Cultural Recovery and Determination of Antimicrobial Susceptibility in *Helicobacter pylori* by Using Commercial Transport and Isolation Media

B. Yuen, R. Zbinden, M. Fried, P. Bauerfeind, M. Bernardi

Abstract

Background: Antimicrobial resistance of *Helicobacter pylori* is the main reason for eradication failure. We have studied the feasibility of a commercial transport medium for cultural recovery and subsequent drug susceptibility testing.

Patients and Methods: From March to December 2000, 79 consecutive gastric biopsies, positive in a rapid urease test, were transferred into a commercial transport medium and sent within 24 hours from the district hospital to the microbiological laboratory for culture and susceptibility testing. A commercial agar plate and an in-house Wilkins-Chalgren agar plate were used for culture. Susceptibility data were compared with data collected from 1992 to 2003 in the University Hospital of Zurich.

Results: Cultural recovery and susceptibility testing of *H. pylori* was successful in 55 of 79 patients. In 17 cases cultural recovery failed because of technical problems (n = 14), long transport time (n = 1) and unknown reason (n = 2). Failure of susceptibility testing (n = 7) was mainly due to fungal overgrowth. Resistance to metronidazole and clarithromycin was found in 15 (27%) and in 12 patients (22%), respectively; resistance to amoxicillin was not observed. Five patients (9%) showed resistance both to metronidazole and to clarithromycin. Eradication therapy failed in all patients with macrolide resistance. Resistance rates were higher in females than in males; 30% vs 12% for clarithromycin and 33% vs 20% for metronidazole. Resistance to metronidazole was significantly lower in Swiss patients (15%) than in non-Swiss patients (39%).

Conclusion: Antimicrobial resistance data can reliably be obtained by sending the biopsy specimen in a commercial transport medium to a microbiological laboratory. This is especially important after eradication failure. Resistance to metronidazole and clarithromycin is highly prevalent and more common in women and non-Swiss patients.

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Introduction

Helicobacter pylori infection, now generally accepted as the cause of chronic gastritis and peptic ulcer disease, has also been linked to gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [1]. Eradication of *H. pylori* is the first therapeutic approach that constitutes a reliable long-term prophylaxis of peptic ulcer relapse [2], accelerates ulcer healing [3], and reduces the rate of ulcer complications [4]. Moreover, eradication of *H. pylori* improves the quality of life of ulcer patients [5]. Over the past 15 years different eradication therapies have been promoted. The first-line therapies of choice are proton pump inhibitor-based triple therapies with clarithromycin, metronidazole, and amoxicillin as the most frequently used antibiotics for curing *H. pylori* infection [1, 6]. In a European survey, drug resistances range from 10% to 50% for metronidazole and up to 15% for clarithromycin, resistance to amoxicillin appears to be uncommon [7]. Apart from noncompliance, antibiotic resistance is the major cause of eradication treatment failure. Susceptibility testing is frequently recommended for correct treatment of *H. pylori* infection, especially when a first treatment attempt has failed [8].

H. pylori is a fastidious, microaerophilic species. Various conditions can affect survival rates of *H. pylori*, e.g. the time lapse between sampling and culture, transport temperature, transport media, duration of exposure to air [9]. The aim of this study was to evaluate the reliability of

B. Yuen, M. Bernardi
Dept. of Internal Medicine, GZO Hospital Wetzikon, Wetzikon, Switzerland
R. Zbinden *(corresponding author)*
Institute of Medical Microbiology, University of Zurich, Gloriastr. 32, 8006 Zurich, Switzerland; Phone: (+41/44) 634-2608, Fax: -4906, e-mail: rzbinden@immv.unizh.ch
M. Fried, P. Bauerfeind
Dept. of Internal Medicine, Division of Gastroenterology and Hepatology, University Hospital Zurich, Switzerland.

