


In vitro activity of delafloxacin against clinical levofloxacin-resistant *Helicobacter pylori* isolates

Victor Luzarraga^{1,2}, Julie Cremniter^{1,2}, Chloé Plouzeau¹, Anthony Michaud¹, Lauranne Broutin¹,
Christophe Burucoa^{1,2} and Maxime Pichon ^{1,2*}

¹Bacteriology Laboratory, Infectious Agents Department, CHU de Poitiers, 2 rue de la Milétrie, 86021 Poitiers, France; ²PHAR2 Pharmacology of Antimicrobial Agents and Antibiotic Resistance, INSERM U1070, Université de Poitiers, Poitiers, France

*Corresponding author. E-mail: maxime.pichon@chu-poitiers.fr

Received 18 April 2024; accepted 12 July 2024

Background: Increasing antibiotic resistance in *Helicobacter pylori* necessitates research on new active molecules. In 2017, delafloxacin, a new fluoroquinolone with chemical properties of activity under acidic conditions, was approved for treatment of community-acquired bacterial pneumonia and acute bacterial skin and soft-tissue infections. Mutations in *gyrA* are responsible for fluoroquinolone resistance, but certain clinical isolates of *H. pylori* appear to display a dual phenotype: resistance to levofloxacin associated with very low delafloxacin MICs.

Objectives: To estimate epidemiological cut-off (ECOFF) values and to identify mutations in the *gyrA* gene, specific to FQ resistance, without increasing the MICs of delafloxacin.

Methods: Clinical strains ($n=231$) were collected in the bacteriology laboratory of Poitiers University Hospital over a 2 year period to determine the ECOFF of delafloxacin. Retrospectively, 101 clinical strains with an levofloxacin-resistant phenotype (MIC > 1 mg/L) were selected from 2018 to 2022 for delafloxacin MIC determination and QRDR (*gyrA*) sequencing.

Results: The estimated ECOFF of delafloxacin was ≤ 0.125 mg/L. No *H. pylori* isolate showed a levofloxacin-sensitive phenotype with a delafloxacin MIC of >0.125 mg/L. Among the levofloxacin-resistant *H. pylori* isolates, 53.5% had delafloxacin MICs of ≤ 0.125 mg/L. The N87I mutation was associated with dual levofloxacin/delafloxacin resistance ($P < 0.001$) in contrast to the N87K and D91N mutations ($P > 0.05$). Mutations D91G and D91Y were not associated with a delafloxacin resistance phenotype ($P > 0.05$).

Conclusions: Delafloxacin seems to be a therapeutic alternative for levofloxacin-resistant strains with greater *in vitro* activity. However, further clinical/biological investigations are required to determine its efficacy in *H. pylori* eradication.

Introduction

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

Levofloxacin-based triple or quadruple therapy can be used as second-line treatment for rescue eradication of *H. pylori* after

failure of clarithromycin-based triple therapy or bismuth quadruple or non-bismuth concomitant quadruple therapy.^{2,3}

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

For example, in Italy, a progressive increase in the rate of resistance to levofloxacin has been reported, initially at 10% in 2017 and up to 37.8% in 2020. The authors also reported a secondary resistance rate of close to 60% in patients with therapeutic failure.⁶ The high level of resistance reported in certain regions has an impact on the therapeutic strategy, resulting in a switch to a more effective bismuth therapy, as demonstrated recently.⁷ Detection of antibiotic resistance in *H. pylori* is routinely

performed phenotypically by determining the MICs of antibiotics used for eradication treatment following the EUCAST/Comité de l'antibiogramme de la Société Française de Microbiologie (CA-SFM) recommendations.⁸ FQ resistance in *H. pylori* strains is mainly due to amino acid substitution in *gyrA* with D91G, D91N, N87K or N87I leading to FQ resistance.^{9,10}

