Effect of an Acidic Environment on the Susceptibility of *Helicobacter pylori* to Trospectomycin and Other Antimicrobial Agents

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The susceptibility of 30 clinical isolates of *Helicobacter pylori* to trospectomycin, ampicillin, metronidazole, clarithromycin, azithromycin and clindamycin under varying pH conditions was evaluated. An acidic environment was shown to affect unfavourably the activity of all the antimicrobial agents tested. This pH effect was most marked for the two macrolides and for clindamycin.

Helicobacter pylori is currently recognised as an important aetiologic factor for chronic active gastritis, nonulcer dyspepsia and peptic ulcer disease (1). Treatment of peptic ulcer disease and nonulcer dyspepsia with antimicrobial agents directed against *Helicobacter pylori* has merited attention ever since it was shown that elimination of *Helicobacter pylori* clearly reduced the relapse rate of peptic ulceration (2, 3). Therefore, attention has been focused on the susceptibility of *Helicobacter pylori* isolates to antimicrobial agents. *Helicobacter pylori* is susceptible to most antimicrobial agents, with the exception of vancomycin, sulfonamides and trimethoprim; yet many of these same agents, such as erythromycin, clindamycin and ciprofloxacin, have proven ineffective when used clinically. There are several hypotheses to account for the failure to eradicate *Helicobacter pylori* from the gastric mucosa and mucus layer, such as loss of activity of the antimicrobial agents in an acidic environment (4, 5), insufficient concentration of the antimicrobial agent in the mucus layer or gastric pits, inadequate formulation of the antimicrobial agent administered, inadequate dosing and duration of therapy and acquired resistance. This study was undertaken to measure the susceptibility of *Helicobacter pylori* to several antimicrobial agents at different pH values.

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Materials and Methods. The 30 *Helicobacter pylori* isolates were obtained from gastric mucosal biopsy specimens from the Free University Hospital, Amsterdam. The identity of each strain was confirmed by Gram stain, oxidase, catalase and urease tests. All isolates were removed from storage at -70°C and subcultured on Belohorizonte agar (6) containing 5 % lysed horse blood. Subcultures were incubated at 37°C under microaerophilic conditions (5 % O_2, 10 % CO_2, 85 % N_2) for 48 h and passaged twice to ensure reliable growth. Inocula were prepared in 0.9 % NaCl and adjusted to a McFarland standard no. 1 (approximately 5×10^8 cfu/ml). The sensitivity plates were inoculated with 40 µl so that the final inoculum contained approximately 2×10^7 cfu/ml. MICs were determined with the E Test (AB Biodisk, Sweden) (7). Columbia agar (Oxoid, UK) plates enriched with 10 % lysed horse blood with different pH values ranging from 5.5 to 7.0 were prepared by adding HCl after sterilisation. The pH was measured with a surface pH meter after solidification of the agar and prior to inoculation of the agar plates. The inoculum was evenly spread with a swab over the surface of the plate. Inoculated plates were allowed to dry before E Test strips were applied to the medium. After application of the E Test strips, plates were incubated at 37°C under a microaerophilic atmosphere for 72 h. The antimicrobial agents tested were trospectomycin, ampicillin, metronidazole, clindamycin, azithromycin and clarithromycin. *Helicobacter pylori* NCTC 11637, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as controls.

Results and Discussion. The susceptibility of the 30 isolates at various pH values is shown in Table 1. An acidic environment affected unfavourably the activity of all the antimicrobial agents tested, particularly that of the macrolides and clindamycin. The MIC90 increased 16- to 47-fold for the two macrolides as the pH was reduced to 5.5. These findings correspond with other research (5). For ampicillin, metronidazole and trospectomycin the MIC90 increased fourfold, threefold and fivefold, respectively. The MIC at pH 5.5 and pH 7.0 for ampicillin in our study differs from the MIC in the study of Grayson et al. (4). Such differences are possible when different bacterial populations are tested. Moreover, we tested a range of

Table 1: MICs (mg/l) for 30 clinical isolates of *Helicobacter pylori* at varying pH values.

