

# Pattern of antibiotic resistance in *Helicobacter pylori*

Preeti Chaudhary, Varsha Gupta, Atul Sachdev

Department of Microbiology  
Government Medical College & Hospital, Sector-32, Chandigarh.

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## ABSTRACT

Antibiotic resistance has been an emerging concern for common bacterial infections worldwide. *Helicobacter pylori*, commonly associated with chronic bacterial infections, is also included in bacteria with drug resistant problems. Its infection, once considered curable, is now becoming a matter of grave concern with rising antibiotic resistant patterns reported worldwide. Resistance is mainly reported to the key antibiotics in the treatment of infection *i.e.* metronidazole and clarithromycin, and to a lesser extent to amoxicillin and tetracyclin, thereby decreasing the cure rates of the combination therapies used. Recently resistance to quinolones has also been reported.

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## INTRODUCTION

*Helicobacter pylori* is a fastidious Gram negative bacillus, found under the mucus layer on the epithelium of the gastric antrum of patients with chronic disease. Chronic infections caused by the organism include chronic gastritis, gastric and duodenal ulcers, mucosa associated lymphoid tumors (MALT) and adenocarcinoma of stomach. The bacterium was incidentally discovered by Marshall and Warren in 1983, and later in 2005 they were awarded the Nobel Prize for the discovery of *H. pylori*.<sup>[1]</sup> The organism is microaerophilic in nature and requires gaseous mixture of CO<sub>2</sub>/O<sub>2</sub>/N<sub>2</sub> in a ratio of 10:5:85% for growth, with high level of humidity. Culture media also require supplementation with 10% debrinated horse blood and selective antibiotic supplement containing vancomycin (10 mg/L), cefsulodin (5 mg/L), trimethoprim (5 mg/L) and amphotericin B (5 mg/L). The culture plates need incubation for 3-5 days at 37°C before considering the culture as negative. Biopsy is the most common specimen used for the culture of organisms. As proton pump inhibitors (PPIs) or antimicrobials inhibit the growth of *H. pylori*, their use reduces the chances of successful culture. Therefore patients should avoid

taking PPIs for at least two weeks and antimicrobials for at least four weeks prior to endoscopy.<sup>[2]</sup> Biopsy specimens should be transported and processed for culture as soon as possible, ideally within six hours. *H. pylori* is further identified based on colony characteristics identified (circular, convex and translucent) in media, cellular morphology (Gram-negative and helix-shaped under the microscope) and positive biochemical tests (urease, catalase and oxidase).

This article is compiled by reviewing various studies to sum up the prevalence of *H. pylori* and the resistant pattern of antibiotics used as combination therapies in order to formulate an opinion regarding treatment of infection in areas with low and high prevalence of *H. pylori*.

### Prevalence and pathogenesis

Prevalence of *H. pylori* infection varies in different geographical regions and ranges from 40% in developed countries to 90% in developing nations.<sup>[3]</sup> Prevalence is reported the least in children, increasing with age and is seen the maximum in the elderly.<sup>[3]</sup> The prevalence of *H. pylori* infection in India is extremely high. Up to 70–90% of patients with duodenal ulcer and 50–80% of patients with dyspepsia or healthy asymptomatic adults harbor the bacteria.<sup>[4-6]</sup>

The infection is acquired feco-orally; the bacterium resides under the layer of mucus of the gastric antrum epithelium and its habitat provides protection from the lethal action of gastric acid, thereby contributing in its pathogenesis. Most of the patients in high prevalence

Corresponding author: Dr. Varsha Gupta  
E-mail: varshagupta\_99@yahoo.com

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area with *H. pylori* are asymptomatic, whereas others are diagnosed with the infection during evaluation of dyspeptic symptoms. *H. pylori* infection may manifest as chronic gastritis, peptic ulcer disease, gastric neoplasms including adenocarcinoma and MALT. Besides its carcinogenic potential, the organism also has the potential to cause recurrent infections. *H. pylori* is also associated with iron deficiency anemia due to alterations in iron absorption and occult blood loss through the development of erosive esophagitis or peptic ulcer disease. It has also been suggested that *H. pylori* may utilize iron itself, further contributing for iron deficiency anemia.<sup>[7]</sup>

Eradication of *H. pylori* is associated with healing of ulcers and prevention of recurrence of infection. It may also cure low grade MALT lymphoma and incidence of gastric carcinoma is also reduced as reported by a meta-analysis study.<sup>[8]</sup> Chey *et al*<sup>[7]</sup> reported 25% reduction in the incidence of gastric cancer and a 67.4% reduction in the development of peptic ulcer disease after treatment.