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a commercial transport medium and a commercial *H. pylori* plate for cultural recovery to allow drug susceptibility testing of *H. pylori*. The antibiotic resistance data obtained were compared with those from the years 1992–2003 of the University Hospital of Zurich.

Patients and Methods

Between March and December 2,000 consecutive patients over age 18, who were referred for upper endoscopy to the gastroenterology division of the hospital Wetzikon, Switzerland, were included in this study. During endoscopy biopsy specimens from antrum and corpus were taken for rapid urease test (HUT test; AstraZeneca, Wedel, Germany) and culture of *H. pylori*. We considered a patient to be *H. pylori* positive when the rapid urease test indicated mucosal colonization. Rapid urease test positive gastric biopsy samples of 79 patients (37 female, 42 male; mean age 52.4 years) were sent to the medical microbiology laboratory of the University of Zurich to determine antimicrobial susceptibility to metronidazole, clarithromycin, and amoxicillin. Detailed epidemiological data, including country of origin and medical history, were obtained from each patient. Nine patients had duodenal ulcer, two patients ventricular ulcer, ten patients erosive duodenitis and 12 patients erosive antral gastritis, respectively. All other 46 patients with a positive urease test had no pathologic endoscopic or histologic findings and were not treated. Patients with active gastric or duodenal ulcers or erosive duodenitis or antral gastritis or with a history of peptic ulcer disease were treated with a 7-day triple therapy (pantoprazol 40 mg, clarithromycin 500 mg, amoxicillin 1,000 mg, each twice daily), followed by a 4-week regimen with the proton pump inhibitor once daily. Compliance of the patients was surveyed by telephone interview 3 days after onset of eradication therapy and after the end of therapy. Then, 4 weeks after the end of proton pump inhibitor therapy, success of *H. pylori* eradication was determined with a ^{13}C -urease breath test (Labco; High Wycombe, Buckinghamshire, UK) or, if endoscopy was indicated, by rapid urease test of an endoscopic biopsy.

Biopsy specimens were transported from the endoscopic unit to the microbiological laboratory within 24 h in a special transport medium (Portagerm pylori®; BioMérieux, Marcy l'Etoile, France) and processed immediately upon receipt. Biopsy specimens were removed from the transport medium and spread over the surface of Wilkins-Chalgren agar (in-house made) supplemented with human blood (65 ml/1,000 ml) and antibiotics (vancomycin 10 mg/l, cefsulodin 2 mg/l, trimethoprim 5 mg/l, actidione 100 mg/l). Plates were incubated for 4 days at 37 °C in a microaerophilic atmosphere (90% N_2, 5% CO_2, 5% O_2) using a gas generator (Campy Gen; Oxoid, Basingstoke, England). Following recognition of a laboratory problem with a too low content of blood in the in-house Wilkins Chalgren agar (33 ml instead of 65 ml/1,000 ml), we included a commercial *H. pylori* plate (Pylori Agar®, BioMérieux, Marcy l'Etoile, France) for cultural recovery.

Translucent colonies of characteristic appearance for *H. pylori* were Gram stained for typical morphology and tested for catalase, oxidase, and urease activities. Susceptibility testing was performed using Etest (AB Biodisk, Solna, Sweden) according to the prescriptions of the manufacturer as described earlier [10]. In brief, an isolate of *H. pylori* was inoculated onto sheep blood agar plates with a suspension of MacFarland 3 and the Etest stripes were applied to the agar surface. Plates were incubated for 3 days in a microaerophilic atmosphere at 37 °C. For interpretation of susceptibility we used the NCCLS breakpoint of ≥ 1 mg/l for clarithromycin [11]; for amoxicillin and metronidazole the breakpoints were ≥ 2 mg/l and ≥ 8 mg/l, respectively [12]. The susceptibility data obtained were compared with the susceptibility results of the years 1992–2003 of the University Hospital of Zurich, Switzerland, tested at the Institute of Medical Microbiology of the University of Zurich.

