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Victor Luzarraga, Julie Cremniter, Chloé Plouzeau, Anthony Michaud,
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1 ***In Vitro* activity of delafloxacin against clinical levofloxacin-resistant *Helicobacter pylori***
2 **isolates**

3 Victor LUZARRAGA^{1,2}, Julie CREMNITER^{1,2}, Chloé PLOUZEAU¹, Anthony MICHAUD¹,
4 Lauranne BROUTIN¹, Christophe BURUCOA^{1,2}, Maxime PICHON^{1,2*}

5 1- CHU de Poitiers, Bacteriology laboratory, Infectious Agents Department, Poitiers, France.

6 2- Université de Poitiers, INSERM U1070, PHAR2 Pharmacology of Antimicrobial agents
7 and Antibiotic resistance, Poitiers, France.

8 *Corresponding author:

9 Maxime PICHON

10 CHU de Poitiers, Bacteriology laboratory, Infectious Agents Department

11 2 rue de la Milétrie

12 86021 Poitiers

13 Mail : maxime.pichon@chu-poitiers.fr

14 Phone: +33 (0) 5 49 44 41 43

15

16 **Running title :** *In Vitro* activity of delafloxacin against *H.pylori*

17

18 **Abstract.**

19 **Introduction.** Increasing antibiotic resistance in *Helicobacter pylori* necessitates research on
20 new active molecules. In 2017, delafloxacin, a new fluoroquinolone (FQ) with chemical
21 properties of activity under acidic conditions, was approved for treatment of community-
22 acquired bacterial pneumonia and acute bacterial skin and soft-tissue infections. Mutations in
23 *gyrA* are responsible for fluoroquinolone resistance, but certain clinical isolates of *H. pylori*
24 appear to display a dual phenotype: resistance to levofloxacin associated with very low
25 delafloxacin MICs. **Objectives.** To estimate Epidemiological Cutoff Values (ECOFF) and to
26 identify mutations in the *gyrA* gene, specific for FQ resistance, without increasing the MICs of
27 delafloxacin. **Methods.** Clinical strains (n=231) were collected in the bacteriology laboratory
28 of Poitiers University Hospital over a 2-year period to determine the ECOFF of delafloxacin.
29 Retrospectively, 101 clinical strains with an levofloxacin-resistant phenotype (MIC > 1 mg/L)
30 were selected from 2018 to 2022 for delafloxacin MIC determination and QRDR (*gyrA*)
31 sequencing. **Results.** The estimated ECOFF of delafloxacin was ≤ 0.125 mg/L. No *H. pylori*
32 isolate showed an levofloxacin sensitive phenotype with a delafloxacin MIC > 0.125 mg/L.
33 Among the levofloxacin-resistant *H. pylori* isolates, 53.5% had MICs of delafloxacin ≤ 0.125
34 mg/L. The N87I mutation was associated with dual levofloxacin/delafloxacin resistance
35 ($p < 0.001$) in contrast to the N87K and D91N mutations ($p > 0.05$). Mutations D91G) and D91Y
36 were not associated with a delafloxacin resistance phenotype ($p > 0.05$). **Discussion and**
37 **conclusion.** delafloxacin seems to be a therapeutic alternative for levofloxacin-resistant strains
38 with a greater *in vitro* activity. However, further clinical-biological investigations are required
39 to determine its efficacy in *H. pylori* eradication.

40 **Introduction**

41 *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacterium that infects the stomach of half
42 of humanity. Often asymptomatic, *H. pylori* infection can nonetheless cause gastroduodenal
43 diseases: gastritis, ulcer or gastric cancer.¹ Recently, the sixth Maastricht/Florence European
44 Consensus Conference (VI) recommended that eradication treatment should be guided by
45 antibiotic-susceptibility testing (AST) results. Currently, treatment is based on a combination
46 of antimicrobial agents according to local recommendations.

47 Levofloxacin-based triple or quadruple therapy can be used as second-line treatment for rescue
48 eradication of *H. pylori* after failure of clarithromycin-based triple therapy or bismuth
49 quadruple or non-bismuth concomitant quadruple therapy.^{2,3}

50 For several years, levofloxacin-based therapies have been compromised by increased
51 fluoroquinolone resistance (over 20% in several countries and up to 50% in some regions).^{4,5}

