JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Regional Review

Antimicrobial resistance in *Helicobacter pylori*: current situation and management strategy in Vietnam

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Abstract

Increasing antimicrobial resistance to key antibiotics in *Helicobacter pylori* has become a main cause of treatment failures in many countries, including Vietnam.

For this reason it is advisable to perform antimicrobial sensitivity tests to provide more focused regimens for *H. pylori* eradication.

However, this approach is generally unavailable for *H. pylori* in Vietnam and the selection of treatment regimens is mainly based on the trend of antibiotic use in the population, resistance development in the region, and history of *H. pylori* eradication of patients.

The aim of this review is to examine the current situation of antimicrobial resistance in Vietnam and suggest management strategies for treatment selection.

Key words: *Helicobacter pylori;* antimicrobial resistance; therapy regimens management.

J Infect Dev Ctries 2015; 9(6):609-613. doi:10.3855/jidc.6942

(Received 30 March 2015 – Accepted 16 May 2015)

Introduction

Helicobacter pylori (*H. pylori*) is an important human pathogen which plays a significant role in the pathogenesis of upper gastrointestinal tract diseases. Actual infection rates is nearly 50% of the world's population and varies geographically, being higher in developing countries [1,2]. *H. pylori* gastritis is etiologically associated with peptic ulcer, primary gastric B-cell lymphoma and gastric carcinoma. Despite a general decline in the incidence of gastric cancer, it remains the fourth most common cancer and the second leading cause of cancer-related deaths worldwide. In 2010, about 21,000 new gastric cancer cases and 10,570 deaths from gastric cancer were estimated in the USA [3]. Recent data showed that the prevalence of *H. pylori* is still high in most countries, not only in South America, Africa and Asia, but also in some areas in Europe where it is estimated higher than 50% [4].

Vietnam is a developing country with high prevalence of *H. pylori* infection (74.6% population) [5] and an intermediate-high risk of gastric cancer (24.4 age-standardized incidence rate per 100,000 population) [3]. In this view a successful eradication therapy appears essential not only to reduce the risk of developing gastric cancer but also to treat other severe related disorders such as peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, iron deficiency anemia, hemorrhage idiopathic thrombocytopenia [6].

The development in *H. pylori* of resistance towards key antibiotics included in the current eradication regimens affects the therapeutic outcome having a substantial influence on treatment failures [7],[8]. Currently data about *H. pylori* antibiotic resistance in Vietnam are still very limited and vary depending on geographic region.

H. pylori antibiotic resistance in Vietnam

The problem of the increase of antibiotic resistance represents the most important factor responsible for the declining success rate of *H. pylori* eradication therapy [9]. Surveillance of *H. pylori* antibiotic resistance is therefore mandatory in order to adapt the antibiotic combination to local resistance patterns [10]. This issue is of particular relevance with regard to clarithromycin, which can induce virtually a 70% loss of effectiveness in the standard triple therapy (proton

pump inhibitor, PPI + clarithromycin + amoxicillin) in patients infected with clarithromycin resistant strains versus susceptible strains [11].

Unfortunately, recent data of *H. pylori* antibiotic resistance in Vietnam showed that the resistance rate to clarithromycin, the key antibiotic for first-line treatment, was very high, in order of 33-34.2% for primary and 73.7% for secondary resistance in adult patients [12,13], and notably, over 50% in children patients [14].

Moreover, resistance rate to levofloxacin, the alternative drug after first-line eradication treatment failure with the standard triple or quadruple therapy, is also remarkably high in Vietnam, ranging from 18.4% in 2008 to 35.6% for primary resistance and 63.2% for secondary resistance in 2014, showing a rapid acquisition of resistance to levofloxacin in nearly 5 years [12,13]. The resistance towards these two key antibiotics occurred in a high background level of resistance to metronidazole varying from 69.9% to 76,1% [12,13].

However, the low resistance to amoxicillin detected in Vietnam indicates that it probably occurred in exceptional cases with less than 2% of reports [12-14].

Interestingly, in a previous study levofloxacin resistance rate was significantly more common in females than in males [13]. This probably reflects the increasing use of fluoroquinolones in females, which may lead to cross-resistance with levofloxacin in Vietnam. Moreover, the resistance to clarithromycin and levofloxacin increased according to the rise of age, which was similar to that reported from a European study describing older age as a risk factor for antibiotic resistance [8].

