Journal of Microbiology (2014) Vol. 52, No. 4, pp. 259–272
DOI 10.1007/s12275-014-3603-5

MINIREVIEW

Nontraditional Therapies to Treat *Helicobacter pylori* Infection

Morris O. Makobongo, Jeremy J. Gilbreath[#], and D. Scott Merrell[*]

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814, USA
[#]Present address: Department of Laboratory Medicine, University of Washington,1959 NE Pacific St., Seattle WA 98195, USA

(Received Nov 18, 2013 / Accepted Dec 16, 2013)

The Gram-negative pathogen *Helicobacter pylori* is increasingly more resistant to the three major antibiotics (metronidazole, clarithromycin and amoxicillin) that are most commonly used to treat infection. As a result, there is an increased rate of treatment failure; this translates into an overall higher cost of treatment due to the need for increased length of treatment and/or the requirement for combination or sequential therapy. Given the rise in antibiotic resistance, the complicated treatment regime, and issues related to patient compliance that stem from the duration and complexity of treatment, there is clearly a pressing need for the development of novel therapeutic strategies to combat *H. pylori* infection. As such, researchers are actively investigating the utility of antimicrobial peptides, small molecule inhibitors and naturopathic therapies. Herein we review and discuss each of these novel approaches as a means to target this important gastric pathogen.

Keywords: *Helicobacter pylori*, novel antibiotics, antimicrobial peptides, small molecule therapeutics, phytoceuticals

Introduction

Helicobacter pylori, a microaerophilic, spiral-shaped, flagellated, Gram-negative bacterium has evolved to colonize the human gastric mucosa. The bacterium was first isolated by Warren and Marshall in 1982 (Warren and Marshall, 1983) and has since been linked with development of severe gastrointestinal diseases such as chronic gastritis, gastric and duodenal ulcers and gastric cancer (Marshall *et al.*, 1984; Isaacson, 1994; Peek and Blaser, 1997). As a result, the World Health Organization International Agency for Research on Cancer has classified *H. pylori* as a group I carcinogen, distinctively making this bacterium the only known bacterial carcinogen (WHO, 1994). Since its discovery, evidence of the role of *H. pylori* as a major human gastrointestinal pathogen has steadily accumulated (Glassman *et al.*, 1990; Isaacson, 1994; Peek and Blaser, 1997). Currently, it is estimated that half of the world's population carry the bacterium (Pounder and Ng, 1995; Dunn *et al.*, 1997; Suerbaum and Josenhans, 2007). However, the prevalence of infection varies widely based on geographic location, age, and socio-economic status (Everhart, 2000). Infection rates are higher in developing countries (50–90%) than in developed countries, where prevalence may be as low as 10–20% (Parsonnet, 1993; Everhart, 2000; Calvet *et al.*, 2013). Lower infection rates have been observed with improved sanitation and hygiene, possibly suggesting fecal contamination as a mode of transmission (Rowland and Drumm, 1995; Bourke *et al.*, 1996). However, other studies have strongly suggested oral-oral or gastro-oral transmission (Rowland and Drumm, 1995). Thus, the route of transmission remains poorly defined and little or nothing is known about the infectious dose required to infect a human during the process of natural transmission.

The current standard antibiotic-based treatments for *H. pylori* infection have recently become less practical for global control and/or eradication because they are not always effective (Bazzoli *et al.*, 2002). This reduced efficacy has largely been attributed to the fact that *H. pylori* has developed resistance to virtually all antibiotics (Jones *et al.*, 2008). Moreover, treatment regimens have evolved from monotherapy to triple, quadruple and sequential therapies that extend up to 14 days of duration and result in high treatment cost as well as significant patient compliance issues; the prolonged treatment duration along with accompanying gastrointestinal discomfort often cause patients to stop taking their medications early or to only take them intermittently. These practices often lead to incomplete eradication and relapse of infection and may enhance the emergence of antibiotic resistance. Indeed, the lack of patient compliance and high *H. pylori* mutation frequencies are the two major factors that drive the development of antibiotic resistance (Malfertheiner, 1993; Versalovic *et al.*, 1996).

Although the current management of *H. pylori* infection involves treatment with antibiotics, clearly novel therapies that target alternative microbial pathways and that are not circumvented by existing bacterial antibiotic resistance strategies would be incredibly useful. In order for these novel therapies to be most effective, they should be simple and act quickly so that the bacteria have less time to develop resistance. These tasks are challenging enough on their own, but there are additional physiological and physical challenges

*For correspondence. E-mail: douglas.merrell@usuhs.edu; Tel.: +1-301-295-1584; Fax: +1-301-295-3773

that must be overcome for any novel therapeutics that will target *H. pylori*. These include the low intra-gastric pH found in the stomach, which may inhibit the effectiveness of therapeutic compounds, as well as the need to either penetrate the gastric mucus layer where *H. pylori* resides or to enter the circulatory system and then reach the bacteria from that route. The challenges associated with developing novel anti-*H. pylori* therapies have necessitated that researches employ increasingly creative strategies. Despite the fact that few of these potential new treatments have currently been tested thoroughly in the clinic, significant research progress in this area has been made over the last several years.

Herein, we discuss current therapies and review several non-traditional strategies that are currently being developed to control *H. pylori* infection. These non-traditional approaches are broadly divided into two main categories: 1) novel "synthetic" means of treatment, which include new classes of antimicrobial peptides and small molecule inhibitors, and 2) "natural" treatment options, which include the use of probiotics and naturopathic therapies to treat *H. pylori* infection.

Current antibiotic therapies

The current recommended first line anti-*H. pylori* regimen is 10–14 days of a triple drug therapy that contains a proton pump inhibitor (PPI), amoxicillin and clarithromycin or metronidazole (Malfertheiner *et al.*, 2007, 2012). As a second-line of treatment, a quadruple drug therapy with a proton pump inhibitor, bismuth salt, tetracycline, and metronidazole can be given for 14 days (Malfertheiner *et al.*, 2007, 2012). Unfortunately, the efficacy of first and second line therapies has fallen; treatment failures have recently been noted in up to 20–30% of patients (70–80% treatment success rate) (Bertoli Neto *et al.*, 2006). This success rate does not reach the suggested "ideal" rate of >90% treatment success, and does not even meet the current requirements that are expected of new antibiotics in development. In situations where the first or second line therapies are not effective in clearing the infection, one of the following treatment regimens may be given: 1) a 14 day concomitant quadruple treatment regimen that consists of a PPI, amoxicillin, clarithromycin and a nitro-imidazole, 2) a 10 day sequential treatment that consists of a PPI plus amoxicillin for the first 5 days followed by a PPI, clarithromycin and a nitro-imidazole for the following 5 days, or 3) a 14 day quadruple therapy supplemented with bismuth (Graham and Fischbach, 2010). A clear downside to these complex regimens is that they employ more antibiotics, which increases the cost of therapy and patient non-compliance.

The driving factor, that has changed how anti-*H. pylori* therapies are administered, is the increase in resistance to first line drugs like clarithromycin and the nitro-imidazoles (*e.g.* metronidazole). Currently, resistance rates for these drugs range from 1.7–28% for clarithromycin and 20–39% for metronidazole (Wong *et al.*, 2003; Molina-Infante *et al.*, 2013). Importantly, a high percentage of metronidazole resistant strains (~85%) are also clarithromycin resistant, which further decreases the effectiveness of treatment regimens that utilize these drugs in combination (Nardone, 2000; Queiroz *et al.*, 2002). Given these current levels of resistance, it is likely that similar degrees of treatment failure will be seen with sequential therapy as have been observed with the standard triple-drug treatment regimens. In contrast to clarithromycin and metronidazole, resistance to amoxicillin is rare and this drug remains effective against most strains of *H. pylori* (Huang and Hunt, 2003). As a result, amoxicillin is one of the few traditional antibiotics that continue to be effective in clearing *H. pylori* infection, albeit in combination therapy.

Taken together, these factors highlight the great need for new antibiotics and/or new classes of antimicrobials that employ novel modes of action to successfully combat *H. pylori* infection. In response to this great need, recent studies have highlighted several novel therapeutic approaches that could potentially be used to develop novel classes of antibiotics. These strategies include the use of natural and synthetic antimicrobial peptides, small molecule inhibitors and naturopathic therapy.

Antimicrobial peptides (AMPs)

Although there are an increasing number of therapeutic options for targeting Gram-positive bacteria, the development of new classes of antibiotics that are effective against Gram-negative bacteria has lagged behind (Livermore, 2009). Gram-negative bacteria owe their resistance to currently used antibiotics largely to the outer membrane (OM) and OM-associated cell surface structures. This structure, which is stabilized by LPS, acts as a permeability barrier that is able to efficiently block many antimicrobial compounds from reaching their intracellular targets (Delcour, 2009). In addition, other key resistance mechanisms such as efflux pump systems that remove antibiotics from the bacterial cell, rely on the ability of the OM to maintain sub-lethal drug concentrations in the periplasm (Nikaido, 2003). Given the importance of these OM components, it is perhaps no surprise that nearly every organism that can be infected with Gram-negative bacteria produces host defense molecules that target this structure. One such group of molecules that target the Gram-negative cell wall is the antimicrobial peptides (AMPs) (Jenssen *et al.*, 2006). Given the increase in antibiotic resistance rates seen in Gram-negative bacteria over the last decade, these molecules have gained a renewed interest as a possible treatment option. As such, natural as well as synthetic AMPs are actively being investigated.

Natural AMPs: AMPs are a core component of the innate immune system of numerous eukaryotes (Andreu and Rivas, 1998). While AMPs are highly conserved among these organisms, and may share cationic and amphipathic properties, these molecules can also maintain a great deal of diversity based on primary sequence, secondary structures and size (Zasloff, 2002). The cationic AMPs have the ability to interact with the anionic bacterial cell wall due to charge electrostatic attractions. However, there is accumulating evidence that suggests that peptide hydrophobic properties also enable interaction and insertion into the hydrophobic core of the cell wall to form transmembrane pores (Lockey and Ourth, 1996; Matsuzaki, 1998). In some instances, the AMP may directly interact with specific glycoproteins or glycolipids in the cell membrane (Breukink *et al.*, 1999).

There are three families of natural antimicrobial polypep-

tides among the AMPs produced in the stomach: elafins, defensins, and cathelicidins. Of the three, defensins and cathelicidins are the two examples of principal human antimicrobial peptides that display activity against *H. pylori*. For example, LL-37, which is a member of the cathelicidin family of peptides that is also known as human cationic antimicrobial peptide 18 (hCAP18), has been demonstrated to show antimicrobial activity against *H. pylori*. LL-37 primarily exerts its direct effect by binding LPS, but also has chemotactic activities that help in the recruitment of inflammatory cells (Hase *et al.*, 2003; Leszczynska *et al.*, 2009). Similar to the cathelicidins, defensin peptides have also been shown to inhibit *H. pylori* (Hamanaka *et al.*, 2001; Uehara *et al.*, 2003). Recent studies have demonstrated that of the four known human β-defensins (HBD), HBD-1, HBD-2, HBD-3, and HBD-4, only HBD-2 and HBD-3 are potent against *H. pylori* (Kawauchi *et al.*, 2006; Nuding *et al.*, 2013). Interestingly, although ineffective against *H. pylori*, HBD-4 is known to be effective against other bacterial species such as *Enterococcus faecalis* (Lee and Baek, 2012); this perhaps suggests that evolution can contribute to the ability of some bacteria to evade some AMPs.

