RESISTANCE TO AMOXICILLIN, CLARITHROMYCIN AND CIPROFLOXACIN OF Helicobacter pylori
ISOLATED FROM SOUTHERN BRAZIL PATIENTS

Simone Ulrich PICOLI(1), Luiz Edmundo MAZZOLENI(2), Heriberto FERNÁNDEZ(3), Laura Renata DE BONA(4), Erli NEUHAUSS(5),
Larisse LONGO(4) & João Carlos PROLLA(6)

SUMMARY

Introduction: Helicobacter pylori is a bacteria which infects half the world population and is an important cause of gastric cancer. The eradication therapy is not always effective because resistance to antimicrobials may occur. The aim of this study was to determine the susceptibility profile of H. pylori to amoxicillin, clarithromycin and ciprofloxacin in the population of Southern Brazil.

Material and methods: Fifty four samples of H. pylori were evaluated. The antibiotics susceptibility was determined according to the guidelines of the British Society for Antimicrobial Chemotherapy and the Comité de l’Antibiogramme de la Société Française de Microbiologie.

Results: Six (11.1%) H. pylori isolates were resistant to clarithromycin, one (1.9%) to amoxicillin and three (5.5%) to ciprofloxacin. These indices of resistance are considered satisfactory and show that all of these antibiotics can be used in the empirical therapy.

Conclusion: The antibiotics amoxicillin and clarithromycin are still a good option for first line anti-H. pylori treatment in the population of Southern Brazil.

KEYWORDS: Helicobacter pylori; Antibiotic resistance; Clarithromycin; Amoxicillin; Ciprofloxacin.

INTRODUCTION

Helicobacter pylori (H. pylori) is a bacterial agent affecting more than 80% of the population of developing countries and therapy schedules have not always been effective in such cases. One of the possible explanations for failures in eradicating it is the bacterial resistance to the used antimicrobial or the utilized antibiotics concentration.

The susceptibility of H. pylori to antibiotics can be quite variable in particular geographical areas of the same country as well as among different countries, being directly influenced by the previous use of these medications. Thus, the success of a scheme of treatment in a community does not enable the generalization of the results. It would be ideal to provide a therapy based on previous knowledge of the microbial resistance rate in a local community, which has been difficult in most centers of developing countries.

Classically, the treatment consists of the association of amoxicillin and clarithromycin with a proton pump inhibitor. This is one of the first choice therapeutic purposes and is recommended by the II Brazilian Consensus on H. pylori. Other antimicrobials, as quinolones, are useful therapeutically, but they are normally considered for second line treatments.

There are few Brazilian studies, and none in Southern Brazil (State of Rio Grande do Sul), that have demonstrated rates of resistance to antibiotics, and not knowing this information has complicated the therapeutic success. This way, this research aims to define the profile of susceptibility of H. pylori to antibiotics widely utilized as a first line treatment, amoxicillin and clarithromycin, and also a second line antibiotic, quinolones, along the population of Rio Grande do Sul, Brazil.

MATERIAL AND METHODS

Patients: A total of 342 patients were included, ranging in age from 18 to 80 years old, with a clinical indication for the realization of upper digestive endoscopy at the Clinical Hospital of Porto Alegre, in Southern Brazil. Individuals excluded from this study were: the ones presenting gastric cancer or those who were undergoing treatment for any kind of cancer, considering their total or partial gastrectomia, cirrhosis in critical condition, decreasing of blood platelets or if they were using anticoagulants, presenting indication of esophageal dilatation or passage of probe, nephropathy in critical condition, esophageal varicose veins or ligation of esophageal varicose veins.

