Gastric Microenvironment Enables Persistence of Helicobacter pylori: a Physician’s Combat Towards Eradication and Directions for the Future

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Infections caused by H. pylori are associated with multiple gastrointestinal diseases. To effectively reduce disease-related morbidity is to ensure the absolute eradication of the organism. The stomach plays a vital role in endowing survival strategies to H. pylori by inducing stress-hardening and cross-resistance, which helps H. pylori resist the acidic gastric ecosystem and administered antibiotics, respectively. An unstable gastric pH can cause phenotypic alteration in the bacteria, which induces a non-proliferative state and makes them refractory to antibiotics. Moreover, the abundance of colonizing bacteria and the formation of biofilm aid in frequent mutual genetic transfers result in antibiotic resistance. Low gastric pH and rapid gastric emptying hinder the dissolution and distribution of antibiotics across the mucosal surface, which reduces the bioavailability and consequently favours the development of secondary resistance. The thick mucus lining of the stomach reduces the accessibility of administered antibiotic to all alcoves of H. pylori, which can be overcome by developing targeted drug delivery systems. Newer drug regimens have shown better eradication rates than standard triple therapy. However, regimens tailored based on conventional culture-sensitivity, molecular methods, or PPI pharmacogenomics are more effective. Probiotic supplementation can improve the treatment outcomes by increasing bacterial clearance and reducing adverse effects. A promising innovative technique would be to use H. pylori-specific bacteriophages for eradication. Additionally, oral vaccination against H. pylori is suggested to play a remarkable role in the prevention and treatment of infection, as shown in animal experiments. The most effective protective measures would be to improve environmental conditions and quality of life. This review provides insight into currently available treatment options, the challenges faced in successful treatment of H. pylori infections, and possible interventions to overcome these challenges in near future.

INTRODUCTION

The stomach, an assumed sterile surface free from microbial habitation (Bizzozero, 1893), was proven to be inhabited following the isolation and demonstration of Helicobacter pylori from the gastric biopsy samples by Warren and Marshall in 1983 (Warren and Marshall, 1983). Since then, medical literature databases have been inundated with thousands of studies, performed to determine the whereabouts of this bacterium every year. Consequently, we now discern the spectrum of gastrointestinal dysfunctions: chronic gastritis, peptic ulcer disease (PUD), gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric carcinoma (Malfettheiner et al., 2009; Kuipers, 1997; IARC monograph, 1994) as being associated with H. pylori infection. Efforts were also focused on instituting modalities to counter these pathologies in the form of reinforcing the gastric wall and killing the causative bacteria. Deep insight into the microbial structure, virulence factors, pathogenesis, and associated pathological states has directed scientists and clinical researchers to design compactly constituted drug regimens comprising antibiotics (amoxicillin, clarithromycin, metronidazole, levofloxacin, etc.), anti-secretory agents (PPIs and H2 blockers), and topical medications (colloidal bismuth preparations) for the eradication of H. pylori. These endeavours should simplify and expedite the process of the absolute eradication of H. pylori from the stomach. However, it proves to be a significant challenge. This bacterial endurance has been attributed to phenotypic and genotypic variations such as the development of drug resistance (Ebinesh and Kailash, 2016; Broutet et al., 2003) and the impotency of antimicrobial agents in the stomach (Vakil and Megraud, 2007; Bloom and Polak, 1980). The role of the stomach and its microenvironment in eradication failure (Table 1) and future prospects for successful eradication will be discussed.

DISCOVERY: HELICOBACTER PYLORI AS A PATHOGEN
Presence of bacteria along the mucosal surface and inside the biofilm formation and resultant drug resistance
Stress-induced phenotypic resistance by survival as a slow grower (dormant form)
Inducing secondary drug resistance due to reduction in bioavailability
Fast gastric emptying resulting in reduced duration of action
Thick mucus lining which prevents access to all niches of bacteria
Poor bioavailability of acid labile antibiotic due to low gastric pH

