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Helicobacter pylori ANTIBIOTIC RESISTANCE IN BRAZIL: clarithromycin is still a good option

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ABSTRACT – ***Context*** - The antibiotic susceptibility is the cornerstone for the eradication therapies of *Helicobacter pylori*. ***Objectives*** - To evaluate the prevalence of primary resistance of *H. pylori* was evaluated in an urban Brazilian population. ***Methods*** - *H. pylori* isolates were obtained from patients submitted to an upper gastrointestinal endoscopy for the evaluation of dyspeptic symptoms. Biopsies from antrum, corpus and fundus were taken to determine the antibiotic susceptibility of *H. pylori* isolates. The minimal inhibitory concentration of furazolidone and bismuth were routinely determined by agar dilution method and the minimal inhibitory for amoxicillin, clarithromycin, tetracycline, levofloxacin, and metronidazole were routinely determined with the E-test. ***Results*** - Fifty-four patients were included. In vitro antimicrobial susceptibility of *H. pylori* strains were obtained from 39 patients. Resistance to metronidazole was detected in 20 patients (51%), to clarithromycin in 3 patients (8%), to levofloxacin in 9 patients (23%) and to bismuth in 2 patients (5%). There was no observed resistance to amoxicillin, tetracycline or furazolidone. ***Conclusion*** - Due to the low amoxicillin and clarithromycin resistance observed in this study, therapies using these antimicrobials remain appropriated first-line *H. pylori* therapy.

HEADINGS – *Helicobacter pylori*. Drug resistance, microbial. Clarithromycin. Amoxicillin.

INTRODUCTION

The therapy of *Helicobacter pylori* (*H. pylori*) infection has been a challenge for clinicians since its discovery in the early 1980s. The challenges go beyond finding the correct combination of antibiotics and manipulation of gastric pH to ensure eradication and include avoiding the development of antimicrobial resistance and ensuring compliance with prescribed drugs[25].

Recent management guidelines from the Maastricht consensus conference[23] and the American College of Gastroenterology[5] recommend first line treatment of *H. pylori* infection with the combination of a proton pump inhibitor (PPI), bismuth, metronidazole and tetracycline, or with a PPI, clarithromycin and amoxicillin.

Is usually accepted that for an eradication treatment regimen to be considered effective, it would need to achieve an intention-to-treat eradication rate in excess of 80% and be successful in more than 90% per protocol treated patients[17].

However, in recent times, eradication rates in practice for many of the first-line treatment regimens has fallen well below these levels, generally due to the connected factors of poor compliance with medication and antibiotic resistance[11, 22]. With failure of first-line therapy, the need for effective second-line therapy is clear[13]. The most common used second-line therapies are bismuth-based and levofloxacin-based ones.

Bismuth-based quadruple therapies usually consist of a PPI, bismuth, tetracycline and metronidazole[3, 9, 14].

In developing countries antimicrobial resistance is considered to be higher than in developed countries. In Brazil, resistance rates as high as 55% for metronidazole and 16% for clarithromycin has been previously reported[15]. Unusual resistance rates to tetracycline (7%) have also been observed[24].

This could, at least partially, explain eradication rates as low as 66% observed with a first-line therapy in Brazil[8]. To overcome metronidazole resistance, furazolidone has been used as an alternative in China and South America, including Brazil. It is inexpensive, resistance is rare and may have topical and systemic action[2, 9, 26, 27].

Since resistance to antimicrobials is a major cause of eradication failure, and may change with time, the monitoring of antimicrobial resistance of *H. pylori* in each domestic area should be warrant, especially for clarithromycin and the commonly applied amoxicillin and metronidazole. Such monitoring is

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also recommended by the Maastricht III consensus, with the statement that clarithromycin-based triple therapy should not be used empirically in the first-line therapy when the local resistance rate is more than 15%-20%[4, 23].

For developing countries, due to the higher and/or unusual resistance rates, this monitoring should probably also include other antimicrobials used in eradication therapy. Therefore, the aim of this prospective study was to evaluate the prevalence of *H. pylori* resistance to clarithromycin, amoxicillin, bismuth, furazolidone, tetracycline, metronidazole and levofloxacin in an urban Brazilian population.