Levofloxacin is the FQ used for the eradication of *H. pylori*. A novel FQ, named 'delafloxacin', has drawn growing interest due to its acid-resistant properties.^{11,12} Delafloxacin is a brand-new non-zwitterionic anionic FQ with unique chemical properties due to its special structure, enabling it to be more effective under acidic conditions and to have dual action against DNA gyrase and type IV topoisomerase.^{13,14} Available in different formulations (for both oral or IV administration) in Europe, America and the Asia-Pacific region population, this molecule has already been used to treat community-acquired bacterial pneumonia.¹⁵ In these clinical situations, delafloxacin has been shown to be effective not only against MRSA and MSSA isolates, but also against levofloxacin-resistant *Staphylococcus aureus*.¹⁶ High microbiological response rates were observed against these isolates, as well as against isolates with documented mutations in the QRDR.¹⁶ These results suggest an *in vitro* difference between delafloxacin and other quinolones. A previous study showed very low delafloxacin MICs for levofloxacin-resistant *H. pylori* strains (MIC₅₀=0.094 and MIC₉₀=0.38 mg/L) but since a clinical breakpoint (CB) has not yet been defined, it is difficult to analyse these results.¹⁷ In the absence of an established CB, the epidemiological cut-off (ECOFF) value can be calculated from the distribution of a WT bacterial population.¹⁸ ECOFFs are based on the phenotypic traits of the microorganism and the antimicrobial agent.¹⁹ The upper end of WT distribution has been defined and applied in the process of evaluating antimicrobial *in vitro* activity.²⁰ Delafloxacin *in vitro* activity against *H. pylori* could be estimated by the ECOFF, without replacing a CB, which would be decisive for *H. pylori* eradication in patients.

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

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

Methods

Strains tested for delafloxacin (ECOFF), Cohort A

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

Characterization of *H. pylori* from gastric biopsy was part of routine laboratory diagnostics, and prospective determination of delafloxacin MIC had been implemented in 2022 as described in this manuscript. Gradient strip range for delafloxacin MIC was in conformity with a 2-fold dilution series from 0.002 to 32 mg/L for ECOFF determination.

Genomic investigation population, Cohort B

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

H. pylori culture, isolation and AST

H. pylori strains from gastric biopsies were isolated using selective media; specifically, Mueller-Hinton (Oxoid, Basingstoke, UK) supplemented with 10% defibrinated horse blood and Skirrow selective supplement (Oxoid) were used. Samples were then incubated in microaerobic conditions for 44 h at 35°C ± 2°C. Strains were identified at the species level by urease activity (bioMérieux, Marcy-l'Étoile, France) and morphological identification by Gram staining (CellaVision, Lund, Sweden). According to national recommendations, both clarithromycin and levofloxacin MICs were determined by using gradient strips (Liofilchem) then interpreted according to the French Society for Microbiology and EUCAST guidelines (considering a CB of 1 mg/L for both agents).²⁴ The proportion of bacterial strains for which quinolone antibiotic therapy would have been recommended was determined by collecting clarithromycin resistance data. Among levofloxacin-resistant strains, low-level resistance was defined as an MIC between 1 and ≤2 mg/L, intermediate-level resistance as an MIC between 3 and ≤24 mg/L, and high-level resistance as MIC > 24 mg/L. In parallel, delafloxacin MICs were determined using the same methodology.²⁵

gyrA sequencing

As indicated previously, the *H. pylori* genome was sequenced to determine the presence of *gyrA* mutations. Primers were 5'-TGA-GCG-TGA-TCA-TAG-GGC-GCG-CTT-TAC-CGG-A-3' and 5'-GGG-GGA-TAG-AAG-TCG-CCA-TCC-CTA-CAG-CGA-TCC-CA-3' (474 bp). Taq DNA Polymerase (Hifi Kapa Hot Start, Roche, Meylan, France) was used for PCR amplification, which was performed in 25 µL final volumes. The PCR profile was as follows: 1 cycle of 3 min at 95°C; 35 cycles of 20 s at 98°C, 15 s at 69°C and 2 min at 72°C; and then 2 min at 72°C. After checking PCR amplification by 1.5% agarose gel electrophoresis, Sanger sequencing was performed (3500Dx, Thermo Fisher, Waltham, USA). The sequencing process was carried out using BigDye™ Terminator v3.1 (Thermo Fisher) (i.e. 1 cycle of 1 min at 96°C and 25 cycles of 5 s at 96°C, 10 s at 50°C, 3 min at 60°C) before analysis on SeqScape version 2.7 (Thermo Fisher). The nucleotide and deduced amino acid sequences of analysed isolates were compared with those of the known *H. pylori gyrA* gene (GenBank accession number: PRJNA1081826).

