

## Review of current diagnostic methods and advances in *Helicobacter pylori* diagnostics in the era of next generation sequencing

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### Abstract

*Helicobacter pylori* (*H. pylori*) infection is highly prevalent in the human population and may lead to severe gastrointestinal pathology including gastric and duodenal ulcers, mucosa associated tissue lymphoma and gastric adenocarcinoma. In recent years, an alarming increase in antimicrobial resistance and subsequently failing empiric *H. pylori* eradication therapies have been noted worldwide, also in many European countries. Therefore, rapid and accurate determination of *H. pylori*'s antibiotic susceptibility prior to the administration of eradication regimens becomes ever more important. Traditionally, detection of *H. pylori* and its antimicrobial resistance is done by culture and phenotypic drug susceptibility testing that are cumbersome with a long turn-around-time. Recent advances in diagnostics provide new tools, like real-time polymerase chain reaction (PCR) and line probe assays, to diagnose *H. pylori* infection and antimicrobial resistance to certain antibiotics, directly from clinical specimens. Moreover, high-throughput whole genome sequencing technologies allow the rapid analysis of the pathogen's genome, thereby allowing identification of resistance mutations and associated antibiotic resistance. In the first part of this review, we will give an overview on currently available diagnostic methods for detection of *H. pylori* and its drug resistance and their implementation in *H. pylori* management. The second part of the review focusses on the use of next generation sequencing technology in *H. pylori* research. To this end, we conducted a literature search for original research articles in English using the terms "*Helicobacter*", "transcriptomic", "transcriptome", "next generation sequencing" and "whole genome sequencing". This review is aimed to bridge the gap between current diagnostic practice (histology, rapid urease test, *H. pylori* culture, PCR and line probe assays) and new sequencing technologies and their

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potential implementation in diagnostic laboratory settings in order to complement the currently recommended *H. pylori* management guidelines and subsequently improve public health.

**Key words:** *Helicobacter pylori*; Advances in diagnostics; Next generation sequencing; Whole genome sequencing; Clinical management

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**Core tip:** With worldwide increasing antibiotic resistance in *Helicobacter pylori* (*H. pylori*), drug resistance phenotypes should be determined prior to the administration of antibiotic eradication regimens. Our literature search yielded studies that focused on the prediction of drug resistance phenotypes in *H. pylori* based on the presence of certain point mutations in the bacterium's genome using next generation sequencing (NGS) technology. Thus, NGS technology may enable the implementation of rapid and accurate genotypic drug susceptibility testing prior to the administration of antimicrobial therapy. This may increase *H. pylori* eradication rates and ultimately improve patient management.

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## HELICOBACTER PYLORI PREVALENCE, EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE

### Current *Helicobacter pylori* prevalence and epidemiology

Initial acquisition of *Helicobacter pylori* (*H. pylori*) occurs primarily during childhood and may persist throughout life<sup>[1]</sup>. Infection with *H. pylori* occurs worldwide, but there are substantial geographic differences in the prevalence of infection between countries<sup>[2]</sup>. Multiple studies have demonstrated that socioeconomic status and ethnic origin of the population are strongly associated with prevalence of *H. pylori* infection<sup>[3-5]</sup>. In Central and Northern Europe, *H. pylori* prevalence, excluding non-European immigrants, was found to be around 24% to 32%<sup>[6-10]</sup>. Studies conducted in Switzerland revealed a *H. pylori* prevalence of 12%-20% in patients born in Switzerland and a prevalence of 27% in immigrants<sup>[5,11]</sup>. *H. pylori* can be divided into relatively distinct populations that are specific for large geographical areas: HpEurope, hpSahul, hpEastAsia, hpAsia2, hpNEAfrica, hpAfrica1 and hpAfrica2<sup>[12-14]</sup>. The most prevalent *H. pylori* populations in Europe are hpEurope and hpNEAfrica<sup>[15]</sup>.

### Management of *Helicobacter pylori* infections

In most patients, *H. pylori* infection stays asymptomatic, but it can progress to various gastrointestinal diseases including chronic active gastritis, peptic or duodenal ulcers, gastric adenocarcinoma and mucosa associated tissue lymphoma<sup>[16]</sup>. Consequently, it is a challenge for physicians to decide who should be tested for *H. pylori* infection and who should be treated. In general, treatment is recommended in case of detection of *H. pylori* infection, even in patients with asymptomatic *H. pylori* gastritis<sup>[17,18]</sup>. This practice is supported by results from a systematic review of six randomized trials evaluating *H. pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic individuals that found a significant reduction in the incidence of gastric cancer<sup>[19]</sup>. However, this conclusion is mostly based on results of one interventional, placebo-controlled trial that was conducted in China<sup>[20]</sup>, a high incidence country for gastric cancer. Therefore, further studies are needed in countries with low prevalence of gastric cancer to evaluate the long-term cost-effectiveness of such interventions. In the United Kingdom, two placebo-controlled trials conducted a *H. pylori* screening and treatment program in the general population that reduced dyspepsia in patients, who receive eradication therapy<sup>[21,22]</sup>. Though, they concluded that targeted eradication strategies in dyspeptic patients may be preferable. Therefore, the main question in clinical practice remains: Who should be tested and consequently treated?

Based on recent research, current guidelines (*i.e.*, fifth Maastricht/Florence consensus report<sup>[23]</sup>) recommend testing for *H. pylori* infection in situations described in **Table 1**.

Adult patients in industrial countries that have been successfully treated for *H. pylori* infection rarely show reinfection (reinfection rate of 2%)<sup>[24]</sup>. Therefore, adequate treatment promises high eradication efficacy (see next chapter for antimicrobial therapy options) without recurrence of *H. pylori* infection. However, there are major challenges in the treatment of *H. pylori* infection including increasing resistance to antibiotics, which will be discussed in detail in the next section, and compliance to therapy. A study performed in Switzerland showed that approximately 89% of the patients treated were considered as “good compliers”, meaning that they consumed more than 85% of the prescribed doses<sup>[25]</sup>. In this study, *H. pylori* eradication was inversely associated with poor compliance ( $P = 0.029$ ) and the major reason mentioned by the patients not complying with the treatment was side effects. Antibiotic therapy indeed has non-negligible, short-term side effects such as diarrhea, nausea, vomiting, bloating and/or abdominal pain. Moreover, it has been shown, and also received media attention, that antibiotic treatment can alter the gut microbiota richness and diversity<sup>[26,27]</sup>, possibly deferring health-conscious patients from following through with antibiotic treatment.

### **Antibiotic resistance in *Helicobacter pylori***

The only currently available efficient treatment against *H. pylori* infection includes the use of antibiotics. Main mechanisms of antibiotic resistance development in *H. pylori* include mutations that impair the capability of antibiotics to bind the ribosomes and interfere with protein synthesis; mutations that affect DNA replication and transcription; mutations that modify penicillin binding proteins, involved in peptidoglycan biosynthesis<sup>[28]</sup>. As *H. pylori* easily develops drug resistance to single antibiotics, combination therapy of several antibiotics is recommended. Combination of antibiotics used in therapy should depend on local drug resistance rates estimated in the respective country. Primary and acquired resistance to clarithromycin and metronidazole has increased globally in the last years, diminishing the effectiveness of conventional first-line treatment regimens and increasing treatment failures due to drug-resistant *H. pylori* strains<sup>[29-33]</sup>. In particular, clarithromycin resistance increased rapidly in several countries, to reach 30% in Japan and up to 50% in China<sup>[32]</sup>. Also in Europe, an increasing trend of clarithromycin resistance in *H. pylori* can be observed with an overall primary clarithromycin resistance rate of 17%<sup>[34]</sup>. However, prevalence of clarithromycin resistance varies from 21% in Austria to 6% in Finland and the Netherlands<sup>[10,35]</sup>. This shows that clarithromycin resistance is strongly variable between neighboring European countries emphasizing the need to examine drug resistance separately in each geographic region to better guide empiric treatment regimens. Metronidazole resistance, although not as important as clarithromycin resistance, also significantly reduces treatment efficiency of the standard triple regimen<sup>[36]</sup>. Overall, metronidazole resistance rates have been increasing in many European countries<sup>[34,37-40]</sup>, ranging from 14% to 33% in Europe<sup>[41-46]</sup>. The general trend towards increasing resistance to first-line antibiotics in *H. pylori* has urged treating physicians to prescribe alternative treatment regimens including tetracycline with a PPI and a bismuth salt or the use of levofloxacin or rifabutin-based treatment regimens<sup>[23,47,48]</sup>. However, these regimens require high patient compliance as antibiotic therapy consist of many tablets that have to be taken daily for 10 to 14 d<sup>[49]</sup>. Incomplete patient adherence to antibiotic therapy is directly associated with further resistance development in *H. pylori*. Although levofloxacin resistance has not been studied as extensively as clarithromycin and metronidazole resistance, there is also a trend towards high primary and secondary levofloxacin resistance in *H. pylori*<sup>[35,38-40,50]</sup>. In contrast, resistance to amoxicillin and tetracycline seems to be negligible in *H. pylori* (0 to 2%) in European countries<sup>[35,40,51-53]</sup>. Though tetracycline resistance does not seem alarming yet in Western Europe, high resistance rates ranging from 5% to 19% were found in Eastern European and Asian countries<sup>[54,55]</sup>, emphasizing the need to prevent further resistance spread in *H. pylori*. Therefore, similar as for first-line *H. pylori* eradication regimens, administration of alternative antimicrobial therapy, especially when levofloxacin based, should be guided by the regional and patient-specific antimicrobial resistance patterns and knowledge about their local effectiveness.

