Antibiotic susceptibilities of *Helicobacter pylori* strains isolated in the Province of Alberta

Diane E Taylor PhD[1], Qin Jiang MSc[1], Richard N Fedorak MD[2]

DE Taylor, Q Jiang, RN Fedorak. Antibiotic susceptibilities of *Helicobacter pylori* strains isolated in the Province of Alberta. Can J Gastroenterol 1998;12(4):295-298. The incidence of antibiotic resistance to amoxicillin, clarithromycin, erythromycin, metronidazole and tetracycline in *Helicobacter pylori* strains isolated from gastric biopsy specimens obtained in Alberta was investigated. Results for all antibiotics were obtained using agar dilution, and in addition to metronidazole, the E test was used. Resistance to amoxicillin and tetracycline was not detected. Metronidazole resistance determined using agar dilution was approximately 12% (95% CI 4% to 26%) when minimal inhibitory concentrations (MICs) were at least 8 µg/mL, but fell to 2% (95% CI 0.1% to 13%) when MICs were set at 32 µg/mL or greater. The E test for metronidazole resistance (MIC 8 µg/mL or greater) yielded a slightly higher percentage of resistant strains compared with agar dilution tests (14%, 95% CI 5% to 29%). One of the 31 strains was resistant to clarithromycin (MIC 8 µg/mL) and erythromycin (MIC 16 µg/mL). Thus, the incidence of resistance to clarithromycin, part of the currently used triple therapy for eradication of *H pylori*, was 3% (95% CI 0.1% to 17%).

Key Words: *Antibiotic resistance, Clarithromycin,* Helicobacter pylori

Sensibilité des souches de *H. pylori* isolées en Alberta, au Canada, à l'endroit des antibiotiques

RÉSUMÉ : Une recherche a porté sur la fréquence des cas de résistance à l'amoxicilline, à la clarithromycine, à l'érythromycine, au métronidazole et à la tétracycline, manifestée par des souches d'*Helicobacter pylori* isolées dans des spécimens de biopsie gastrique prélevés en Alberta. Les résultats pour tous les antibiotiques ont été obtenus à l'aide de dilutions sur gélose et, en plus du métronidazole, le test E a été utilisé. On n'a décelé aucune résistance à l'amoxicilline ni à la tétracycline. La résistance au métronidazole, déterminée à l'aide d'une dilution sur gélose, a été d'environ 12 % (IC 95 %, 4 à 26 %) lorsque les concentrations minimales inhibitrices (CMI) étaient au moins 8 µg/mL, mais a diminué jusqu'à 2 % (IC 95 %, 0,1 à 13 %) lorsque les CMI ont été établies à ≥32 µg/mL. Le test E pour la résistance au métronidazole (CMI ≥8 µg/mL) a donné lieu à un pourcentage légèrement plus élevé de souches résistantes en comparaison avec les tests de dilution sur gélose (14 %, IC 95 %, 5 à 29 %). L'une des 31 souches s'est révélée résistante à la clarithromycine (CMI = 8 µg/mL) et à l'érythromycine (CMI = 16 µg/mL). Donc, la fréquence des cas de résistance à la clarithromycine actuellement utilisée en trithérapie pour l'éradication de *H. pylori* s'est révélée être à 3 % (IC 95 %, 0,1 à 17 %).

Helicobacter pylori is associated with chronic gastritis, duodenal and gastric ulcers, and gastric cancer, and is, therefore, a major public health concern (1). The United States National Institutes of Health Consensus Conference on the role of *H pylori* in peptic ulcers (2) recommends treatment of *H pylori* infection for all patients with acute or recurrent duodenal or gastric ulcers in whom *H pylori* infection is present. Similar guidelines were recently published for Can-

Department of Medical Microbiology and Immunology[1]; Division of Gastroenterology, Department of Medicine[2], University of Alberta, Edmonton, Alberta

Correspondence and reprints: Dr Diane E Taylor, Department of Medical Microbiology and Immunology, 1–28 Medical Sciences Building, University of Alberta, Edmonton, Alberta T6G 2H7. Telephone 403-492-4777, fax 403-492-7521, e-mail diane.taylor@ualberta.ca

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TABLE 1
Antibiotic susceptibilities of *Helicobacter pylori* strains isolated in Alberta

