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Detection of Mutation of PBP1 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 FBDT, SKN and NDC managed the analyses of the study. Authors NG, 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 the gene *pbp1*, conferring resistance to amoxicillin of *Helicobacter pylori*, from gastric biopsies in Abidjan (Côte d'Ivoire).
Place and Duration: Between August 2015 and February 2016, gastric biopsies were collected in the endoscopy room from adult patients in the Gastroenterology Department at the Hospital and University Center of Cocody (Abidjan) and then stored. From October to December 2016, laboratory tests were performed in the Bacteriology-virology department, the molecular biology platform of the Institute Pasteur of Côte d'Ivoire, and the sequencing platform Eurofins (Cochin, France).
Methodology: *Helicobacter pylori* DNA was extracted directly from the stored gastric biopsies. The detection of the gene *pbp1* in *Helicobacter pylori* was done through conventional PCR and the DNA

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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 database with accession numbers ranging from AY 743230.1 to AY 743236.1.

Results: Thirteen out of fifty-six *pbp1* genes, conferring resistance to amoxicillin, were sequenced. The substitution of Lysine for Leucine at position 102 (K102L) was predominant in 7 of them, about 53.8%. A Substitution at position 62 of glycine by alanine (G62A) was also found in 6 of them (about 46.2%). In addition, 5 of the 13 strains (or 38.5%) all had Lysine substitutions. They are F45K, D54K, H60K, and I117K.

Conclusion: The presence of several mutations in the gene pbp1 of *H. pylori* might be a dominant factor in the resistance to amoxicillin of *H. pylori*, which needs further investigations.

Keywords: Helicobacter pylori; pbp1 gene; amoxicillin; mutation; Abidjan.

1. INTRODUCTION

Helicobacter pylori infection (*H. pylori*) is one of the most common chronic conditions. It affects more than 80% of the population in developing countries and treatment regimens have not always been effective [1,2]. One possible reason for the failures in eradication is the bacterial resistance to antimicrobial used or concentration of antibiotics used. The reaction of *H. pylori* to antibiotics, directly influenced by the previous use of these drugs, varies between the regions of the same country and between different countries as well. Thus, the success of a treatment regimen in a community does not allow for the standardization of its results. In general, clinicians in developing countries apply the therapeutic protocols from the European international consensus due to the absence or scarcity of data on the levels of resistance in these countries. Conventionally, the treatment consists in combining two or three antibiotics taken from amoxicillin (AML), clarithromycin (CLR), and metronidazole MTZ) with a proton pump inhibitor. This is one of the leading therapeutic methods recommended by the French Helicobacter Study Group (GEFH). It would be ideal to provide therapy based on prior knowledge of microbial resistance in a local community but this is difficult in most developing countries. In Côte d'Ivoire, data on antibiotic resistance and its causes is scarce even. In addition, out of the three antibiotics combined for the treatment of *H. pylori* infection, AML seemed ineffective due to problems of resistance. In 2004, the highest rate of resistance found in France was less than 1% [3], and less than 2% in Brazil in 2014 [4] (no resistance was found in São Paulo, Brazil [5]). However, a recent study conducted in Côte d'Ivoire by Diplo et al. [6] reports a high resistance rate of *H. pylori* to AML of 58.2%.

The purpose of this study is to determine the presence of mutations in the gene *pbp1* conferring resistance to AML of *H. pylori* from gastric biopsies in Ivorian patients.

2. MATERIALS AND METHODS

2.1 Sample Preparation

First, gastric biopsies were collected in the endoscopy room from adult patients in the Gastroenterology Department of the Hospital and University Center of Cocody (Abidjan) from August 2015 to February 2016, then stored at - 4 Fahrenheit degrees in the Bacteriology-Virology department [6]. By conventional PCR, 56 DNA samples of the gene pbp1 of *H. pylori* were extracted directly from gastric biopsies and stored at - 4 Fahrenheit degree. 13 of them were sequenced.

PCR and samples preparation was performed at the Molecular biology platform of the Institute Pasteur of Côte d'Ivoire between October to December 2016. The whole sample was purified using a QIAquick PCR purification kit (Qiagen® GmbH, Hilden, Germany). The genome size of the 13 DNA samples was measured by using a NanoDrop Lite spectrophotometer (Thermo Fischer Scientific®, USA).

Table 1. Concentration and volume used for sample

Sample type	Product length	Sample concentration	Sample volume
Purified PCR Products	300-1000 bp	5 ng/ µl	15 µl

2.2 Premixed Sample Preparation before Sending

Each amplicon of AML resistance gene (pbp1) produced by PCR was aliquoted in an Eppendorf tube of 2 mL at 13 µl of purified DNA with either concentration given in Table 1 for 2 µl of primer with a concentration of 10 pmol/µl (10 µM). The total volume of the premixed sample was 17 µl.

