**Helicobacter pylori eradication – the alternatives beyond antibiotics**

M. Roxo-Rosa\(^{a,1,2}\), M. Oleastro\(^3\) and F. F. Vale\(^{4,5}\)

1Center for Biodiversity, Functional & Integrative Genomics (BioFIG), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal
2Departamento de Genética Humana, Instituto Nacional Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal
3Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal
4Faculdade de Engenharia, Universidade Católica Portuguesa, Estrada Octávio Pato, 2635-631 Rio de Mouro, Portugal
5Centro de Estudos do Ambiente e do Mar (CESAM/FCUL), Faculdade de Ciências da Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal

*Helicobacter pylori* is one of the most common gastrointestinal bacterial pathogens among humans, affecting over 50% of the world’s population. Yet, the prevalence of infection is highly dependent on the socioeconomic status of the country, varying from 20 to over 80% of the population, with higher rates for developing countries [1]. Almost invariably, colonization of the human gastric mucosa by this Gram-negative bacterium starts in childhood and elicits an acute host immune response. As it is, however, inefficient in bacteria clearance, it opens the door for sustained infection and chronic gastric inflammation throughout the patient life. Although often asymptomatic, most patients experience dyspeptic symptoms in result to the long-term *H. pylori*-dependent gastritis and, later in the adulthood, 15-20% of them end up developing peptic ulcer disease (gastric or duodenal) and 2-5% gastric cancer (adenocarcinoma or mucosa-associated lymphoid tissue lymphoma) [2].

Despite the ongoing discussion on which infected patients should be treated, all colonized patients or just those with overt symptoms of disease, the high prevalence rates worldwide and the real risk of severe gastric diseases’ development demand the need for good strategies for *H. pylori* eradication. Currently, this is managed by the use of a triple therapy, involving the co-administration of two antibiotics (with macrolides, fluoroquinolones, amoxicillin, nitroimidazoles and tetracycline among the most prescribed ones) and ranitidine bismuth and/or a proton pump inhibitor [3]. However, as for other bacteria, during the last decades we have been observing the continuous growth of the number of antibiotic resistant *H. pylori* strains, especially in countries in which antibiotic prescription is more common. Stressing the urgent need for alternative therapies, the still growing antibiotic resistance rate already leads to treatment failure in about 20% of the *H. pylori*-infected patients, but depending on the therapeutic schema and strain resistance pattern, the failure rate can reach 70% [4]. This chapter focuses on the state of the art of alternative strategies for combating *H. pylori* infection, not involving the use of antibiotics, currently under investigation, namely vaccines [5,6], bacterial photodynamic inactivation [7,8], phage therapy [9,10] and the use of probiotics [11].

**Keywords** *Helicobacter pylori*; vaccines; photodynamic inactivation; phage therapy; probiotics

1. *Helicobacter pylori* infection

In the early 1980s, Barry Marshall and Robin Warren reported the successful isolation and culture of a spiral bacterial species from the human stomach, whose presence was closely associated with mucosal inflammation [12]. This discovery revolutionized our understanding of upper gastrointestinal tract diseases and triggered a revolution in the treatment of gastritis and peptic ulcer.

The bacterium, previously named *Campylobacter pyloridis*, and later on *Helicobacter pylori*, is a gastric Gram-negative bacterium belonging to the phylum Proteobacteria, Epsilonproteobacteria class, Campylobacterales order and *Helicobacteraceae* family. Currently, the genus *Helicobacter* is composed by more than a dozen gastric species, that have been detected in different animals, such as cats, dogs, pigs and primates, although the zoonotic potential of these species is still unclear [13].

All these bacteria are microaerophilic, flagellated, spiral and urease positive, allowing the colonization of the harsh stomach environment. *H. pylori* is the human major pathogen type species of the *Helicobacter* genus, affecting over 50% of the world’s population. The prevalence of infection is highly dependent on the socioeconomic status of the country, varying from 20 to over 80% of the population, with higher rates for developing countries, which are characterized by poor sanitary conditions, crowded living conditions and lack of clean water [1]. In Western countries, infection is associated more with lower socioeconomic groups with a prevalence of the infection ranging from 10–60% [14]. Risk factors include increasing age, large family size and low socioeconomic conditions [15-18].