The primary resistance is most likely the consequence of treatments administered to the patient for other types of infection, such as clarithromycin for respiratory infections; levofloxacin for urinary infections and metronidazole for intestinal parasites, periodontal, and gynecologic diseases which are common in developing countries. The abuse of these drugs for self-medication may be the main factor of high resistance rate in Vietnam. In such cases, the antibiotic used as a monotherapy may have selected resistant *H. pylori* mutants and therefore it may be inefficient in achieving complete eradication

After treatment failure with clarithromycin-based triple therapy, the risk of finding a strain resistant to clarithromycin is approximately 65% [11]. It is in the same range after failure of a levofloxacin based triple therapy [15].

A recent report from Vietnam showed that the secondary resistance of *H. pylori* to clarithromycin and levofloxacin was 73,7% and 63,2%, respectively [13], this was consistent with other studies conducted in Poland and Korea [16,17]. After several treatment attempts, it was possible to find strains resistant to three, four antibiotics in 14.5% to 15.2% and 1.1% to 1.9% cases respectively, and multidrug resistance in over 56% of cases [12,13]. Furthermore, triple drug and multidrug resistance in secondary resistant strains were significantly higher than in primary resistant strains [13].

H. pylori therapy regimens currently used in Vietnam

H. pylori infections are commonly acquired during early childhood [18] and colonization persists lifelong unless antibiotic treatment is administered, as natural clearance of the infection is rare [19]. All consensus statements agree that whenever *H. pylori* is diagnosed it should be cured if possible because *H. pylori* eradication can reduce the risk for gastric cancer.

Treatment requires combination of at least two antibiotics to kill the bacteria and anti-acid medications to ensure their effectiveness in the stomach.

For infections caused by several other bacterial pathogens, standard method using culture and antimicrobial susceptibility testing are available, thus an appropriate antibiotic therapy can be promptly administered. This approach is generally unavailable for *H. pylori*, and decisions about the choice of antibiotic treatment is therefore based on the knowledge of antibiotic use in the population, resistance development in the region, and history of *H. pylori* eradication of patients [10].

Treatment of *H. pylori* infection in Vietnam follows the latest international guideline (the Maastricht IV/ Florence Consensus Report) [6]. It is currently recommended to split first-line empiric therapy into two large groups: populations with low (< 20%) and with high resistance (≥ 20%) to clarithromycin. For these groups, the acceptable resistance levels are set as 20%. The recommendations and therapies available for the eradication of *H. pylori* are mentioned as first-, second-, and third- line treatments, according to clarithromycin resistance [6] as summarized in Table 1.

The latest IV Maastricht consensus recommends that PPI-clarithromycin-containing triple therapy without prior susceptibility testing should be abandoned in areas where clarithromycin resistance

Table 1. *Helicobacter pylori* treatment regimens according to clarythromicin resistance.

Treatment	Population with low resistance to clarithromycin	Population with high resistance to clarithromycin
First-line	**PPI-based triple therapy** PPIs, omeprazole or equivalent (20mg/12h) + clarithromycin (500mg/12h) + amoxicillin (1g/12h), for 7- 14 days. In cases of allergy to penicillin, metronidazole is an option to replace amoxicillin	**Bismuth-based quadruple therapy** PPI, bismuth subsalicylate (525mg, 4 times daily), + metronidazole (250mg 4 times daily) + tetracycline (500mg 4 times daily), for 10-14 days or **Non bismuth-based quadruple therapy** Sequential therapy: PPI + amoxicillin (1g twice daily) for 5 days, followed by a PPI + clarithromycin + metronidazole (500 mg twice daily) for 5 days or Concomitant therapy: simultaneous administration of PPI + metronidazole, clarithromycin and amoxicillin for 10 days
Second-line	**Bismuth-based quadruple therapy** or **Non bismuth-based quadruple therapy** or **Levofloxacin containing triple therapy** PPI (20mg b.d.) + levofloxacin (250mg b.d.) + amoxycillin (1g b.d.) for 10 days	**Levofloxacin containing triple therapy**
Third-line	Treatment should be guided by individual antimicrobial susceptibility testing	

recorded is higher than 20% [6]. Therefore, according to this advice, the use of clarithromycin should be dissuaded in Vietnam as first-line treatment of *H. pylori* infection without a preliminary assessment of drug susceptibility. This is true for metronidazole due to the high rate of primary resistance recorded, although standard susceptibility testing for metronidazole lacks reproducibility *in vivo* [20].

In addition, considering the high rate of primary resistance (35.6%) [13], levofloxacin should not be considered as an alternative drug for *H. pylori* eradication in this country. Similar to clarithromycin, it had about 45% loss of effectiveness in the triple therapy PPI + levofloxacin + amoxicillin in case of levofloxacin resistant strains versus susceptible strains [15].