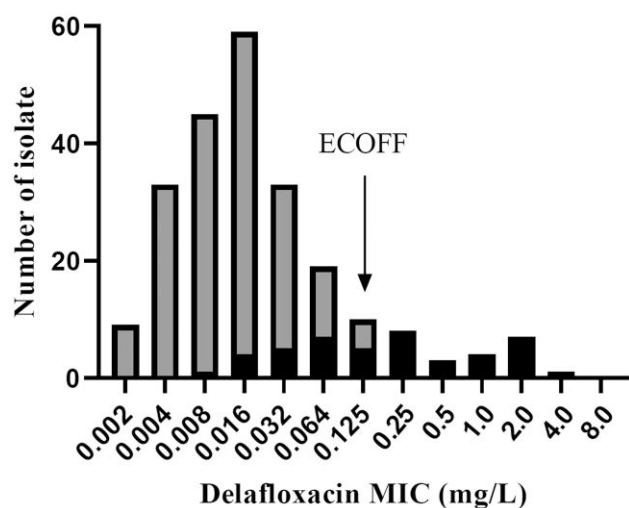


Figure 1. Microbiological distribution of 231 *H. pylori* isolates (Cohort A) for calculating delafloxacin ECOFF associated with levofloxacin phenotype. The measured ECOFF of ≤ 0.125 mg/L (black arrow) corresponds to a distribution of 99.0% (according to ECOFFinder_XL_2010_v2.1 software). Levofloxacin-susceptible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

Statistical analyses

Statistical analyses were performed using GraphPad Prism 9.0.0 software (Dotmatics, Boston, USA). Quantitative variables were compared using the non-parametric Mann-Whitney test or the z-test. Qualitative variables were compared using a chi-squared test or Fisher's exact test. Correlation tests were performed using Spearman's non-parametric test. For each statistical test, a *P* value of <0.05 was considered as significant (0.01 as highly significant).

Results

Estimation of delafloxacin ECOFF value and in vitro delafloxacin activity (Cohort A)

Using Cohort A ($n=231$ strains), analysis of delafloxacin MIC results led to an estimation of a delafloxacin ECOFF 99% at 0.125 mg/L using both CLSI ECOFFinder_XL_2010_v2.1 software and graphical analysis (Figure 1). All strains of Cohort A presenting a delafloxacin MIC below this threshold were considered as susceptible strains. Delafloxacin and levofloxacin MIC₅₀ and MIC₉₀ values were 0.016 and 0.125 mg/L versus 0.125 and ≥ 32 mg/L, respectively. Levofloxacin-resistant strains all presented delafloxacin MICs of ≥ 0.016 mg/L. No clinical isolates with delafloxacin MIC > 0.125 mg/L have been found to be susceptible to levofloxacin (Figure 1).

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

In vitro delafloxacin activity in levofloxacin-resistant H. pylori isolates (Cohort B)

Among Cohort B (composed of levofloxacin-resistant strains), 24 strains (24/101; 23.8%) have been considered as low-level

Table 1. *H. pylori* strains: clarithromycin and levofloxacin resistance among delafloxacin-susceptible isolates

<i>H. pylori</i> antibiotic susceptibility phenotype ($n=231$)	No. of isolates (%)
CLA-R	41 (17.7)
CLA-R and LVX-R	9 (3.9)
CLA-R and LVX-R and DLX-S	5 (2.2)

CLA, clarithromycin; LVX, levofloxacin; DLX, delafloxacin; R, resistant; S, susceptible.

