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

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LM Best, DJM Haldane, GS Bezanson, SJO Veldhuyzen van Zanten. *Helicobacter pylori*: Primary susceptibility to clarithromycin in vitro in Nova Scotia. *Can J Gastroenterol* 1997; 11(4):298-300. Resistance to antimicrobial agents is a major determinant of the efficacy of regimens to eradicate *Helicobacter pylori*. Clarithromycin (CLA) has become one of the most commonly used antibiotics for treatment of *H pylori* infection. In this study, the rate of primary resistance to CLA in *H pylori* isolated from patients was determined. One hundred sixty-two strains were recovered from patients before treatment. Strains were grown and inoculated onto Mueller-Hinton agar with 7% sheep blood. CLA epsilometer gradient agar diffusion test (E test) strips were used to test for susceptibility. Appropriate control organisms were tested to validate the assay. Plates were incubated at 37°C in a microaerophilic atmosphere for up to five days. E test results were easy to interpret. Strains were considered resistant if the minimum inhibitory concentration (MIC) was 2 µg/mL or greater. Three strains were resistant (two strains with MIC 8 µg/mL and one strain with MIC 12 µg/mL), and 159 strains were sensitive (MICs ranged from less than 0.016 to 0.38 µg/mL). Ninety per cent of the strains had MICs of 0.023 µg/mL. Primary resistance was 1.8%. These susceptibility data support the use of CLA for the treatment of *H pylori* in the Nova Scotia population.

Key Words: Clarithromycin, *Helicobacter pylori*

Helicobacter pylori : sensibilité à la clarithromycine in vitro

RÉSUMÉ : La résistance aux agents antimicrobiens est un facteur déterminant de l'efficacité des schémas visant l'éradication d'*Helicobacter pylori*. La clarithromycine est devenue l'un des antibiotiques les plus couramment utilisés en traitement de l'infection à *H. pylori*. Dans cette étude, le taux de résistance primaire à la clarithromycine manifestée par des isolats de *H. pylori* provenant de patients a été mesuré. Cent soixante-deux souches provenant de patients avant leur traitement ont été analysées. Les souches ont été mises en croissance, puis inoculées sur une gélose Mueller Hinton avec sang de mouton à 7 %. Des bandelettes de test E ont été utilisées pour vérifier la sensibilité des isolats. Les organismes pathogènes témoins appropriés ont servi à valider les analyses. Les plaques ont été mises en incubation à 37 °C dans une atmosphère microaéroophile pendant jusqu'à cinq jours. Les résultats du test E ont été faciles à interpréter. Les souches étaient résistantes si la concentration minimale inhibitrice (CMI) était d'au moins 2 µg/mL. Trois souches se sont révélées résistantes, les CMI de 8 µg/mL (n = 2) et CMI 12 µg/mL (n = 1) et 159 souches ont été sensibles (éventail 0,016 à 0,38 µg/mL). Quatre-vingt-dix pour cent des souches avaient des CMI de 0,023 µg/mL ou moins. Le taux de résistance primaire a été de 1,8 %. Ces données sur la sensibilité appuient l'emploi de la clarithromycine dans le traitement de *H. pylori* au sein de la population néo-écossaise.

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

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Figure 1) Epsilometer test results with a strain of *Helicobacter pylori* that is susceptible to clarithromycin at a minimum inhibitory concentration of less than 0.016 µg/mL

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. Bi-

Figure 2) Three strains of *Helicobacter pylori* had minimum inhibitory concentrations (MIC) greater than 2 µg/mL, which is the cutoff value for clarithromycin (CLA) susceptibility. Three (1.8%) of 162 *H pylori* strains were resistant to CLA. MIC of 90% of the strains was 0.023 µg/mL or less

opsies 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 10⁸ 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 to occur 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.

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