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TOPIC HIGHLIGHT

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Biofilms and *Helicobacter pylori*: Dissemination and persistence within the environment and host

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Core tip: The ability of *Helicobacter pylori* (*H. pylori*) to form biofilms is fundamental to its pathogenicity. Research into the mechanisms behind *H. pylori* resuscitation from coccoid to virulent spiral forms will aid a better understanding into infection recurrence in the host and the external environment.

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Abstract

The presence of viable *Helicobacter pylori* (*H. pylori*) in the environment is considered to contribute to the levels of *H. pylori* found in the human population, which also aids to increase its genetic variability and its environment adaptability and persistence. *H. pylori* form biofilms both within the *in vitro* and *in vivo* environment. This represents an important attribute that assists the survival of this bacterium within environments that are both hostile and adverse to proliferation. It is the aim of this paper to review the ability of *H. pylori* to form biofilms *in vivo* and *in vitro* and to address the inherent mechanisms considered to significantly enhance its persistence within the host and in external environments. Furthermore, the dissemination of *H. pylori* in the external environment and within in the human body and its impact upon infection control shall be discussed.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is an opportunistic pathogen that plays an important role in the aetiology of peptic and gastric ulcers. *H. pylori* primarily colonizes the antral part of the stomach whereby they either adhere to the walls of the stomach or simply remain in a planktonic, free-floating state. This bacterium has been reported to spread from the stomach to the intestine where it is then secreted in faeces[1]. Furthermore, *H. pylori* infection is known to be associated with nausea and vomiting which can lead to the spread of this pathogen to the oral cavity, leading to the colonisation of gingival and dental plaques[2].

H. pylori has been reported to colonise over half the world's population with clinical signs of infection only manifesting in less than 20% of these individuals[3]. Nevertheless, the majority of these individuals are colonised with *H. pylori* for life unless eradication using appropriate chemotherapeutic agents is successful. Lifelong colo-

nisation seems to be due to the ability of some strains of *H. pylori* to both adapt to the host's immunological responses and to also withstand the constantly changing gastric environment. In genetically predisposed individuals, colonisation with *H. pylori* is reported to heighten the risk of developing cancer[4].

H. pylori can be described as Gram negative, spiral- (S-shape) or cocci-shaped bacteria. It has been reported to exist in three forms, the viable and culturable spiral form, the viable but non-culturable (VBNC) coccoid form, which are less virulent, and the non-viable degenerative *H. pylori* form[5]. It is their spiral shape that is thought to enhance their colonisation of the gastric mucosa. Whilst generally considered microaerophilic, there is now evidence that *H. pylori* can grow in humidified aerobic conditions[6].

The colonisation of *H. pylori* and its effect on resident gastric microbiota is relatively unknown. A study by Bik *et al*[7] assessed the human gastric microbiota from 23 gastric biopsy samples using small subunit 16S rDNA clone library method and subsequently found that the presence of *H. pylori* had no effect on the microbial profile of the gut[7]. A recent study investigated the effects of *H. pylori* on the gastric microbiota in a Rhesus macaque model. The authors found no significant impact upon the non-Helicobacter taxa after *H. pylori* challenge[8]. However it appears that the microbial profile of the gut may have an effect on the degrees of pathogenicity of *H. pylori*. A germ-free gastric cancer mouse model showed less symptoms of disease and a later onset of neoplasia upon *H. pylori* infection when compared to those mice with a typical gastric microbiota profile[9].

As an avid coloniser of the gastric mucosa *H. pylori* must possess a number of characteristics that include flagella, adhesions, urease production, and biofilm forming ability[10,11]. The importance of the biofilm forming potential of *H. pylori* is fundamental to its pathogenicity. The formation of a biofilm is a virulence mechanism that aids in the enhancement and longevity of *H. pylori* in "unfriendly" and hostile environments, such as in the human stomach and the natural environment.