PATIENTS

H. pylori isolates were obtained from patients with gastritis, duodenal ulcer and gastroesophageal reflux disease detected during upper gastrointestinal endoscopy for the evaluation of dyspeptic symptoms.

Biopsies from antrum, corpus and fundus were taken to determine the presence of *H. pylori* by the rapid urease test, culture and histological examination.

Patients were selected from the Outpatient Clinical Gastroenterology Unit of São Paulo University Medical School. Exclusion criteria were: age younger than 18 years or older than 80 years, prior eradication treatment, treatment with inhibitors of acid secretion during the 2 weeks and antibiotics 4 weeks before the study, gastrointestinal malignancy, pregnancy or lactation and long-term use of corticosteroids or nonsteroidal anti-inflammatory drugs.

The study was performed in accordance with the declaration of Helsinki, and was approved by the institutional Ethics Review Board for Clinical Research. Written informed consent was obtained from each volunteer prior to entering the study.

METHODS

H. pylori culture and minimal inhibitory concentration (MIC) determination biopsy specimens obtained during endoscopy were immediately transported in saline solution to the microbiology laboratory. *H. pylori* isolates were obtained by inoculating the specimens into selective and non-selective media followed by incubation for 3-5 days at 37°C under microaerophilic conditions, as previously described[24]. The colonies were identified by Gram staining and by oxidase, catalase and urease production. *H. pylori* strains were stored at -70°C in brain heart infusion (BHI) broth containing glycerol 30%.

The MIC of furazolidone and bismuth were routinely determined by agar dilution using Mueller-Hinton (Difco, Sparks, MD, USA) plates supplemented with 5% sheep blood (BioTrading), according to the protocols of the National Committee for Clinical Laboratory Standard (NCCLS). Two microliters of a 2.0 MacFarland bacterial suspension was inoculated on plates containing twofold serial dilutions of furazolidone and bismuth (Sigma Aldrich Chemie). Isolates were considered to be resistant when the MIC of furazolidone exceeded 4 mg/L and 8 mg/L for bismuth.

The MIC for amoxicillin, clarithromycin, tetracycline, levofloxacin, and metronidazole were routinely determined with the E-test (AB Biodisk, Solna, Sweden). Inocula were prepared from a fresh *H. pylori* culture grown routinely for 2 days on BHI agar plates (Difco, Sparks, MD, USA) plates supplemented with 5% sheep blood (BioTrading). Mueller-Hinton plates containing 7% lysed horse blood, were inoculated with approximately 2x 108 colony-forming unit in 20 µl of 0.9% NaCl, the plates were dried for 3 to 4 min, and then the E-test strips were applied to the agar surface. The plates were incubated at 37°C under microaerophilic conditions, and 3 days later the MIC was determined by the intercept of the zone of inhibition with the graded E-test strip. The isolates were considered resistant when the MICs of amoxicillin was <0.5 µg/mL, <1 µg/mL for clarithromycin, levofloxacin and tetracycline, <8 µg/mL for metronidazole and <16 µg/mL for bismuth. As control, the American type culture collection (ATCC 43504) susceptible strain was always included.

RESULTS

Fifty-four patients were included in study. Thirty-seven were females and 17, males. The age range was 22 to 72 years and median age, 46.6 years. In vitro antimicrobial susceptibility of *H. pylori* strains were obtained from 39 patients. Resistance to metronidazole was detected in 20 patients (51%), to clarithromycin in 3 patients (8%), to levofloxacin in 9 patients (23%) and to bismuth in 2 patients (5%). There was no observed resistance to amoxicillin, tetracycline or furazolidone (Table 1).

TABLE 1. *H. pylori* antimicrobial resistance among the studied population

Antimicrobials	Resistance n (%)	Susceptible n (%)
Amoxicillin	0	39 (100%)
Clarithromycin	3 (8%)	36 (92%)
Metronidazole	20 (51%)	19 (49%)
Tetracycline	0	39 (100%)
Levofloxacin	9 (23%)	30 (77%)
Bismuth	2 (5%)	37 (95%)
Furazolidone	0	39 (100%)

DISCUSSION

The key determinants of the outcome of eradication therapy for *H. pylori* infection are compliance and the presence of pretreatment antibiotic resistance of the isolate[18]. Antimicrobial resistance varies by geographical region and is highly influenced by patterns of antimicrobial use within a population[22].