*H. pylori* is elegantly well adapted for a lifelong colonization of the gastric mucosa of humans, using complex strategies to maintain an inflammation of the gastric epithelium, while limiting the extent of the immune response in order to prevent its elimination. Besides the unusual high degree of genetic variability, due to its natural competence for transformation and for conjugative transfer of genomic islands and a high recombination rate [19], some factors have been identified as critical for colonization and persistence of infection such as the urease, flagella and adhesins. Then,
once having escaped the gastric acidity and have reached the gastric mucus layer and the epithelium, the bacterium delivers its virulence factors, CagA and VacA, respectively encoded by the cytotoxin-associated gene A and the vacuolating cytotoxin, the two major toxins which will damaged the gastric mucosa and causing disease (reviewed in [20]).

Infection by \(H.\) \(pylori\) is usually acquired during childhood [21]. Most infected individuals remain asymptomatic throughout life while \(H.\) \(pylori\) infection always causes a chronic active gastritis. In some cases, this gastric inflammation may evolve toward more severe diseases such as duodenal or gastric ulcers, gastric mucosa-associated lymphoid tissue lymphoma or non-cardia gastric adenocarcinomas [2]. In 1994, \(H.\) \(pylori\) infection was classified as a type 1 carcinogen by the International Agency for Research on Cancer, a World Health Organization agency [22]. Studies have also associated \(H.\) \(pylori\) infection to diverse extragastric non-malignant diseases (involving the cardiovascular, hepatobiliary, dermatological, immunological, and haematological systems).

\(H.\) \(pylori\) infection can be difficult to treat and requires the use of several antimicrobials. Currently, a triple therapy is considered the most effective treatment for \(H.\) \(pylori\) eradication. This involves the combined intake of a proton pump inhibitor (PPI) with amoxicillin and one of two antibiotics, clarithromycin or metronidazole (or tinidazole), given for 10 days according to the European recommendations for \(H.\) \(pylori\) eradication [3,23]. However, the most recent data show that this combination fails in about 20% of the patients, but depending on the antibiotic it can reach 70% of unsuccessful [4]. Alternative regimens using different combinations of the same antibiotics can increase the rate of treatment, such as the sequential treatment which includes a 5-day period with PPI-amoxicillin, followed by a 5 day period with PPI-clarithromycin-metronidazole (or tinidazole [24]); the three antibiotics given concomitantly together with a PPI [25]; levofloxacin containing triple therapy [26]; and the bismuth-containing quadruple therapy (bismuth salts, metronidazole, and tetracycline plus PPI [27]).

However, the rapid worldwide emergence of resistant strains to clarithromycin and metronidazole, besides contributing to a decreased effectiveness of the first-line treatment, is causing the emergence of resistant strains to second-line drugs, and, for example for levofloxacin, rising levels of resistance have been reported worldwide [28,29]. Accordingly, a recent large multicentre study showed high levels of \(H.\) \(pylori\) resistance to clarithromycin, metronidazole and levofloxacin. Moreover, it showed that resistance to antibiotics was uneven distributed among European countries, with resistance rates for clarithromycin and levofloxacin being significantly higher in Western/Central and Southern Europe (>20%) than in Northern European countries (<10%), reflecting different antibiotic policies. It was also noted that in many countries the high rate of clarithromycin resistance no longer allows its empirical use in standard anti-\(H.\) \(pylori\) regimens [4].

2. Therapeutic alternatives against \(H.\) \(pylori\)

The antibiotic era, a period of massive misuse of antibiotics that extends for almost 100 years, since the discovery of penicillin in 1928 by Sir Alexander Fleming [30], may be reaching an end because of the rapidly increasing emergence of resistance among several species of pathogenic bacteria, including \(H.\) \(pylori\). This opened doors to an intense research over the last two decades in the search for alternatives to bacterial eradication. Included in the most exploited alternative approaches to combat \(H.\) \(pylori\) infections are: the development of efficient anti-\(H.\) \(pylori\) vaccines, which has proven to be a hard goal to achieve; the photodynamic inactivation, an old promising approach that was forgotten for decades; the phage therapy, which began to be studied before the discovery of antibiotics, but was forgotten until recently; the use of probiotics, a new fashion in fighting pathogens and promoting health, using other bacteria or their derivates. Additionally, as briefly discussed at the end of the chapter, important efforts have also been recently spent in the study of the anti-microbial and anti-inflammatory effect of natural compounds against \(H.\) \(pylori\) infection.

