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Susceptibilities to Different Antibiotics of *Helicobacter pylori* Strains Isolated from Patients at the Pediatric Medical Center of Tehran, Iran

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Antibiotic susceptibility testing of 70 pediatric *Helicobacter pylori* isolates was performed by using screening agar and disk diffusion methods. Resistance to metronidazole and tinidazole was 72 to 79% and 71 to 81% by modified disk diffusion and 77% and 78% by screening agar, respectively. Susceptibilities to amoxicillin, ampicillin, clarithromycin, tetracycline, erythromycin, and ciprofloxacin were 58, 69, 75, 68, 68, and 65%, respectively.

Resistance to antibiotics among *Helicobacter pylori* isolates is prevalent worldwide. The rate of metronidazole resistance is on the rise, and it varies according to the population studied. Resistance to amoxicillin and tetracycline has also been observed, and there are reports of high rates of resistance to clarithromycin (1–2, 4, 8–11, 16). Most antibiotic resistance studies have been done on *H. pylor*i isolates from adults; fewer data are available for children, particularly those in developing countries. The purpose of this study was to assess the susceptibility of pediatric *H. pylori* isolates to commonly used antibiotics, by using the modified disk diffusion method (MDDM) and the screening agar method (SAM).

During 1997 to 2000, biopsies from 250 children (median age, 9.4 years; range, 3 to 15 years) experiencing recurrent abdominal pain, nausea, and vomiting were used for bacterial culturing. Biopsies were placed within a modified Campy-Thio medium, composed of thioglycolate base medium (Difco), 10% sheep blood (SB), 8 mg of polymyxin B per liter, 2 mg of amphotericin B per liter, and 6 mg of vancomycin (Fluka) per liter and incubated at 37°C under a microaerophilic atmosphere. After 2 to 3 days, 20 μl was used to streak Campy blood agar plates containing brucella agar base (Difco), 10% SB, and antibiotics. Isolates were conserved in skim milk–15% glycerol–10% fetal calf serum at −80°C, after identification by gram staining and biochemical analysis

For antimicrobial susceptibility assays, a suspension was adjusted to a turbidity approximating that of a McFarland no. 3 standard, spread on SB–Mueller-Hinton agar (SBMHA) plates containing 8 mg of metronidazole or tinidazole per liter, and incubated for 3 days (5, 13). Isolates were considered susceptible or resistant if similar results were obtained from three experiments. If similar results were observed in two experiments out of three, the isolates were considered susceptible or resistant but were designated as mixed populations. For the MDDM, disks containing 4, 8, 16, 32, and 64 μg of metronidazole and tinidazole (prepared from pure powders; Sigma), clarithromycin (15 μg; Becton Dickinson), amoxicillin (25 μg), ampicillin (10 μg), erythromycin (15 μg), tetracycline (30 μg), and ciprofloxacin (5 μg) from Padtan Tab and BioMerieux were used. Bacterial suspensions were spread on SBMHA plates, disks were added, and the diameter of the zone of inhibition was measured after 3 days. Criteria for nitroimidazole susceptibility were inhibitory zones of ≥15 to 16 mm or ≥26 mm (3, 9, 12). Quality control was ensured by using organisms from the American Type Culture Collection, including *Pseudomonas aeruginosa* ATCC 85327, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *H. pylori* ATCC 26695.

Of the culture-positive subjects, 98% showed gastritis, gastric ulcer, duodenitis, duodenal ulcer, and gastroduodenal ulcer; no gastric carcinoma was observed. The susceptibility pattern obtained for nitroimidazole is described in Table 1. No difference was found in the results for disks containing increasing concentrations of antibiotic. By the SAM, 23 and 22% of isolates were susceptible to metronidazole and tinidazole. The rates of mixed populations were 7% (SAM) and 12% (MDDM) for metronidazole and 4% (SAM) and 7% (MDDM) for tinidazole. The highest agreement between the results of the SAM and those of the MDDM for nitroimidazole was observed in the case of inhibitory zones of ≥26 mm. The susceptibility patterns for other antibiotics are described in Table 2. By SAM, the susceptibility of isolates to 8 mg of amoxicillin per liter according to European *H. pylori* breakpoints (9, 16) was 51%. The rates of mixed populations for amoxicillin, ampicillin, tetracycline, erythromycin, clarithromycin, and ciprofloxacin were 12, 10, 13, 7, 8, and 18%. Cross-reactivity between metronidazole and tinidazole was ~100%. All strains that were resistant to ampicillin were also resistant to amoxicillin, and strains that were resistant to clarithromycin were also resistant to erythromycin. Of interest, of 16 isolates susceptible to metronidazole (23% by the SAM), 14 were also susceptible to other antibiotics tested. The multiresistance pattern of metronidazole-resistant isolates is described in Table 3.