Antimicrobial agent	MIC_{90} (μg/mL)*	Range (μg/mL)	% resistant†
Amoxicillin	≤0.0125	≤0.0125-0.1	0
Clarithromycin	≤0.0125	≤0.00625-8	3
Erythromycin	0.125	≤0.0125-16	3
Metronidazole	1	0.5-32	12
Tetracycline	1	0.5-2	0

*Antibiotic susceptibilities were determined by agar dilution as described in 'Materials and Methods'; 31 strains were tested against all antibiotics except for metronidazole for which 42 strains were tested. *Minimal inhibitory concentration [MIC] for 90% of strains; †Strains with metronidazole MICs at least 8 μg/mL were defined as resistant*

ada (3). Initially, the most effective treatment regimen was the triple combination of bismuth, metronidazole and tetracycline, which achieved eradication rates of more than 80% (1). Concern over the prevalence of metronidazole-resistant *H pylori* and poor compliance due to side effects led to the development of other therapies such as dual therapy combining the proton pump inhibitor omeprazole with amoxicillin or with clarithromycin (1). More recently, triple therapies consisting of omeprazole in combination with two antibiotics – clarithromycin and either metronidazole or amoxicillin – have been shown to eradicate *H pylori* in more than 90% of patients (see reference 1 for review). Antibiotic resistance adversely affects the success rate of various anti-*H pylori* therapies (4). It is important for clinicians using therapies for eradication of *H pylori* to be aware of the prevalence of antibiotic resistance within their community. We undertook this study to monitor a representative sample of *H pylori* strains isolated in Alberta from 1992 to 1995 for resistance to the antibiotics amoxicillin, clarithromycin, metronidazole and tetracycline, which are most commonly used in anti-*H pylori* regimens.

MATERIALS AND METHODS

H pylori strains were isolated from gastric biopsies obtained from patients examined at the University of Alberta Hospital endoscopy unit (Edmonton, Alberta) using methods described previously (5-7). All patients had endoscopically proven gastritis or additional symptoms of duodenal or gastric ulcer, or nonulcer dyspepsia. None of the patients was treated with antibiotic therapy for eradication of *H pylori* before biopsy. Information on treatment with antibiotics for other conditions was not available. Strains of *H pylori* were isolated from biopsies obtained between 1992 and 1995, and 31 were randomly selected for antimicrobial susceptibility testing. *H pylori* strain HP439, which is resistant to metronidazole (minimal inhibitory concentration [MIC] 64 μg/mL), was used as positive control in tests of susceptibility to metronidazole. An additional 11 randomly selected strains were tested for resistance to metronidazole.

MICs of antibiotics were determined using an agar dilution method for most tests in this study. Serial twofold dilutions of antibiotics were made with sterile water as follows: amoxicillin (Sigma) 0.0125 to 1 μg/mL; clarithromycin (Bayer, Leverkusen, Germany) 0.00625 to 32 μg/ mL; erythromycin (Sigma) 0.0125 to 64 μg/mL; metronidazole (Sigma) 0.5 to 64 μg/ml; and tetracycline (Sigma) 0.5 to 8 μg/mL. For each plate, 1 mL each of diluted antibiotic was mixed with 24 mL of supplemented BHI-YE media (animal serum 5%, Mores). Plates were dried briefly before use. A 48 h culture of each strain was suspended in BHI broth and was adjusted to a concentration equal to McFarland standard #3. A 5 μL drop of each such cell suspension was immediately placed on the agar plate. For each concentration tested, MIC was determined on three separate occasions. Metronidazole resistance was also tested using the E test method (Epsilometer gradient agar diffusion test strips, AB Biodisk, Solna, Sweden).

BHI-YE supplemented agar plates were spread evenly with a swab soaked in the same cell suspension used for the agar dilution method. An E test strip was placed in the centre of each plate. All plates were incubated under microaerobic conditions as described previously (5). Results were recorded after 72 h incubation.

Confidence intervals were calculated using the normal approximation of the binomial distribution (8).

