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Helicobacter pylori culture: from bench to bedside

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Abstract

Helicobacter pylori (*H. pylori*) infection is a widespread infection that causes various gastroduodenal diseases and some extraintestinal disorders. Curing this infection remains challenging for clinicians, mainly because of bacterial resistance towards the few available antibiotics. Therefore, as for other infectious diseases, therapeutic approaches should be opportunely designed using the principles of antimicrobial stewardship. Theoretically, only susceptibility-based antimicrobial therapy should be considered as appropriate for treating this infection. Unfortunately, *H. pylori* owns some particular characteristics that make the infection slightly peculiar. More specifically, it is "fastidious" about growing in culture, and its isolation is not easily achieved, even in dedicated laboratories that, to make matters worse, are only scantily spread among countries. We examined the pros and cons of bacterial culture for antibiotic susceptibility testing before different therapy lines, and its applicability in the real clinical life.

Keywords *Helicobacter pylori*, antibiotic, culture, resistance, susceptibility testing

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Introduction

Although its prevalence is decreasing, *Helicobacter pylori* (*H. pylori*) still infects millions of persons worldwide, and the infection is generally lifelong unless a specific therapy is performed [1]. Indeed, the probability of spontaneous disappearance or eradication with antibiotic therapies administered for other infections is negligible. *H. pylori* causes both gastrointestinal (non-ulcer dyspepsia, peptic ulcers, gastric lymphoma, and cancer) diseases, and some extragastric disorders, including idiopathic thrombocytopenic purpura and idiopathic iron deficiency anemia [2,3]. During the 40 years since its rediscovery, no proposed therapy has been able to cure the infection in all treated patients [4]. On the contrary, the success rate of dual and triple therapies, first introduced in the 1990s, progressively decreased, so that more complex regimens—i.e., bismuth-free and bismuth-based quadruple regimens—were pioneered in the course of last 2 decades [5]. Both primary and secondary bacterial resistance towards the few available antibiotics are deemed to play the major role in therapy failure [6]. Therefore, knowing the susceptibility status before administering antibiotics is certainly advantageous. Nonetheless, *H. pylori* is a microbe that is "fastidious" about being cultured from gastric biopsies, and its isolation is not easily achieved, even in dedicated laboratories that, to make matters worse, are only scantily spread among different countries. Therefore, while helpful for surveillance of primary bacterial resistance in *H. pylori* isolates performed in specific epidemiological studies, the use of bacterial culture for clinical practice has been questioned [7]. The concept that *H. pylori* is an infection for which therapy is not opportunely designed using the principles of antimicrobial stewardship was recently renewed, suggesting that the only appropriate antimicrobial therapy should be a susceptibility-based treatment [8]. In other words, gastroenterologists should learn and implement in clinical practice the approach suggested by infectious disease specialists [9].

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Bacterial culture: pros and cons

When to perform culture-based susceptibility testing for therapeutic purposes?

The concept of culture-based susceptibility testing for guiding *H. pylori* therapy was officially introduced in the

first European guidelines, published in 1997, as an option to manage patients with persistent infection following at least one therapeutic attempt [10]. In the updated guidelines of 2007 and 2012, culture was advised for assembling a third-line therapy in every case, or for second-line therapy, whenever endoscopy was performed for another reason [11,12]. Moreover, it was recommended even before first-line treatment whenever a clarithromycin-based therapy was chosen in those areas where primary clarithromycin resistance was >15-20% [11,12]. These statements were substantially confirmed in the last updated guidelines delivered in 2017 [13]. Susceptibility testing before second- or third-line therapies was also endorsed by the last Italian (2015) guidelines, whilst its use only before third-line treatment was suggested by Spanish (2016), Irish (2017), Greek (2020), Canadian (2016), American (2017 and 2018), and Japanese (2019) guidelines [14-21]. Moreover, the Kyoto global consensus (2015) encourages culture, ideally every time endoscopy is performed, but no clear indication was provided about the precise step in which it is strongly recommended in clinical practice [22].

In summary: (a) there is substantial agreement among guidelines in recommending culture-based antibiotic susceptibility testing for tailoring third-line treatment following 2 therapy failures; and (b) culture should be attempted, whenever possible, every time an endoscopy is performed in patients with suspected *H. pylori* infection.

How successful is culture-based antibiotic susceptibility testing?