Despite the sustained potency of natural AMPs against bacteria, some bacterial pathogens have also developed resistance to many of these AMPs (Guo *et al.*, 1998; Peschel *et al.*, 2001; Jin *et al.*, 2004). The mechanisms utilized by bacteria to evade natural AMPs have been well-documented (Shafer *et al.*, 1998; Peschel, 2002) and include 1) repulsion of AMPs by reducing the net negative charge of the bacterial cell wall via covalent modification of anionic molecules such as teichoic acids, phospholipids or lipid A (Tran *et al.*, 2006); 2) pumping out AMPs through energy-dependent efflux pumps (Shen *et al.*, 2010); 3) altering membrane fluidity (Bayer *et al.*, 2000; Andra *et al.*, 2011); and/or 4) cleaving AMPs with OM proteases (Guina *et al.*, 2000; Schmidtchen *et al.*, 2002). Outside of these types of potential resistance mechanisms, a major barrier to implementing the use of natural AMPs to treat *H. pylori* infection is the fact that many of the AMPs undergo proteolytic cleavage by both the host digestive components as well as by bacterial enzymes (Schmidtchen *et al.*, 2002). Despite the fact that resistance to AMPs has been observed, because these molecules have successfully been a key component in combating bacterial infection for millions of years, investigators have come to realize that natural AMP structures may serve as the basis for designing new synthetic AMPs that may overcome some of the challenges seen with natural AMPs.

Synthetic AMPs: Oligo acyl lysyl peptides (OAKs): Recently, several synthetic mimetics of natural AMPs have been developed for use as antimicrobials (Basile *et al.*, 2006; Radzishevsky *et al.*, 2008; Makobongo *et al.*, 2009, 2012). Among the best characterized of those mimetics is a class of copolymer compounds referred to as oligo-acyl-lysyl (OAK) peptides. OAKs consist of tandem repeats of alternating acyl chains and lysine residues, a novel design that mimics the primary structure and function of natural AMPs, but does not form stable secondary structures (Radzishevsky *et al.*, 2008; Rotem and Mor, 2009; Zaknoon *et al.*, 2009; Rotem *et al.*, 2010). Physicochemical studies have shown that the activity of OAKs is derived from the peptide's optimized size, charge, hydrophobicity and amphipathic organization (Brogden, 2005; Radzishevsky *et al.*, 2007; Sarig *et al.*, 2008; Epand *et al.*, 2009). OAKs have broad spectrum antibacterial activity and have recently been shown to be highly potent against *H. pylori in vitro* (Makobongo *et al.*, 2009) and *in vivo* (Makobongo *et al.*, 2012). Like natural AMPs, OAKs have the ability to target both the bacterial cell wall as well as intracellular components (Makobongo *et al.*, 2012). Although the specific target(s) of the OAKs in the cell membrane(s) of Gram-negative bacteria is unknown, it is believed that the peptides interact with LPS and permeabilize the cell via pore formation (Makobongo *et al.*, 2012). In addition, some OAKs such as $C_{12}K\text{-}2\beta_{12}$ (Makobongo *et al.*, 2012) and $C_{12}K\text{-}5\alpha_8$ (Rotem *et al.*, 2008) may have the ability to enter the cell and bind to nucleic acids. Consistent with this idea, the $C_{12}K\text{-}2\beta_{12}$ OAK was shown to have dual modes of action against *H. pylori in vitro;* at high concentrations, the peptide results in irreversible pore formation, whereas at lower concentrations the peptide reaches the intracellular compartment and binds to RNA and DNA (Makobongo *et al.*, 2012). An attractive feature of using OAKs as antimicrobials is that the peptides are devoid of known proteolytic cleavage sites, which makes these molecules resistant to enzymatic cleavage. In addition, OAKs such as $C_{12}K\text{-}2\beta_{12}$ have the advantage of multiple nonspecific modes of action, which may limit the ability of *H. pylori* to develop resistance.

Although there are no clinical data regarding the effectiveness of OAKs and other synthetic AMPs in treating *H. pylori* infection, data from animal models suggest that these molecules could be a promising alternative to the current treatment options. In a recent study, therapeutic treatment of *H. pylori*-infected Mongolian gerbils 1 week post-infection with sequential daily doses of $C_{12}K\text{-}2\beta_{12}$ resulted in a significant reduction in stomach colonization burden (Makobongo *et al.*, 2012). Other studies also suggest the possibility of synergistic activity when OAKs are used in combination with conventional antibiotics (Makobongo *et al.*, 2009). Thus, OAKs may also be useful in combination therapies to treat *H. pylori* infection. Taken *en masse*, data indicate that synthetic AMPs may have a promising future as potential novel therapies that can be used to treat *H. pylori* infection.

Small molecule inhibitors as anti-*H. pylori* therapies

Another group of therapeutics that is currently being considered for treating *H. pylori* infection is composed of small molecule inhibitors. Characteristically, small molecule therapeutic activity is based on the ability to inactivate or alter the function of bacterial enzymes. In order to be effective, the small molecule target needs to be conserved among all strains, be ubiquitously expressed, and should not be present in humans. To date, several candidate small molecules have been evaluated for activity against *H. pylori* (Table 1).

Of the small molecules that have been tested, perhaps the best characterized is SQ109 (Protopopova *et al.*, 2005; Makobongo *et al.*, 2013). This drug, *N*-geranyl-*N*'-(2-adamantyl) ethane-1, 2-diamine, was originally developed as an antituberculosis therapeutic and has been shown to be both safe and well-tolerated in three human safety trials (Horwith *et al.*, 2007; National Institute of Allergy and Infectious Diseases (NIAID), 2010). During the development of SQ109 as an

Table 1. Small molecule inhibitors

Small molecule (name)	*H. pylori* target	Effective dose		References
		IC_{50}	MIC	
N-geranyl-*N*′-(2-adamantyl) ethane-1,2 diamine (SQ109)	Unknown	ND	6–10 μM	Makobongo *et al.* (2013)
Pyrazolopyrimidinediones	MurI	ND	1–64 μM	de Jonge *et al.* (2009)
Pyridodiazepines	MurI	1.7 μM	0.5 μg/ml	Geng *et al.* (2009)
Sulfonamides/Sulfamates	β-Carbonic anhydrase	54–105 nM	ND	Nishimori *et al.* (2007)
Thiadiazolidine-3, 5-dione (CHIR-1)	Cagα ATPase	0.45 μM	ND	Hilleringmann *et al.* (2006)
Pyrimidopyridone/Cyanothiophene	AddAB	13–96 μM	ND	Amundsen *et al.* (2008)
N-(4-Aminobutyl)-2-fluoro-ethanimidiamide (ABFA)	Agmatine deaminase	6.8 μM	ND	Jones *et al.* (2010)
N-(4-Aminobutyl)-2-chloro-ethanimidiamide (ABCA)	Agmatine deaminase	0.87 μM	ND	Jones *et al.* (2010)
8-Hydroxy-7-(6-sulfonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid	Shp2 protein tyrosine phosphatase (PTP)	0.269–0.367 μM	ND	Chen *et al.* (2006)
Compound A	Glutamate racemases	1.4 μM	4 μg/ml	Lundqvist *et al.* (2007)

IC_{50}, inhibitory concentration 50%; MIC, minimum inhibitory concentration; ND, not determined

anti-tuberculosis treatment, pharmacokinetic studies revealed that the drug was present in high concentrations in the stomach (Jia *et al.*, 2005, 2006); this finding prompted further studies to determine whether SQ109 could be used as an anti-*H. pylori* drug. A recent publication (Makobongo *et al.*, 2013) evaluated the efficacy of SQ109 against a panel of laboratory derived and clinically isolated *H. pylori* strains *in vitro*. In that study, all *H. pylori* strains tested were highly susceptible to SQ109. In addition to being effective at relatively low concentrations, the drug retained the ability to kill the bacteria at low pH, and was effective at killing slow growing or static bacteria. These qualities make SQ109 an attractive option for treating *H. pylori,* since the bacteria reside in or near a low pH environment and are typically slow growing *in vivo.* In a molar-to-molar comparison, SQ109 was more effective than commonly used therapeutic antibiotics such as amoxicillin and metronidazole. As with the currently used antibiotics, a key determinant in the effectiveness of any potential new antimicrobial is whether or not the target organism is able to develop resistance to the drug. Importantly, preliminary *in vitro* studies indicate *H. pylori* resistance rates to SQ109 are low (Makobongo *et al.*, 2013). Although the precise mechanism of action of this drug against *H. pylori* is unknown, when taken together with the previously established clinical safety trials that indicate that the drug is well tolerated in humans (National Institute of Allergy and Infectious Diseases (NIAID), 2010). The *in vitro* studies suggest that SQ109 could potentially be used as an anti-*H. pylori* monotherapy. Indeed, a Phase IIA and a Phase IIB clinical trial (SQ109 alone and SQ109 plus a proton pump inhibitor [PPI], respectively) have been conducted; results suggest SQ109 was more effective when used with a PPI. Based on these results a Phase IIC clinical trial of SQ109 plus conventional antibiotics is currently being conducted (Sequella, 2003).

A key component of survival of all microorganisms is maintaining the integrity of the cell wall. This requirement has long been exploited as a microbial weakness by natural antimicrobials as well as those designed in the laboratory. For example, the β-lactam class of antibiotics (e.g. penicillins, cephalosporins, carbapenams, and monobactams) inhibits a key step in cell wall biosynthesis resulting in bacterial killing. Because cell wall synthesis in bacteria is a complex process that involves multiple enzyme-dependent biosynthetic reactions, there are several enzymes that could potentially serve as targets for small molecules. One such enzyme that has been targeted by multiple small molecules is the glutamate racemase (MurI). MurI functions to convert L-glutamate to D-glutamate (van Heijenoort, 2001), which is an essential step in peptidoglycan formation.

Within the past several years, there have been several screens to identify *H. pylori* MurI inhibitors (Lundqvist *et al.*, 2007; de Jonge *et al.*, 2009; Geng *et al.*, 2009). Using a high-throughput screening technique (Lundqvist *et al.*, 2007), de Jonge and colleagues identified a novel group of pyrazolopyrimidinediones that exhibit *H. pylori*-specific anti-MurI activity (de Jonge *et al.*, 2009). The effective dose of the six compounds that were analyzed ranged from 1–64 μM (reported as MIC_{90}), and activity depended on the specific analogue tested as well as the strain of *H. pylori* examined. One of the pyrazolopyrimidinedione compounds was also tested against a broad range of non-*Helicobacter* bacterial species; interestingly the tested molecule (referred to as compound D in that study) displayed no antimicrobial activity against any of the other bacterial species; thus, the drug shows specificity for *H. pylori* MurI. Spontaneous resistance to the pyrazolopyrimidinedione compounds in *H. pylori* was relatively low. While these *in vitro* studies suggest that this group of pyrazolopyrimidinedione compounds could be used to combat *H. pylori* infections, studies that evaluate the drug's safety and pharmacokinetic properties are needed to determine the full utility of pyrazolopyrimidinediones in anti-*H. pylori* therapy.

Given the importance of the MurI enzyme in cell wall biosynthesis, it is perhaps not surprising that multiple small molecule compounds have been developed to target MurI. Based on the same high throughput screen that led to the discovery of the pyrazolopyrimidinedione compounds (Lundqvist *et al.*, 2007; de Jonge *et al.*, 2009), another group of small molecules called pyridodiazepine amines, were also selected as highly specific anti-*H. pylori* MurI inhibitors (Geng *et al.*, 2009). These molecules, a group of pyridodiazepine amines, share several characteristics with the pyrazolopyrimidinediones. For example, pyridodiazepine amines display a low *H. pylori* MurI inhibitory concentration (IC_{50} = 1.7 μM), as well as low MICs against *H. pylori* in culture

(Geng *et al.*, 2009). Furthermore, the pyridodiazepine amines are also highly selective for *H. pylori,* and are not effective against other pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis.* These findings, combined with the data from the evaluation of the pyrazolopyrimidinedione compounds, suggest that MurI is an excellent target for *H. pylori*-specific antimicrobial therapy. However, similar to the pyrazolopyrimidinediones, further studies are needed to fully evaluate the utility of the pyridodiazepine amines as an anti-*H. pylori* therapy.

