Gut Pathogens

REVIEW

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Antibiotic resistance in *Helicobacter pylori*: a genetic and physiological perspective

Rania G. Elbaiomy[1], Xiaoling Luo[2], Rong Guo[2], Shiyuan Deng[1], Meifang Du[1], Ahmed H. El-Sappah[3,4], Mohammed Bakeer[5,6], Mahmoud M. Azzam[7], Ahmed A. Elolimy[8*], Mahmoud Madkour[9], Zaixin Li[1*] and Zhi Zhang[1*]

Abstract

The identification of *Helicobacter pylori* (*H. pylori*) infection as the primary etiology of gastroduodenal diseases represents a significant advancement in the field of gastroenterology. The management of these diseases has undergone a substantial transformation, and antibiotic treatment is now universally applicable. *H. pylori* has been the subject of numerous investigations to determine the prevalence of antibiotic resistance. However, many of these studies are limited, particularly regarding the number and representativeness of the strains assessed. Genetic and physiological modifications, such as gene mutations, efflux pump alterations, biofilm formation, and coccoid formation, contribute to the observed resistance. Our review focuses on the emergence of antibiotic-resistant strains, particularly emphasizing the various modifications of *H. pylori* that confer this resistance. In conclusion, we elucidate the challenges, potential solutions, and prospects in this field, providing researchers with the knowledge necessary to overcome the resistance exhibited by *H. pylori*.

Keywords *H. pylori*, Efflux pump systems, Biofilm, Resistance, Clarithromycin, Penicillin

*Correspondence:
Ahmed A. Elolimy
elolimy@uaeu.ac.ae
Zaixin Li
492747726@qq.com
Zhi Zhang
zhangzhi@suse.edu.cn
[1] Department of Biological Engineering, Sichuan University of Science & Engineering, Zigong 643000, China
[2] Department of Gastroenterology, FuShun People's Hospital, Zigong 643000, China
[3] School of Agriculture, Forestry and Food Engineering, Yibin University, Yibin 644000, Sichuan, China
[4] Department of Genetics, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
[5] Division of Hematology and Medical Oncology, Mayo Clinic, Jacksonville, FL, USA
[6] Division of Internal Medicine-Clinical Hematology, Al-Azhar University, Cairo 11765, Egypt
[7] Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia
[8] Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, Abu Dhabi 15551, United Arab Emirates
[9] Animal Production Department, National Research Centre, Dokki 12622, Giza, Egypt

Introduction

Helicobacter pylori (*H. pylori*) is a prevalent gram-negative microaerobic pathogen especially in developing countries [1]. Recent estimates place the *H. pylori* infection rate in developing nations near fifty percent [2]. The reported infection rates of *H. pylori* in West Asia, Africa, and South America are 66.6%, 70.1%, and 69.4%, respectively [3]. The colonization of gastric mucosa by *H. pylori* in 50–70% of the worldwide population raises the likelihood of developing chronic gastritis, peptic ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4]. In the absence of an effective vaccine, the primary method of reducing bacterial transmission, healing gastric lesions in infected individuals, and preventing the development of gastric cancer is to treat the *H. pylori* infection [5]. Moreover, in contrast to prior notions of *H. pylori* management, a substantial transformation has occurred since the 2015 Kyoto *H. pylori* conference consensus, which advised the eradication of all *H. pylori* infections unless there exist compelling justifications for non-eradication, including co-morbidities, high rates of re-infection within the nation, or conflicting societal health priorities [6]. As illustrated in Fig. 1, the main modes of intra-individual transmission encompass the gastro-oral, oral-oral, and fecal–oral routes [7]. Other methods of disease dispersion include iatrogenic transmission and breastfeeding. Small nucleotide sequences can reveal transmission pathways and directions between individuals due to the substantial genetic diversity of *H. pylori*. Seven-gene multilocus sequence typing [8] and,

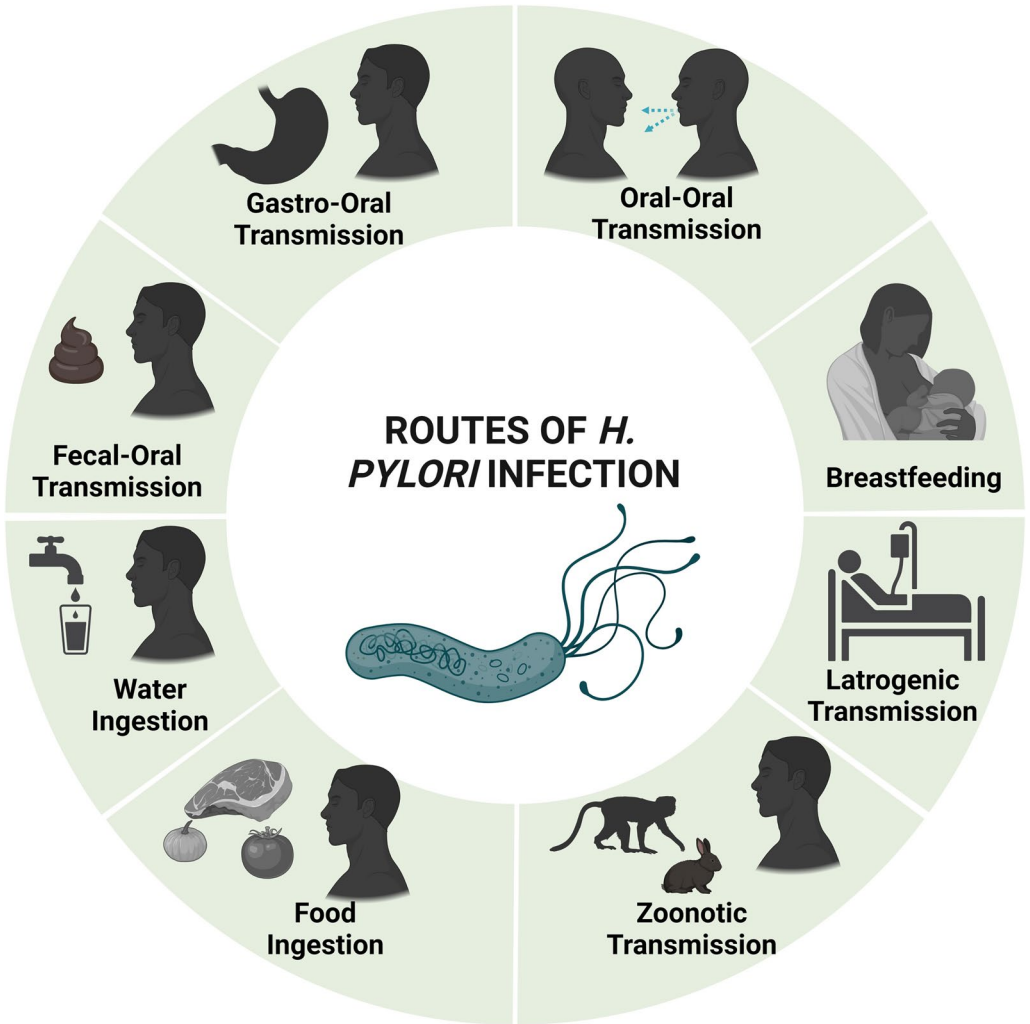

Fig. 1 Proposed modes of transmission for *H. pylori*. Five of the suggested routes exemplify direct transmission between individuals (breastfeeding, iatrogenic, oral-oral, gastro-oral, and fecal–oral), whereas the other three necessitate an environmental reservoir in the intervening stages. This figure was created using BioRender

more recently, whole-genome sequence analysis [9] have facilitated the comprehension of how *H. pylori* disseminates within families, providing valuable insights for confronting epidemiological challenges.

H. pylori is treated with a variety of antibiotics, including amoxicillin (AMX), clarithromycin (CLR), metronidazole (MNZ), tetracycline (TET), furazolidone (FR), levofloxacin (LVX), and rifabutin (RFB), which are administered in combination. The AMX, a β-lactam antibiotic, affects bacterial cell wall formation [10]. However, resistance may develop via changes in penicillin-binding proteins.The CLR, a macrolide, suppresses protein synthesis by targeting the 23S rRNA; nevertheless, resistance is usually caused by point mutations in the 23S rRNA gene. The MNZ, a nitroimidazole, acts intracellularly to destroy bacterial DNA, but resistance develops via changes in the *rdxA* and *frxA* genes [11]. The TET binds to the 30S ribosomal subunit and inhibits translation, with resistance related to changes in the 16S rRNA gene [12]. The FR, a nitrofuran, produces reactive intermediates that harm bacterial components, although resistance mechanisms are poorly known. The LVX, a fluoroquinolone, inhibits DNA gyrase and topoisomerase IV; however, resistance develops via *gyrA* mutations. The RFB inhibits RNA polymerase, but mutations in the *rpoB* gene may provide resistance [10]. The rising resistance of *H. pylori* to various antibiotics highlights the need for customized therapy, innovative antimicrobial drugs, and other treatment options to improve eradication rates [13].

