Helicobacter pylori: prevalence of antimicrobial resistance in clinical isolates

C. HOOTON*, C. DEMPSEY§, J. KEOHANE†, S.O'MAHONY‡, O. CROSBIE‡ and B. LUCEY*

*Departments of *Microbiology, †Medicine and ‡Gastroenterology, Cork University Hospital; and §Department of Biological Sciences, Cork Institute of Technology, Cork, Ireland*

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ABSTRACT

This study aims to determine the *in vitro* susceptibility of *Helicobacter pylori* to clarithromycin, metronidazole, amoxycillin and tetracycline, the four antibiotics commonly used in eradication therapies. These data are used to evaluate the efficacy of current empiric treatment of *H. pylori* infection in the Southern Region of Ireland. Culture is performed on gastric biopsy samples obtained from 147 consecutive patients undergoing gastroscopy for investigation of dyspepsia. Susceptibility testing to metronidazole, clarithromycin, amoxycillin and tetracycline is performed on the isolates by Etest. Isolates demonstrating clarithromycin resistance are subjected to polymerase chain reaction (PCR) amplification and nucleotide sequence analysis to identify the presence of point mutations in the peptidyltransferase region of the 23S rRNA gene previously associated with resistance to clarithromycin. Prevalence of *H. pylori* in the population studied was 31% (45 isolates). Antimicrobial resistance to metronidazole and clarithromycin was detected in nine (20%) and four (8.9%) of the isolates, respectively. A single isolate demonstrated co-resistance to metronidazole and clarithromycin (2.2%). No resistance was detected to either amoxycillin or tetracycline. The low level of resistance demonstrated among this group of isolates indicates that the empiric treatment currently in place in the Southern Region of Ireland is likely to be successful.

KEY WORDS: Drug resistance, microbial.
Helicobacter pylori.

Introduction

Helicobacter pylori is now recognised as the principal causative agent of peptic ulcer disease, as approximately 95% of patients with duodenal ulcers and about 80% of patients with gastric ulcers are infected with this bacterium.[1] In addition, *H. pylori* is associated with the aetiology of gastric cancer and cancer of the mucosa-associated lymphoid tissue (MALT).[2] Treatment of *H. pylori* is widely accepted as an important goal in the management of peptic ulcer disease and in patients with gastric MALT lymphoma.[3]

International guidelines recommend one-week triple therapy, comprising a proton pump inhibitor in combination with two antibiotics.[4–6] The macrolide clarithromycin is a key component in most triple therapy regimens. First-line therapies, which include clarithromycin in combination with either amoxycillin or metronidazole, have been reported to achieve eradication rates of 70–95%.[7–9]

Quadruple therapy, consisting of a proton pump inhibitor plus metronidazole, tetracycline and an antibacterial bismuth compound, is recommended by the Maastrict 2–2000 consensus report to treat patients in whom first-line therapy has failed. This regime has a reported efficacy of 75–90%.[4]

Bacterial resistance is one of the main factors contributing to treatment failure.[3] A number of studies have reported 11–70% resistance to metronidazole in western Europe.[3,8] Clarithromycin resistance occurs with a prevalence up to 15%.[8,10] Reported resistance to amoxicillin and tetracycline is rare.[3] The development of clarithromycin resistance in *H. pylori* is recognised as a key factor in treatment failure.

Resistance to this antibiotic is reported to be due predominantly to distinct point mutations in the peptidyltransferase region of the 23S rRNA gene, which results in reduced binding of the antibiotic to the ribosomes, and consequently the macrolide cannot prevent protein synthesis. The most common mutations occur where the adenine residues at positions 2142, 2143 and 2144 are replaced by guanine or cystosine (A2142G/C, A2143G/C, A2144G).[10,11] A mutation at position 2717, where the thymine residue is replaced by cytosine, has been reported recently by Fontana *et al*.[12]

This study aims to determine the *in vitro* susceptibility of *H. pylori* to clarithromycin, metronidazole, amoxycillin and tetracycline, four antibiotics commonly used in eradication therapies. These data will be used to evaluate the efficacy of current empiric treatment of *H. pylori* infection in the Southern Region of Ireland.

Correspondence to: Carmel Hooton
Email: hootonc@shb.ie

Material and methods

Ethical approval

Ethical approval was sought from, and granted by, the Clinical Research Ethics Committee, University College Cork, Ireland. The purpose of the study was explained to all patients and signed consent was received prior to participation.