Statistical significance for two independent classes was assessed by the χ^2 test or Fisher's exact test if numbers smaller than five were present.

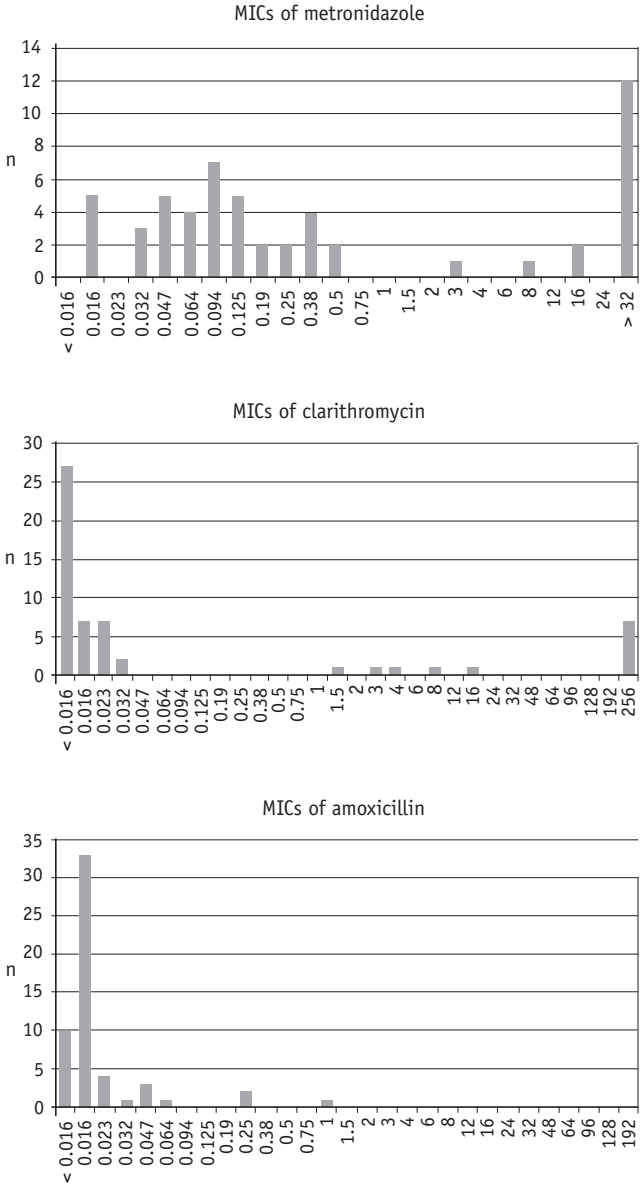

Figure 1. MICs of the 55 strains of *H. pylori* isolated in the district hospital.

Results

Culture and susceptibility testing was successful in 55 of 79 patients. Antimicrobial resistance against metronidazole was present in 15 strains (27%), resistance against clarithro-

mycin was found in 12 strains (22%). Five patients (9%) showed resistance both to metronidazole and to clarithromycin. Resistance against amoxicillin was not observed. MICs of metronidazole, clarithromycin, and amoxicillin are shown in figure 1. For 25 of the 33 patients undergoing eradication therapy, cultural recovery was successful and drug susceptibility data were obtained. Eradication of *H. pylori* was successful in all patients (N=15) with susceptible *H. pylori* strains and in five of six patients with metronidazole resistant strains, whereas eradication failed in two patients with clarithromycin-resistant strains and in two patients with double resistance. ^{13}C -urease breath test indicated successful eradication therapy in all eight patients with negative culture results.

Geographic origin and sex of the patients were the main factors affecting the results of antibiotic resistance. In Swiss patients, metronidazole resistance was less frequent (15%, n = 4), compared to non-Swiss patients (39%, n = 11; $p < 0.05$, Fisher's exact test), originating mainly from the Mediterranean area, e.g. Italy, Balkans, and Africa. Clarithromycin resistance was present in five Swiss patients (19%) and in seven non-Swiss patients (25%). In female patients resistance against metronidazole was found in ten (33%) and against clarithromycin in nine (30%) cases, compared to five (20%) and three (12%) cases in male patients, respectively.

