Antimicrobial Susceptibility Testing for *Helicobacter pylori* Is Now Widely Available: When, How, Why

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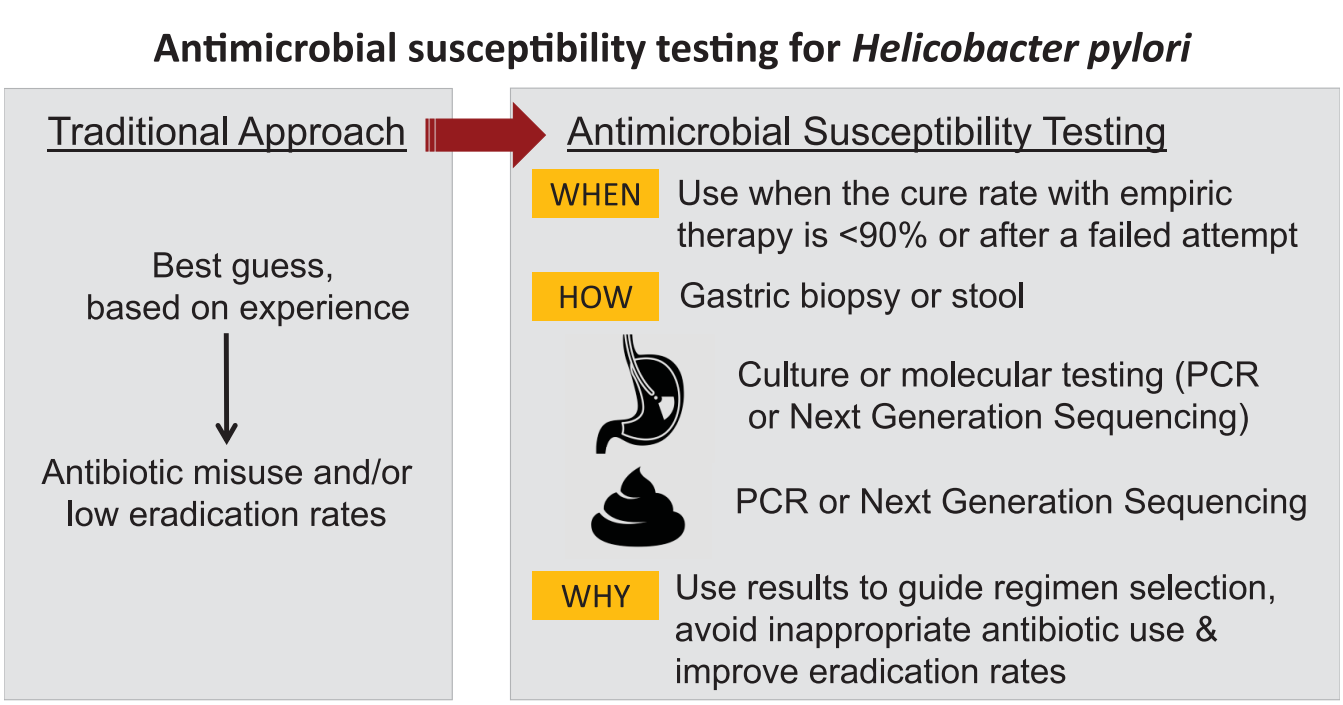

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INTRODUCTION

Helicobacter pylori gastritis is now recognized as an important transmissible infectious disease involving the stomach (1). Increasing rates of antimicrobial resistance worldwide have led to declining *H. pylori* eradication success, necessitating antimicrobial resistance testing as an important tool in *H. pylori* management. Susceptibility testing for *H. pylori* has now become widely available in the United States, allowing for full utilization of susceptibility-based antimicrobial therapy (Table 1).

Treatment success requires the use of optimized regimens that are defined as consistently achieving high cure rates (e.g., ≥95%) in adherent patients with susceptible infections (2,3). Optimized treatment regimens are shown in Table 2 (4). These are best used as susceptibility-based therapies. The decision to use an optimized regimen empirically should be based on knowledge of the local susceptibility patterns and include monitoring treatment outcomes to ensure the regimen remains highly effective locally (2,3). Effectiveness must be evaluated in near real-time by routinely obtaining posttreatment test-of-cure results which serves to

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Table 1. Where to obtain *Helicobacter pylori* susceptibility testing in the United States