2.3 Sequencing Primers

The primers used were PBP1F (CACGAGCACCGGTAAGATTT). 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 the concentration of primers with wobble bases must be calculated according to the following formula: nX x ConcPrimer.

n = number of wobble bases according to IUPC code, X = number of wobble bases within the 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 database with accession numbers AY 743230.1 through AY 743236.1.

3. RESULTS

The gene *pbp1* conferring resistance to AML detected in thirteen biopsies were sequenced. Substitution of Lysine for Leucine at position 102 (K102L) was predominant (53.8%). Substitution at position 62 of glycine by alanine (G62A) was also found in 6/13 (46.2%). Among sequences, 5/13 (38.5%) had amino acid substitutions by Lysine. These are F45K, D54K, H60K and I117K (Table 2).

Fig. 1 shows the amino acid alignment of the synthesized neo sequences to the reference sequences. The frequencies of appearance of the mutations are also shown in Fig. 2.

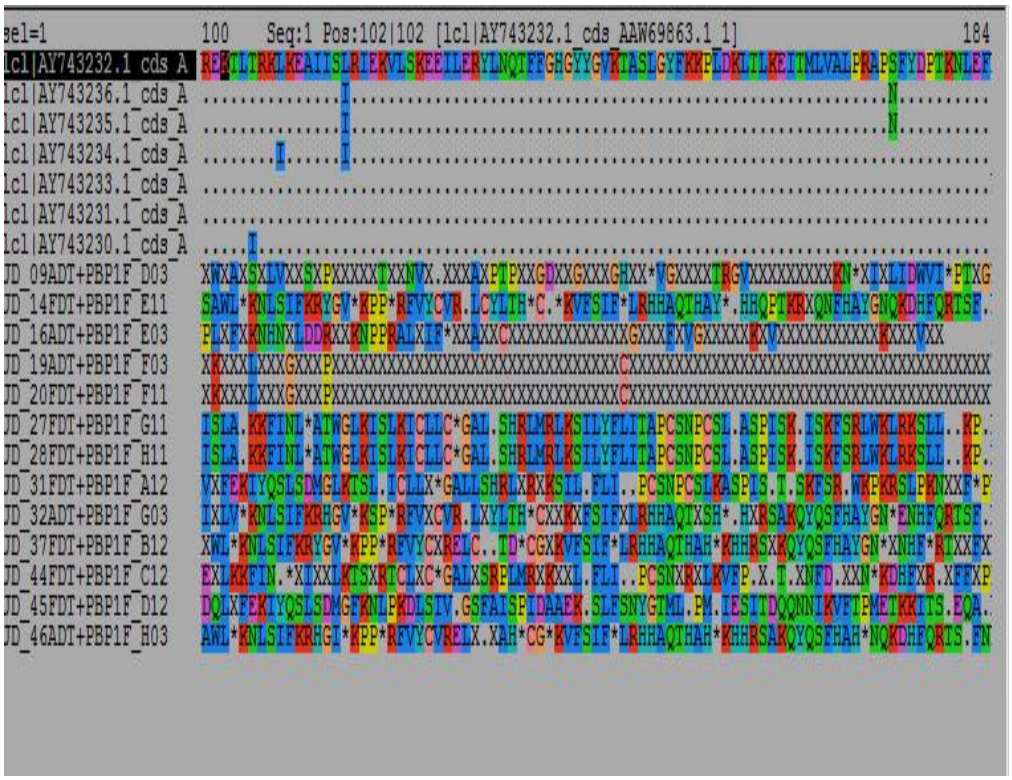

Fig. 1. Alignment of the amino acids pbp1 of selected strains resistant to antibiotics

Table 2. Different types of mutation identified in the gene *pbp1* sequences

H. pylori strains	Gene	Amino acid change		Type of mutation
		Effective (n)	Frequency (%)	
09ADT 14FDT	*pbp1*	K102L (n=7)	53,8	Substitution
16ADT 19ADT		G62A (n=6)	46,2	
20FDT		F45K, D54K, H60K et I117K (n=5)	38,2	
27FDT 28FDT 31FDT 32ADT 37FDT 44FDT 45FDT 46ADT		V12F, I24R, D29L, R33K, F34S, A36L, E40I, I41P, P42R, R44L, D232K (n=4)	30,8	

