Helicobacter pylori Antimicrobial Resistance in a Pediatric Population From the New England Region of the United States

Helicobacter pylori (*H pylori*) infection remains common worldwide, with an estimated overall prevalence of 33% in pediatric population.[1] Growing data demonstrate a global rise in the prevalence of *H pylori* antimicrobial resistance.[2]

Pediatric gastroenterology guidelines strongly recommend obtaining *H pylori* antimicrobial susceptibility before prescribing treatment when possible.[3] Because of several reasons, including limited availability of gastric biopsy culture, U.S. antimicrobial susceptibility data are limited.[4,5] Clarithromycin and levofloxacin resistance rates of 15%–30% have been reported from single-center small adult studies.[6–8] However, data in children are lacking.

We determined the rate of *H pylori* antimicrobial resistance in formalin-fixed paraffin embedded gastric tissue from pediatric patients from 2 New England tertiary care centers using a novel next generation sequencing (NGS)-based molecular method with proven reliability in adult population.[9] We compared results with gastric biopsy culture antimicrobial susceptibilities via agar dilution in a subset of patients and evaluated the impact of antimicrobial resistance on eradication rates of empiric therapies.

Electronic medical records of patients with *H pylori* diagnostic codes evaluated at Boston Children's Hospital, Boston, Massachusetts and Hasbro Children's Hospital, Providence, Rhode Island between January 2015 and September 2019 were reviewed. We included patients with *H pylori* identified on gastric biopsy who had tissue blocks available for analysis. Demographic variables, first *H pylori* treatment, and eradication results were collected. *H pylori* antimicrobial resistance profiles were obtained via NGS-based molecular method using PyloriAR (American Molecular Laboratories, Vernon Hills, IL). Off-site specialty laboratory performed *H pylori* gastric biopsy culture (Supplementary Methods).

We included 274 patients (102 from Boston,172 from Providence). Mean age was 12.7 $\pm$ 4.6 years; 151 (55%) were female, 164 (60%) were white, and 95 (35%) were Hispanic. Twenty-three cases (23/274, 8.4%) were excluded from the analysis because of insufficient *H pylori* DNA. One hundred nine cases (109/251, 43.4%) were resistant to at least 1 antimicrobial. Clarithromycin and metronidazole were the antimicrobials with the highest resistance rates, 23.5% and 22%, respectively.

Treatment data were available in all 235 treated patients. The most common treatment regimens included proton pump inhibitor (PPI)-clarithromycin-amoxicillin (53.2%), PPI-metronidazole-amoxicillin (23%), and PPI-tetracycline-metronidazole-bismuth (6%). Pediatric societal recommendations guided dosing and duration of treatment.[3] Eradication data were available for 61.3% of treated patients (144/235). The overall eradication rate was 73.6% (106/144). Quadruple therapies had the highest eradication rates ($\geq$90%). Eradication rates for triple therapies were less than 75%.

Table 1 shows antimicrobial resistance rates, treatment regimens, and eradication rates. Resistance profiles by treatment are provided in Supplementary Table 1.

Among the 92 patients who received clarithromycin-containing regimens, strains with clarithromycin resistance were statistically significantly higher among eradication-failures (13/22, 59.1%) compared with eradication successes (10/70, 14.3%; $P < .01$). Among the 62 patients who received metronidazole-containing regimens, strains with metronidazole resistance were statistically significantly higher among patients with eradication failures (9/18, 50.0%) than eradication successes (6/44, 13.6%; $P = .002$). Among the 6 patients who received metronidazole-containing regimens with eradication success and metronidazole-resistant strains, 3 regimens (50%) were quadruple therapy, and all 6 patients received $\geq$1 other antimicrobials to which the strain was susceptible.

The rate of culture growth was 34.5% (20/58); 15 patients had antimicrobial-susceptibility data (9 clarithromycin-resistant, 6 clarithromycin-sensitive). There was no reported resistance to other antimicrobials. There was 100% concordance between clarithromycin resistance as assessed by agar dilution and NGS-based method.

This report represents one of the most extensive U.S. pediatric studies on the efficacy of a NGS-based technique in characterizing microbial resistance rates in *H pylori* from gastric biopsies. Predictably, our cohort had the highest resistance rate to clarithromycin (23.5%) and metronidazole (21.9%). These rates are similar to the

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Table 1. Antimicrobial Resistance, Treatment Regimens, and Eradication Rates