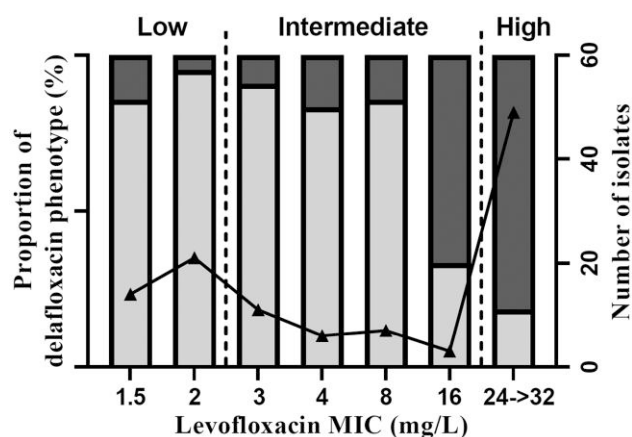


Figure 2. Proportions of the delafloxacin phenotypes according to the levofloxacin MIC among 101 *H. pylori* isolates. Based on the levofloxacin MIC (x-axis) for the number of strains (right axis) and the proportion of delafloxacin-resistant strains (left axis). Delafloxacin-susceptible and -resistant strains have been indicated in grey and black, respectively.

resistant strains, 29 (29/101; 28.7%) have been considered as intermediate-level resistant strains and 48 (48/101; 47.5%) have been considered as high-level resistant strains.

Among this cohort, 54 (54/101; 53.4%) strains were considered as susceptible to delafloxacin according to the previously determined ECOFF. The proportion of delafloxacin-resistant isolates positively correlates with the levofloxacin MIC ($P<0.05$) so that delafloxacin-resistant isolates (MIC > 0.125 mg/L) were more frequent in isolates with higher levofloxacin MICs (≥ 16 mg/L) ($P<0.01$) (Figure 2). Delafloxacin MIC₅₀ and MIC₉₀ values (0.125 and 0.75 mg/L, respectively) were at least 32 times lower than levofloxacin MIC₅₀ and MIC₉₀ values (16 and > 32 mg/L, respectively).

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

Levofloxacin-resistant *H. pylori* strains were sequenced, and several mutations were found in the *gyrA* QRDR (Table 2). Among these strains, 84 (84/101; 83.2%) presented a single-point polymorphism and 14 strains (14/101; 13.9%) a double-point polymorphism. Nine polymorphisms in *gyrA* were identified, leading to substitution of the amino acids 87, 91, 92, 99 and 129. Position 87 was the most commonly substituted (62/98;

63.3%) and substitution N87I ($n=29$; 29.6%) was the most frequent. In the whole cohort, delafloxacin MICs were significantly lower than levofloxacin MICs only for substitution at positions 87 and 91 ($P<0.01$) without significant difference in delafloxacin MIC means between strains harbouring substitutions in positions 91 or 87 ($P>0.05$). However, among delafloxacin-resistant strains, substitution at position 87 (36/62; 58.1%) was over-represented compared with position 91 (14/45; 31.1%) ($P<0.01$). Moreover, the N87I substitution was associated with higher delafloxacin MICs (at least three times more; $P<0.01$) than other mutations, whereas the N87K substitution could not be associated with any delafloxacin phenotype ($P>0.05$). Similarly, at position 91, the D91N substitution was more frequently associated with delafloxacin-susceptible strains

($P<0.05$), while the D91G substitution could not be associated with any delafloxacin phenotype ($P>0.05$) (Figure 3).

Mutations at position 87, particularly N87K and N87I, were associated with intermediate to high levels of resistance, whereas mutations at position 91, such as D91N and D91G, were associated with low to intermediate levels of resistance to levofloxacin (Figure S1, available as [Supplementary data](#) at JAC Online).