H. pylori was first found to demonstrate an ability to form *in vitro* biofilms in the early and late 1990s with solid evidence of this ability reported by Stark *et al*[12] in 1999. More recent reports on the ability of *H. pylori* to form biofilms within *in vitro*[13,14] and *in vivo* environments, specifically the gastric mucosa, have now been demonstrated[14-16]. In particular the *H. pylori* strain TK1402 isolated from a patient with duodenal and gastric ulcers has been shown to have very strong biofilm forming ability both inside and outside the host[14,15,17-20]. In this mode of growth it is likely that *H. pylori* is protected from external perturbations[18,21].

Biofilms can develop on both biotic and abiotic surfaces through the conversion of microorganisms in a free-floating or planktonic state, to a sessile state, where they become attached onto a surface. Once microorganisms attach onto a surface they proliferate, produce extracellular polymeric substance (EPS) and become firmly attached to that surface. The matrix of the biofilm is known to be composed of polysaccharides, extracellular DNA (eDNA), lipids and proteins that form the "house" of the biofilm[22,23]. It is the biofilm and the ability of microorganisms to form biofilms that form an essential element, aiding in their persistence, survivability, and recalcitrance to antimicrobial interventions and the hosts immune response. Furthermore the ability of pathogenic microbes to survive within diverse and hostile environments is enhanced significantly when growing within a biofilm. Growth within a biofilm is known to cause and exacerbate infections and is responsible for prolonging infection, leading to chronicity[24].

A biofilm is dynamically and structurally complex and is often referred to as a "living organism" due to its ability to adapt to external perturbations. Of particular concern with biofilms of public health significance is the fact that sections of biofilms can easily detach or shear off, enabling these sections or individual bacteria to recolonise other surfaces. Detachment or dissemination from the biofilm can be achieved by the dispersal of single cells or the detachment/shedding of large cellular aggregates. Both situations constitute a concern to public health particularly where fluid resides, as microbial dissemination is enhanced *e.g.*, catheters, blood stream, drinking water[24]. Further to this there is growing evidence that within a biofilm the horizontal transfer of genes can occur, leading to large variations in *H. pylori* strains, particularly in one host, enhancing their survival and immune evasion. Moreover, gene transfer *in situ* has an important role to play in immunological effectiveness and eradication of pathogens by the host[25]. In addition to this it is well documented that when microorganisms are growing within a biofilm they have increased tolerance to antimicrobial agents[26].

It is the aim of this paper to review the ability of *H. pylori* to form biofilms *in vivo* and *in vitro* and to address the inherent mechanisms considered to significantly enhance its persistence within the host and in external environments. Furthermore, the dissemination of *H. pylori* in the external environment and within in the human body and its impact upon infection control shall be discussed.

TRANSMISSION OF *H. PYLORI*

The routes of transmission of *H. pylori* are said to occur via an array of different pathways[27,28]. Although *H. pylori* are considered to be pathogens commonly associated with the human stomach, Brown proposed that *H. pylori* are able to survive in environments that are external to that of the human stomach[29]. Dental plaque has also been reported to contain *H. pylori;* however, in plaque, *H. pylori* are thought to only exist in a transient state[30-32]. Young *et al*[2] reported that both the spiral and viable coccoid form of *H. pylori* are present in the oral cavity. Souto and Colombo found *H. pylori* in a subgingival bio-

film in 11% of periodontally healthy patients compared to 50% of patients suffering from periodontitis[33]. The authors proposed that biofilm formation in the oral cavity should be considered as a potential reservoir for *H. pylori*.

There is building evidence to suggest that *H. pylori* may reside in potable water systems[11]. In general, waterborne bacteria can adhere to surfaces by aggregating matrix to form biofilms[26]. Information regarding the exact ecological niche where *H. pylori* reside and persist outside of the human host is limited. Despite this, there is growing evidence that external reservoirs of *H. pylori* may exist, potentially aiding transmission to the host. Furthermore there are also reports that *H. pylori* may have, as part of its life cycle, a zoonotic component. However, further scientific evidence of cultivability will be required to fully support this area.