## **CURRENT *HELICOBACTER PYLORI* DIAGNOSTICS**

Several diagnostic methods are available for detecting *H. pylori* infections. They can be broadly classified as invasive and non-invasive methods depending on the need to retrieve a gastric biopsy from the patient. For *H. pylori* detection, endoscopy is

Table 1 Who to test, summary of the recommendations from the fifth Maastricht/Florence consensus report<sup>[23]</sup>

Recommendations from the fifth Maastricht/Florence consensus report	
Dyspepsia	Subject to regional <i>Helicobacter pylori</i> prevalence, not applicable to patients with alarm symptoms or older patients
Peptic ulcers	Especially in aspirin and nonsteroidal anti-inflammatory drugs users with history of peptic ulcer
Gastritis	Especially in long-term proton-pump-inhibitor users
Gastric cancer	In individuals at increased risk of gastric cancer
MALToma	In individuals with localized early-stage MALToma
Iron deficiency anemia, idiopathic thrombocytopenic purpura, vitamin B12 deficiency	

employed in combination with histology and/or culture from the gastric biopsy specimen. The major limitation of endoscopic examination is its relative invasiveness and that only a small portion of the gastric mucosa can be explored. Therefore, assessment of multiple gastric biopsy specimens is necessary to provide a global picture of *H. pylori* infection in the stomach<sup>[56,57]</sup>. When an endoscopy is indicated, *H. pylori* can be detected by histology, rapid urease test (RUT), culture and polymerase chain reaction (PCR)-based tests using gastric biopsy specimens<sup>[58,59]</sup>. The accuracy of histology depends on a number of factors like the pathologist's experience, density of *H. pylori* colonization in the gastric mucosa, the quality and quantity of the clinical specimen and subjective assessment of tissue changes.

The RUT is based on detecting urea produced by *H. pylori*, and results are obtained within minutes to hours. The RUT is a cheap, rapid and generally highly specific assay, but its sensitivity is affected if less than  $10^4$  bacterial cells are present in the gastric biopsy, most probably leading to false-negative results. In some instances, RUT specificity may be negatively affected by the presence of other urease producing bacteria like *Staphylococcus capitis urealiticum* in the stomach that can lead to false-positive test results<sup>[60]</sup>. Commercially available RUTs (e.g., HpFast, CLOTest, HpOne) have reported specificities from 95% to 100%, but their sensitivity is moderate (85% to 95%)<sup>[17,61,62]</sup>.

Successful isolation and cultivation of *H. pylori* from gastric biopsy specimens is a challenging task that is affected by a number of factors like the quality of the clinical specimen, occurrence of microbial commensal flora in clinical specimens, time interval between sampling and culture and inappropriate transport conditions (temperature, duration of air exposure, etc.). Furthermore, *H. pylori* culture requires highly trained laboratory personnel and takes up to 7 d until samples can be reported as negative and up to 2 wk until *H. pylori* has grown and an antibiogram can be provided to the treating physician. *H. pylori* culture from gastric biopsy specimens typically has a sensitivity greater 90% and a specificity of 100%, when performed under optimal conditions<sup>[63]</sup>. *H. pylori* culture from clinical specimens obtained by non-invasive procedures, such as gastric juice, saliva and stool, is challenging and hampered by low sensitivity<sup>[64-66]</sup>, and therefore not recommended in routine diagnostics<sup>[67]</sup>. With the global emergence of antibiotic resistant *H. pylori* isolates and subsequently increasingly failing empiric first-line therapies, bacterial culture and phenotypic drug susceptibility testing (DST) remains a crucial diagnostic method for antibiotic resistance surveillance and management of antibiotic treatment failures. However, it is not recommended to do a full phenotypic DST before administration of first-line treatment as: (1) An invasive endoscopy is required to obtain gastric biopsy specimens from the patient; (2) It is time consuming and costly; and (3) Less invasive, molecular based methods are also able to detect clarithromycin resistance that is momentarily the main cause of empiric treatment failure in European countries.

Due to these drawbacks, numerous attempts have been made to develop non-invasive diagnostic methods that avoid endoscopy. Classically, non-invasive tests for *H. pylori* detection include stool antigen assays, serology and the frequently used urea breath test (UBT)<sup>[68,69]</sup>. Antigen tests have been widely used for *H. pylori* detection in clinical specimens like gastric juice, saliva, urine and stool<sup>[70-72]</sup>. However, antigen detection methods, may suffer from poor specificity and sensitivity<sup>[70,71,73]</sup>. Different stool antigen tests have been developed to detect *H. pylori* in stool specimens with a sensitivity and specificity of 85% to 95%<sup>[17]</sup>. The UBT is the most frequently used point-of-care test in the clinic with a sensitivity and specificity of 85% to 95%<sup>[17,74]</sup>.

One limitation of the beforehand presented non-invasive diagnostic methods is that they can solely detect *H. pylori* but do not provide information on the drug susceptibility of the bacterium. With increasing clarithromycin resistance rates in

*H. pylori*, rapid and accurate methods that can simultaneously detect *H. pylori* and assess its clarithromycin susceptibility offer high added value<sup>[10,44,75]</sup>. Clarithromycin resistance in *H. pylori* is attributable, in a majority of cases, to three single point mutations (A2146C, A2146G and A2147G) in the 23S rRNA gene that can be accurately detected by PCR<sup>[76-79]</sup>. At the moment, there are a number of molecular assays commercially available for *H. pylori* and clarithromycin resistance detection, such as the *H. pylori* ClariRes (Ingenetix, Vienna, Austria), the Allplex *H. pylori* and ClariR (Seegene, Korea), the Lightmix<sup>®</sup>*H. pylori* (TIBMolbiol, Germany) and the *H. pylori* Taqman<sup>®</sup> real-time PCR assay (Meridian Bioscience, United States). These assays mostly combine real-time PCR with melting curve analysis and are highly specific and rapid (< 2 h) molecular methods that can be applied to gastric biopsy and stool specimens<sup>[77,78]</sup>. Moreover, they can distinguish the three most common point mutations (A2146G, A2147G and A2146C) in the 23S rRNA gene, which allows to genotypically distinguish low- and high-level clarithromycin resistance<sup>[77]</sup>. However, several studies found rather low sensitivity (ranging from 63% to 84%) of *H. pylori* detection from stool specimens using the ClariRes assay when compared to stool antigen test and *H. pylori* culture from gastric biopsy specimens<sup>[80-82]</sup>. Another study validating the *H. pylori* Taqman<sup>®</sup> real-time PCR assay in stool specimens reported higher sensitivity of 93.8%<sup>[79]</sup>. Therefore, *H. pylori* and clarithromycin resistance detection directly from stool specimens may strongly depend on the DNA extraction method and the PCR assay used. Consequently, no general statement on the diagnostic performance of PCR from stool can be made. One limitation of PCR assays is, however, that they can just provide resistance information for clarithromycin. At the moment, there is only one line probe assay (the Genotype HelicoDR assay; Hain Life Sciences, Germany) commercially available that enables the detection of the most common point mutations in the 23S rRNA (A2146G, A2147G and A2146C) and the *gyrA* gene (N87K, D91G, D91N, D91Y) to determine clarithromycin and levofloxacin susceptibility, respectively. The Genotype HelicoDR assay has been reported to accurately detect *H. pylori* and clarithromycin resistance from gastric biopsy specimens<sup>[83]</sup>, but low concordance between *H. pylori* and clarithromycin resistance detection from biopsy and stool specimens was found<sup>[84]</sup>. Moreover, the Genotype HelicoDR assay has a long turn-around-time of 6 h compared to real-time PCR assays.

In sum, non-invasive molecular testing from stool would have the following advantages: (1) No invasive endoscopy is required; (2) Specimens can be stored longer and do not require immediate processing; (3) Batching of specimens is possible; (4) *H. pylori* detection and genotypic clarithromycin susceptibility screening can be done within one working day (< 4 h); (5) Detection of hetero-resistance in specimens is achievable when more than one *H. pylori* strain is present in a clinical specimen; (6) Automated DNA extraction and real-time PCR analysis offers a high degree of standardization and reproducibility. However, further studies are needed that assess the diagnostic performance of optimized DNA extraction procedures and non-invasive stool PCRs (ideally targeting the 23S rRNA and *gyrA* gene) in comparison to *H. pylori* culture based phenotypic DST from gastric biopsy specimens.

## HELICOBACTER PYLORI TREATMENT AND PROPHYLAXIS

As *H. pylori* antibiotic therapy is mostly based on clarithromycin, clarithromycin resistance is the major determinant of antimicrobial treatment: In countries with low clarithromycin resistance (*i.e.*, < 15%), current first-line standard regimens for *H. pylori* eradication are a proton pump inhibitor (PPI)-based triple therapy (with clarithromycin in combination with metronidazole or amoxicillin) or a bismuth quadruple therapy<sup>[23,85,86]</sup>. The second line therapy should then be the bismuth-quadruple (if not used as first-line therapy) or a triple therapy containing fluoroquinolones. Choice of third-line therapy should be guided by phenotypic DST or genotypic determination of drug resistance (associated costs for antibiotic therapy are listed in Tables 2 and 3; approximate drug prices from Germany).

In countries with high clarithromycin resistance (*i.e.*, > 15%), metronidazole resistance, although clinically less relevant, should be considered. If metronidazole resistance is low, a triple therapy with PPI, amoxicillin and metronidazole can be applied. If the dual resistance for clarithromycin and metronidazole is low, a bismuth quadruple or a concomitant non-bismuth quadruple therapy should be used. However, if the dual resistance is high, bismuth containing quadruple therapies should be used<sup>[23]</sup>.

