Original article

Helicobacter pylori antibiotic resistance patterns among Egyptian children and predictors of resistance

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A R T I C L E I N F O

A B S T R A C T

Background: *Helicobacter pylori (H. pylori)* is a spiral-shaped Gram-negative bacteria that typically colonizes the stomach in early childhood. It is common worldwide, with higher rates in developing countries. Treatment eradication using first-line treatment regimens is declining, highlighting the importance of regional antibiotic resistance data to guide *H. pylori* treatment regimens. This study aimed at evaluation of antibiotic resistance patterns in *H. pylori* strains affecting pediatric patients and the predictors of this resistance. **Patients and methods:** This study included thirty pediatric patients who underwent endoscopic evaluation of their upper gastrointestinal symptoms at Ain Shams University pediatric hospital's gastroenterology unit. Five gastric biopsies were taken (from the corpus and antrum) for histopathology, rapid urease test and culture and sensitivity **Results:** According to culture and sensitivity results, resistance to metronidazole was the highest (86.7%), followed by clarithromycin (50%). Levofloxacin and tetracycline had the lowest resistance (6.7% and 13.3%, respectively). Excess fat consumption was associated with resistance to clarithromycin and amoxicillin. While duodenal nodularity and duodenal ulcer were associated with resistance to levofloxacin and tetracycline respectively. **Conclusion:** current *H. pylori* eradication regimen in Egypt, should be revised according to the local antibiotic susceptibility data. Further studies are needed to assess predictors for resistance to antibiotics.

Introduction

In developing countries, *Helicobacter pylori (H. pylori)* infects nearly 50% of children by the age of five [1]. According to the World Organization for Gastroenterology, the prevalence of *H. pylori* in Egyptian children aged three years is 50% [2,3]. Higher rates (72%) were found in Egyptian schoolchildren [3,4].

According to the last update of ESPAGHAN/NASPAGHAN guidelines,

noninvasive tests e.g. *H. pylori* stool antigen test and urea breath test cannot be relied upon alone for initial diagnosis of *H. pylori* infection. Invasive gastric biopsy-based methods, e.g., positive bacterial culture alone or evidence of *H. pylori* gastritis on histopathology plus rapid urease test or a molecular-based test should confirm the infection [5]. To achieve at least a 90% eradication rate, they also recommended obtaining antimicrobial susceptibility for the infecting *H. pylori* strain(s) and

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evaluating the effectiveness of the locally prescribed regimens [5].

In order to cut down on procedures and treatments and to stop the emergence of antibiotic resistance, successful eradication is crucial. Unfortunately, eradication rates are decreasing in children receiving the usual triple therapy regimen. Antibiotic resistance is one factor contributing to this decline [6].

According to a systematic review by **Fekadu** *et al.*, the overall *H. pylori* eradication rate in Egypt was 82% [7]. Comparable eradication rates were documented in Egyptian children 69.2%- 84.6% [8], and 80.5%- 84.7% [9]. The eradication rate differs according to the regimen used, duration of treatment, and local susceptibility patterns [7]. Other factors contributing to eradication rates include genetic factors e.g. genetic polymorphism in genes affecting the metabolism of proton pump inhibitors that will have an impact on gastric acidity and subsequently on acid-sensitive antimicrobials e.g. amoxicillin [9].

Despite the high prevalence of *H. pylori* infection in Africa, no regional guidelines for *H. pylori* treatment options exist, and cumulative data from subsequent publications addressing susceptibility patterns can aid in the development of such guidelines [10].

To the best of our knowledge in the last five years, few studies assessed susceptibility patterns of *H. pylori* in Egypt. They included adult Egyptian patients and showed an alarming high resistance pattern, especially for metronidazole and amoxicillin the members of recommended first-line therapy [11, 12].

The primary goal of this study was to assess the antibiotic susceptibility of *H. pylori* strains isolated from Egyptian pediatric patients; the secondary goal was to identify clinical predictors of antibiotic resistance.

Methods

This cross-sectional study included thirty pediatric patients over the age of five who presented to the gastroenterology unit at Ain Shams University Children's Hospital in Egypt for endoscopic evaluation of upper gastrointestinal symptoms such as hematemesis, vomiting, and abdominal pain and tested positive for *H. pylori* infection via rapid urease test, histopathology, and culture.