The success rate of culture was evaluated in different settings of clinical practice, from before first-line therapy to following more treatment failures. In a large Korean study, where the prevalence of *H. pylori* infection in the general population is high, antibiotic susceptibility testing was achieved in only 247 (13.3%) of 1862 unselected naïve patients undergoing upper endoscopy in whom bacterial culture was attempted [23]. This would mean that bacterial culture was useless in as many as 9 of every 10 cases who underwent upper endoscopy. Therefore, to perform culture for tailoring first-line therapy in unselected patients seems to be a prohibitive approach from the cost-efficacy point of view. This is particularly compelling for western countries and other areas where the prevalence of infection in the general population is distinctly lower than in Korea.

When antibiotic susceptibility testing was attempted in patients with a documented infection by another test, the success rate varied according to the setting. In detail, the pooled-data analysis of 51 studies showed that *H. pylori* strains were isolated overall in 80.7% of 7889 patients, with success rates of 78.1%, 77.5%, 86.3%, and 86.6% before first-, second-, third-line or more therapies, respectively [24]. Therefore, the attempt to acquire information on bacterial resistance failed in more than 20% of cases, even though the infection was present. This probability should be taken into account and clearly disclosed to the patient, who may have to undergo (and pay for) a second, and most likely clinically unrewarding, endoscopic examination solely to take gastric biopsies for culture.

In summary: (a) the procedure of culture-based antibiotic susceptibility in unselected patients before first-line treatment is actually not feasible; and (b) the attempt to culture in patients with a documented persistent infection following at least one treatment fails in at least 2 of every 10 cases.

How do results of antibiotic susceptibility testing impact on therapy success?

A recent systematic review of comparative studies showed that *H. pylori* infection was cured in 89.9% of 2052 patients treated with antibiotic tailored therapies and in 77.6% of 2516 patients receiving empiric therapy (P<0.001) [24]. However, in the sub-analysis, acceptably high eradication rates with tailored therapies were achieved only when the approach was applied before either first- (91.6% vs. 78.2%) or second-line (91.2% vs. 79%) therapies, but not before third-line treatment (79% vs. 70%), that is for the step suggested in all current guidelines. Beyond this observation, it is clinically relevant to note that the therapeutic advantage is mainly due to the low efficacy of empiric therapies used as a comparator, rather than a notably high success rate of antibiotic-tailored therapies, which is above 90%. The evidence that a similar cure rate may be achieved with current quadruple regimens, empirically administered as first-line therapies, further weakens the real advantage of culture in clinical practice [25,26]. Indeed, for other infectious diseases, the use of an antibiotic therapy tailored to bacterial susceptibility testing is expected to achieve bacterial eradication in virtually all patients, but this seems not to be the case for *H. pylori* infection. The same phenomenon is expected when antibiotic susceptibility is genetically assessed using a culture-free, polymerase chain reaction (PCR)-based tool, that overcomes the culture limitation of difficult bacterial growth [27]. On the other hand, a recent randomized controlled trial found that, as second- or third-line treatment, therapy guided by antimicrobial susceptibility testing was not significantly superior to the costless therapy guided by personal medication history, according to the intention to treat analysis (78.1% vs. 74.3%) [28].

In summary: (a) the use of culture-based therapies following 2 failures, as suggested in current guidelines, allows us to achieve an eradication rate <80%; and (b) even using therapies tailored to antibiotic susceptibility as first- or second-line treatments, the cure rate remains about 90%, similar to that of current empirically used quadruple regimens.

Why does susceptibility-tailored therapy fail *in vivo*?

There are different factors that could provide potential explanations for the discrepancy in effectiveness seen when considering the *in vitro* and *in vivo* results of antibiotic susceptibility testing. In culture, the activity of antibiotics is

generally tested at a pH of 7, a value not achieved in the peculiar niche where *H. pylori* survives in the stomach, even when high-dose proton pump inhibitors (PPI) are used [29]. In contrast to metronidazole and tetracycline, the activity of amoxicillin, levofloxacin and, to a lesser extent, clarithromycin is deeply impaired if pH values are <6 [30]. Therefore, a bacterial strain tested as susceptible *in vitro* could actually behave as resistant *in vivo*. Another factor that might affect the discrepancy between *in vivo* and *in vitro* results could be the presence of a heteroresistant status—namely, the coexistence of susceptible and resistant strains, either intra-niche (i.e., at the same gastric site) or inter-niche (antrum and gastric body)—that has been clearly demonstrated for *H. pylori* infection [31,32]. Generally, only 1-2 gastric biopsies are taken to perform culture in clinical practice. Therefore, it may happen that only one type of strain is casually sampled, providing an inaccurate estimation of the real intra-niche resistance status *in vivo*. Similarly, when only antral biopsies are sampled, the potential status of inter-niche heteroresistance is missed.