Vaccines against *H. pylori* have only recently been given serious consideration. In animal models, initial vaccination tried oral immunization with *H. pylori* bacterial lysate and cholera toxin as adjuvant<sup>[87]</sup>. Later on, intranasal and rectal delivery systems

Table 2 Standard clarithromycin-based triple regimens using metronidazole or amoxicillin and associated costs

	Drug	Dose	Costs (EUR) per dose	Costs (EUR) for 7 d	Costs (EUR) for 14 d
Standard triple regimen (with metronidazole)	Clarithromycin	500 mg (twice daily)	1.1	15.4	30.8
	Metronidazole	500 mg (three times daily)	0.6	13.1	26.2
	Pantoprazole (Proton-pump inhibitor)	Standard dose (twice daily)	0.1	1.6	3.2
<b>In total</b>			<b>1.8</b>	<b>30.1</b>	<b>60.2</b>
Standard triple regimen (with amoxicillin)	Clarithromycin	500 mg (twice daily)	1.1	15.4	30.8
	Amoxicillin	1 g (twice daily)	1.4	20.2	40.4
	Pantoprazole (Proton-pump inhibitor)	Standard dose (twice daily)	0.1	1.6	3.2
<b>In total</b>			<b>2.6</b>	<b>37.2</b>	<b>74.4</b>

allowed reducing the required amount of purified antigen compared to oral immunization. Given the proposed mechanism of action involving the cellular immune system<sup>[88]</sup>, parenteral immunization with *H. pylori* antigens has been shown to result in a significant protection from infection in mice models<sup>[89]</sup>. However, although clinical trials in human on immunization with *H. pylori* proteins have shown adaptive immune mechanisms, they have since failed to consistently reduce bacterial load<sup>[90]</sup>. More studies are needed in this area.

Due to increasing antibiotic resistances and side effects of antibiotics, alternative therapies are of great interest. Probiotics have been shown to have positive effects on eradication rates, prevention of adverse reactions and antibiotic-associated diarrhea when combined to eradication therapies. A recent systematic review and meta-analysis on probiotics as adjunct therapy found 19 randomized controlled trials, all showing positive effects on at least one of the above-mentioned aspects<sup>[91]</sup>. However, it appears that the number of meta-analysis on the topic exceeds the number of original publications, that, even in randomized controlled fashion show large detail variance<sup>[92]</sup>. Interestingly, a large and well performed meta-analysis showed that probiotic dose, duration, number of strains and duration of antibiotic treatment did not affect the benefits conferred by probiotic adjunction<sup>[93]</sup>, reducing the scientific plausibility of this intervention based on current publications. The fifth Maastricht/Florence consensus rapport acknowledges probiotics as beneficial in its report, but evaluates the level of evidence as low to moderate with weak grade of recommendation. That being said, probiotics alone, not in combination with antibiotics, have not been shown to efficiently eradicate *H. pylori*<sup>[94]</sup>. We as others conclude that "more data are definitely needed to assess the direct efficacy of probiotics against *H. pylori*"<sup>[23]</sup>.

Licorice root is a botanical product frequently used in Chinese medicine. It has detoxifying, antiulcer, anti-inflammatory, anti-viral and anticarcinogenic properties<sup>[95]</sup>. A randomized controlled trial on 120 *H. pylori* positive dyspeptic patients (with or without peptic ulcer) assessed the effect of licorice in addition to clarithromycin-based triple regimen. They showed that treatment response was 83.3% in the licorice-group compared to 62.5% in the control group ( $P = 0.018$ ). When distinguishing between peptic ulcer disease and non-ulcer dyspepsia, significantly better response to treatment was only observed in patients with peptic ulcer ( $P = 0.034$ )<sup>[96]</sup>.

Several other plant-based products are used for the treatment of gastrointestinal disorders. Some of them have been mentioned as influencing *H. pylori* infections such as garlic, cranberry juice, oregano or broccoli sprouts (non-exhaustive list)<sup>[97]</sup>. However, few studies have identified the active ingredient or its mechanism of action and dose/response or exposure level are not understood. Possible safety issues as well as impact of resistance on efficacy of phyto-therapeutic agents has to be addressed. Moreover, one review article mentioned the possibility of phage therapy against *H. pylori*<sup>[98]</sup>.

## NEXT GENERATION SEQUENCING AND *HELICOBACTER PYLORI*

In order to get an overview on original research studies that focused on the characterization of *H. pylori* by next generation sequencing (NGS) a PubMed

**Table 3** Alternative antibiotic *Helicobacter pylori* eradication therapy using quadruple or levofloxacin-based regimens and associated costs

Regimen	Drug	Dose	Costs(EUR) per dose	Costs (EUR) for 7 d	Costs (EUR) for 14 d
Bismuth quadruple regimen	Tetracycline	500 mg (four times daily)	0.6	12	16.8
	Metronidazole	500 mg (three times daily)	0.6	18.6	26.2
	Pantoprazole (Proton-pump inhibitor)	Standard dose (twice daily)	0.1	2.2	3
	Bismuth Subsalicylate	Standard dose (three times daily)	0.3	10	14
<b>In total</b>			<b>1.6</b>	<b>42.8</b>	<b>60</b>
Levofloxacin-based regimen	Levofloxacin	500 mg (once daily)	2.7	27	38
	Amoxicillin	1 g (twice daily)	1.4	28.8	40.4
	Pantoprazole (Proton-pump inhibitor)	Standard dose (twice daily)	0.1	2.2	3.1
<b>In total</b>			<b>4.2</b>	<b>58</b>	<b>81.5</b>
Concomitant regimen	Clarithromycin	500 mg (twice daily)	1.1	22	30.8
	Amoxicillin	1 g (twice daily)	1.4	28.8	40.4
	Metronidazole	500 mg (three times daily)	0.6	2.2	3
	Pantoprazole (Proton-pump inhibitor)	Standard dose (twice daily)	0.1	18.6	26.04
<b>In total</b>			<b>3.2</b>	<b>72</b>	<b>100</b>

MEDLINE and EMBASE literature search was conducted without any time constraints. Inclusion criteria: (1) Original research manuscripts; (2) Characterization of clinical human *H. pylori* isolates; (3) Use of second and/or third generation sequencing technologies. Exclusion criteria: (1) Reviews, case reports, comments, letters; (2) Characterization of non-human *H. pylori* isolates; (3) Original research manuscripts that did not use second or third generation sequencing technology. First, the terms “*Helicobacter pylori* AND transcriptome OR transcriptomic” were searched and yielded 134 results, of which 12 were original research articles meeting the inclusion criteria (Table 4). Second, a PubMed, MEDLINE and EMBASE search using the terms “*Helicobacter pylori* AND next generation sequencing” was done that yielded 102 results, of which 19 met the inclusion criteria (Table 5). And finally, a PubMed, MEDLINE and EMBASE search with the terms “*Helicobacter pylori* AND whole genome sequencing” was done that yielded 89 results, of which 15 met the inclusion criteria (Table 6).

The literature search mostly yielded basic research studies investigating a broad spectrum of fundamental mechanism in *H. pylori* like the presence of genes associated with bacterial virulence<sup>[99-104]</sup> and biofilm formation<sup>[105,106]</sup>, the characterization of methylases<sup>[107]</sup>, nudix hydrolases<sup>[108]</sup>, restriction-modification (R-M) systems<sup>[109]</sup>, exo- and endo-ribonucleases<sup>[110]</sup>; and the transcriptional response of *H. pylori* to exposition to different salt concentrations<sup>[111]</sup>, different pH conditions<sup>[112]</sup>, heat shock<sup>[113]</sup> and chemical agents like bismuth<sup>[114]</sup> and nickel<sup>[115]</sup>. Most of these studies initially depleted ribosomal RNA, either using RiboZero eukaryotic rRNA depletion treatment (Epicentre, Illumina)<sup>[112,113,116]</sup> or a rRNA modified capture hybridization approach<sup>[110]</sup>, and performed sequencing on an Illumina platform (Illumina, United States). More applied studies focused on two main topics: The composition of the gut microbial community in patients with *H. pylori* associated gastrointestinal pathology<sup>[117-123]</sup> or the association between genotypic and phenotypic drug resistance in *H. pylori*<sup>[124-133]</sup>.

The assessment of the human gut microbiome in health and disease is a hot topic in medical sciences. Changes in the gut microbial composition after *H. pylori* infection may induce pathogenesis and various disorders. However, studies investigating changes in the microbial community composition after *H. pylori* infection have generated conflicting results. While some studies could not detect any significant changes in the taxonomic composition of the gut microbiota<sup>[134-136]</sup>, studies using NGS technology have reported increased abundance of the families *Xanthomonadaceae* and *Enterobacteriaceae*, and the genera *Spirochaetae*, *Streptococcus*, *Lactobacillus*, *Granulicatella*, *Prevotella* and *Veillonella* in response to *H. pylori* infection<sup>[137-139]</sup>. Moreover, some studies showed that antibiotic use to eradicate *H. pylori* affected the

Table 4 A PubMed, MEDLINE and EMBASE search using the terms “*Helicobacter pylori* AND transcriptome OR transcriptomic” yielded 12 original research studies