52 For example in Italy, a progressive increase in the rate of resistance to levofloxacin has been
53 reported: initially at 10% in 2017 and up to 37.8% in 2020. The authors also reported a
54 secondary resistance rate of close to 60% in patients with therapeutic failure.⁶ The high level of
55 resistance reported in certain regions has an impact on the therapeutic strategy, resulting in a
56 switch to a more effective bismuth therapy, as demonstrated recently.⁷ Detection of antibiotic
57 resistance in *H. pylori* is routinely performed phenotypically by determining the antibiotics
58 MIC used for eradication treatment following the EUCAST-CA-SFM recommendations.⁸
59 Fluoroquinolone (FQ) resistance in *H. pylori* strains is mainly due to amino acid substitution in
60 *gyrA* with D91G, D91N, N87K or N87I leading to fluoroquinolone resistance.^{9,10}

61 Levofloxacin is the fluoroquinolone used for the eradication of *H. pylori*, a novel
62 fluoroquinolone, named delafloxacin, has drawn growing interest due to its acid-resistant
63 properties.^{11,12} Delafloxacin is a brand-new non-zwitterionic anionic FQ with unique chemical
64 properties due to its special structure, enabling it to be more effective under acidic conditions
65 and to have a dual action against DNA-gyrase and type IV topoisomerase.^{13,14} Available in
66 different formulations (for both oral or intravenous administrations) in Europe, America and
67 the Asia-Pacific region population, this molecule has already been used to treat community-
68 acquired bacterial pneumonia.¹⁵ In these clinical situations, delafloxacin has been shown to be
69 effective not only against methicillin-resistant and methicillin-susceptible *S. aureus* isolates,
70 but also on levofloxacin-resistant *S. aureus*.¹⁶ High microbiological response rates were
71 observed against these isolates, as well as against isolates with documented mutations in the
72 quinolone resistance-determining region (QRDR).¹⁶ These results suggest an *in vitro* difference
73 between delafloxacin and other quinolones. A previous study showed very low delafloxacin

74 MIC among levofloxacin-resistant *H. pylori* strains (MIC₅₀ 0.094 and MIC₉₀ 0.38 mg/L) but
75 since a clinical breakpoint has not yet been defined, it is difficult to analyze these results.¹⁷ In
76 the absence of an established clinical breakpoint, Epidemiological Cut-Off value (ECOFF) can
77 be calculated from the distribution of a wild-type bacterial population.¹⁸ ECOFFs are based on
78 the phenotypic traits of the microorganism and the antimicrobial agent.¹⁹ The upper end of wild-
79 type distribution has been defined and applied in the process of evaluating antimicrobial *in vitro*
80 activity.²⁰ Delafloxacin *in vitro* activity on *H. pylori* could be estimated by ECOFF, without
81 replacing a clinical breakpoint (CB), which would be decisive for *H. pylori* eradication in
82 patients.

83 In the literature, some pharmacokinetic (PK) and pharmacodynamic (PD) properties of the drug
84 have been described. These include oral bioavailability of 58.8%, with plasma protein binding
85 of 84% and half-life ranged from 4.2 to 8.5 hours following oral administration, with no
86 difference regarding age and sex Monte Carlo simulation analyses using clinical PK and non-
87 clinical PK/PD data revealed high probability of target attainment for MIC values ≤ 0.5 mg/L
88 with intravenous and oral doses of 300 mg and 450 mg, respectively. These doses were chosen
89 for the phase 3 studies.^{21,22} It is important to exercise caution in light of the paucity of
90 information in the literature on the intragastric passage of this molecule, which complicates
91 interpretation of data on its effectiveness against *H. pylori*.

92 Although the relationship between fluoroquinolone phenotypes and resistance-conferring *gyrA*
93 mutations is established, MIC of delafloxacin and levofloxacin in *H. pylori* strains did not
94 always appear to be equivalent, suggesting that some *gyrA* mutations have not yet been
95 determined about delafloxacin resistance. The aim of the present study was to compare
96 delafloxacin and levofloxacin activity against *H. pylori* strains, based on ECOFF and CB
97 values, respectively, and to identify, among *H. pylori*, *gyrA* mutations associated with
98 levofloxacin resistance (MIC > 1 mg/L), that could impact on delafloxacin MIC.

99 **Methods**

100 ***Strains tested for delafloxacin Epidemiological Cut-OFF Value (ECOFF), Cohort A.***

101 An ECOFF estimation was set up to assess the *in vitro* activity of delafloxacin. CLSI software
102 ("ECOFFinder_XL_2010_v2.1") was used.²³ For this purpose, a prospective distribution of
103 delafloxacin MIC obtained by gradient strip Etest method (Liofilchem, Roseto degli Abruzzi,
104 Italy) was carried out on a cohort ("Cohort A") of *H. pylori* clinical strains (n=231), isolated
105 from patient biopsies between 2022 and 2023 at the University Hospital of Poitiers.

