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Primary antibiotic resistance and its relationship with *cagA* and *vacA* genes in *Helicobacter pylori* isolates from Algerian patients

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ABSTRACT

The epidemiology of *Helicobacter pylori* resistance to antibiotics is poorly documented in Africa and especially in Algeria. The aim of our study was to determine the antibiotic resistance rates, as well as its possible relationship with *Vac*A and *Cag*A virulence markers of isolates from Algerian patients. One hundred and fifty one *H. pylori* isolate were obtained between 2012 and 2015 from 200 patients with upper abdominal pain. Antimicrobial susceptibility testing was performed for amoxicillin, clarithromycin, metronidazole, ciprofloxacin, rifampicin and tetracycline. Molecular identification of *H. pylori* and the detection of *vacA* and *cagA* genes were performed using specific primers. We found that *H. pylori* was present in 83.5% of collected biopsies, 54.9% of the samples were *cagA* positive, 49.67% were *vacA s1m1*, 18.30% were *vacA s1m2* and 25.49% were *vacA s2m2*. Isolates were characterized by no resistance to amoxicillin (0%), tetracycline (0%), rifampicin (0%), a high rate of resistance to metronidazole (61.1%) and a lower rate of resistance to clarithromycin (22.8%) and ciprofloxacin (16.8%). No statically significant relationship was found between *vagA* and *cagA* genotypes and antibiotic resistance results ($p > 0.5$) except for the metronidazole, which had relation with the presence of *cagA* genotype ($p = 0.001$).

Introduction

Helicobacter pylori (*H. pylori*) is a gram negative spiral chapped bacterium, described in 1982 by Marshal and Warren. It specifically infects the gastric mucosa. This discovery had changed all data about gastro-duodenal disorders. *H. pylori* infects approximately fifty per cent of the world's population, it is an important risk factor in chronic gastritis, peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid

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tissue (MALT) lymphoma. It chronically infects most people in the developing countries, and also remains a significant pathogen in industrialized countries.[1] In Algeria, its prevalence is average 80% in peptic ulcer.[2] Fortunately, many *H. pylori* associated pathologies can be prevented or cured by the eradication of the bacterium.

Until now, in Algeria eradication of *H. pylori* is recommended by a triple therapy which includes amoxicillin, clarithromycin or metronidazole combined with proton pump inhibitors (PPI) for 7–10 days.[3] It is effective for 79–85% of patients but after the publication of the Maastricht V, Toronto and Spanish Consensus about management of *H. pylori* infection and its treatment in adults,[4,5] recommendations for eradication, therapy in Algeria should be changed, at least in length in order to gain an eradication >=90% of treated patients.

Eradication efforts fail for a significant proportion of patients in Algeria for several reasons, including bacterial resistance to clarithromycin. Several studies have demonstrated that primary resistance to clarithromycin is a major factor for therapeutic failure; the rate of resistance to clarithromycin is significantly increasing and the high rate of the resistance to metronidazole leads to avoid the combination of these two antibiotics in the treatment regimens.[3] In another way, results of some studies have suggested that the eradication rate in patients with gastritis is lower than in those with peptic ulcer diseases.[6] Since the worldwide increase of the drug resistance rates represents a problem of relevance, some researches have been conducted on antibiotic resistance relationship with bacterial genetic factors such as the cytotoxin associated gene A (*cag*A) and vaculating cytotoxin gene A (*vac*A).[7,8]

Because of the limited data about the resistance of *H. pylori* to antibiotics in Algeria, the aim of this study was to investigate the resistance rates of amoxicillin, clarithromycin, metronidazole, tetracycline, ciprofloxacin and rifampicin, and to study the relation between *H. pylori* genotypes and the resistance to antibiotics.

Materials and methods patients

From 2012 to 2015, four Hospitals were involved in the present study: Mustapha Pacha University Hospital (Algiers), Halouche clinic of Gastroenterology (Chlef), First November 1954 University Hospital of Oran and Sidi Belabess University Hospital. Two hundred patients with upper abdominal pain from four different regions in Algeria (Algiers, Chlef, Oran and Sidi Belabess), were enrolled in the present study (91 males and 109 females). The patients were Algerian citizens aging from 18 to 86 years and provided written informed consent before endoscopy. Patients either receiving proton pump inhibitory drugs or have been treated with antimicrobials were excluded from the study. All subjects underwent endoscopy to obtain two antrum biopsies from each patient. The first was used for screening of *H. pylori* positive specimens by a rapid urease test (RUT). While the second piece was placed in 1 mL of sterile phosphate buffer saline solution and was transported immediately for the isolation of *H. pylori*. This study protocol was approved by the Algerian national ethics committee.

Bacterial culture

H. pylori was isolated from grinded gastric biopsy samples on brain heart infusion (BHI) agar (bioMérieux) supplemented with 10% defibrinated horse blood, 0.4% IsoVitaleX, 5 mg/L of trimethoprim, 5 mg/L of cefsulodin, 10 mg/L of vancomycin, and 8 mg/L of amphotericin B. The plates were incubated at 37 °C under microaerobic conditions for 7 days or more. *H. pylori* was identified by colony aspect, microscopic morphology, positive urease, catalase and oxidase tests. Strains were stored at −80 °C in BHI broth with 20% glycerol.[9,10]

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined using the E-test and agar dilution methods as recommended by EUCAST. The tested antibiotics were amoxicillin (AMX), clarithromycin (CLA), ciprofloxacin (CIP), metronidazole (MTZ), rifampicin (RIF) and tetracycline (TET). The breakpoints used to classify isolates as susceptible or resistant according to the MIC value according to EUCAST: the isolate is resistant to AMX if MIC >0.12 mg/L, resistant to CLA if MIC >0.5 mg/L, resistant to CIP if MIC >1 mg/L; resistant to MTZ if MIC >8 mg/L; resistant to RIF if MIC >1 mg/L and resistant to TET if MIC >1 mg/L.[11] Mueller–Hinton agar supplemented with 7% defibrinated horse blood (bioMérieux) was used as the culture medium. An *H. pylori* culture suspension with a turbidity equivalent of a 3.0 McFarland standard was used to inoculate the plates containing serial dilutions of antibiotics: CLA, 0.016–256 mg/L; AMX, 0.016–32 mg/L; MTZ, 0.016–256 mg/L; TET, CIP and RIF, 0.016–32 mg/L (bioMérieux), or plates without antibiotics onto E-test strips of each antibiotic tested were applied (bioMérieux). The plates were incubated at 37 °C for 3 days under microaerophilic conditions.[11]

DNA extraction and purification

Total genomic DNA was extracted using commercial kits: Magazorb DNA mini-prep kit (Promega) for biopsies and Wizard Genomic DNA Purification Kit (Promega) for strains according to the manufacturer's guidelines. Genomic DNA from biopsies and strains was stored at −20 °C.

Molecular detection of H. pylori

Molecular detection of *H. pylori* in biopsy DNA and strain identification were performed using the primers in Table 1. The 25 μL reaction mixture consisted of 1× PCR buffer, 1.5 mM magnesium chloride, 200 μM of each dNTP, 20 pmol of each primer and 1 U hot start Taq DNA polymerase (Promega). Amplification was carried out in an Eppendorf Mastercycler gradient using the cycling parameters in Table 1. The PCR products were separated on a 2% agarose gel and 100 bp ladder was used as DNA molecular weight standard.[12,13]

The cagA gene detection

The presence of the genotype *cag*A was determined by PCR using primers in Table 1. DNA sample from *H. pylori J99* strain was used as positive control and sterile distilled water was

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