On the other hand, a misclassification of antibiotic resistance may occur even using culture-free genetic-based tools, such as PCR-based methods [33]. Indeed, only a few point mutations have been tested, but some others are emerging [34], and their impact on antibiotic efficacy *in vivo* may differ among different mutations. For instance, among *H. pylori* clarithromycin-resistant strains, those harboring the A2143G mutation—but not the A2142G—significantly lowered the cure rate [35]. Therefore, some bacterial strains are classified as clarithromycin resistant *in vitro*, because of the A2142G point mutation, but an acceptable eradication rate could be achieved *in vivo*. Moreover, by assessing only the genotypic resistance as a simple effect of rRNA point mutations, the potential role of efflux pumps in causing phenotypic resistance is ignored [36-38].

Another aspect to be considered is that *H. pylori* strains are classified *in vitro* as resistant according to a definite cutoff of minimal inhibitory concentration (MIC) values [39]. However, the level of resistance has been observed to play a role in predicting the efficacy of a specific therapy. For instance, the presence of dual resistance towards clarithromycin (MIC levels >0.5 mg/L) and metronidazole (MIC values >8 mg/L) significantly reduces the efficacy of sequential therapy only when the MIC values were >8 mg/L for clarithromycin and >32 mg/L for metronidazole [40]. Thus, several *H. pylori* strains classified as resistant *in vitro*, based on the standard cutoff, actually behave as susceptible *in vivo*, at least following some effective therapy regimens. This could depend on a synergism among different antibiotics *in vivo*, as demonstrated also for primary metronidazole resistance, which can be surmounted by higher doses, longer therapy duration or coadministration of clarithromycin [8]. Similarly, *in vitro* levofloxacin resistance is generally overcome by a combination of levofloxacin with tetracycline (88.9% eradication rate), but not with amoxicillin (50% cure rate) [41]. Therefore, the *in vitro* resistance in *H. pylori* strains should not be interpreted as an on/off effect, *a priori* discarding a still useful antibiotic.

In summary: Because of various factors, *H. pylori* strains tested as susceptible *in vitro* may behave as resistant *in vivo*, and *vice versa*.

How frequently is antibiotic susceptibility testing performed in the real life?

In June 2013, a European, non-interventionist registry (Hp-EuReg) was created to collect data on the management of *H. pylori* infection performed in the participating centers. An analysis of data from 21,801 patients who underwent upper endoscopy up to December 2020 revealed that culture was performed in only 3974 (9.5%) individuals [42]. Surprisingly, culture was performed much more (71.7%) frequently in naïve patients than in previously treated patients, as currently suggested by guidelines. Moreover, it should be noted that a large majority of the cases (N=2360) were collected in Italy, whilst other countries contributed only a few cases, such as Spain (N=454), Norway (N=368), Greece (N=248), Slovenia (N=211), Israel (N=110), or very few cases, such as France (N=45), and Ireland (N=40). When we consider that these data were collected in tertiary centers with interest in *H. pylori* for scientific purpose, we might expect that *H. pylori* culture is even more rarely performed in real clinical practice. Notably, an antibiotic susceptibility registry containing regional data across North America is still lacking [43], and it has been recently stated that culture is performed only by the Mayo Clinic laboratory and a few other major commercial laboratories [8]. This severely impacts facilities in clinical practice.

In summary: *H. pylori* culture is currently performed in only a few tertiary centers, limiting its application in real clinical practice.

Concluding remarks

According to Kyoto consensus recommendations, *H. pylori* is an infectious disease and therefore it should be managed as other infections are, even when patients have no symptoms and irrespectively of complications [22]. However, *H. pylori* owns some particular characteristics, which make the infection slightly peculiar: 1) it can survive in an acidic milieu as well as under the mucous layer, where it lives in a specific biological niche in the stomach; 2) it can colonize gastric mucosa patchily; 3) it may migrate with flagella from the antrum to the body of the stomach under certain microenvironment conditions; 3) it is a microaerophile and, therefore, is "fastidious" about growing in culture. Finally, despite being Gram negative, *H. pylori* is highly susceptible to penicillin. Therefore, it is a "quite unique bacterium", and culture is really challenging (Table 1).