Discussion

Delafloxacin is a novel FQ with higher activity in acidic environments and lower MICs compared with other molecules of this family.¹³ Currently, there is no evidence to support the use of delafloxacin in treating *H. pylori* infections, as there are no validated

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

Mutation	N (%)	Delafloxacin			Phenotype	
		MIC range (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	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 ^a	3 (21.4)	0.032–0.094	0.064	0.094	3 (100.0)	1 (—)
N87I and other ^b	4 (28.6)	0.19–>32	0.38	>32	0 (—)	4 (100.0)
N87T and other ^c	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)

^aA92T ($n=2$) and P151S ($n=1$).

^bD91G ($n=1$), D91N ($n=1$), D91Y ($n=1$) and D161N ($n=1$).

^cD91N ($n=3$), D91N+L167I ($n=1$), D91N+D155G ($n=1$) and D91Y ($n=1$).

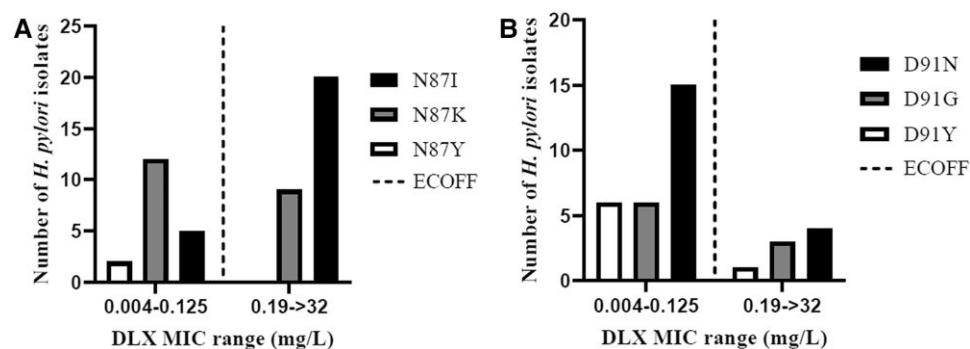


Figure 3. Distribution of single point mutations detected in *gyrA* (QRDR) in levofloxacin-resistant *H. pylori* strains according to delafloxacin MIC. (a) Levofloxacin-resistant strains with mutations at position 87 according to delafloxacin phenotype; (b) levofloxacin-resistant strains with mutations at position 91 according to delafloxacin phenotype. Delafloxacin MICs were classified into two ranges on either side of the measured ECOFF for this antibiotic.

CBs for this bacterium. One previous study observed lower MICs of delafloxacin compared with levofloxacin against *H. pylori* isolates without a cut-off value to interpret MICs as susceptible or resistant.¹⁷ Herein, to establish an ECOFF for delafloxacin, *H. pylori* MICs were obtained, using gradient test strips, for isolates from a large clinical cohort ($n = 231$). Whilst the broth micro-dilution method is considered as the reference for determining an ECOFF, the correlation between this method and gradient test strips was found to be outstanding for levofloxacin, clarithromycin and amoxicillin.^{26,27} Since the aggregated MIC distribution must include at least five valid MIC distributions (generated in separate laboratories), the present study allows only a tentative ECOFF (TECOFF) value according to EUCAST definition, in the absence of such a multicentre study (harder to set up). In doing so, interlaboratory variability was not calculated since delafloxacin MICs were determined only at a single centre. Other studies are required to determine interlaboratory variability.

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

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

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

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

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

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

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

In conclusion, these *in vitro* findings allowed us to estimate a delafloxacin TECOFF of 0.125 mg/L. For now, eradication treatment using antibiotics are based on the recommendations for guided treatment by the results of the antibiotic susceptibility testing (i.e. association of clarithromycin, amoxicillin and metronidazole as first-line treatment and association of levofloxacin, amoxicillin and metronidazole as second-line therapy). In the present study, it could be suggested that up to half of the strains that could be considered resistant to both the first and second-line treatments could be susceptible to delafloxacin (5/9; 55.5%; Table 1), suggesting that delafloxacin could be a potential therapy in place of levofloxacin. Indeed, *in vitro*, delafloxacin has lower MICs than levofloxacin, and some mutations responsible for levofloxacin resistance seem to have a lower impact on delafloxacin phenotype. Nevertheless, establishing CBs remains crucial to evaluation of delafloxacin's potential as an alternative to levofloxacin.

