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Universal High-Level Primary Metronidazole Resistance in *Helicobacter pylori* Isolated from Children in Egypt

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Antimicrobial susceptibility testing was performed on 48 isolates of *Helicobacter pylori* recovered from Egyptian children undergoing routine endoscopies. The isolates were universally highly resistant to metronidazole, but resistance to other tested antimicrobial agents was rare (4% for clarithromycin, erythromycin, and azithromycin resistance versus 2% for ciprofloxacin and ampicillin resistance). Use of metronidazole for the treatment of *H. pylori* in Egypt should be avoided.

Helicobacter pylori is currently recognized as one of the most common chronic bacterial infections worldwide (6). While the majority of infections are asymptomatic (12, 13), the association of *H. pylori* colonization of the stomach with chronic gastritis, peptic ulcer disease, and gastric malignancies is now well documented in both adults and children (11, 18). Eradication of bacteria is effective in healing peptic ulcers, preventing ulcer relapses, and potentially decreasing the risk of progression to gastric carcinoma (7, 9, 16). Current practice dictates treatment of symptomatic individuals with a regimen containing two antimicrobial agents along with a proton pump inhibitor (18). For successful eradication of bacteria, it is imperative that the clinician be aware of the current antimicrobial susceptibility profiles of isolates within the region. Therefore, this study was initiated to determine antimicrobial susceptibility patterns among *H. pylori* isolates recovered from children in Egypt.

Children aged 2 to 17 years requiring endoscopy for evaluation of their gastrointestinal complaints were enrolled in this study. Prior to endoscopy, written informed consent was obtained from the parent of the study subject allowing their child to be enrolled. Children who had taken antimicrobials, antacids, H_2 blockers, proton pump inhibitors, or bismuth subsalicylate within the 4 weeks prior to endoscopy were excluded from the study. Similarly, children with a history of infection with *H. pylori*, a known bleeding disorder, or previous endoscopy were also excluded from the study. At the time of endoscopy, a gastric antral biopsy sample was obtained for culture and stored in normal saline on ice until delivered to the laboratory within 2 to 3 h of collection. The biopsy sample was ground using a sterile, disposable plastic pestle and inoculated onto Columbia agar (Campy-Pak Systems; Becton Dickinson, BBL, Cockeysville, Md.) plates enriched with 5% sheep blood, one of which was supplemented with antimicrobials to selectively inhibit growth of bacteria other than *H. pylori*. Cultures were incubated at 37°C under microaerophilic conditions. *H. pylori* isolates were harvested, suspended in tryptic soy broth with 15% glycerol, and stored frozen at −70°C pending further testing.

Antimicrobial susceptibilities to erythromycin, clarithromycin, azithromycin, ciprofloxacin, ampicillin, and metronidazole were tested by using antimicrobial impregnated strips (E-test; AB Biodisk, Solna, Sweden). *H. pylori* isolates were thawed, inoculated onto Columbia agar, and incubated as outlined above. After incubation, bacteria were harvested, suspended in sterile saline to McFarland standard 3 per the manufacturer's instructions, and then inoculated onto Columbia agar plates enriched with 5% sheep blood. Plates were allowed to dry for 15 min at room temperature prior to addition of E-test strip.

After 5 days of incubation, the MICs were determined as the point where the elliptical zone of complete inhibition of all bacterial growth, including hazes and isolated colonies, intersected the MIC scale on the strip. When growth occurred along the entire strip, the MIC was reported as greater than the highest value on the reading scale. Alternatively, when the inhibition ellipse was below the strip and did not intersect it, the MIC was reported as less than the lowest value on the reading scale. Antimicrobial resistance to metronidazole was defined as an MIC of $\geq$8 μg/ml, while resistance to erythromycin, clarithromycin, azithromycin, and ciprofloxacin was defined as an MIC of $\geq$1 μg/ml and resistance to ampicillin was defined as an MIC of $\geq$0.5 μg/ml. Results of the E-test were quality controlled for each run using *H. pylori* ATCC 43504.

Between June 2002 and February 2003, 104 children (58 males and 46 females; mean age, 7 years 2 months; range, 2 to17 years) were enrolled in the study, and *H. pylori* was isolated from 48 of the patients.