Antimicrobial agent	pH 7.0			pH 6.5			pH 6.0			pH 5.5		
	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range	MIC50	MIC90	Range
Ampicillin	< 0.016	< 0.016	< 0.016 – 0.047	< 0.016	0.016	< 0.016 – 0.047	0.016	0.048	< 0.016 – 0.047	0.023	0.064	< 0.016 – 0.064
Metronidazole	0.125	12	0.023 – > 32	0.25	32	0.047 – > 32	0.75	32	0.064 – > 32	0.5	32	0.064 – > 32
Azithromycin	< 0.016	0.094	< 0.016 – > 256	0.125	0.25	< 0.016 – > 256	0.375	0.75	< 0.016 – > 256	0.5	1.5	0.023 – > 256
Clarithromycin	< 0.016	< 0.016	< 0.016 – > 256	< 0.016	0.032	< 0.016 – > 256	0.064	0.25	< 0.016 – > 256	0.25	0.75	0.047 – > 256
Clindamycin	0.375	0.75	< 0.016 – > 256	0.75	1.5	0.094 – > 256	1.5	4	0.38 – > 256	4	16	0.5 – > 256
Trospectomycin	0.187	0.375	< 0.016 – 0.5	0.375	0.75	0.032 – 1	0.5	1.5	0.094 – 2	0.75	2	0.125 – 3

pH values different from that used by Grayson et al. (4) and also used another method for susceptibility testing (E Test). As for the effect of lowering the pH value from 7.0 to 5.5 on the MIC for ampicillin, we obtained results comparable with those of Grayson et al. (4). In the case of metronidazole we found many resistant strains in the bacterial population tested, which was not anticipated. Our results on the effect of lowering the pH on the MIC for metronidazole were also comparable with those of Grayson et al. (4).

The two most active antimicrobial agents against *Helicobacter pylori* are ampicillin and clarithromycin, with the MIC90 being lowest at a pH of 5.5. Talley et al. (8) demonstrated a stable pH gradient in vivo across the stomach wall in *Helicobacter pylori* gastritis, the pH being approximately 5.5 at the surface of the mucosa. Most *Helicobacter pylori* strains colonising the stomach are found in the mucus layer and gastric pits and on the gastric mucosa. In this environment the pH is 5.5. Despite good in vitro susceptibility of *Helicobacter pylori* to many antimicrobial agents, it is difficult to eradicate *Helicobacter pylori* from the gastric mucosa with common therapeutic strategies. The MIC90 of all the antimicrobial agents tested increases in an acidic environment. There is little data available on the concentrations of antimicrobial agents in the mucus layer itself. Despite adequate antimicrobial concentrations in the gastric mucosa, eradication of *Helicobacter pylori* has not been successful (9, 10). The most important factor in eradication and prevention of reinfection is probably the establishment of adequate concentrations in the mucus layer. The concentrations achieved with azithromycin in the mucus layer (11) are known to be lower than the MIC90 values we determined at a pH of 5.5. Therefore, it is not surprising that azithromycin fails to eradicate *Helicobacter pylori* from the human stomach (12). Such low concentrations in situ subsequently contribute to the rapid emergence of resistance.

Trospectomycin was evaluated in this study because of its potential role as a therapeutic agent in infections caused by *Helicobacter pylori* (13). Trospectomycin is a spectinomycin analogue which acts by binding to ribosomal 30 S-subunits, thus inhibiting protein synthesis. Trospectomycin is poorly absorbed after oral administration and consequently reaches high concentrations in the gastrointestinal tract. Following infusion of a 1 g dose the average peak concentration in serum was 81 mg/l and the half-life in serum was 2.2 h. Trospectomycin readily penetrates most tissues

and is eliminated very slowly; i.e. the half-life in tissue is approximately three days (14). On the basis of pharmacokinetic studies in humans, the breakpoint for trospectomycin is considered to be 16 mg/l (Novak et al., 27th ICAAC, 1987, Abstract no. 271). In this study the MIC90 at a pH of 5.5 was 2 mg/l. Simultaneous administration of bismuth subcitrate enhances the activity of trospectomycin against *Helicobacter pylori* in vitro (15). Studies have yet to be undertaken to measure the concentrations of trospectomycin that can be achieved in gastric mucosa, mucus and gastric juice after parenteral and oral administration. Should there be evidence of adequate concentrations in gastric mucus, clinical trials with trospectomycin in *Helicobacter pylori* gastritis are indicated.

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Rapid Detection of *Campylobacter jejuni* and *Campylobacter coli* Isolated from Clinical Specimens Using the Polymerase Chain Reaction

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Seventeen *Campylobacter* strains isolated from 16 children hospitalised with acute diarrhea were analysed by in vitro enzymatic amplification using two sets of oligonucleotide primers specific for *Campylobacter jejuni* and *Campylobacter coli*, respectively. Thirteen strains (76 %) were identified as *Campylobacter jejuni* and four strains (24 %) as *Campylobacter coli*. Subsequent bacteriological identification confirmed the identity of the same 13 *Campylobacter jejuni* strains and the 4 *Campylobacter coli* strains. Thus, these PCR methods enabled rapid and specific detection of all the *Campylobacter jejuni*

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