5. **Grasmick AE, Naito N, Bruckner DA:** Clinical comparison of the AutoMicrobic System Gram-Positive Identification Card, API-Staph-Ident, and conventional methods in the identification of coagulase-negative *Staphylococcus* spp. Journal of Clinical Microbiology 1983, 18: 1323–1328.
6. **Gruer LD, Bartlett R, Ayliffe GAJ:** Species identification and antibiotic sensitivity of coagulase-negative staphylococci from CAPD peritonitis. Journal of Antimicrobial Chemotherapy 1984, 13: 577–583.
7. **Marsik FJ, Brake S:** Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. Journal of Clinical Microbiology 1982, 15: 640–645.
8. **Sewell CM, Clarridge JE, Young EJ, Guthrie RK:** Clinical significance of coagulase-negative staphylococci. Journal of Clinical Microbiology 1982, 16: 236–239.
9. **Spencer RC:** Infections in continuous ambulatory peritoneal dialysis. Journal of Medical Microbiology 1988, 27: 1–9.
10. **West TE, Walshe JJ, Krol CP, Amsterdam D:** Staphylococcal peritonitis in patients on continuous peritoneal dialysis. Journal of Clinical Microbiology 1986, 23: 809–812.
11. **Almeida RJ, Jorgensen JH, Johnson JE:** Evaluation of the AutoMicrobic System Gram-Positive Identification Card for species identification of coagulase-negative staphylococci. Journal of Clinical Microbiology 1983, 18: 438–439.
12. **Ruoff KL, Ferraro MJ, Jerz ME, Kissling J:** Automated identification of gram-positive bacteria. Journal of Clinical Microbiology 1982, 16: 1091–1095.
13. **Lindsay JA, Riley TV:** Susceptibility to desferrioxamine: a new test for the identification of *Staphylococcus epidermidis*. Journal of Medical Microbiology 1991, 35: 45–48.
14. **Boswell TC, Wilcox MH, Spencer RC:** Susceptibility of coagulase-negative staphylococci to desferrioxamine. Journal of Hospital Infection 1991, 19: 295–296.

Effect of pH Variation on the Susceptibility of *Helicobacter pylori* to Three Macrolide Antimicrobial Agents and Temafloxacin

G.J. Malanoski[1,3], G.M. Eliopoulos[1,3]*, M.J. Ferraro[2,3], R.C. Moellering Jr.[1,3]

The in vitro susceptibility of 27 clinical isolates of *Helicobacter pylori* to erythromycin, clarithromycin, azithromycin and temafloxacin under various pH conditions was evaluated. Clarithromycin (MIC90 0.03 μg/ml) was found to be significantly more active than either erythromycin (MIC90 0.125 μg/ml) or azithromycin (MIC90 0.25 μg/ml) at a neutral pH. Lowering the pH to 5.75 resulted in a loss in efficacy from 8- to 32-fold for all three macrolides studied. The MIC90 of clarithromycin (0.5 μg/ml) remained lower than those of azithromycin (2 μg/ml) and erythromycin (4 μg/ml). No synergism or antagonism was observed with combinations of clarithromycin and temafloxacin at either the neutral or lower pH values.

The association between peptic ulcer disease and the presence of *Helicobacter pylori* has been well established (1, 2). As a result, much emphasis has been placed on the treatment of gastritis and ulcer disease with regimens which include antimicrobial agents. *Helicobacter pylori* is susceptible in vitro to a variety of antibiotics, including penicillins, cephalosporins, tetracyclines, macrolides and quinolones, as well as to bismuth compounds (2–7). This has led to clinical trials utilizing antimicrobials alone or in combination with these other compounds, with varying degrees of success. The discordance between the in vitro activity and in vivo effectiveness of antimicrobial agents is not completely understood, but may relate in part to diminished activity of these drugs at a reduced pH (8).

[1] Department of Medicine, Division of Infectious Diseases, New England Deaconess Hospital, 185 Pilgrim Road, Boston, Massachusetts, 02215, USA.
[2] Massachusetts General Hospital, Boston, Massachusetts, 02114, USA.
[3] Harvard Medical School, Boston, Massachusetts, 02115, USA.

In the present study, 27 clinical isolates of *Helicobacter pylori* were evaluated for in vitro susceptibility to erythromycin, clarithromycin, azithromycin and temafloxacin under various pH conditions. The effect in vitro of combinations of clarithromycin with temafloxacin also was investigated at neutral and lower pH ranges.