Another essential enzyme that has been targeted by small molecule therapeutics is the *H. pylori* β-carbonic anhydrase (Nishimori *et al.*, 2007). This enzyme, which catalyzes the hydration of carbon dioxide to bicarbonate and a proton, is thought to play an important role in urea and bicarbonate metabolism in *H. pylori*. Due to the essential nature of these metabolic intermediates in the ability of *H. pylori* to survive low pH, disrupting these pathways at the level of carbon dioxide hydration is an attractive strategy. Consistent with this line of thinking, a screen against the purified recombinant *H. pylori* β-carbonic anhydrase enzyme identified several potent anti-β-carbonic anhydrase molecules, that belonged to the sulfonamide and sulfamate classes of inhibitors (Nishimori *et al.*, 2007). Notably, while these *in vitro* enzyme inhibition studies are suggestive, the lack of *in vivo* efficacy data makes it difficult to predict how effective these compounds will actually be against *H. pylori.* Despite this uncertainty, the fact that many of these compounds are already proven to be safe and well tolerated in humans should prove to be beneficial if they are pursued as therapies.

Most traditional antibiotic agents target essential cellular components or pathways whose loss results in cellular stasis or death. A fairly recent approach to treating infections is to target bacterial components or processes that are important for virulence of the organism rather than basic cellular function. An attractive feature of this approach is that because the drug targets are not essential for viability *per se,* the likelihood of selecting for and enriching compensatory mutations that confer drug resistance is presumably less likely. In the case of *H. pylori,* this approach was applied to identify compounds that inhibit the activity of Cagα, which is the VirB11-type ATPase required for translocation of the CagA oncoprotein (Hilleringmann *et al.*, 2006). A high throughput screen directed against purified Cagα identified three compounds that inhibited activity of the ATPase. All three compounds (CHIR-1, -2, and -3) were highly effective in *in vitro* enzymatic assays, with IC_{50} values of <1 μM. Two of the three compounds are thiadiazolidine-3, 5-diones, and are closely related structurally. From that pair, CHIR-1 was the most potent. Consistent with inhibition of the Cagα ATPase, *H. pylori* cells treated with CHIR-1 display an accumulation of the CagA protein; the protein is not translocated into target host cells. Furthermore, CHIR-1 significantly decreased CagA-dependent IL-8 secretion from host cells that were co-cultured with *H. pylori.* Combined, these *in vitro* studies indicate that CHIR-1 acts as an effective means to prevent translocation of CagA into host cells. In addition to effectively inhibiting the Cagα ATPase and CagA secretion *in vitro,* CHIR-1 treatment also significantly reduced the *H. pylori* gastric colonization load in a murine infection model, which suggests that the drug is available within the gastric mucosa (Hilleringmann *et al.*, 2006). Currently, further work is needed to fully demonstrate the effectiveness of CHIR-1, -2, and -3 as therapeutics.

A key determinant in the successful persistence of *H. pylori* within the host is the ability to repair DNA damage. One of the major enzymes that facilitates repair of DNA breaks and homologous recombination in *H. pylori* is the helicase-nuclease enzyme AddAB (Amundsen *et al.*, 2008). As a result of this enzyme's essentiality, as well as the high prevalence of this enzyme among bacteria, high-throughput screens have been used to identify inhibitors of AddAB activity (Amundsen *et al.*, 2008). Several compounds that effectively and selectively inhibited AddAB enzyme activity have been identified. Two of the most potent inhibitors (CID 1045135, a pyrimidopyridone; CID 2295461, a cyanothiophene) inhibited the double-stranded exonuclease activity of AddAB at relatively low concentrations (Table 1). Subsequent analysis of molecules structurally related to the potent pyrimidopyridone and cyanothiophene compounds allowed the identification of two additional inhibitors of AddAB activity, CID 697851 (a cyanothiophene) and CID 1517823 (a pyrimidopyridone). Of this set of compounds, CID 697851 was the most effective AddAB inhibitor tested, with an IC_{50} of 13 μM. Similar to the other compounds, CID 697851 inhibited the AddAB exonuclease activity, but not helicase activity. While the specific mechanism of inhibition has not been determined for these small molecules, these findings perhaps suggest a common mode of action between these inhibitors. As with most of the anti-*H. pylori* small molecules described to date, the *in vitro* results with these pyrimidopyridone and cyanothiophene compounds are promising. However, whether or not these small molecule inhibitors would be effective treatment for *H. pylori* infection will require further testing.

Other common targets for small molecule therapeutics are often components involved in metabolic pathways. One such factor that has been targeted in *H. pylori* is the agmatine deaminase enzyme (HpAgD), which catalyzes the formation of *N*-carbamoyl putricine and ammonia from agmatine (Jones *et al.*, 2010). Though not considered a *bona fide* virulence factor, because the substrate of agmatine deaminase stimulates the innate immune system, this enzyme may play a role in disease pathogenesis by affecting the levels of agmatine *in vivo* (Jones *et al.*, 2010). Using purified recombinant HpAgD, Jones and colleagues designed and characterized two potent haloacetamidine-based HpAgD inhibitors: *N*-(4-aminobutyl)-2-fluoro-ethanimidamide (referred to as ABFA), and *N*-(4-aminobutyl)-2-cholo-ethanimidamide (referred to as ABCA) (Jones *et al.*, 2010). Both of these rationally designed compounds share the same mode of action, namely modification of the catalytic Cys324 residue within the HpAgD active site. The IC_{50} values for these compounds are in the low micromolar range (~0.87 μM for ABCA, ~6.8 μM for ABFA), and both are highly selective for HpAgD (Jones *et al.*, 2010). Combined with good water solubility and irreversible inactivation of HpAgD, these small molecules may have potential as possible anti-*H. pylori* therapeutics. However, despite strong *in vitro* inhibition of enzyme activity, neither of these compounds has yet been tested against *H. pylori* in culture. Thus, it remains unclear

whether successful inhibition of HpAgD and the resulting increased levels of agmatine would affect *H. pylori* growth, survival and/or disease pathogenesis.

Although the number of small molecules that have been tested against *H. pylori* is relatively small, there are several important enzymes/proteins that could potentially be targeted in the future. One such protein is flavodoxin, a small redox protein that serves as the electron acceptor for the

Table 2. Representative phytotherapy examples

Phytotherapeutical extracts	Active/ Principle constituent(s)	Effective dose		*H. pylori* Target/Mechanism	References
		IC_{50}	MIC		
Cinnamon extract	Cinnamic aldehyde, eugenol and terpenes	ND	0.04–1.0 mg/ml	Anti-inflammatory effects-block the production of IL-1, IL-6 and the TNF-α	Nir *et al.* (2000), Bergonzelli *et al.* (2003)
Broccoli sprouts	Sulforafane	ND	2–4 μg/ml (MIC_{90}); Plant: 0.125 mg/ml (w/v)	Anti-inflammatory effects-IL-1β, IL-8, and TNF-α; Reduce colonization and attenuate gastritis	Fahey *et al.* (2002), Yanaka *et al.* (2009), Keenan *et al.* (2012)
Manuka honey	Sugar	ND	ND	High viscosity and osmotic potential due to sugar content	al Somal *et al.* (1994), Ndip *et al.* (2007), Keenan *et al.* (2012)
Omega-3 oil/fatty acids	Docosahexaenoic acid Omega-3 Linolenic acid	ND	100–500 μM (MIC_{90}) $1.8–5 \times 10^{-4}$ M	Anti-inflammatory effects and anti-*H. pylori* adhesion to epithelial gastric cells	Thompson *et al.* (1994), Keenan *et al.* (2012)
Essential oils (cloves, oregano, aniseed, mint, carrot seed)	α-Bisabolol	0.017–0.146 μg/ml	ND	Anti-inflammatory and anti-ulcerogenic effects	Bergonzelli *et al.* (2003), Cwikla *et al.* (2010), Silverio *et al.* (2013)
Curcuma longa (turmeric)	Curcumin	ND	6.25–50 μg/ml	Anti-inflammatory effects-suppress TNF-induced NF-κB activation and IL-8 release	Mahady *et al.* (2002), De *et al.* (2009), Koosirirat *et al.* (2010), Vitor and Vale (2011)
Anisomeles indica (Labiatae)	Ovatodiolide (OVT), pedalitin, scutellarein 7-O-beta-d-glucuronide methyl ester, and acteoside	ND	0.06 μM (OVT); 50–100 μg/ml (Extracts)	Anti-inflammatory effects-inhibits bacterial LPS induced NF-kB activation, iNOS, NO, TNF-α, IL-12, and IL-8	Rao *et al.* (2009), Lien *et al.* (2013)
Cyrtocarpa procera Kunth (Anacardiaceae)	Unknown	ND	7.81 μg/ml	Anti-inflammation and bacteriostatic and bactericidal	Escobedo-Hinojosa *et al.* (2012)
Rutaceae family, Citrus fruits-lemon peels, leaves, pulp etc.	Pierene, β-pinene, geranyloxycoumarin Auraptene, Limonene	ND	75–500 μg/ml	Anti-inflammation and anti-colonization	Rozza *et al.* (2011), Sekiguchi *et al.* (2012)
Calophyllum brasiliense (Clusiaceae)	Unknown	<25 μg/ml	ND	Modulation of endogenous antioxidant systems	Lemos *et al.* (2012)
Bridelia micrantha (Euphorbiaceae)	Alkaloids, flavonoids, steroids, tannins and saponins	ND	0.0048–0.625 mg/ml	Inhibition of DNA gyrase and FabZ enzyme	Zhang *et al.* (2008), Okeleye *et al.* (2011)
Sclerocarya birrea (Anacardiaceae)	Essential oils, with terpinen-4-ol, pyrrolidine, aromadendrene and α-gurjunene in order of abundance.	310 μg/ml to 2500 μg/ml	MIC_{50} = 0.004–6.3 μg/ml	Increased bacterial cell membrane fluidity and permeability	Njume *et al.* (2011)
Enantia chlorantha (Annonaceae)	Unknown	ND	MIC = 0.39 mg/ml; MBC = 1.56 mg/ml;	Anti-gastritis	Tan *et al.* (2010)
Pelargonium sidoides extract (Eps 7630)	Glucuronic acid-enriched polysaccharides, tannin-like proanthocyanidin	ND	0.001 to 10 mg/ml	Anti-bacterial adhesins	Wittschier *et al.* (2007a and 2007b)
Allium sativum L. *(Garlic)*	Thiosulfinates (allicin)	ND	40 μg/ml	Membrane lipid, total inhibition of RNA synthesis	Sivam *et al.* (1997), Sivam (2001)

IC_{50}, inhibitory concentration 50%; MIC, minimum inhibitory concentration; ND, not determined

pyruvate-oxidoreductase enzyme complex (POR) (Cremades *et al.*, 2005). In addition to being essential for *H. pylori* survival, this enzyme is not found in humans, which makes flavodoxin an attractive therapeutic target. *In vitro* studies using purified *H. pylori* flavodoxin suggest that the binding site in this enzyme is accessible to water and can bind small molecules such as benzylamine (Cremades *et al.*, 2005); however, no studies have been performed to determine whether this water accessible site could actually be exploited as a therapeutic target.

Another enzyme that is crucial to the *in vivo* survival of *H. pylori* is urease. This enzyme converts urea to carbon dioxide and ammonia, which buffers the environmental pH and enables *H. pylori* to survive pH stress. Given the available structural information (Ha *et al.*, 2001) and the fact that *H. pylori* urease has been extensively characterized (Dunn *et al.*, 1990; Mobley *et al.*, 1995; McGee and Mobley, 1999; McGee *et al.*, 2002; Nolan *et al.*, 2002), this enzyme seems like a reasonable target for future rational drug design.