Test	Laboratory	Web address	Catalog #
Culture	AURP Laboratories	https://ltd.aruplab.com/Tests/Pub/2006686	2006686
Culture	Mayo Clinical Laboratories	https://www.mayocliniclabs.com/test-catalog/Overview/62769	HELIS
Culture	QUEST	https://testdirectory.questdiagnostics.com/test/test-detail/8395/helicobacter-pylori-culture?cc=MASTER	369949
Culture	Labcorp	https://www.labcorp.com/tests/180885/i-helicobacter-pylori-i-culture	18085
Culture	Microbiology Specialists Inc.	https://microbiologyspecialists.com/helicobacter-pylori-testing/	058, 238
Reflex stool by polymerase chain reaction	Mayo Clinical Laboratories	https://www.mayocliniclabs.com/test-catalog/Overview/607594	HPFRP
Next-generation sequencing	American Molecular Laboratories	http://amlaboratories.com/testing-services/helicobacter-pylori-detection-antibiotic-resistant-analysis/	PyloriAR™/AmHPR®
Reflex stool by next-generation sequencing	American Molecular Laboratories	http://amlaboratories.com/testing-services/helicobacter-pylori-detection-antibiotic-resistant-analysis/	PyloriAR™/AmHPR®

both validate the clinician's current practices and inform when a change to a more effective treatment regimen is required. A decline in cure rates would prompt clinicians to obtain susceptibility data until a new proven locally-effective regimen was identified, which could then be used empirically. Ideally, test-of-cure data should be shared to provide local/regional information regarding treatment effectiveness (2,3). Formal reporting mechanisms to do this is yet to be established in the United States.

Currently, successful empiric therapies most often use antibiotics for which resistance is rare (e.g., in the United States, tetracycline, amoxicillin, and rifabutin; Table 2). Despite high rates of metronidazole resistance, bismuth quadruple therapy resistance can generally be overcome by increasing the metronidazole dose to 1,500 to 2,000 mg/d using the standard 14-day treatment duration (5–8). Currently, suitable empiric regimens include bismuth quadruple therapy and rifabutin triple therapy (Table 2; Figure 1). Theoretically, proton pump inhibitor or vonoprazan plus amoxicillin dual therapy are candidates for an empiric first-line choice, especially in Asia, but the regimen has not yet been optimized for use in Western countries. We predict that after such optimization, vonoprazan dual therapy will likely become a preferred therapy. Importantly, obtaining high cure rates with any therapy requires that patients adhere to the details of the regimen (Table 3). The importance of patient education in achieving high cure rates cannot be overemphasized (Table 4).

HOW TO ORDER/OBTAIN SUSCEPTIBILITY TESTING FOR *H. PYLORI*

As noted in Table 1, susceptibility testing is currently available from a number of diagnostic laboratories, making it possibly to restrict the prescription of clarithromycin, levofloxacin, or metronidazole triple therapies to confirmed susceptible infections. It now behooves clinicians to explore how to add *H. pylori* susceptibility testing to their practices. An e-mail or phone call to one of the listed facilities generally leads to a rapid response that includes a requisition and collection instructions. We suggest ordering susceptibility testing for the 6 commonly used antibiotics: amoxicillin, metronidazole, tetracycline, levofloxacin, clarithromycin, and rifabutin. Note, some culture laboratories do not yet offer rifabutin susceptibility testing but include rifampicin among their variety of tests. Rifampicin is not a valid surrogate for rifabutin in *H. pylori* treatment and should not be substituted.

Culture and susceptibility testing is available using fresh or frozen gastric biopsies. Molecular testing can be performed on the same material and, in addition, on formalin-fixed gastric biopsies that had been used for histology. Importantly, molecular testing can also be performed on stools.

Gastric biopsies

At least 2 biopsies (1 from the corpus and 1 from the antrum) should be taken, preferably using large cup forceps. These can be placed in the same bottle. If the culture is positive, susceptibility testing is reflexively performed and reported. Traditionally, gastric biopsies have been placed in a transport medium (e.g., brucella broth with 20% glycerol or Portagerm pylori [bioMerieux, Durham, NC] or an equivalent) and shipped overnight. However, Quest, Labcorp, and AURP require samples in saline, which generally results in a reduced proportion of cases with successful growth. Currently, only Microbiology Specialists Inc. provides brucella broth with 20% glycerol for transport. The sample in the brucella broth is immediately frozen, preferably by a quick freeze at −70 to −80 °C (although −20 °C will suffice), and shipped overnight on dry ice. Kept frozen, the sample will be stable for up to a week before culturing. If maintained in a −70 to −80 °C freezer, the sample will remain viable for months, if not years, and can be shipped on dry ice when convenient.