Biopsy sampling and bacterial strains: The samples were collected between January 2011 and January 2012. Three gastric antral biopsy
specimens were obtained for each patient during endoscopy. One of them underwent a rapid urease test, the other was sent to histological examination, and the other for culture. For the cultivation, the biopsy specimens were sent to the laboratory within three hours of being collected, in Eppendorf sterile tubes containing 0.1 mL of physiological solution. Each biopsy specimen was seeded onto Agar Belo Horizonte (Probac of Brazil, Brazil) and incubated in microaerobic conditions (Microaerobac, Probac of Brazil), at 37 °C, for five days. The growth suggestive of *H. pylori* (small, circular and bright colonies) was identified through universally accepted phenotypic proofs: morphology characteristic in Gram’s stained smears (Gram negative rod curved or in “S”), positive urease, positive catalase and positive oxidase.\(^{12,23}\)

The cultures correspondent to *H. pylori* were subcultured in Agar Columbia Chocolate (Oxoid, United Kingdom) along with 0.1 mL of Brain Heart Infusion broth (Himedia, India) and incubated in microaerobic conditions (Microaerobac, Probac of Brazil), stored at 37 °C, during three days. The abundant and fresh growth of the bacteria was utilized for antimicrobial susceptibility tests.

**Determination of antimicrobial susceptibility:** The susceptibility to antibiotics was defined in 54 strains with culture positive according to the standards of the British Society for Antimicrobial Chemotherapy (BSAC)\(^3\) and the Committee of l’Antibiogramme of la Société Française de Microbiologie (CA-SFM)\(^8\). Several colonies were suspended from *H. pylori* of each sample in some sterile diluent until equivalence to 1 (1.9) to 2 (20) of each sample in some sterile diluent until equivalence to 1 (1.9) to 2 (20).

The rate of resistance to CIP was 5.5% (3 samples) (Table 1). One strain resistant to CIP presented simultaneous resistance to CLA (Table 2) (MIC > 256 µg/mL).

**DISCUSSION**

This study demonstrated that the rates of resistance of *H. pylori* to antibiotics usually utilized as first line therapy were satisfactory, with rates practically null for AMO and low for CLA and for CIP.

The therapeutic failure for eradication treatment of *H. pylori* infections may be multifactorial, but the antimicrobial resistance is the main reason for the treatment failure.\(^{14}\) The susceptibility of *H. pylori* to antibiotics has demonstrated variations between different locations and it has been influenced by the previous use of these drugs. Therefore, it becomes relevant to base the treatment on previous knowledge about the antimicrobial resistance rate in the local community, promoting the most rational use of antibiotics.

In 8 (14.8%) of 54 strains of *H. pylori*, the resistance was found in at least one of the tested antibiotics (Table 2), and the lowest frequency of this event was associated with AMO (1.9%). Currently, the world rates of resistance to this antibacterials have been low and, for this reason, the same has been frequently utilized in the combined first line therapy. In countries of Latin America, rates of resistance to AMO were reported as less than 4%, being 3.8% in Colombia, 2.2% in Paraguay and 2.3% in Chile. There was no resistance to this antibiotic in Venezuela, São Paulo/Brazil and in any other Colombian work. The susceptibility to AMO has been also high in other countries as Germany, Spain, Philippines and Tunisia. Even if the susceptibility of *H. pylori* to AMO may be very satisfactory, it is important to monitor it, once high resistance rates have been found in some locations.\(^1\)
Fortunately, beyond the rate of resistance to AMO found in this study being low (1.9%), the level of expression of such resistance was also underestimated, not overcoming the MIC of 2 µg/mL. On the other hand, in Chile, despite of rates of resistance to AMO being low (2.3%) strains with expressive level of resistance were found with MIC greater than 256 µg/mL.23

In this study, six strains (11.1%) of _H. pylori_ were resistant to CLA. World data has demonstrated that the rates of resistance to CLA presented geographical variations. Data reviewed by WANG et al. (2000) has indicated levels of resistance of 9.1% in Japan, from 6.1 to 12.6% in the United States and less than 15% in Europe.23

In Latin America, many studies reported variable resistance frequencies to CLA, ranging between 2.2% and 17.7% in Colombia,22,29 between 9.1%23 and 20%21 in Chile and 2.2% in Paraguay.29 In Brazil, rare studies realized have also demonstrated variable rates: in São Paulo from 8%6 to 16%,23, in Belo Horizonte 17.3%29 and 16.5% in Recife.22