The ability to form biofilms and the cell morphology and architecture formed depends greatly on the support material. To date however, in potable water supplies there is not enough substantial evidence that *H. pylori* within the viable state, plays a role in the development of a biofilm. Despite this, there is significant evidence that, in terms of epidemiological evidence, the risk of acquiring *H. pylori* increases in individuals who drink well water and river water or swim in pools, streams and rivers in particular[27,34-37]. Consequently, environmental water is considered a risk for the acquisition of *H. pylori* and therefore *H. pylori* biofilms in these environments should be a very important consideration when investigating reservoirs of source. The association of *H. pylori* with biofilms in water distribution systems can offer bacteria protection from disinfection and protozoan predation[38]. The challenge however, remains to determine the importance of waterborne *H. pylori*. It may be possible that a specifically adapted form of *H. pylori*, or simple *H. pylori* within a biofilm, may be required for persistence and transmission[39].

Although based on scientific logic, if *H. pylori* is able to survive and persist outside of the human host, its ability to develop a biofilm and survive within a biofilm may well help to answer fundamental questions regarding acquisition and potential eradication, particularly in the developing world.

THE DETECTION OF *H. PYLORI* IN THE ENVIRONMENT AND HOST

The ability of *H. pylori* to transform from a highly virulent, spiral shaped bacteria to a less virulent, non-culturable coccoid state, presents difficulties in the successful detection of this bacterium in both an environmental and clinical setting. In particular, the VBNC coccoid state is thought to arise under less favourable conditions, making the identification of *H. pylori* from water sources, particularly *H. pylori* within biofilms, unlikely using traditional culture methods.

Molecular methods such as real-time polymerase chain reaction (PCR) have been used to identify *H. pylori* in both spiral and coccoid states. Linke *et al*[40] used real-time PCR to target the ureA subunit of the *H. pylori* urea gene to identify *H. pylori* in drinking water biofilms. This *in vitro* study demonstrated successful identification of *H. pylori* from biofilms in silicone tubing, an imitation of drinking water systems. The study not only highlighted the capability of *H. pylori* to form biofilms in such a system but also emphasised the potential of using real-time PCR as a viable detection method. Although it is clear that more research into the identification of different strains of *H. pylori* using this method should be considered.

In terms of identification within the host, a very recent research paper by Fontenete *et al*[41] demonstrated the use of fluorescent *in situ* hybridisation (FISH) to identify *H. pylori* in culture and human gastric biopsies. This study mimicked *in vivo* conditions using gastric biopsies and modified the FISH method by replacing toxic chemicals, giving rise to the opportunity of using this method, given further development and trails, in *in vivo* situations.

THE ABILITY OF *H. PYLORI* TO FORM A BIOFILM

The ability of *H. pylori* to form a biofilm has been documented for over 15 years with biofilm growth heightened in environments which are composed of high carbon:nitrogen ratios[12,39]. The ability of *H. pylori* to develop biofilms has been reported in many *in vitro* studies[12-14,17,42,43]. The specific knowledge regarding the ability of *H. pylori* to form biofilms has been made possible by observations using microscopic techniques in particular scanning electron microscopy (SEM) specifically on glass but also on other materials[17].

Further to this, Yonezawa *et al*[18] reported that the *H. pylori* strain TK1402 (isolated from a Japanese patient with both duodenal and gastric ulcers) was able to form a biofilm but was dependent on the flagella, their ability to form cellular aggregates, and its ability to produce outer membrane vesicles. This ability to form biofilms has been shown to be modulated by quorum sensing molecules; in particular the LuxS proteins have been identified in *H. pylori*[44].