It was believed that the microenvironment of stomach was unsuitable for microbial existence (Bizzozero, 1893). Towards the end of 19th century, few scientists recorded the occurrence of spiral-shaped bacilli in the stomach of animals (Bizzozero, 1893), following which a parallel observation was made in the stomach of humans suffering from peptic ulcer disease (Pel, 1899). Considering these spiral-shaped bacteria to be the cause of PUD, the patients underwent treatment with high doses of bismuth (Pel, 1899), which is one of the first known antimicrobial compounds. However, the idea that PUD was caused by these bacteria was disproven due to the presence of these spiral-shaped bacteria in the stomach of healthy individuals (Kusters et al., 2006). In the late 20th century, a series of remarkable experiments were staged by Warren and Marshall to demonstrate, isolate, and characterize the properties of this bacterium (Marshall et al., 1987; Marshall et al., 1985; Marshall et al., 1985; Marshall and Warren, 1984). In 1987, Marshall et al. and Morris and Nicholasen described an acute gastric inflammation and a long-standing gastritis respectively following autoinfection of H. pylori. In 1991, Morris again recorded a long-standing gastritis in human participants that was treated successfully with doxycycline and bismuth subsalicylate (Morris et al., 1991). Initially, this spiral-shaped bacterium was named “gastric Campylobacter- like organism” or “Campylobacter pyloridis”. Goodwin (Godwin et al., 1989) described the divergent properties of the genus Campylobacter, after which it was termed “Helicobacter pylori”. CURRENT MANAGEMENT OF H. PYLORI INFECTIONS

Extensive scientific investigations have ascertained a direct association of H. pylori as the causative agent for chronic gastritis, PUD and gastric malignancies. Chronic gastritis was the first sequel of colonization, followed by PUD and malignancies. It should also be noted that H. pylori-associated gastroduodenal disease impart a high incidence of relapse (Rauwss and Tygat, 1990; Axon, 1991) which further enhances the jeopardy of developing a malignancy. IARC has classified H. pylori as a group 1 carcinogen (Testerman and Morris, 2014) which also encompasses radiations and smoking, accounting for 25% of all infection-associated malignancies and 5.5% of all cancers (Mbulaiteye et al., 2009). An absolute eradication will certify a colossal drop in H. pylori-associated morbidity and mortality. Bearing this fact in mind, European (Malfertheiner et al., 2002) and Canadian (Hunt et al., 1999) guidelines endorsed a “triple therapy regimen,” roping in amoxicillin/ metronidazole, clarithromycin, and a proton pump inhibitor (PPI) for a scheduled period of 14 days and was once widely adopted across the globe (Hoffman and David, 2001) to eradicate the bacteria. Owing to the emergence of drug resistance in the recent years, triple therapy regimen is recommended for regions with a resistance rate of less than 20% and with susceptibility to clarithromycin infections (Malfertheiner et al., 2007). Greater frequency of drug resistance is generally encountered in Africa and East Asia, which is hypothesized to be a result of prevalence of multiple genotypic variant strains (Kaleb et al., 2007; Graham et al., 2009). Recently, various new drug regimens (incorporating antibiotics such as amoxicillin, metronidazole, clarithromycin, rifabutin, levofloxacin and furazolidone) have been introduced and are being increasingly utilized (Basu et al., 2011; Hsu et al., 2008). The newer drug regimens comprise of four first-line regimens (sequential, concomitant, bismuth-containing quadruple and hybrid regimens), two second-line regimens (bismuth-containing quadruple and levofloxacin-based regimens) and three third-line regimens (levofloxacin-based quadruple, rifabutin-based quadruple, and furazolidone-based quadruple regimens). The latter of which surpassed the standard triple regimen therapy in terms of eradication rates (Chua et al., 2011; Gisbert et al., 2010; Wu et al., 2010; Malfertheiner et al., 2012; O’Morain et al., 2003; Laine et al., 2003; Phillips et al., 2001; Choy et al., 2007; Nishizawa et al., 2009; Van Der Poorten and Katelaris, 2007; M’egraud and Lehours, 2007). Though the prevalence of drug resistance exhibits worldwide diversity, according to a systematic review by Francesco and colleagues (Francesco et al., 2010), the global prevalence of resistance in H. pylori to clarithromycin, metronidazole, amoxicillin, tetracycline, levofloxacin and rifabutin was estimated to be 17.2%, 26.7%, 11.2%, 5.9%, 16.9% and 5.4%, respectively, while multidrug resistance was 9.6%. The surge in H. pylori’s drug resistance is a consequence of the widespread use of agents like amoxicillin and clarithromycin for respiratory tract infections (M’egraud, 2004) and is also correlated with increased antibiotic consumption (Perez Aldana et al., 2002). This has intensified the antibiotic resistance burden, which has been a major contributor to the failure to eradicate H. pylori.

