Helicobacter pylori: Primary susceptibility to clarithromycin in vitro in Nova Scotia

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Helicobacter pylori is associated with gastritis, duodenal and gastric ulcers, and gastric cancer. Successful treatment of H pylori infection cures gastritis and ulcer disease, and may prevent H pylori-associated gastric cancer (1-3). Eradication is difficult because many of the organisms reside deep in the protective gastric mucosal layer, where it is difficult to achieve bactericidal antibiotic concentrations. An important reason for treatment failure is primary or acquired resistance to one or more of the antibiotics. The most successful regimens to eradicate H pylori use a...
proton pump inhibitor or a bismuth compound with two antimicrobials (4-7). Of the currently used antibiotic regimens, those that include clarithromycin (CLA) are most effective with cure rates exceeding 90% (7-10). The failure of CLA treatment regimens is primarily the result of infection with CLA-resistant strains of *H pylori* (11,12).

Antibiotic susceptibility tests demonstrate, under defined in vitro conditions, the minimum inhibitory concentration (MIC) at which an agent will inhibit the growth of bacteria. MIC values are used to predict in vivo sensitivity or resistance of organisms. The National Committee for Clinical Laboratory Standards has not yet published a reference antimicrobial susceptibility testing method for *H pylori*. CLA epsilometer gradient agar diffusion tests (E tests, AB Biodisk, Solna, Sweden) and agar dilution tests yield comparable CLA results, but E tests result in better discrimination between sensitive and resistant strains, are easier to perform and are widely used to determine MIC values (13-17).

The objective of our study was to determine MIC and rate of primary resistance to CLA in strains of *H pylori* isolated from our patient population.

**PATIENTS AND METHODS**

**Patients:** One hundred and sixty-two pretreatment strains of *H pylori*, from patients seen at the Victoria General Hospital Site, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, were tested. All patients had endoscopically proven gastric or duodenal ulcers or nonulcer dyspepsia. Biopsies were cultured on Wilkins-Chalgren agar (Oxoid) with 7% sheep blood, identified by morphological and biochemical tests and frozen at −20°C in porcine mucin. Patients were between 16 and 93 years of age. This was a retrospective study, and previous treatment with macrolide antibiotics was not assessed.

**Susceptibility testing:** The *H pylori* strains, frozen at −20°C in porcine mucin, were subcultured to Mueller-Hinton (Oxoid) agar with 7% sheep blood and incubated at 37°C under humid, microaerophilic (5% oxygen, 85% nitrogen, 10% carbon dioxide) conditions for four days. E tests were carried out according to the manufacturer’s instructions. A cotton tipped swab was used to inoculate each *H pylori* strain into Brucella broth with 5% fetal calf serum. Turbidity was adjusted to yield 10⁷ to 10⁹ colony-forming units per mL. A fresh swab was dipped into the suspension, squeezed out and streaked in three directions on Mueller Hinton agar with 7% sheep blood (MHB). The plates were allowed to dry under microaerophilic conditions before carefully adding E test strips with a MIC range of 0.016 to 256 µg/mL. The plates were incubated as above and interpreted according to the manufacturer’s instructions on the second, third, fourth and fifth days. When bacterial growth was distinctly visible, MIC was calculated from the point of intersection between the zone edge and the E test strip, with care taken to assess diffuse endpoints, mutant colonies and resistance. *H pylori* strains with a CLA MIC 2 µg/mL or less were considered resistant (11).

The control strains American Type Culture Collection (ATCC) 29212 *Streptococcus pneumoniae* (defined range CLA MIC 0.03 to 0.12 µg/mL) and ATCC 49247 *Haemophilus influenzae* (CLA MIC 4 to 16 µg/mL), were suspended in Brucella broth, turbidity was adjusted as above, and a fresh
swab was used to streak S pneumoniae on MHB plates and H influenzae on Haemophilus test plates. Control plates were allowed to dry before adding E test strips and incubating S pneumoniae aerobically at 37°C and H influenzae in 5% carbon dioxide at 37°C. MICs were determined, as above, after 24 h.

RESULTS
CLA MICs obtained with the ATCC control strains were within the defined ranges.
E tests were easy to perform and easy to read despite the translucent nature of H pylori (Figure 1). Growth was visible after two days, and clearly defined MIC endpoints were observed and recorded after three days. The endpoints did not change with additional incubation.

Three H pylori strains were resistant (1.8%) and 159 were sensitive (98.2%), and MICs of 90% of the strains were 0.023 µg/mL or less (Figure 2).

DISCUSSION
CLA is one of the most effective antibiotics for treatment of H pylori infection (7-10). It is a macrolide antibiotic that inhibits bacterial RNA-dependent protein synthesis and has a similar antimicrobial spectrum to erythromycin, but is more acid-stable and has better tissue penetration and fewer gastrointestinal side effects (18).

CLA is rapidly absorbed in the gastrointestinal tract, is stable in gastric acid, has a long half-life and is well tolerated (18). Used in combination with omeprazole, the antibiotic concentration in the antral mucosa and mucus layer appears to increase significantly (19).

For H pylori susceptibility testing, E tests have yielded results that are equivalent to, but more precise than, agar dilution and disk diffusion results (13-17). The E test strip effectively diffuses a continuous concentration gradient of an antimicrobial agent into the agar medium and indicates the MIC where the zone of inhibition intersects the strip. The precision and simplicity of the test have led to its widespread use.

CLA resistance appears because of point mutations in 23S rRNA, which alter the ribosomal target (20). In countries where macrolide antibiotics are commonly used, CLA resistance ranges from 2% to 60% and is increasing (11,12). Therefore, it is important to monitor closely susceptibility to observe any increase in primary resistance and to ensure that CLA is being used to treat only CLA-sensitive strains. Recently, we identified two patients who did not respond to triple therapy with omeprazole, metronidazole and CLA. The primary H pylori cultures from both patients were CLA-sensitive, but post-treatment CLA MICs were greater than 256 µg/mL.

Primary CLA resistance in H pylori strains is currently 1.8% for our population. These data support the use of CLA for treatment of H pylori in Nova Scotia.

REFERENCES
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