Quorum sensing within H. pylori biofilms

Quorum sensing is an intercellular method of communication between microorganisms using chemical signalling. Quorum sensing molecules can be enzymes or peptides depending upon the signalling system. The accumulation of these signalling molecules leads to an interaction with cytoplasmic DNA-binding receptor proteins such as the *lux* protein family, whereby quorum sensing genes are modulated. Quorum sensing molecules however do not always bind to receptor proteins intracellularly; peptide molecule binding can occur on cell membranes whereby signal transduction leads to gene

regulation[45].

In the case of *H. pylori*, this bacterium expresses a homolog of the luxS gene, a gene responsible for the production of the quorum sensing molecule, autoinducer 2 (AI-2)[45]. The *H. pylori luxS* homolog has been implicated in bacterial attachment. Cole *et al*[13] revealed a two-fold increase in the biofilm formation of *H. pylori luxS* mutants when compared to the wild-type control. The authors concluded that in some strains of *H. pylori*, a mutation in quorum sensing signalling actually increases biofilm formation[13]. Later work by Rader *et al*[46] demonstrated defective motility in *luxS* mutants and highlighted the importance of quorum sensing AI-2 molecules as a regulator of flagella-associated genes in *H. pylori*. Further work by Rader *et al*[47] revealed that the release of AI-2 molecules acts as a chemorepellent for *H. pylori*. At this stage, the authors hypothesised that this action may cause *H. pylori* to break away from the majority of the bacterial population, avoiding niche competition and encouraging *H. pylori* dispersal. In the context of both external environments and within the clinical setting of the host, quorum sensing within a *H. pylori* biofilm may encourage dispersal, a mechanism that may induce the likelihood of transmission to and from an external environment and the host, and dissemination within the host.

H. PYLORI GROWTH WITHIN BIOFILMS: THE IMPORTANCE OF COCCOID FORMS AND RESUSCITATION

Understanding the growth of H. pylori

In vitro studies are an important starting point in the understanding of the dynamics of *H. pylori* growth within a biofilm. In light of this, the study of *H. pylori* in biofilms present challenges in the laboratory; nevertheless, the growth of *H. pylori* has been documented to behave differently in different growth conditions.

Bessa *et al*[48] assessed the growth of *H. pylori* in four types of liquid culture medium to assess the physiological behaviour and growth standardisation of *H. pylori*. *H. pylori* in free-living and biofilm modes of growth were assessed in Brucella broth, brain heart infusion broth and Ham's F-12 medium supplemented with 2% fetal calf serum and Ham's F-12 without serum. Free-living growth was monitored for 72 h in each medium and characterised for bacterial density, cultivability, viability and morphology. Biofilm formation in the same medium was evaluated for biomass production, colony forming unit (CFU) counts and microscopic visualisation. Afterward, using Ham's F-12, the effect of amoxicillin and clarithromycin at sub-minimum inhibitory concentrations (sub-MICs) was evaluated on *H. pylori* biofilm formation and luxS gene expression. Differences in free-living growth were observed between the culture medium supplemented with serum and Ham's F-12 without serum. Biofilm formation was significantly dependent on the growth media used. Ham's F-12 appeared to be a good medium to support both growth phenotypes of *H. pylori*. Moreover, sub-MICs of antibiotics increased the biofilm formation and affected the luxS gene expression[48]. Optimising the growth conditions of *H. pylori*, especially in the biofilm mode, will be helpful to perform more accurate in-depth studies that will increase the knowledge about *H. pylori* biofilms, which should be a target to eradicate resistant infection. Humidified conditions with 5%-7% oxygen and 7%-10% CO_2 with some H_2 or 10% CO_2 are also reported to be ideal for the growth of *H. pylori*[49]. However, the expression of catalase and superoxide dismutase (SOD), allows *H. pylori* to persist in higher levels of oxygen[50,51].

H. pylori biofilms, VBNC coccoid phenotypes and resuscitation

The emergence of VBNC pathogens has been of much interest in recent years due to the notion that this state is a form of survival and protection.