Antimicrobial (n = 251)	Resistance, % (n)
Resistance to ≥1 antibiotics	43.4 (109)
Clarithromycin	23.5 (59)
Metronidazole	21.9 (55)
Fluoroquinolones	8.4 (21)
Rifabutin	3.6 (9)
Amoxicillin	2.4 (6)
Tetracycline	<1 (1)
Co-resistance patterns (n = 29 isolates resistant to >1 antimicrobials)	
Clarithromycin-metronidazole	n = 10
Clarithromycin-rifabutin	n = 1
Clarithromycin-amoxicillin	n = 1
Clarithromycin-tetracycline	n = 1
Metronidazole-fluoroquinolones	n = 4
Metronidazole- rifabutin	n = 2
Clarithromycin-metronidazole-fluoroquinolones	n = 4
Clarithromycin-amoxicillin-fluoroquinolones	n = 2
Metronidazole-fluoroquinolones-rifabutin	n = 1
Clarithromycin-metronidazole-amoxicillin-fluoroquinolone	n = 2
Clarithromycin-metronidazole-fluoroquinolone-rifabutin	n = 1

Treatment regimen (total [%])	Eradication success (%)
All treatment regimens (144 [100])[a]	
Triple regimens	
PPI-clarithromycin-amoxicillin (78 [54.2])	58 (74.4)
PPI-metronidazole-amoxicillin (29 [20.1])	19 (65.5)
PPI-clarithromycin-metronidazole (5 [3.5])	3 (60.0)
PPI-clarithromycin-amoxicillin clavulanate (1 [0.7])	1 (100.0)
PPI-amoxicillin-doxycycline (1 [0.7])	1 (100.0)
PPI-metronidazole-bismuth (1 [0.7])	0 (0)
Quadruple regimens	
PPI-amoxicillin-metronidazole-bismuth (8 [5.6])	4 (50.0)
PPI-tetracycline-metronidazole-bismuth (11 [7.6])	10 (90.9)
PPI-doxycycline-metronidazole-bismuth (1 [0.7])	1 (100.0)
PPI-clarithromycin-amoxicillin-metronidazole (5 [3.5])	5 (100.0)
PPI-clarithromycin-amoxicillin-bismuth (1 [0.7])	1 (100.0)
PPI-clarithromycin-metronidazole-tetracycline (1 [0.7])	1 (100.0)
PPI-clarithromycin-metronidazole-bismuth (1 [0.7])	1 (100.0)

PPI, proton pump inhibitor.
[a]Dual regimen PPI-amoxicillin (1 [0.7], 100% eradication).

calculated U.S.-pooled prevalence and in small single-center studies in U.S. adults.[6–8] Resistance rates to other antimicrobials were low as expected, because these antibiotics are infrequently used in children. Fluoroquinolone resistance rate was 8.4% compared with the 37% U.S.-pooled prevalence. Similarly, resistance to rifabutin (9, 3.6%), amoxicillin (6, 2.4%), and tetracycline (1, <1%) were low. None of the patients received rifabutin, and only one received fluoroquinolones.

The overall eradication rate was 73.6%, with only quadruple therapies achieving the recommended goal for effective therapies of ≥90%. The eradication rates were lower in patients with resistant *H pylori* strains when compared with non-resistant. Metronidazole resistance significantly impacted eradication success. It is possible that the decreased efficacy of metronidazole in the members of our cohort with metronidazole resistance was in part due to lower dose of metronidazole or the reduced use of bismuth in metronidazole-containing regimens.

NGS-based technique successfully characterized antimicrobial resistance in 92% of cases, even with the limitations of isolating DNA from paraffin blocks. The method also correlated very well with clarithromycin resistance, when compared with agar dilution. Poor culture growth due to the difficulties with isolation and cultivation of *H pylori* constitutes a significant problem in the United States. Only a few centers have availability of gastric biopsy culture on-site and instead rely on off-site specialty labs.[4,5] NGS-based technique can also be performed in stool,[10] further highlighting its value in pediatric population where noninvasive procedures are preferred.

Limitations of our study include the regional nature of the study population, the complex population seen at tertiary care centers, which could potentially increase rates of antimicrobial resistance because of increased exposure to antibiotics (related or unrelated to *H pylori*), and the low rate of confirmation of eradication. In addition, the relatively small number of patients who underwent culture (the current gold standard) limits our ability to assess the validity of this assay.

In conclusion, NGS-based technique provides a novel and potentially clinically useful method of characterizing resistance rates to clarithromycin and metronidazole on formalin-fixed paraffin embedded gastric biopsies. The presence of identified resistance to clarithromycin and metronidazole by NGS-based technique corresponds with lower eradication rates to common first-line regimens in children. The method is also easier and more "user friendly" than culture, although at the current time it is not reimbursable by most insurances. Although additional studies are needed to establish the correlation between NGS-based method and agar dilution in children, our preliminary data suggest the 2 tests yield similar results. The development of an easy way to ascertain antimicrobial resistance may revolutionize the treatment of *H pylori* by identifying a resistant population that would most benefit from quadruple therapy as initial treatment. Future studies should report on resistance and eradication rates in other U.S. regions to confirm the validity of the current clinical practices and modification of recommendations accordingly.