Patients who received antibiotics thirty days before endoscopy, as well as those who received proton pump inhibitors fourteen days before endoscopy, were excluded from the study [13].

The study protocol was approved by the Faculty of Medicine Research Ethics Committee (Ethical approval number FMASU MSO30/2022) and adheres to the Helsinki Declaration regulations. Before the children were enrolled in the study, their parents or guardians provided informed consent. All materials for culture, identification, and susceptibility testing, were prepared and used following the manufacturer's guidelines (Oxoid-England).

All study participants were subjected to thorough medical history taking stressing on their dietetic history, history of previous treatment of *H. pylori*, history of metronidazole taken for any reason, and full examination.

All patients had upper gastrointestinal endoscopy. Five gastric biopsies were obtained from each patient: two were taken from the corpus for histopathology and culture, and three were taken from the antrum, one for histopathology, the second for a rapid urease test HelicotecUT®Plus (Strong Biotech Corporation, Taipei, Taiwan), and the third for culture [14]. The biopsies were sent for culture only when the rapid urease test proved positive within 30 minutes. All endoscopic findings were recorded.

Culture of the samples

Samples for culture that were taken from the antrum and corpus were placed into sterile test tubes with 0.5 ml of saline solution. Biopsies were sent for culture as soon as possible after endoscopy. If there was a delay, samples were kept in Brain-Heat Infusion broth and glycerol 10% at 4°C [15].

Biopsy specimens were crushed perfectly before being cultured on Colombia agar medium containing horse blood and *H. pylori* Selective Supplement (Dent). Plates were placed in anaerobic jars with CampyGen™ envelope and cultured at 37°C in the incubator. After 96 hours, the jar was opened, and the plates were examined for growth. If no or only low growth was observed, plates were put back into the jar with a fresh CampyGen™ envelope for additional 48 to 72 hours. Bacterial colonies were identified as *H. pylori* based on colonial morphology, Gram stain, positive urease, catalase, and oxidase tests [16].

Antimicrobial susceptibility testing

A suspension equal to the 3 McFarland was prepared for each isolate and streaked on Mueller-Hinton agar medium supplemented with 10 % horse blood. Disk diffusion test was done using amoxicillin 10 μg, metronidazole 5 μg, tetracycline 30 μg, levofloxacin 5 μg and clarithromycin 15 μg disks.

The E-test strips for the antibiotics: amoxicillin, tetracycline, and metronidazole were used to determine the minimal inhibitory concentration (MIC). After 48 hours of incubation at the same conditions for primary culture, results were interpreted according to previously published breakpoints [15,17,18] as follows: For disk diffusion test: Metronidazole: susceptible $\geq$ 21 mm, intermediary 16–21 mm, and resistant < 16 mm, amoxicillin susceptible $\geq$ 25 mm and amoxicillin resistant < 25 mm. Resistance was determined by a zone of growth inhibition $\leq$ 30 mm for clarithromycin and tetracycline. Inhibition diameters <12 mm was considered resistant to levofloxacin.

For MIC testing by E test, isolates were considered resistant if (MIC $\geq$ 2 μg/mL to amoxicillin, $\geq$ 4 μg/mL to tetracycline, and $\geq$ 8 μg/mL to metronidazole). Disk diffusion and E-test susceptibility results were recorded. If there was a disagreement, the E- test results for the tested antibiotics were recorded as the susceptibility result **(Supplementary Figure 1)**.

Statistical analysis

SPSS statistical package, version 22, was used for data analysis. Descriptive analyses were carried out, using the mean and standard deviation for the quantitative variable and frequency and percentages for the qualitative one. Independent "t" tests were employed for parametric data and Mann-Whitney tests were utilized for non-parametric data to compare the means of the two groups. The correlation between two numerical parameters within the same group was evaluated using Pearson correlation coefficients.

Results

The study included 30 patients. The age of patients ranged from 5-16 years, with a mean age of 9.47±3.08 years old, about 60% of them were males while females represented (40%). The duration of symptoms ranged from 1-36 months, persistent vomiting was the most common presenting symptom in 43.3% of patients, while the percentage of patients with hematemesis was 36.7%, and those with abdominal pain represented 20% of the cases.

