

Antimicrobial Susceptibilities of *Helicobacter pylori* Isolates under Microaerophilic Atmospheres Established by Two Different Methods

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The MICs of clarithromycin, amoxicillin, and metronidazole for 150 *Helicobacter pylori* isolates were determined using the AnaeroPack system and were compared with those determined using a microaerophilic incubator. The MICs of clarithromycin, amoxicillin, and metronidazole determined under both microaerophilic atmospheres were mostly within one twofold dilution for 146 (97.3%), 150 (100%), and 149 (99.3%) of the isolates, respectively.

Helicobacter pylori is the major causative pathogen of active chronic gastritis and peptic ulcers (6, 13), and infection with *H. pylori* is associated with an increased risk of developing gastric cancer (10). The National Institutes of Health (Bethesda, Md.) have subsequently recommended the routine treatment of patients with *H. pylori* infection with antibiotics to eradicate the causative pathogen (9). Clarithromycin and metronidazole are representative antibiotics which are used to eradicate *H. pylori* (2, 4). However, for some patients antibacterial therapy fails to eradicate the pathogen because of the frequent development of resistance to clarithromycin and metronidazole (11), and this may lead to relapse or recurrent infection. Testing for the susceptibility of *H. pylori* isolates to these antibiotics is the first step to successful therapy for patients with *H. pylori* infections. MICs of test antibiotics for *H. pylori* are determined by the agar dilution method according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (7). Since *H. pylori* is fastidious and slow-growing, for MIC determination it takes 3 to 4 days at 35°C for visible colonies to form in rich culture medium under a microaerophilic atmosphere. However, very few clinical microbiology laboratories are equipped with a microaerophilic incubator. For the present study, a small jar enclosed with a recently developed microaerophilic gas-generating system (AnaeroPack; Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan) was tested for MIC determination of antibiotics for *H. pylori*. The AnaeroPack can generate CO₂ by simply the cutting of one end of the gas pack. The manufacturer's instructions show that after 24 h of incubation the AnaeroPack can produce a suitable atmosphere for the growth of *H. pylori* (O₂, 4.7%; CO₂, 9.2%) in a closed jar. For the present study, the MICs of clarithromycin, amoxicillin, and metronidazole for 150 *H. pylori* isolates were determined using the AnaeroPack and were compared with those determined with a conventional microaerophilic

incubator (Tabai Espec Co., Osaka, Japan). One hundred fifty strains of *H. pylori* were isolated from gastric biopsy specimens from patients with gastritis or peptic ulcers in 1999. After the specimens were cultured using selective agar medium as described previously (12), the isolates were stored at –80°C in brucella broth (Difco Laboratories, Detroit, Mich.) containing 10% dimethyl sulfoxide and 10% horse serum until use. The susceptibility of *H. pylori* isolates to the three antibiotics was determined under the microaerophilic atmospheres established by the two different methods. The results were expressed as the MICs at which 50% of the isolates were inhibited (MIC_{50s}), the MIC_{90s}, and the MIC ranges of three antibiotics for 150 isolates.

The MIC_{50s}, MIC_{90s}, and MIC ranges of clarithromycin for the 150 *H. pylori* isolates were equivalent between the two microaerophilic atmospheres (Table 1). Similar results were obtained for the MIC_{50s}, MIC_{90s}, and MIC ranges of amoxicillin and metronidazole in both culture atmospheres. Table 2 shows the correlation between the individual MICs of clarithromycin for the *H. pylori* isolates obtained under microaerophilic atmospheres generated by the two different methods. The MICs of clarithromycin determined by both methods were the same for 99 (66.0%) of the 150 isolates, but those for 35 (23.3%) and 3 (2.0%) isolates were one and two twofold dilutions lower, respectively, when isolates were grown in the AnaeroPack than when they were grown in the incubator. In contrast, the MICs of clarithromycin for 12 (8.0%) and 1 (0.7%) isolate were one and two twofold dilutions higher when isolates were grown in the AnaeroPack than when isolates were grown in the incubator. However, the MICs of clarithromycin for 47 (31.3%) of the 150 isolates only differed by one twofold dilution between the two systems. In general, there was a close correlation between the MICs of clarithromycin for *H. pylori* isolates determined under microaerophilic atmospheres established by the two different methods. The same MICs of amoxicillin were obtained in both groups for 127 (84.7%) of the 150 isolates, but those for 19 (12.7%) and 4 (2.7%) isolates were within one twofold dilution higher and lower, respectively, when isolates were grown in the AnaeroPack than when

