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Detection of New Mutations in 23S rRNA Gene of Helicobacter Pylori in Gastric Biopsies in Abidjan

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CVMG and FBDT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KN and SNDC managed the analyses of the study. Author NG, AFY, AFY, AJD and MD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: The purpose of this study is to determine the presence of mutations in 23S rRNA gene, conferring resistance to clarithromycin of *Helicobacter pylori* from gastric biopsies in Abidjan (Côte d'Ivoire).
Place and Duration: Between August 2015 and February 2016, gastric biopsies were collected from adult patients in endoscopy room in Gastroenterology Department of Hospital and University Center of Cocody (Abidjan), then stored. From October to December 2016, laboratory tests were performed in Bacteriology-Virology department, molecular biology platform of Institute Pasteur of Côte d'Ivoire, and sequencing platform at Eurofins (Cochin, France).

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Methodology: *Helicobacter pylori* DNA was extracted directly from stored gastric biopsies. Detection of 23S rRNA gene of *Helicobacter pylori* resistance to clarithromycin was done through conventional PCR and was quantified using a NanoDrop® spectrophotometer, Lite (Thermo Fischer Scientific, USA), followed by sequencing from Eurofins, MWG / operon (Cochin, France). The reference strains used for sequence comparison were selected from NCBI's Genbank's database with the accession number U27270.1.
Results: A new unreported T> C substitution was identified at 100% (3/3) at position 2616 (T2616C) compared to the reference strain.
Conclusion: The presence of a constant mutation, not yet described in 23S rRNA gene does require monitoring when the frequency of this mutation is probably responsible for future generations of mutant clones.

Keywords: Helicobacter pylori; 23S rRNA gene; clarithromycin; mutations; abidjan.

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) is a major cause of gastroduodenal disorders and diseases such as dyspepsia, type B gastritis, and duodenal ulcer [1-5]. Recommendations for treatment of *H. pylori* infection which developed by the French study group Helicobacter (GEFH) that consist of two types of treatments, namely a front-line treatment that includes a triple therapy for 14 days combining inhibitor proton pumps (PPIs). Another is two antibiotics for amoxicillin (AML), clarithromycin (CLR) and metronidazole (MTZ), based on results of the antibiogram or Polymerase Chain Reaction (PCR) against these antibiotics. Second-line treatment is a probable treatment including quadruple therapy bismuth (salt of bismuth + metronidazole + tetracycline) during ten days or a concomitant quadruple therapy (IPP + AML + MTZ + CLR) during 14 days [6]. Clarithromycin is one of the most effective microbial agents used in the treatment of *H. pylori* infection [7]. Thus, development of CLR resistance is a major cause of failure of *H. pylori* treatment [8,9]. Resistance rate to CLR of 26.5% in Côte d'Ivoire [10] is much higher than resistance rate in other African countries (13%) where the gene was tested [11]. CLR resistance of *H. pylori* is related to point mutations in 23s rRNA gene that reduce the affinity of antibiotic for its ribosome target or change the methylation site. The most nucleotide substitutions frequently reported in 23S rRNA gene responsible for resistance to CLR in clinical isolates of *H. pylori* are 2142A> G, 2143A> G, 2116A> G, and 2182T> C [12-15]. The purpose of this study is to investigate the presence of point mutations in *23S rRNA* gene of *H. pylori* in gastric biopsies of Ivorian patients with regards to observed therapeutic failures in this molecule [16,17].

2. MATERIALS AND METHODS

2.1 Sample Preparation

Three of twenty-six samples of *23S rRNA* gene of *H. pylori* detected by PCR conventional were sequenced [10]. First, gastric biopsies were collected in endoscopy room from adult patients in Gastroenterology Department of Hospital and University Center of Cocody (Abidjan) from August 2015 to February 2016, then stored at -4 Fahrenheit degrees in Bacteriology-Virology department. PCR and samples preparation was performed at Molecular biology platform of Pasteur Institute of Côte d'Ivoire from October to December 2016. The whole was purified using a QIAquick PCR purification kit (Qiagen® GmbH, Hilden, Germany). The genome size of three genes was measured by using a NanoDrop Lite spectrophotometer (Thermo Fischer Scientific®, USA).

2.2 Premixed Sample Preparation

Each amplicon of 23S rRNA gene produced by PCR was aliquoted in an Eppendorf tube of 2 mL at 13 µL of 23S rRNA gene purified with either concentration given in Table 1 for twoµL of primer with a concentration of 10 pmol/UL (10 UM). The total volume of premixed sample was 17 UL.