Therefore, currently in Vietnam first-line strategies such as bismuth-based quadruple therapy (a combination of a PPI, bismuth subsalicylate 525mg four times daily, and 2 antibiotics, metronidazole 250mg four times daily and tetracycline 500mg four times daily, for 10-14 days) or non bismuth-based quadruple therapy [6] with sequential therapy (the combination of a PPI and amoxicillin 1g twice daily for 5 days, followed by a PPI and clarithromycin plus metronidazole or tinidazole 500 mg twice daily for 5 days) or concomitant therapy (simultaneous administration of 3 antibiotics metronidazole, clarithromycin, amoxicillin and a PPI for 10 days) may be more effective.

Susceptibility Testing prior treatment: feasible for clinical setting?

Phenotypic methods

It is important to perform antibiotic susceptibility testing prior retreatment based on a case-by-case approach, if possible, in patients in whom more than two treatments have failed. Currently, the determination of resistance of *H. pylori* by culture and susceptibility testing is still the basic and more accurate method, although certain molecular techniques have appeared and replaced phenotypic methods [20].

Until now, the agar dilution assay proposed by the CLSI [20], is usually considered the reference method compared to other techniques, being the most accurate, but it is difficult to perform routinely. The E-test method has the advantage of being a quantitative method, it is adapted to slow-growing bacteria like *H. pylori* and has a good correlation with the agar dilution method [21]. Unfortunately, the E-test is economically impractical for clinical laboratory use when testing individual isolates, particularly in Vietnam.

On the contrary, the disk diffusion method is the simplest and the most economic for routine susceptibility testing but it is not well standardized for slow-growing bacteria like *H. pylori* [22]. Interestingly, some studies demonstrated a close relationship between inhibition diameter of disk diffusion method and minimum inhibitory concentration (MIC) by E-test [22-24].

Genotypic detection of resistance

The traditional culture test for antibiotics susceptibility requires 10–14 days, and is not routinely performed in clinical settings. MIC-based individualized treatment for *H. pylori* infection is not prevalent among clinicians.

Molecular techniques able to determine bacterial resistance to some antibiotics within a few days may have advantages [20].

Recently, sequencing analysis of *23S rRNA* gene related to clarithromycin resistance in strains from Vietnamese patients showed point mutations in all clarithromycin-resistant strains with a great diversity, in which quadruple mutations being the most represented with different combinations. Mutations in *23S rRNA* gene occurred especially in secondary resistant strains and strains with quadruple and quintuple mutations exhibited significantly higher MIC than strains with less mutations [13]. In this study, 85.7% of clarithromycin-resistant strains showed A2143G mutation which seems to play a major role in clarithromycin resistance but any A2142G/C point mutation was found. The absence of the A2142G/C mutation in Vietnamese strains may be due to geographical differences. It is similar to many studies conducted in other Asian countries with main point mutation at A2143 and absence at A2142 [25,26]. Moreover, mutations T2182C, A2223G, T2244C, A2302G, C2195T, C1953T were also common in Vietnamese strains [13].

Mutations in *gyrA* gene related to levofloxacin resistance in Vietnamese strains showed that hot-spot mutations were seen at Asp-91 and Asn-87 accounting for 80% of resistant strains with a variation of amino acid substitutions; moreover, the MIC of levofloxacin in strains harboring Asn-87 mutation was higher than in those with Asp-91 mutation [13].

Alongside with characteristics of point mutations in gene related to clarithromycin and levofloxacin in Vietnamese *H. pylori* strains, the molecular techniques for antibiotic susceptibility testing can focus on detection of mutations at A2143G in the *23S rRNA* gene and Asn-87 or Asp-91 in GyrA, such as PCR-RFLP and real-time PCR, dual-priming oligonucleotide-based multiplex PCR, DNA-strip test.

Conclusion

The findings in Vietnam highlight a very high rate of primary and secondary resistance to clarithromycin, metronidazole and levofloxacin in *H. pylori* strains.

This is of concern and suggests two important principles when prescribing a therapy in Vietnam: 1) clarithromycin-based or metronidazole-based triple therapy might not be useful as first-line therapies; 2) levofloxacin-based triple therapy should not be used as an alternative treatment.

Therefore, first-line strategies such as bismuth-based quadruple or non bismuth-based quadruple therapy should be recommended for Vietnamese infected patients.

Point mutations of *H. pylori* genes related to clarithromycin and levofloxacin resistance with common position at A2143G in *23S rRNA* gene and at Asn-87 or Asp-91 in *gyrA* gene in Vietnamese strains. In the near future, molecular methods to detect these common mutations may be implemented more widely in Vietnam.

Acknowledgements

This review is dedicated to Professor Piero Cappuccinelli, a great teacher of science and life, in honour of his personal commitment to Vietnam.

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Conflict of interests: No conflict of interests is declared.