Small molecule therapeutics represent a potentially important means of controlling *H. pylori* infection. However, there are several major hurdles that must first be overcome if these compounds are to be used clinically. The most important hurdle for many of the compounds described above is to determine whether *in vitro* enzyme inhibition translates to bacterial killing or stasis of bacterial growth. For the compounds that have established desirable MIC and/or MBC values, the next hurdle would be to determine whether the drugs are safe in animal models and to evaluate pharmacokinetics and bioavailability *in vivo.* Finally, after all of those requirements are successfully met, the most significant hurdle is to bring the drug into human safety and efficacy trials. Of all the small molecules discussed here, only SQ109 and the carbonic anhydrase inhibitors have been proven safe in humans, and have established pharmacokinetic and bioavailability data. As a result, these compounds seem most likely to make it into the market as anti-*H. pylori* therapeutics. Though the clinical utility of these compounds in treating *H. pylori* infection is still unclear, the data gathered so far indicate that these drugs may represent one of the better novel anti-therapeutic strategies.

Naturopathic therapy

Over the past two decades, the popularity of complementary and alternative medicine (CAM) has been increasing worldwide. As a result, in Europe and North America the development of CAM has been harmonized with training and regulation of practitioners (Fisher and Ward, 1994; Eisenberg *et al.*, 1998). Currently, it is estimated that in developed countries between 30–70% of patients use some form of CAM or naturopathic therapy to supplement their medical needs (Fisher and Ward, 1994; MacLennan *et al.*, 1996; Eisenberg *et al.*, 1998). In thinking about *H. pylori* infection, there is a growing body of evidence that suggests that certain types of CAM may provide an effective treatment method to control infection or disease symptoms. Broadly speaking those studies that appear most promising can be broken into two categories: probiotics and phytotherapy. Of these two, the potential use of probiotics to promote human health and prevent disease has gained an enormous following (Varbanova *et al.*, 2011; Hungin *et al.*, 2013). Those studies that have been focused specifically on *H. pylori* have recently been summarized (Vitor and Vale, 2011). As such, rather than reiterating those results, we will focus our remaining discussion on the use of phytotherapy; those that are interested in the use of probiotics for treatment of *H. pylori* infection should examine the work of Vitor and Vale (2011).

Phytotherapy

Broadly defined, phytotherapy refers to the use of plants and/or their extracts as medicines or health-promoting agents, whereas phytoceutical refers to any plant or plant product that shows activity on biological systems (Vitor and Vale, 2011). Whereas, some plant extracts are used in more traditional medicines, typically phytotherapy involves the utilization of the plant or extract in its simplest or least processed form so as to preserve the active components in their natural state (Urr, 2003). The therapeutic use of plant-derived products, which is sometimes also referred to as herbal medicine has been practiced for centuries, and is still practiced in many parts of the world today. However, despite its popularity, many critics of phytotherapy exist. Concerns include the lack of systematic scientific research and large-scale randomized clinical trials as a means to prove effectiveness, as well as issues with standardization, quality and safety (Posadzki *et al.*, 2013). Despite these concerns, modern production and analytical technologies are being used as a means to standardize the production and formulation methods of certain forms of phytotherapy. The remainder of this review will briefly highlight some of those phytotherapy approaches that have been investigated as *H. pylori* therapies (Table 2); given the substantial literature on this topic and space limitations, our list will not be exhaustive, but will attempt to give the reader a "flavor" of the types of studies being pursued.

Herbs and spices (oregano, chilli pepper, garlic, cloves, turmeric/curcumin): One of the most popular forms of phytotherapy involves the use of herbs and spices. Indeed, remedies continue to be used in a variety of cultures to treat a wide range of disorders (Nanji *et al.*, 2003; Nicoll and Henein, 2009). Scientific investigation into how these spices, herbs or their extracts work at the biological level has shown that many of them possess antimicrobial and anti-inflammatory activities (Pozharitskaya *et al.*, 2010). Among these studies, several have identified specific anti-*H. pylori* compounds from specific phytoceuticals. The isolated products include phenolics from *Decalepis hamiltonii* (Srikanta *et al.*, 2007), flavonoids in *Cistus laurifolius* leaves (Ustun *et al.*, 2006), triterpenoids from *Pteleopsis suberosa* stem bark (De Leo *et al.*, 2006), quinones from *Tabebuia impetiginosa* Martius ex DC (Taheebo) (Park *et al.*, 2006) and carotenoids from golden delicious apple peel (Molnar *et al.*, 2005, 2010).

In addition to those studies that looked for specific biologically active components of the phytoceutical, several preclinical *in vivo* experiments and clinical studies have demonstrated that certain herbs and spices have anti-ulcer properties mediated through their anti-*H. pylori* effects. For example, ginger rhizome extract has been shown to have gastro-protective activity via promotion of gastric mucin regeneration, increased expression of antioxidant enzymes

and inhibition of *H. pylori* growth (Mahady *et al.*, 2005; Nanjundaiah *et al.*, 2011; Haniadka *et al.*, 2013). Similarly, cinnamon extracts have been demonstrated to inhibit *H. pylori* growth and affect enzymatic activity of urease (Tabak *et al.*, 1999), which is essential for *H. pylori* colonization. Similarly, turmeric/curcumin (Koosirirat *et al.*, 2010), chili (Lee *et al.*, 2007b), and oregano (Lin *et al.*, 2005) have been shown to have varying levels of bacteriocidal effects against *H. pylori*. Furthermore, garlic extracts and its active ingredient, allicin, have been shown to contain *H. pylori* growth inhibitory properties when tested *in vitro* (Canizares *et al.*, 2002, 2004), *in vivo* in animal models (Iimuro *et al.*, 2002), as well as in clinical trials (Martin and Ernst, 2003; Gail *et al.*, 2007). Moreover, several studies have shown a reduced risk of gastric cancer with increased consumption of allium containing vegetables such as onions and garlic (Jonkers *et al.*, 1999); this effect may possibly be due to an effect on *H. pylori.* Indeed, some studies have suggested that a combination regimen of garlic and omeprazole may be a more efficacious eradication treatment than the conventional quadruple therapy used to treat *H. pylori* infection (Fani *et al.*, 2007). Clearly, further clinical evaluations will be needed to determine the effectiveness of this as well as other herbs and spices as potential *H. pylori* therapeutics.

Cruciferous vegetables (cabbages, cabbage sprouts and broccoli): Cruciferous vegetables such as cabbages and broccoli contain sulphoraphane, which is a biologically active isothiocyanate (Warin *et al.*, 2009). Sulphoraphane has been shown to inhibit *H. pylori* growth *in vitro* (Johansson *et al.*, 2008; Moon *et al.*, 2010), and has further been demonstrated to reduce *H. pylori* colonization and attenuate gastritis in both mice and humans infected with the bacterium (Yanaka *et al.*, 2009; Yanaka, 2011).

Korean red ginseng and green tea: Korean red ginseng and green tea have been widely studied for their medicinal applications. Red ginseng contains ginsenosides that show inhibitory activities against *H. pylori* (Bae *et al.*, 2001), as well as the ability to block development of *H. pylori*-induced cytotoxicity, gastritis, ulcer and cancer through attenuation of 5-LOX expression (Park *et al.*, 2007; Lee *et al.*, 2008). On the other hand, the inhibitory effects of green tea against *H. pylori* have been attributed to the presence of acidic polysaccharides, epigallocatechin gallate (EGCG) and polyphenols as the active ingredients (Yanagawa *et al.*, 2003). Green tea carbohydrates containing uronic acid have been shown to have the capacity to selectively block adhesion of *H. pylori* to host cell surface without interfering with adherence of commensal bacteria such as *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Escherichia coli*, or *Staphylococcus epidermidis* (Lee *et al.*, 2009). Given that the adherence of *H. pylori* to gastric mucosal cells is a crucial event in colonization and infection, blocking adherence may be a powerful strategy to control infection. Furthermore, protection against *H. pylori*-induced gastric epithelial cytotoxicity, which is considered to be the hallmark of ulceration, by green tea EGCG (Yanagawa *et al.*, 2003; Lee *et al.*, 2004; Song and Seong, 2007), as well as inhibition of *H. pylori* VacA by polyphenols (Tombola *et al.*, 2003), indicate that green tea may protect against *H. pylori*-induced disease. Indeed, the protective effects of green tea polyphenols or catechins against development of gastritis and associated gastric damages have been demonstrated in mice (Ruggiero *et al.*, 2007; Stoicov *et al.*, 2009), rats (Lee *et al.*, 2005), and Mongolian gerbils (Matsubara *et al.*, 2003). Despite these promising results, clinical studies will be required to determine whether these findings can be translated to humans.

Extracts of oils, resveratrol, beta-carotene: Additional phytoceuticals that have been shown to have anti-*H. pylori* effects include extracts of oils, resveratrol, and beta-carotene. Similar to green tea, garlic oil, olive oil, mastic oil, pine nut oil, and chamomile oil each contain polyphenols that have been shown to possess potent bactericidal effects (Romero *et al.*, 2007; O'Gara *et al.*, 2008; Eftekhar *et al.*, 2009), antioxidant properties that can reduce inflammation via inhibition of neutrophil activation (Kottakis *et al.*, 2009), and even the ability to inhibit the production of *H. pylori* urease (Shikov *et al.*, 2008). Similar to the oil polyphenols, resveratrol is a polyphenol that is mainly found in wine; resveratrol has been shown to have a potent inhibitory effect against *H. pylori* (Mahady *et al.*, 2003) and the ability to suppress gastritis in *H. pylori*-infected mice (Ruggiero *et al.*, 2007). In comparison, beta-carotene, which was mainly obtained from carrots, has been shown to inhibit oxidant-mediated activation of inflammatory signaling through MAPK, NF-kB and AP-1. This in turn results in suppression of iNOS and COX-2 expression in *H. pylori*-infected human gastric epithelial cells (Jang *et al.*, 2009). Given the promising effects of these various phytoceuticals, it is possible that some of these compounds could be exploited to reduce *H. pylori* colonization, inflammation and gastric mucosal damage.

Flavonoids and vitamins: Flavonoids and vitamins owe their anti-*H. pylori* activities mainly to their antioxidant properties; oxidative stress plays a significant role in exacerbating mucosal damage during *H. pylori* infection. Flavonoids have been suggested to block *H. pylori*-induced inflammation in human gastric cancer cells via their ability to target NF-kB and MAPK (Ustun *et al.*, 2006; Lee *et al.*, 2007a). Similarly, several studies have shown that powerful antioxidant vitamins such as α-tocopherol (vitamin E) and ascorbic acid (vitamin C) could significantly reduce oxidative stress-induced mucosal damage (Oh *et al.*, 2005). Moreover, it has been shown that the addition of vitamin C or vitamins C and E to the clarithromycin-amoxicillin-omeprazol triple therapy regimen significantly increased *H. pylori* eradication rates (Sezikli *et al.*, 2009), as well as might further reduce the needed dosage of clarithromycin by 50% (Kaboli *et al.*, 2009). Furthermore, data from a case-control study of the effect of vitamin E and C intake on the risk of gastric cancer demonstrated that *H. pylori*-infected patients who had a high intake of the vitamins showed a lower risk of developing gastric cancer than those who had no vitamin supplements (Kim *et al.*, 2005a, 2005b). Thus, vitamin supplementation may be effective for the treatment of *H. pylori*-induced disease.

Conclusion

It is clear that antibiotic resistant *H. pylori* is rapidly emerging in many areas. Given that this pathogen infects more

than half of the population of the world, this emergence is a potentially serious threat to public health. Since the treatment regime for *H. pylori* is already complicated, it is unclear how a radical change in the overall use of traditional antibiotics can be instituted. Furthermore, even if a revision of antibiotic utilization was to happen, since *H. pylori* is genetically endowed with a high frequency of recombination and mutation (Kraft and Suerbaum, 2005), it is unclear if such a change would be effective. The emergence of antibiotic resistance has sadly prompted a longer duration of treatment and the use of more antibiotics. The unfortunate effect of these changes is a perpetuation of a cycle of antibiotic-induced side effects, non-compliance, treatment failures, and resistant microorganisms. Moreover, these changes do not relieve the underlying selective pressure that drives the development of antibiotic resistance. Thus, a radical shift in the treatment of *H. pylori* is needed in order to prevent conventional antibiotics from becoming obsolete. In this review, we have attempted to highlight three potential areas that are being explored in order to identify novel drugs that could be used to combat *H. pylori* infection: antimicrobial peptides, small molecule inhibitors and naturopathic therapy.