The VBNC coccoid form of *H. pylori* is formed during stress and starvation[52]; therefore it is in this form in which *H. pylori* is thought to reside in biofilms.

It has been reported that atmospheric conditions enhance the formation of VBNC coccoid *H. pylori* which has been suggested to resemble the same characteristics of persister cells documented in biofilms[11,53]. Furthermore, these cells then have the ability to resuscitate and lead to infection recurrence[54,55]. Cellini *et al*[20] identified the presence of *H. pylori* in gastric mucosa biopsies of patients treated for *H. pylori* infection. In this study, patients were identified as harbouring *H. pylori* through culture methods or, if non-culturable, the molecular method, RT-PCR. Scanning electron microscopy (SEM) of biopsies from patients with culturable samples, revealed prevalent spiral forms, nonetheless, co-existant with coccoid forms embedded within a matrix. In non-culturable cases, SEM showed the presence of coccoid clusters in a matrix that was shown to be a biofilms, through the further identification of the *luxS* quorum sensing gene[20]. This study highlighted the importance of *H. pylori* biofilms, the presence of coccoid forms within the biofilm and resistance. Furthermore, it provided insight into the prevalence of coccoid forms in the gastric mucosa. With this is mind, it is important to focus research on the identification of these VBNC coccoid forms, and more importantly, understand the mechanisms behind recalcitrant coccoid states and how they can phenotypically shift into more virulent spiral forms.

The resuscitation of a pathogen in a VBNC state is of great clinical importance, given the extensive dormancy within the host for years before infection recurrence; thus the host is incorrectly diagnosed as infection-free. Therefore it is important to distinguish between viable and culturable pathogens and VBNC states in order understand the mechanism behind reactivation. Such detection methods can include Live/Dead assays and RT-PCR[40,56,57]. There have been several reported factors

that induce resuscitation in a number of pathogenic species of bacteria such as temperature shifts, peptidoglycan hydrolases and the release of human norepinephrine following tissue injury[58].

Earlier studies such as research by Cellini *et al*[59], stressed the importance of evaluating the survival potential of VBNC coccoid *H. pylori*. In this study, *H. pylori* ATCC 43504 was grown *in vitro* until a VBNC coccoid state was achieved, whereby "resuscitation" was then attempted using heat, pH and sonication shock methods. Unfortunately the authors were not confident in whether true resuscitation actually occurred, or whether it was simply a re-growth of undetected culturable cells. Richards *et al*[60] sought to create a modified resuscitation broth containing serum and lysed erythrocytes for *H. pylori* in the VBNC state. The resuscitation of *H. pylori* was recorded and the assessment of a gene involved in growth repression (*cdrA*) showed low expression in resuscitated *H. pylori*. These results show that although the *cdrA* gene is probably not responsible for loss of cultivability in *H. pylori*, the modified broth can be successfully used to resuscitate and therefore explore other possible mechanisms.

THE SURVIVAL AND PERSISTENCE OF *H. PYLORI* AND BIOFILMS

The ability to *H. pylori* to persist as a infectious entity and resist the armoury of antimicrobials employed to eradicate it, is considered to be due to both genetic variability but in addition, the ability of *H. pylori* to form biofilms which significantly aids its survival[15,16,18,61]. The formation of a biofilm by *H. pylori* has been shown to enable its protection from fluctuations in pH due to its ability to over produce EPS[12,62]. Siavoshi *et al*[6] set up a study to identify two mucoid strains of *H. pylori* and compare their growth under aerobic and microaerobic conditions with that of a control *H. pylori* strain. The authors found that the EPS produced by the two strains could serve as a physical barrier to reduce the oxygen diffusion and uptake of antibiotics into the bacterial cell. The EPS aimed to protect them against the increasing levels of oxygen, osmotic stress, acidic pH, host immune system, and antibiotics. The authors concluded that production of EPS by *H. pylori* could be an adaptation mechanism that facilitates bacterial survival and growth. This survival strategy would prevent bacterial removal by the host defence factors and antimicrobial therapy. Furthermore it would aid the persistent and long-term infection of *H. pylori* in the stomach and possibly the environment.