Study	Main finding	Method	Sequencing	Ref.
1	<p><b>Objective</b></p> <p>Characterization of the MTase JHP1050 in <i>H. pylori</i></p> <p><b>Main finding</b></p> <p>The MTase JHP1050, which methylates GCGC sequences, was found to be highly conserved in all analyzed <i>H. pylori</i> strains, with a nucleotide sequence identity &gt; 87%. Absence of <sup>m5</sup>C methylation had a significant effect on <i>H. pylori</i> growth, led to a significant reduction in DNA uptake capacity, and reduced the bacterial protection against an excess of copper</p>	<i>H. pylori</i> 26695 culture grown in liquid medium to log phase	Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)  RNA-sequencing on an Illumina HiSeq platform (Illumina, United States; 1 × 50 bp)	Estibariz <i>et al</i> <sup>[107]</sup>
2	<p><b>Objective</b></p> <p>Analyzing the impact of bismuth on a diverse array of intracellular pathways in <i>H. pylori</i></p> <p><b>Main finding</b></p> <p>Bismuth influences multiple metabolic pathways and suppresses energy production in <i>H. pylori</i> through disruption of the central carbon metabolism of the bacterium. Bismuth initially perturbs the citric acid cycle and then urease activity, followed by the induction of oxidative stress and inhibition of energy production, and in the meantime, induces extensive down-regulation in the <i>H. pylori</i> metabolome</p>	<i>H. pylori</i> 26695 culture grown in liquid medium to early log phase	RNA-sequencing on a HTSeq v0.6.1 platform <sup>[148]</sup>	Han <i>et al</i> <sup>[114]</sup>
3	<p><b>Objective</b></p> <p>Transcriptomic analysis to assess the process of biofilm formation in <i>H. pylori</i></p> <p><b>Main finding</b></p> <p><i>H. pylori</i> biofilm cells displayed a distinct transcriptomic profile. Lower metabolism and stress responses, likely associated with the microenvironment generated in the <i>H. pylori</i> biofilm, could be determinants of antimicrobial tolerance and involved in the persistence and survival of <i>H. pylori</i>. However, there were no specific genes up- or downregulated that are specific for biofilm formation, suggesting that there is no biofilm-specific set of genes expressed. However, genes encoding flagellar filaments were upregulated in biofilm cells and formed an integral part of the biofilm matrix</p>	<i>H. pylori</i> grown on non-selective solid agar media	Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina) RNA-sequencing on an Illumina NextSeq platform (Illumina)	Hathroubi <i>et al</i> <sup>[106]</sup>



4	<p><b>Objective</b></p> <p>Transcriptional analysis of <i>H. pylori</i> gene expression under high salt conditions</p> <p><b>Main finding</b></p> <p>Differential expression of multiple genes encoding outer membrane proteins, including adhesins (SabA, HopA and HopQ) and proteins involved in iron acquisition (FecA2 and FecA3) was observed. Transcript levels of <i>sabA</i>, <i>hopA</i> and <i>hopQ</i> were increased, whereas transcript levels of <i>fecA2</i> and <i>fecA3</i> were decreased under high-salt conditions. Functions associated with the up- and downregulated genes included acetone metabolism, acid survival, flagellar synthesis and iron transport</p>	<p><i>H. pylori</i> strain 7.13 was grown in liquid medium to mid exponential phase (OD of 0.5)</p>	<p>Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)</p> <p>RNA-sequencing on an Illumina HiSeq 3000 platform (Illumina; 2 × 75 bp)</p>	Loh <i>et al</i> <sup>[111]</sup>
5	<p><b>Objective</b></p> <p>Transcriptional analysis of <i>H. pylori</i> gene expression under different pH conditions</p> <p><b>Main finding</b></p> <p>About 250 genes were induced, and an equal number of genes were repressed at acidic pH. Genes encoding for antioxidant proteins, flagellar structural proteins, type-IV secretion system (T4SS)/Cag-pathogenicity island, F<sub>0</sub>F<sub>1</sub>-ATPase, and proteins involved in acid acclimation were highly expressed at acidic pH</p>	<p><i>H. pylori</i> strain G27 grown in liquid medium, followed by adaptation of the pH (3.0, 4.5, 6.0, 7.4, 8.0)</p>	<p>Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)</p> <p>RNA-sequencing on an Illumina HiSeq 2500 platform (Illumina; 1 × 50 bp)</p>	Marcus <i>et al</i> <sup>[112]</sup>
6	<p><b>Objective</b></p> <p>Characterization of the heat shock protein repressor (HspR) binding sites in <i>H.pylori</i></p> <p><b>Main finding</b></p> <p>HspR is involved in the regulation of different crucial cellular functions through a limited number of genomic binding sites. There is high sequence conservation in the HAIR motif (an HspR-associated inverted repeat of <i>Streptomyces</i> spp.) among <i>H.pylori</i> strains. Site-directed mutagenesis demonstrated that the HAIR motif is fundamental for HspR binding and that additional nucleotide determinants flanking the HAIR motif are required for complete binding of HspR to its operator sequence spanning over 70 bp of DNA</p>	<p>Different <i>H. pylori</i> strains grown in liquid medium to mid exponential phase (OD = 0.7) with/without heat shock treatment</p>	<p>Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)</p> <p>RNA-sequencing on an Illumina GAIIX platform (Illumina; 1 × 85 bp)</p>	Pepe <i>et al</i> <sup>[113]</sup>
7	<p><b>Objective</b></p>	<p>Gastric biopsy specimens from patients with <i>H. pylori</i> infection and premalignant tissue changes</p>	<p>Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)</p>	Thorell <i>et al</i> <sup>[116]</sup>

	<p>Analysis of the composition of the transcriptionally active microbial community and <i>H.pylori</i> gene expression in gastric biopsy specimens from patients with <i>H. pylori</i> infection and premalignant tissue changes</p> <p><b>Main finding</b></p> <p>Although <i>H. pylori</i> infection did not change the bacterial diversity, <i>H. pylori</i> abundance was positively correlated with the presence of <i>Campylobacter</i>, <i>Deinococcus</i> and <i>Sulfurospirillum</i>. Quantification of <i>H. pylori</i> gene expression found high expression of genes involved in pH regulation and nickel transport</p>		<p>RNA-Sequencing on an Illumina HiScanSQ platform (Illumina; 2 × 100 bp)</p>	
8	<p><b>Objective</b></p> <p>Characterization of the Nickel dependent transcriptional regulator (NikR) in <i>H. pylori</i></p> <p><b>Main finding</b></p> <p>NikR not only regulates metal-ion transporters but also virulence factors, non-coding RNAs, as well as toxin-antitoxin systems in response to nickel stimulation</p>	<p><i>H. pylori</i> strains grown in liquid medium and treated with high nickel (500 μM Ni2+) concentrations</p>	<p>Depletion of ribosomal RNA (RiboZero, Epicentre, Illumina)</p> <p>RNA-sequencing on an Illumina MiSeq platform (Illumina; 1 × 76 bp)</p>	<p>Vannini <i>et al</i><sup>[115]</sup></p>
9	<p><b>Objective</b></p> <p>Characterization of Nudix hydrolases in <i>H. pylori</i></p> <p><b>Main finding</b></p> <p><i>H. pylori</i> encodes two proteins resembling Nudix enzymes. One of them, HpRppH, is an RNA pyrophosphohydrolase that triggers RNA degradation in <i>H. pylori</i>, whereas the other, HP0507, lacks such activity. Transcriptional analysis revealed at least 63 potential HpRppH targets in <i>H. pylori</i></p>	<p><i>H. pylori</i> strains grown to mid exponential growth phase (OD = 0.7)</p>	<p>RNA-sequencing on an Illumina HiSeq 2000 platform (Illumina; 1 × 97 bp)</p>	<p>Bischler <i>et al</i><sup>[108]</sup></p>
10	<p><b>Objective</b></p> <p>Characterization of the exo- and endoribonuclease RNase J in <i>H. pylori</i> and its putative targets</p> <p><b>Main finding</b></p> <p>Strong depletion of RNase J led to a massive increase in the steady-state levels of non-rRNAs. mRNAs and RNAs antisense to open reading frames. In contrast, non-coding RNAs expressed in the intergenic regions were much less affected by RNase J depletion. This suggests that RNase J is a major RNase involved in degradation of most cellular RNAs in <i>H.pylori</i></p>	<p><i>H. pylori</i> strains grown in liquid medium to mid exponential phase (OD of 0.5)</p>	<p>Depletion of ribosomal RNA by a rRNA modified capture hybridization approach from MICROBExpress kit (Ambion, Invitrogen, Life Technologies)</p> <p>RNA-sequencing on an Illumina HiSeq 2000 platform (Illumina; 1 × 50 bp)</p>	<p>Redko <i>et al</i><sup>[110]</sup></p>
11	<p><b>Objective</b></p> <p>Analysis of methylated DNA sites throughout the <i>H. pylori</i> genome for several closely related <i>H. pylori</i> strains</p> <p><b>Main finding</b></p>	<p><i>H. pylori</i> strains (HPYF1 and HPYF2) grown to mid exponential phase (OD of 0.4)</p>	<p>RNA-sequencing on an Illumina HiSeq 2000 platform (Illumina)</p>	<p>Futura <i>et al</i><sup>[149]</sup></p>

12	<p>Overall, the methylome was highly variable among closely related <i>H. pylori</i> strains. DNA sequence motifs for methylation could be assigned to a specific homology group of the target recognition domains in the specificity-determining genes for Type I and other restriction-modification systems. Knocking out one of the Type I specificity genes led to transcriptome changes</p> <p><b>Objective</b></p> <p>Characterization of the transcriptome of <i>H. pylori</i>, and construction of a genome-wide map of <i>H.pylori</i> transcriptional start sites and operons</p> <p><b>Main finding</b></p> <p>Discovery of hundreds of transcriptional start sites within operons, and opposite to annotated genes, indicating that complexity of gene expression from the small <i>H. pylori</i> genome is increased by uncoupling of polycistrons and by genome-wide antisense transcription. An unexpected number of approximately 60 small RNAs including the epsilon-subdivision counterpart of the regulatory 6S RNA and associated RNA products, and potential regulators of cis- and trans-encoded target messenger RNAs were discovered</p>	<p><i>H. pylori</i> grown on non-selective solid agar media</p>	<p>Total RNA was digested with DNase I Sharma <i>et al</i><sup>[150]</sup></p> <p>RNA-sequencing was performed on a Roche 454 FLX platform (Roche, Basel, Switzerland) and on a Genome Analyzer II platform (Illumina; 1 × 76 bp)</p>
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Their objective, employed sequencing method and main finding is briefly described in the table. *H. pylori*: *Helicobacter pylori*.

proliferation of the gut microbial community and altered microbial diversity<sup>[140-142]</sup>, though; species diversity recovered to pre-treatment levels upon long-term follow-up<sup>[141,142]</sup>. This encourages the calculation of a microbial dysbiosis index based on the abundances of certain gut microbial taxa in *H. pylori* infected patients<sup>[143]</sup>. The dysbiosis index enables to distinguish patients with different *H. pylori* associated gastrointestinal pathologies<sup>[138]</sup>, and may potentially help to guide patient management.