While the results for each of these three possible therapies look promising, each will clearly require more studies with animals as well as human clinical trials to determine their overall effectiveness. Furthermore, it is possible that even if they are unsuccessful on their own, some of these therapies could potentially be used in combination with a single traditional antibiotic; this strategy could prolong the successful use of conventional antibiotics by sensitizing *H. pylori* to these drugs. Each of these possibilities remains to be explored. In the end, it is clear that successful treatment of *H. pylori* and associated gastric disease will require an integrated method of patient management that takes into account prophylactic vaccinations, public health education and hygiene, efficient and cheap early diagnosis, and effective drugs.

Acknowledgements

Research in the laboratory of Dr. D. Scott Merrell is supported by grants R073PW from Uniformed Services University of Health Sciences (USUHS), 60393-300411-7.20 from the United States Military Cancer Institute and by R01AI065529 from the National Institutes of Health (NIH). Contents of this manuscript are the sole responsibility of the authors and do not necessarily represent the official views of the NIH, USUHS, the US Department of Defense, or the federal government.

References

al Somal, N., Coley, K.E., Molan, P.C., and Hancock, B.M. 1994. Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *J. R. Soc. Med.* **87**, 9–12.

Amundsen, S.K., Fero, J., Hansen, L.M., Cromie, G.A., Solnick, J.V., Smith, G.R., and Salama, N.R. 2008. *Helicobacter pylori* AddAB helicase-nuclease and RecA promote recombination-related DNA repair and survival during stomach colonization. *Mol. Microbiol.* **69**, 994–1007.

Andra, J., Goldmann, T., Ernst, C.M., Peschel, A., and Gutsmann, T. 2011. Multiple peptide resistance factor (MprF)-mediated resistance of *Staphylococcus aureus* against antimicrobial peptides coincides with a modulated peptide interaction with artificial membranes comprising lysyl-phosphatidylglycerol. *J. Biol. Chem.* **286**, 18692–18700.

Andreu, D. and Rivas, L. 1998. Animal antimicrobial peptides: an overview. *Biopolymers* **47**, 415–433.

Bae, E.A., Han, M.J., Baek, N.I., and Kim, D.H. 2001. *In vitro* anti-*Helicobacter pylori* activity of panaxytriol isolated from ginseng. *Arch. Pharm. Res.* **24**, 297–299.

Basile, A., Senatore, F., Gargano, R., Sorbo, S., Del Pezzo, M., Lavitola, A., Ritieni, A., Bruno, M., Spatuzzi, D., Rigano, D., and *et al.* 2006. Antibacterial and antioxidant activities in *Sideritis italica* (Miller) Greuter et Burdet essential oils. *J. Ethnopharmacol.* **107**, 240–248.

Bayer, A.S., Prasad, R., Chandra, J., Koul, A., Smriti, M., Varma, A., Skurray, R.A., Firth, N., Brown, M.H., Koo, S.P., and *et al.* 2000. *In vitro* resistance of *Staphylococcus aureus* to thrombin-induced platelet microbicidal protein is associated with alterations in cytoplasmic membrane fluidity. *Infect. Immun.* **68**, 3548–3553.

Bazzoli, F., Pozzato, P., and Rokkas, T. 2002. *Helicobacter pylori*: the challenge in therapy. *Helicobacter* **7** Suppl 1, 43–49.

Bergonzelli, G.E., Donnicola, D., Porta, N., and Corthesy-Theulaz, I.E. 2003. Essential oils as components of a diet-based approach to management of *Helicobacter* infection. *Antimicrob. Agents Chemother.* **47**, 3240–3246.

Bertoli Neto, J.L., Lourenco, L.G., Bertoli, C.F., Ulbrich, F.S., Sabbi, A.R., and Bueno, A.G. 2006. Evaluation of the efficacy of triple therapy regimen for *Helicobacter pylori* eradication in gastrectomized patients with gastric adenocarcinoma. *Gastric Cancer* **9**, 291–294.

Bourke, B., Jones, N., and Sherman, P. 1996. *Helicobacter pylori* infection and peptic ulcer disease in children. *Pediatr. Infect. Dis. J.* **15**, 1–13.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O.P., Sahl, H.G., and de Kruijff, B. 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. *Science* **286**, 2361–2364.

Brogden, K.A. 2005. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.* **3**, 238–250.

Calvet, X., Ramirez Lazaro, M.J., Lehours, P., and Megraud, F. 2013. Diagnosis and epidemiology of *Helicobacter pylori* infection. *Helicobacter* **18** Suppl 1, 5–11.

Canizares, P., Gracia, I., Gomez, L.A., Martin de Argila, C., Boixeda, D., Garcia, A., and de Rafael, L. 2004. Allyl-thiosulfinates, the bacteriostatic compounds of garlic against *Helicobacter pylori*. *Biotechnol. Prog.* **20**, 397–401.

Canizares, P., Gracia, I., Gomez, L.A., Martin de Argila, C., de Rafael, L., and Garcia, A. 2002. Optimization of *Allium sativum* solvent extraction for the inhibition of *in vitro* growth of *Helicobacter pylori*. *Biotechnol. Prog.* **18**, 1227–1232.

Chen, L., Sung, S.S., Yip, M.L., Lawrence, H.R., Ren, Y., Guida, W.C., Sebti, S.M., Lawrence, N.J., and Wu, J. 2006. Discovery of a novel shp2 protein tyrosine phosphatase inhibitor. *Mol. Pharmacol.* **70**, 562–570.

Cremades, N., Bueno, M., Toja, M., and Sancho, J. 2005. Towards a new therapeutic target: *Helicobacter pylori* flavodoxin. *Biophys. Chem.* **115**, 267–276.

Cwikla, C., Schmidt, K., Matthias, A., Bone, K.M., Lehmann, R., and Tiralongo, E. 2010. Investigations into the antibacterial activities of phytotherapeutics against *Helicobacter pylori* and *Campylobacter jejuni*. *Phytother. Res.* **24**, 649–656.

de Jonge, B.L., Kutschke, A., Uria-Nickelsen, M., Kamp, H.D., and Mills, S.D. 2009. Pyrazolopyrimidinediones are selective agents for *Helicobacter pylori* that suppress growth through inhibition

of glutamate racemase (MurI). *Antimicrob. Agents Chemother.* **53**, 3331–3336.

De Leo, M., De Tommasi, N., Sanogo, R., D'Angelo, V., Germano, M.P., Bisignano, G., and Braca, A. 2006. Triterpenoid saponins from Pteleopsis suberosa stem bark. *Phytochemistry* **67**, 2623–2629.

De, R., Kundu, P., Swarnakar, S., Ramamurthy, T., Chowdhury, A., Nair, G.B., and Mukhopadhyay, A.K. 2009. Antimicrobial activity of curcumin against Indian *Helicobacter pylori* and also during mice infection. *Antimicrob. Agents Chemother.* **53**, 1592–1597.

Delcour, A.H. 2009. Outer membrane permeability and antibiotic resistance. *Biochim. Biophys. Acta.* **1794**, 808–816.

Dunn, B.E., Campbell, G.P., Perez-Perez, G.I., and Blaser, M.J. 1990. Purification and characterization of urease from *Helicobacter pylori*. *J. Biol. Chem.* **265**, 9464–9469.

Dunn, B.E., Cohen, H., and Blaser, M.J. 1997. *Helicobacter pylori*. *Clin. Microbiol. Rev.* **10**, 720–741.

Eftekhar, F., Nariman, F., Yousefzadi, M., Hadiand, J., and Ebrahimi, S.N. 2009. Anti-*Helicobacter pylori* activity and essential oil composition of *Thymus caramanicus* from Iran. *Nat. Prod. Commun.* **4**, 1139–1142.

Eisenberg, D.M., Davis, R.B., Ettner, S.L., Appel, S., Wilkey, S., Van Rompay, M., and Kessler, R.C. 1998. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* **280**, 1569–1575.

Epand, R.F., Sarig, H., Mor, A., and Epand, R.M. 2009. Cell-wall interactions and the selective bacteriostatic activity of a miniature oligo-acyl-lysyl. *Biophys. J.* **97**, 2250–2257.

Escobedo-Hinojosa, W.I., Del Carpio, J.D., Palacios-Espinosa, J.F., and Romero, I. 2012. Contribution to the ethnopharmacological and anti-*Helicobacter pylori* knowledge of *Cyrtocarpa procera* Kunth (Anacardiaceae). *J. Ethnopharmacol.* **143**, 363–371.

Everhart, J.E. 2000. Recent developments in the epidemiology of *Helicobacter pylori*. *Gastroenterol. Clin. North Am.* **29**, 559–578.

Fahey, J.W., Haristoy, X., Dolan, P.M., Kensler, T.W., Scholtus, I., Stephenson, K.K., Talalay, P., and Lozniewski, A. 2002. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. *Proc. Natl. Acad. Sci. USA* **99**, 7610–7615.

Fani, A., Fani, I., Delavar, M., Fani, P., Eshrati, B., and Elahi, M. 2007. Combined garlic-omeprazole versus standard quadruple therapy for eradication of *Helicobacter pylori* infection. *Indian J. Gastroenterol.* **26**, 145–146.

Fisher, P. and Ward, A. 1994. Complementary medicine in Europe. *BMJ* **309**, 107–111.

Gail, M.H., Pfeiffer, R.M., Brown, L.M., Zhang, L., Ma, J.L., Pan, K.F., Liu, W.D., and You, W.C. 2007. Garlic, vitamin, and antibiotic treatment for *Helicobacter pylori*: a randomized factorial controlled trial. *Helicobacter* **12**, 575–578.

Geng, B., Basarab, G., Comita-Prevoir, J., Gowravaram, M., Hill, P., Kiely, A., Loch, J., MacPherson, L., Morningstar, M., Mullen, G., and *et al.* 2009. Potent and selective inhibitors of *Helicobacter pylori* glutamate racemase (MurI): pyridodiazepine amines. *Bioorg. Med. Chem. Lett.* **19**, 930–936.

Glassman, M.S., Dallal, S., Berezin, S.H., Bostwick, H.E., Newman, L.J., Perez-Perez, G.I., and Blaser, M.J. 1990. *Helicobacter pylori*-related gastroduodenal disease in children. Diagnostic utility of enzyme-linked immunosorbent assay. *Dig. Dis. Sci.* **35**, 993–997.

Graham, D.Y. and Fischbach, L. 2010. *Helicobacter pylori* treatment in the era of increasing antibiotic resistance. *Gut* **59**, 1143–1153.

Guina, T., Yi, E.C., Wang, H., Hackett, M., and Miller, S.I. 2000. A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar *typhimurium* promotes resistance to alpha-helical antimicrobial peptides. *J. Bacteriol.* **182**, 4077–4086.

Guo, L., Lim, K.B., Poduje, C.M., Daniel, M., Gunn, J.S., Hackett, M., and Miller, S.I. 1998. Lipid A acylation and bacterial resistance against vertebrate antimicrobial peptides. *Cell* **95**, 189–198.

Ha, N.C., Oh, S.T., Sung, J.Y., Cha, K.A., Lee, M.H., and Oh, B.H. 2001. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nat. Struct. Biol.* **8**, 505–509.

Hamanaka, Y., Nakashima, M., Wada, A., Ito, M., Kurazono, H., Hojo, H., Nakahara, Y., Kohno, S., Hirayama, T., and Sekine, I. 2001. Expression of human beta-defensin 2 (hBD-2) in *Helicobacter pylori* induced gastritis: antibacterial effect of hBD-2 against *Helicobacter pylori*. *Gut* **49**, 481–487.

