Evolution of resistance to metronidazole and clarithromycin in 
*Helicobacter pylori* clinical isolates from Spain

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The aim of this study was to determine the frequency of resistance to amoxycillin, tetracycline, metronidazole and clarithromycin in 282 *Helicobacter pylori* clinical isolates from Spain and to evaluate the evolution of resistance over the five years of this study. The overall percentage of resistance was 19.9% for metronidazole and 3.5% for clarithromycin. Resistance to metronidazole rose from 9% in 1991 to 21.6% in 1995, although 33.3% resistance was found in 1993. Clarithromycin resistance was not detected in 1991 or 1992 and the rate was 4%, 3.4% and 4.4% in 1993, 1994 and 1995, respectively. No amoxycillin or tetracycline resistance was found.

Introduction

*Helicobacter pylori* is a Gram-negative curved and microaerophilic bacillus found in the human stomach, associated with gastritis, duodenal and gastric ulcer; recently it has been considered to be a risk factor in the development of gastric cancer. A variety of antimicrobial agents display good activity against *Helicobacter pylori* in vitro, but when used as single agents in clinical studies, they do not eradicate the organism. Several regimens have been proposed in the treatment of the pathology produced by *H. pylori* combining two or three drugs. They include one or two antibiotics and other compounds such as bismuth salts, H2 receptor antagonists and proton pump inhibitors such as omeprazole, lansoprazole and pantoprazole. Amoxycillin, tetracycline, metronidazole and clarithromycin are the most common antimicrobial agents used for the treatment of *H. pylori* infection.

The aim of this study was to determine the rate of resistance to amoxycillin, tetracycline, metronidazole and clarithromycin in *H. pylori* isolates. The strains were collected over five years and the evolution of resistance to metronidazole and clarithromycin was determined.

Materials and methods

Microorganisms

A total of 282 *H. pylori* clinical isolates were obtained from gastric biopsies taken by endoscopy. The microorganisms were identified using conventional tests such as colony and Gram’s stain morphology and oxidase, catalase and urease positive tests and stored in Trypticase Soy Broth plus 15% glycerol at −80°C until used. As metronidazole and clarithromycin have been used for treatment of *H. pylori* infections and because development of resistance during treatment has been reported, only the pretreatment isolates were included in this study. Fifteen isolates were obtained in 1991, 19 in 1992, 24 in 1993, 88 in 1994 and 136 in 1995. Fifty-six isolates were obtained from women, 130 from men, 54 from girls and 42 from boys. *H. pylori* NCTC 11637 was used as control in the susceptibility tests.

Antibiotics

Amoxycillin, tetracycline, metronidazole and clarithromycin, diluted in the appropriate solvent, in two-fold dilutions ranging from 128 to 0.008 mg/L were studied. Resistance was detected by an agar dilution method using Mueller–Hinton agar supplemented with 7% horse blood. As metronidazole and clarithromycin have been used for treatment of *H. pylori* infections and because development of resistance during treatment has been reported, only the pretreatment isolates were included in this study.

Determination of the MIC

Resistance was detected by an agar dilution method using Mueller–Hinton agar supplemented with 7% horse blood. *H. pylori* 48 h cultures were inoculated in Brain Heart Infusion with 10% fetal calf serum and incubated at 37°C in 5% CO2 for 48 h. Plates containing the antibiotics were inoculated using a Steer replicator (approximately 106 cfu/spot) and incubated for 2–5 days at 37°C in a CO2 incubator. Bacteria were considered resistant when the MIC was ≥8 mg/L for metronidazole and ≥4 mg/L for clarithromycin. For amoxycillin and tetracycline the NCCLS recommendations were followed.

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Results and discussion

The $\text{MIC}_{50}$, $\text{MIC}_{90}$, range and the percentage of resistance for the antibiotics tested are shown in Table I. Amoxycillin and clarithromycin were the most active antimicrobial agents against $H.\, pylori$ with $\text{MIC}_{50}$s of 0.008 mg/L and $\text{MIC}_{90}$s of 0.064 mg/L followed by tetracycline with an $\text{MIC}_{90}$ of 0.5 mg/L. No amoxycillin or tetracycline resistance was found in our clinical isolates.

Table II shows the percentage of strains resistant to metronidazole and clarithromycin according to the year of isolation. An overall resistance rate of 19.9% for metronidazole and 3.5% for clarithromycin was found in the $H.\, pylori$ clinical isolates included in this study. Resistance to metronidazole rose from 9% in 1991 to 21.6% in 1995, although a maximum of 33.3% in 1993 was found. Clarithromycin resistance was not detected in 1991 and 1992 and was 4% in 1993, 3.4% in 1994 and 4.4% in 1995.

In 56 $H.\, pylori$ strains obtained from women the proportion of strains resistant to metronidazole was 25% but no resistance to clarithromycin was found. In 130 strains from men, 21.5% metronidazole and 3.1% clarithromycin resistance was detected. The frequency of resistance detected in strains from girls and boys was 13% and 16.2% respectively for metronidazole and 7.4% and 4.7% respectively for clarithromycin.

Resistance to metronidazole and clarithromycin has been reported, both primary and after treatment, and has caused failures in the eradication of the microorganism. As in this study amoxycillin resistance has not been found and tetracycline resistance remains very low (<1%).

The overall resistance rate to metronidazole of 19.9% found in our study is lower than the rates found in Belgium (37%) and Amsterdam (41%), but higher than the 12.5% previously reported in our hospital. An increase in resistance to metronidazole from 30% to 48.3% in a 4 year period has been reported elsewhere. Among patients born in the UK, it has been reported that women were more likely to harbour metronidazole-resistant $H.\, pylori$ strains than men (54% compared with 18%).

Resistance to clarithromycin in $H.\, pylori$ strains was observed in untreated Spanish patients for the first time in 1993, probably due to the increase in the use of the new macrolides. Clarithromycin resistance has also increased in Belgium, rising from 1.7% to 10.5%, and in Ireland it has been reported to be present in 4.5% of strains.

The results of this study are clearly important in choosing the most appropriate treatment for $H.\, pylori$ infection.

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References

Metronidazole/clarithromycin resistance in *H. pylori*


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