2.1. Vaccines

As with other infectious diseases, making a vaccine against \(H.\) \(pylori\) seems to be an obvious priority in the search for alternatives to antibiotics. In the 1990s, it was thought that this task would be easily achieved, not only because of the knowledge on \(H.\) \(pylori\) biology available at the time, but also because of the already vast experience in the vaccines field, accumulated with the development of vaccines against other pathogens. However, all the spent efforts failed so far, which lead us to share with others [6] the feeling that the prospects for producing an efficient vaccine against \(H.\) \(pylori\) seem further away than they did 15 years ago. Rendering it more difficult is the marked decline on the invested funds from large pharmaceutical companies. Indeed, even considering that a vaccine should be the most cost-effective approach to deal with this microorganism, the decline in the prevalence of \(H.\) \(pylori\) infection observed in the last decade in developed countries [31], is driving the investor’s attention to the research on other human diseases.

In the early 1990’s, as soon as the \(Helicobacter felis\) mouse model became available [32], several studies were made in order to prove that vaccination could protect against this gastric infection [33-35]. The findings of these authors contributed to the early enthusiasm of the field, as mice immunization was considered well succeeded when used prophylactically [33,34], i.e., prior to infection, but also therapeutically after established infection [35]. However, more accurate methods for quantification of \(H.\) \(felis\), developed later on, revealed that immunization led to significantly
reduced colonization levels, but did not achieve complete and sustained eradication of these bacteria in mice [36]. In the late 1990’s, the scientific community’s expectations were again defrauded with the studies using primates. Indeed, although some authors claimed a significant reduction in the number of bacteria colonizing the stomach of rhesus monkeys, therapeutically immunized with some anti-\textit{H. pylori} vaccine candidates [37,38], others, using similar formulations, failed to detect an effect on bacterial load, only achieving some reduction in the levels of gastritis [39]. Moreover, when similar experiments were repeated in rhesus monkeys that had never been infected with \textit{H. pylori} before, no effect at all was observed on gastric bacterial colonization, nor on gastric pathological changes [40].

Since then, different formulations (including whole cell and recombinant-antigens’ based vaccines), adjuvants and delivery routes have been tested on animal models, offering them a significant (although not complete) protection against experimental infections (reviewed in [6]). Most of the tested vaccines were based in \textit{H. pylori} key virulence factors, and on abundant and/or surface exposed proteins. These include urease [37-45], known for its crucial role in regulating the pH during colonization, the well-established virulence factor CagA in conjugation with the VacA toxin and the neutrophil activating protein [46]. Other examples of tested proteins are: the antioxidant proteins catalase [47] and thiolperoxidase [48]; some heat shock proteins (Hsp) (namely HspB) [49]; the flagellar sheath protein putative N-acetylatedaminylactose-binding hemagglutinin [50]; and also some adhesins [51]. In addition, vaccines composed of predicted immunodominant CD4+ T cell epitopes of some of these \textit{H. pylori} proteins have been tested [52]. Even considering that some have reached clinical trials [46], none resulted yet effective for human use.

Rendering difficult the development of effective vaccines is the aforementioned high degree of genetic variability among \textit{H. pylori} strains, required for the full adaptation of the bacterium to the host’s stomach [19]. Thus, acting as a quasisspecies each individual host harbours a unique \textit{H. pylori} strain [53,54], a fact that has been exploited for a variety of studies regarding human population migrations [55]. Co-infection with multiple closely related clones of \textit{H. pylori} is also a possibility [19]. This fact, underlies the considerable inter-individual variation of the humoral recognition pattern among different infected patients and \textit{H. pylori} isolates, suggesting both variations on the host immune responses and on the antigenic pattern among strains [56]. Indeed, by studying the immunoproteome of a group of heterogeneous \textit{H. pylori} strains, isolated from Portuguese patients differing in age, gender and \textit{H. pylori}-associated gastric diseases, we have recently presented clear evidence of the variability of the antigenic pattern among \textit{H. pylori} strains. We demonstrated that genetic variability among strains is not reflected in their proteome but, instead, in their immunoproteome [5].