Antimicrobial susceptibility study
A total of 147 consecutive patients undergoing gastroscopy for investigation of dyspepsia at Cork University Hospital, Ireland, were enrolled in the study during the period January 2004 to January 2005, inclusive. Dyspepsia was defined as a symptom complex of epigastric pain or discomfort that may include symptoms such as heartburn, excessive belching, acid regurgitation, upper abdominal bloating and nausea.[13]

Culture
For the purpose of the study, two additional biopsies (one from the antrum and one from the body of the stomach) were taken for culture. The biopsies were introduced immediately into transport medium (Portagerm pylori, bioMérieux, France) and sent directly to the microbiology laboratory. On receipt, the samples were stored in the laboratory at 4°C. All samples were cultured within one hour of endoscopy.

Biopsies were transferred to 0.5 mL Brucella Broth (Becton Dickinson, UK) using a sterile loop, and homogenised for 10–20 sec at 10,000 rpm using an electric tissue grinder (Ultra Turax T25).[14,15] The homogenate was cultured on two selective media (Columbia agar and brain-heart infusion [BHI] agar; Oxoid, UK) enriched with 10% horse blood, and two non-selective media (BHI supplemented with Dent selective agent [Oxoid], and Pylori agar [bioMérieux]). Inoculated plates were placed in an anaerobic jar (lids uppermost) and incubated at 37°C under microaerobic conditions (Gas paks, Oxoid) for up to 14 days.[16]

Plates were examined for growth on the third day and then daily thereafter. *H. pylori* was identified on the basis of typical macroscopic and microscopic morphology, and positive urease, catalase and oxidase reactions.

Susceptibility testing
Antimicrobial susceptibility tests were performed on all *H. pylori* isolates by Epsilometer test (Etest, AB Biodisk, Solna, Sweden) using a standardised and previously validated method.[17] Isolates were considered to be resistant to clarithromycin (CLA) when the minimum inhibitory concentration (MIC) was >1 µg/mL, as defined by the National Committee for Clinical Laboratory Standards (NCCLS).[18]

In the absence of standard breakpoints for metronidazole (MTZ), amoxycillin (AMX) and tetracycline (TE), the breakpoints used to define a resistant strain in this study were based on previous publications. Resistance to MTZ, AMX and TE were defined as MIC values of >8 µg/mL, >0.5 Ìg/mL and >2.0 µg/mL, respectively.[14,17,19] *H. pylori* NCTC 12822 (susceptible to all four antibiotics) was included as a control.

Detection of mutations
Genomic DNA from isolates resistant to clarithromycin by Etest was subjected to semi-nested polymerase chain reaction (PCR) amplification, as outlined by Fontana *et al*.[12] The amplicons of the 783-bp region obtained from the semi-nested PCR underwent nucleotide sequencing (MWG Biotech, UK). DNA sequences were identified as part of the 23S rRNA gene of *H. pylori* by comparison with the current databases using the BLAST suite of programmes on the NCBI website (www.ncbi.nlm.nih.gov/BLAST).[20] The nucleotide sequence for each isolate and the GenBank reference sequence (AB088054) were aligned using ClustalW (www.2ebi.ac.uk/clustalw/) for the purpose of identifying mutations among the test isolates.

Results

Antimicrobial susceptibility
A total of 147 patients were included in the study. *H. pylori* was isolated from 45/147 (31%) samples after an average incubation period of six days. Results of the susceptibility study are presented in Table 1. Antimicrobial resistance to metronidazole was detected in nine (20%) of the isolates. Resistance to clarithromycin was detected in four (8.9%) of the isolates. A single isolate demonstrated co-resistance to metronidazole and clarithromycin (2.2%) with MICs >256 µg/mL in each case. No resistance was detected to either amoxycillin or tetracycline.

Detection of mutations
Analysis of the DNA sequence of the 783-bp amplicon identified the A2143G mutation in two isolates. No mutations currently associated with clarithromycin resistance were detected in the remaining two isolates.