The antimicrobial susceptibility testing results are presented in Table 1. High-level resistance to metronidazole was present in all 48 *H. pylori* isolates. In contrast, 44 of the 48 isolates were susceptible to all other antimicrobial agents tested. Of the remaining four isolates, one was resistant to ciprofloxacin (MIC of >256 μg/ml), another was resistant to ampicillin (MIC of >256 μg/ml), and two were resistant to the macrolides (erythromycin, clarithromycin, and azithromycin) tested. With the exception of metronidazole, the MIC of each of the antimicrobials tested was within a narrow range, and most were

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TABLE 1. MICs and resistance rates for *H. pylori* isolates recovered from 48 study children

E-test MIC (μg/ml)	Resistance rate[a] (no. of isolates [%])					
	AM	EM	CH	AZ	CI	MZ
<0.016	2 (4)	4 (8)	30 (63)			
0.016–<0.094	39 (81)	31 (65)	16 (33)	34 (71)	22 (46)	
0.094–<0.5	6 (13)	11 (23)		12 (25)	24 (50)	
0.5–<1.0					1 (2)	
1.0–<4.0			2 (4)	1 (2)		
4.0–<48						
48–≥256	1 (2)	2 (4)		1 (2)	1 (2)	48 (100)
Total resistant (no. of isolates [%])	1 (2)	2 (4)	2 (4)	2 (4)	1 (2)	48 (100)

[a] Resistance rates of *H. pylori* isolates to ampicillin (AM), erythromycin (ER), clarithromycin (CH), azithromycin (AZ), ciprofloxacin (CI), and metronidazole (MZ). The resistance MICs of the antimicrobial agents were as follows: ≥0.5 μg/ml for AM; ≥1 μg/ml for EM, CH, AZ, and CI; and ≥8 μg/ml for MZ.

well below the cutoff point used to determine susceptibility (Table 1).

In this study group of Egyptian children, *H. pylori* was cultured from 46%. Since patients were enrolled from a population evaluated at a pediatric gastroenterology clinic, it is possible that the prevalence of infection with *H. pylori* is higher than in the general population in Egypt. However, the prevalence is within the range reported from much of the developing world as well as previous research in Egypt (3, 10, 14, 20). Additionally, we have demonstrated that the antimicrobial resistance patterns for *H. pylori* isolates tested in the present study are consistent with other studies from the region and the developing world in general (1, 5, 21).

The universal high-level primary resistance of *H. pylori* to metronidazole was noteworthy in this study. Our findings are consistent with previous reports, including studies in the Middle East where metronidazole resistance was between 60 and 80% (2, 4, 21). The design of the present study did not allow for determination of the etiology of the high level of metronidazole resistance. However, we hypothesize that the widespread use of metronidazole in Egypt for treatment of numerous infectious diseases likely led to the development of resistance to the drug. Studies have found the mechanism of metronidazole resistance to be due to inactivation of the gene that encodes an oxygen-insensitive NADPH nitroreductase (*rdxA*) (8), but the present study is unable to corroborate the findings, as we did not test for the presence of *rdxA* in the *H. pylori* isolates. Regardless of the reason, it is clear that metronidazole should not be included in treatment regimens for *H. pylori* in Egypt.

With the exception of metronidazole, *H. pylori* isolates were highly sensitive to all antimicrobials tested. Clarithromycin resistance rates over 20% have been reported in some areas of the world (15, 19), so it was surprising that only one *H. pylori* isolate was resistant to this agent. However, prior studies in the region have found similar low levels of resistance to clarithromycin (17). While the reason for the low level of resistance is not certain, it may be partly due to clarithromycin being available in Egypt for 18 months at the time the present study was initiated. Further surveillance will be needed to determine if resistance to clarithromycin increases within the region in the future.

In conclusion, this study emphasizes that resistance rates of *H. pylori* isolates differ considerably from one geographical region to another. In vitro data would suggest that treatment regimens incorporating antimicrobials other than metronidazole would be effective in eradicating *H. pylori* in Egypt. Nonetheless, in vivo results will need to be carefully monitored to ensure treatment is effective. Continued surveillance to allow for the early detection of development of antimicrobial resistance is critical and will need to be an ongoing endeavor.

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