In the 1st month of the study, determination of susceptibility was successful in ten of 11 isolates. Subsequently culture of *H. pylori* failed in 14 patients because two lots of the in-house Wilkins Chalgren agar were produced with an incorrect low content of human blood. During this period, determination of drug susceptibility was not successful in an additional six cases because subcultivation failed due to fungal overgrowth. Following identification and correction of the laboratory problem a new commercial plate, i.e. Pylori agar (BioMérieux), was chosen to be evaluated at the same time. In 33 cases the commercial plate was compared to the standard in-house Wilkins Chalgren agar. Culture with the commercial *H. pylori* plate was successful in 32 cases (97%), and in 29 cases (88%) with the in-house *H. pylori* plate. During the time with correct agar plates three culture results were negative; once because the transport time from the endoscopic unit to the microbiological laboratory was too long (4 days) and in two cases because of unresolved reason. In one case subcultivation failed.

Data on antimicrobial susceptibility of *H. pylori* collected at the University Hospital Zurich in the years 1992–2003 are shown together with the data of the district hospital in table 1. The fluctuation in the number of samples submitted for culture indicates nonrepresentative patient selection. In the years with a low number of examined patients, susceptibility testing was performed primarily in selected patients, e.g. patients after eradication therapy failure. Thus, the data have to be interpreted with caution. Nevertheless, our data indicate that resistance to clarithromycin shows an increasing trend over the years.

Discussion

Isolation of *H. pylori* from gastric biopsy specimen is a difficult procedure that is affected by a number of factors, such as presence of oropharyngeal flora, time lapse between sampling and culture, inappropriate transport temperature, duration of exposure to air. The present study indicates that survival rates of *H. pylori* are excellent when using a commercially available transport medium and cul-

Table 1
***Helicobacter pylori* antimicrobial resistance by year in the university hospital (1992–2003) and in a district hospital (2000).**

	Total patients	Positive culture (%)	Susceptibility testing	Metronidazole resistance (%)	Clarithromycin resistance (%)	Amoxicillin resistance
1992	163	91 (56)	82	64 (78)	-	-
1993	314	149 (47)	130	93 (72)	-	-
1994	288	107 (37)	94	43 (46)	-	-
1995	82	42 (51)	34	14 (41)	-	-
1996	48	22 (45)	22	8 (36)	2 (9)	0
1997	256	90 (35)	76	28 (36)	11 (14)	0
1998	69	24 (34)	24	15 (62)	7 (29)	0
1999	19	8 (42)	7	4 (57)	5 (71)	0
2000	21	10 (48)	10	5 (50)	6 (60)	0
2001	25	14 (56)	11	9 (82)	5 (45)	1
2002	34	18 (53)	14	9 (64)	7 (50)	0
2003	27	13 (48)	13	6 (46)	6 (46)	1
2000	79[a]	55 (70)	55[b]	15 (27)	12 (22)	0

[a] Consecutive patients with positive rapid urease test (HUT test); [b] Positive culture and susceptibility testing

turing within 24 h. These results are in line with earlier reports [13, 14]. The reliability of the transport medium is especially important for district hospitals or practitioners who have to send the biopsy specimen to an external microbiological laboratory. Due to the fastidious nature of *H. pylori*, primary isolation from biopsy specimen can be difficult. During parallel testing a commercial pylori agar plate was found to be superior to the in-house pylori agar.