Survival and persistence in the environment

H. pylori in the viable and culturable form has been shown to survive > 10 d, whereas the VBNC coccoid form has been reported to survive for up to 1 year in fresh water[63]. Within distilled water West *et al*[64] reported that *H. pylori* can survive > 14 d, similar to that in saline, and > 7 d in sea water. More recent studies have shown that *H. pylori* can survive in deep ground water[65]. Interestingly numerous studies have reported that *H. pylori* are able to survive within a cultivable state for numerous weeks in water and other natural systems when compared to that of growth in nutrient rich conditions. The adaptation of *H. pylori* in different environments is reported to be intrinsic and consequently this may assist in the survival of the bacterium in the diverse environments outside of the human host. This potential persistence in the environment may not only be due to its ability to form biofilms but also its ability to survive within a community of other microorganisms within a polymicrobial ecosystem. This ability to survive hostile environments is made possible by a number of factors mentioned above but also by the ability of *H. pylori* to produce peptides[15].

An environment that has been reported to aid the survival of *H. pylori,* is that of water or more specifically in reference to public health, potable water - an oligotrophic environment that contrasts significantly to that of the gastric mucosa.

Mackay *et al*[66] and Park *et al*[67] colleagues first provided evidence that biofilms in water distribution systems may harbour *H. pylori*. Within this study *H. pylori* incorporated itself into a laboratory-scale biofilm and persisted for over 8 d. Further to this Bunn *et al*[68] utilised 16S rDNA sequences and provided further evidence that *H. pylori* can survive in biofilms within water. Azevedo *et al*[21] and Bragança *et al*[69], have also shown that *H. pylori* may be present on pipe samples in drinking water systems which remain adhered and grow as biofilms. However, in this study it was found that a lack of recovery using culturable techniques occurred quickly over time indicating that *H. pylori* quickly enters a non-culturable state in more "hostile" environments to that of the gastric mucosa. The survival of *H. pylori* in well water has also been documented, suggesting this is related to the ability of *H. pylori* to integrate into biofilms[69,70]. Substratum material used in conjunction with both domestic and distribution systems are known to be one of the factors affecting the growth of biofilms. Subsequently, Azevedo *et al*[21] showed that *H. pylori* was able to adhere to different plumbing materials. Watson *et al*[57] also demonstrated a close link between *Helicobacter* DNA in showerhead biofilm used in domestic households.

All the research findings above support the concept that water may provide a route for the transmission of *H. pylori* outside of the human host.

Survival and persistence within the host

H. pylori has been detected and isolated from different regions of the human body. These have included gastric biopsies, gastric juice, dental plaque, saliva, bile and faecal matter, indicating its ability to colonise surfaces either transiently or in the case of the gastric mucosa, permanently[2,15,16,71]. The viable spiral-shaped *H. pylori* are referred to as more virulent and therefore infectious whereas the less virulent coccoid form have a reduced

ability to colonise and induce inflammation and disease; an effect that has been observed in animal models[5].

Biofilms are reported to serve as population-level virulence factors. Consequently this will enable the resident bacteria to acquire virulence attributes[25]. Biofilms provide ideal areas for bacterial horizontal gene transfer, which will help the production and provide a source of related strains, but with different antigenic and virulence profiles. Ultimately this will help to confuse the host immune system providing the bacterial community with a means to confuse and overwhelm the host's immune system[72].

Grande *et al*[73] investigated that persistence of *H. pylori* might be associated with genetic variability and biofilm development. The researchers investigated the interaction between two clinical strains of *H. pylori* so they could understand the balance between strains that could co-exist in the same niche to be cooperative/competitive in their colonisation.