Also strongly impairing the effectiveness of the tested vaccines, is the fact that \textit{H. pylori} guarantees its survival and persistence throughout the life of its host, by using a set of molecular mechanisms to constantly evade the host immune response (reviewed in [57]). Among such successful strategies of bacterial evasion is the relatively anergic lipopolysaccharide of the \textit{H. pylori} cell wall, which mimics the molecular structures of the host cells surface, through the expression of host-related Lewis antigens [58]. Another camouflage used by \textit{H. pylori}, is the expression of proteins at its surface which specifically bind to host-secreted proteins, e.g., bacterial plasminogen-binding proteins (PgbA and PgbB), allowing the bacterium to be coated with host proteins [59]. Moreover, \textit{H. pylori} uses mechanisms of phase variation to switch between different allelic variants of genes encoding its virulence factors, which allows this organism to adapt to the varying conditions in the niche over time [57,60]. Perhaps the most ingenious evasion mechanism proposed to be used by \textit{H. pylori} is the “altruist autolysis”. Accordingly, in culture some bacteria suffer a programmed autolytic process, releasing their cytoplasmic proteins that are subsequently adsorbed to the external surface of the neighbouring viable cells, ensuring them a range of protective proteins, such as urease [61]. If occurring \textit{in vivo}, such mechanism could also offer a protective effect against host antibodies as these would no longer be able to strongly agglutinate viable \textit{H. pylori}, because the loosely attached surface proteins would detach, removing the antibodies from the bacterial surface (reviewed in [6]).

Taking all together, it is our conviction that the development of efficient anti-\textit{H. pylori} vaccines, for both prophylactic and therapeutic purposes, relies on the fully understanding of the interactions between this pathogen and its host immune system.

2.2. Bacterial photodynamic inactivation

The use of the solar light as a medical tool dates back the ancient Greece, Egypt and India. It was, however, long forgotten, until the early twentieth century when the modern Western civilization, by the Danish Niels Finsen and the Germans Oscar Raab and Herman von Tappeiner, rediscovered the benefits of phototherapy (reviewed in [62]). It was Herman von Tappeiner who, in 1904 in his pioneering studies in photobiology, introduced the term “photodynamic action”. Although it is not clear why he called the process “dynamic”, a term that others tried unsuccessfully to replace [63], it persisted through the “photodynamic therapy” (PDT), a promising tool in modern cancer treatment. Underlying this clinical method was the discovery of haematoporphyrin ability to accumulate in tumours, together with its phototoxic effect on cancer cells. Currently, PDT is based on the administration of nontoxic, or of low-toxicity in the dark, photosensitive molecules, usually referred to as photosensitizers, that, once irradiated with light at appropriated wavelength, are activated and, in the presence of O₂, trigger a destructive action in biological systems, leading to cell death [64].
Figure 1 briefly describes the energy shifts suffered by photosensitizers during PDT. Upon absorbing light, the photosensitizer reaches its singlet excited state (1PS), a highly reactive energetic state from which the photosensitizer may decay by fluorescence to its ground state (PS), or by electronic “intersystem crossing” to its long-lived triplet excited state (3PS). In this highly reactive triplet state, the photosensitizer reacts with any molecule in its microenvironment via electron or energy transfer, two competing pathways called type I and type II reactions, respectively [65].

![Diagram illustrating the energy shifts of the photosensitizer during PDT. PS, photosensitizer ground state; 1PS, photosensitizer singlet state; 3PS, photosensitizer triplet state; , light at appropriate wavelength.](image)

Type I reactions (Eq. 1) are redox reactions, in which the photosensitizer in its excited triplet state exchanges an electron with a neighbouring molecule (i.e., any biomolecule (A), the molecular oxygen (O₂), or another molecule of photosensitizer also in its triplet state), originating several types of cytotoxic free radicals (PS⁺⁺, PS−−, A⁺⁺ and O₂⁻⁻). Alternatively, in type II reactions (Eq. 2), the photosensitizer at its triplet state transfers its energy (not electrons) to the molecular oxygen O₂, originating the high-energetic singlet oxygen (1O₂). Due to their short lifetimes and diffusion paths, all the reactive species originated by both types I and II reactions further interact with biomolecules in their immediate vicinity, leading to severe cell damage and, ultimately, death (Fig. 1). Both type I and type II photochemical reactions occur simultaneously, in a ratio that is dependent on the photosensitizer itself, on its cellular and sub-cellular localization and on surrounding medium [65].

