

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

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### 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<sup>16</sup>. 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<sup>13</sup>. 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<sup>14</sup>.

Classically, the treatment consists of the association of amoxicillin and clarithromycin with a proton pump inhibitor<sup>21</sup>. This is one of the first choice therapeutic purposes and is recommended by the II Brazilian Consensus on *H. pylori*<sup>5</sup>. 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 gastrectomy, 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

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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 on to 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<sup>22,23</sup>.

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)<sup>3</sup> and the Committee of l'Antibiogramme de la Société Française de Microbiologie (CA-SFM)<sup>6</sup>. Several colonies were suspended from *H. pylori* of each sample in some sterile diluent until equivalence to pattern 3 of McFarland standard. The suspensions were inoculated with sterile swabs onto the surface of Agar Mueller Hinton (Oxoid, United Kingdom) 10% horse blood until drying. The antibiotics applied were: E-test amoxicillin strips of and E-test clarithromycin strips, both with gradient of concentration of 0.016 until 256 µg/mL and disks of ciprofloxacin 5 µg. The plates were incubated at 37 °C, in microaerobic conditions (Microaerobac, Probac of Brazil), from three to four days. The minimum inhibitory concentrations (MIC) of amoxicillin (AMO) and clarithromycin (CLA) were read in the point where the zone of elliptic inhibition intercepted the E-test strip. The diameter of the inhibition zone was measured for ciprofloxacin (CIP), promoted by the diffusion of the antibiotic contained in the disk.

The criteria for the definition of resistance according to each standard were the following: AMO (MIC > 1 µg/mL) and CLA (MIC > 1 µg/mL)<sup>5</sup>, CIP (diameter of inhibition zone < 20 mm)<sup>6</sup>.

The Committee of Ethics in Research of the Hospital de Clínicas de Porto Alegre approved this study (number 07-654), and all the participants signed an informed consent.

## RESULTS

The resistance to CLA was detected in six of 54 (11.1%) strains of *H. pylori* (Table 1), considering that three of them expressed high level of resistance to antibiotic, with MICs equal or greater than 256 µg/mL. The other strain presented MICs of 8, 24 and 32 µg/mL for this antibiotic.

The resistance to AMO was found in only one strain (1.9%) of *H. pylori* (Table 1) and being compatible to a low level of resistance expression (MIC 2 µg/mL). The strain resistant to AMO presented simultaneous resistance to CLA (Table 2) (MIC 24 µg/mL).

**Table 1.**  
Profile of susceptibility of 54 isolates of *H. pylori* to antibiotic

Antibiotics	Resistant isolates of <i>H. pylori</i> n (%)	MIC (µg/mL)
Amoxicillin (AMO)	1 (1.9)	< 0.016 - 2
Clarithromycin (CLA)	6 (11.1)	< 0.016 - > 256
Ciprofloxacin (CIP)	3 (5.5)	Non-applicable

MIC: Minimum Inhibitory Concentration; µg/mL: microgram/milliliter.

**Table 2.**  
Resistance profiles of eight *H. pylori* strains with some resistance to antibiotics

Resistance profiles	n
Only Amoxicillin	0
Only Clarithromycin	4
Only Ciprofloxacin	2
Clarithromycin+Amoxicillin	1
Clarithromycin+Ciprofloxacin	1

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<sup>14</sup>. 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<sup>14</sup>, 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 antibacterial 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<sup>29</sup>, 2.2% in Paraguay<sup>10</sup> and 2.3% in Chile<sup>23</sup>. There was no resistance to this antibiotic in Venezuela<sup>30</sup>, São Paulo/Brazil<sup>9</sup> and in any other Colombian work<sup>2</sup>. The susceptibility to AMO has been also high in other countries as Germany<sup>34</sup>, Spain<sup>8,18</sup>, Philippines<sup>7</sup> and Tunisia<sup>20</sup>. 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<sup>1</sup>.

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 undermost, 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<sup>23</sup>.

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<sup>33</sup>.

In Latin America, many studies reported variable resistance frequencies to CLA, ranging between 2.2% and 17.7% in Colombia<sup>2,15,29</sup>, between 9.1%<sup>23</sup> and 20%<sup>31</sup> in Chile and 2.2% in Paraguay<sup>10</sup>. In Brazil, rare studies realized have also demonstrated variable rates: in São Paulo from 8%<sup>9</sup> to 16%<sup>25</sup>, in Belo Horizonte 17.3%<sup>19</sup> and 16.5% in Recife<sup>17</sup>.

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%<sup>24</sup>. The literature has considered that antibiotics which rates of resistance have overcome 20% must not be used in the therapy of anti-*H. pylori*<sup>11</sup>.

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<sup>23</sup>.

Ciprofloxacin (CIP) may be prescribed to individuals allergic to AMO or to those that present failure in the triple therapy<sup>26</sup>. The world levels of resistance to CIP have been relatively low, reaching 2.4% in Teheran/Iran<sup>27</sup>, 5.7% in Chile<sup>23</sup>, 7.9% in Spain<sup>28</sup> and 9.5% in Germany<sup>12</sup>. 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%)<sup>32</sup>, Iran (35%)<sup>1</sup> and Portugal (21%)<sup>4</sup>.

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.

## ACKNOWLEDGEMENTS

This research was supported by grants from the Fundo de Incentivo a Pesquisa e Eventos (FIPE).

## 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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Received: 6 June 2013

Accepted: 17 October 2013