Interestingly *H. pylori* are a species that are very genetically diverse. To date it has not been possible to isolate two identical DNA patterns from different hosts[74]. This of course is significant in evading the immune response from the host and consequently will favour the survival of *H. pylori*. Such a difference may explain the long-term colonisation that occurs in some hosts. There is a high level of genetic recombination within biofilms[75]. It is within the biofilm that horizontal gene transfer can occur as evident by high levels of eDNA detected in *H. pylori* biofilms[76]. As the biofilm is highly tolerant to the host's immune response, the availability of eDNA which is evident in the biofilm matrix could then be acquired by other *H. pylori*. This may therefore lead to the development of highly virulent strains of *H. pylori* in the host leading to their persistence.

ROLE OF BIOFILMS IN DISSEMINATION AND DISPERSAL OF *H. PYLORI* IN THE NATURAL ENVIRONMENT AND THE HOST

The dissemination of *H. pylori* is thought to occur through person-to-person contact but it is now also evident as demonstrated above, that *H. pylori* may also reside in drinking water systems. Whether in planktonic or biofilm form, albeit in the human stomach or external water supplies; the spread of this bacterium in such adverse environments is inevitable. With *in vitro* evidence of *H. pylori* residing in these environments in biofilm form, it is important to contemplate another method of dissemination. Not only do biofilms demonstrate increased resistance towards antimicrobials; biofilms possess another mechanism that greatly impacts upon transmission and dissemination within the host. "Dispersal" is a mechanism whereby members of the microbial community within a biofilm, detach and attach to new surfaces, effectively colonising a new site[77]. It is highly possible that dispersal has great impact on the dissemination of *H. pylori* not only within the host but also in the external environment, increasing the likelihood of transmission.

Dispersal can be described in three stages; the first being the detachment of bacterial cells from the biofilm, followed by the translocation of cells to a new site and finally the attachment of these cells to the new surface[77]. Given the adverse and hostile environment both outside and within the host, the dispersal of *H. pylori* may seem like an unavoidable process. However, in many microbial biofilms, dispersal is thought to be a carefully controlled mechanism. Bacterial cells that reach the end of their biofilm life cycle become differentiated and highly motile. These dispersal cells are specialised in that they are regulated by the intracellular molecule cyclic-di-GMP (c-di-GMP). In general, it is thought that a reduction in c-di-GMP leads to dispersal. Furthermore, genes that are associated with motility such as the flagellum are up-regulated[78].

In terms of *H. pylori* dispersal within biofilms, research to support this mechanism in both the environment and in the human body is lacking. Evidence that does indicate that this is a likely occurrence in *H. pylori* biofilms relate to that of *H. pylori* motility within biofilms.

It has been known for over a decade now that motility is essential for the survival and successful colonisation of *H. pylori* within the host[79].

As mentioned earlier, research by Rader *et al*[47] showed that the presence of AI-2 quorum sensing molecules that can be synthesised by *H. pylori*, act as a chemorepellent, affecting motility. Therefore the formation of *H. pylori* biofilms within the host and in the environment, whereby quorum sensing is likely to occur, may encourage the dispersal of cells from the biofilm and thus new sites of infection.

H. PYLORI ERADICATION

Environmental eradication of H. pylori

Early research by Baker *et al*[80] has shown that *H. pylori* demonstrates resistance to low dosages of free chlorine that ordinarily kill the coliforms such as *Escherichia coli*. Consequently areas in water distribution systems may not prevent the entry and potential proliferation of *H. pylori* in water. Further studies by Mazari-Hiriart *et al*[81] and Moreno *et al*[82] have demonstrated that drinking water treatments employed to date may be ineffective particularly when *H. pylori* are present in the coccoid shape, which is a well known VBNC and a potentially infective state of *H. pylori*.