RESULTS

Susceptibility test results as determined by the agar dilution method are shown in Table 1. The majority of the *H pylori* strains examined were highly susceptible to amoxicillin (MIC 0.0125 μg/mL or less). Only one strain of 31 tested had a greater MIC (0.1 μg/mL). The antibiotic history of the individual from whom this *H pylori* strain was taken was not available. Likewise, susceptibility to tetracycline was observed in 31 strains of *H pylori* tested with MIC_{90} 1 μg/mL. Two *H pylori* strains were slightly more resistant with tetracycline MICs of 2 μg/mL.

Almost all *H pylori* strains of the 31 tested were susceptible to macrolide antibiotics clarithromycin (MIC_{90} 0.0125 μg/mL or less) and erythromycin (MIC_{90} 0.125 μg/mL). One strain, UA1182, was found to be resistant to clarithromycin (MIC 8 μg/mL) and to erythromycin (16 μg/mL).

Metronidazole resistance defined as 8 μg/mL or greater was noted in five of 42 *H pylori* strains (12%, 95% CI 4% to 26%) isolated from the Alberta population during 1992 to 1995, as determined by the agar dilution method. An *H pylori* control culture with a known metronidazole MIC of 64 μg/mL (HP439) was tested at the same time. The E test was also used to determine MICs of metronidazole for 42 strains. With the use of the E test, 6 strains (14%, 95% CI 5% to 29%) were identified as resistant with MICs of 8 μg/mL or greater. These strains included the five identified as resistant to metronidazole by agar dilution testing plus an additional strain. Therefore, the difference between prevalence of resistance as determined by agar dilution testing and the E test is not considered statistically significant. However,

when MICs of at least 32 μg/mL were used as the cut-off with the agar dilution method, one of 42 strains was resistant (2%, 95% CI 0.1% to 3%). In contrast, the E test identified four of 42 strains as resistant (9.5%, 95% CI 3% to 23%).

DISCUSSION

Treatment of patients with various antimicrobial agents in combination therapies for eradication of *H pylori* (1) has resulted in an increase in resistance to some antibiotics. Although resistance to amoxicillin has rarely been reported, a few *H pylori* strains resistant to tetracycline have appeared recently (9,10). Resistance to neither antibiotic was observed in this study.

The prevalence of clarithromycin-resistant *H pylori* is considered to be low (less than 5%) in most countries, but as high as 10% in France and Belgium, as reported in the review by Goddard and Logan (11). In a study done in The Netherlands, the incidence of clarithromycin resistance in pretreatment isolates was less than 1.5%, whereas in Italy resistance was reported to be 6% (10). In Canada, clarithromycin resistance was reported to be 1% in patents from Montreal, Quebec (12) and 1.8% in patients from Halifax, Nova Scotia (13).

In our study, one strain of *H pylori*, UA1182, exhibited cross-resistance to clarithromycin and erythromycin with MICs of 8 and 16 μg/mL, respectively. Our study indicates that 3% (95% CI 0.1% to 17%) of the *H pylori* strains isolated from gastric biopsies in Alberta are clarithromycin-resistant, but our sample size of the *H pylori* isolate tested was small (n=31). It is possible that the prevalence of clarithromycin resistance in Alberta strains could be lower than 3% if a larger sample of *H pylori* strains were tested.

One difficulty encountered in comparing incidence of antibiotic resistance in *H pylori* is the great variability in the MICs taken as breakpoints of resistance. Table 2 (14-22) lists some examples of MIC breakpoints used to define resistance to metronidazole and clarithromycin taken from several recent studies. Regarding clarithromycin, the majority of our strains, 30, were susceptible based on the most stringent criteria defined in these previous studies.

Resistance to clarithromycin, associated with cross-resistance to another macrolide, erythromycin, has been shown to depend on chromosomal mutation (7,14,23). Both of the two rRNA gene copies encoding 23S rRNA are usually mutated. Mutations occur in the *H pylori* 23S rRNA at coordinates (adenosine) A2042 or A2043, replacing the nucleoside with guanosine (G). We observed that mutations of A2042→G resulted in high level resistance to clarithromycin with MIC of 32 μg/mL and erythromycin cross-resistance at 256 μg/mL. In contrast, intermediate resistance – MIC 0.5 to 1 μg/mL and MIC 64 to 128 μg/mL for clarithromycin and erythromycin, respectively – were associated with A2043→G substitutions (7). In the present study, we observed moderate level resistance to clarithromycin and erythromycin in one strain of *H pylori*. The mutation present in the resistant isolate has not yet been determined and will be the subject of a separate study. The patient from whom this *H pylori* strain was obtained had not apparently been treated with a regimen containing clarithromycin for eradication of *H pylori* before isolation of the strain, but reported visiting the Middle East on a regular basis and may have acquired the strain during foreign travel.