106 Characterization of *H. pylori* from gastric biopsy was part of routine laboratory diagnostics,
107 and prospective determination of delafloxacin MIC had been implemented in 2022 as described

108 in this manuscript. Etest range for delafloxacin MIC was in conformity with a two-fold dilution
109 series from 0.002 mg/L to 32 mg/L for ECOFF determination.

110 ***Genomic Investigation population, Cohort B.***

111 To study the correlation between delafloxacin/levofloxacin MIC values and *gyrA* mutations,
112 clinical isolates of levofloxacin-resistant *H. pylori* (n=101, 98 patients) collected at the
113 University Hospital of Poitiers (Poitiers, France) between 2018 and 2022 were retrospectively
114 selected ("Cohort B"). For each strain, *gyrA* gene were sequenced and both the delafloxacin and
115 levofloxacin MIC have been analysed accordingly.

116 ***H. pylori culture, isolation, and antibiotic susceptibility testing***

117 *H. pylori* strains from gastric biopsy were isolated using selective media. Specifically, Mueller-
118 Hinton (Oxoid, Basingstoke, England) supplemented with 10% defibrinated horse blood and
119 Skirrow selective supplement (Oxoid) were used. Samples were then incubated in microaerobic
120 conditions for 44 hours at 35 °C +/- 2°C. Strains were identified at the species level by urease
121 activity (BioMérieux, Marcy-l'Étoile, France) and morphological identification by Gram-
122 staining (CellaVision, Lund, Sweden). According to national recommendations, both
123 clarithromycin and levofloxacin MIC were determined by using Etest (Liofilchem) then
124 interpreted according to the French Society for Microbiology and EUCAST guidelines
125 (considering a CB of 1 mg/L for both MIC)²⁴. The proportion of bacterial strains for which
126 quinolone antibiotic therapy would have been recommended was determined by collecting
127 clarithromycin resistance data. Among levofloxacin-resistant strains, low-level resistances
128 were defined as a MIC between 1 and ≤ 2 mg/L, intermediate level of resistances as a MIC
129 between 3 and ≤ 24 mg/L, and high-level resistances as a MIC > 24 mg/L. In parallel,
130 delafloxacin MIC were determined using the same methodology²⁵.

131 ***GyrA sequencing***

132 As indicated previously, *H. pylori* genome was sequenced to determine presence of *gyrA*
133 mutations. Primers were 5'-TGA-GCG-TGA-TCA-TAG-GGC-GCG-CTT-TAC-CGG-A-3'
134 and 5'-GGG-GGA-TAG-AAG-TCG-CCA-TCC-CTA-CAG-CGA-TCC-CA (size of 474 bp).
135 Taq DNA Polymerase (Hifi Kapa Hot Start, Roche, Meylan, France) was used for PCR
136 amplification, which was performed in 25 µL final volume. The PCR profile was as follows: 1
137 cycle of 3 min at 95°C; 35 cycles of 20 sec at 98°C, 15 sec at 69°C and 2 min at 72°C; and then
138 2 min at 72 °C. After checking PCR amplification by 1.5% agarose gel electrophoresis, Sanger
139 sequencing was performed (3500Dx, ThermoFisher, Waltham, USA). The sequencing process
140 was carried out using Bigdye™ Terminator v3.1 (ThermoFisher) (*i.e.*, 1 cycle of 1 min at 96
141 °C and 25 cycles of 5 sec at 96 °C, 10 sec at 50°C, 3 min at 60°C) before analysis on Seqscape

142 version 2.7 (ThermoFisher). The nucleotide and deduced amino acid sequences of analyzed
143 were compared with those of the known *H. pylori gyrA* gene (GenBank accession number:
144 PRJNA1081826).

145 **Statistical analyses**

146 Statistical analyses were performed using GraphPad Prism 9.0.0 software (Dotmatics, Boston,
147 USA). Quantitative variables were compared using the non-parametric Mann-Whitney test or
148 the Z-test. Qualitative variables were compared using a Chi² test or Fisher's exact test.
149 Correlation tests were performed using Spearman's non-parametric test. For each statistical test,
150 a p-value < 0.05 was considered as significant (0.01 as highly significant).

151 **Results**

152 **Estimation of delafloxacin Epidemiological Cut-Off Value and *in vitro* delafloxacin** 153 **activity (Cohort A).**

154 Using Cohort A (n=231 strains), analysis of delafloxacin MIC results led to estimate a
155 delafloxacin ECOFF 99% at 0.125 mg/L using both CLSI ECOFFinder_XL_2010_v2.1
156 software and graphical analysis (Figure 1). All strains of Cohort A presenting a delafloxacin
157 MIC below this threshold were considered as susceptible strains. Delafloxacin and levofloxacin
158 MIC₅₀ and MIC₉₀ were 0.016 and 0.125 mg/L *versus* 0.125 and ≥32 mg/L, respectively.
159 levofloxacin-resistant strains all presented delafloxacin MIC of ≥ 0.016 mg/L. No clinical
160 isolates with delafloxacin MIC > 0.125 mg/L have been found susceptible to levofloxacin
161 (Figure 1).