The alternative to culture is susceptibility testing using molecular methods, such as next-generation sequencing (NGS) from American Molecular Laboratories (AML). AML provides specimen containers with a preservative medium that can be sent

Table 2. Currently available and effective *Helicobacter pylori* therapies in the United States

Empiric therapies	
Bismuth quadruple therapy Bismuth subsalicylate q.i.d. 14 d	Bismuth (e.g., Pepto-Bismol) 2 tablets or 2 capsules q.i.d. 30 min before meals, tetracycline HCl 500 mg, and metronidazole 500 mg 30 min after meals q.i.d. plus a PPI, 30 min b.i.d. before breakfast and with the evening meal (see PPI recommendations below)
Pylera. 3-in 1 formulation of bismuth quadruple therapy with bismuth citrate) metronidazole and tetracycline 14-d	Give combination tablets 4 times daily (with meals and at bedtime) plus a PPI 30 min before breakfast (see PPI recommendations below). If the pharmacist will only dispense a 10-d supply, use 10 d or consider using 14-d generic bismuth quadruple therapy instead (see above)
Rifabutin triple therapy. 14-d	Rifabutin 150 mg b.i.d. 30 after breakfast and the evening meal, amoxicillin 1 g t.i.d. 30 after breakfast, the evening meal, and bedtime plus 40 mg of esomeprazole or rabeprazole 30 min before breakfast and the evening meal (see PPI recommendations below).
Talicia 3-in 1 formulation of rifabutin/ amoxicillin/omeprazole triple therapy. 14-d	4 capsules t.id., as directed by the package insert
Therapies only effective as susceptibility-based therapy. Do not use empirically unless proven to cure >90% locally	
Clarithromycin triple therapy. 14-d	Clarithromycin 500 mg b.i.d., amoxicillin 1 g b.i.d. 30 min after meal plus a PPI b.i.d. 30 min before breakfast and the evening meal (see PPI recommendations below)
Metronidazole triple therapy. 14-d	Metronidazole 500 mg b.i.d., amoxicillin 1 g b.i.d., 30 min after meal plus a PPI b.i.d. 30 min before breakfast and the evening meal (see PPI recommendations below)
Levofloxacin triple therapy. 14-d[a]	Levofloxacin 500 mg in a.m., amoxicillin 1 g b.i.d., 30 min after meal plus a PPI b.i.d. 30 min before breakfast and the evening meal (see PPI recommendations below)
PPI recommendations PPI should preferably be a PPI which is minimally affected by CYP2C19 metabolism (i.e., rabeprazole or esomeprazole) and at least 20 mg per dose (preferably 40 mg) of rabeprazole or esomeprazole b.i.d.	
Therapies containing unnecessary antibiotics that should not be used Regimens that include at least 1 antibiotic that offers no therapeutic benefit and serve to increase global antimicrobial resistance include concomitant, hybrid, reverse hybrid, sequential therapies and vonoprazan clarithromycin and amoxicillin triple therapy (3).	

b.i.d., 2 time daily; HCl, hydrochloride; PPI, proton pump inhibitor; q.i.d., 4 times daily; t.i.d., 3 times daily.
[a]The US Food and Drug Administration recommends fluoroquinolones be used as a last choice because of the risk of serious side effects.
Table adapted from reference (4), with permission.

immediately for *H. pylori* diagnosis (PyloriDx) and, if positive, the specimen will reflexively be tested for susceptibility. Alternately, the protected preserved specimen can be retained up to 2 weeks at room temperature and be sent for susceptibility testing only if the histology is positive or equivocal.

Formalin-fixed gastric biopsies

AML offers NGS susceptibility testing using formalin fixed gastric tissue blocks (sections can be sent, although care must be taken not to contaminate them while sectioning) (9,10).

Stools

Susceptibility testing using stool samples has the advantage of obviating the need for endoscopy and gastric biopsy (11). It is important to follow the detailed instructions provided by the laboratory to which the samples are sent; typically, <1 g of formed stool is sufficient. AML offers a reflex option for stool antigen or polymerase chain reaction testing using NGS to reflexively test stool for the 6 commonly prescribed antibiotics. Mayo Clinic Laboratories offers reflex stool testing for clarithromycin resistance.