TABLE 2
Variability in breakpoints used to define resistance to metronidazole and clarithromycin in *Helicobacter pylori*

Antibiotic	MIC breakpoint (μg/mL)*	Geographic origin of strains	Reference
Metronidazole	≥4	Peru	18
	>4	Canada/Europe	19
	≥64	United States	20
	≥8	Norway	21
	≥16	Canada (i) joint study†	17
	>8	(ii) Montreal, Quebec	12
	>8	(iii) Halifax, Nova Scotia	17
	>32	Belgium	15
	>32	Italy	10
	>32	Netherlands	22
Clarithromycin	≥0.125	Peru	18
	≥2	Canada/Europe	19
	≥4	Canada (i) joint study†	16
	>2	(ii) Montreal, Quebec	12
	>2	(iii) Halifax, Nova Scotia	16
	>8	Italy	10
	≥2‡	United States	14

**Strains with minimal inhibitory concentrations (MICs) with the breakpoints shown were regarded as resistant; †Montreal, Edmonton and Toronto contributed* H pylori *to this Canadian study; ‡An MIC breakpoint of 2 μg/mL was regarded as intermediate resistance*

Wide geographic variation in the prevalence of metronidazole-resistant *H pylori* has been observed, ranging from 20% to 80% in Western Europe, to approximately 100% in Africa (15,24). In Canada, the prevalence of resistance has been reported to vary, being 32% in a combined study of the cities of Montreal, Toronto and Edmonton (18) using agar dilution testing. A study in Montreal reported 20% in Quebec using agar dilution (12) and another in Halifax reported 38% in Nova Scotia using the E test (17).

As is clear from Table 2, the decision on what metronidazole MIC defines resistance has a bearing on the level of resistance. Taking at least 8 μg/mL to define resistance, we obtained an incidence of resistance of 12% in Alberta using agar dilution and 14% using the E test results. If however, a MIC 32 μg/mL or greater is used to define metronidazole resistance, only one of 42 strains (2%) would be considered metronidazole-resistant. The E test identified four of 42 strains (9.5%) as resistant to metronidazole. The Sanford Guide to Antimicrobial Therapy (25) gives peak serum level achievable for metronidazole as 2.5 to 13 μg/mL with the

mean as 6.2 μg/mL for a 250 mg oral dose. Therefore, 8 μg/mL metronidazole appears to be the most appropriate cut-off value in relation to treatment response. In contrast, for clarithromycin the peak serum level achievable for an oral dose of 500 mg is 2 to 3 μg/mL (25). A cut-off value of 2 or 4 μg/mL to define resistance may be most appropriate for clarithromycin.

The E test is rapid and convenient, and some investigators have reported excellent correlation of E test results with those obtained by standard susceptibility testing methods for *H pylori* (9,15,26). Nevertheless, the E test for metronidazole has been shown to yield somewhat greater numbers of resistant strains than either agar or broth dilution methods, especially when the E test MIC values fall between 8 and 32 μg/mL (10,27). Our results confirm this finding. The explanation for the discrepancies between metronidazole MICs for the E test and other methods were not determined in this or previous studies (10,27).

Our study highlights that standardization of MIC breakpoints in reporting studies of antimicrobial resistance in *H pylori* would be an important advance.

CONCLUSIONS

H pylori isolates from biopsies obtained from individuals in Alberta were highly susceptible to amoxicillin and tetracycline. Resistance to clarithromycin and erythromycin was identified in one of 31 *H pylori* isolates (3%). At present, amoxicillin, clarithromycin and possibly tetracycline remain useful as part of combination therapies for eradication of *H pylori*. Resistance to metronidazole was also found to be fairly low at 12%, where resistance is defined as 8 μg/mL or greater, and 2%, where resistance is defined as 32 μg/mL or greater. The level of resistance of *H pylori* in Alberta should continue to be monitored for the emergence of resistant isolates so that eradication therapy can be chosen wisely.

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