P0521

Validation by site-directed mutagenesis of mutations described as associated with clarithromycin resistance in *Helicobacter pylori*

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Introduction

Helicobacter pylori (*H. pylori*) infects the stomach of **half of humanity**. Often asymptomatic, its carriage can **cause gastroduodenal pathologies** and/or lead to **gastric cancer**. The **sixth Maastricht/Florence European Consensus Conference (VI)** [1] recommended its eradication **based on antibiotic-susceptibility testing** (AST) **results**. AST can be performed by detecting **mutations conferring antibiotic resistance in *23S rRNA* gene, *gyrA*, *plp1*, *rdxA/frxA*, *rpoB* and *16S rRNA* gene** for clarithromycin, levofloxacin, amoxicillin, metronidazole rifamycin, tetracyclines or metronidazole resistance.

Nevertheless, **many mutations have been described whose link with resistance has not always been formally proven** because these mutations have been described with concomitant mutations whose role is known.

Objective

To validate main mutations in *23S rRNA* gene for clarithromycin resistance by producing site-specific mutants of antibiotic-susceptible reference strain.

Methods

After an **extensive review of the literature**, nucleotide mutations described as involved in clarithromycin resistance for which there is a need to determine their impact on antibiotic resistance were selected for the site-directed mutagenesis step.

Briefly, a **wild-type strain of *H. pylori* (J99)** was **transformed by site-directed mutagenesis** using its natural transformation properties (Figure 1).

After **culturing the transformed strain on agar plates supplemented or not with antibiotics**, mutants were **validated by Sanger Sequencing** and their **AST profile was determined for clarithromycin** as recommended by the EUCAST procedure (Figure 2).

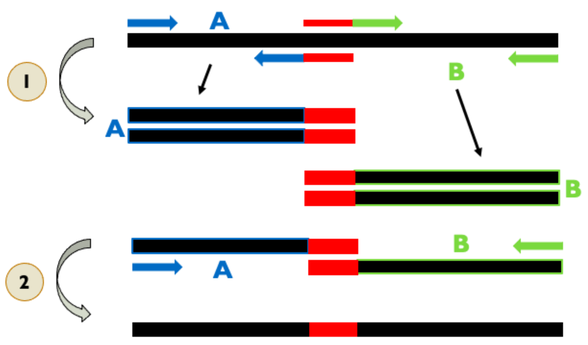

Figure 1. Site-directed mutagenesis, two-step PCR (as described by [2]).

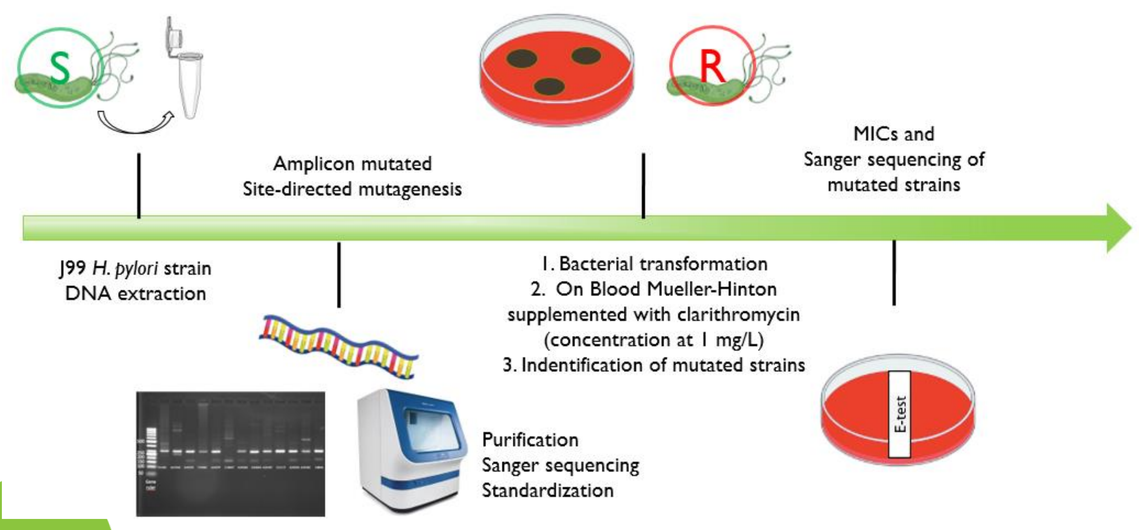

Figure 2. Process for the production of a clarithromycin-resistant J99 strain by transformation of a wild type J99 strain from a mutated PCR product by site-directed mutagenesis.