THE EFFECT OF SUSCEPTIBILITY TESTING ON ENDOSCOPY PRACTICES

Although primary susceptibility-based therapy is a good option, we anticipate the use of a mixed empiric and susceptibility-based approach, depending on the local success of first-line empiric therapies and previous treatment attempts (Figure 1). Biopsies for *H. pylori* culture placed in saline or a transport medium, such as brucella broth with glycerol, must immediately be sent for culture. Successful culture is dependent on the handling and shipment of biopsies. If culture success of known positive samples is <80% when sent in saline, we recommend switching to a laboratory that provides brucella broth with glycerol or another proven-effective transport medium. As noted above, biopsies frozen at −70 °C in brucella broth with glycerol remain stable for months. For molecular testing with NGS, the specimen should be placed in the

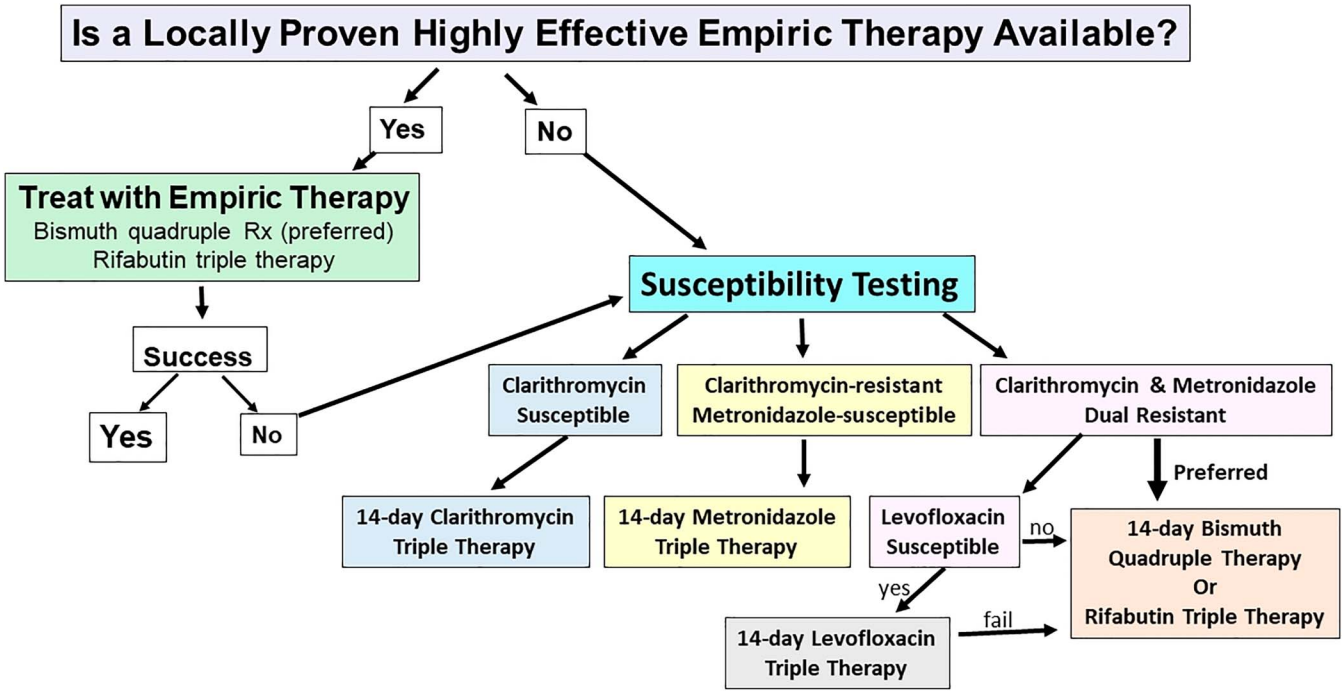

Figure 1. Proposed algorithm for the selection of *Helicobacter pylori* regimen based on knowledge of the results of empiric first-line therapies and the results of susceptibility testing.

special transport media provided for molecular testing and can be held at room temperature until the results of histology are available.

SUMMARY

The widespread availability of *H. pylori* susceptibility testing now renders the low cure-rate empiric therapies (e.g., triple or quadruple therapies containing clarithromycin, metronidazole, or levofloxacin) obsolete. The exception is the use of high-dose metronidazole in 14-day bismuth quadruple therapy. Susceptibility-based therapy, when combined with optimized regimens, will reliably achieve high cure rates, provided the patient is well-advised and motivated to be adherent with the regimen (Table 4 lists some common-sense guidelines).