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\text{Type I reactions (electron transfer)} \begin{align*}
3\text{PS} + 3\text{PS} &\rightarrow \text{PS}^{++} + \text{PS}^{--} \\
3\text{PS} + \text{A} &\rightarrow \text{A}^{++} + \text{PS}^{--} \\
\text{PS}^{--} + \text{O}_2 &\rightarrow \text{PS}^{++} + \text{O}_2^{--}
\end{align*}
\]  

(1)

\[
\text{Type II reactions (energy transfer)} \begin{align*}
3\text{PS} + \text{O}_2 &\rightarrow \text{PS} + 1\text{O}_2
\end{align*}
\]  

(2)

By now, PDT and various photosensitizers, with porphyrin derivatives among the most widely used (reviewed in [66]), have regulatory approval for treatment of several malignant and pre-malignant conditions, including Barrett’s oesophagus and gastric cancer [65,67-69]. Interestingly, its application has also been shown to be effective in inactivation of viruses [70], bacteria [7,71,72], fungi [73] and protozoa [74]. In fact, it was already in 1900 that Oscar Raab accidentally discovered that cells of Paramecium caudatum were inhibited by the dye acridine orange under illumination, an approach that was immediately put in practice to treat skin infections (reviewed in [75]). However, overshadowed by the discovery of penicillin in 1928 by Sir Alexander Fleming [30], this was forgotten for decades. Strongly motivated by the worldwide rise in bacterial resistance to antibiotics [71], the photodynamic inactivation (PDI) of bacteria has gain a new breath and has been widely explored in the past two decades (reviewed in [66]), with the studies suggesting that PDI does not induce bacterial resistance [71,76,77] and its efficiency is independent of the pattern of antibiotic resistance of the strain [78,79].

Gram-positive bacteria are in general sensitive to PDI [80]. However, its successful application in the eradication of Gram-negative strains has been more challenging. Indeed, with their additional lipid membrane, located externally to the peptidoglycan network, strongly impairing the electrostatic attraction and the access of the photosensitizer to the bacterial cytoplasm, Gram-negative bacteria are not efficiently inactivated with the classically negatively and non-charged photosensitizers [81]. Nevertheless, the use of such photosensitizers in the presence of permeabilization agents (such as CaCl₂ and Tris-EDTA) or, alternatively, the use of cationic photosensitizers are considered efficient for in vitro PDI eradication of Escherichia coli [72,82-88]. Current research efforts focus on the scrutiny of novel families of photosensitizers and on the study of their structural features that somehow potentiate their antimicrobial effects for in vivo use. In fact, physical properties such as the number and type of charge, its distribution over the molecule and the
presence of long hydrocarbon chains, have an influence on the hydrophilicity of the photosensitizer and, therefore, on its cellular distribution and PDI effectiveness [7,89]. Moreover, differences in photochemical properties can lead to differences in $\text{O}_2$ production and decay, when the photosensitizers are taken up by bacterial cells [72].

With its broad spectrum of action, PDI has conducted to promising results in treating animal models [90] and human infections [91-93]. For example, compared with standard endodontic treatment, PDI was shown to be a better approach in the elimination of bacterial biofilms in root canals, and, even better results were obtained by combining these two therapies, a procedure that is already in clinical trials [91,92]. However, notwithstanding the bactericidal potential of photosensitizers, for treatment of bacterial infections it should be considered their interaction with surrounding healthy tissues of the host. Indeed, in general, such molecules are not specifically or exclusively accumulated by bacteria and are structurally similar to those used in PDT (in cancer treatment), emphasizing the importance of evaluating the intracellular distribution of photosensitizers in eukaryotic cells, namely human cells [7]. PDI cannot be used to treat systemic infections [71] as photosensitizer accumulation in healthy host cells: nuclei (a common feature among cationic photosensitizers), increases the risk of DNA damage, mutations and carcinogenesis; mitochondria, is likely to induce apoptosis; lysosomes or endosomes, leads to permeabilization of these organelles, with the consequent release of their content to the cytosol, where the photosensitizer can sensitize tubulin to photodamage (reviewed in [94]).

Although still limited, data on the in vitro, ex vivo and in vivo use of PDI in eradication of $H.\text{ pylori}$ are promising and supportive of it as a clinical alternative to antibiotics [8,71,93,95-97]. Already in 1996, Millson et al. [97] concluded that Helicobacter mustelae can be efficiently killed on explanted ferret gastric mucosa following topical administration of methylene blue or toluidine blue O (>0.75 mg/kg) and subsequent eradication with appropriate laser (20 and 200 J/cm², respectively). After these findings, a prospective clinical trial, carried out in 13 infected patients, showed that the oral administration of 5-aminolevulinic acid (20 mg/kg), a metabolic precursor of protoporphyrin IX in the heme biosynthetic pathway (Fig. 2), followed by endoscopic irradiation of the gastric antrum with blue (410 nm, 50 J/cm²) or white (10 J/cm²) light, lead to a greater eradication of $H.\text{ pylori}$ in illuminated areas compared to control zones [98]. However, the use of these drugs is not innocuous to the stomach lining, since it is also absorbed by human cells.