Haniadka, R., Saldanha, E., Sunita, V., Palatty, P.L., Fayad, R., and Baliga, M.S. 2013. A review of the gastroprotective effects of ginger (*Zingiber officinale* Roscoe). *Food Funct.* **4**, 845–855.

Hase, K., Murakami, M., Iimura, M., Cole, S.P., Horibe, Y., Ohtake, T., Obonyo, M., Gallo, R.L., Eckmann, L., and Kagnoff, M.F. 2003. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against *Helicobacter pylori*. *Gastroenterology* **125**, 1613–1625.

Hilleringmann, M., Pansegrau, W., Doyle, M., Kaufman, S., MacKichan, M.L., Gianfaldoni, C., Ruggiero, P., and Covacci, A. 2006. Inhibitors of *Helicobacter pylori* ATPase Cagalpha block CagA transport and cag virulence. *Microbiology* **152**, 2919–2930.

Horwith, G., Einck, L., Protopopova, M., and Nacy, C. 2007. Phase 1 safety and pharmacokinetics of SQ109, a new diamine antituberculosis drug. Meeting Abstract, 45th IDSA Annual Meeting, 2007 Oct 4–7; San Diego, CA, USA. Sequella, Inc., Rockville, MD.

Huang, J.Q. and Hunt, R.H. 2003. The evolving epidemiology of *Helicobacter pylori* infection and gastric cancer. *Can. J. Gastroenterol.* **17** Suppl B, 18B–20B.

Hungin, A.P., Mulligan, C., Pot, B., Whorwell, P., Agreus, L., Fracasso, P., Lionis, C., Mendive, J., Philippart de Foy, J.M., Rubin, G., and *et al.* 2013. Systematic review: probiotics in the management of lower gastrointestinal symptoms in clinical practice - an evidence-based international guide. *Aliment Pharmacol. Ther.* **38**, 864–886.

Iimuro, M., Shibata, H., Kawamori, T., Matsumoto, T., Arakawa, T., Sugimura, T., and Wakabayashi, K. 2002. Suppressive effects of garlic extract on *Helicobacter pylori*-induced gastritis in Mongolian gerbils. *Cancer Lett.* **187**, 61–68.

Isaacson, P.G. 1994. Gastric lymphoma and *Helicobacter pylori*. *N. Engl. J. Med.* **330**, 1310–1311.

Jang, S.H., Lim, J.W., and Kim, H. 2009. Beta-carotene inhibits *Helicobacter pylori*-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in human gastric epithelial AGS cells. *J. Physiol. Pharmacol.* **60** Suppl **7**, 131–137.

Jenssen, H., Hamill, P., and Hancock, R.E. 2006. Peptide antimicrobial agents. *Clin. Microbiol. Rev.* **19**, 491–511.

Jia, L., Noker, P.E., Coward, L., Gorman, G.S., Protopopova, M., and Tomaszewski, J.E. 2006. Interspecies pharmacokinetics and *in vitro* metabolism of SQ109. *Br. J. Pharmacol.* **147**, 476–485.

Jia, L., Tomaszewski, J.E., Hanrahan, C., Coward, L., Noker, P., Gorman, G., Nikonenko, B., and Protopopova, M. 2005. Pharmacodynamics and pharmacokinetics of SQ109, a new diamine-based antitubercular drug. *Br. J. Pharmacol.* **144**, 80–87.

Jin, T., Bokarewa, M., Foster, T., Mitchell, J., Higgins, J., and Tarkowski, A. 2004. *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. *J. Immunol.* **172**, 1169–1176.

Johansson, N.L., Pavia, C.S., and Chiao, J.W. 2008. Growth inhibition of a spectrum of bacterial and fungal pathogens by sulforaphane, an isothiocyanate product found in broccoli and other cruciferous vegetables. *Planta Med.* **74**, 747–750.

Jones, J.E., Causey, C.P., Lovelace, L., Knuckley, B., Flick, H., Lebioda, L., and Thompson, P.R. 2010. Characterization and inactivation of an agmatine deiminase from *Helicobacter pylori*. *Bioorg. Chem.* **38**, 62–73.

Jones, K.R., Cha, J.H., and Merrell, D.S. 2008. Who's winning the war? Molecular mechanisms of antibiotic resistance in *Helicobacter pylori*. *Curr. Drug Ther.* **3**, 190–203.

Jonkers, D., van den Broek, E., van Dooren, I., Thijs, C., Dorant, E., Hageman, G., and Stobberingh, E. 1999. Antibacterial effect of garlic and omeprazole on *Helicobacter pylori*. *J. Antimicrob. Chemother.* **43**, 837–839.

Kaboli, S.A., Zojaji, H., Mirsattari, D., Talaie, R., Derakhshan, F., Zali, M.R., and Sheikhvatan, M. 2009. Effect of addition of vitamin C to clarithromycin-amoxicillin-omeprazol triple regimen on *Helicobacter pylori* eradication. *Acta Gastroenterol. Belg.* **72**, 222–224.

Kawauchi, K., Yagihashi, A., Tsuji, N., Uehara, N., Furuya, D., Kobayashi, D., and Watanabe, N. 2006. Human beta-defensin-3 induction in *H. pylori*-infected gastric mucosal tissues. *World J. Gastroenterol.* **12**, 5793–5797.

Keenan, J.I., Salm, N., Wallace, A.J., and Hampton, M.B. 2012. Using food to reduce *H. pylori*-associated inflammation. *Phytother. Res.* **26**, 1620–1625.

Kim, D.S., Lee, M.S., Kim, Y.S., Kim, D.H., Bae, J.M., Shin, M.H., and Ahn, Y.O. 2005a. Effect modification by vitamin C on the relation between gastric cancer and *Helicobacter pylori*. *Eur. J. Epidemiol.* **20**, 67–71.

Kim, H.J., Kim, M.K., Chang, W.K., Choi, H.S., Choi, B.Y., and Lee, S.S. 2005b. Effect of nutrient intake and *Helicobacter pylori* infection on gastric cancer in Korea: a case-control study. *Nutr. Cancer* **52**, 138–146.

Koosirirat, C., Linpisarn, S., Changsom, D., Chawansuntati, K., and Wipasa, J. 2010. Investigation of the anti-inflammatory effect of *Curcuma longa* in *Helicobacter pylori*-infected patients. *Int. Immunopharmacol.* **10**, 815–818.

Kottakis, F., Kouzi-Koliakou, K., Pendas, S., Kountouras, J., and Choli-Papadopoulou, T. 2009. Effects of mastic gum *Pistacia lentiscus* var. *Chia* on innate cellular immune effectors. *Eur. J. Gastroenterol. Hepatol.* **21**, 143–149.

Kraft, C. and Suerbaum, S. 2005. Mutation and recombination in *Helicobacter pylori*: mechanisms and role in generating strain diversity. *Int. J. Med. Microbiol.* **295**, 299–305.

Lee, S.H. and Baek, D.H. 2012. Antibacterial and neutralizing effect of human beta-defensins on *Enterococcus faecalis* and *Enterococcus faecalis* lipoteichoic acid. *J. Endod.* **38**, 351–356.

Lee, J.S., Kim, H.S., Hahm, K.B., Sohn, M.W., Yoo, M., Johnson, J.A., and Surh, Y.J. 2007a. Inhibitory effects of 7-carboxymethyloxy-3′,4′,5-trimethoxyflavone (DA-6034) on *Helicobacter pylori*-nduced NF-kappa B activation and iNOS expression in AGS cells. *Ann. NY Acad. Sci.* **1095**, 527–535.

Lee, I.O., Lee, K.H., Pyo, J.H., Kim, J.H., Choi, Y.J., and Lee, Y.C. 2007b. Anti-inflammatory effect of capsaicin in *Helicobacter pylori*-infected gastric epithelial cells. *Helicobacter* **12**, 510–517.

Lee, J.S., Oh, T.Y., Kim, Y.K., Baik, J.H., So, S., Hahm, K.B., and Surh, Y.J. 2005. Protective effects of green tea polyphenol extracts against ethanol-induced gastric mucosal damages in rats: stress-responsive transcription factors and MAP kinases as potential targets. *Mutat. Res.* **579**, 214–224.

Lee, J.H., Shim, J.S., Chung, M.S., Lim, S.T., and Kim, K.H. 2009. *In vitro* anti-adhesive activity of green tea extract against pathogen adhesion. *Phytother. Res.* **23**, 460–466.

Lee, S.Y., Shin, Y.W., and Hahm, K.B. 2008. Phytoceuticals: mighty but ignored weapons against *Helicobacter pylori* infection. *J. Dig. Dis.* **9**, 129–139.

Lee, K.M., Yeo, M., Choue, J.S., Jin, J.H., Park, S.J., Cheong, J.Y., Lee, K.J., Kim, J.H., and Hahm, K.B. 2004. Protective mechanism of epigallocatechin-3-gallate against *Helicobacter pylori*-nduced gastric epithelial cytotoxicity via the blockage of TLR-4 signaling. *Helicobacter* **9**, 632–642.

Lemos, L.M., Martins, T.B., Tanajura, G.H., Gazoni, V.F., Bonaldo, J., Strada, C.L., Silva, M.G., Dall'oglio, E.L., de Sousa Junior, P.T., and Martins, D.T. 2012. Evaluation of antiulcer activity of chromanone fraction from *Calophyllum brasiliesnse* Camb. *J. Ethnopharmacol.* **141**, 432–439.

Leszczynska, K., Namiot, A., Fein, D.E., Wen, Q., Namiot, Z., Savage, P.B., Diamond, S., Janmey, P.A., and Bucki, R. 2009. Bactericidal activities of the cationic steroid CSA-13 and the cathelicidin peptide LL-37 against *Helicobacter pylori* in simulated gastric juice. *BMC Microbiol.* **9**, 187.

Lien, H.M., Wang, C.Y., Chang, H.Y., Huang, C.L., Peng, M.T., Sing, Y.T., Chen, C.C., and Lai, C.H. 2013. Bioevaluation of *Anisomeles indica* extracts and their inhibitory effects on *Helicobacter pylori*-mediated inflammation. *J. Ethnopharmacol.* **145**, 397–401.

Lin, Y.T., Kwon, Y.I., Labbe, R.G., and Shetty, K. 2005. Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. *Appl. Environ. Microbiol.* **71**, 8558–8564.

Livermore, D.M. 2009. Has the era of untreatable infections arrived? *J. Antimicrob. Chemother.* **64** Suppl 1, i29–36.

Lockey, T.D. and Ourth, D.D. 1996. Formation of pores in *Escherichia coli* cell membranes by a cecropin isolated from hemolymph of *Heliothis virescens* larvae. *Eur. J. Biochem.* **236**, 263–271.

Lundqvist, T., Fisher, S.L., Kern, G., Folmer, R.H., Xue, Y., Newton, D.T., Keating, T.A., Alm, R.A., and de Jonge, B.L. 2007. Exploitation of structural and regulatory diversity in glutamate racemases. *Nature* **447**, 817–822.

MacLennan, A.H., Wilson, D.H., and Taylor, A.W. 1996. Prevalence and cost of alternative medicine in Australia. *Lancet* **347**, 569–573.

Mahady, G.B., Pendland, S.L., and Chadwick, L.R. 2003. Resveratrol and red wine extracts inhibit the growth of CagA+ strains of *Helicobacter pylori in vitro*. *Am. J. Gastroenterol.* **98**, 1440–1441.

Mahady, G.B., Pendland, S.L., Stoia, A., Hamill, F.A., Fabricant, D., Dietz, B.M., and Chadwick, L.R. 2005. *In vitro* susceptibility of *Helicobacter pylori* to botanical extracts used traditionally for the treatment of gastrointestinal disorders. *Phytother. Res.* **19**, 988–991.

Mahady, G.B., Pendland, S.L., Yun, G., and Lu, Z.Z. 2002. Turmeric (*Curcuma longa*) and curcumin inhibit the growth of *Helicobacter pylori*, a group 1 carcinogen. *Anticancer Res.* **22**, 4179–4181.