Susceptibility-based therapy can be used for initial therapy. Alternately, an empiric-therapy-first strategy can be used but only if the empiric therapy selected has been confirmed to be highly effective locally. Both susceptibility-based and empiric-therapy-first strategies require monitoring using a test-of-cure to provide feedback to confirm continuing success. Use of an empiric-therapy-first approach must be based on the willingness to abandon empiric therapy if its

Table 3. Guidelines for prescribing successful *Helicobacter pylori* therapies

Take an antibiotic use history and if available review previous prescriptions to identify the antibiotics where resistance is likely.
Prescribe only therapies that are proven to be effective locally (i.e., cure rates ≥90%) or preferably highly effective locally (i.e., cure rates of ≥95%).
The rules of thumb regarding therapy include only use antibiotics to which the organism is susceptible. Antibiotic doses and dosing frequency are based on the local results. A duration of 14-d is best. Esomeprazole or rabeprazole 40 mg b.i.d. are preferred because they are more potent and minimally affected by CYP2C19 metabolism.
Do not prescribe clarithromycin, metronidazole, or levofloxacin for *H. pylori* infections unless susceptibility has been confirmed. The exceptions are use of metronidazole in bismuth quadruple therapy and confirmed excellent outcomes locally with these triple therapies.
Quinolones (e.g., levofloxacin) have recently been associated with severe long-term side effects and should not be prescribed unless a) susceptibility is confirmed and b) no other options are available.
Resistance to tetracycline, amoxicillin, and rifabutin are still rare
Therapies that contain unneeded antibiotics (e.g., concomitant, sequential, hybrid, reverse hybrid, and vonoprazan clarithromycin triple therapies). Should not be prescribed as the unneeded antibiotic (most often clarithromycin) unnecessarily contributes to increased global antimicrobial resistance
Perform test-of-cure after every treatment to provide continuing feedback regarding current effectiveness.
Share test-of-cure results with partners and colleagues so as to contribute to the local and regional experience regarding which *H. pylori* therapies are locally effective vs ineffective.
Successful use of an empiric therapy is critically dependent on monitoring its effectiveness and the willingness to abandon an empiric therapy if its effectiveness declines.
Susceptibility data must be coupled with optimized therapy to achieve their full potential

Table 4. Methods to enhance the effectiveness of *Helicobacter pylori* therapy

Take a detailed medical and antibiotic use history and provide adequate time for office visits.
Explain in simplistic terms the effects of the infection on the stomach, the potential outcomes of the infection, and how cure of the infection results in healing of the damage, prevention of ulcers and ulcer recurrences, and greatly reducing the risk of gastric cancer.
Provide a clear written description of the complexities of the regimen chosen and the necessity for adherence to the full treatment schedule
Provide a clear written description of the medications and plan for dosing and, if possible, providing appropriate containers (pill boxes or blister packs) arranged according to the dosing plans in relation to meals and bedtime.
Emphasize that the medications are taken concurrently for the full 14-d period and to not start to take the medications until all of the drugs have been received.
Describe the adverse effects that are commonly expected as a consequence of the treatment, such as feeling unwell with nausea, headaches, taste disturbances, and loose or dark stool.
Provide written instructions in a language that can be read and understood for patients where English is not their first language.
To ensure adherence, provide a contact available after hours and weekends that can answer questions.
Monitor adherence by discussion and by pill counting during treatment if necessary.
A test-of-cure by breath or stool antigen/PCR test should be performed 4 or more weeks after therapy and off PPIs for at least 2 wk to ensure cure and provide feedback on the local effectiveness of the therapy used. H2 receptor antagonists can be substituted during the PPI abstinence period.
Test-of-cure results should be shared with colleagues and institutions locally to provide information regarding local susceptibility patterns.

PCR, polymerase chain reaction; PPI, proton pump inhibitor.
Adapted from reference (13), with permission.

effectiveness declines. Patients with a history of previous treatment failure should receive susceptibility-based therapy. It is important to note that clarithromycin, metronidazole, and levofloxacin triple therapies remain highly effective as susceptibility-based therapies (12). Finally, the US Food and Drug Administration warning about serious long-term side effects of quinolone therapy dictate that fluoroquinolones be used only in susceptibility-based therapies.

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CONFLICTS OF INTEREST

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Potential competing interests: D.Y.G. is a consultant for RedHill Biopharma and Phathom Pharmaceuticals regarding novel *H. pylori* therapies and has received research support for culture of *Helicobacter pylori*; he is also a consultant for DiaSorin regarding *H. pylori* diagnostics and with Otsuka Japan regarding novel breath tests; he has ongoing collaborative research projects with American Molecular Laboratories regarding molecular diagnostics for *H. pylori*; and he was the PI of an international study of the use of antimycobacterial therapy for Crohn's disease. S.F.M. is a consultant for Takeda, has served on advisory boards for RedHill Biopharma and Phathom Pharmaceuticals regarding novel *H. pylori* therapies, and has received research support from American Molecular Laboratories regarding molecular diagnostics for *H. pylori*.

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