H. pylori's antibiotic resistance is crucial given that it is one of the most common causes of bacterial infections worldwide, affecting millions of people each year [14]. Furthermore, overuse or misuse of antibiotics may contribute to the development of antimicrobial resistance in *H. pylori* and other bacteria, creating serious public health concerns [15]. The World Health Organization has designated *H. pylori* as a serious threat to human health because of its resistance to most current treatment procedures [16].

The molecular processes behind antibiotic resistance in *H. pylori* infection are diverse and complicated [17]. Several strategies have been proposed to assist *H. pylori* antibiotic resistance, including genetic defects within the bacterium and physiological changes that may increase efflux pump synthesis in bacterial cells [10]. Another potential resistance mechanism is cellular adaptation connected to biofilm or coccoid formation, which prevents drugs from entering bacterial cells [18]. Numerous resistance routes may synergistically confer multidrug resistance (MDR) in *H. pylori*, complicating eradication attempts and emphasizing the need for innovative treatments [19]. In conclusion, we highlight the obstacles, prospective solutions, and possibilities in this sector, providing researchers with the knowledge essential to overcome the resistance displayed by *H. pylori*.

Emerging antibiotic-resistant strains

Lower income, lower education level, housing congestion, rural residence, ethnicity, use of tanks for water, smoking, alcohol consumption, consumption of raw vegetables or spicy meals, inadequate living conditions, and hygiene practices are all risk factors for *H. pylori* infection, particularly in Asia [3, 21]. There is a growing prevalence of *H. pylori* infection in developing nations, as indicated by the data presented in Fig. 2 [22, 23]. The prevalence of infection in underdeveloped countries is approximately 25%, which is higher than in Western countries such as the United States, England, and Canada [24]. Nevertheless, it is worth noting that around 85% of individuals who were infected with *H. pylori* did not experience any associated issues or symptoms. Geographic variations have been discovered to be connected with specific putative virulence genes of *H. pylori* [24]. Compared to high-income countries (21.7%), infection rates among children and adults were higher in low- and middle-income countries or regions (43.2%) during the period from 2014 to 2020 [25]. However, this disparity was diminished in the Western Pacific region [20]. In terms of the age factor, the infection rates of children are lower than those of adults, and they are higher in developing rural areas than in metropolitan areas. Despite advancements in sanitation and socioeconomic conditions, 34% of children worldwide were discovered to have *H. pylori* infections between 2014 and 2020 [20, 25].

H. pylori is eradicated by combining two to three antibiotics, including a restricted number of options such as AMX, CLR, MNZ, TET, LVX, and RFB, with an acid-suppressant, with or without bismuth [26, 27]. In contrast, the rate of effective *H. pylori* eradication has declined over the past few decades, coinciding with the rise in antibiotic resistance [15, 16, 28–30]. *H. pylori* antibiotic resistance is especially concerning, given that it is one of the leading causes of bacterial infections across the globe, impacting millions of individuals annually [3, 31]. The molecular pathways responsible for antibiotic resistance in *H. pylori* infection are varied and intricate [32].

Antibiotic resistance in *H. pylori* has arisen as a major public health concern, with significant variation depending on location [10]. The incidence of antibiotic resistance is influenced by a variety of factors, including antibiotic use, healthcare laws, and regional economic situations [33]. In Asia, more than half of *H. pylori* infections are resistant to drugs, including CLR, MNZ, and LVX [34]. This is mostly due to poor antibiotic usage and the frequency of self-medication. In Africa, a similar

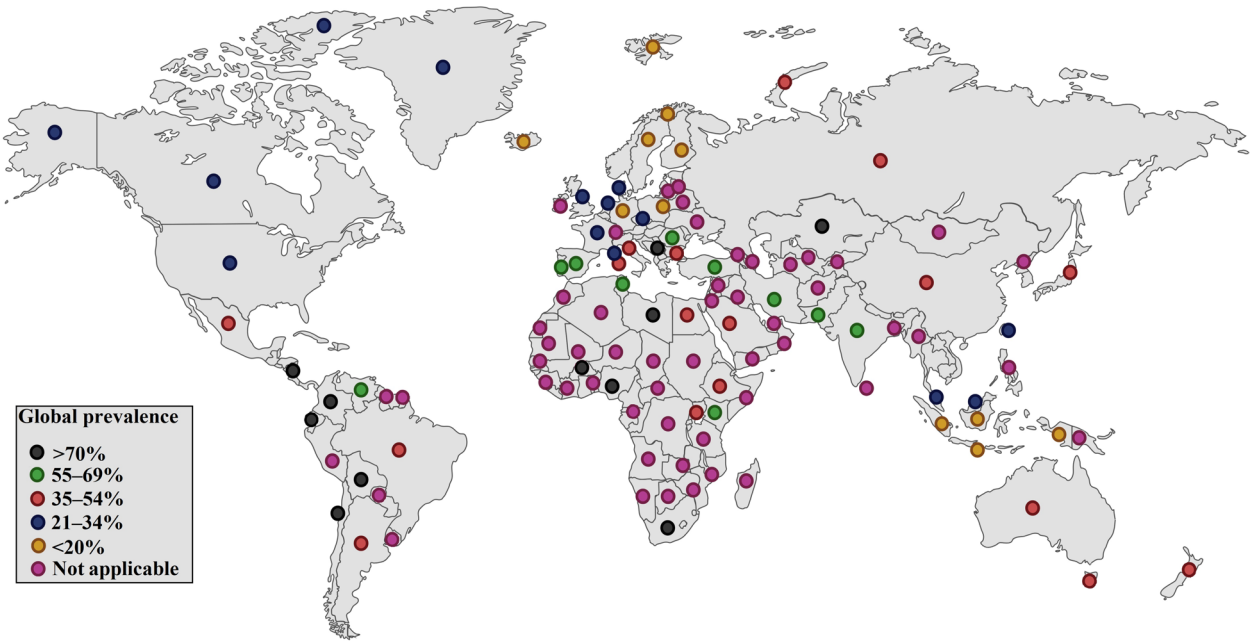

Fig. 2 A global map illustrating the global distribution of *H. pylori* infection. The predominance has been visually shown using areas that are encircled with colors. The data categories are as follows: black (70%), green (55–69%), red (35–54%), blue (21–34%), brown (20%), and purple (not applicable) [22, 23]. This map was created using BioRender

scenario persists, with high resistance, notably to MNZ. This is most likely owing to the widespread use of MNZ to treat parasitic illnesses, as well as the lack of tight antibiotic use regulations [35]. However, Europe has a diverse topography of resistance. Southern and Eastern Europe frequently have higher rates than Northern and Western Europe, which may reflect differences in antibiotic prescribing policies among physicians [36]. Antibiotic resistance is spreading across the Americas, notably in Latin America, where MDR illnesses are becoming more common [37]. In contrast, Oceania has lower rates of resistance, possibly due to stricter antibiotic usage and prescribing practices [38].

Resistance in *H. pylori* can take several forms, ranging from resistance to a single antibiotic, such as CLR or MNZ, to dual resistance, which usually includes both CLR and MNZ [10]. This dual resistance severely reduces the efficacy of standard *H. pylori* therapy. However, there is growing worry about the development of the MDR, which is defined as resistance to three or more antibiotics. These multidrug-resistant *H. pylori* strains are far more difficult to treat with traditional methods, prompting the development of new therapeutic techniques.

H. pylori can acquire resistance to treatment through two main mechanisms: primary resistance and secondary resistance [39]. Primary resistance develops in people who have not previously undergone therapy [40]. This sort of resistance is typically caused by an overuse of antibiotics in a community. It is especially common in areas where antibiotics are easily available over the counter or prescribed for diseases unrelated to bacterial infections. People who have previously sought but failed to remove *H. pylori* develop secondary resistance [39]. This might happen when the treatment regimen is poor or when people do not take their medications as advised. Repeated exposure to ineffective treatments creates pressure, facilitating the establishment of super-resistant strains and complicating the eradication of the sickness [41].