#### Methods used for *H. pylori* detection

Methods used for the detection of *H. pylori* are histology, rapid urease test (RUT), culture, polymerase chain reaction (PCR) and various serology tests.<sup>[9]</sup> Histology was the first method used for the detection of *H. pylori*. Presence of typical bacteria along with inflammatory reaction was suggestive of *H. pylori* infection. Detection of urease production has been used as a surrogate marker of *H. pylori* infection, as this organism is a strong and rapid urease enzyme producer.<sup>[9]</sup> The organism can be cultured but it is microaerophilic and requires complex media for growth. Culture of the organism is a tedious, time-consuming procedure, and unnecessary for routine diagnosis of *H. pylori* infection because other non-invasive tests can detect evidence of the organism in a majority of the patients.<sup>[9]</sup> Advantage of the culture method is that it allows the testing of the antibiotic sensitivity of *H. pylori*.

Molecular methods are widely used for the detection of bacterium as well as the characterization of pathogenic genes and specific mutations associated with antimicrobial resistance. The conserved genes used for detection of *H. pylori* are urease operon: *urea*<sup>[10]</sup> and *glmM*, also known as *ure C*,<sup>[11]</sup> or the 16S rRNA,<sup>[12,13]</sup> 23S rRNA<sup>[14,15]</sup> and *hsp60*<sup>[16]</sup> gene. There are many modifications of the PCR for increasing the sensitivity of detection e.g. nested or semi-nested PCR using internal primer targeting conserved gene (heat shock protein;

Hsp60) which increases the specificity and sensitivity up to 100%<sup>[16,17]</sup> (approximately 3 organisms) after the nested cycles of amplification.<sup>[16]</sup> New methods like liquid phase DNA-enzyme immunoassay<sup>[18,19]</sup> and the reverse dot blot line probe assay (LiPA)<sup>[20]</sup> have also been proposed to increase its specificity and sensitivity. There are reports suggesting use of reverse transcription PCR (RT-PCR) which successfully shows the viability of the bacterium.<sup>[21, 22]</sup> The PCR technology may also be used to target pathogenic and resistant genes of *H. pylori*.

#### Antibiotics and Resistance Patterns

Several options are available to test the antimicrobial susceptibility testing of *H. pylori*. The disc diffusion method though cost-effective and widely used for antimicrobial susceptibility testing for most of the bacterial isolates, have not yet been defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for *H. pylori*.<sup>[23]</sup> Both EUCAST and the British Society for Antimicrobial Chemotherapy recommend E-test strips, a culture-based antimicrobial susceptibility testing for *H. pylori* as it enables the quantitative determination of the minimum inhibitory concentration of an antimicrobial agent required to inhibit bacterial growth.<sup>[24]</sup> Besides these phenotypic methods, molecular methods are reliable alternatives to culture as genetic identification of mutations in *H. pylori* directly from biopsy samples and culture material is rapid, thereby enabling to report the diagnosis the same-day. Molecular testing has been recommended to detect *H. pylori* genes and resistant genes in clarithromycin and quinolone. These mutations can be detected by a number of PCR based molecular methods. Several molecular testing kits are commercially available for the detection of clarithromycin resistance, including the MutaREAL *H. pylori* kit<sup>[25]</sup> (Immunodiagnostik; Bensheim, Germany), the ClariRes real-time PCR assay<sup>[26]</sup> (Ingentix; Vienna Austria) and the Seeplex ClaR-*H. pylori* ACE detection system<sup>[27]</sup> (Seegene; Eschborn, Germany). Accumulating evidence has demonstrated that the presence of mutations detected by molecular tests correlates well with culture-based susceptibility testing. A recently developed method i.e. GenoType HelicoDR assay (Hain Lifescience; Nehren, Germany) enables determination of resistance. This is highly accurate with a sensitivity and specificity of 94-100% and 86-99% respectively for clarithromycin resistance and 83-87% and 95-98.5% respectively for quinolone resistance.<sup>[28,29]</sup> In spite of good sensitivity and specificity, the majority of molecular tests available do not detect resistance based on rare genetic mechanisms. Therefore phenotypic methods should be paired along to ensure

the results.

Various treatment regimes including dual, triple and quadruple therapies are used for the eradication of *H. pylori*. Eight antibiotic drugs are available to treat *H. pylori* infection *i.e.* amoxicillin, tetracyclin, clarithromycin, metronidazole, levofloxacin, tinidazole, furazolidine and rifabutin. Adjuvant drugs *i.e.* PPI, H<sub>2</sub>-blockers and bismuth containing agents enhance the performance of antimicrobials.