Discussion

H. pylori is the most common pathogen to infect humans around the world, occurring in 40–50% of the populations in developed countries and in 80–90% of the populations in developing countries.[1,2] Over the last decade a rapid and progressive decline in the incidence and prevalence of colonisation by *H. pylori* has been observed in Western developed nations.[3,21,22] This decline has been attributed to several factors, including the standardisation of treatment regimes for *H. pylori* eradication, improved standards of living, and smaller family size.[3] Nonetheless, *H. pylori* has been associated with increasing morbidity due to empiric treatment failure.[3,22]

The primary factor influencing treatment success is the organism's susceptibility to the antimicrobial agents used in eradication, in particular clarithromycin and metronidazole.[23,24] The importance of susceptibility testing for the prevention of treatment failure has been highlighted recently in several studies, where success rates of more than

Table 1. Antimicrobial resistance of 45 *H. pylori* isolates cultured from endoscopic biopsy specimens.

Antibiotic	MIC values (µg/mL) Resistant	Resistance *n* (%)	MIC range (µg/mL)
Clarithromycin	>1.0	4 (8.9)	32–>256
Metronidazole	>8.0	9 (20)	>256
Amoxycillin	>0.5	0 (0.0)	<0.016–0.047
Tetracycline	>2.0	0 (0.0)	<0.016–0.094
Total		12 (26.6)	

MIC: Minimum inhibitory concentration.

95% have been reported when susceptibility of *H. pylori* to antibiotics was determined prior to the first course of treatment.[14,25,26] A consequence of a high eradication rate is a low occurrence of secondary resistance.

Currently, few laboratories undertake routine culture and susceptibility testing of *H. pylori*. This is due mainly to cost and also to the availability of alternative non-invasive methods of diagnosis.[27]

In the absence of routine susceptibility testing, it is advisable that regional surveillance programmes should be implemented to monitor the local levels of antimicrobial resistance and to guide local empirical treatment strategies. First-line treatment regimens should include clarithromycin, and in regions where clarithromycin resistance is above 15%, a regimen based on metronidazole should be used.[4,28]

The present study aimed to determine the level of antimicrobial resistance to clarithromycin, metronidazole, amoxycillin, and tetracycline, four antibiotics commonly used for eradication, in the local community. The findings indicate that the overall level of antimicrobial resistance to *H. pylori* is low in the population studied.

The incidence of clarithromycin resistance (8.9%) is comparable to that reported worldwide, where rates of up to 13% have been documented.[3,27,28] The low level of clarithromycin resistance detected in the present study is also similar to that found in two previous Irish studies, where rates of 3.4–4.5% were documented.[29,30]

Data obtained for metronidazole susceptibility in this study indicate a lower than average rate of resistance (20%), as figures of 11–70% have been cited for western Europe.[3,8] Only one isolate exhibited dual resistance to both metronidazole and clarithromycin, where MIC values in both cases were >256 μg/mL. In this case, the patient had previously being treated unsuccessfully with both metronidazole and clarithromycin. This finding correlates well with reports that the isolation of strains with dual resistance often occurs following treatment failure.[28,29]

The results obtained for amoxycillin and tetracycline susceptibilities agreed with those of previous studies, confirming that the incidence of resistance to these antibiotics is extremely low.[3,27]

In order to identify mutations in the four isolates that demonstrated phenotypic resistance to clarithromycin by Etest, the semi-nested PCR products of each isolate were sequenced with a GenBank 23S rRNA gene reference sequence (AB088054). The A2143G mutation was identified in two of the isolates only. No mutation was detected in either of the other isolates. In each case, the MIC observed by Etest was >256 μg/mL, and first-line eradication therapy, which included clarithromycin, had failed in all four patients.

It may be noted that all four isolates demonstrated clearly distinguishable fingerprints when DNA amplification fingerprinting analysis (data not shown) was performed according to the method outlined by Taylor *et al*.[31] One possible reason for phenotypic resistance unaccompanied by recognisable mutation in two of the clarithromycin-resistant isolates may be explained by the presence of an efflux mechanism.

Bina *et al*. have identified the presence of portions of three genes with homology to potential restriction nodulation division (RND) efflux systems in *H. pylori* NCTC 11637.[32] However, further work is required to elucidate the importance of such efflux mechanisms in *H. pylori* resistance.

In conclusion, the results of the present study indicate that an empiric treatment policy based on clarithromycin is likely to be successful in 91% of cases. In cases of treatment failure, follow-up therapy with metronidazole is likely to achieve up to 98% eradication. However, the performance of regular, local surveillance studies is advisable, in order to monitor antimicrobial resistance rates. This may ensure the continued efficacy of local treatment regimens and prevent an increase in the rate of treatment failure. □

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