Materials and Methods. The 27 *Helicobacter pylori* isolates were obtained from gastric mucosal biopsy specimens from the Massachusetts General and New England Deaconess Hospitals, Boston, MA. All isolates were removed from storage at -70 °C and subcultured on brucella agar containing 5 % horse blood (BBL Microbiology Systems, USA). Subcultures were incubated at 35 °C under microaerophilic conditions (BBL Campy Pak Plus) for 96 hours and for two passages to ensure reliable growth.

Antimicrobial agents utilized included erythromycin (Eli Lilly, USA), clarithromycin (Abbott Laboratories, USA) azithromycin (Pfizer Laboratories, USA) and temafloxacin (Abbott Laboratories). MICs were determined by an agar dilution technique in which serial two-fold dilutions of antimicrobials were added to brain heart infusion agar (Difco Laboratories, USA) supplemented with 7 % lysed horse blood (3). The pH was measured using a surface pH probe and adjusted to a pH range of 5.7–6.0 and 7.8–8.0 by the addition of hydrochloric acid or sodium hydroxide. Inocula were prepared by suspending each isolate in brain heart infusion broth to a density of a MacFarland no. 1 standard (5×10^8 cfu/ml). A multiprong inoculating device was used to place approximately 5×10^5 cfu per spot onto the prepared antimicrobial containing media. Following four days of incubation at 35 °C under microaerophilic conditions, plates were examined for growth. The MIC was defined as the lowest concentration of antimicrobial that inhibited colony growth. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as control strains.

Serial two-fold dilutions of clarithromycin and temafloxacin were added in combination to brain heart infusion agar supplement with 7 % lysed horse blood for evaluation of synergism or antagonism. Interactions were considered synergistic if the FIC index was ≤ 0.5 and antagonistic if the FIC index exceeded 4.

Results and Discussion. Table 1 summarizes the in vitro susceptibility of the 27 isolates of *Helicobacter pylori* at various pH ranges. At a neutral pH of 7.4, clarithromycin (MIC90 0.03 µg/ml) was significantly more active than either erythromycin (MIC90 0.125 µg/ml) or azithromycin (MIC90 0.25 µg/ml). The MIC90 increased 8- to 32-fold for the three macrolides as the pH was reduced to 5.75. However, the MIC90 of clarithromycin (0.5 µg/ml) remained well within clinically achievable concentrations and lower than those of azithromycin (2 µg/ml) and erythromycin (4 µg/ml). At a pH of 7.91, the exquisite in vitro activity of clarithromycin was maintained (MIC90 ≤ 0.015 µg/ml) and remained significantly greater than that of erythromycin (MIC90 0.125 µg/ml).

Temafloxacin alone had moderate activity against *Helicobacter pylori* (MIC90 2.0 µg/ml) at a neutral pH but also lost activity at the lower pH. When this agent was used in combination with clarithromycin at the neutral and lower pH ranges, neither synergism nor antagonism could be demonstrated, all FIC index values falling between 0.625 and 3 (data not shown). Although temafloxacin has been withdrawn from clinical use since this study was performed, these data which fail to show a significant interaction between macrolides and a quinolone may be useful in the design of future combination regimens.

There has been a discrepancy between the in vitro activity and the clinical efficacy of several antimicrobial agents in the clearance of *Helicobacter pylori* from the gastric mucosa in the presence of peptic ulcer disease (9, 10). The local environ-

Table 1: MICs (µg/ml) for 27 clinical isolates of *Helicobacter pylori* at varying pH values.

Antimicrobial agent	pH 5.75			pH 7.4			pH 7.91		
	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90
Erythromycin	0.25 –>4	2	4	0.06 –>4	0.125	0.125	0.06 –>4	0.125	0.125
Clarithromycin	0.06 –>4	0.25	0.5	<0.015 –>4	0.015	0.03	<0.015 –2	<0.015	<0.015
Azithromycin	0.125 –>4	2	2	0.25 –>4	0.25	0.25		ND	
Temafloxacin	0.25 –4	1	4	0.25 –2	1	2		ND	

ND = no data.

ment of the gastric mucosa may play a crucial role in this observation, particularly with respect to pH variability (2). McNulty et al. (11) have shown that erythromycin and ciprofloxacin were ineffective despite high gastric mucosal concentrations. Also, failure to eradicate *Helicobacter pylori* during treatment with azithromycin alone has been associated with development of resistance to this agent (12). Whether reduced activity of this drug under local pH conditions might have contributed to the frequency with which resistant clones can be selected is an interesting point of speculation. Emergence of resistance during treatment is yet another reason why combination regimens appear particularly attractive for treatment of this condition.