To address the growing problem of *H. pylori* bacteria acquiring antibiotic resistance, it is critical to build monitoring systems tailored to local sites [10]. These techniques are critical for monitoring the progression of resistance and supporting clinicians in selecting the best therapy. Furthermore, it is critical to create campaigns that encourage the prudent use of antibiotics. This will prevent unnecessary antibiotic use and lessen the pressures that contribute to resistance. Using molecular tests to determine antibiotic susceptibility before starting therapy may increase the chance of successful eradication by allowing doctors to tailor medicine to each patient [42]. Failure to respond quickly and collectively will accelerate *H. pylori* resistance to antibiotics, reducing treatment efficacy, raising the risk of serious gastrointestinal problems, and putting significant pressure on global healthcare systems.

Fundamental mechanisms driving drug resistance in *H. pylori*

Physiological modifications

Physiological effects describe the alterations or reactions that occur in living organisms due to interactions with their surroundings. These impacts are crucial for maintaining life and may happen immediately.

Efflux pump system

Efflux pumps are proteins that transfer substances across bacteria's cytoplasmic membrane. They regulate the interior environment by extruding poisonous compounds, quorum sensing molecules (autoinducers), biofilm matrix subunits, and bacterial virulence factors. They are regarded as one of the most frequent resistance mechanisms among a wide variety of harmful bacteria [44]. In the early 1990s, the discovery of MDR pumps in *E. coli* and *Pseudomonas aeruginosa*, represented by the resistance-nodulation-division (RND) superfamily exporters, significantly improved our knowledge of resistance mechanisms [45–47]. In contrast, the role of efflux pumps in *H. pylori* resistance to MNZ was first demonstrated in 2005 [48]. At present, bacteria possess six distinct efflux pump families: the ATP-binding cassette (ABC) superfamily, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), the proteobacterial antimicrobial compound efflux (PACE) family, the RND superfamily, and the multidrug and toxin extrusion (MATE) family [44, 49].

Primary transporters need biological energy tos move substrate across the membrane, while passive transporters do not [50–52]. The ABC superfamily has a key active transporter that has been widely studied. In contrast, several different superfamilies exhibit secondary active transport. These are MFS, SMR, RND, and MATE. While several efflux pump families have been investigated, more research is needed on *H. pylori* efflux pumps. Gene analysis revealed 144 putative translocases in *H. pylori,* all of which were proven to belong to the RND, MFS, MATE, and ABC families (Fig. 3A) [53, 54].

Until now, most *H. pylori* transporters are not well described in their processes, structures, and activities [54, 55]. Surprisingly, little is known about *H. pylori* SMR transporters. The prevalence of efflux genes in bacterial genomes raises the topic of the significance of SMR pumps in *H. pylori* multidrug resistance. Identifying particular EPIs is crucial for efficiently eliminating *H. pylori* due to their structural similarity and extensive substrate specificity [56]. To improve the efficacy of present *H. pylori* therapies, efflux pumps must be classified, and particular translocases identified.

Efflux pumps are proteins that transfer substances across the bacterial cytoplasmic membrane, regulating the interior environment by extruding poisonous compounds, quorum sensing molecules (autoinducers), biofilm formation molecules, and bacterial virulence factors [57, 58]. They are regarded as one of the most frequent resistance mechanisms among a wide variety of harmful bacteria [59].

In the early 1990s, the discovery of the MDR pumps in *E. coli* and *Pseudomonas aeruginosa*, represented by the RND superfamily exporters, significantly improved our knowledge of resistance mechanisms [60, 61]. In contrast, the role of efflux pumps in *H. pylori* resistance to MNZ was first demonstrated in 2005 [11]. At present, bacteria possess six distinct efflux pump families: the ABC superfamily, the SMR family, the MFS, the PACE family, the RND superfamily, and the MATE family [62].

Efflux pumps can be classified based on their energy source. Primary transporters, such as those in the ABC superfamily, require biological energy to move substrates across the membrane, whereas passive transporters do not [63]. The ABC superfamily has been widely studied as a key active transporter. In contrast, several other superfamilies, including MFS, SMR, RND, and MATE, exhibit secondary active transport [11].

Although several efflux pump families have been investigated, more research is needed on *H. pylori* efflux pumps [54]. Gene analysis has revealed 144 putative translocases in *H. pylori*, all of which belong to the RND, MFS, MATE, and ABC families [54]. However, most *H. pylori* transporters remain poorly described in terms of their processes, structures, and activities [64]. Notably, little is known about *H. pylori* SMR transporters. The prevalence of efflux genes in bacterial genomes raises the question of the significance of SMR pumps in *H. pylori* multidrug resistance.

Identifying specific efflux pump inhibitors (EPIs) is crucial for efficiently eliminating *H. pylori* due to their structural similarity and extensive substrate specificity [54]. To improve the efficacy of current *H. pylori* therapy efflux pumps must be classified, and specific translocases must be identified. Efflux pumps can expel a wide range of drugs and have been identified as key components of multidrug resistance in various bacteria [65], including *H. pylori* [54].

Additionally, efflux pumps contribute to biofilm antimicrobial resistance, with evidence found in multiple bacteria, including *Pseudomonas aeruginosa* [66], *E. coli* [67], and *Candida albicans* [68]. Yonezawa et al. [69] observed that in *H. pylori* clinical MDR strains (C-MDR), biofilm cells expressed more efflux pump genes than planktonic cells. While certain efflux transporters have

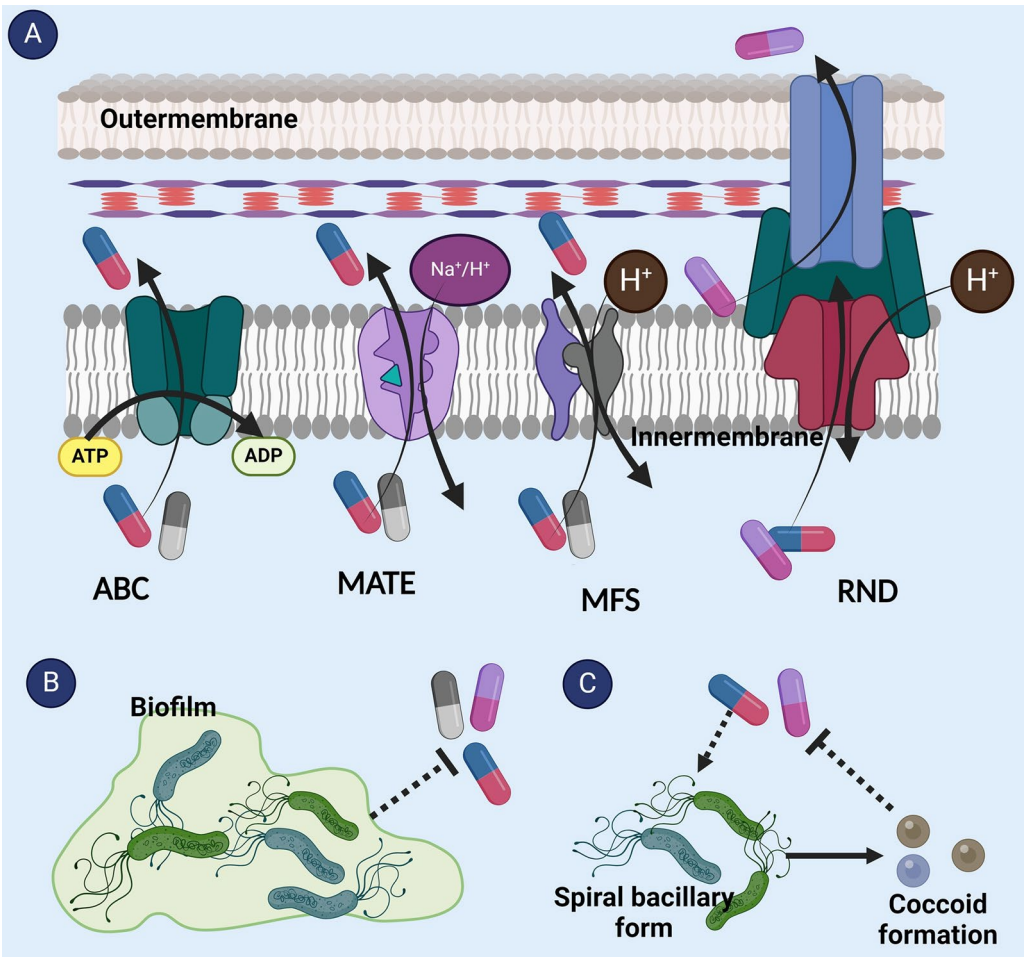

Fig. 3 Summary of physiological changes involved in *H. pylori* antibiotic resistance, **A** The four confirmed multidrug efflux pumps families which have been associated with bacterial drug resistance: resistance-nodulation-division (RND), major facilitators (MFS type), drug/metabolite transporters (DMT type), ATP-binding cassette (ABC) transporters, extrusion of various medications and hazardous substances (MATE category), (**B**) biofilm formation, and (**D**) coccoid formation. This figure was created using BioRender

been discovered in *H. pylori* [70]their activities, particularly in *H. pylori* biofilm formation, require further investigation.