In this study, we report the susceptibility to commonly used antibiotics of pediatric *H. pylori* isolates from Iran. A high rate of resistance to nitroimidazole was found throughout the study; these results are similar to frequencies reported for other developing countries (14). In Iran, metronidazole is used

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TABLE 1. Susceptibility rates of 70 *H. pylori* isolates to metronidazole and tinidazole by the MDDM (using disks of 4, 8, and 16 μg) and the SAM

Category[a]	Susceptibility rate (%)					
	Metronidazole			Tinidazole		
	4 μg	8 μg	16 μg	4 μg	8 μg	16 μg
S by MDDM[b]	28	28	28	29	31	30
S by MDDM[c]	21	21	21	19	19	19
R by MDDM[b] R by SAM	76	76	75	79	78	77
R by MDDM[c] R by SAM	79	79	79	80	80	80

[a] S, susceptible; R, resistant. Susceptibility by the SAM was 23 and, 22% for metronidazole and tanidazole, respectively.
[b] Diameter of inhibitory zone, ≥15 mm.
[c] Diameter of inhibitory zone, ≥26 mm.

to treat diarrheal disease, and *H. pylori* strains obtained from adults are also highly resistant to metronidazole (6). Among antibiotics examined, clarithromycin was the most effective, but 21% resistance was unexpected, since clarithromycin is not currently used in Iran. This resistance could be related to cross-reactivity between erythromycin and clarithromycin, which implies that if a strain is resistant to one macrolide it becomes resistant to all others (15). Resistance to amoxicillin was observed in 27% of the isolates, reflecting the importance of its use in our country, especially in children. A high level of resistance to amoxicillin was also observed in *Shigella* spp., *Salmonella* spp., and *Campylobacter jejuni* isolates in Iran (7). Resistance to amoxicillin was also observed in Mexican children (14). In isolates from this work, moderate resistance to tetracycline and ciprofloxacin was observed. Ciprofloxacin has been proposed for treatment of the infections noted above (7), but tetracycline is not frequently used in our pediatric medical centers; this resistance could be related to genetic acquisition of resistance due to a multidrug resistance mechanism (11). Few studies have reported the rate of multiresistance in *H. pylori* isolates (4, 11, 14). In our study, the lowest rate of multiresistance was observed for nitroimidazole plus ciprofloxacin plus tetracycline (Table 3), signifying that treatment regiments could also contain ciprofloxacin as an antibiotic unrelated to the others.

In conclusion, we documented a high rate of resistance to nitroimidazole, a considerable resistance to amoxicillin, ciprofloxacin, tetracycline, erythromycin, and ampicillin, and the least resistance to clarithromycin. We confirm the need for culture and susceptibility testing to define resistance and/or multiresistance patterns of pediatric *H. pylori* isolates before selection of a treatment regimen.

TABLE 2. Susceptibility rates of 70 isolates to selected antibiotics by the MDDM

Antibiotic	% of isolates		
	Susceptible	Intermediate	Resistant
Amoxicillin	58	15	27
Ampicillin	69	10	21
Tetracycline	68	12	20
Erythromycin	68	12	20
Clarithromycin	75	04	21
Ciprofloxacin	65	15	20

TABLE 3. Multiresistance pattern of 54 metronidazole-resistant strains

Antibiotic(s)[a]	No. of multiresistant strains	Resistance (%)
AMX[b]	18	26
AMX + CLA	10	14.5
AMX + TET	8	11.5
AMX + CIP	3	04
CLA[c]	14	20
CLA + TET	10	14.5
CLA + CIP	3	4
CLA + CIP + TET	2	3
TET	14	20
CIP	14	20
TET + CIP	2	3
AMX + CLA + TET	5	7
AMX + CLA + CIP	3	4

[a] AMX, amoxicillin; CLA, clarithromycin; TET, tetracycline; CIP, ciprofloxacin.
[b] Also resistant to ampicillin.
[c] Also resistant to erythromycin.