Makobongo, M.O., Einck, L., Peek, R.M.Jr., and Merrell, D.S. 2013. *In vitro* characterization of the anti-bacterial activity of SQ109 against *Helicobacter pylori*. *PLoS One* **8**, e68917.

Makobongo, M.O., Gancz, H., Carpenter, B.M., McDaniel, D.P., and Merrell, D.S. 2012. The oligo-acyl lysyl antimicrobial peptide $C_{12}K\text{-}2\beta_{12}$ exhibits a dual mechanism of action and demonstrates strong *in vivo* efficacy against *Helicobacter pylori*. *Antimicrob. Agents Chemother.* **56**, 378–390.

Makobongo, M.O., Kovachi, T., Gancz, H., Mor, A., and Merrell, D.S. 2009. *In vitro* antibacterial activity of acyl-lysyl oligomers against *Helicobacter pylori*. *Antimicrob. Agents Chemother.* **53**, 4231–4239.

Malfertheiner, P. 1993. Compliance, adverse events and antibiotic resistance in *Helicobacter pylori* treatment. *Scand. J. Gastroenterol.* **196**, 34–37.

Malfertheiner, P., Megraud, F., O'Morain, C.A., Atherton, J., Axon, A.T., Bazzoli, F., Gensini, G.F., Gisbert, J.P., Graham, D.Y., Rokkas, T., and *et al.* 2012. Management of *Helicobacter pylori* infection–the Maastricht IV/ Florence Consensus Report. *Gut* **61**, 646–664.

Malfertheiner, P., Megraud, F., O'Morain, C., Bazzoli, F., El-Omar, E., Graham, D., Hunt, R., Rokkas, T., Vakil, N., and Kuipers, E.J. 2007. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III consensus report. *Gut* **56**, 772–781.

Marshall, B.J., Warren, J.R., McGechie, D.B., Francis, G.J., Utley, P.J., Rogers, P.A., and Glancy, R.J. 1984. Unidentified curved

bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* **1**, 1311–1315.

Martin, K.W. and Ernst, E. 2003. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *J. Antimicrob. Chemother.* **51**, 241–246.

Matsubara, S., Shibata, H., Ishikawa, F., Yokokura, T., Takahashi, M., Sugimura, T., and Wakabayashi, K. 2003. Suppression of *Helicobacter pylori*-induced gastritis by green tea extract in *Mongolian gerbils*. *Biochem. Biophys. Res. Commun.* **310**, 715–719.

Matsuzaki, K. 1998. Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim. Biophys. Acta* **1376**, 391–400.

McGee, D.J., Coker, C., Testerman, T.L., Harro, J.M., Gibson, S.V., and Mobley, H.L. 2002. The *Helicobacter pylori* flbA flagellar biosynthesis and regulatory gene is required for motility and virulence and modulates urease of *H. pylori* and *Proteus mirabilis*. *J. Med. Microbiol.* **51**, 958–970.

McGee, D.J. and Mobley, H.L. 1999. Mechanisms of *Helicobacter pylori* infection: bacterial factors. *Curr. Top Microbiol. Immunol.* **241**, 155–180.

Mobley, H.L., Island, M.D., and Hausinger, R.P. 1995. Molecular biology of microbial ureases. *Microbiol. Rev.* **59**, 451–480.

Molina-Infante, J., Romano, M., Fernandez-Bermejo, M., Federico, A., Gravina, A.G., Pozzati, L., Garcia-Abadia, E., Vinagre-Rodriguez, G., Martinez-Alcala, C., Hernandez-Alonso, M., and *et al.* 2013. Optimized nonbismuth quadruple therapies cure most patients with *Helicobacter pylori* infection in populations with high rates of antibiotic resistance. *Gastroenterology* **145**, 121–128.

Molnar, P., Deli, J., Tanaka, T., Kann, Y., Tani, S., Gyemant, N., Molnar, J., and Kawase, M. 2010. Carotenoids with anti-*Helicobacter pylori* activity from Golden delicious apple. *Phytother. Res.* **24**, 644–648.

Molnar, P., Kawase, M., Satoh, K., Sohara, Y., Tanaka, T., Tani, S., Sakagami, H., Nakashima, H., Motohashi, N., Gyemant, N., and Molnar, J. 2005. Biological activity of carotenoids in red paprika, Valencia orange and Golden delicious apple. *Phytother. Res.* **19**, 700–707.

Moon, J.K., Kim, J.R., Ahn, Y.J., and Shibamoto, T. 2010. Analysis and anti-Helicobacter activity of sulforaphane and related compounds present in broccoli (*Brassica oleracea* L.) sprouts. *J. Agric Food Chem.* **58**, 6672–6677.

Nanji, A.A., Jokelainen, K., Tipoe, G.L., Rahemtulla, A., Thomas, P., and Dannenberg, A.J. 2003. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. *Am. J. Physiol. Gastrointest Liver Physiol.* **284**, G321–327.

Nanjundaiah, S.M., Annaiah, H.N., and Dharmesh, S.M. 2011. Gastroprotective effect of ginger rhizome (*Zingiber officinale*) extract: role of gallic acid and cinnamic acid in H(+), K(+)-ATPase/*H. pylori* inhibition and anti-oxidative mechanism. *Evid. Based Complement. Alternat. Med.* **2011**, 249487.

Nardone, G. 2000. Risk factors for cancer development in *Helicobacter pylori* gastritis. *Dig. Liver Dis.* **32** Suppl 3, S190–192.

National Institute of Allergy and Infectious Diseases (NIAID). 2010. posting date. Dose escalation study of SQ109 in healthy adult volunteers. US National Institutes of Health, ClinicalTrials.gov online. ClinicalTrials.gov identifier NCT00866190. Available from URL: http://www.clinicaltrials.gov [Accessed 2010 Jun 28] [Online.]

Ndip, R.N., Malange Takang, A.E., Echakachi, C.M., Malongue, A., Akoachere, J.F., Ndip, L.M., and Luma, H.N. 2007. *In-vitro* antimicrobial activity of selected honeys on clinical isolates of *Helicobacter pylori*. *Afr. Health Sci.* **7**, 228–232.

Nicoll, R. and Henein, M.Y. 2009. Ginger (*Zingiber officinale* Roscoe): a hot remedy for cardiovascular disease? *Int. J. Cardiol.* **131**, 408–409.

Nikaido, H. 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* **67**, 593–656.

Nir, Y., Potasman, I., Stermer, E., Tabak, M., and Neeman, I. 2000. Controlled trial of the effect of cinnamon extract on *Helicobacter pylori*. *Helicobacter* **5**, 94–97.

Nishimori, I., Minakuchi, T., Kohsaki, T., Onishi, S., Takeuchi, H., Vullo, D., Scozzafava, A., and Supuran, C.T. 2007. Carbonic anhydrase inhibitors: the beta-carbonic anhydrase from *Helicobacter pylori* is a new target for sulfonamide and sulfamate inhibitors. *Bioorg. Med. Chem. Lett.* **17**, 3585–3594.

Njume, C., Afolayan, A.J., Green, E., and Ndip, R.N. 2011. Volatile compounds in the stem bark of *Sclerocarya birrea* (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of *Helicobacter pylori*. *Int. J. Antimicrob. Agents* **38**, 319–324.

Nolan, K.J., McGee, D.J., Mitchell, H.M., Kolesnikow, T., Harro, J.M., O'Rourke, J., Wilson, J.E., Danon, S.J., Moss, N.D., Mobley, H.L., and *et al.* 2002. *In vivo* behavior of a *Helicobacter pylori* SS1 nixA mutant with reduced urease activity. *Infect. Immun.* **70**, 685–691.

Nuding, S., Gersemann, M., Hosaka, Y., Konietzny, S., Schaefer, C., Beisner, J., Schroeder, B.O., Ostaff, M.J., Saigenji, K., Ott, G., and *et al.* 2013. Gastric antimicrobial peptides fail to eradicate *Helicobacter pylori* infection due to selective induction and resistance. *PLoS One* **8**, e73867.

O'Gara, E.A., Maslin, D.J., Nevill, A.M., and Hill, D.J. 2008. The effect of simulated gastric environments on the anti-*Helicobacter* activity of garlic oil. *J. Appl. Microbiol.* **104**, 1324–1331.

Oh, T.Y., Yeo, M., Han, S.U., Cho, Y.K., Kim, Y.B., Chung, M.H., Kim, Y.S., Cho, S.W., and Hahm, K.B. 2005. Synergism of *Helicobacter pylori* infection and stress on the augmentation of gastric mucosal damage and its prevention with alpha-tocopherol. *Free Radic. Biol. Med.* **38**, 1447–1457.

Okeleye, B.I., Bessong, P.O., and Ndip, R.N. 2011. Preliminary phytochemical screening and *in vitro* anti-*Helicobacter pylori* activity of extracts of the stem bark of *Bridelia micrantha* (Hochst., Baill., Euphorbiaceae). *Molecules* **16**, 6193–6205.

Park, B.S., Lee, H.K., Lee, S.E., Piao, X.L., Takeoka, G.R., Wong, R.Y., Ahn, Y.J., and Kim, J.H. 2006. Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. *J. Ethnopharmacol.* **105**, 255–262.

Park, S., Yeo, M., Jin, J.H., Lee, K.M., Kim, S.S., Choi, S.Y., and Hahm, K.B. 2007. Inhibitory activities and attenuated expressions of 5-LOX with red ginseng in *Helicobacter pylori*-infected gastric epithelial cells. *Dig. Dis. Sci.* **52**, 973–982.

Parsonnet, J. 1993. *Helicobacter pylori* and gastric cancer. *Gastroenterol. Clin. North Am.* **22**, 89–104.

Peek, R.M.Jr. and Blaser, M.J. 1997. Pathophysiology of *Helicobacter pylori*-induced gastritis and peptic ulcer disease. *Am. J. Med.* **102**, 200–207.

Peschel, A. 2002. How do bacteria resist human antimicrobial peptides? *Trends Microbiol.* **10**, 179–186.

Peschel, A., Jack, R.W., Otto, M., Collins, L.V., Staubitz, P., Nicholson, G., Kalbacher, H., Nieuwenhuizen, W.F., Jung, G., Tarkowski, A., and *et al.* 2001. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. *J. Exp. Med.* **193**, 1067–1076.

Posadzki, P., Watson, L., and Ernst, E. 2013. Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews. *Eur. J. Clin. Pharmacol.* **69**, 295–307.

Pounder, R.E. and Ng, D. 1995. The prevalence of *Helicobacter pylori* infection in different countries. *Aliment Pharmacol. Ther.* **9** Suppl 2, 33–39.

Pozharitskaya, O.N., Shikov, A.N., Makarova, M.N., Kosman, V.M., Faustova, N.M., Tesakova, S.V., Makarov, V.G., and Galambosi, B. 2010. Anti-inflammatory activity of a HPLC-fingerprinted aqueous infusion of aerial part of *Bidens tripartita* L.

Phytomedicine **17**, 463–468.

Protopopova, M., Hanrahan, C., Nikonenko, B., Samala, R., Chen, P., Gearhart, J., Einck, L., and Nacy, C.A. 2005. Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. *J. Antimicrob. Chemother.* **56**, 968–974.

Queiroz, D.M., Dani, R., Silva, L.D., Santos, A., Moreira, L.S., Rocha, G.A., Correa, P.R., Reis, L.F., Nogueira, A.M., Alvares Cabral, M.M., and *et al.* 2002. Factors associated with treatment failure of *Helicobacter pylori* infection in a developing country. *J. Clin. Gastroenterol.* **35**, 315–320.

Radzishevsky, I.S., Kovachi, T., Porat, Y., Ziserman, L., Zaknoon, F., Danino, D., and Mor, A. 2008. Structure-activity relationships of antibacterial acyl-lysine oligomers. *Chem. Biol.* **15**, 354–362.