Forty years following the rediscovery of *H. pylori*, we still have only 6 antibiotics to set up an effective therapeutic combination. Consequently, we should take care before discarding an antibiotic *a priori* because of suspected primary antibiotic resistance, by simply predicting the

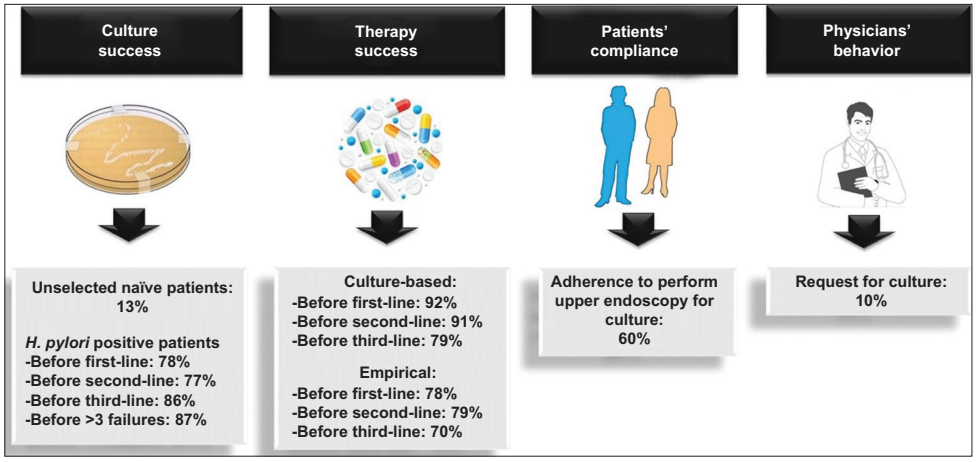

Figure 1 A synopsis of available data
H. pylori, Helicobacter pylori

Table 1 Factors limiting *Helicobacter pylori* culture

Factor	Comments
Need for endoscopy	Upper endoscopy is repeated only to perform bacterial culture without providing additional diagnostic yield in already investigated patients
Sample transport	Gastric biopsy specimens need to be collected in a specific medium and transported in dedicated laboratories
Sensitivity	Bacterial culture fails in at least 20% of infected patients when tested under optimal conditions, as occurs in clinical trials. An even lower sensitivity can be expected in clinical practice
Antibiotics tested	Limited to only a few antibiotics. Clarithromycin and metronidazole resistance is largely predictable if previously used. The prevalence of resistance to amoxicillin and tetracycline remains quite low, even if previously used
Discrepancy *in vitro/in vivo*	Bacterial strains tested as susceptible *in vitro* could actually behave as resistant *in vivo* and *vice versa*
Therapeutic yield	Success rates of culture-tailored therapy regimens range from 79-90%
Dedicated laboratories	Not available in all hospitals/cities

in vivo effect at bench side [44]. Likewise, even evidence of proven antibiotic resistance should be interpreted with caution. Indeed, it has been calculated that in order to obtain reliable information on the effectiveness of a specific therapy regimen, some 98-144 patients with resistant strains need to be studied [45], a sample size virtually never included in a therapeutic trial or in systematic reviews. Similarly, beyond the cutoff, the real values of MICs were largely neglected in clinical trials, despite their potential role in affecting therapy success [40]. On the other hand, when a tetracycline–amoxicillin combination, namely 2 drugs with no or a very low (<3%) primary resistance rate in *H. pylori* isolates [41], was empirically administered for 7 or 14 days, the eradication rate was as low as 35-43% [46,47]. This seems to indicate that factors other than bacterial resistance and/or drug combinations play an important role in *H. pylori* eradication [48]. Another relevant aspect limiting the availability of antibiotic susceptibility testing in clinical practice is the patient's compliance. *H. pylori* culture is not as simple as urine or sputum culture, and it implies a further invasive (and costly) endoscopy. Notably, when the compliance rate for undergoing upper endoscopy for purposes of *H. pylori* culture was evaluated, only 60% of patients accepted the procedure [49]. Therefore, to apply the principles of standard antimicrobial stewardship and to use only susceptibility-based treatments is largely not practicable for managing *H. pylori* worldwide (Fig. 1). The World Health Organization claimed *H. pylori* as a pathogen with a high-priority need for new antimicrobial drugs [50]. While waiting for other molecules, a potential solution of the problem could be to attempt optimizing the use (dose, frequency, duration, combination, etc.) of the few available antibiotics, also opportunely powering their action in the gastric acid, namely with a deep acid inhibition. The imminent delivery of potassium-competitive acid blockers, novel molecules more powerful than PPI in reducing gastric acid secretion, may represent an advantage in this field [51]. However, the best vonoprazan-based regimen for treating *H. pylori* in western populations has still to be established.

Finally, it has been suggested that more studies were required to demonstrate the theoretical expected superiority of antimicrobial susceptibility testing-guided therapy over empiric therapy [24,43]. However, this goal is difficult to achieve, as these studies are very expensive. Indeed, they require a very large sample size in order to avoid possible type II error [45]. Furthermore, it is necessary to combine these trials with a health economic evaluation to verify that the antimicrobial susceptibility testing-guided therapy is cost-

effective. In the meantime, the only acceptable battle is not between infectious diseases specialists and gastroenterologists, but against *H. pylori*!

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