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REFERENCES

1. **Alarcon, T., D. Domingo, and Lopez-Brea.** 1999. Antibiotic resistance problems with *Helicobacter pylori*. Int. J. Antimicrob. Agents **12:**19–26.
2. **Bontems, P., J. M. Devaster, L. Corvaglia, L. A. Dezofi, C. Van Den Borre, S. Goutier, J. P. Butzler, and S. Cadranel.** 2001. Twelve years observation of primary and secondary antibiotic-resistant *Helicobacter pylori* strains in children. Pediatr. Infect. Dis. J. **20:**1033–1038.
3. **Boyanova, L.** 1999. Comparative evaluation of two methods for testing metronidazole susceptibility of *Helicobacter pylori* in routine practice. Diagn. Microbiol. Infect. Dis. **35:**33–36.
4. **Boyanova, L., I. Stancheva, Z. Spassova, N. Katzarov, I. Mitove, and R. Koumanova.** 2000. Primary and combined resistance to four antimicrobial agents in *Helicobacter pylori* in Sofia, Bulgaria. J. Med. Microbiol. **49:**415–418.
5. **Chaves, S., M. Gadaho, R. Tenereiro, and J. Cabrita.** 1999. Assessment of metronidazole susceptibility in *Helicobacter pylori*: statistical validation and error rate analysis of breakpoints determined by the disk diffusion test. J. Clin. Microbiol. **37:**1628–1631.
6. **Fakheri, H., R. Malekzadeh, S. Merat, M. Khatibian, A. Fazel, B. Z. Alizadeh, and S. Massarrat.** 2001. Clarithromycin vs. furazolidone in quadruple therapy regimens for the treatment of *Helicobacter pylori* in a population with a high metronidazole resistance rate. Aliment. Pharmacol. Ther. **15:**411–416.
7. **Falsafi, T., E. Abdi-Ali, F. Mobasheri, and M. Najafi.** 1999. Drug resistance to *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. in pediatric infections. Iranian J. Pediatr. **11:**20–27.
8. **Graham, D. Y., and W. A. Qureshi.** 2000. Antibiotic-resistant *Helicobacter pylori* infections and its treatment. Curr. Pharm. **6:**1537–1544.
9. **Ioven, M. R., M. Romano, A. P. Pilloni, B. Giordano, F. Montella, S. Caliendo, M. A. Tufano.** 1999. Prevalence of antimicrobial resistance in eighty clinical isolates of *Helicobacter pylori*. Chemotherapy **45:**8–14.
10. **Kalach, N., M. Bergeret, P. H. Benhamou, C. Dupont, and J. Raymond.** 2001. High level of resistance to metronidazole and clarithromycin in *Helicobacter pylori* strains in children. J. Clin. Microbiol. **39:**394–397.
11. **Kwon, D. H., M. P. Dore, J. J. Kim, M. Kato, M. Lee, J. Y. Wu, and D. Y. Graham.** 2003. High-level beta-lactam resistance associated with acquired multidrug resistance in *Helicobacter pylori*. Antimicrob Agents Chemother. **47:**2169–2178.
12. **Midolo, P. D., J. Turnidge, J. R. Lambert, and J. M. Bell.** 1995. Validation of a modified Kirby-Bauer disk diffusion method for metronidazole susceptibility testing of *Helicobacter pylori*. Diagn. Microbiol. Infect. Dis. **21:**135–140.
13. **National Committee for Clinical Laboratory Standards.** 2000. Methods for

dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5, 5th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14. **Torre, J., M. Camorlinga-Ponce, G. Pérez-Pérez, A. Madrazo-De la Garza, M. Dehesa, G. Gonzales-Valencia, and O. Munozet.** 2001. Increasing multidrug resistance in *Helicobacter pylori* strains isolated from children and adults in Mexico. J. Clin. Microbiol. **39:**22677–22680.
15. **Versalovic, J., D. Shortridge, K. Kibler, M. V. Griffy, J. Beyer, R. K. Flamm, S. K. Tanaka, D. Y. Graham, and M. F. Go.** 1996. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrobial. Agents Chemother. **40:**1232–1234.
16. **Wu, H., X. D. Shi, H. T. Wang, and J. X. Liu.** 2000. Resistance of *Helicobacter pylori* to metronidazole, tetracycline and amoxicillin. J. Antimicrob. Chemother. **46:**121–123.