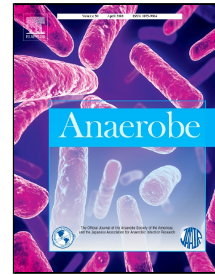


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A multicenter survey of antimicrobial susceptibility of *Prevotella* species as determined by Etest methodology



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1 **A multicenter survey of antimicrobial susceptibility of *Prevotella* species as determined by**

2 **Etest methodology**

3

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45 **Highlights**

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

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67 **Abstract**

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

85

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

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92 **Introduction**

93

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

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

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

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

123

124 **Materials and methods**

125

126 Bacterial isolates collected for the studies

127

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

141

142 Antimicrobial susceptibility testing

143

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

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

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

172

173 **Results**

174

175 Clinical isolates of *Prevotella* spp.

176

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

183 Patient age range was 1 to 91 years (mean age, 43; median age, 45 years). Specimens
184 were collected from various clinics. The majority of patients (30.1%) were treated at surgical
185 departments, followed by dentistry (17.3%) and departments of internal medical (14.2%). The
186 proportion of patients from gynecology and urology departments was 5.9%. The strains were
187 mainly isolated from abscesses (37.4%) and wounds (18.1%). Other major categories of
188 clinical samples were from intraoral infections (17.4 %), biopsy (7.7%) and sterile body fluids
189 (7.7%).

190

191 Antimicrobial susceptibility testing results

192

193 The MIC ranges, MIC₅₀, MIC₉₀, the proportions of susceptible, non-susceptible and resistant
194 isolates of 508 *Prevotella* spp. to 12 different antimicrobials is presented in Table 2. All
195 *Prevotella* isolates were susceptible to piperacillin/tazobactam, imipenem, meropenem,
196 tigecycline and metronidazole. Ampicillin/sulbactam and ceftiofur also showed good activity.
197 However, for both antimicrobials intermediate resistance was encountered in 0.4% of the
198 *Prevotella* isolates. Non-susceptibility to ampicillin, clindamycin, tetracycline and
199 moxifloxacin (resistant isolates and those with intermediate resistance taken together) was
200 detected at different rates; 51.2%, 33.7% 36.8% and 18.3%, respectively (Table 2).

201 Ampicillin non-susceptible *Prevotella* isolates were isolated from pulmonary samples
202 (76.9 %), abscesses (61.1%), bone biopsies (58%) and wounds (56.5%). The highest
203 prevalence of ampicillin non-susceptible strains was detected among isolates from orthopedic
204 clinics (83.3%), followed by dermatology (75%), medical departments (66.6%) and dentistry
205 (26.1%) (data not shown).

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

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

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

217 MICs of erythromycin ranged between < 0.016 and > 256 mg/L, with a MIC₅₀ of 2
218 mg/L and a MIC₉₀ of >256 mg/L. The distribution of erythromycin MIC's was also
219 evaluated because erythromycin is an alternative for penicillin (in case of penicillin allergy) in
220 various types of infections. There were more isolates with a high MIC (≥ 4 mg/L) for
221 erythromycin among ampicillin non-susceptible *Prevotella* spp. compared with all the
222 *Prevotella* isolates (Figure 1).

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

233 A total of 49 isolates were resistant to three or more antimicrobials. At the species
234 level, multidrug-resistant isolates were mostly found among *P. bivia* isolates. While only 5.9
235 % of the *P. bivia* isolates proved to be susceptible to all tested antimicrobials, 27.1% of the *P.*
236 *bivia* isolates showed resistance to three or more antimicrobials. See Table 4 for distribution
237 of multidrug-resistant *Prevotella* isolates encountered in the different countries.

238 The prevalence of *Prevotella* species non-susceptible to ampicillin, clindamycin,
239 tetracycline and moxifloxacin varied considerably between countries (Table 5). The highest
240 non-susceptibility rate was detected among isolates from Kuwait. These isolates were non-
241 susceptible to ampicillin (92.9%), clindamycin (64.3%), tetracycline (57.1%) and
242 moxifloxacin (43.1%). However, this country submitted the lowest number of strains, namely
243 14 isolates. Among the isolates collected from Germany, the prevalence of isolates non-
244 susceptible to ampicillin, clindamycin and moxifloxacin was found to be low compared to the
245 other countries, 5.9%, 14.7% and 8.8%, respectively (Table 5). The percentage of multidrug-
246 resistant isolates was also the highest among the isolates from Kuwait (28.6%). No multidrug-
247 resistance was found among the German isolates (Table 4).