Furthermore, recent findings by Gong [71] suggest that various resistance gene point mutations may have different effects on efflux pump gene expression. Knockout of efflux pump genes may delay or prevent antibiotic resistance gene changes to some extent and may also reverse phenotypic resistance to CLR and MNZ in specific strains. Additionally, Alfaray et al. [72] identified 29 putative efflux pump-related antimicrobial resistance (AMR) genes in *H. pylori*, which were generally classified as part of the Core Genome of *H. pylori* (ARG-CORE). The distribution of antimicrobial resistance genes (ARGs) is consistent across geographical areas and *H. pylori* populations. However, certain ARGs exhibit distinct distributions and are found only in specific regions or populations.

Biofilm formation

A biofilm is a cluster of microbial cells intricately organized and encircled by a matrix they produce independently [73]. This matrix establishes a boundary between the biofilm and its environs [74]. This arrangement enhances the survival of microbial producers and facilitates their collection in challenging environments [75]. In contrast to planktonic forms, the biofilm state of microbial cells is characterized by a distinct rate of proliferation, metabolism, and morphology, as well as a modified gene expression profile [76]. The term "biofilm" has been extensively employed in environmental microbiology due to the prevalence of biofilms in natural environments [77]. At present, there is a greater emphasis on their presence within the human body, as they have the capacity to influence the onset of disorders and the maintenance of good health [78]. Biofilms are responsible for over 80% of all human infections and 60% of chronic ones, according

to the US National Institutes of Health and the US Centers for Disease Control and Prevention [79].

Biofilm is approximately one thousand times more resistant to antibiotics and other antimicrobial agents than planktonic forms [80–82]. Various factors, including persister cells and a reduced growth rate, influence this phenotype. Additionally, the mobility of antimicrobial substances in the encircling environment is restricted by a dense matrix layer [73]. Numerous scientific investigations have demonstrated a correlation between the composition of the biofilm matrix and antibiotic resistance [73, 83, 84]. Additionally, there is evidence that the prevalence of antibiotic resistance to specific antibiotic groups [85–87] or even multidrug resistance [73, 88–91] is associated with the thickness and intensity of biostructure production. Consequently, certain scientists believe that the primary factor in developing an effective antimicrobial treatment is the comprehension of the mechanisms that drive the growth and maturation of biofilms [73, 75].

The physiological mechanisms responsible for producing *H. pylori* biofilms are primarily unclear [92]. The scientific community has long denied bacterial biofilms, leading to our current predicament [93, 94]. Fortunately, this impasse has been gradually resolved over the past five years, leading to the publication of substantial research that delineates the biofilm forms of this bacterium [95]. According to original research on the subject, biofilm *H. pylori* forms exhibit significantly higher resistance levels to numerous antibiotics that are employed in clinical settings (Fig. 3B) [96–99]. The proteins and extracellular DNA (eDNA) that constitute *H. pylori*'s biofilm matrix have also been identified in publications [73, 100]. These results may explain the previously observed decrease in antibiotic sensitivity [101]. The scientific community was motivated to investigate this critical issue further by these findings, which confirmed the previous hypothesis that biofilm substantially impacted the elimination of *H. pylori* [95]. Ultimately, research conducted by Krzyżek et al., [73]and [102] revealed that clinical *H. pylori* strains that exhibited resistance to CLR had a wide range of phenotypic traits that may be interpreted as the capacity to create robust biofilms.

The coccoid formation

Gram-negative rods frequently generate coccoid forms in response to stress, such as starvation [103]. Electron microscopy investigations have demonstrated that *H. pylori* can assume three distinct forms [104–106]. The transformation of *H. pylori* from the bacillary to the coccoid form in response to in *vitro* exposure to diverse antimicrobial agents has been demonstrated in numerous studies (Fig. 3C) [107]. A dissolved cell membrane and degenerative organelles characterize the nonviable coccoid forms. The viable coccoid is frequently more compact and diminutive than the degenerative coccoid forms. According to West et al., [108], the culturable variants of *H. pylori* can be cultured in artificial seawater for a period exceeding seven days and in distilled water and salinity for a period exceeding fourteen days. Shahamat et al., [109] conducted a study that indicated that certain varieties of *H. pylori* can survive in pure water for more than ten days, while nonculturable coccoid *H. pylori* can survive for up to a year. Over seven days, the identical *H. pylori* strain maintained in a nutrient-rich medium did not perform as well as that maintained in natural saline or deep groundwater at 4 °C [110]. As per their research, culturable genotypes of *H. pylori* can persist in water for up to a week and perform better in environments devoid of artificial nutrients than in artificially wealthy ones. The spiral forms likely did not contaminate the fractions then, as the nonculturable coccoid form survived for months.

Antibiotic concentrations such as CLA, MNZ, ERY, and AMX can cause this morphological alteration [111, 112]. AMX, effective against *H. pylori* in vitro, has the greatest induction effect [111, 113]. However, morphological analysis of the cultures reveals that bacillary forms diminish in favor of coccoid forms [111, 113]. Berry et al. [111] discovered that AMX cleared bacillary *H. pylori* with a 10×MIC but also promoted coccoid forms. Coccoid forms have different penicillin-binding protein profiles than bacillary forms, making them less likely to respond to β-lactam antibiotics [114]. Thus, after eradication, certain *H. pylori* cells may become coccoid and resistant to antibacterial treatments. This may explain recurrence and treatment failure [115]. The coccoid form, like the bacillary form, encodes important virulence genes, including *ureA*, *ureB*, *hpaA*, *vacA*, *cagA*, *cagE*, and *babA* [112, 116]. This long-term expression is likely to play a role in persistent severe gastrointestinal problems.

A genetic mutation

Significant contributors to the antibiotic resistance mode are mutations in critical functional genes of *H. pylori* [117]. Consequently, most of these gene mutations that confer resistance to a particular class of antibiotic have been discussed (Fig. 4). The following are *H. pylori* genes whose mutations contribute to the bacteria's antibiotic resistance.

PBP

Bacterial cell division is a complicated multimolecular process that requires *PBPs* to synthesize new peptidoglycan during cell wall elongation and septum formation [118]. The *PBPs* change and polymerize peptidoglycan, the part of the bacterial cell wall that bears the brunt of the stress [119, 120]. *PBPs*, in conjunction with