Twenty percent of patients (n=6) had a previous history of receiving the standard treatment of *H. pylori* (amoxicillin, clarithromycin, and proton pump inhibitor). While 23 (76.7%) patients received metronidazole before due to suspected parasitic infections.

Endoscopic findings included lower-end esophagitis (30%), gastric erythema (90%), gastric nodularity (73.3%), gastric ulcer (3.3%), duodenal erythema (36.7%), duodenal nodularity (30%), and duodenal ulcer in (13.3%) of patients.

Results of culture and sensitivity for all samples have shown that overall resistance to metronidazole was 86.7%, followed by clarithromycin resistance, which was 50%, then amoxicillin resistance, which was 20%. Tetracycline resistance was shown to be 13.3% and finally, levofloxacin resistance was 6.7%. While primary resistance in the naive 24 samples who did not receive any treatment to *H. pylori* previously showed less percentage of resistance as shown in **table (1)**.

Previous intake of triple therapy for *H. pylori* once before, not based on culture, was not a predictor of resistance. However, previous intake of metronidazole to treat gastroenteritis was significantly higher among the metronidazole-resistant group as shown in **table (2)**. Logistic regression analysis showed also that previous intake of metronidazole increased the risk of metronidazole resistance with an odd's ratio of 16.5 (*p* value=0.02) (95% CI: 1.35-201).

Comparisons between patients resistant to each antibiotic and those sensitive to it, as regards sociodemographic and dietary habits, were done, to find out predictors for resistance. The resistant group to amoxicillin showed higher mean age as compared to the sensitive group, however, this showed no significant difference using the logistic regression analysis *p* = 0.073, 95% CI (0.97-2.0). Excess intake of fat was significantly higher among the group resistant to amoxicillin and clarithromycin. There were no gender differences between sensitive and resistant groups, **table (3)**.

On the other hand, comparing endoscopic findings revealed that there was a significant difference between the group sensitive and the group resistant to levofloxacin, as regards duodenal nodularity which was present in 25% of the former

and 100% of the latter. Duodenal ulcer was also associated with tetracycline resistance. No other endoscopic findings were found between the groups. Full details of this comparison are found in the **supplementary table (1).** All statistically significant variables associated with resistance were fitted into logistic regression analysis which demonstrated that the presence of duodenal ulcer was a risk factor for tetracycline resistance as shown in **table (3).** (Odd's ratio=75.00, *p* value=0.005, 95% CI (3.66-1535.9).

Table 1. Comparing antimicrobial susceptibility between patients who received previous treatment of *H. pylori* to naïve patients.

Antimicrobial	Susceptibility	Previous treatment of *H. pylori*		*p*-value†
		Yes n=6	No n=24	
Amoxicillin	Sensitive	4 (16.7%)	20 (83.3%)	0.361
	Resistant	2 (33.3%)	4 (66.7%)	
Clarithromycin	Sensitive	1 (6.7%)	14 (93.3%)	0.068
	Resistant	5 (33.3%)	10 (66.7%)	
Tetracycline	Sensitive	5 (19.2%)	21 (80.8%)	0.788
	Resistant	1 (25%)	3 (75%)	
Levofloxacin	Sensitive	5 (17.9%)	23 (82.1%)	0.272
	Resistant	1 (50%)	1 (50%)	
Metronidazole	Sensitive	0 (0.0%)	4 (100.0%)	0.283
	Resistant	6 (23.1%)	20 (76.9%)	

†: Fisher's exact test.

Table 2. Comparing previous intake of metronidazole between patients sensitive to metronidazole and those resistant to it.

		Metronidazole		*p* value*
		Sensitive	**Resistant**	
		No. = 4	**No. = 26**	
Metronidazole taken previously to treat gastroenteritis	No	3 (75.0%)	4 (15.4%)	0.009
	Yes	1 (25.0%)	22 (84.6%)	

*Chi-square test

Table 3. logistic regression analysis for predictors of resistance to tested antimicrobials.