INHERENT AND ACQUIRED POTENTIALS OF H. PYLORI THAT INFLUENCE THERAPEUTIC OUTCOMES

A conspicuous element of the H. pylori’s survival strategies is its...
ability to resist the stomach’s high acidity despite it being non-acidophilic. Pyrosequencing of the small unit 16sRNA has led provided insight on normal gut flora and has established the predominance of *H. pylori* in the stomach (Necchi et al., 2007; Friedrich, 2008). The average recorded pH of the stomach is 1.4 (Bloom and Polak, 1980), but it is said to vary somewhere between pH 4 and 6.5 (McLachlan et al., 1989). Occasional “acid shocks”, or transients peaks of acidity, are known to occur (Schade et al., 1994). Though the urease enzyme is believed to confer acid resistance necessary for survival (Burne and Chen, 2000), it does not aid the bacteria in withstanding acid shock (Bijlsma et al., 2000; Stingl et al., 2002). Hence, varying pHs provoke a perturbation in the spatial alignment of the bacteria, promoting resistance (Schreiber et al., 2004). It may also be a consequence of stress. Acidic pHs of 4 - 6.5 (normal gastric pH) is a sublethal environmental stress which, during prolonged exposure, induces the production of stress proteins that protect the bacteria from stress, stress hardening, and cross resistance. Stress hardening is a phenomenon by which repeated or prolonged exposure to sublethal levels of some form of stress makes the bacteria resistant to even lethal levels of the same stress (Rowan, 1999). This phenomenon holds true for *H. pylori’s* resistance to acid shocks, since the bacteria has been in contact with a sublethal acidic pH (4 - 6.5) for a long duration, it also develops resistance to the lethal pH levels (1 - 3.5 during acid shocks). Moreover, it renders the bacteria resilient to other forms of stress including antibiotics (McDowell, 2004; Bremer and Kramer, 2000; Hastings et al., 2004; Ebinesh et al., 2018), making them cross-resistant (Alekshun and Levy, 1997; Archer, 1996; Ma et al., 1995; Velkov et al., 1999). These phenotypic variations are due to stress-induced genomic plasticity, causing an increase in the frequency of random mutagenesis and gene transfer (Ebinesh et al., 2018; Velkov et al., 1999; Gougeon et al., 2000; McMahon et al., 2007). Another phenotypic variation recorded in *H. pylori* is its ability to oscillate between an active proliferative state and a dormant non-proliferative state in pace with the microenvironment. As most of the antibiotics are only active against actively replicating clone of bacteria, this phenotypic resistant state serves as another impediment for eradication (Scott et al., 1998).

*H. pylori* are uniformly distributed throughout the surface of gastric mucosa (Genta et al., 1994) which indicates a heavy bacterial load (Graham, 1998). Basic statistical application reveals that a high bacterial load is associated with a high rate of spontaneous mutation and a healthy drug resistant clone (innate and acquired resistance) (Graham DY and Fischbach L, 2010). Moreover, a large inoculum would also reduce the efficacy of administered antibiotic agent (Genta RM et al, 1994). *H. pylori* possess the ability to form biofilms, which makes eradication tougher by facilitating gene transfer and thus harnessing drug resistance (M’egraud F et al, 1991). Presence of multiple adhesins for binding to mucosal epithelial cells (Graham DY, 1998) and both intra and extra cellular habituation of *H. pylori* (Engstrand L et al, 1997) increases the risk of eradication failure by expanding the degree of virulence and being inaccessible to antibiotic agents respectively (the latter will be discussed extensively under the next heading).