162 Note that in Cohort A, clarithromycin-resistance rate was estimated to 17.7% (n=41) (Table
163 1.). Considering a delafloxacin ECOFF of 0.125 mg/L, five isolates (2%) of *H. pylori* were
164 considered susceptible to this antibiotic, even if double-resistant to clarithromycin and
165 levofloxacin.

166 ***In vitro* delafloxacin activity in levofloxacin-Resistant *H. pylori* isolates (Cohort B).**

167 Among the Cohort B (composed of levofloxacin-resistant strains), 24 strains (24/101; 23.8%)
168 have been considered as low-level resistant strains, 29 (29/101; 28.7%) have been considered
169 as intermediate-level resistant strains, and 48 (48/101; 47.5%) have been considered as high-
170 level resistant strains.

171 Among this cohort, 54 (54/101; 53.4%) strains were considered as susceptible to delafloxacin
172 according to the previously determined ECOFF. The proportion of delafloxacin-resistant
173 isolates positively correlate with the levofloxacin MIC (p<0.05) so that delafloxacin-resistant
174 isolates (MIC > 0.125 mg/L) were more frequent in isolates with higher levofloxacin MIC (≥

175 16 mg/L) ($p < 0.01$) (Figure 2.). Delafloxacin MIC₅₀ and MIC₉₀ (0.125 and 0.75 mg/L) were at
176 least 32-times lower than those of levofloxacin MIC₅₀ and MIC₉₀ (16 and >32 mg/L).

177 **Delafloxacin phenotype of strains and *gyrA* QRDR mutations (Cohort B).**

178 Levofloxacin-resistant *H. pylori* strains were sequenced, and several mutations were found in
179 the *gyrA* QRDR (Table 2). Among these strains, 84 strains (84/101; 83.2%) presented a single
180 point polymorphism, and 14 strains (14/101; 13.9%) a double point polymorphism. Nine
181 polymorphisms on *gyrA* were identified, leading to substitution of the amino acid 87, 91, 92,
182 99, and 129. Position 87 was the most substituted (62/98; 63.3%) and substitution N87I (n=29;
183 29.6%) was the most frequent. In the whole cohort, delafloxacin MIC were significantly lower
184 than levofloxacin MIC only for substitution at positions 87 and 91 ($p < 0.01$) without significant
185 difference in delafloxacin MIC means between strains harboring substitution in positions 91 or
186 87 ($p > 0.05$). However, among delafloxacin-resistant strains, substitution at position 87 (36/62;
187 58.1%) was over-represented compared with position 91 (14/45; 31.1%) ($p < 0.01$). Moreover,
188 the N87I substitution was associated with higher delafloxacin MIC (at least 3 times more;
189 $p < 0.01$) than other mutations, whereas the N87K substitution could not be associated with any
190 delafloxacin phenotype ($p > 0.05$). Similarly, at position 91, the D91N substitution was more
191 frequently associated with delafloxacin-susceptible strains ($p < 0.05$), while the D91G
192 substitution could not be associated with any delafloxacin phenotype ($p > 0.05$) (Figure 3).

193 Mutations at position 87, particularly N87K and N87I, were associated with intermediate to
194 high levels of resistance, whereas mutations at position 91, such as D91N and D91G, were
195 associated with low to intermediate level of resistance to levofloxacin (Figure S1).

196 **Discussion.**

197 Delafloxacin is a novel FQ with higher activity in acidic environments and lower MIC
198 compared to other molecules of this family.¹³ Currently, there is no evidence to support the use
199 of delafloxacin in treating *H. pylori* infections, as there are no validated clinical breakpoints for
200 this bacterium. One previous study observed lower MIC of delafloxacin compared to
201 levofloxacin against *H. pylori* isolates without cutoff value to interpret MIC as susceptible or
202 resistant.¹⁷ Herein, to establish an ECOFF for delafloxacin, *H. pylori* MIC were obtained using
203 Etest, from a large clinical cohort (n=231). Whilst the broth microdilution method is considered
204 as the reference for determining an ECOFF, the correlation between this method and Etest was
205 found to be outstanding for levofloxacin, clarithromycin and amoxicillin.^{26,27} Since the
206 aggregated MIC distribution must include at least five valid MIC distributions (generated in a
207 separate laboratory), the present study allows only a Tentative Epidemiological Cut-Off value
208 (TECOFF) according to EUCAST definition, in absence of such a multicenter study (harder to

209 set up). Doing so, inter-laboratory variability was not calculated since delafloxacin MICs were
210 determined only at a single center. Other studies are required to determine inter-laboratory
211 variability.