Acknowledgements

We wish to thank Jeffrey Ashram, a native English speaker, for his extensive reviewing of the English language in the manuscript.

Funding

This study was supported by internal funding.

Transparency declarations

The authors declare no conflict of interest regarding the present publication.

Supplementary data

Figure S1 is available as [Supplementary data](#) at JAC Online.

References

- Wang F, Meng W, Wang B et al. *Helicobacter pylori*-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014; **345**: 196–202. <https://doi.org/10.1016/j.canlet.2013.08.016>
- Malfertheiner P, Megraud F, Rokkas T et al. Management of *Helicobacter pylori* infection: the Maastricht VI/Florence consensus report. *Gut* 2022; [gutjnl-2022-327745](https://doi.org/10.1136/gutjnl-2022-327745). <https://doi.org/10.1136/gutjnl-2022-327745>
- Mégraud F, Alix C, Charron P et al. Survey of the antimicrobial resistance of *Helicobacter pylori* in France in 2018 and evolution during the previous 5 years. *Helicobacter* 2021; **26**: e12767. <https://doi.org/10.1111/hel.12767>
- Thung I, Aramin H, Vavinskaya V et al. Review article: the global emergence of *Helicobacter pylori* antibiotic resistance. *Aliment Pharmacol Ther* 2016; **43**: 514–33. <https://doi.org/10.1111/apt.13497>
- Savoldi A, Carrara E, Graham DY et al. Prevalence of antibiotic resistance in *Helicobacter pylori*: a systematic review and meta-analysis in World Health Organization regions. *Gastroenterology* 2018; **155**: 1372–82.e17. <https://doi.org/10.1053/j.gastro.2018.07.007>
- Losurdo G, Giorgio F, Pricci M et al. *Helicobacter pylori* primary and secondary genotypic resistance to clarithromycin and levofloxacin detection in stools: a 4-year scenario in southern Italy. *Antibiotics (Basel)* 2020; **9**: 723. <https://doi.org/10.3390/antibiotics9100723>
- Bujanda L, Nyssen OP, Ramos J et al. Effectiveness of *Helicobacter pylori* treatments according to antibiotic resistance. *Am J Gastroenterol* 2024; **119**: 646–54. <https://doi.org/10.14309/ajg.0000000000002600>
- Soci t  Francaise de Microbiologie, EUCAST. Comit  de l'antibiogramme de la Soci t  Francaise de Microbiologie, Recommandations 2017. 2017. https://www.sfm-microbiologie.org/wp-content/uploads/2019/02/CASFMV1.0_MARS.2017.pdf
- Garcia M, Raymond J, Garnier M et al. Distribution of spontaneous *gyrA* mutations in 97 fluoroquinolone-resistant *Helicobacter pylori* isolates collected in France. *Antimicrob Agents Chemother* 2012; **56**: 550–1. <https://doi.org/10.1128/AAC.05243-11>
- Glocker E, Berning M, Gerrits MM et al. Real-time PCR screening for 16S rRNA mutations associated with resistance to tetracycline in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2005; **49**: 3166–70. <https://doi.org/10.1128/AAC.49.8.3166-3170.2005>
- Nilius AM, Shen LL, Hensey-Rudloff D et al. In vitro antibacterial potency and spectrum of ABT-492, a new fluoroquinolone. *Antimicrob Agents Chemother* 2003; **47**: 3260–9. <https://doi.org/10.1128/AAC.47.10.3260-3269.2003>
- Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. *Expert Rev Anti Infect Ther* 2010; **8**: 59–70. <https://doi.org/10.1586/eri.09.113>
- Candel FJ, Pe uelas M. Delafloxacin: design, development and potential place in therapy. *Drug Des Devel Ther* 2017; **11**: 881–91. <https://doi.org/10.2147/DDDT.S106071>
- Van Bambeke F. Delafloxacin, a non-zwitterionic fluoroquinolone in phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy. *Future Microbiol* 2015; **10**: 1111–23. <https://doi.org/10.2217/fmb.15.39>
- G mez-Zorrilla S, Sendra E, Horcajada JP. A profile of delafloxacin in the treatment of adults with community-acquired bacterial pneumonia. *Expert Rev Clin Pharmacol* 2022; **15**: 671–88. <https://doi.org/10.1080/17512433.2022.2100346>