Curiously, the nature seems to encourage the use of PDI in eradication of $H.\text{ pylori}$ infection. In fact, this Gram-negative bacterium has a natural ability to accumulate photoactive porphyrins, namely protoporphyrin IX and coproporphyrin (by-products of the endogenous heme biosynthesis, Fig. 2) [96]. Since protoporphyrin IX maximally absorbs light at a wavelength of 410 nm, efficient $H.\text{ pylori}$ killing is possible just by low fluence of violet/blue light (375 - 425 nm) [96,100] or of broad-spectrum conventional white endoscopic light [8], without the need of any added photosensitizer. Moreover, the localization of the infection in the gastric mucosa facilitates the endoscopic access for light delivery. As shown by Ganz et al. [100], the delivery of blue light (405 nm, 40 J/cm²) to a 1 cm diameter spot in the gastric antrum, via optical fiber passed through an upper gastrointestinal endoscope, lead to the local death of 90% of the $H.\text{ pylori}$ colony forming units in infected patients. This is a promising procedure that was shown to be safe and feasible for whole-stomach treatment [93]. Recent data have shown that the bactericidal effect of protoporphyrin IX is mediated by cell membrane injury [8] and that of methylene blue is via DNA damage, which is further potentiated by

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**Fig. 2** $H.\text{ pylori}$ endogenous heme biosynthetic pathway [99]. Enzymes catalyzing each reaction are indicated by the respective “open reading frame” in the $H.\text{ pylori}$ 26695 (reference strain, ATCC 700392) genome: HP0163, delta-aminolevulinic acid dehydratase; HP0237, porphobilinogen deaminase; Hp1224, uroporphyrinogen-III synthase; Hp0604, uroporphyrinogen decarboxylase; HP0665, coproporphyrinogen III oxidase; ?, presumed protoporphyrinogen IX dehydrogenase (not yet identified in the genome of the strain $H.\text{ pylori}$ 26695); and HP0376, ferrochelatase.
the fact that, in contrast to other Gram-negative bacteria, such as *E. coli* [101], *H. pylori* expresses fewer genes to repair phototoxicity-induced DNA damage [95].

Nevertheless, none of the mentioned prospective pilot trials [93,96,100] achieved complete and sustained eradication of *H. pylori*, stressing the need of further studies for efficient PDI eradication of this bacterium.

2.3. Phage therapy

The concept of phage therapy was introduced long before the golden era of antibiotics. However, the discoveries of Sir Alexander Fleming [30] on penicillin effects, led to the ceasing of the research on phage therapy, at least in Western countries. Actually, it continued in the ex-Union of Soviet Socialist Republics and East-European countries, remaining unknown for the West until the end of communism political regimes. Due to the antibiotic resistance crisis phage therapy is experimenting a renaissance period [10]. Phage therapy consists on the use of all lytic bacteriophages or their lytic proteins, to induce the lysis of the host bacterium cell and, thus, eliminating the infecting agent, regardless of the existence of antibiotic resistance [102]. The phage life cycle can be either lytic or lysogenic (Fig. 3), with some phages presenting just the lytic cycle (lytic phages), while others having one or the other cycle (temperate phages, *i.e.*, phages that can switch from the lytic to the lysogenic cycle). The first steps of phage infection are similar for both cycles, *i.e.*, adsorption and injection of the phage nucleic acid into the host cell. The next phases in lytic phages are: synthesis of phage nucleic acid and proteins; phage assembly; and phage release of the host cell, usually leading to lysis of the latter. The remaining steps for lysogenic phages consist in the integration of the phage nucleic acid within the host bacterium genome, becoming a prophage, which is replicated with the host cell machinery [103]. An induction event, such as radiation or a SOS response, can produce the switch from the lysogenic to the lytic cycle (Fig. 3) [104]. Phages control bacterial populations in the wild and can be potentially used as hygiene measures in food production facilities and hospitals, to treat bacterial infections in humans, animals and crops [10].