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2023.02.026.

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Supplementary Methods

PyloriAR (American Molecular Laboratories, Vernon Hills, IL) is an assay that evaluates DNA mutations or variances in the *H pylori* genome associated with resistance to fluoroquinolones, metronidazole, clarithromycin, amoxicillin, tetracycline, and rifabutin. We obtained five 10-mm sections from the formalin-fixed paraffin-embedded gastric biopsies collected for histopathology. Sections were sent to American Molecular Laboratories for processing. Briefly, sections were digested with lysis buffer and proteinase K, and whole DNA extracted with Qiagen spin columns and QIACube devices. A real-time polymerase chain reaction was performed to quantitatively determine *H pylori* presence by targeting 23S rRNA. For samples with sufficient *H pylori* DNA, targeted genes from the *H pylori* genome were amplified and enriched, and then paired-end sequencing libraries were sequenced with the Illumina-MiSeq platform (Illumina, Inc, San Diego, CA). The generated reads were mapped to the reference of *H pylori* 26695 genome sequence to analyze DNA mutations or variances responsible for the resistance to corresponding antimicrobials.

Gastric biopsy culture specimens from Boston Children's Hospital were sent to off-site specialty lab ARUP Laboratories via courier service within the same day of biopsy procurement. Briefly, once received tissue was ground in 1 mL TSB to homogenize and subsequently inoculated on 2 Brucella agar plates. The plates are incubated at 33°C–37°C in microaerophilic conditions. Plates were examined at 3, 5, and 7 days looking for characteristic colonies. Identification was performed by matrix-assisted laser desorption ionization time-of-flight—mass spectrometry or 16S sequencing. If positive growth occurred, susceptibility testing was added. The sample then was sent by ARUP Laboratories to Mayo Laboratories (test code: ZMMLS/8073), which conducted antimicrobial susceptibilities by means of agar dilution method. The susceptibility breakpoint values for *H pylori* isolates used in assessing resistance to a given antibiotic are as follows (μg/mL): amoxicillin >0.25, clarithromycin >1, metronidazole >8, levofloxacin >1, and tetracycline >4.

Supplementary Table 1. Percentage of Antimicrobial Resistance by Type of Treatment Regimens and Eradication Results

Treatment regimen/eradication result	No. of strains	% Resistance (n)					
		Cla	Met	Lev	Tet	Amox	Rif
PPI-clarithromycin-amoxicillin							
Success	58	13.8 (8)	22.4 (13)	10.3 (6)	0	0	3.4 (2)
Failure	20	55.0 (11)	20.0 (4)	10.0 (2)	0	5.0 (1)	0
PPI-metronidazole-amoxicillin							
Success	19	0	10.5 (2)	0	0	0	5.2 (1)
Failure	10	20.0 (2)	50.0 (5)	20.0 (2)	0	10.0 (1)	0
PPI-clarithromycin-metronidazole							
Success	3	33.3 (1)	33.3 (1)	0	0	0	0
Failure	2	100.0 (2)	0	0	0	0	0
PPI-clarithromycin-amoxicillin clavulanic							
Success	1	0	100.0 (1)	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-amoxicillin-doxycycline							
Success	1	0	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-amoxicillin							
Success	1	100.0 (1)	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-metronidazole-bismuth							
Success	0	N/A	N/A	N/A	N/A	N/A	N/A
Failure	1	100 (1)	100 (1)	100 (1)	0	0	100 (1)
PPI-amoxicillin-metronidazole-bismuth							
Success	4	50.0 (2)	50.0 (2)	50.0 (2)	0	25.0 (1)	25.0 (1)
Failure	4	100 (4)	50.0 (2)	0	0	0	0
PPI-tetracycline-metronidazole-bismuth							
Success	10	30.0 (3)	10.0 (1)	10.0 (1)	0	0	10.0 (1)
Failure	1	100 (1)	100 (1)	0	0	0	0
PPI-doxycycline-metronidazole-bismuth							
Success	1	0	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-clarithromycin-amoxicillin-metronidazole							
Success	5	20.0 (1)	0	0	0	0	20.0 (1)
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-clarithromycin-amoxicillin-bismuth							
Success	1	0	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-clarithromycin-metronidazole-tetracycline							
Success	1	0	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A
PPI-clarithromycin-metronidazole-bismuth							
Success	1	0	0	0	0	0	0
Failure	0	N/A	N/A	N/A	N/A	N/A	N/A

NOTE. Doses and frequencies of medications for all treatment regimens followed the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition 2016 revised clinical guidelines.

Amox, amoxicillin; Cla, clarithromycin; Met, metronidazole; PPI, proton pump inhibitor; Rif, rifabutin; Tet, tetracycline.