	Predictor variables	*p*-value	Odds ratio (OR)	95% C.I. for OR	
				Lower	Upper
Amoxicillin resistance	Age in years	0.073	1.402	0.970	2.027
	Excess intake of fat	0.013	14.00	1.741	112.551
Clarithromycin resistance	Excess intake of fat	0.044	9.333	1.958	90.94
Metronidazole resistance	Previous Metronidazole intake	0.028	16.500	1.353	201.290
Tetracycline resistance	Duodenal ulcer	0.005	75.00	3.662	1535.998

* All statistically significant variables associated with resistance were fitted into logistic regression analysis, herein significant results are presented

Discussion

Helicobacter pylori antimicrobial resistance to commonly used antibiotics is on the rise, with susceptibility patterns varying even within the same country. Data on the true burden in Africa are scarce, as are data on antibiotic resistance prevalence in children [19,20].

The antibiotic resistance in our study has been found to be (86.7%) for metronidazole, (50%) for clarithromycin, and (20%) for amoxicillin, while resistance rates for tetracycline and levofloxacin were (13.3%) and (6.7%) respectively.

Metronidazole resistance was significantly related to the previous intake of metronidazole. The rate of primary metronidazole resistance in *H. pylori* isolated from children ranges from 14.8% 21 to 95.0% [22]. Similar high rates of resistance were detected in Africa [23]. The noticed association between metronidazole resistance and previous intake of metronidazole may be related to the use of metronidazole for gynecological, dental, and parasitic-related infectious diseases [24]. The pediatric population in Egypt is frequently prescribed metronidazole for the treatment of parasitic infections [17].

Previous antibiotic susceptibility testing in Egypt revealed that 48 samples from pediatric patients (2–17 years old) had strong phenotypic metronidazole resistance; 100% [25]. However, in a different study that used molecular techniques to analyze metronidazole resistance and included 70 adult patients, the *rdxA* gene deletion responsible for metronidazole resistance, was found in 62.9% of the samples [26]. In fact, this difference in results may be due to different age groups as well as different methods of estimation of resistance [10]. Additional research revealed that, while metronidazole resistance has increased in African nations, it has remained mostly unchanged in Asian and North American nations [27], and has even been reported to be decreasing in Europe [28].

Because of the high level of metronidazole resistance in Egypt and other African countries, it is no longer a viable option in metronidazole-based triple therapy. This is especially important for children under the age of twelve, for whom no rescue treatment is currently available [29].

According to the current study, 50% of isolates were resistant to clarithromycin. Recent investigations have found that children are more likely than adults to be resistant to metronidazole

and clarithromycin [30,31]. Younger children experience infectious disorders, especially respiratory tract infections, more frequently than older ones, which increases their risk of exposure to new macrolides [29]. Clarithromycin resistance has been linked to outpatient long-acting macrolide use [32].

Surprisingly, just 4% of Egyptian children in Sherif and Associates' study in 2004 had clarithromycin resistance [25]. In contrast, the A2142G mutation (a hallmark of clarithromycin resistance) was discovered in nearly 56% of the cases when the *H. pylori* 23S rRNA V domain was examined, since in Egypt macrolides are commonly used to treat upper respiratory tract infections [26]. The effectiveness of triple regimens based on clarithromycin is significantly reduced by the presence of clarithromycin-resistant strains by 47–70% [33].

Amoxicillin resistance was determined to be 20% in the current investigation. Africa has a 40.87% resistance rate to amoxicillin. The low or even absent prevalence of amoxicillin resistance in some regions of Europe and North America may be due to their governments' restrictions on the use of antibiotics to treat infectious diseases. In contrast, particularly in Asia and South America, where these antibiotics can be purchased without a prescription, the prevalence of *H. pylori* amoxicillin resistance appears to be rising [34].

According to data from the European Registry of *H. pylori* Infection in Pediatrics, 1%, 25%, and 17.7% of patients, respectively, have primary resistance to amoxicillin, clarithromycin, and metronidazole [28]. Additionally, it had been demonstrated that having been born outside of Europe increased one's likelihood of harboring a metronidazole-resistant strain by roughly four times [35].