Taking into account the differences between lytic and lysogenic cycles, it is rather easy to understand that lytic phages are more suitable for phage therapy. Indeed, in the case of lysogenic phages, infection does not lead to cell lysis in the majority of times (Fig. 3).

Concerning *H. pylori*, the description of phages is still a resumed topic in the literature, although it is growing. The firsts descriptions were made by electron microscopy soon after the discovery of *H. pylori* in the early eighties [105,106]. In the next decade, there were just two additional publications from the same group [107,108], describing the spontaneous release of phages from the *Siphoviridae* family. Although these two studies provided a brief description of the genomic map of the phage, the information available at that time did not allow concluding whether it is a lytic phage, or a temperate phage. Fifteen years later, another description of a temperate phage of *H. pylori*, induced by UV, came out [104]. In the previous two years, there was a burst of information available from genome sequencing projects.
of \( H. \text{pylori} \) strains, that provided until now the sequences of four complete [9,109,110] and three remnant prophages [111,112]. Although there is no available information on the nature of the life cycle of the described \( H. \text{pylori} \) phages, it is correct to affirm that there is no description of lytic phages. Certainly the screening for lytic phages in this bacterium reservoir could led to their identification, but to our knowledge there are only a couple of studies addressing this issue [104,113].

Taking into account the absence of a currently identified lytic phage in \( H. \text{pylori} \), the approach that considers the use of phage lytic proteins appears to be more suitable for phage therapy (Fig. 3). This approach is applicable for any phage, since the minimum request is the existence of a lysin, the protein responsible for the host bacterial cell wall lysis. The advantage of using lysins resides in the fact that: they are specific for the bacteria species, which means that these proteins would not disturb the gut microbiota; and there is no description of resistance to bacterial lysins [114]. The most effective way of producing high concentrations of lysins is based on classical molecular biology methods (cloning, expression and purification of the protein) [115]. The main drawback of using lysins against \( H. \text{pylori} \) is that being a Gram-negative, lysins are not capable of crossing its outer membrane from the outside. Indeed, in the nature, lysins hydrolyze the cell wall from the inside, not from the outside as would be the case in a therapeutic scenario. A modification of the lysine, or its encapsapsulation in a drug delivery system, could help to overcome this limitation. There are some reports of lysins’ modifications that can cross the outer membrane of other Gram-negative bacteria, such as \( Yersinia \text{pestis} \) [116]. Therefore, phage therapy against \( H. \text{pylori} \) is a promising approach that should be further exploited.

2.4. Probiotics

Probiotics are live organisms or produced substances that are orally administered to promote health [11]. Probiotics may be administered alone or in conjunction with antibiotics. The mode of action of probiotics against \( H. \text{pylori} \) is not completely ascertained. The most feasible mode of action is by stimulation of the immune system, turning it more ready to fight the infection by \( H. \text{pylori} \). Alternatively, probiotics may compete with \( H. \text{pylori} \) for nutrients and habitat, and may lead to \( H. \text{pylori} \) toxins inhibition. This last hypothesis would certainly diminish the bacterial load, which have been associated with disease development [117], turning easier its eliminations by the immune system and/or antibiotics. Probiotics may also contribute for patient compliance with the therapy, because they tend to diminish the side effects of antibiotics, namely by preservation of gut microbiota, improving eradication rates [11,118-121]. Several randomized controlled trials showed a decrease in the frequency and severity of antibiotic side effects, even considering that an improvement in eradication rate was not always observed [11,121].

The most common probiotics used in clinical trials are \( Saccharomyces \text{boulardii} \) and \( Lactobacillus \) strains [11]. Nevertheless, others have been tested, specially preparations of multiple strains of different species, such as \( Bacillus \text{subtilis} \) and \( Streptococcus \text{faecium} \) [122], \( B. \text{animalis} \) and \( L. \text{casei} \) [123], \( Lactobacillus \) and \( Bifidobacterium \) [124], among others. Three recent meta-analysis concluded that the yeast \( S. \text{boulardii} \) and the bacterium \( Lactobacillus \) increases eradication rates and decreases overall therapy-related side effects, particularly diarrhoea [125-127]. Indeed, \( Lactobacillus \) strains are known to make part of the transient gastric flora [128].