One limitation of using DNA based techniques for the determination of bacterial abundances and the calculation of a dysbiosis index is that all microorganisms, which are present in the human gut, are detected, including dead bacteria and DNA contaminations (*e.g.*, from incautious sampling, unsterile laboratory procedures or from extraction and sequencing chemicals). In contrast, using meta-transcriptomics allows to investigate only the viable and transcriptionally active proportion of the human gut microbial community. A recently conducted study by Thorell *et al*<sup>[116]</sup> nicely demonstrated that meta-transcriptomics is a very sensitive method that can be applied directly on gastric biopsy specimens. They found that gastric biopsies from patients initially classified as *H. pylori* uninfected by conventional methods, contained actively replicating *H. pylori*, though in lower numbers than biopsy specimens initially scored as *H. pylori* positive. This suggests that *H. pylori* may be present in low abundance in individuals, in whom conventional methods have failed to detect *H.pylori*. It would be interesting to investigate if these patients develop *H. pylori* infection at a later stage and which circumstances may trigger or reinforce infection. Moreover, it would be interesting to investigate if probiotic treatment in these patients would reduce the transcriptionally active *H. pylori* population to undetectable levels, potentially preventing disease development.

The other research studies identified in our literature review focused on using NGS

Table 5 A PubMed, MEDLINE and EMBASE search using the terms “*Helicobacter pylori* AND next generation sequencing” yielded 19 original research studies

Study	Main finding	Method	Sequencing	Ref.
1	<p><b>Objective</b></p> <p>Assessment of the correlation between the microbial gut community composition and the degree of inflammatory cell infiltration, endoscopic findings and the gastrointestinal disorders symptom severity index (PAGI-SYM)</p> <p><b>Main finding</b></p> <p>Histological and endoscopic gastritis was associated with the abundance of <i>H. pylori</i> and that of commensal bacteria in the stomach. The abundances of <i>Variovorax paradoxus</i> and <i>Porphyromonas gingivalis</i> were correlated with histological gastritis, but not with endoscopic or symptomatic gastritis. The total PAGI-SYM score showed a stronger correlation with the duodenal microbiota (<i>Prevotella nanceiensis</i> and <i>Alloprevotella rava</i>) than with the gastric microbiota (<i>H. pylori</i>, <i>Neisseria elongate</i>, and <i>Corynebacterium segmentosum</i>)</p>	DNA extraction from gastric biopsy specimens	Targeted 16S rRNA sequencing on an Ion S5XL platform (Thermo Fisher Scientific, United States)	Han <i>et al</i> <sup>[117]</sup>
2	<p><b>Objective</b></p> <p>Resistance to metronidazole, rifaximin, rifabutin, furazolidone, garenoxacin and sitafloxacin was investigated in Indonesian <i>H. pylori</i> strains</p> <p><b>Main finding</b></p> <p>Furazolidone-, rifabutin-, and sitafloxacin-based therapies might be considered as alternative regimens to eradicate metronidazole and clarithromycin resistant <i>H. pylori</i> in Indonesian patients. Moreover, sitafloxacin but not garenoxacin should be considered for eradication of levofloxacin-resistant <i>H. pylori</i> strains</p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina MiSeq platform (Illumina, United States)	Miftahussurur <i>et al</i> <sup>[128]</sup>
3	<p><b>Objective</b></p> <p>Detection of <i>H. pylori</i> mutations that are known to confer resistance to clarithromycin, levofloxacin, and tetracycline directly from formalin-fixed paraffin-embedded (FFPE) gastric biopsy specimens using next generation sequencing</p> <p><b>Main finding</b></p>	DNA extraction from gastric FFPE tissue blocks	16S rRNA targeted sequencing on an Ion Torrent (Thermo Fischer) platform	Nezami <i>et al</i> <sup>[132]</sup>

	<p>Therapy failure correlated with the number of mutated genes: no failure in cases with no mutations (0/15), 19% (5/27) failure in cases with one gene mutation, and 69% (11/16) failure in cases with more than one mutated gene. Common 23S rRNA mutations (A2146G or A241G) were present in 88% (14/16) of failed cases as opposed to only 10% (4/42) of eradicated cases (<math>P &lt; 0.001</math>). NGS can be used on clinical specimens collected during standard of care testing to detect mutations that correlate with increased risk of treatment failure</p>			
4	<p><b>Objective</b> Assessment of the changes in the microbial esophageal community composition in Chinese patients with reflux esophagitis and healthy volunteers using metagenomic high-throughput DNA sequencing</p> <p><b>Main finding</b> Moderate changes in the microbial community composition were found in patients with reflux esophagitis and compared with the healthy volunteers. At the phylum level, only <i>Bacteroidetes</i> differed between the groups, being less abundant in the reflux esophagitis group. The overall number and diversity of species tended to be lower in reflux esophagitis patients, but there were no significant differences between the groups. Three genera, <i>Prevotella</i>, <i>Helicobacter</i> and <i>Moraxella</i>, were obviously depleted in reflux esophagitis patients</p>	DNA extraction from gastric biopsy specimens	16S rRNA targeted sequencing on an Illumina HiSeq 2500 platform (Illumina; 2 × 250 bp)	Yu <i>et al</i> <sup>[118]</sup>
5	<p><b>Objective</b> Characterization of <i>H. pylori</i>-induced alterations in the gastric and tongue coating microbiota and evaluation of potential impacts on human health in patients with chronic gastritis</p> <p><b>Main finding</b></p>	DNA extraction from gastric biopsy specimens	16S rRNA targeted sequencing on an Illumina MiSeq platform (Illumina)	Zhao <i>et al</i> <sup>[119]</sup>

	<p>Significant alterations of the gastric microbiota were found in <i>H. pylori</i>-positive (<i>cagA</i>-positive) samples represented by a decrease in bacterial diversity, a reduced abundance of <i>Roseburia</i> and increased abundances of <i>Helicobacter</i> and <i>Haemophilus</i>. At the community level, functions involved in biofilm formation, mobile element content, and facultative anaerobiosis were significantly decreased in the microbial community in <i>H. pylori</i>-positive subjects. Presence of CagA was linked to an increased proportion of Gram-negative bacteria in the stomach, thereby contributing to an up regulation of lipopolysaccharide biosynthesis</p>			
6	<p><b>Objective</b> Characterization of the microbial community in patients suffering from gastritis and gastric cancer</p> <p><b>Main finding</b> The gastric carcinoma microbiota was characterized by reduced microbial diversity, decreased abundance of <i>H. pylori</i> and the enrichment of other bacterial genera, mostly represented by intestinal commensals. The combination of these taxa into a microbial dysbiosis index revealed that dysbiosis can be used to discriminate between gastritis and gastric carcinoma. Analysis of the functional features of the microbiota was compatible with the presence of a nitrosating microbial community in carcinoma</p>	<p>DNA extraction from gastric biopsy specimens or surgical specimens of non-neoplastic gastric mucosa adjacent to the tumor</p>	<p>16S rRNA targeted sequencing on an Ion PGM Torrent platform (Thermo Fischer Scientific)</p>	<p>Ferreira <i>et al</i><sup>[138]</sup></p>
7	<p><b>Objective</b> Assessment of the changes in the gut microbiome after <i>H. pylori</i> eradication therapy in teenagers</p> <p><b>Main finding</b> Alpha diversity revealed that both species richness and evenness were recovered to pre-treatment levels at 2 mo after <i>H. pylori</i> eradication therapy. Although <i>H. pylori</i> eradication therapy caused short-term dysbiosis, microbial diversity was restored in healthy teenagers</p>	<p>DNA extraction from stool specimens</p>	<p>16S rRNA targeted sequencing on an Illumina MiSeq platform (Illumina)</p>	<p>Gotoda <i>et al</i><sup>[142]</sup></p>
8	<p><b>Objective</b> Assessment of the association between <i>H. pylori</i> infection and the abundance of <i>Lactobacillus</i> species in the gut microbial community in Japanese patients</p> <p><b>Main finding</b></p>	<p>DNA extraction from stool specimens</p>	<p>16S rRNA targeted sequencing on an Illumina MiSeq platform (Illumina; 2 × 300bp)</p>	<p>Iino <i>et al</i><sup>[120]</sup></p>

9	<p>The relative abundance of <i>Lactobacillus</i> in <i>H. pylori</i>-infected subjects with severe atrophic gastritis was higher compared with patients with mild atrophic gastritis and without atrophic gastritis (<math>P &lt; 0.001</math>) and non-infected subjects (<math>P &lt; 0.001</math>). The proportion of <i>Lactobacillus salivarius</i> was high in <i>H. pylori</i>-infected subjects while that of <i>Lactobacillus acidophilus</i> was high in non-infected subjects</p>			
	<p><b>Objective</b></p> <p>Determination of the sequences of virulence genes (<i>cagA</i> and <i>vacA</i>) and seven housekeeping genes by next generation sequencing</p> <p><b>Main finding</b></p> <p>All <i>H. pylori</i> strains were considered Western-type, and 73.2% of them carried <i>cagA</i>. Patients infected with <i>cagA</i>-positive strains had more severe histological scores than patients infected with <i>cagA</i>-negative strains. Thus, the low incidence of gastric cancer in Bangladesh might be attributable to the high proportion of less-virulent <i>H. pylori</i> genotypes</p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina MiSeq platform (Illumina)	Aftab <i>et al</i> <sup>[103]</sup>
10	<p><b>Objective</b></p> <p>Assessment of the bacterial microbiome in a total of 30 homogenized and frozen gastric biopsy samples from eight geographic locations.</p> <p><b>Main finding</b></p> <p><i>H. pylori</i> infection of the gastric habitat dominates the gastric microbiota in most patients and is associated with a significant decrease of the microbial alpha diversity. Moreover, some bacterial genera like <i>Actinomyces</i>, <i>Granulicatella</i>, <i>Veillonella</i>, <i>Fusobacterium</i>, <i>Neisseria</i>, <i>Helicobacter</i>, <i>Streptococcus</i>, and <i>Prevotella</i> were associated with the presence of <i>H. pylori</i></p>	DNA extraction from gastric biopsy specimens	16S rRNA targeted sequencing on an Illumina MiSeq platform (Illumina; 1 × 500 bp)	Klymiuk <i>et al</i> <sup>[139]</sup>
11	<p><b>Objective</b></p> <p>Characterization of levofloxacin, metronidazole, clarithromycin, amoxicillin and tetracycline resistance in <i>H. pylori</i> isolated from 158 dyspeptic patients in Santo Domingo</p> <p><b>Main finding</b></p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina MiSeq platform (Illumina)	Miftahussurur <i>et al</i> <sup>[124]</sup>