212 Delafloxacin MICs were determined following the French national recommendations, and a
213 TECOFF breakpoint of 0.125 mg/L was determined, which aligns with the EUCAST
214 breakpoints for other bacteria such as *Escherichia coli* (MIC \leq 0.125 mg/L).²⁸ To obtain robust
215 findings, delafloxacin MICs were performed in duplicate for values close to the TECOFF
216 breakpoint (Figure 1 and Table 1).

217 A previous study has shown low MICs for delafloxacin on clinical isolates of *H. pylori* resistant
218 to levofloxacin, supporting the current findings. In our study, both delafloxacin and
219 levofloxacin MIC₅₀ and MIC₉₀ observed from cohort A were the same as those already
220 observed.²⁹ For the cohort B, delafloxacin MIC₅₀ and MIC₉₀ were 0.125 and 0.75 mg/L,
221 respectively, close to other studies.¹⁷ This very small difference could be explained by the large
222 collection of strains in the present cohort.

223 This study assessed delafloxacin *in vitro* activity in a cohort of 101 *H. pylori* levofloxacin-
224 resistant using the delafloxacin TECOFF derived from *H. pylori* consecutive clinical strains.
225 Mutations at positions 87 and 91 were associated with levofloxacin resistance, as indicated
226 previously, even if the distribution of mutations in this study differs from that observed in
227 France in 2012.⁹ Within Cohort B, high levofloxacin MICs (\geq 16 mg/L) have been associated
228 with high delafloxacin MIC (Figure 2). Concerning N87I, 29.6% of all mutations, was
229 associated with high delafloxacin MICs, suggesting that this mutation may induce resistance to
230 delafloxacin. On the contrary, low levofloxacin MIC and low delafloxacin MIC appeared to be
231 associated with a substitution at position 91 (especially with the D91N mutation being the most
232 frequent, *i.e.* 71.5%). Finally, the N87K mutation was detected in both delafloxacin-resistant
233 (n=9) and delafloxacin-susceptible isolates (n=15), suggesting that this substitution alone is not
234 sufficient to confer resistance to delafloxacin. As previously published, the present study
235 demonstrated that D91 mutations, which are prevalent in the literature (with frequencies
236 ranging from 11.6% to 13.96% in some studies) and for which delafloxacin confers a net
237 benefit, remained less frequent than N87K mutations (with frequencies ranging from 37.5% to
238 52.35%) for which this molecule is also ineffective. This epidemiological data indicates that
239 the clinical benefit of using this molecule in situations of levofloxacin resistance may be limited
240 by up to one-third or one-half.^{30,31}

241 The sequencing of *gyrA* has allowed to study the nucleotide coordinates between 89 and 562
242 (on a 474-long sequence). The N87T substitution was identified once in conjunction with a

243 single point mutation and five times in association with substitution at position 91. Thus, the
244 increased delafloxacin MIC in these six isolates could be attributed to the D91N mutation (n=5)
245 and the D91Y mutation (n=1). Indeed, in *H. pylori* J99 strain, N87T occurred naturally and has
246 been linked to elevated susceptibility to fluoroquinolones.³²

247 Other mutations (including D99N, A129T, A92T, D161N, P151S) were observed in the present
248 study. The D99N mutation was observed only once (associated with quinolone resistance, with
249 levofloxacin MIC greater than 32 mg/L).^{33,34} In the current study, the A129T-harboring strain
250 presented delafloxacin MIC of 0.19 mg/L, categorizing it as resistant according to TECOFF.
251 In the literature, previous studies have reported A92T and A129T mutations among
252 levofloxacin-resistant isolates.³⁵ However, it is challenging to attribute a precise role to these
253 three mutations, as they have been identified only in a limited number of isolates and lack
254 sufficient data. These observations suggested that other mutations occurring beyond the
255 sequenced regions, which have not been described yet, may be responsible for resistance.
256 Additionally, mutations could be observed outside the QRDR region, and other resistance
257 mechanisms, such as efflux pumps or *gyrB* substitutions, may contribute to antibiotic resistance
258 in *H. pylori*.^{34,36–39}

259 Furthermore, it might be inferred that the introduction of mutations could alter the resistance
260 phenotype of quinolones (including delafloxacin). Nevertheless, bacterial transformation
261 experiments using directed mutagenesis from a wild-type *H. pylori* strain are necessary to
262 robustly confirm the impact of A92T, D99N and A129T mutations on antibiotic resistance as
263 demonstrated with mutations described in 23S rDNA and *gyrA* genes.^{38,40–42}

