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In-vitro susceptibility of *Helicobacter pylori* to ampicillin, clarithromycin, metronidazole and omeprazole

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The in-vitro activities of omeprazole and three antimicrobial agents against 89 clinical isolates of *Helicobacter pylori* from a population with duodenal ulcer disease were determined by an agar dilution method. Resistance rates were 20% for metronidazole (MIC > 8 mg/L), 1% for clarithromycin (MIC > 2 mg/L) and zero for ampicillin (MIC > 8 mg/L). Omeprazole was relatively active against *H. pylori in vitro* (MIC ≤ 8 mg/L).

Introduction

Numerous reports have demonstrated the strong association between *Helicobacter pylori* and the development of duodenal ulcer, chronic active gastritis, gastric carcinoma and MALT lymphoma.[1] It is a difficult infection to eradicate, but remains an important goal of therapy since the frequency of ulcer relapse is reduced with its elimination.[1] Multiple antibiotic regimens have been used, including combinations of tetracycline, amoxycillin, metronidazole and bismuth, but they have been fraught with significant adverse effects such as nausea, vomiting and diarrhoea.[2] More recently, clarithromycin in combination with omeprazole and other antibiotics has been advocated in the treatment of duodenal ulcer.[2]

Patients infected with metronidazole-resistant *H. pylori* strains have a lower eradication rate than patients infected with metronidazole-susceptible strains.[3] It is, therefore, important to determine local patterns of resistance in order to select an appropriate treatment regimen. The study was undertaken to determine the in-vitro susceptibility of *H. pylori* to three antimicrobial agents and omeprazole in the Montreal area of Canada.

Materials and methods

Eighty-nine isolates of *H. pylori*, originally from gastric antral biopsy samples from adults suffering from duodenal ulcer disease, were studied. These patients were evaluated at the Montreal General Hospital, Montreal, Canada between 1991 and 1994 and did not receive any prior antibiotics for treatment of *H. pylori*. The isolates were identified by Gram's stain and by oxidase, catalase and urease tests.[4] The organisms were stored frozen at −70°C, thawed, subcultured on to 5% sheep blood agar and incubated at 37°C under microaerophilic conditions. The isolates were passaged twice to ensure reliable growth and purity.

The MICs were determined by an agar dilution method[5] using Mueller–Hinton agar (Oxoid Ltd, Basingstoke, UK) supplemented with 5% horse blood and containing two-fold dilutions of antimicrobial agents. The inocula were prepared by suspending organisms in Mueller–Hinton broth (Oxoid) and adjusting the turbidity equivalent to that of a 1.0 McFarland standard. The organisms were then inoculated on to the agar plates with a Cathra replicator, delivering 2 μL samples for a final bacterial inoculum of 10^4 cfu per spot. The bacterial inoculum was confirmed by colony counts. Agar plates were incubated at 37°C under microaerophilic conditions for 48 h. The antimicrobial agents tested were ampicillin (0.015–16 mg/L), clarithromycin (0.03–4 mg/L), metronidazole (0.06–16 mg/L) and omeprazole (8–512 mg/L). These were kindly provided by the manufacturers as pure powder preparations with known potency. Reference strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *H. pylori* ATCC 43504 were used as controls.

Isolates were considered to be resistant when the MIC was >8 mg/L for either ampicillin or metronidazole and >2 mg/L for clarithromycin. There are no established guidelines for omeprazole MIC breakpoints.

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Confidence intervals were calculated using the normal approximation of the binomial distribution.[6] For comparison of proportions, Fisher's exact test was used.[6]

Results

The Table summarizes the results of susceptibilities of *H. pylori* to the chemotherapeutic agents tested; these are expressed as MICs for 50% and 90% of the isolates. Metronidazole resistance was found in 20% (95% CI, 13–31%) of isolates. All were susceptible to ampicillin. Resistance to clarithromycin was demonstrated in 1% (95% CI, 0–6%) of isolates. Isolates were inhibited by 8 mg/L of omeprazole, but this agent did not show any activity against the *S. aureus* and *E. coli* reference strains.