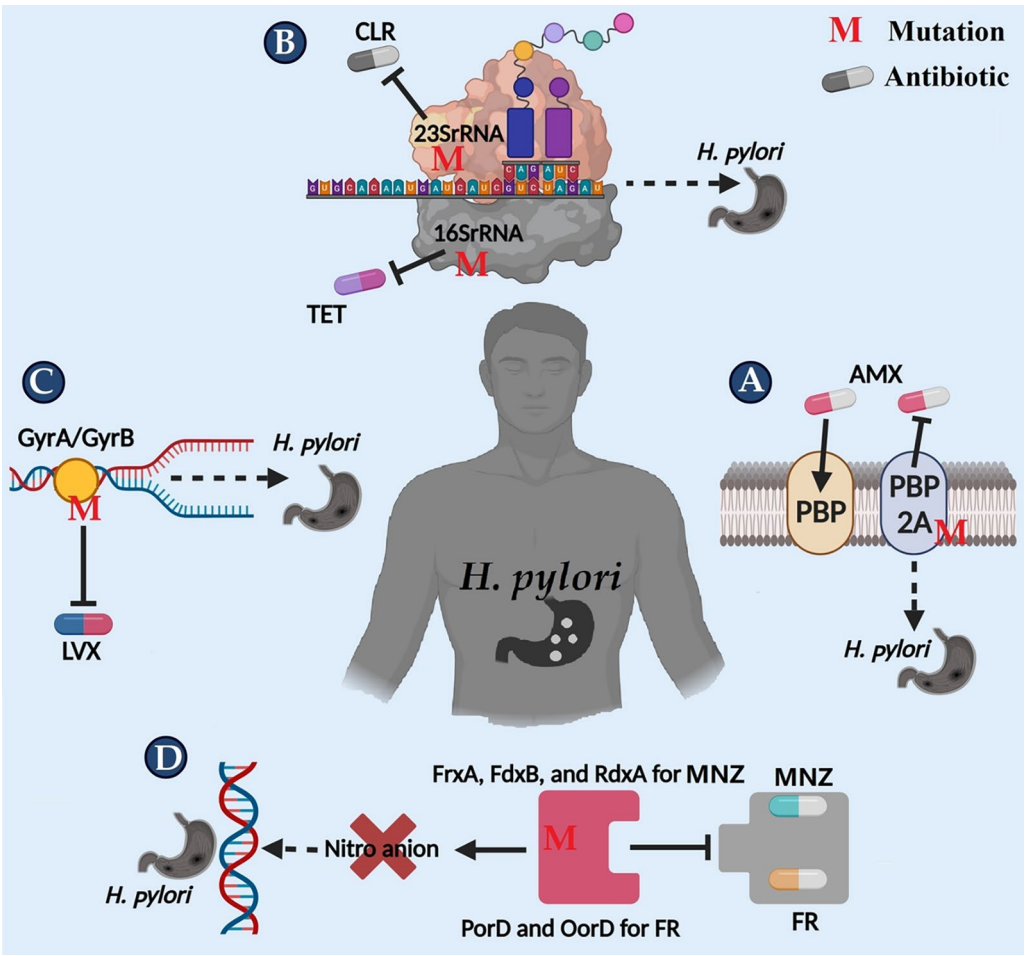

Fig. 4 Summary of genetic changes (genes mutations) involved in *H. pylori* antibiotic resistance; The mutation of the penicillin-binding proteins (*PBP*) gene is a prevalent method by which *H. pylori* strains obtain resistance to amoxicillin (AMX), (**B**) The tetracycline (TET) resistance identified in *H. pylori* is due to point mutations in the 16S rRNA gene that prevent TET from binding to the primary binding site of the 16S ribosome. The presence of point mutations in the 23S rRNA gene is indicative of clarithromycin (CLR) resistance, (**C**) Resistance to levofloxacin (LVX) may be caused by mutations in the quinolone resistance determining region (QRDR), which contains the genes *gyrA* and *gyrB*, which encode bacterial gyrase subunits and finally, (**D**) Resistance mechanisms of *H. pylori* towards furazolidone (FR) are predominantly attributed to single nucleotide mutations in the target genes of *rdxA*, *frxA*, and *fdxB*. Conversely, resistance to metronidazole (MNZ) is caused by alterations in *porD* and *oorD*. This figure was created using BioRender

cytoskeleton proteins that regulate cell morphology, impact the visual characteristics of the peptidoglycan exoskeleton [120]. According to genetic and microscopic investigations, class A and class B PBPs have distinct morphological roles, which point to differential protein localization and interactions with certain cell components as the mechanism of shape determination [120].

AMX, a semisynthetic penicillin with broad-spectrum β-lactam antibiotic properties, is commonly employed as the initial treatment in current therapeutic regimens for eradicating *H. pylori* [55]. The AMX inhibits the formation of cell wall mucins in *H. pylori* cells by binding to *PBPs*, leading to cell enlargement and cracking [55]. In *H. pylori* strains, mutation of *PBP* genes is a frequently seen mechanism for developing resistance to AMX [55]. Researchers have discovered nine distinct *PBPs* in *H. pylori*. These include three PBPs with large molecular weights, namely *PBP1* (66 kDa), *PBP2* (63 kDa), and *PBP3* (60 kDa), as well as six *PBPs* with low molecular weights known as *PBP4-9* [55]. *PBP1A*, a subtype of *PBP1*, has glycosyltransferase and acyltransferase functions and is primarily associated with resistance against AMX [121]. A mutation in the *PBP1A* gene causes *H. pylori* to be resistant to the antibiotic AMX, as shown by Okamoto et al. [122]. Three crucial regions in the transpeptidase domain of *PBP1A* are $STGK_{338–341}$, $SKN_{402–404}$,

and $KTG_{555–557}$ [123]. Also, G591K and A480V in *PBP1A* are critical mutations linked to AMX resistance, as shown by Islam et al. [121] via the preparation and analysis of recombinants. Monique and her colleagues transferred these three fragments to cultures susceptible to AMX, and they discovered that transferring the last two fragments resulted in a moderate level of drug resistance in the sensitive strains. Changes in amino acids, specifically S402G (found in $SKN_{402–404}$), E406A, S417T, S414R, T555S (found in $KTG_{555–557}$), and N561Y, are what make *H. pylori* resistant [124]. Additionally, it was believed that low-molecular-weight *PBP*s were associated with *H. pylori* resistance [55]. For instance, PBPD, a PBP with a low molecular weight, is either absent or expressed at low levels in certain strains resistant to AMX [124].

16S rRNA

The RNA component of the 30S subunit of a bacterial ribosome is known as 16S ribosomal RNA, abbreviated as 16S rRNA. Forming the bulk of the SSU structure, it binds to the Shine-Dalgarno sequence [125]. Structure modeling and sequence analysis [126] show that the 16S rRNA gene sequence comprises many different and conserved regions. Point mutations in *H. pylori*'s 16S rRNA impede TET attachment to the 16S ribosome's major binding site, a common TET resistance mechanism [127]. There is a high level of resistance to TET (MIC > 8 μg/mL) because there are three mutations (AGA965–967TTC) in the 16S rRNA sequence that are thought to bind TET. On the other hand, resistance at a low level (MIC ≤ 4 μg/mL) is caused by either double or single mutations [128]. Gerrits et al. [129] found a mutation (AGA926–928) in the 16S rRNA gene of *H. pylori* that made the minimum inhibitory concentration (MIC) of TET higher. Furthermore, they observed the A939C mutation in TET-resistant organisms that lacked AGA926-928 mutations. This indicates that other changes in the 16S rRNA gene are associated with TET resistance.

23S rRNA

Traditionally, in bacteria and archaea, the genes responsible for generating the RNA components of the ribosome are arranged inside a single operon in the 16S-23S-5S sequence.

The CLR inhibits bacteria's protein production by attaching to the peptidyl transferase ring in the V region of the 23S rRNA subunit [130]. This implies that changes in the 23S rRNA gene could provide CLR resistance [131]. Researchers have extensively explored the relationship between 23S rRNA mutations and resistance to CLR [132]. Mutations at A2142G, A2142C, and A2143G can provide high-level resistance to CLR [133, 134]. In contrast, CLR-resistant bacteria with lower CLR minimum inhibitory concentration (MIC) levels have unique point mutations, such as T2717C. The 23S rRNA also showed modifications such as A2115G, G2141A, A2144G, T2182C, and T2289C [135]. However, there is insufficient data to establish a link between these mutations and CLR resistance. Furthermore, it has been demonstrated that certain gene mutations have synergistic effects when combined with 23S rRNA changes. For example, it has been demonstrated that two mutations in genes other than 23S rRNA, namely *rpl22* and *infB*, had synergistic effects when paired with mutations in the V region of 23S rRNA.

gyrA and gyrB

DNA gyrase is a tetrameric enzyme composed of two A and two B subunits encoded by the *gyrA* and *gyrB* genes [136]. Mutations in the quinolone resistance determination region (QRDR) of *gyrA* can alter the DNA helicase's interaction with quinolone antibiotics, resulting in LVX resistance [137, 138]. Changes in encoded amino acids caused by mutations in the *gyrA* gene have been shown to be concentrated in amino acids N87K, N87L, N87I, N87A, D91G, D91N, D91A, D91Y, and D91H [137, 139–141]. Some *gyrA* mutations are complicated, involving other amino acid changes, such as D91N + L45F, D91Y + L45F, D91N + 130 K, D91G + A55S + G60S, and N87A + A88N + V65I [55]. A55S, D86N, A88P, A88V, R130K, and R295H are additional amino acid changes found in some *H. pylori*-resistant strains, indicating that *H. pylori* have other mutations linked to LVX resistance in addition to those that affect amino acids 87 and 91 [140, 141]. Furthermore, mutations in *gyrB* have been found in LVX-resistant strains, including E463K, S479G, D481E, and R484K, but their significance is unknown [43, 142, 143].