264 In conclusion, these *in vitro* findings allowed us to estimate a delafloxacin TECOFF of 0.125
265 mg/L. For now, eradication treatment using antibiotics are based on the recommendations for
266 guided treatment by the results of the antibiotic susceptibility testing (*i.e.*, association of
267 clarithromycin, amoxicillin, and metronidazole as first-line treatment and association of
268 levofloxacin, amoxicillin, and metronidazole as second-line therapy). In the present study, it
269 could be suggested that delafloxacin could catch up with half of the strains (5/9; 55,5%; Table
270 1) that could be considered as resistant to both the first and second-line treatments, suggesting
271 that delafloxacin could be a potential therapy in place of levofloxacin. Indeed, *in vitro*,
272 delafloxacin has lower MIC than levofloxacin, and some mutations responsible for levofloxacin
273 resistance seem to have a lower impact on delafloxacin phenotype. Nevertheless, establishing
274 clinical breakpoints remains crucial to evaluation of delafloxacin potential as an alternative to
275 levofloxacin.

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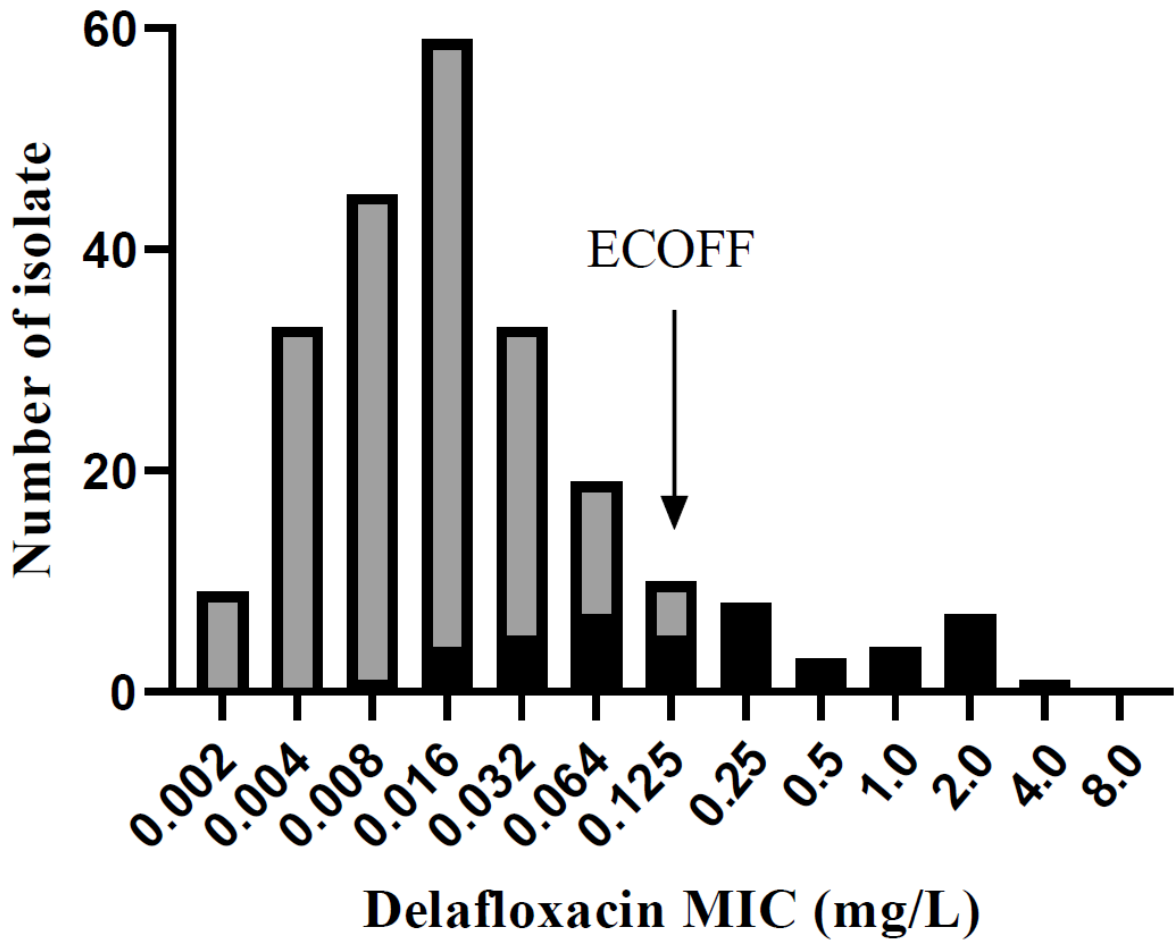
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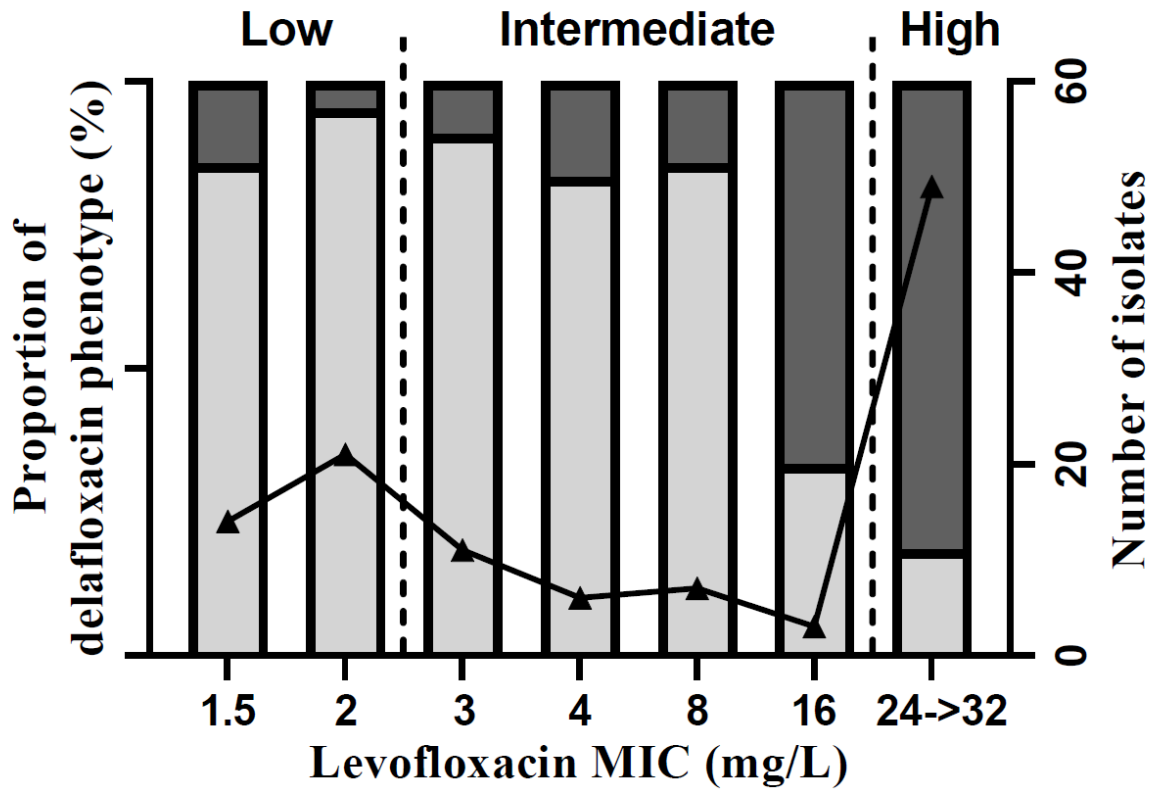
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395 **Figure:**