One week triple therapies combining a PPI such as omeprazole and a low dose of two antibiotics (metronidazole or amoxicillin plus clarithromycin) are the overall most effective eradication regimens with eradication rates of about 90%.<sup>[30]</sup> Though cure rate after treatment is up to 90%, a matter of concern is the rising antimicrobial resistance for mainly three antibiotics *i.e.* Clarithromycin, metronidazole and levofloxacin. These being the key antibiotics for treatment of *H. pylori*, they have reduced the success rate of treatment from 90 to 70% in the past decade. Antibiotic resistance is associated with both genetic alteration and biofilm formation.<sup>[31]</sup>

Reports of *H. pylori* resistance have been published from different geographic regions worldwide with different ranges. Prevalence of clarithromycin, metronidazole and levofloxacin resistance is increasing over time with higher reports from developing countries than developed countries.

Clarithromycin is a second generation macrolide. Resistance to clarithromycin is the major reason for decrease in susceptibility as it is the most effective antibiotic of combination therapy against *H. pylori*. Studies from European countries have reported resistance in clarithromycin ranging from 31 to 36.7% (2012-13) which is much higher than that reported in 2008-09 (7% to 21%).<sup>[31]</sup> Clarithromycin resistance has almost doubled during the past 10 years.<sup>[31]</sup> Studies from India have reported varying results. Clarithromycin was reported as sensitive in all the strains tested from Kolkata<sup>[32]</sup> and Delhi<sup>[33]</sup> although other studies have reported resistant strains. The resistant strains reported were 96% from Hyderabad,<sup>[34]</sup> 91% from Mumbai,<sup>[34]</sup> 58.8% from Gujarat<sup>[34]</sup> and 10% from Chandigarh.<sup>[33]</sup> Clarithromycin resistance is due to point mutations in 23S rRNA genes, different nucleotide positions involved are 2143 (A2143G) and 2144 (A2144G). About 90% of the cases of primary clarithromycin resistance in western countries are due to A2143G, A2142G and A2142C mutations.<sup>[35]</sup>

Mutation varies with geographical area. Resistance is most commonly due to the use of macrolides in treatment of respiratory tract infections. Mcmohan *et al*<sup>[31]</sup> in his study demonstrated a statistical significant relationship between patients with previous use of antibiotics and subsequent isolation of resistant *H. pylori* strains. This study also includes metronidazole. Resistance to metronidazole has remained high in the past decade ranging from 35.5% to 45.5%.<sup>[31]</sup> Resistance reported in Arctic countries ranges from 42 — 66%.<sup>[36]</sup> Studies from India show comparatively higher resistance rate. A study from Kolkata has reported 85% resistant strains. High metronidazole resistance has also been reported from other states like Delhi, Lucknow and Mumbai.<sup>[32]</sup> Pandya *et al*<sup>[34]</sup> has reported 83.8% resistance in Gujarat, 37.5% in Delhi and 38.2% resistant strains from Chandigarh. A multicentric study from South India has reported 100% resistant strains from Hyderabad, 88.2% from Chennai and 68% from Lucknow.<sup>[37]</sup>

Metronidazole resistance of *H. pylori* is closely related to the indiscriminate use of this antibiotic as it is easily available and cheap. It is used as an anti-parasitic, in the treatment of genital and dental infections, and also in anaerobic infections. Resistance varies geographically, corresponding to its use. A high rate of resistance up to 90%, is seen in African countries while it is around 95% to 100% in India and China, 45% in Spain, 22.5% in Norway and 3.3% to 4.9% in Japan.<sup>[3, 31, 33]</sup>

Varied resistance pattern for amoxicillin has also been reported from India and abroad. Mainly low prevalence is reported, except for a few studies from India were 72.5%, 73% and 80% resistance is reported from Gujarat, Mumbai and Hyderabad.<sup>[37]</sup> All sensitive strains have been reported from Kolkata, North India (Lucknow, Delhi and Chandigarh) and south India (Chennai). Low amoxicillin resistance (0.7%) has been reported from Europe.<sup>[38]</sup> Other studies have also indicated that the rates of resistance to amoxicillin are < 1% in China, Bahrain, Malaysia, Bhutan and Vietnam but are >10% in Japan.<sup>[31]</sup> Low prevalence was also reported in Southeast Asia, Latin America and Brazil. Thus the inclusion of this antibiotic in empirical eradication regimens can still be considered.<sup>[31]</sup>