**THE STOMACH AND THERAPEUTICS: THE RELATIONSHIP AND ITS IMPLICATIONS**

An important factor that challenges absolute eradication is the fact that the stomach is *H. pylori’s* habitat. A major determinant of the outcome of antibiotic therapy is the drug concentration at the site of action. The pH of the stomach is lower than the optimal pH for the action of drugs, thus limiting the diffusion and distribution of antimicrobial agent across the mucosal surface (Vakil and Megraud, 2007; Bloom and Polak, 1980; Graham and Borsch, 1990; Borsch and Graham, 1991; Hunt, 1993). Hence, the bioavailability of antibiotic agents decreases and the bacteria are exposed to sublethal doses (Graham, 1998) of antibiotics, culminating in secondary drug resistance. The development of secondary resistance to clarithromycin and metronidazole has been known to impair the progress and outcome of therapy (Boltin et al., 2015). Low gastric pH is a major barrier to the action of many drugs especially those like clarithromycin, which are acid labile. However, the action of tetracycline is instead supplemented by acidic pH and that of bismuth and metronidazole is not influenced (Grayson et al., 1989; Goodwin and McNulty, 1992). Erratic gastric pH bestows a state of phenotypical resistance upon the bacteria where the bacilli do not multiply and thus the antibiotics seldom work (Scott et al., 1998). Therefore, the co-administration of acid secretion inhibitors (like PPIs and H2 blockers) should result in a decrease in eradication failure (Moayyedi et al., 1995; Unge et al., 1989).

*H. pylori* are known to reside on the mucosal surface and inside the epithelial cells (Engstrand et al., 1997). Persistence of the bacilli along the epithelial surface leaves a theoretical advantage for rapid eradication, as the inaccessibility can be attributed to the presence of thick mucus membrane along the mucosal surface (Wu et al., 2012). The active flow of the antibiotic across the mucus lining is needed to ensure eradication (Midolo et al., 1996), which when compromised, results in recolonization and reinfection that further complicates the issue (Atherton et al., 1995).

Gastric emptying and the distribution of antibiotics across the mucosa are the two other parameters that determine post-therapeutic outcomes and can be maneuvered (Graham, 1998). The administration of a drug with food delays gastric emptying, provides wider distribution, and dilutes acidity (Graham and Borsch, 1990; Graham and Evans, 1990). However, a major drawback in administering antibiotics with food is the unpredictable molecular interactions and possible reduction in the local availability of the antibiotic. The grinding action of stomach during digestion generates friction between the gastric mucosa and the bulk of food, bringing about mucosal desquamation and mucus secretion, thereby exposing the bacteria to a higher concentration of the drug and thus enhancing antimicrobial activity (Grant et al., 1953; Willems, 1988). The form of the administered drug influences the distribution of the drug throughout the mucosal surface. Capsules may get lodged in the rugal folds of the stomach and may only produce a local action (Graham, 1998). Tablets have a relatively better dis-
solution and distribution. Liquid forms have the highest surface area of distribution, but are emptied faster into the duodenum. Tablets and capsules can be designed for a sustained depot delivery while liquid forms cannot be designed to be extended release forms (Goodwin and McNulty, 1992). Colloidal bismuth preparations bind to the mucus and give off extended and sustained action which renders them potent adjuvants especially in case of managing reinfection or relapse. Unfortunately, the hypothetical advantage of substituting liquid formulation of amoxicillin for capsules was documented to possess no difference in outcome (Marshall, 1993), after which no attempts were directed to evaluate the efficacy of other antibiotics.

**IMPELLING PROSPECTS AND FUTURE DIRECTIONS**

In comparison to the standard triple therapy, newer regimens are now known to yield higher eradication rates despite their specific disadvantages (Hsu et al., 2011; Malfertheiner et al., 2011; Liou et al., 2010; Toracchio et al., 2005). Other strategies that may work would be pretreatment drug sensitivity-based tailored regimens, bacteriophage-mediated selective clearance, probiotic supplementation, and the development of potent vaccines.