The results of our study show antimicrobial resistance rates of 22% for clarithromycin and 27% for metronidazole, respectively. In a study by *Maggi-Solcà* et al. performed in the southern part of Switzerland resistance rates were as high as 12% for clarithromycin (13% with a breakpoint of 1 mg/l according to NCCLS [11]) and 29% for metronidazole [12]. Our epidemiological data showed an insignificant higher prevalence of resistant *H. pylori* strains in female compared to male patients. These data correspond to results of other studies [1, 15, 16]. With respect to metronidazole, this may be due to treatment of genital tract infections (e.g. trichomoniasis, bacterial vaginosis). Resistance to clarithromycin may be provoked by the use of erythromycin in pregnancy or by the use of macrolides for chlamydial or non-gonococcal urethritis/cervicitis. A significant difference in metronidazole resistance was observed between Swiss patients (15%) and non-Swiss patients (39%), whereas there was no difference in clarithromycin resistance between these two groups. In accordance with our results, an analysis of data from 11 European countries showed that rates of resistance to metronidazole were higher in non-white patients from northern Africa and Mediterranean countries than in patients from northern European countries [17].

The systematic drug susceptibility testing of culturally recovered *H. pylori* revealed a high prevalence of resistance to clarithromycin. So far only a few studies have examined the development of clarithromycin resistance in *H. pylori*. A study conducted in the USA by *Vakil* et al. [18] showed an increase in clarithromycin resistance from 4% in 1993–94 to 12.6% in 1995–96. A 9-year study of the evolution of clarithromycin resistance in Spanish children by *Lopez-Brea* et al. [19] showed an increase in clarithromycin resistance from 2.3% in 1991–93 to 21% in 1994–96 and 28% in 1997–99. The increase in clarithromycin resistance might be due to the widespread use of macrolides, especially for respiratory tract infections. In Switzerland macrolides are the second most prescribed antibiotics for community acquired pneumonia [20]. Data concerning metronidazole resistance rates are conflicting. A UK study by *Parsons* et al. [16] showed a significant increase in metronidazole resistance from 1994/95 (20.8%) to 1995/96 (46.7%), but thereafter resistance rates appeared to have reached a plateau. In a recently published US meta-analysis by *Meyer* et al. [15] metronidazole resistance tended to decrease over time. One reason for this apparent decrease may be a result of changes in susceptibility testing methods.

Although the number of patients undergoing eradication therapy was small, our study demonstrates that antimicrobial resistance has an important impact on *H. pylori* eradication rates. All patients with susceptible *H. pylori* strains have been successfully eradicated, whereas eradication therapy failed in all patients with clarithromycin resistance. Recent recommendations suggest that the optimal regimen for primary treatment of *H. pylori* is a combination of a proton pump inhibitor with two of the following antibiotics: amoxicillin, clarithromycin or a nitroimidazole (either metronidazole or tinidazole) [21]. With such triple therapies, cure rates of 80–85% can be achieved [22]. In the presence of antimicrobial resistance cure rates are markedly reduced. A meta-analysis of the effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on the outcome of eradication therapy showed a reduction of almost 40% in strains with metronidazole resistance and 55% in strains with clarithromycin resistance [23]. In these patients standard triple therapy often leads to treatment failure with selection of *H. pylori* strains that have acquired dual resistance and are difficult to eradicate [8]. To avoid development of dual resistance, it was proposed not to combine clarithromycin and metronidazole in therapeutic regimens [6]. The increasing problem of antibiotic resistance is a major argument to restrict *H. pylori* eradication therapy to those patients with adequate indication. Drug susceptibility testing should be done to prevent treatment failure and the emergence of multiple-resistant *H. pylori* strains [24].

In conclusion, antimicrobial resistance can be reliably determined in a district hospital by using the commercial transport medium Portagerm pylori. Resistance to metronidazole and clarithromycin is highly prevalent and more common in women and non-Swiss patients. A continuous surveillance of *H. pylori* susceptibility to antibiotics at the national or regional level is needed. The role of antimicrobial susceptibility testing is of increasing importance in treating *H. pylori* infection, especially after eradication failure.

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