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TABLE 1. Susceptibility of 150 *H. pylori* isolates to clarithromycin, amoxicillin, and metronidazole determined under microaerophilic atmospheres established by two different methods

Microaerophilic atmosphere method	Results of MIC determinations (µg/ml)								
	Clarithromycin			Amoxicillin			Metronidazole		
	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀	MIC range
Incubator	0.06	0.12	≤0.015–64	≤0.015	0.06	≤0.015–0.25	2	4	0.5–64
AnaeroPack	0.06	0.12	≤0.015–32	≤0.015	0.06	≤0.015–0.25	2	4	0.5–64

they were grown in the incubator. No isolates showed a difference in amoxicillin MICs of more than one twofold dilution in either group. For metronidazole, the same MICs were obtained for 111 (74.0%) of the 150 isolates by the two methods, but the MICs for 33 (22.0%) and 1 (0.7%) isolate were one and two twofold dilutions higher, respectively, when the isolates were grown in the AnaeroPack. In contrast, the MICs for 5 (3.3%) isolates grown in the AnaeroPack were lower than those for isolates grown in the incubator.

As mentioned above, *H. pylori* is a fastidious organism which only grows under microaerophilic atmospheres. According to the *Clinical Microbiology Procedures Handbook* (3), an atmosphere of 10% CO₂, 5% O₂, and 85% N₂ is necessary to culture *H. pylori*, like that for *Campylobacter* spp. Various types of microaerophilic systems, including microaerophilic incubators and gas-generating systems, have been used to generate these conditions. The former require high-cost laboratory equipment, with gas tanks which also require high maintenance, but can produce and control an appropriate atmosphere for bacterial growth. The latter include several gas-generating systems which are now available, but some of these systems cannot produce a sufficient microaerophilic atmosphere for the optimal growth of some microaerophilic bacteria because of an insufficient supply of each gas at the required pressure. However, macrolide antibiotics such as clarithromycin are inactivated by long incubation in a high CO₂ atmosphere, and as a result, the MICs of macrolides for *H. pylori* isolates often become higher (1, 5). In the guidelines established by the NCCLS (8), the MIC interpretive standard of clarithromycin for *H. pylori* is defined as follows: sensitive, ≤0.25 µg/ml; intermediate, 0.5 µg/ml; and resistant, ≥ 1 µg/ml. When a MIC is estimated to be two twofold dilutions greater than the real

value on the basis of the interpretive standard, the susceptibility of *H. pylori* to clarithromycin changes from sensitive to resistant. Therefore, the control of the precise conditions is important for the susceptibility testing of *H. pylori*.

The MICs of clarithromycin for 150 *H. pylori* isolates under microaerophilic atmospheres established by using the microaerophilic incubator corresponded well to those under atmospheres established by using the AnaeroPack.

In conclusion, the AnaeroPack was a convenient and easy way to produce a microaerophilic atmosphere that met the guidelines established by the NCCLS and resulted in appropriate conditions for the growth of *H. pylori*. The AnaeroPack was successfully used for the determination of the MICs of clarithromycin, amoxicillin, and metronidazole for *H. pylori* isolates.

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TABLE 2. Correlation between MICs of clarithromycin, amoxicillin, and metronidazole for 150 *H. pylori* isolates determined under microaerophilic atmospheres established by two different methods

Antibiotic	No. of isolates for which MICs determined by both methods differed by the indicated no. of twofold dilutions ^a				
	-2	-1	0	+1	+2
Clarithromycin	3	35	99	12	1
Amoxicillin		4	127	19	
Metronidazole		5	111	33	1

^a For -1 and -2, MICs determined in the AnaeroPack were one and two twofold dilutions lower than those determined in the incubator; for 0, the two methods showed the same MICs; for +1, and +2, MICs determined in the AnaeroPack were one and two twofold dilutions higher than those determined in the incubator.