**New drug regimens**

The newer drug regimens promise improved treatment outcomes. Most of the novel drug regimens are levofloxacin-based, meaning they are relatively more efficacious (Liou et al., 2010). Widespread use of levofloxacin for other indications might result in per- vasive resistance. Hence, levofloxacin-based regimens should be reserved for second-line treatment only. Rifabutin is a rifamycin-S derivative which has proven to potentially act against *H. pylori* activity (Van Der Poorten and Katelaris, 2007; Toracchio et al., 2005). Extensive non-judicial use of rifabutin also results in the development of resistance. Furthermore, it is also known to induce cross resistance against rifampin (a potent anti-tubercular drug) (Heep et al., 1999; Suzuki et al., 2009; Glocker et al., 2007) which puts the developing nations with high tuberculosis prevalence at risk. Therefore, use of rifabutin-based therapeutics should be judicial with accord to the local prevalence of tuberculosis. The hope for any new regimen is to yield eradication rates of more than 90%; however, this also relies on patient adherence and compliance. Consequential to long term treatment, all regimens go with questionable adherence for the completion of the entire course. Dispensing drugs in patient-friendly plasticized convenience packs would improve patient adherence to the regimens. Framing a universally acceptable standard regimen is infeasible due to phyl- ogeographic variations among the prevalent strains leading to wide distinctions in worldwide antimicrobial susceptibility (Yamaoka, 2010; Yamaoka et al., 2008). Availability of local surveillance data on regional susceptibility patterns can drive the implementation of nation-wide efficient management protocols for better therapeutic outcomes. Another barrier for eradication is the inaccessibility of antibiotics to all the niches of *H. pylori* due to the presence of bacteria along the mucosal surface and within the epithelial cells, which can be overcome by developing targeted delivery systems for transcellular and paracellular coverage.

**Tailored therapeutic regimens**

The current convention is to assess the antibiotic sensitivity of the pathogen following two courses of unsuccessful treatment (Malfertheiner et al., 2012). Exposure of the bacteria to sublethal doses of antibiotics for two relatively long courses may result in secondary resistance (Boltin et al., 2015) and so, pretreatment assessment of antibiotic susceptibility may be a superior option. Modalities for tailoring therapy can be made based on culture, susceptibility assessment by molecular methods, or pharmacogenomics. A systematic review comparing the results of 5 RCTs has established a baseline for culture-guided regimen over the standard regimen (Wenzhen et al., 2010) but conventional culture-sensitivity testing is invasive, time consuming, expensive, and unreliable as it does not accurately reflect the drug’s *in vivo* activity (Gisbert et al., 2011). PCR and other PCR-based molecular techniques like restriction fragment length polymorphism (RFLP), DNA enzyme immunoassay (DEIA), oligonucleotide ligation assay (OLA), line probe assay (LPA), and real time PCR can be used to determine sensitivity (Lehours et al., 2011; M’egraud and Lehours, 2007; Schabereiter Grunter et al., 2004). For example, 23Sr RNA sequencing is done to assess clarithromycin susceptibility (Versalovic et al., 1996; Taylor et al., 1997). Molecular diagnostics can be run on traditional biopsy specimens and even on samples obtained by oro-gastric brush and gastric wash which are minimally invasive or stool samples, which are noninvasive (Kawai et al., 2008; Graham et al., 2005; Baba et al., 2011; Lottspeich et al., 2007). Moreover, these methods are time-saving when compared to conventional culture-sensitivity that consumes almost 10-14 days (7-10 days for culture, 2-4 days for sensitivity testing) and are more accurate. Fluorescent *in situ* hybridization (FISH) is another molecular method to detect the presence of resistant genotypes on wax-embedded sections (Yilmaz and Demiray, 2007; Yilmaz et al., 2007). Pharmacogenomic-based tailoring of anti-*H. pylori* treatment is done to optimize the action of PPIs by evaluating the activity of PPI-metabolizing microsomal enzyme CYP2C19, which is known to influence the action of other drugs (Goddard et al., 1996; Grayson et al., 1989; Sim et al., 2006; Furuta et al., 2001) and has been documented to yield better results when used as a parameter for tailoring along with clarithromycin susceptibility testing by molecular methods (Furuta et al., 2007). However, pharmacogenomics of CYP2C19 is only known to affect the metabolism of lansoprazole (Zhao et al., 2008) while other PPIs are not influenced (Lee et al., 2010; Pan et al., 2010). Development and promotion of simpler sampling techniques, rapid molecular drug sensitivity testing kits that are cost-effective and easily accessible, and PPI pharmacogenomics-based tailored drug regimens (Papastergiou et al., 2014) would be future aims.