Clarithromycin and amoxicillin resistance were low (3.1% and 1.6%), and no resistance to tetracycline was found. In contrast, metronidazole and levofloxacin resistance were high (82.8% and 35.9%). Most levofloxacin-resistant *H. pylori* strains had an amino acid substitution at codon 87 or 91 in the *gyrA* gene. Many different *rdxA* and *frxA* mutations in metronidazole-resistant *H. pylori* strains were found without synergistic effects. Novel mutations in *dppA*, *dppB*, *fdxA* and *fdxB*, irrespective of *rdxA* and *frxA* mutations were associated with different levels of metronidazole resistance in *H. pylori*

12	<p><b>Objective</b></p> <p>Assessment of the influence of antimicrobials on both, the gut microbiota community composition and the plasma ghrelin level in <i>H. pylori</i>-infected patients, who underwent eradication therapy (amoxicillin, clarithromycin and proton-pump inhibitors)</p> <p><b>Main finding</b></p> <p>The <i>Bacteroidetes:Firmicutes</i> (B:F) ratio was significantly increased 3 months after than before antibiotic treatment (<math>P &lt; 0.01</math>). A significant decrease in the concentration of active ghrelin (<math>P &lt; 0.01</math>) in the plasma was observed before and 3 mo after antibiotic therapy</p>	DNA extraction from stool specimens	16S rRNA targeted sequencing on an Illumina MiSeq platform (Illumina; 2 × 300 bp)	Yanagi <i>et al</i> <sup>[121]</sup>
13	<p><b>Objective</b></p> <p>Comparison of <i>cagA</i> and <i>vacA</i> sequences of <i>H. pylori</i> strains isolated from patients with gastric cancer and MALT lymphoma in Japan</p> <p><b>Main finding</b></p> <p>Conventional genotyping of <i>cagA</i> and <i>vacA</i> showed no significant difference between patients with gastric cancer and MALT lymphoma. When comparing full protein sequences of CagA and VacA, four novel loci were found on CagA, and three loci were detected on VacA. Significant differences were observed at one CagA locus between gastritis and MALT lymphoma <i>H. pylori</i> strains, and at one VacA locus between gastritis and gastric cancer <i>H. pylori</i> strains</p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina HiSeq 2000 and MiSeq platform (Illumina; 2 × 150 bp and 2 × 300 bp)	Hashinaga <i>et al</i> <sup>[100]</sup>
14	<p><b>Objective</b></p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina MiSeq platform (Illumina)	Miftahussurur <i>et al</i> <sup>[126]</sup>



Characterization of levofloxacin, metronidazole, clarithromycin, amoxicillin and tetracycline resistance in *H. pylori* isolates from Indonesia

**Main finding**

Clarithromycin, amoxicillin and tetracycline resistance were low (9.1%, 5.2% and 2.6%). In contrast, high resistance rates to metronidazole (46.7%) and levofloxacin (31.2%) were found. Metronidazole resistant *H. pylori* strains showed different *rdxA* amino acid substitutions, and the 23S rRNA A2147G mutation occurred in clarithromycin resistant *H. pylori*. However, one clarithromycin resistant *H. pylori* strain had a novel mutation in *rpl22* without an A2147G mutation. Amino acid exchanges at N87 and/or D91 of *gyrA* were associated with levofloxacin-resistance

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**Objective**

Characterization of *H. pylori* strains isolated from 146 patients in Kathmandu, Nepal

**Main finding**

*H. pylori* was not resistant to amoxicillin and tetracycline. In contrast, metronidazole resistance was extremely high (88.1%), while clarithromycin resistance was modestly high (21.4%). Most of metronidazole resistant *H. pylori* strains had diverse *rdxA* and *frxA* mutations. Novel mutations in *dppA* (A212, Q382 and I485) and *dapF* (L145, T168, E117, V121, R221) aside from missense mutations in *rdxA* were found in metronidazole resistant *H. pylori* strains. Amino acid exchanges at N87 and/or D91 in *gyrA* were predominantly found in levofloxacin-resistant *H. pylori* strains

*H. pylori* culture from gastric biopsy specimens

Sequencing on an Illumina MiSeq platform (Illumina)

Miftahussurur *et al*<sup>[127]</sup>

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**Objective**

Characterization of wildtype and metronidazole resistant *H. pylori* reference strain 26695 in order to elucidate the molecular basis of metronidazole resistance and the involved genes in *H. pylori*

**Main finding**

A metronidazole-resistant strain was cultured from the metronidazole-susceptible *H. pylori* reference strain 26695 by exposure to low concentrations of metronidazole

Sequencing on an Illumina HiSeq 2000 platform (Illumina; 2 × 90 bp)

Binh *et al*<sup>[129]</sup>

	<p>Mutated sequences in <i>rdxA</i> were successfully transformed into the <i>H. pylori</i> reference strain 26695, and the transformants showed resistance to metronidazole. Transformed <i>H. pylori</i> isolates containing a single mutation in <i>rdxA</i> showed a low MIC (16 mg/L), while those containing mutations in both <i>rdxA</i> and <i>frxA</i> showed a higher MIC (48 mg/L). Moreover, mutations in <i>rpsU</i> may play a role in metronidazole resistance</p>			
17	<p><b>Objective</b></p> <p><i>H. pylori</i> strains were isolated from the members of five families to investigate the microevolution and adaptation of the <i>H. pylori</i> genome using next generation sequencing and multi-locus sequence typing</p> <p><b>Main finding</b></p> <p>Detection of nucleotide substitutions revealed likely transmission pathways involving children. Nonsynonymous mutations were found in virulence-related genes (<i>cag</i>, <i>vacA</i>, <i>hcpDX</i>, <i>tnfa</i>, <i>ggt</i>, <i>htrA</i> and the collagenase gene), outer membrane protein (OMP) genes and other cell surface-related protein genes, signal transduction genes and restriction-modification genes</p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina platform (Illumina)	Furuta <i>et al</i> <sup>[104]</sup>
18	<p><b>Objective</b></p> <p>Elucidating the biological significance of the restriction-modification (R-M) system in the physiology and pathogenesis of <i>H. pylori</i></p> <p><b>Main finding</b></p> <p>Strain UM032 contains a relatively large number of R-M systems, including some MTase activities with novel specificities. Specifically, 17 methylated sequence motifs corresponding to 1 Type I and 16 Type II R-M systems were found</p>	<i>H. pylori</i> strain UM032 was grown on non-selective agar medium	Sequencing on a RS instrument (Pacific Biosciences, United States; yielding > 300 × average genome coverage) and on an Illumina MiSeq platform (Illumina; 2 × 300 bp)	Lee <i>et al</i> <sup>[109]</sup>
19	<p><b>Objective</b></p> <p>Assessment of the prevalence of <i>H. pylori</i> infection and evaluation of human migration patterns in the remote areas of North Sulawesi using next-generation sequencing and multi-locus sequence typing</p> <p><b>Main finding</b></p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina HiSeq 2000 platform (Illumina; 2 × 90bp)	Miftahussurur <i>et al</i> <sup>[122]</sup>

*H. pylori* prevalence was low (14.3% in adults and 3.8% in children). One *H. pylori* strain carried East Asian type *cagA* (ABD type), *vacA* s1c-m1b, *iceA1*, *jhp0562*-positive/ $\beta$ -(1,3), *oipA* "status-on". Phylogenetic analyses showed that the strain belonged to hspMaori type, a major type observed in native Taiwanese and Maori tribes

Their objective, employed sequencing method and main finding is briefly described in the table. *H. pylori*: *Helicobacter pylori*.

technology for the detection of drug resistance mutations in *H. pylori* and their correlation with phenotypic drug resistance<sup>[124-133]</sup>. Collectively, these studies show that clarithromycin resistance is based on point mutations at nucleotide positions A2146 and A2147 in the 23S rRNA gene<sup>[125-127,133]</sup>. Additional mutations in *rpl22* and *infB* were reported in clarithromycin-resistant *H. pylori* strains without 23S rRNA mutations<sup>[126,127]</sup>. However, some methodological concerns should be considered. First, multiple gastric biopsy specimens should be used for *H. pylori* culture in order to enhance sensitivity and detect *H. pylori* sub-populations. Second, multiple colonies should be picked from agar plates for DNA extraction and library preparation to not miss drug resistant sub-populations. Third, *H. pylori* strains should be sequenced with sufficient coverage to detect hetero-resistance. Fourth, multiple susceptible and resistant *H. pylori* strains should be sequenced to distinguish naturally occurring polymorphisms from mutations potentially conferring drug resistance.