Radzishevsky, I.S., Rotem, S., Bourdetsky, D., Navon-Venezia, S., Carmeli, Y., and Mor, A. 2007. Improved antimicrobial peptides based on acyl-lysine oligomers. *Nat. Biotechnol.* **25**, 657–659.

Rao, Y.K., Fang, S.H., Hsieh, S.C., Yeh, T.H., and Tzeng, Y.M. 2009. The constituents of *Anisomeles indica* and their anti-inflammatory activities. *J. Ethnopharmacol.* **121**, 292–296.

Romero, C., Medina, E., Vargas, J., Brenes, M., and De Castro, A. 2007. *In vitro* activity of olive oil polyphenols against *Helicobacter pylori*. *J. Agric. Food Chem.* **55**, 680–686.

Rotem, S. and Mor, A. 2009. Antimicrobial peptide mimics for improved therapeutic properties. *Biochim. Biophys. Acta.* **1788**, 1582–1592.

Rotem, S., Radzishevsky, I.S., Bourdetsky, D., Navon-Venezia, S., Carmeli, Y., and Mor, A. 2008. Analogous oligo-acyl-lysines with distinct antibacterial mechanisms. *FASEB J.* **22**, 2652–2661.

Rotem, S., Raz, N., Kashi, Y., and Mor, A. 2010. Bacterial capture by peptide-mimetic oligoacyllysine surfaces. *Appl. Environ. Microbiol.* **76**, 3301–3307.

Rowland, M. and Drumm, B. 1995. *Helicobacter pylori* infection and peptic ulcer disease in children. *Curr. Opin. Pediatr.* **7**, 553–559.

Rozza, A.L., Moraes Tde, M., Kushima, H., Tanimoto, A., Marques, M.O., Bauab, T.M., Hiruma-Lima, C.A., and Pellizzon, C.H. 2011. Gastroprotective mechanisms of Citrus lemon (Rutaceae) essential oil and its majority compounds limonene and beta-pinene: involvement of heat-shock protein-70, vasoactive intestinal peptide, glutathione, sulfhydryl compounds, nitric oxide and prostaglandin E. *Chem. Biol. Interact.* **189**, 82–89.

Ruggiero, P., Rossi, G., Tombola, F., Pancotto, L., Lauretti, L., Del Giudice, G., and Zoratti, M. 2007. Red wine and green tea reduce *H. pylori*- or VacA-induced gastritis in a mouse model. *World J. Gastroenterol.* **13**, 349–354.

Sarig, H., Rotem, S., Ziserman, L., Danino, D., and Mor, A. 2008. Impact of self-assembly properties on antibacterial activity of short acyl-lysine oligomers. *Antimicrob. Agents Chemother.* **52**, 4308–4314.

Schmidtchen, A., Frick, I.M., Andersson, E., Tapper, H., and Bjorck, L. 2002. Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. *Mol. Microbiol.* **46**, 157–168.

Sekiguchi, H., Takabayashi, F., Irie, K., and Murakami, A. 2012. Auraptene attenuates gastritis via reduction of *Helicobacter pylori* colonization and pro-inflammatory mediator production in C57BL/6 mice. *J. Med. Food* **15**, 658–663.

Sequella 2013, posting date. SQ109 for the Treatment of *Helicobacter pylori* infection-Clinical Development Status: Phase 2 Trials. Sequella. Accessed March 07, 2013 [Online.]

Sezikli, M., Cetinkaya, Z.A., Sezikli, H., Guzelbulut, F., Tiftikci, A., Ince, A.T., Gokden, Y., Yasar, B., Atalay, S., and Kurdas, O.O. 2009. Oxidative stress in *Helicobacter pylori* infection: does supplementation with vitamins C and E increase the eradication rate? *Helicobacter* **14**, 280–285.

Shafer, W.M., Qu, X., Waring, A.J., and Lehrer, R.I. 1998. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/nodulation/division efflux pump family. *Proc. Natl. Acad. Sci. USA* **95**, 1829–1833.

Shen, C.J., Kuo, T.Y., Lin, C.C., Chow, L.P., and Chen, W.J. 2010. Proteomic identification of membrane proteins regulating antimicrobial peptide resistance in *Vibrio parahaemolyticus*. *J. Appl. Microbiol.* **108**, 1398–1407.

Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G., and Kvetnaya, A.S. 2008. Antibacterial activity of *Chamomilla recutita* oil extract against *Helicobacter pylori*. *Phytother. Res.* **22**, 252–253.

Silverio, M.S., Del-Vechio-Vieira, G., Pinto, M.A., Alves, M.S., and Sousa, O.V. 2013. Chemical composition and biological activities of essential oils of *Eremanthus erythropappus* (DC) McLeisch (Asteraceae). *Molecules* **18**, 9785–9796.

Sivam, G.P. 2001. Protection against *Helicobacter pylori* and other bacterial infections by garlic. *J. Nutr.* **131**, 1106S–1108S.

Sivam, G.P., Lampe, J.W., Ulness, B., Swanzy, S.R., and Potter, J.D. 1997. *Helicobacter pylori--in vitro* susceptibility to garlic (*Allium sativum*) extract. *Nutr. Cancer* **27**, 118–121.

Song, J.M. and Seong, B.L. 2007. Tea catechins as a potential alternative anti-infectious agent. *Expert. Rev. Anti. Infect. Ther.* **5**, 497–506.

Srikanta, B., Siddaraju, M., and Dharmesh, S. 2007. A novel phenol-bound pectic polysaccharide from *Decalepis hamiltonii* with multi-step ulcer preventive activity. *World J. Gastroenterol.* **13**, 5196–5207.

Stoicov, C., Saffari, R., and Houghton, J. 2009. Green tea inhibits *Helicobacter* growth *in vivo* and *in vitro*. *Int. J. Antimicrob. Agents* **33**, 473–478.

Suerbaum, S. and Josenhans, C. 2007. *Helicobacter pylori* evolution and phenotypic diversification in a changing host. *Nat. Rev. Microbiol.* **5**, 441–452.

Tabak, M., Armon, R., and Neeman, I. 1999. Cinnamon extracts' inhibitory effect on *Helicobacter pylori*. *J. Ethnopharmacol.* **67**, 269–277.

Tan, P.V., Boda, M., and Etoa, F.X. 2010. *In vitro* and *in vivo* anti-*Helicobacter/Campylobacter* activity of the aqueous extract of *Enantia chlorantha*. *Pharm. Biol.* **48**, 349–356.

Thompson, L., Cockayne, A., and Spiller, R.C. 1994. Inhibitory effect of polyunsaturated fatty acids on the growth of *Helicobacter pylori*: a possible explanation of the effect of diet on peptic ulceration. *Gut* **35**, 1557–1561.

Tombola, F., Campello, S., De Luca, L., Ruggiero, P., Del Giudice, G., Papini, E., and Zoratti, M. 2003. Plant polyphenols inhibit VacA, a toxin secreted by the gastric pathogen *Helicobacter pylori*. *FEBS Lett.* **543**, 184–189.

Tran, A.X., Whittimore, J.D., Wyrick, P.B., McGrath, S.C., Cotter, R.J., and Trent, M.S. 2006. The lipid A 1-phosphatase of *Helicobacter pylori* is required for resistance to the antimicrobial peptide polymyxin. *J. Bacteriol.* **188**, 4531–4541.

Uehara, N., Yagihashi, A., Kondoh, K., Tsuji, N., Fujita, T., Hamada, H., and Watanabe, N. 2003. Human beta-defensin-2 induction in *Helicobacter pylori*-infected gastric mucosal tissues: antimicrobial effect of overexpression. *J. Med. Microbiol.* **52**, 41–45.

Urr, P. 2003. Medicinal Plants and Their Utilization. United Nations Industrial Development Organization (UNIDO) and the International Centre for Scienceand High Technology (ICS). UNIDO Publication, ICS-UNIDO, AREA Science Park, Padriciano 99, 34012 Trieste, Italy.

Ustun, O., Ozcelik, B., Akyon, Y., Abbasoglu, U., and Yesilada, E. 2006. Flavonoids with anti-*Helicobacter pylori* activity from *Cistus laurifolius* leaves. *J. Ethnopharmacol.* **108**, 457–461.

van Heijenoort, J. 2001. Recent advances in the formation of the bacterial peptidoglycan monomer unit. *Nat. Prod. Rep.* **18**, 503–519.

Varbanova, M., Schulz, C., and Malfertheiner, P. 2011. *Helicobacter*

pylori and other gastric bacteria. *Dig. Dis.* **29**, 562–569.

Versalovic, J., Shortridge, D., Kibler, K., Griffy, M.V., Beyer, J., Flamm, R.K., Tanaka, S.K., Graham, D.Y., and Go, M.F. 1996. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori. Antimicrob. Agents Chemother.* **40**, 477–480.

Vitor, J.M. and Vale, F.F. 2011. Alternative therapies for *Helicobacter pylori*: probiotics and phytomedicine. *FEMS Immunol. Med. Microbiol.* **63**, 153–164.

Warin, R., Chambers, W.H., Potter, D.M., and Singh, S.V. 2009. Prevention of mammary carcinogenesis in MMTV-neu mice by cruciferous vegetable constituent benzyl isothiocyanate. *Cancer Res.* **69**, 9473–9480.

Warren, J.R. and Marshall, B. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **1**, 1273–1275.

WHO. 1994. Schistosomes, Liver Flukes and *Helicobacter pylori*. Monographs on the evaluation of carcinogenic risks to humans. *Lyon. (IARC)* **61**, 177–240.

Wittschier, N., Faller, G., and Hensel, A. 2007a. An extract of *Pelargonium sidoides* (EPs 7630) inhibits in situ adhesion of *Helicobacter pylori* to human stomach. *Phytomedicine* **14**, 285–288.

Wittschier, N., Lengsfeld, C., Vorthems, S., Stratmann, U., Ernst, J.F., Verspohl, E.J., and Hensel, A. 2007b. Large molecules as anti-adhesive compounds against pathogens. *J. Pharm. Pharmacol.* **59**, 777–786.

Wong, W.M., Gu, Q., Wang, W.H., Fung, F.M., Berg, D.E., Lai, K.C., Xia, H.H., Hu, W.H., Chan, C.K., Chan, A.O., and *et al.* 2003. Effects of primary metronidazole and clarithromycin resistance to *Helicobacter pylori* on omeprazole, metronidazole, and clarithromycin triple-therapy regimen in a region with high rates of metronidazole resistance. *Clin. Infect. Dis.* **37**, 882–889.

Yanagawa, Y., Yamamoto, Y., Hara, Y., and Shimamura, T. 2003. A combination effect of epigallocatechin gallate, a major compound of green tea catechins, with antibiotics on *Helicobacter pylori* growth *in vitro*. *Curr. Microbiol.* **47**, 244–249.

Yanaka, A. 2011. Sulforaphane enhances protection and repair of gastric mucosa against oxidative stress *in vitro*, and demonstrates anti-inflammatory effects on *Helicobacter pylori*-infected gastric mucosae in mice and human subjects. *Curr. Pharm. Des.* **17**, 1532–1540.

Yanaka, A., Fahey, J.W., Fukumoto, A., Nakayama, M., Inoue, S., Zhang, S., Tauchi, M., Suzuki, H., Hyodo, I., and Yamamoto, M. 2009. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected mice and humans. *Cancer Prev. Res.* (*Phila*). **2**, 353–360.

Zaknoon, F., Sarig, H., Rotem, S., Livne, L., Ivankin, A., Gidalevitz, D., and Mor, A. 2009. Antibacterial properties and mode of action of a short acyl-lysyl oligomer. *Antimicrob. Agents Chemother.* **53**, 3422–3429.

Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. *Nature* **415**, 389–395.

Zhang, L., Liu, W., Hu, T., Du, L., Luo, C., Chen, K., Shen, X., and Jiang, H. 2008. Structural basis for catalytic and inhibitory mechanisms of beta-hydroxyacyl-acyl carrier protein dehydratase (FabZ). *J. Biol. Chem.* **283**, 5370–5379.