Short chain fatty acids and bacteriocins proteins have been implicated in the inhibition of \( H. \text{pylori} \) by lactic acid bacteria [11]. The short chain fatty acids’ antimicrobial activity could be due to the inhibition of urease activity. In turn, bacteriocins are a heterogeneous group of proteins, lethal to bacteria of the same species, other than the producing bacteria [11]. The short chain fatty acids’ antimicrobial activity could be due to the inhibition of urease activity. In turn, bacteriocins are a heterogeneous group of proteins, lethal to bacteria of the same species, other than the producing bacteria [11]. The release of heat-stable bacteriocins with anti-\( H. \text{pylori} \) activity was previously identified in \( Lactobacillus \) strains, among others [11,128].

Although there are some controversial results in the use of probiotics from clinical trials [11], the application of probiotics appears to improve the eradication rate of \( H. \text{pylori} \), even if indirectly. The main question nowadays is how to use probiotics and include them in the regimen against \( H. \text{pylori} \), with the maximum advantage for the patient [130].

Similar alternatives to fight gastrointestinal diseases are based on the administration of probiotics, a non-digestible food ingredient favourable to the growth and/or activity of indigenous probiotic bacteria, and thus benefit the host. These usually are oligosaccharides, such as fructo-oligosaccharides, inulin, galacto-oligosaccharides, and soybean oligosaccharides, and complex polysaccharides that constitute dietary fiber [131,132]. For instance some infant-formula contain probiotic oligosaccharides [131]. There are clinical trials addressing safety issues of prebiotics [133], but not their action in \( H. \text{pylori} \) infection. Other alternatives are: symbiotics, a product that contain probiotics and prebiotics, which may have a synergetic effect and that may be a supplement, or be present in functional food as a food additive; postbiotic, a metabolic by-product produced by a probiotic microorganism that favours the host; functional food, a modified food or food ingredient that, by containing probiotics or prebiotics, provides a health benefit beyond the nutrients it contains [131]. Examples of the latter are: live-culture yogurt that contains probiotic bacteria, prebiotics, and other dietary nutrients; or human milk that contains oligosaccharides (prebiotics) and may contain some naturally occurring bifidobacteria (probiotics) [131]. Inflammation is widely recognized as a risk factor for gastric \( H. \text{pylori} \) associated diseases, notably gastric cancer, and certain foods, applied as a non-antimicrobial approach, may reduce the inflammation that is a major risk factor for \( H. \text{pylori} \) associated diseases [134].
2.5. Natural compounds

The use of plant extracts as medicines is an ancient human practice and several compounds appear to show antimicrobial activity against \textit{H. pylori} (reviewed in [11]). The studies focusing on alternative therapies should be endured to assure different therapeutic approaches in multi-resistant \textit{H. pylori} strains. In the context of \textit{H. pylori} infection and the associated inflammation, ultimately is the main cause of mucosal damage and severe disease, other therapeutic approaches should also be envisaged, with the use of natural occurring substances that target both the microbe and the associated-inflammation. Is this category fall the resveratrol, a phytoalexin found mostly in grapes, peanuts, cranberries, and strawberries, and the curcumin, a phytochemical derived from \textit{Curcuma longa}. The remarkable anticarcinogenic, anti-inflammatory and antioxidant properties of both these natural compounds are well documented in the literature [135,136]. Moreover, they both exhibit antimicrobial activity against \textit{H. pylori} [137,138]. Despite their low bioavailability and the preclinical studies suggesting higher efficiency if higher levels of exposure are achieved, they are suitable for the development of novel chemical analogs and/or micro/nanotechnology-based formulations, representing an interesting nutritional approach in the combat of \textit{H. pylori} infection and associated-disease.

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3. Conclusion

In nowadays antibiotic resistance is a significant problem for treatment of diseases caused by virtually all known infectious bacteria. The gastric pathogen \textit{H. pylori} is no exception to this rule and during the last years we have observed a continuous and worrying increase of resistant strains. This is related with the bad use of antibiotics that work as a selective agent of resistant strains, a fact supported by the highest prevalence of resistant strains in countries in which antibiotic prescription is also more common. The growing rate of bacterial resistance to antibiotics can soon made humanity enter in a period, similar to the one we have before the discovery of antibiotics. Thus, we need to focus on finding and exploring alternative anti-\textit{H. pylori} combating strategies with clinical applicability, such as the ones revisited in this chapter. These strategies should be further exploited, while limiting antibiotics prescription to a minimum to make us win some years.

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