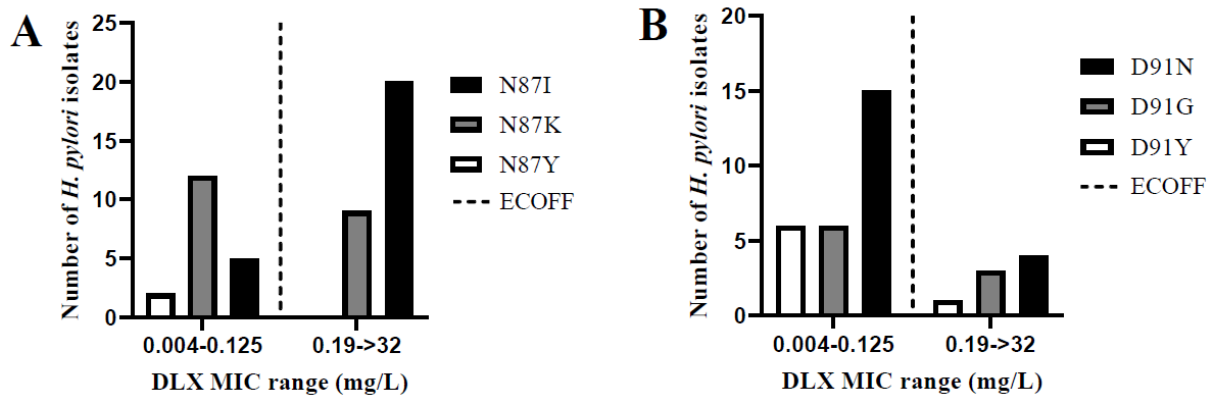


396

397 **Figure 1. Microbiological distribution of 231 *H. pylori* isolates (Cohort A) for calculating delafloxacin**
398 **ECOFF associated with levofloxacin phenotype.** The measured ECOFF ≤ 0.125 mg/L (black arrow)
399 corresponds to a distribution of 99.0% (according to "ECOFFinder_XL_2010_v2.1" software).
400 Levofloxacin-susceptible and resistant strains have been indicated in gray and black respectively.