Results

The site-directed mutagenesis PCR products are sequenced to show the presence of the mutation before the processing step. (Figure 3).

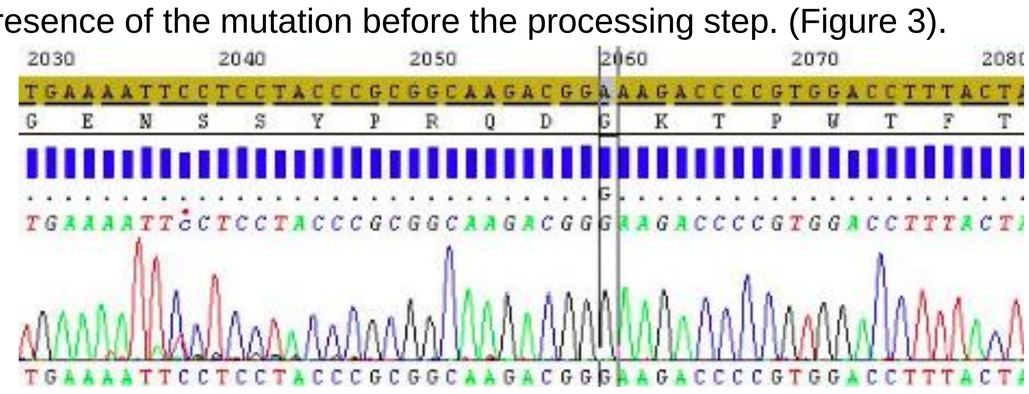

Figure 3. Sanger sequencing of a PCR product by site-directed mutagenesis containing the A2058G (A2142G) mutation before the transformation step. Aligment with J99.

Mutations that do not confer resistance to clarithromycin are not detected on clarithromycin plates, suggesting that the mutation does not confer resistance to clarithromycin. The results obtained (on *23S rRNA* gene, a gene involved in clarithromycin resistance) validated some mutations (including the well-described A2142/2143 mutations as a positive control) and excluded others (Table 1). These results are obtained in triplicate.

Table 1. Mutations studied and results about their clarithromycin phenotype.
20 mutations were studied such as C977A>G>T, G2141A, T2190C, C2195T, T2711C, C2759T [3], A2142C>G>T, A2143C>G>T [2], G1949A, C1953T, T>C2182, A2223G, C2759T [4], A2144G [3,5], C2926T [6].

	Resistance	Susceptible		
Mutations	**A2142C** **A2142G** **A2142T** **A2143C** **A2143G**	C977A>G>T G1949A C1953T G2141A A2143T	A2144G T2182C T2190C C2195T	A2223G T2711C C2759T C2926T

Discussion

The present study reinforced the **robustness of *23S rRNA* gene mutations described as associated with antibiotic resistance in *H. pylori***. Indeed, Sanger sequencing of mutated amplicons obtained by site-directed mutagenesis prior to bacterial transformation reinforces the absence of cultured clarithromycin resistance-conferring mutated strains on clarithromycin supplemented plates. As culture of *H. pylori* remains fastidious, AST realization is not performed in all laboratories. The five mutations conferring clarithromycin resistance were previously studied by site-directed mutagenesis by Wang *et al*., but our study expanded the panel of mutations studied, suggesting that clarithromycin resistance was limited to only a few mutations that could be detected by molecular biological techniques.

Conclusion

The objective of this study is to demonstrate that most of the mutations observed in the *23S rRNA* gene do not confer resistance to clarithromycin. Finally, **20 mutations were studied** but **only five mutations confer resistance** to clarithromycin such as A2142C>G>T and A2143C>G. The same approach is **currently applied to quinolone resistance (*gyrA*-associated mutation) as this treatment is the second-line antibiotic** in case of clarithromycin-resistant strain. In the absence of a WHO database, this type of validation is essential to optimize the clinical management of infected patients, in a context of increasing antibiotic resistance in *H. pylori*.

Reference

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