Levofloxacin resistance in *H. pylori* has been found associated with amino acid exchanges at codon 87 and/or 91 in the *gyrA* gene<sup>[124-127]</sup>. No synergistic effect on levofloxacin resistance has been found in *H. pylori* strains that carried additional mutations in *gyrB*. Rifampicin resistance has been reported to be associated with amino acid exchanges in the rifampicin resistance determining region of the *rpoB* gene<sup>[125]</sup>. In contrast, the prediction of metronidazole resistance based on genotypic information remains challenging for *H. pylori*. Most metronidazole resistant *H. pylori* strains have been reported to carry multiple *rdxA* and *frxA* mutations<sup>[124,125,127]</sup>; though, also metronidazole resistant strains without mutations in *rdxA* and/or *frxA* were reported<sup>[125]</sup>. Moreover, frameshift and resistance mutations in *rdxA* and *frxA* have also been reported in metronidazole susceptible *H. pylori* strains<sup>[125]</sup>. Collectively, this suggests that more studies are needed investigating the association between polymorphisms detected in *rdxA*, *frxA*, *mdaB*, *omp11* and *rpsU* and other genes and phenotypic metronidazole resistance. NGS may also be used to detect novel mutations associated with metronidazole resistance in genes such as *dapF*, *dppA*, *dppB*, *fdxA*, and *fdxB*<sup>[124,127]</sup>.

Another area of application of NGS technology is the investigation of mutations associated with resistance against new antibiotic agents like the rifamycins rifabutin and rifaximin, the quinolones garenoxacin and sitafloxacin and the nitrofurantoin antimicrobial agent furazolidone<sup>[128]</sup>. Analysis of *rpoB* sequences of rifaximin resistant *H. pylori* strains showed amino acid exchanges at I837, A2414, K2068, Q2079 in *rpoB*, while amino acid exchanges at N87 and D91 in *gyrA* were associated with high levofloxacin resistance in *H. pylori*. No *H. pylori* strain was phenotypically resistant to garenoxacin or sitafloxacin, suggesting a higher genetic barrier to resistance development, and that multiple mutations or synergistic effects may be required to infer resistance against these antibiotics. NGS can further be employed for the identification of putative candidate mutations in phenotypically resistant *H. pylori* strains without known resistance mutations<sup>[129,130]</sup>; and to identify efflux pump genes that may be involved in the development of drug resistance in *H. pylori*<sup>[131]</sup>.

## CONCLUSION AND POTENTIAL FUTURE DIRECTIONS

During the last years, antibiotic resistance in *H. pylori* has continuously increased, also in Western and Central Europe, where antibiotic resistance has been traditionally considered low. This alarming trend leads to question the usefulness of the currently employed "test-and-treat" strategy and to consider determining *H. pylori*'s antibiotic resistance prior to eradication therapy in order to achieve better treatment efficiency. When considering the current costs for *H. pylori* eradication regimens (Table 2 and Table 3; approximate drug prices from Germany), depending on local

Table 6 A PubMed, MEDLINE and EMBASE search using the terms “*Helicobacter pylori* AND whole genome sequencing” yielded 15 original research studies

Study	Main finding	Method	Sequencing	Ref.
1	<p><b>Objective</b></p> <p>Investigation of <i>H. pylori</i> evolution during infection and population dynamics inside the gastric environment</p> <p><b>Main finding</b></p> <p>Phylogenetic analyses suggested location-specific evolution and bacterial migration between gastric regions. Migration was significantly more frequent between the corpus and the fundus than with the antrum, suggesting that physiological differences between antral and oxyntic mucosa contribute to spatial partitioning of <i>H. pylori</i> populations. Associations between <i>H. pylori</i> gene polymorphisms and stomach niches suggested that chemotaxis, regulatory functions and outer membrane proteins contribute to the specific adaptation to the antral and oxyntic mucosa</p>	DNA extraction from biopsy specimens	Sequencing on an Illumina HiSeq 2500 platform (Illumina, United States; 2 × 50 bp)	Ailloud <i>et al</i> <sup>[123]</sup>
2	<p><b>Objective</b></p> <p>Single nucleotide polymorphisms (SNPs) were detected in <i>H. pylori</i> isolates by whole genome sequencing and their correlation with phenotypic resistance to clarithromycin, metronidazole, tetracycline, levofloxacin and rifampicin was assessed</p> <p><b>Main finding</b></p> <p>Overall, there was high congruence of &gt; 99% between phenotypic drug susceptibility testing results for clarithromycin, levofloxacin, and rifampicin and SNPs identified in the 23S rRNA, <i>gyrA</i> and <i>rpoB</i> genes. However, it was not possible to infer a resistance phenotype for metronidazole based on the occurrence of distinct SNPs in <i>rdxA</i> and/or <i>frxA</i></p>	<i>H. pylori</i> culture from gastric biopsy specimens	Sequencing on an Illumina MiSeq platform (Illumina; 2 × 150 bp)	Lauener <i>et al</i> <sup>[125]</sup>
3	<p><b>Objective</b></p> <p>Characterization of polymorphisms in Clarithromycin resistant and susceptible <i>H. pylori</i> strains using whole genome sequencing</p> <p><b>Main finding</b></p>	<i>H. pylori</i> strains grown on solid non-selective agar media	Sequencing on an Illumina HiSeq platform (Illumina; 2 × 150 bp)	Chen <i>et al</i> <sup>[133]</sup>

	<p>No mutations known to be associated with clarithromycin resistance, except for the controversial T2182C mutation, were detected. Single nucleotide variants (SNVs) in multidrug efflux transporter genes and HP0605 were significantly different between clarithromycin resistant and susceptible <i>H. pylori</i> strains. No significant difference in SNVs of membrane proteins of the RND family or MFS (HP1181) family were found</p>			
4	<p><b>Objective</b></p> <p>Characterization of the binding ability, adhesion modes, and growth of <i>H. pylori</i> strains isolated from pediatric patients with abdominal pain, gastritis, gastric or duodenal ulcers</p> <p><b>Main finding</b></p> <p>Increased adhesion capacity of pediatric peptic ulcer disease (PUD) <i>H. pylori</i> strains to human gastric mucins compared to the non-ulcer dyspepsia (NUD) strains both at neutral and acidic pH, regardless if the mucins were positive for Lewis b (Leb), Sialyl-Lewis x (SLe<sub>x</sub>) or LacdiNAc. In addition to <i>babA</i> positive strains being more common among PUD associated strains, <i>H. pylori babA</i> positive strains bound more avidly to gastric mucins than NUD <i>babA</i> positive strains at acidic pH. Binding to Leb was higher among <i>babA</i> positive PUD <i>H. pylori</i> strains compared to NUD strains at neutral, but not acidic, pH. PUD derived <i>babA</i>-knockout mutants had attenuated binding to mucins and Leb at acidic and neutral pH, and to SLe<sub>x</sub> and DNA at acidic pH</p>	<p><i>H. pylori</i> strains isolated from patients with abdominal pain, gastritis, gastric or duodenal ulcers</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina)</p>	<p>Quintana-Hayashi <i>et al</i><sup>[151]</sup></p>
5	<p><b>Objective</b></p> <p><i>H. pylori</i> was continuously cultured <i>in vitro</i> under low iron or high salt conditions to characterize <i>fur</i> genetic variation. Moreover, <i>fur</i> sequence variation was assessed in 339 clinical <i>H. pylori</i> strains</p> <p><b>Main finding</b></p>	<p><i>H. pylori</i> strain B128 isolated from a gastric biopsy of a patient with gastric ulceration was challenged with low/high salt concentrations</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina)</p>	<p>Noto <i>et al</i><sup>[99]</sup></p>

6	<p>Exposure to low iron or high salt selected for a specific single nucleotide polymorphism in the <i>fur</i> gene (FurR88H) in <i>H. pylori</i>. Among the isolates examined, 17% of <i>H. pylori</i> strains isolated from patients with premalignant lesions harbored the FurR88H variant, compared to only 6% of <i>H. pylori</i> strains from patients with non-atrophic gastritis. These results indicate that specific genetic variation arises within <i>H. pylori</i> strains during in vivo adaptation to conditions conducive for gastric carcinogenesis</p>	<p><i>H. pylori</i> strains were cultured on solid non-selective agar media</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 300 bp)</p>	<p>Zhang <i>et al</i><sup>[152]</sup></p>
	<p><b>Objective</b> Comparison of homogenization <i>vs</i> enzymatic digestion protocols for DNA extraction from gastric, esophageal and colorectal biopsies and survey of the microbial content and composition using whole genome sequencing</p>	<p><b>Main finding</b></p>	<p>Neither method demonstrated preferential extraction of any particular clade of bacteria, nor significantly altered the detection of Gram-positive or Gram-negative organisms. However, although the overall microbial community composition remained very similar and the most prevalent bacteria could be detected effectively using either method, the precise community structure and microbial abundances between the two methods were different. The homogenization extraction method provided higher microbial DNA content and higher read counts from human tissue biopsy samples of the gastrointestinal tract</p>	
7	<p><b>Objective</b> Whole genome sequencing and comparative analysis of three <i>H. pylori</i> strains isolated from three Arab patients</p>	<p><i>H. pylori</i> strains isolated from gastric biopsies of patients suffering from dyspepsia</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 300 bp)</p>	<p>Kumar <i>et al</i><sup>[153]</sup></p>
	<p><b>Main finding</b></p>	<p>The three genomes clustered along with HpEurope strains in the phylogenetic tree comprising various <i>H. pylori</i> lineages. The three genomes possessed a complete <i>cag</i>-pathogenicity island with an AB-C type EPIYA motif</p>		
8	<p><b>Objective</b></p>	<p><i>H. pylori</i> strains were isolated from gastric biopsy specimens of patients with chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 300 bp)</p>	<p>Ogawa <i>et al</i><sup>[101]</sup></p>