401
 402 **Figure 2. Proportion of the delafloxacin phenotype according to the levofloxacin MIC among 101 *H.***
 403 ***pylori* isolates.** Based on the levofloxacin MIC for, the number of strains (right axis) and the delafloxacin-
 404 resistant strains proportion (left axis) were considered. delafloxacin-susceptible and resistant strains have
 405 been indicated in gray and black respectively.



406
 407 **Figure 3. Distribution of single point mutations detected in *gyrA* (QRDR) from levofloxacin-resistant**
 408 ***H. pylori* strains according to delafloxacin MIC.** A. levofloxacin-resistant strains with mutations at
 409 position 87 according to delafloxacin phenotype; B. levofloxacin-resistant strains with mutations at position
 410 91 according to delafloxacin phenotype. delafloxacin MIC were classified into 2 ranges on either side of the
 411 measured ECOFF for this antibiotic.

412
 413 **Tables:**

414 **Table 1. *H. pylori* strains clarithromycin and levofloxacin-resistant among delafloxacin-susceptible**
 415 **isolates.**

<i>H. pylori</i> antibiotic susceptibility phenotype (n= 231)	No. of isolate (%)	
<i>H. pylori</i> CLA-R	41	(17.7 %)
<i>H. pylori</i> CLA-R and LVX-R	9	(3,9%)
<i>H. pylori</i> CLA-R and LVX-R and DLX-S	5	(2,2%)

416 CLA-R: Clarithromycin-resistant; LVX-R: Levofloxacin-resistant; DLX-S: Delafloxacin-susceptible.

417 **Table 2. QRDR mutations in levofloxacin-resistant *H. pylori* isolates (n=101) according to delafloxacin**
 418 **MIC.**

Mutation	N (%)	Delafloxacin						
		MIC range (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Phenotype			
					S (n; %)	R (n; %)		
Absence of mutation (n = 3)								
	3 (100)	0.016-2	0.19	2	1	33.3	2	66.6
Single point mutation (n = 84)								
N87K	21 (25.0)	0.032-4	0.125	0.50	12	57.1	9	42.9
N87I	25 (29.8)	0.032-3	0.38	1.5	5	20.0	20	80.0
N87Y	2 (2.3)	0.016-0.047	-	-	2	100.0	0	-
N87T	1 (1.2)	0.75	-	-	0	-	1	100.0
D91N	19 (22.6)	0.032-0.5	0.094	0.5	15	78.9	4	21.1
D91Y	7 (8.3)	0.023-0.094	0.032	1.0	6	85.7	1	14.3
D91G	9 (10.7)	0.0016-2	0.064	0.38	6	66.7	3	33.3
Double point mutation (n = 14)								
N87K and other*	3 (21.4)	0.032-0.094	0.064	0.094	3	100.0	1	-
N87I and other [§]	4 (28.6)	0.19->32	0.38	>32	0	-	4	100.0
N87T and other [°]	6 (42.9)	0.004-0.19	0.064	0.19	4	66.7	2	33.3
D99N + A129T	1 (7.1)	0.19	-	-	0	-	1	100.0

419 * A92T (n=2) and P151S (n=1)

420 [§] D91G (n=1), D91N (n=1), D91Y (n=1) and D161N (n=1)

421 [°] D91N (n=3), D91N+L167I (n=1), D91N+D155G (n=1) and D91Y (n=1)

422

423 **Supplementary data**

424 **Figure S1. Distribution of single point mutations detected in *gyrA* (QRDR) from levofloxacin-resistant**
 425 ***H. pylori* clinical strains according to levofloxacin resistance level. A.** Distribution of single point
 426 mutations at position 87 according to levofloxacin resistance; B. Distribution of single point mutations at
 427 position 91 according to levofloxacin resistance. Resistance level of levofloxacin definition: MIC of ≤ 2
 428 mg/L, low-level resistance; MIC of 3 to 16 mg/L, intermediate level of resistance; MIC of 24 to ≥ 32 mg/L,
 429 high-level resistance.