Grayson et al. (8) observed a 2- to 8-fold loss in efficacy of six antimicrobial agents, including ampicillin, clindamycin, erythromycin and ciprofloxacin when the pH was lowered to a range of 5.7–6.0. The MIC90 often increased beyond clinically feasible serum concentrations. However, a pH below this range failed to support adequate growth of *Helicobacter pylori* for reliable susceptibility testing. The results of the present investigation suggest that the relative preservation of in vitro activity at a reduced pH may favorably influence the clinical efficacy of clarithromycin in the treatment of peptic ulcer disease. In fact, Logan et al. (13) have shown that eradication of this organism was maintained in 20 of 25 patients four weeks following the conclusion of combination therapy with clarithromycin and omeprazole, a proton pump inhibitor which also directly suppresses *Helicobacter pylori* in vitro. Larger clinical trials with clarithromycin in combination with acid reducing compounds appear to be warranted.

References

1. **Rollason TP, Stone J, Rhodes JM:** Spiral organisms in endoscopic biopsies of the human stomach. Journal of Clinical Pathology 1984, 37: 23–26.
2. **Bayerdorffer E, Ottenjann R:** The role of antibiotics in *Campylobacter pylori* associated peptic ulcer disease. Scandinavian Journal of Gastroenterology, 1988, 142, Supplement: 93–100.
3. **Shungu DL, Nalin DR, Gilman RH, Gadebusch HH, Cerami AT, Gill C, Weissberger B:** Comparative susceptibilities of *Campylobacter pylori* to norfloxacin and other agents. Antimicrobial Agents and Chemotherapy 1987, 31: 949–950.
4. **Czinn S, Carr H, Aronoff S:** Susceptibility of *Campylobacter pyloridis* to three macrolide antibiotics (erythromycin, roxithromycin [RU 28965], and CP 62,993) and rifampin. Antimicrobial Agents and Chemotherapy 1986, 30: 328–329.
5. **Hardy DJ, Hanson CW, Hensey DM, Beyer JM, Fernandes PB:** Susceptibility of *Campylobacter pylori* to macrolides and fluoroquinolones. Journal of Antimicrobial Chemotherapy 1988, 22: 631–636.
6. **Nagate T, Numata K, Hanada K, Kondo I:** The susceptibility of *Campylobacter pylori* to antiulcer agents and antibiotics. Journal of Clinical Gastroenterology 1990, 12, Supplement 1: 135–138.
7. **Czinn SJ, Carr H, Hupertz V:** Susceptibility of *Campylobacter pylori* to newer cephalosporin antibiotics. Chemotherapy 1989, 35: 164–167.
8. **Grayson ML, Eliopoulos GM, Ferraro MJ, Moellering RC:** Effect of varying pH on the susceptibility of *Campylobacter pylori* to antimicrobial agents. European Journal of Clinical Microbiology & Infectious Diseases 1989, 8: 888–889.
9. **McNulty CAM, Gearty JC, Crump B, Davis M, Donovan IA, Melikian V, Lister DM, Wise R:** *Campylobacter pyloridis* and associated gastritis: investigator blind, placebo controlled trial of bismuth salicylate and erythromycin ethylsuccinate. British Medical Journal 1986, 293: 645–649.
10. **Stone JW, Wise R, Donovan IA, Gearty J:** Failure of ciprofloxacin to irradicate *Campylobacter pylori* from the stomach. Journal of Antimicrobial Chemotherapy 1988, 22: 92–93.
11. **McNulty CA, Dent JC, Ford GA, Wilkinson SP:** Inhibitory antimicrobial concentrations against *Campylobacter pylori* in gastric mucosa. Journal of Antimicrobial Chemotherapy 1988, 22: 729–738.
12. **Glupczynski Y, Burette A:** Failure of azithromycin to eradicate *Campylobacter pylori* from the stomach because of acquired resistance during treatment. American Journal of Gastroenterology 1990, 85: 98–99.
13. **Logan RPH, Gummett PA, Hegarty BT, Walker MM, Baron JH, Misiewicz JJ:** Clarithromycin and omeperazole for *Helicobacter pylori*. Lancet 1992, 340: 239.