	<p>Characterization of <i>cagL</i> and <i>cagI</i> in <i>H. pylori</i> isolated from patients in Southeast Asia with chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer</p> <p><b>Main finding</b></p> <p><i>CagL</i> motifs were highly conserved among the <i>H. pylori</i> isolates. <i>CagL</i> E59 and I234 in the C-terminal motif were more common in <i>H. pylori</i> isolates from gastric cancer patients. The <i>CagI</i> C-terminal motif was completely conserved across all <i>H. pylori</i> isolates</p>			
9	<p><b>Objective</b></p> <p>Characterization of the expression of <i>virB</i> genes, encoding parts of the type-IV secretion system (T4SS)/Cag-pathogenicity island, in <i>H. pylori</i> strains isolated from Western patients with different gastrointestinal malignancies</p> <p><b>Main finding</b></p> <p>The region spanning from <i>virB2</i> to <i>virB10</i> constitutes an operon, whose expression is increased in the adherent fraction of bacteria during infection, as well as in both adherent and nonadherent fractions at acidic conditions.</p>	<p><i>H. pylori</i> strains were isolated from gastric biopsy specimens of patients with non-ulcer dyspepsia, gastric ulcer and duodenal ulcer</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 150 bp)</p>	<p>Silva <i>et al</i><sup>[102]</sup></p>
10	<p><b>Objective</b></p> <p>Characterization of <i>H. pylori</i> strains isolated in Nicaragua</p> <p><b>Main finding</b></p> <p>The Nicaraguan isolates showed a phylogenetic relationship with West African <i>H. pylori</i> isolates in whole genome sequence comparison and with Western and urban South- and Central-American isolates using multi-locus sequence analysis. A majority (77%) of the isolates carried the cancer-associated virulence gene <i>cagA</i> and also the s1/i1/m1 allele of the vacuolating cytotoxin gene that is linked to increased severity of disease. Moreover, it was found that Nicaraguan isolates have a blood group-binding adhesin (<i>babA</i>) variant highly similar to previously reported <i>babA</i> sequences from Latin America <i>H. pylori</i> isolates</p>	<p><i>H. pylori</i> culture from gastric biopsy specimens</p>	<p>Sequencing on an Illumina HiScanSQ platform (Illumina; 2 × 100 bp)</p>	<p>Thorell <i>et al</i><sup>[154]</sup></p>
11	<p><b>Objective</b></p> <p>Characterization of genes associated with biofilm formation in <i>H. pylori</i></p> <p><b>Main finding</b></p>	<p><i>H. pylori</i> reference strain 26695, <i>cagA</i> and Cag-pathogenicity island deletion mutants were cultured</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina)</p>	<p>Wong <i>et al</i><sup>[105]</sup></p>

	<p>Genes identified to be associated with biofilm formation in <i>H. pylori</i> included alpha (1,3)-fucosyltransferase, flagellar protein, 3 hypothetical proteins, outer membrane proteins and a Cag-pathogenicity island protein (CagPAI). These genes play a role in bacterial motility, lipopolysaccharide synthesis, Lewis antigen synthesis, adhesion and/or the type-IV secretion system (T4SS). Deletion of <i>cagA</i> and CagPAI confirmed that CagA and T4SS were involved in <i>H. pylori</i> biofilm formation</p>			
12	<p><b>Objective</b></p> <p><i>H. pylori</i> was isolated from a gastroscopic antral biopsy specimen of a 53-year-old male patient with chronic gastritis. Whole genome sequencing was applied to these isolates, and bioinformatic tools were used to investigate the within host evolution of <i>H. pylori</i> isolates</p> <p><b>Main finding</b></p> <p>The <i>H. pylori</i> genomes fall into two clades, reflecting colonization of the stomach by two distinct strains. The lineages have accumulated diversity during an estimated 2.8 and 4.2 yr of evolution. Around 150 clear recombination events between the two clades were found. Imputed ancestral sequences also showed evidence of recombination between the two strains prior to their diversification, and it was estimated that they have both been infecting the same host for at least 12 yr</p>	<p><i>H. pylori</i> was isolated from a gastroscopic antral biopsy specimen of a patient with chronic gastritis</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 150 bp)</p>	<p>Cao <i>et al</i><sup>[155]</sup></p>
13	<p><b>Objective</b></p> <p>Whole genome sequencing of 12 clarithromycin resistant and 7 susceptible <i>H. pylori</i> strains was done to identify novel genetic factors that reduce susceptibility to clarithromycin</p> <p><b>Main finding</b></p> <p>In clarithromycin resistant <i>H. pylori</i> strains specific point mutations in the 23S rRNA gene were found. In addition, genetic variants of four gene clusters (hp0605-hp0607, hp0971-hp0969, hp1327-hp1329, and hp1489-hp1487) of efflux pumps homologues, which have been previously implicated in multi-drug resistance, were found</p>	<p>Nineteen <i>H. pylori</i> clinical isolates were isolated from gastric epithelium biopsy specimens</p>	<p>Sequencing on an Illumina MiSeq platform (Illumina; 2 × 300 bp)</p>	<p>Iwamoto <i>et al</i><sup>[131]</sup></p>



14	<p><b>Objective</b></p> <p>Investigation of the occurrence of genetic mutations that contribute to amoxicillin resistance in <i>H. pylori</i> when exposed to increasing concentrations of amoxicillin <i>in vitro</i></p> <p><b>Main finding</b></p> <p>Using a whole genome sequencing approach, mutations in a number of genes were identified, notably <i>pbp1</i>, <i>pbp2</i>, <i>hefC</i>, <i>hopC</i> and <i>hofH</i>. Mutations in <i>pbp1</i>, <i>hefC</i>, <i>hopC</i>, <i>hofH</i>, and possibly <i>pbp2</i>, contributed to <i>H. pylori</i> high-level amoxicillin resistance</p>	<p><i>H. pylori</i> reference strain 26695 was used as amoxicillin-sensitive reference strain and as parental strain to create <i>in vitro</i> resistant <i>H. pylori</i> isolates</p>	<p>Sequencing on an Illumina Genome Analyzer (Illumina)</p>	<p>Qureshi <i>et al</i><sup>[130]</sup></p>
15	<p><b>Objective</b></p> <p>Next generation sequencing of the <i>H. pylori</i> reference strains J99 and 26695 and bioinformatic analysis of the sequencing data using publicly available algorithms. The accuracy of the coding sequence assemblies was compared to the originally published sequences</p> <p><b>Main finding</b></p> <p>With the Ion Torrent PGM, an inherently high-error rate in the raw sequencing data was found. With the Illumina MiSeq, significantly more non-covered nucleotides were found when using Illumina Nextera XT compared to the Illumina Nextera library preparation method. The most accurate de novo assemblies were found when using the Nextera technology. However, extracting an accurate multi-locus sequence type was inconsistent compared to the Ion Torrent PGM. The Cag-pathogenicity island failed to assemble onto a single contig in all technologies but was more accurate using the Nextera technology. The Illumina MiSeq Nextera method was found as the most accurate method for whole genome sequencing of <i>H. pylori</i> and de novo assembly of its genome</p>	<p><i>H. pylori</i> reference strain 26695 and <i>H. pylori</i> strain J99 were grown on solid non-selective agar media</p>	<p>Sequencing on a PGM (Ion Torrent, Thermo Fischer Scientific, United States) and an Illumina MiSeq platform (Illumina)</p>	<p>Perkins <i>et al</i><sup>[136]</sup></p>

Their objective, employed sequencing method and main finding is briefly described in the table. *H. pylori*: *Helicobacter pylori*.

resistance rates, initial molecular determination of *H. pylori* drug susceptibility may be cost efficient, especially, when considering that costs for PCR assays (< 20 EUR) and WGS (< 100 EUR) have consistently decreased over the last years. In contrast, endoscopy (100-250 EUR) and *H. pylori* culture-based phenotypic DST (80-100 EUR) remains costly.

However, in order to determine drug resistance phenotypes prior to the administration of antibiotics, resistance information must be more rapidly available, ideally with non-invasive methods that do not require endoscopy. Diagnostic methods, like line probe assays or culture based phenotypic DST, that provide drug

resistance information have long turn-around-times and require a gastric biopsy that can just be obtained by invasive endoscopy. In contrast, currently available, non-invasive diagnostic methods can only detect resistance mutations in the 23S rRNA gene of *H. pylori* (e.g., PCR from stool). This may be insufficient in areas with high metronidazole resistance or if levofloxacin- or rifampicin-based regimens have to be administered to patients. Our literature search yielded studies that focused on the prediction of drug resistance phenotypes based on the presence of certain point mutations in the *H. pylori* genome. However, all of these studies used culture *H. pylori* isolates or DNA extraction from gastric biopsy specimens. In an effort to decrease turn-around-times and apply diagnostic workflows that do not require endoscopy, future studies should aim at detecting *H. pylori* and associated resistance mutations directly from clinical specimens (gastric biopsies or stool) using meta-genomic and/or meta-transcriptomic sequencing. Our literature search yielded primary research articles that have successfully applied WGS directly on gastric biopsies for the detection of *H. pylori*<sup>[116,132,144,145]</sup>. One major limitation for the cost-effectiveness and feasibility of clinical meta-genomic and meta-transcriptomic sequencing has always been the rather big amounts of RNA or DNA required for subsequent library preparation and high human DNA background requiring deep sequencing. There has been a tremendous development in this area, and in-house developed<sup>[146,147]</sup> and commercial protocols [e.g., RiboZero (Illumina), RiboGold (Illumina), MIC-ROBExpress (Ambion, Invitrogen)] are becoming available for the depletion of human DNA or the enrichment of bacterial DNA prior to performing WGS, thereby increasing the efficiency and cost-effectiveness of NGS due to less human DNA background in clinical specimens.

In conclusion, NGS technology has opened up new avenues for the characterization of complex microbial communities, including those associated with *H. pylori* associated gastrointestinal disease. Particularly exciting is the promise of culture-independent approaches to *H. pylori* detection and assessment of antibiotic resistance. In the diagnostic laboratory, NGS may enable the implementation of rapid and accurate genotypic DST prior to the administration of antimicrobial therapy for *H. pylori* eradication.

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