Therefore, the selection of a regimen among those recommended for *H. pylori* eradication, must consider the local data of antimicrobial resistance[4]. We evaluated the primary resistance of *H. pylori* to amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin, bismuth and furazolidone in 39 patients, from the city of São Paulo. These drugs are the most used in *H. pylori* eradication therapy in Brazil[6].

Resistance to amoxicillin, tetracycline and furazolidone has been already reported in different countries, including Brazil[7, 10, 20, 24], with a potential negative impact in treatment outcome. We were not able to detected resistant strains to these antimicrobials among our clinical isolates (Table 1).

These findings confirm the role of these agents in eradication therapy. Although clarithromycin resistance is considered an emerging problem worldwide, the observed resistance to clarithromycin in the evaluated population was surprisingly low (8%), if we considerer previous reports from Brazil[8, 15]. The finding reinforces the major role of this drug for *H. pylori* eradication in Brazil.

The increasing resistance to clarithromycin has lead to the empiric use of levofloxacin as a surrogate drug for this antimicrobial. A triple therapy with levofloxacin, amoxicillin and a PPI has already been suggested as an alternative first-line therapy[18]. The resistance rate encountered by us (23%) is similar to that observed in Korea (21.5%)[19] and does not support its use in the studied population.

Unexpectedly, two strains (5%) were found to resistant to bismuth. Although it is usually accepted that there is no resistance to bismuth compounds, it shall be stressed that this concept is based mainly in older studies, which evaluated only few strains, and mostly published as abstracts[1, 21]. By the way, resistance to bismuth subcitrate has already been described in Chilean patients[16].

These findings strongly support the need for further evaluation of resistance to bismuth among patients undergoing eradication therapy, since bismuth compounds are recommended not only for second-line treatment but also as a first-line therapy in many third-world countries[6, 12, 22].

Due to the low amoxicillin and clarithromycin resistance in our area, therapies using these antimicrobials remain appropriate first-line *H. pylori* therapy. Due to the resistance rate observed for levofloxacin, this drug shall not be used in our population. Further studies are also required to confirm the findings of resistance to bismuth.

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RESUMO – ***Contexto*** - A susceptibilidade aos antibióticos é a pedra fundamental dos tratamentos de erradicação do *Helicobacter pylori*. ***Objetivo*** - Avaliar a prevalência da resistência primária do *H. pylori* aos antibióticos em uma população urbana do Brasil. ***Métodos*** - As cepas do *H. pylori* foram obtidas de pacientes submetidos a endoscopia digestiva para avaliação de sintomas dispépticos. Biopsias do antro, corpo e fundo gástrico foram realizadas para determinar a susceptibilidade das cepas do *H. pylori* aos antibióticos. A concentração inibitória mínima da furazolidona e do bismuto foram determinadas rotineiramente pelo método da diluição em Agar e a concentração inibitória mínima da amoxicilina, claritromicina, tetraciclina, levofloxacina e do metronidazol foram determinadas pelo E-test. ***Resultados*** - Cinquenta e quarto pacientes foram incluídos no estudo. Destes, a susceptibilidade das cepas do *H. pylori* in vitro foi determinada em 39 pacientes. Resistência ao metronidazol foi detectada em 20 pacientes (51%), à claritromicina em 3 pacientes (8%), à levofloxacina in 9 pacientes (23%) e ao bismuto em 2 pacientes (5%). Não foi observada resistência à amoxicilina, tetraciclina e à furazolidona. ***Conclusão*** - Devido à baixa resistência, observada neste estudo para a amoxicilina e a claritromicina, os tratamentos que usam estes antibióticos permanecem apropriados como esquemas de primeira linha para a erradicação do *H. pylori*.

DESCRITORES – *Helicobacter pylori*. Resistência microbiana a medicamentos. Claritromicina. Amoxilina.

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