porD and oorD

Pyruvate-ferredoxin oxidoreductase POR is an enzyme that catalyzes the thiamine pyrophosphate (TPP)-dependent oxidative decarboxylation of pyruvate, resulting in acetyl-CoA and CO_2 [144]. In contrast to pyruvate dehydrogenase, a widely distributed enzyme that catalyzes the same activity, POR can also catalyze the opposite reaction, namely the reductive carboxylation of acetyl-CoA, if a suitably low-potential electron donor is available [145]. POR is present in archaea, bacteria, and anaerobic protozoans. It belongs to the 2-oxoacid oxidoreductase (OR) family, catalyzes the oxidative decarboxylation of 2-oxoacids into acyl- or aryl-CoA derivatives [146]. The 2-oxoglutarate (-ketoglutarate) oxidoreductase (OOR) catalyzes the conversion of -ketoglutarate to succinyl-CoA and CO_2 [146, 147]. The FR resistance mechanisms in *H. pylori* primarily stem

from mutations in *porD* and *oorD* [55]. Su et al. [148], found G353A, A356G, and C357T changes in the *porD* genes of FR-resistant isolates. They also found A041G, A122G, and C349A(G) changes in the *oorD* gene. The six mutations in *H. pylori*'s *porD* and *oorD* genes result in six amino acid alterations related to FR resistance [55]. Research is identifying more mutations as potentially associated with FR resistance, including C347T, C347G, and C346A in *porD*, and A78G, A112G, A335G, C156T, and C165T in *oorD* [149].

RdxA, frxA, and frxA

Mutations that inactivate the *rdxA* gene, which encodes an oxygen-insensitive NADPH nitroreductase, and/or the *frxA* gene, which encodes a NADPH: flavin oxidoreductase, are the primary causes of MNZ resistance. Subsequently, mutations in the *fdxB* gene, which encodes a ferredoxin-like protein, result from frameshift mutations, insertions, and deletions [150]. The *rdxA* activity significantly impacts nitroreductase activity, which is critical for MNZ antibiotic action [151]. Changes in the *rdxA* gene are the most important factor because they can make *H. pylori* resistant to MNZ without changing *frxA*. Studies have shown that *frxA* frameshift mutations happen simultaneously in resistant and sensitive strains [152]. As a result, *frxA* mutations are thought to facilitate rdxA-mediated MNZ resistance rather than give MNZ resistance in and of themselves [153]. A recent investigation discovered numerous significant changes in the *rdxA* gene of MNZ-resistant strains [154], whereas mutations in the *fdxB* gene are less common. Furthermore, *fdxB* disruption paired with an inactive *rdxA* gene increases MNZ resistance [155]. Alternative resistant strains are characterized by substituting different amino acids in their complete *rdxA* sequences [152]. Zhang et al. [151] sequenced the whole genome of 238 clinical *H. pylori* strains and found that only the R16H/C and M21A amino acid alterations in *rdxA* were significant contributors to MNZ resistance, while mutations in the other 15 locations were solely associated with *H. pylori* origin.

rpoB

The RFB, a semi-synthetic compound derived from rifamycin S (RFP), is predominantly employed to treat tuberculosis [156]. Rifamycinoid (RBU) exhibits superior efficacy against both Gram-positive and Gram-negative bacteria compared to RFP [157]. It stops transcription when RBU and other RFP compounds bind to the b-subunit of the DNA-dependent RNA polymerase (*rpoB*) [158]. Mutations in four distinct areas of the *H. pylori rpoB* gene may be responsible for the effects [159, 160]. The regions are named Cluster I (codons 525–545), Cluster II (codon 585), Codon 149, and Codon 701[161]. Prior studies have shown that resistant laboratory *H. pylori* mutations exclusively occur in cluster I and cluster II of the *rpoB* gene [159, 160]. These mutations are similar to those that cause resistance in *Escherichia coli* and *Mycobacterium tuberculosis* [162].

The substitution of codon 149 from GTC to TTC has been demonstrated to lead to significant resistance to RBU. On the other hand, various random mutations introduce different amino acids and result in varying resistance levels. The substitution of codon 701 from CGC to CAH frequently results in modest resistance [163]. In addition, another study discovered that most strains resistant to RFP have specific genetic abnormalities in the *rpoB* gene, which lead to alterations in codons 530, 540, and 545 [158]. These changes cause resistance to develop not only to RFP but also to RBU. Previous research has shown that three specific codons in this area—525 (L525I), 530 (D530G/D530E/D530V/D530N), and 540 (H540Y/H540N)—are the main places where mutations happen that make the bacteria very resistant [55]. Furthermore, strains exhibiting high resistance levels have observed other mutations, such as I586N and L547F [164].

Challenges, potential solutions, and future directions

At present, treatment-induced *H. pylori* elimination is beset by a number of obstacles [165]. Strategies for eradicating *H. pylori* have experienced significant changes since its discovery in 1983 [166]. The principal cause of this is the escalating incidence of antibiotic-resistant strains, which renders many medications ineffective within clinical environments [167]. Antibiotic resistance among multidrug-resistant microorganisms has been recognized as an additional barrier to clinical eradication therapy [168]. Furthermore, the progression towards antibiotic resistance in *H. pylori* is continuous and ever-changing.

The degree and characteristics of antibiotic resistance and sensitivity significantly influence the effectiveness of eradication therapy in clinical patients [169]. Consequently, regular modifications to antibiotic dosages are necessary in order to preserve their therapeutic effectiveness. Unfortunately, there is a scarcity of dependable epidemiological studies that consistently monitor the risk of antibiotic resistance among the local population in a number of regions [170]. This research must form the basis for the choice of empirical antimicrobial medications. Eradication therapy is less effective when patients disobey medical advice and adhere to atypical prescription regimens prescribed by specialists [171].

There are a lot of factors that contribute to patients following their *H. pylori* eradication plans (Fig. 5). Several