Table 1. Concentrations and volumes used for samples preparation

Sample type	Product length	Sample concentration	Sample volume
Purified PCR Products	300-1000 bp	Five ng/ µl	15 µl

2.3 Sequencing Primers

Primers used were CLA1995FW (GTAACTATAACGGTCCTAAG). Exactly 10 pmol/µl primer concentration was required per sequencing reaction. Each primer had to have a total volume of 15 µl (double distilled water or 5mM Tris-HCl); 5 µl of volume in primers was required for every additional sequencing reaction, and concentration of primers with wobble bases must be calculated according to following formula: nX x Conc Primer.

n = number of wobble bases within a wobble according to IUPC code, X = number of wobble bases within primer sequence. [e.g. 1 V (AGC) = 31 x 10 pmol/µl; 2 V (AGC) (AGC) = 32 x 10 pmol/µl]

Sequencing was performed at Eurofins, MWG / operon (Cochin, France). Alignment of neo-synthesized nucleotide sequences was performed using Bioedit® and Seaview® 64-bit software (France). Reference strains used for sequence comparison were selected from NCBI Genbank's database with the accession number U27270.1.

3. RESULTS

3.1 Prevalence of Mutation Points

Three *23S rRNA* gene conferring resistance to CLR was sequenced. A new unreported T> C substitution was identified at position 2616 (T2616 C) compared to the sample strain in 100% (3/3) of identified strains. This mutation regarding amino acid was silent (same sense) at position 872 and relates to serine (S872S). A point mutation C883S / N has also been described at 75% (2/3).

4. DISCUSSION

Overall, the most frequently reported nucleotide substitutions within 23S rRNA gene responsible for CLR resistance in *H. pylori* clinical isolates are 2142A> C, 2142A> G, and 2143A> G (with 2116A> G and 2182T> C) [18-21]. The current study could not identify the substitutions described above. However, a new nucleotide substitution (2616 T> C) in three 23S rRNA genes conferring resistance to CLR. These types of mutations generally go unnoticed but are delicate and must be monitored especially because they could create mutant clones in future generations with or without silent mutations. Indeed, during replication process in eukaryotes, a fragment of the DNA is passed onto the offspring. In this case, the given fragment carrying the mutation will induce the synthesis of a complementary new strand, different from that of the mother cell. This new synthesis, consequently, leads to the synthesis of the DNA of the mutated individual from the insertion of a new link. The appearance of such mutations could lead to the creation of "hyper-resistant" clones. However, since nucleotide substitutions are not always linked to antibiotic resistance associated with the genes, they may not be responsible for drug resistance [22]. Thus, other studies including mutagenic transformation analyses are necessary to justify any direct association of this new nucleotide variation in studied genes and the ability to confer resistance to *H. pylori* as suggested by Raseed et al. [23].

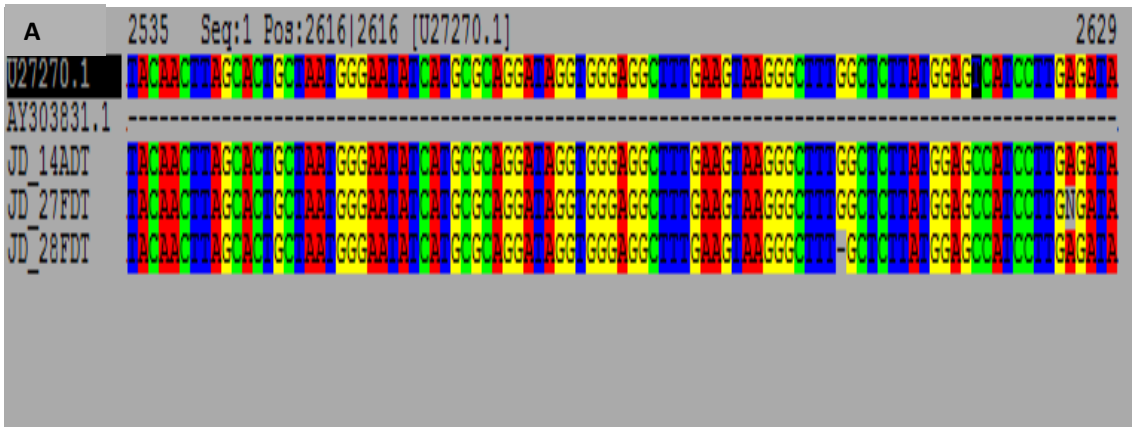

Fig. 1. Alignment of the amino acids *23S rRNA* of selected strains resistant to CLR
A: Visualization of T2616C mutation in 23S rRNA gene

5. CONCLUSION

This study revealed a 2616 T> C point mutation not yet described in *H. pylori* 23S rRNA gene. This mutation could be one of the causes of CLR resistance of this bacterium in Côte d'Ivoire although research for similar mutations should be sought. Detection of such mutation requires monitoring as the frequency of this mutation is probably responsible for future generations of mutant clones.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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