In this study, levofloxacin resistance was 6.7%. Quinolones are only used in children in limited circumstances; quinolones may be particularly useful in treating infections caused by multidrug-resistant organisms in immunocompromised patients who do not respond to standard antibiotic therapy [36]. It has been used also as an alternative to clarithromycin in some regions due to the marked increase in clarithromycin resistance, and it has been used frequently to treat urinary tract infections in adults, and thus levofloxacin resistance has increased over the past 3 years [34]. However, levofloxacin resistance in

children is relatively low in comparison with adults [37], due to the limited use in this age group [32].

In the current study, tetracycline resistance was 13.3%. Except for Africa, which recorded a resistance of 50%, the frequency of tetracycline resistance remains extremely low (less than 7.4%) [38]. However, management failure owing to tetracycline resistance has been reported [24].

Tetracyclines are not recommended for pediatric patients due to their negative side effects. It can, however, be used to treat *H. pylori* in children over the age of eight as part of bismuth quadruple therapy [14, 39, 40].

The cause of antimicrobial resistance, particularly in developing countries, is multifactorial. It may be related to unregulated consumption of antimicrobials in the form of self-prescription, incomplete course of treatment, or non-indicated use, in addition to lack of adequate diagnostic facilities and the use of antimicrobials in animal production [41]. Based on the latest guidelines, to achieve eradication, it is recommended that the first-line triple therapy includes a proton pump inhibitor plus *H. pylori* susceptible antimicrobials based on culture and sensitivity results. When resistance to both clarithromycin and metronidazole is detected, the antimicrobials suggested might include metronidazole and higher dose ampicillin [5]. In light of the results of the current study, this regimen may be recommended as an empirical first-line treatment when the susceptibility results are not available.

Despite considering the agar dilution method the gold standard for detecting *H. pylori* susceptibility to antibiotics, it is impractical to rely on in routine laboratory practice. In the present study, we used both the E-test and disk diffusion test for testing isolates` susceptibility to antibiotics (except for levofloxacin and clarithromycin). E test showed a good correlation with disk diffusion test results for detecting susceptibility. These findings are in concordance with **Fukazawa *et al*.** [42] and, **Mishra *et al*.** [43] who find non-significant difference between the two methods. However, some other research preferred the E-test over the disk diffusion test due to relative stability in prolonged incubation [15].

In the current study, predictors of antibiotic resistance included firstly, excess fat intake for amoxicillin and clarithromycin, Many hypotheses were postulated to link dietary components and their effect on microbiome composition or colonocyte metabolism to antimicrobial resistance [44,45], yet a formal diet assessment with body composition through a body mass analyzer should be considered before accepting those results, secondly, endoscopic criteria, especially for duodenal mucosal affection (nodularity and ulcer), increasing risk of levofloxacin and tetracycline respectively, and finally previous intake of metronidazole and its highly significant influence on metronidazole resistance.

De Francesco *et al* [34] stated that primary clarithromycin resistance is significantly higher in female patients than in male patients and non-ulcer dyspepsia patients than in peptic ulcer patients. Another study addressing predictors found no difference in metronidazole, clarithromycin, and amoxicillin resistance rates between males and females, children and adults, or ulcer disease isolates and non-ulcer dyspepsia isolates [46]. Moreover, a study for European children found no relation between antibiotic susceptibility and peptic ulcer disease [29]. Considering these observations may aid in formulating first-line therapy in clinical practice.

Conclusion

Despite the importance of culture and sensitivity for decision-making in the treatment of *H. pylori* infection, unfortunately, it is laborious, relatively expensive, and time-consuming. The lack of standardized reference standards for practical susceptibility, and the great variance in recommended inoculum and culture conditions, all aid more to the problem. This study together with similar studies will help to guide treatment regimens for *H. pylori* infection in Egypt and the Middle East since the eradication rates of *H. pylori* have been decreasing recently due to antibiotic resistance, while data remain scarce.

This study was limited to only 30 children in a monocenter in Cairo, thus we cannot extrapolate their results, however; it reports the local resistance, in a country with limited information in that field. Moreover, patients who failed primary treatment consisted only a small portion of the study, and thus proper comparisons cannot be done.

Conflict of interest
There are no conflicts of interest to declare.

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