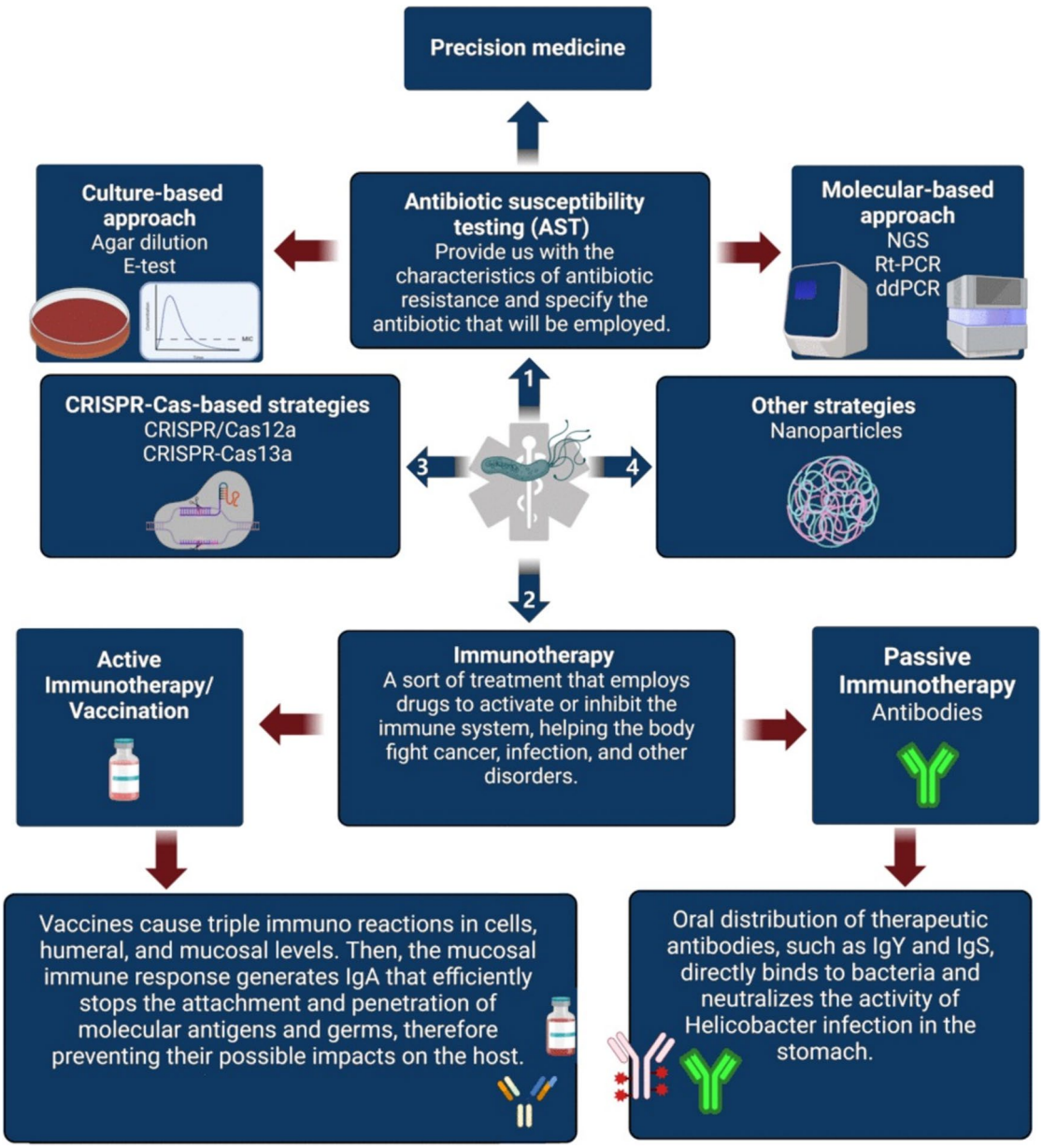

Fig. 5 Treatment strategies for antibiotic resistance to Helicobacter. Antibiotic susceptibility testing (AST) is vital for identifying bacterial resistance patterns and guiding antibiotic selection. This testing may be done using two methods: culture-based procedures like agar dilution and the E-test, or molecular techniques like Next-Generation Sequencing (NGS), Reverse Transcription PCR (Rt-PCR), and Droplet Digital PCR (ddPCR). Following the information gained by AST, numerous therapeutic strategies are investigated. Immunotherapy is critical in this situation, since it works by modifying immune system activity. This might include active immunotherapy approaches like vaccination, which boosts immune responses in cells and mucosal surfaces, resulting in the production of IgA antibodies that keep infections from sticking to and entering tissues. Immunotherapy, which includes the oral delivery of therapeutic antibodies such IgY and IgG, may help treat *H. pylori* in the stomach. CRISPR-Cas tools, such as CRISPR/Cas12a and CRISPR-Cas13a, which allow for precise gene editing to tackle antibiotic-resistant organisms. Furthermore, nanoparticle therapies should not be dismissed as a feasible option. Integrating these approaches marks a big step forward in precision medicine, aiding the battle against diseases that are more resistant to traditional treatments

research and clinical practice advocate for multi-antibiotic, multi-pronged elimination regimens that regulate stomach pH [172]. Because of this intricacy, both the doctor and the patient face difficulties. The persistence of socioeconomic conditions, variations in medication accessibility, and disparities in the prevalence of *H. pylori* infection across regions remain impediments to developing effective treatment strategies for *H. pylori* eradication

[173]. Drug susceptibility testing is essential in the battle against this pathogen, as an increasing number of patients are developing resistance to commonly prescribed medications, including the multidrug-resistant strain of *H. pylori* [174].

Preventive strategies:

Currently, antibiotic susceptibility testing (AST) technologies for *H. pylori* are largely classified as molecular-based and culture-based approaches [169, 175]. The agar dilution method, gradient diffusion susceptibility testing (E-test), broth microdilution method, and disc diffusion method are culture-based procedures that have historically been used as gold standards for *H. pylori* AST [176, 177]. These methods determine antibiotic sensitivity or resistance based on the antibiotic's minimum inhibitory concentration (MIC) under test [39, 169, 178].. Agar dilution is a reliable approach that can be used to test many *H. pylori* strains simultaneously and has been proposed as a reference assay for evaluating the correctness of other methods [178, 179]. The E-tests may quantify the disc diffusion technique and show a significant association with the agar dilution method for the majority of antibiotics, except for MNZ, which does not undergo anaerobic preincubation on the plates [180, 181]. In the regular clinical setting, the E-test outperforms agar dilution regarding cost and time [181].

Although culture-based approaches are presently the most successful for AST detection, they need stomach biopsy samples, which are only available via invasive endoscopic procedures. Furthermore, the technique is expensive, labor-intensive, and time-consuming, with results anticipated within one to two weeks since their dependability is heavily impacted by the samples given and the testing settings [182].

Molecular-based approaches work by identifying particular mutations in *H. pylori* that encode resistance mechanisms. They often provide advantages such as increased standardization and repeatability, quicker testing times (often resulting in findings the same day), and employing specimens collected using non-invasive procedures that do not need rapid processing [169]. Molecular-based approaches are classified into two main categories: next-generation sequencing (NGS) techniques and polymerase chain reaction (PCR)-based tests.

There are numerous diagnostic methodologies, each of which has its advantages and disadvantages [165]. The selection of tests is contingent upon the hospital's medical requirements, the test availability, and the instruments available. There are numerous varieties of tests. Both invasive and noninvasive techniques can be employed to conduct diagnostic testing. Endoscopy, histopathological analyses, rapid urea tests, cultures, and PCR tests are examples of invasive techniques, while serological, stool antigen, and breath tests are examples of noninvasive strategies [165, 183]. PCR, real-time PCR, fluorescence in situ hybridization, and peptide mass fingerprinting are common molecular instruments employed in addition to invasive and noninvasive procedures. It is essential to have accurate diagnostic tests (DT) to maintain the quality of care by preventing the administration of ineffective therapies and/or excessive treatments [184]. In the context of infectious diseases, molecular DT has the potential to enhance the reliability of outcomes yielded by histology, microbiology culture, and monoclonal assays.

The PCR has a high sensitivity and specificity for detecting *H. pylori* in patients with chronic gastritis or nonpeptic ulcer hemorrhage, with a significant proportion of patients diagnosed with dyspepsia testing positive for *H. pylori* [185, 186]. PCR also identifies low-density infections in a significant proportion of patients with dyspepsia, unlike traditional methods. In a study of histologically negative gastritis, 49% of patients with persistent mucosal inflammation tested positive for *H. pylori* [187, 188]. PCR can identify active infection in healthy individuals previously ruled out for *H. pylori* using conventional testing methods [189, 190]. PCR can also help identify *H. pylori* in medical situations where a diagnosis is important but hard to obtain, such as peptic ulcer hemorrhage, gastric cancer, or MALT lymphoma [191]. This latter discovery also implies that low-density infections might be associated with more virulent strains and, as such, might not be harmless.

Digital PCR (dPCR) is the most recent advancement in PCR technology that is commercially available [192]. The dPCR is a quantitative technology that exhibits enhanced sensitivity compared to real-time or conventional PCR techniques without compromising specificity [193, 194]. dPCR serves as a valuable tool in identifying infectious agents across various sample types, particularly in identifying and genotyping resistance genes associated with *H. pylori* infection [195–197]. Furthermore, researchers have used it to determine the significance of CYP219 polymorphisms in determining the efficacy of triple therapy [198]. Droplet digital PCR (ddPCR) is an approach that utilizes the production of water–oil emulsion particles to conduct dPCR [184]. ddPCR involves fractionating the PCR reaction of a single sample into 20,000 droplets, which are subsequently subjected to end-point PCR. Every droplet amplifies the template molecules, and a fluorescence detector determines the number of positive droplets.

A novel stool-based method for the detection, quantification, and partial genotyping of *H. pylori* was devised by Talarico et al. [199]. This method utilizes ddPCR, which

enables absolute quantification and increased sensitivity through PCR partitioning. The stool-based ddPCR assays are a noninvasive, sensitive method for detecting, quantitating, and partially genotyping *H. pylori*. In comparison to serology and stool antigen tests, the stool-based *H. pylori* 16S ddPCR assay demonstrated a sensitivity of 84% and 100% and a specificity of 100% and 71% [199].