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

263

264 **Discussion**

265

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

276 The first Europe-wide study about the antimicrobial susceptibility of *Prevotella* spp.,
277 together with other Gram-negative anaerobes, was carried out in 1995-1996. A total of 488
278 isolates were studied. These isolates represented nine *Prevotella* species, *P. bivia* being most
279 prevalent, from 13 European countries. Although there were differences between the species,
280 the highest resistance rates were in *P. bivia*, *Prevotella* species were highly resistant to
281 penicillin and tetracycline [19]. The most recent representative surveillance studies have
282 indicated high rates of penicillin resistance among *Prevotella* species [4, 5]. In the present
283 study, a total of 51.2% of isolates were found to be non-susceptible to ampicillin, though
284 susceptibility profiles do vary by *Prevotella* species, source of isolation and geographic
285 origin. In previous studies, some authors observed that two thirds of *Prevotella* isolates were
286 penicillin resistant, and some researchers reported resistance rates of > 90% in certain areas
287 [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

313 2.1-fold in Bulgaria over six years because of a decrease in tetracycline consumption [3, 29].
314 The present study shows the variability of tetracycline non-susceptibility patterns among
315 *Prevotella* isolates originating from different countries ranging from 24% to 59%. Non-
316 susceptibility to tetracycline was associated with national antimicrobial consumption and
317 possibly with previous antimicrobial treatments.

318 Moxifloxacin is recommended to adult patients for the treatment of various types
319 infections, caused by *Prevotella* species and a broad range of other anaerobes and aerobes [2].
320 In earlier studies, clinical isolates were fully susceptible to moxifloxacin and over time,
321 resistance rates have increased up to 36% [30, 30, 4, 31]. There is very little comparable data
322 on moxifloxacin resistance from European countries. Two multicenter surveys conducted in
323 Belgium showed that *Prevotella* non-susceptibility to moxifloxacin showed highly similar rates
324 in 2004 (24%) and 2011–12 (23%) [29, 23]. Papaparaskevas et al. [32] found that 42% of the
325 141 *Prevotella* spp. tested were non-susceptible to moxifloxacin. This resistance was
326 particularly frequent among *P. oralis* and *P. bivia* isolates, for which moxifloxacin resistance
327 rates were 62% and 71%, respectively. In our study, 18.3% of all tested *Prevotella* isolates
328 were non-susceptible to moxifloxacin. The rates of non-susceptibility to moxifloxacin in
329 Belgian and Greek isolates in this study were lower than the rates from the previous
330 Belgian and Greek studies, 15.6% and 11.2% respectively. The differences in non-
331 susceptibility rates may be due to differences in the number of isolates, the kind of *Prevotella*
332 species, and the origin of the strains that were tested. In our study, non-susceptibility to
333 moxifloxacin is shown to vary considerably between countries, ranging from 6.6% to 43.1%.
334 This suggests that moxifloxacin should be used cautiously in the empirical treatment of
335 anaerobic infections involving *Prevotella* spp.

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

338 organisms were also *P. bivia* strains. As member of the human microbiota, *P. bivia* may
339 represent a reservoir for resistance genes, and may contribute to resistance gene transmission
340 [1]. This data makes highlights the need to study the pathogenesis and resistance mechanisms
341 of *P. bivia* in the future.

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

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

363

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368 **Transparency Declaration**

