002->32	0.25	>32	83.9	16.1
***P. histicola* (11)**					
Ampicillin	<0.016- >256	0.032	4	81.8	18.2
Clindamycin	<0.016- >256	0.016	>256	81.8	18.2
Tetracycline	<0.016- 32	0.5	1	90.9	9.1

Moxifloxacin	0.002-1	0.25	0.5	100	0.0
***P. intermedia* (21)**					
Ampicillin	<0.016- >256	0.25	24	57.1	42.9
Clindamycin	<0.016- >256	<0.016	>256	66.7	33.3
Tetracycline	<0.016- 16	0.125	8	85.0	15.0
Moxifloxacin	<0.002-1	0.125	0.25	100	0.0
***P. melaninogenica* (44)**					
Ampicillin	<0.016- >256	2	>256	32.6	67.4
Clindamycin	<0.016- >256	<0.016	>256	83.7	16.3
Tetracycline	0.038-32	1	16	70.5	29.5
Moxifloxacin	<0.002->32	0.5	32	79.5	20.5
***P. nanceiensis* (13)**					
Ampicillin	<0.016- >256	16	>256	38.5	61.5
Clindamycin	<0.016- >256	<0.016	>256	76.9	23.1
Tetracycline	<0.016-16	0.5	4	92.3	7.7
Moxifloxacin	0.19->32	8	>32	23.1	76.9
***P. nigrescens* (62)**					
Ampicillin	<0.016- >64	0,094	64	66.1	33.9
Clindamycin	<0.016- >256	<0.016	>256	82.3	17.8

Tetracycline	<0.016- >256	1	8	90,3	9,7
Moxifloxacin	<0.002- >32	0.125	0.5	96.8	3.2
***P. oris* (16)**					
Ampicillin	<0.016- >64	8	>256	37.5	62.5
Clindamycin	<0.016- >256	<0.016	>256	56.2	43.8
Tetracycline	0.09-32	0.064	16	56.2	43.8
Moxifloxacin	0.09->32	0.25	0.5	93.8	6.2
***P. salivae* (11)**					
Ampicillin	<0.016- >256	0.064	64	72.7	27.3
Clindamycin	<0.016- >256	<0.016	0.064	91	9
Tetracycline	<0.016- 16	0.05	4	91	9
Moxifloxacin	0.06-8	0.038	1	91	9
Other *Prevotell*a spp.[a] (30)					
Ampicillin	<0.016- >256	0.25	128	53.4	46.7
Clindamycin	<0.016- >256	<0.016	>256	63.4	36.7
Tetracycline	<0.016- 32	4	16	50	50
Moxifloxacin	0.06-32	0.5	2	93.3	6.7

[a]*P. buccalis* (4), "*P. conceptionensis*" (1), *P. corporis* (5), *P. oralis* (7), *P. timonensis* (9), *P. veroralis* (4)

539 Table 4. Number of multidrug-resistant (MDR) *Prevotella* isolates found in different countries

Countries (no. of isolates tested/no. of strains with MDR)	Number of strains with resistance patterns*				
	AMP+CL+TE	AMP+TE+MOX	AMP+CL+MOX	CL+TE+MOX	AMP+CL+TE+MOX
Austria (29/4)	2	0	1	1	0
Belgium (45/4)	3	0	1	0	0
Croatia (33/5)	4	0	0	1	0
Denmark (45/3)	1	1	0	0	1
France (45/4)	4	0	0	0	0
Germany (34/0)	0	0	0	0	0
Great Britain (45/2)	1	0	1	0	0
Greece (18/3)	2	0	0	0	1
Hungary (47/11)	3	1	3	0	4
Kuwait (14/4)	3	1	0	0	0
The Netherlands (45/1)	1	0	0	0	0
Slovenia (29/2)	0	0	2	0	0
Turkey (79/6)	2	1	0	0	3
Altogether (508/49)	26	4	8	2	9

540 * Only resistant strains were evaluated, AMP ampicillin; CL clindamycin; TE tetracycline; MOX moxifloxacin;

Table 5. Percentage of the non-susceptible *Prevotella* isolates to 4 antimicrobials submitted by the different countries, in comparison with the consumption for systemic use of antimicrobials in the community and the hospital sector in European Union countries, expressed in DDD / 1000 inhabitants / day in 2015 (data available for eleven of the countries participating in the study) [16]

Countries (no. of isolates tested)	Ampicillin		Clindamycin		Tetracycline		Moxifloxacin	
	Non-susceptible (%)	Consumption of β-lactams	Non-susceptible (%)	Consumption of MLS*	Non-susceptible (%)	Consumption of tetracyclin	Non-susceptible (%)	Consumption of quinolons
Austria (29)	31	6.6	51.7	3.06	24.4	0.99	31	1.32
Belgium (45)	53.3	16.47	31.1	3.68	59	2.04	15.6	2.6
Croatia (33)	75.8	11.9	42.4	3.1	57.6	1.14	21.2	1.5
Denmark (45)	28.9	10.67	11.1	1.84	24.4	1.61	24.4	0.49
France (45)	71.1	18.83	33.3	3.23	35.6	3.26	6.6	1.6
Great Britain (45)	40	8.87	20	3.1	33.4	4.97	15.5	0.46
Germany (34)	5.9	4.55	14.7	2.4	24.3	1.97	8.8	1.33
Greece (18)	83.4	14.62	55.6	7.5	50	2.58	11.2	2.66
Hungary (47)	55.4	7.07	48.9	3.3	42.6	1.21	31.9	2.71
Kuwait (14)	92.9	-	64.3	-	57.1	-	43.1	-
Netherlands (45)	44.4	4.35	13.3	1.39	28.9	2.25	11.1	0.77
Slovenia (29)	51.7	9.61	48.3	1.86	40.7	0.42	13.8	1.16
Turkey (79)	59.5	-	40.5	-	25	-	17.7	-

*MLS; Macrolides, lincosamides and streptogramins