Quinolones generally are not effective antibiotics against *H. pylori*, but levofloxacin or ciprofloxacin could be given in combination with amoxicillin as the second line antibiotic in strains resistant to clarithromycin and metronidazole. Initially the eradication success using

new regimen containing levofloxacin was as high as 90%, but same studies have reported increasing quinolone resistance.<sup>[39,40,41]</sup> In Europe, levofloxacin resistance rates have reached 18% and 18.8% rates have been reported from Germany in 2013.<sup>[38,42,43]</sup> Resistance rates are low (8.8%) in Taiwan, but higher in Brazil (23%), Iran (72.5%) and India (50%).<sup>[44]</sup> Ciprofloxacin resistance was 1-8% in Western countries.<sup>[45,46]</sup> Reports from India also show low level resistance. Hyderabad and Mumbai reported zero level ciprofloxacin resistance but 20% resistance rate has been reported from Lucknow.<sup>[47,48,49]</sup> A multicentric study from north India reported 2.7% ciprofloxacin resistance.<sup>[33]</sup> Resistance again is due to the use of quinolones both in respiratory tract infections and urinary tract infections.

Tetracyclin resistance ranging from 1.9 to 6.0% has been reported in various studies.<sup>[50,51,52]</sup> In a multicentric study from India, tetracyclin resistance was 4% from Hyderabad strains whereas Mumbai and Lucknow reported 27% and 10% tetracyclin resistance respectively.<sup>[47,49]</sup>

Rapidly increasing prevalence of antibiotic resistance has called for an urgent need to think about newer antibiotics such as furazolidone. Triple therapy with furazolidone-based regimens cannot achieve ideal eradication rates therefore it cannot be considered as a suitable option for first-line treatment. Quadruple furazolidone-containing regimens including probiotic is recommended, which could achieve more than 90% eradication rates.<sup>[53]</sup> A study from Iran has reported resistance rate to furazolidone between 0 to 4.5%.<sup>[36]</sup> Datta *et al*<sup>[32]</sup> has reported no resistant strain to this antibiotic from Kolkata, India. Till date no furazolidone resistance has been reported from any part of the world except 13.8% resistance from Gujarat and Mumbai, India.<sup>[34]</sup> This antibiotic is not used in developed countries due to its purported carcinogenic effect which is not proven yet.<sup>[54,55]</sup>

Multi drug resistant (MDR) strains have also been reported in various studies, Pandya *et al*<sup>[34]</sup> have reported 85.1% MDR *H. pylori* strains resistant to metronidazole, clarithromycin, and tetracyclin. A multicentric study from north India reported 31.6% resistant strains for metronidazole, amoxicillin and clarithromycin.<sup>[33]</sup> No MDR *H. pylori* strain has been isolated from Delhi.<sup>[33]</sup> In addition, 18.1% of the strains in a study from Turkey were found to harbor clarithromycin, metronidazole and levofloxacin resistance.<sup>[31]</sup>

Various attempts have been made to improve the

efficacy of standard treatments in use, e.g adding bismuth, increasing the dose of PPI, prolonging treatment duration and introducing new antibiotics, but none proved to be fruitful. An attractive option was to introduce probiotics in standard regimens. Studies have reported variable results, but currently there is evidence to support the use of probiotic supplementation to standard triple therapy.<sup>[56,57]</sup> This benefit is particularly reported in patients with recurrent infection and those with history of antibiotic side effects.<sup>[58]</sup> Further studies are ongoing to establish the evidence of probiotics for their significant benefit in the *H. pylori* infection.

Guidelines have been developed in the United States and Europe (areas with low prevalence) for the management of *H. pylori* infection. 'Test and treat' strategy is applied to those with dyspepsia, but these guidelines will not be appropriate in high prevalence areas like India. A group of international experts performed a targeted literature review and formulated an expert opinion for evidence-based benefits and harms for screening and treatment of *H. pylori* in high-prevalence countries. They concluded that in Arctic and Asian countries where *H. pylori* prevalence exceeds 60%, treatment of persons with *H. pylori* infection should be limited only to instances where there is a strong evidence of direct benefit in reduction of morbidity and mortality, associated peptic ulcer disease and MALT lymphoma and that the test- and-treat strategy may not be beneficial for those with dyspepsia in high prevalent areas.<sup>[35]</sup>

To provide effective treatment, susceptibility testing of antibiotics is very important, but *H. pylori* being microaerophilic, its culture and susceptibility testing needs different laboratory set up which is difficult and costly in developing countries like India. Therefore surveillance studies on *H. pylori* resistance patterns at regional and national levels are recommended. Periodic review of reports with antimicrobial susceptibility pattern can at least provide general guidelines for effective and successful eradication of *H. pylori*.

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