**Probiotics and prebiotics**

Probiotics counteract the colonization and growth of pathogenic bacteria by releasing antibacterial substances, competing for receptor-binding sites, reinforcing the gut wall resistance, and modulating the immune response (Lesbros Pantoflickova et al., 2007).
They also reduce the adverse effects of drug therapy (Papastergiou et al., 2014; Wilhelm et al., 2011). Supplementation of probiotics has recently garnered attention and has been found to be an effective adjuvant when administered with standard triple therapy (Du et al., 2012), sequential regimen (Efrati et al., 2012), and levofloxacin-based regimens (Ojetti et al., 2012) but not with others (Manfredi et al., 2012, Shavakhi et al., 2013; Navarro Rodriguez et al., 2013). Lactobacillus spp., Bifidobacterium spp., B. clausii and Saccharomyces boulardii are few of the organisms that are known to have a beneficial role in *H. pylori* eradication (Shavakhi A et al, 2013). *Lactobacillus* spp. and *Bifidobacterium* spp. have been documented to be superior adjuvants with reference to multi-genera combinations in aiding *H. pylori* eradication and reducing adverse effects of antibiotics (Zheng et al., 2013; Zou et al., 2009; Wang et al., 2013). *Saccharomyces boulardii* supplementation reduced the incidence of antibiotic-induced diarrhea (Szajewska et al., 2010). Studies evaluating the effect of the addition of lactoferrin to probiotics concluded that addition of lactoferrin had no added advantages (de Bortoli et al., 2007). Though these suggest the beneficial effect of probiotic administration, efforts should be initiated to characterize the mechanisms of probiotic action to standardize the dose and frequency of administration and the incorporation of probiotics in the scheduled eradication regimens.

**Phage-directed elimination**

An ideal regimen would be one which discriminately kills only *H. pylori*. Bacteriophage-mediated elimination strategies are a novel modality which easily deals with the risk of antibiotic resistance (Ebinesh and Kailash, 2016). Being pathogen-specific, it results in selective elimination of all clones of *H. pylori*, including those which are drug resistant and those which are not. A major disadvantage of this strategy is the spontaneous development of bacterial resistance to phage infection which, if asphyxiated, can yield promising therapeutic modality for the management of any bacterial infection including *H. pylori*.

**Role of vaccines**

*H. pylori* vaccines have been proven to be beneficial in animal models. Human trials have not been initiated until now to establish their potency. Animal models have shown that infection with *H. pylori* induce Th1 cell response, causing inflammation that does not exhibit any kind of resistance to infection (Mohammadi et al., 1996). A healthy immune response requires the activation of Th2 cell-evoked immunity which can be elicited by using Th2 cell response-inducing adjuvants like cholera toxin and Freund’s adjuvant. Evidence suggest that oral vaccination stimulates secretory IgA and serum IgG synthesis in mice (Ferrero et al., 1995; Kuipers et al., 2004). Successful efforts suggested the provocation of Th2 cell mediated cellular response and bacterial elimination following oral immunization with *H. pylori* urease in mice suffering from *H. felis* infection (Saldinger et al., 1998). Hence, *H. pylori* vaccinations can prevent infection, cure an existing infection, and can also be used as adjuvants to antibiotic therapy (Corthesy Theulaz et al., 1996; Crabtree, 1998; Doidge et al., 1994; Ghiara et al., 1997; Hone and Hackett, 1989). Utilization of vaccines as an adjuvant to antibiotic therapy will reduce the bacterial load by Th2 cell-mediated immune response and reduce the chances of nurturing drug resistance. All these interpretations have been made using the documented outcomes of the experiments on animal models. Accordingly, human trials should establish reliable information on the utility of vaccines in prevention and management of *H. pylori* infection (Johansson et al., 2004; Keller and Michetti, 2001; Michetti et al., 1999). *H. pylori*-associated diseases are prevalent in populations of low socioeconomic status, poor hygiene, and living conditions (Herbath et al., 2001; Woodward et al., 2000), the improvement of which would be the finest initiative for primordial prevention (Veldhuyzen van Zanten, 1995).

**CONCLUSION**

The main challenge for the eradication of *H. pylori* is the low gastric pH, which instils survival benefits in the bacteria and reduces the bioavailability of antibiotic agents used for treatment. However, the newer drug regimens have a higher rate of absolute eradication. Tailored therapeutic regimens are effective if simpler sampling techniques and inexpensive molecular methods are introduced. Probiotic supplementation has relatively higher eradication rates but require standardization. Bacteriophage-mediated selective elimination of *H. pylori* is a promising innovation if ways to mitigate development of bacterial resistance are familiarized. Preventive and therapeutic oral vaccination provides high hopes if proven effective by clinical trials. A superior way of prevention would be to improve the environmental and sanitary conditions of human survival.

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