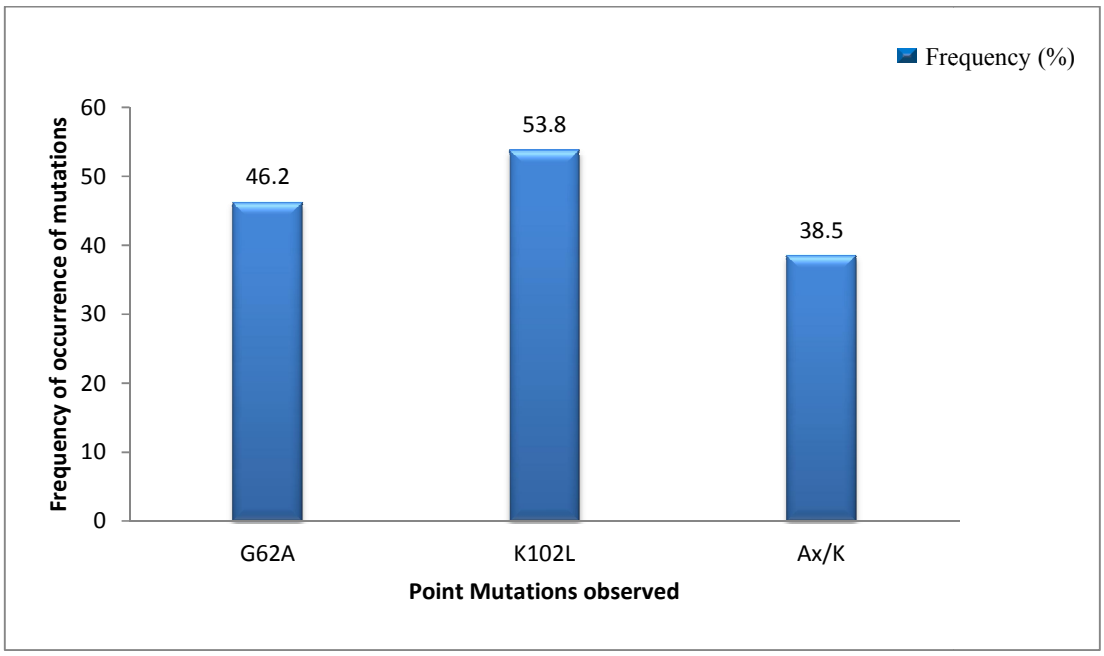

Fig. 2. Frequency of occurrence of point mutations in the gene *pbp1* of selected strains resistant to antibiotics

4. DISCUSSION

The substitutions described in this study have not been reported in previous studies, as AML resistance rates are very rare or non-existent in other countries [3,7] such as Brazil [4] and Israel [8].

Currently, global AML resistance levels are low; therefore, this molecule is frequently used in combined first-line therapy. The Sensitivity to AML has also been high in other countries such as Germany [9], Spain [10], Philippines [11] and even in Africa, around Tunisia [12]. In Latin American countries, the AML resistance rates were below 4% (3.8% in Colombia [13], 2.2% in Paraguay [14] and 2.3% in Chile [15]). There was no resistance to this antibiotic in Venezuela [16]. The high levels of resistance have been found in some places [17], particularly in Côte d'Ivoire, with a molecular prevalence of 58.2%, according to Diplo et al. [6]. Under these conditions, it is important to monitor these levels of resistance.

Amino acid substitutions were found mostly at two positions (K102L and G62A), 53.8% and 46.2% respectively, in AML resistance DNA sequences. These substitutions differ from those described by Rasheed et al. [18] in Pakistan (D535N, S543R and T556S) with a predominance of D535N and other previous studies as well [19-21]. In the current study, the most common amino acid substitution found in pbp1, which is also responsible for AML resistance, was K102L. The differences in mutation sites observed in two different countries of a same African continent would indicate the ability of the *H. pylori* to adapt to its host and its environment [22,23]. These results would also be related to the consumption of this antibiotic by the population. Indeed, AML is widely-used in human medicine, especially in first-line treatments of mild respiratory infections in adults and in children in Ivory Coast [24,25]. Also, the extensive use of AML against other pathogens in people with chronic *H. pylori* infection may stimulate an increased frequency of mutation in *H. pylori*, thus inducing the emergence of resistance against AML and other antibiotics [26].

5. CONCLUSION

These results encourage a review of the therapeutic protocol including the use of AML in Ivorian hospitals. The presence of several mutations in the gene pbp1 of H. pylori might be a dominant factor in the resistance to amoxicillin of *H. pylori*, which needs further investigations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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