The diversity in the rates of resistance to CLA may be attributed to different frequencies of the utilization of this antibiotic in different world geographical areas. It has been proved that the previous use of macrolides, as erythromycin and azithromycin, have induced cross-resistance to CLA. On the other hand, the resistance to CLA decreased the efficacy of the antibiotic therapy and it has been the main risk factor for therapeutic failure. In lineages of _H. pylori_, sensitive to this antibacterial, the rates of eradication have approximated to 88%.24 The literature has considered that antibiotics which rates of resistance have overcome 20% must not be used in the therapy of anti-_H. pylori_.11

Among the six _H. pylori_ strains resistant to CLA reported in this study, three presented high level of resistance to this antibiotic, with MICs equal or greater than 256 µg/mL. Such finding has suggested the previous utilization of macrolides by the population evaluated in our study, generated cross-resistance to CLA in very expressive levels. Still, Chilean work carried out by OTTH et al. revealed that all eight samples of _H. pylori_ resistant to CLA had maximum MICs of 64 µg/mL.23

Ciprofloxacin (CIP) may be prescribed to individuals allergic to AMO or to those that present failure in the triple therapy.26 The world levels of resistance to CIP have been relatively low, reaching 2.4% in Teheran/Iran27, 5.7% in Chile,23, 7.9% in Spain28 and 9.5% in Germany.12 Similarly, the data of this study also revealed a reduced rate of _H. pylori_ resistance to CIP (5.5%). This finding has revealed the possibility of its employment in eradication therapy, in case the utilization of antibiotics not belonging to the first line treatment may be necessary in the population of Rio Grande do Sul, Brazil. On the other hand, the highest resistance rates to CIP have been reported in China (55.7%)23, Iran (35%)4 and Portugal (21%)4.

Due to the low rates of resistance to antibiotics found in this study, AMO and CLA may be used empirically in anti-_H. pylori_ therapy in the State of Rio Grande do Sul, respecting the recommendations of the II Brazilian Consensus Conference on _H. pylori_. However, due to the simultaneous resistance to more than one antimicrobial in two isolates of bacteria (one to CLA+AMO and the other to CLA+CIP), it has been suggested the necessity of establishing a surveillance system in order to prevent treatment failures and the spread of resistant strains. This surveillance would also be important to establish whether these resistant strains represent sporadic cases or correspond to an usual behavior in a given geographical region.

**RESUMO**

**Resistência à amoxicilina, claritromicina e ciprofloxacina de *Helicobacter pylori* isolados de pacientes do Sul do Brasil**

**Introdução:** *Helicobacter pylori* é uma bactéria que infecta metade da população mundial e é considerada importante causa de câncer gástrico. A terapia de erradicação nem sempre é eficaz, pois pode ocorrer a resistência aos antimicrobianos. Este estudo determinou a sensibilidade de _H. pylori_ frente à amoxicilina, claritromicina e ciprofloxacina na população do Sul do Brasil. **Material e métodos:** Foram avaliadas 54 amostras de _H. pylori_. A sensibilidade aos antibióticos foi determinada segundo as orientações da British Society for Antimicrobial Chemotherapy e do Comité de l’Antibiogramme de la Société Française de Microbiologie. **Resultados e discussão:** Sete (13%) isolados de _H. pylori_ foram resistentes à claritromicina, um (1,9%) à amoxicilina e três (5,5%) à ciprofloxacina. Estes índices são satisfatórios e demonstram que esses antibióticos podem ser utilizados na terapia empírica. **Conclusão:** Os antibióticos amoxicilina e claritromicina ainda são uma boa opção no tratamento de primeira linha anti-*H. pylori* na população do Sul do Brasil.

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**AUTHOR CONTRIBUTIONS**

Simone Ulrich Picoli collected data and wrote the article. Luiz Edmundo Mazzoleni critically reviewed the article. Heriberto Fernández oriented the culture and sensitivity test and critically reviewed the article. Laura Renta De Bona, Erli Neuhauss and Larisse Longo assisted in data collection. João Carlos Prolla guided work and critically reviewed the article.

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