369 The authors declare that they have no conflicts of interest.

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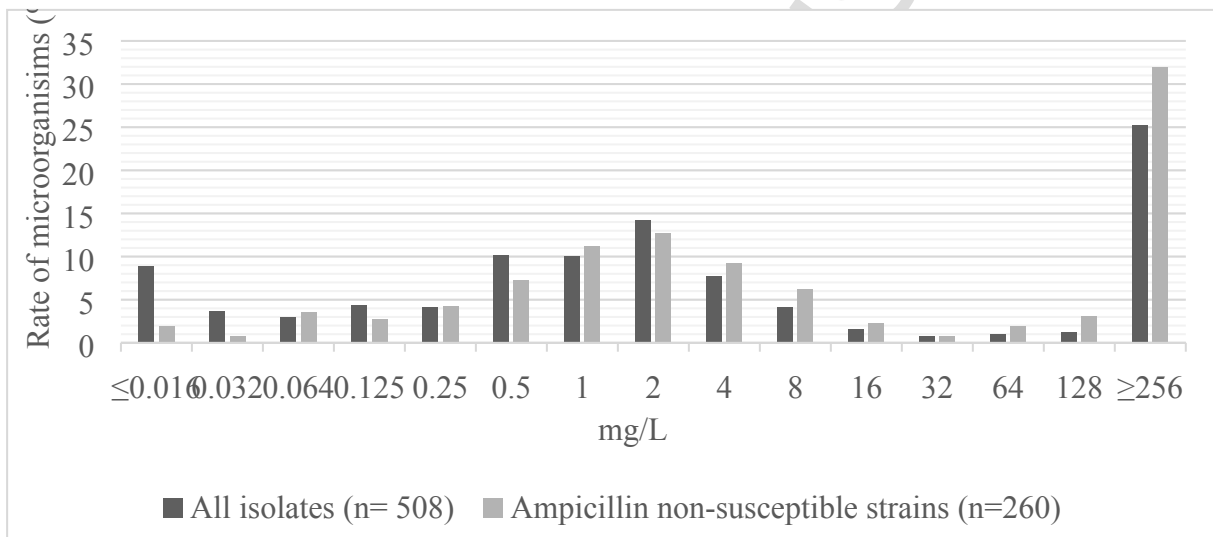
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Figure 1. Erythromycin MIC distribution among *Prevotella* species.



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

<i>Prevotella</i> spp. (n)	Countries* (no of isolates)												
	AT	BE	HR	DK	FR	DE	GB	GR	HU	KW	NL	SI	TR
<i>P. baroniae</i> (13)		1	1		2		1		5		3		
<i>P. bergensis</i> (13)		2		5	3						1	2	
<i>P. bivia</i> (118)	7	10	16	4	9	7	7	6	23	12	8		9
<i>P. buccae</i> (68)	5	7	2	8	5	5	4	2	6		9	3	12
<i>P. buccalis</i> (4)		2					1					1	
" <i>P. conceptionensis</i> "(1)									1				
<i>P. corporis</i> (5)		1					1	1			2		
<i>P. denticola</i> (57)	4	3		3	9	5		1	7		9	5	11
<i>P. disiens</i> (31)	4	3	7	1	1	2		2		1	2	4	4
<i>P. histicola</i> (11)		1				6			1		2		1
<i>P. intermedia</i> (21)	1	1				1	8	2				1	7
<i>P. melaninogenica</i> (44)	1	2	5	8	3	2		2	2		3	6	10
<i>P. nanceiensis</i> (13)	1	1		7	1							3	
<i>P. nigrescens</i> (62)	3	3		1	3	1	22	1	2	1	4	1	20
<i>P. oralis</i> (7)					3	1					1	1	1
<i>P. oris</i> (16)	2		2	3	3			1			1	1	3
<i>P. salivae</i> (11)		1		5		3	1					1	
<i>P. timonensis</i> (9)	1	6			2								
<i>P. veroralis</i> (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, MIC₅₀, MIC₉₀ 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, ^bby the CLSI, and ^cby FDA,

515 ^dNA indicates that clinical breakpoints are unavailable in both EUCAST and CLSI guidelines

516 ^eIntermediate and resistant categories together.

517 ^f only intermediate resistant strains were found

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535 Table 3. MIC range, MIC₅₀, MIC₉₀ 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
<i>P. baroniae</i> (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
<i>P. bergensis</i> (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
<i>P. bivia</i> (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

<i>P. buccae</i> (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
<i>P. denticola</i> (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
<i>P. disiens</i> (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
<i>P. histicola</i> (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
<i>P. intermedia</i> (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
<i>P. melaninogenica</i> (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
<i>P. nanceiensis</i> (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
<i>P. nigrescens</i> (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
<i>P. oris</i> (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
<i>P. salivae</i> (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 <i>Prevotella</i> 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

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

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;

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

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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	-

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547 *MLS; Macrolides, lincosamides and streptogramins