- McCurdy S, Lawrence L, Quintas M et al. In vitro activity of delafloxacin and microbiological response against fluoroquinolone-susceptible and nonsusceptible *Staphylococcus aureus* isolates from two phase 3 studies of acute bacterial skin and skin structure infections. *Antimicrob Agents Chemother* 2017; **61**: e00772-17. <https://doi.org/10.1128/AAC.00772-17>
- Boyanova L, Markovska R, Medeiros J et al. Delafloxacin against *Helicobacter pylori*, a potential option for improving eradication success? *Diagn Microbiol Infect Dis* 2020; **96**: 114980. <https://doi.org/10.1016/j.diagmicrobio.2019.114980>
- Turnidge J, Kahlmeter G, Kronvall G. Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clin Microbiol Infect* 2006; **12**: 418–25. <https://doi.org/10.1111/j.1469-0691.2006.01377.x>
- EUCAST. Setting breakpoints for new antimicrobial agents. EUCAST SOP 1.4. 2021. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/2021/EUCAST_SOP_1.4_Setting_breakpoints_new_agents_20211202.pdf
- Kahlmeter G, Turnidge J. How to: ECOFFs—the why, the how, and the don'ts of EUCAST epidemiological cutoff values. *Clin Microbiol Infect* 2022; **28**: 952–4. <https://doi.org/10.1016/j.cmi.2022.02.024>
- Hoover RK, Alcorn H, Lawrence L et al. Delafloxacin pharmacokinetics in subjects with varying degrees of renal function. *J Clin Pharmacol* 2018; **58**: 514–21. <https://doi.org/10.1002/jcph.1041>
- Hoover R, Hunt T, Benedict M et al. Safety, tolerability, and pharmacokinetic properties of intravenous delafloxacin after single and multiple doses in healthy volunteers. *Clin Ther* 2016; **38**: 53–65. <https://doi.org/10.1016/j.clinthera.2015.11.019>
- CLSI. ECOFFinder: Free MS Excel Spreadsheet Calculator. <https://clsi.org/meetings/susceptibility-testing-subcommittees/ecoffinder/>
- Soci t  Francaise de Microbiologie, EUCAST. Comit  de l'antibiogramme de la Soci t  Francaise de Microbiologie, Recommandations 2022. 2022. https://www.sfm-microbiologie.org/wp-content/uploads/2022/05/CASFM2022_V1.0.pdf
- Miyachi H, Miki I, Aoyama N et al. Primary levofloxacin resistance and *gyrA/B* mutations among *Helicobacter pylori* in Japan. *Helicobacter* 2006; **11**: 243–9. <https://doi.org/10.1111/j.1523-5378.2006.00415.x>
- Tang X, Shen Y, Song X et al. Reassessment of the broth microdilution method for susceptibility testing of *Helicobacter pylori*. *J Infect Dis* 2022; **226**: S486–92. <https://doi.org/10.1093/infdis/jjac389>
- Glupczynski Y, Broutet N, Cantagrel A et al. Comparison of the E test and agar dilution method for antimicrobial susceptibility testing of *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 2002; **21**: 549–52. <https://doi.org/10.1007/s10096-002-0757-6>
- EMA. Summary of Product Characteristics, Quofenix. https://www.ema.europa.eu/en/documents/product-information/quofenix-epar-product-information_en.pdf
- Remy JM, Tow-Keogh CA, McConnell TS et al. Activity of delafloxacin against methicillin-resistant *Staphylococcus aureus*: resistance selection and characterization. *J Antimicrob Chemother* 2012; **67**: 2814–20. <https://doi.org/10.1093/jac/dks307>
- Tran VH, Nguyen TMN, Le PTQ et al. Current status of *Helicobacter pylori* resistance to clarithromycin and levofloxacin in Vietnam: results from molecular analysis of gastric biopsy specimens. *J Glob Antimicrob Resist* 2024; **36**: 76–82. <https://doi.org/10.1016/j.jgar.2023.12.026>
- Wei W, Wang Z, Li C et al. Antibiotic resistance of *Helicobacter pylori* in Nanjing, China: a cross-section study from 2018 to 2023. *Front Cell Infect Microbiol* 2023; **13**: 1294379. <https://doi.org/10.3389/fcimb.2023.1294379>
- Cattoir V, Nectoux J, Lascols C et al. Update on fluoroquinolone resistance in *Helicobacter pylori*: new mutations leading to resistance and first description of a *gyrA* polymorphism associated with hypersusceptibility.