Endoscopy with histology or culture, rapid urease testing (RUT), and molecular assays on clinical specimens are all established procedures for detecting *H. pylori*. Nevertheless, identifying *H. pylori* necessitates the development of a nucleic acid detection platform that is both precise and ultrasensitive [200].

The development of CRISPR-Cas13a-based antibacterial nucleocapsids, known as CapsidCas13a(s), that are capable of sequence-specifically targeting the microorganisms [201]. CRISPR-Cas13a-based antimicrobials have the potential to address the issue of antimicrobial-resistant organisms and may be employed to combat *H. pylori* resistance [202]. Wang et al. [203] validated and developed PCR-Cas13a as a promising method for the detection of *H. pylori* using the DNA of 84 clinical strains and 71 clinical specimens. This PCR-based CRISPR assay has a broad range of potential applications for detecting *H. pylori* and other slow-growing pathogens, as evidenced by their findings. While CapsidCas13a(s) has enormous potential for bacterial gene discovery [201], it does come with a few downsides, such as: (1) For each bacterial species and gene, a unique CapsidCas13a must be developed. (2) The data interpretation depends on the growth of bacteria, which may cause a delay in receiving the test results. (3) It becomes useless when the target gene cannot be translated or the bacteria cannot be cultured.

In contrast, Zhang et al. [204] developed a CRISPR/Cas12a-assisted array to screen for genotypes and detected *H. pylori* concentrations. This array can potentially be an effective tool for diagnosing and preventing *H. pylori* infection-related disorders and for large-scale clinical screening.

Curative strategies

Vaccination has emerged as a more viable approach to the clinical management of *H. pylori* infection, either to prevent infection or to treat an already established infection [165]. Vaccination is likely effective against drug-resistant strains and may potentially restrict the development of drug-resistant *H. pylori* [205]. As demonstrated in experimental models, immunization can also interrupt transmission and protect against reinfection. Although the prevalence of *H. pylori* infection in the developed world appears to be decreasing, estimates from a mathematical model suggest that eradicating *H. pylori* in the United States will take more than a century [165]. Indeed, a recent study evaluating the cost of *H. pylori* disease against the potential benefits of a vaccine suggests a highly favorable cost–benefit ratio [206].These changes impact the strength of the immunological and inflammatory responses of the host.

Despite extensive study, there is currently no vaccine available for *H. pylori* infections. Several vaccine candidates have proceeded to early-stage clinical trials, but none have reached the market [207]. The bacterium's genetic diversity and the difficulties of eliciting a significant immune response provide challenges for the development of an effective *H. pylori* vaccine [208]. Especially, a recent bibliometric analysis noted that research on the *H. pylori* vaccine is currently under development and new antigen combinations are under investigated as viable prospects. These findings are currently being investigated; thus, no vaccine has completed the necessary clinical trials to be made public. As a result, although immunization against *H. pylori* remains a viable option for preventing infections and associated stomach diseases, it is critical to understand that no such vaccine is now available, and research is ongoing to address the existing challenges [165].

It is being looked into whether oral vaccinations against gastrointestinal pathogens like *E. coli*, *V. cholerae*, and *H. pylori* are better than injection-based vaccines because they are safer and boost immune responses at mucosal sites [209–211]. However, administering vaccines orally is challenging due to unfavorable conditions in the gastrointestinal tract [211]. Currently, approved human oral vaccines primarily use attenuated viruses or pathogenic bacteria as carriers, which may regain their virulent properties. Lactic acid bacteria (LAB) have considerable promise as a carrier for oral vaccinations due to their generally recognized safe status, resistance to stomach acid, stability, and ability to activate both innate and adaptive immunity [212]. Research has focused on developing adjuvants and methods to administer oral immunizations, targeting M cells to ensure effectiveness. However, challenges remain, such as finding new ligands and methods for delivering immune responses to mucosal surfaces [213–215]. On the other hand, passive vaccination with orally delivered anti-*H. pylori* antibodies may be an alternate therapy for *H. pylori*. Research has demonstrated the effectiveness of this technique in preventing and treating a wide range of infections, such as rotavirus, *Candida albicans*, *Clostridium difficile*, and *Campylobacter jejuni* [216].

Additionally, the oral delivery of therapeutic antibodies that directly neutralize bacterial infections, such as egg yolk immunoglobulin Y (IgY) [217] and bovine immunoglobulins (Igs) [218] Earlier research has shown

that giving egg yolk IgY by mouth can help with passive immunization, which is crucial for stopping and treating bacterial, viral, and parasitic diseases in the gut [219]. As a result, the prospective benefits of employing egg yolk IgY specific to *H. pylori* in eradication may be worthwhile. Shin et al. [220] confirmed that egg yolk IgY against *H. pylori* whole-cell lysates inhibited *H. pylori* growth and the formation of inflammatory cells in Mongolian gerbils with *H. pylori* in their stomachs. More research by Suzuki et al. [221] showed that people who had *H. pylori* and were given anti-*H. pylori* urease IgY for four weeks had significantly lower C-urea breath test values than before treatment than before treatment. Recently, these subjects reported no serious side effects throughout the treatment period [222]. In a separate study, Borhani et al. [223] examined how well anti-*H. pylori* neutrophil-activating protein (NAP) IgY stopped *H. pylori* from sticking to stomach cancer cells. They discovered that anti-*H. pylori* NAP IgY may effectively inhibit *H. pylori* adherence to AGS cells. The data suggest that egg yolk IgY might be a viable option for preventing and treating *H. pylori* infection, especially in those who have established antibiotic resistance.

In contrast, bovine immunoglobulins (Igs) are responsible for preventing and treating numerous gastrointestinal infections, including *H. pylori* [218]. The Igs have been classified into three types: IgG, IgA, and IgM. Furthermore, IgG is classified into two subclasses: IgG1 and IgG2. The major function of immunoglobulins is to agglutinate bacteria, neutralize poisons, and inactivate viruses. IgA is capable of neutralizing viruses and bacterial toxins, as well as protecting against proteolysis. IgM outperforms other immunoglobulins in terms of complement fixation, viral neutralization, and bacterial agglutination. IgG may also tolerate gastric digestion. Numerous animal investigations have shown that bovine antibodies to *H. pylori* may prevent and even eliminate *H. pylori* infection by lowering the bacterial load [224, 225]. Breastfeeding protects babies from getting an early *H. pylori* infection in humans. This suggests that the passive delivery of immunoglobulin (Ig) A antibodies affects how *H. pylori* colonizes [226].

The protective effect of hyperimmune bovine IgG was demonstrated for certain pathogens in vivo animal models, including *H. pylori* [218, 227, 228].In conclusion, Peypar et al. [229] comprehensive evaluations of 76 publications demonstrated that immunoglobulin Y is more efficient than bovine antibodies in treating *H. pylori* infection.

On the other hand, prior research has established the efficacy of metallic nanoparticles (NPs), such as those made of zinc, silver, *H. pylori*, or silver, in eradicating a wide variety of bacteria [230]. The phenomena above are ascribed to established mechanisms, which include nonoxidative stress, metal ion release, and oxidative stress [231]. A bactericidal effect can be attained through the use of NPs in deficient concentrations, thereby significantly impeding the capacity of bacteria to develop resistance [232]. AgNPs, specifically those obtained from biological sources such as algae, plants, fungi, and bacteria, possess distinct benefits compared to alternative metallic NPs [233, 234]. These advantages stem from the controlled particle size, shape, and monodispersity facilitated by their preparation methods, resulting in decreased preparation time. This is of particular significance when considering environmentally sustainable approaches prior.

Conclusion

Resistance of *H. pylori* to antibiotics is linked to various clinical complications. Among the bacterial factors contributing to *H. pylori* resistance are mutations, biofilms, coccoid form, and efflux pumps. *H. pylori* commonly employ gene modification strategies to circumvent bactericidal effects, specifically targeting genes associated with nucleic acid synthesis, rRNA coding, and cell wall formation. Biological activity and efflux pump systems are processes that are intricately intertwined. The initiation of efflux pump activation and concurrent biofilm formation, which leads to the development multidrug-resistant (MDR) bacteria, significantly complicates eradication therapy.

Finally, antibiotic susceptibility testing, various oral immunotherapy and vaccines, and the new technological tools used to manage *H. pylori* infection have been discussed.

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Author contributions

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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