Of the original 89 patients, 63 (71%) agreed to undergo a repeat endoscopy at 3 months. Of the 63 patients, 13 had metronidazole-resistant isolates and 50 had metronidazole-susceptible isolates. Four of the 13 patients (31%) with metronidazole-resistant strains and four of the 50 patients (8%) with metronidazole-susceptible strains failed therapy ($P < 0.05$).

Discussion

One of the strengths of our study is that an agar dilution method was used. This method is not always practical to perform in routine laboratories but it is relatively inexpensive and *H. pylori* grows readily on solid media. Other methods have been used for susceptibility testing for *H. pylori*, including disc diffusion, broth microdilution and the Etest. These methods generally perform well but have certain limitations. The broth microdilution method is limited by the difficulty in growing *H. pylori* in liquid media. The Etest is expensive and has been reported to give discrepancies in the interpretation of 'susceptible' or 'resistant' when testing for metronidazole.[7] Disc diffusion is easy to perform but does not give an actual MIC.[7]

In addition, our study included a large number of isolates and clinical information was available for the majority of them (71%). In our study, the prevalence of metronidazole resistance was 20% (95% CI, 13–31%). Hence, this prevalence must be considered when opting for a treatment regimen that includes metronidazole as it may result in a failure to eradicate *H. pylori*; we showed that patients with metronidazole-resistant isolates were more likely to fail therapy that included metronidazole than patients with metronidazole-susceptible isolates. Worldwide, resistance of *H. pylori* to metronidazole has been reported, with rates ranging from 5% to 90%.[8] The surveillance of metronidazole resistance is important because this agent is commonly used in many treatment regimens. In particular, triple therapy consisting of bismuth, metronidazole and ampicillin or tetracycline is commonly used to eradicate *H. pylori* infections.[2] Other regimens include omeprazole, metronidazole and clarithromycin.[2] The mechanism of metronidazole resistance may be related to poor drug penetration, decreased nitro-reduction within the organism or enhanced DNA repair mechanisms.[8]

Omeprazole is a drug currently available for the treatment of peptic ulcer disease; it is a mammalian proton pump inhibitor. Omeprazole inhibits the H^+,K^+-ATPase located on the surface membrane of gastric-acid producing cells in the human stomach.[9] This study shows that omeprazole has some in-vitro activity against *H. pylori* (MIC for all isolates was $\leq$8 mg/L), whereas the reference strains were not inhibited by omeprazole (MICs $\geq$ 512 mg/L). This may in part explain the role of omeprazole in *H. pylori* eradication *in vivo*.

Clarithromycin is a macrolide with pharmacokinetic properties better than those of the closely related drug erythromycin. It is more stable than erythromycin in an acid milieu, penetrates in high concentrations into gastric tissue and mucus and has a longer half-life. It is widely used in combination with a proton pump inhibitor with or without a second antibiotic.[2] However, clarithromycin-resistant *H. pylori* have already been reported; the rate of clarithromycin resistance in France and Belgium is approximately 10%.[8] Clarithromycin resistance is mediated by post-transcriptional adenylation and point mutations within the 23S ribosomal RNA.[10] This results in a decrease in affinity of clarithromycin for the 23S ribosome component and thus diminishes antimicrobial activity. Our study shows that the prevalence of clarithromycin resistance in *H. pylori* sampled from patients living in Montreal, Canada, is low. As inclusion of clarithromycin has been shown to result in effective eradication of *H. pylori*, its use should result in a high rate of cure of duodenal ulcers in our region.

Table. In-vitro susceptibility of 89 isolates of *H. pylori*

Antimicrobial agent	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	No. of resistant isolates (%)
Ampicillin	$\leq$0.015–0.5	$\leq$0.015	0.125	0 (0)
Clarithromycin	$\leq$0.03–>4	0.06	0.125	1 (1)
Metronidazole	0.5–>16	2	>16	18 (20)
Omeprazole	$\leq$8	$\leq$8	$\leq$8.0	

Antibiotic resistance is an important factor in the treatment of *H. pylori* infection because treatment failures can be anticipated. Hence, it is important to monitor the prevalence of antibiotic resistance in order to select an effective treatment regimen.

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