- Int J Antimicrob Agents* 2007; **29**: 389–96. <https://doi.org/10.1016/j.ijantimicag.2006.11.007>
- 33** Saranathan R, Levi MH, Wattam AR *et al.* *Helicobacter pylori* infections in the Bronx, New York: surveying antibiotic susceptibility and strain lineage by whole-genome sequencing. *J Clin Microbiol* 2020; **58**: e01591–19. <https://doi.org/10.1128/JCM.01591-19>
- 34** Chu A, Wang D, Guo Q *et al.* Molecular detection of *H. pylori* antibiotic-resistant genes and molecular docking analysis. *FASEB J* 2020; **34**: 610–8. <https://doi.org/10.1096/fj.201900774R>
- 35** Tshibangu-Kabamba E, Ngoma-Kisoko PDJ, Tuan VP *et al.* Next-generation sequencing of the whole bacterial genome for tracking molecular insight into the broad-spectrum antimicrobial resistance of *Helicobacter pylori* clinical isolates from the Democratic Republic of Congo. *Microorganisms* 2020; **8**: 887. <https://doi.org/10.3390/microorganisms8060887>
- 36** Lee JW, Kim N, Nam RH *et al.* Mutations of *Helicobacter pylori* associated with fluoroquinolone resistance in Korea. *Helicobacter* 2011; **16**: 301–10. <https://doi.org/10.1111/j.1523-5378.2011.00840.x>
- 37** Wang L-H, Cheng H, Hu F-L *et al.* Distribution of *gyrA* mutations in fluoroquinolone-resistant *Helicobacter pylori* strains. *World J Gastroenterol* 2010; **16**: 2272–7. <https://doi.org/10.3748/wjg.v16.i18.2272>
- 38** Rimbara E, Noguchi N, Kawai T *et al.* Fluoroquinolone resistance in *Helicobacter pylori*: role of mutations at position 87 and 91 of GyrA on the level of resistance and identification of a resistance conferring mutation in GyrB. *Helicobacter* 2012; **17**: 36–42. <https://doi.org/10.1111/j.1523-5378.2011.00912.x>
- 39** Rhie SY, Park JY, Shin T-S *et al.* Discovery of a novel mutation in DNA gyrase and changes in the fluoroquinolone resistance of *Helicobacter pylori* over a 14-year period: a single center study in Korea. *Antibiotics (Basel)* 2020; **9**: 287. <https://doi.org/10.3390/antibiotics9060287>
- 40** Wang G, Taylor DE. Site-specific mutations in the 23S rRNA gene of *Helicobacter pylori* confer two types of resistance to macrolide-lincosamide-streptogramin B antibiotics. *Antimicrob Agents Chemother* 1998; **42**: 1952–8. <https://doi.org/10.1128/AAC.42.8.1952>
- 41** Burucoa C, Landron C, Garnier M *et al.* T2182C mutation is not associated with clarithromycin resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2005; **49**: 868; author reply 868–70. <https://doi.org/10.1128/AAC.49.2.868-870.2005>
- 42** Pichon M, Luzarraga V, Burucoa C. Mutations associated to antibiotic-resistant *Helicobacter pylori*: it is time to validate sequencing observations. *J Clin Med* 2022; **11**: 4293. <https://doi.org/10.3390/jcm11154293>