Baker *et al*[80] (2002) and Johnson *et al*[83] (1997) demonstrated that *H. pylori* is inactivated by chlorine. However, their studies and conclusions did not recover culturable cells but reported only on the VBNC state. A more recent study by Moreno *et al*[82] also demonstrated the survival of *H. pylori* but again only in the VBNC

state. Unfortunately all these studies did not take into account the survival and association of *H. pylori* in biofilms and the tolerance when grown as part of a biofilm[84]. A later study by Gião *et al*[85] demonstrated that viable *H. pylori* can survive in the viable state in biofilms. The efficacy of chlorine treatment on a biofilm that contained this bacterium was investigated further. In later studies, Gião *et al*[85] found that using a specific peptide nucleic acid (PNA) probe it could be demonstrated that *H. pylori* persist inside biofilms that had been exposed to chlorine at 0.2 and 1.2 mg/L. This occurred for at least 26 d. In this study, no culturable cells were recovered. However when viability stains were employed *H. pylori* was observed suggesting that it could survive within a biofilm at this concentration of chlorine[86].

If *H. pylori* are disseminated into the water cycle and allowing them to enter water distribution systems, it is possible that routinely used water treatment methods and disinfectants presently employed may not be as effective as once thought. This seems to be due to the ability of *H. pylori* to survive within a biofilm.

Eradication within the host

The first-line therapy for the eradication of *H. pylori* involves the combination of a proton pump inhibitor in conjunction with either clarithromycin (CLR) or metronidazole, and amoxicillin[87-89]. The antibiotic CLR is a macrolide antibiotic that is known to bind to the 50S subunit of the bacterial ribosome and thereby inhibiting the translation of peptides, leading to the inhibition of growth. However, of growing significance to *H. pylori* eradication is the increasing problem of CLR-resistance[88-92].

H. PYLORI RESISTANCE WITHIN BIOFILMS

There are growing reports regarding the resistance of *H. pylori* to clarithromycin, the common antibiotic which is used in its eradication in the human host[88]. The occurrence of CLR resistant *H. pylori* is very common with ranges being reported between 10% to 30%[93,94]. The basis of resistance is a point mutation in the domain V loop of the 23S rRNA gene (commonly an adenine-to-guanine transition at position 2142 or 2143)[88,90-96].

Furthermore Yonezawa *et al*[97] investigated the effects of *H. pylori* biofilm formation *in vitro* on clarithromycin (CLR) susceptibility. Within this study CLR susceptibility of intermediate (2-d) and mature (3-d) *H. pylori* biofilms on glass coverslips was determined. Concentrations of CLR applied to the biofilm ranged from 0.03 to 0.5 mg/mL. It was found that the biomass of the *H. pylori* biofilm increased after treatment with CLR at minimum inhibitory concentration levels by up to 4-fold (2-d biofilm) and 16-fold (3-d biofilm). In addition to this the minimum bactericidal concentrations of CLR against cells in a biofilm was higher (1.0 mg/mL) for the biofilm-grown cells when compared with the planktonic cells (0.25 mg/mL). Furthermore the expression of efflux pump genes significantly increased in the biofilm cells. Overall, this study demonstrated that *H. pylori* biofilm formation decreases the susceptibility to CLR. In addition it was found that *H. pylori* CLR resistance mutations were generated more frequently in biofilms than in planktonic cells. *H. pylori* has numerous constitutive genes which may help to rapidly neutralise oxidative antimicrobials. The rapid expression of constitutive enzymes may help to assist the survival of *H. pylori* in the environment. A survival strategy is the formation of coccoid phenotype.

CONCLUSION

The ability to grow and proliferate within a biofilm is significant to the longevity, survival and also dissemination of *H. pylori*. Growth within a biofilm is a significant risk factor in both its eradication and treatment and therefore its persistence both within the host and the environment. Within this state, its recalcitrance is enhanced and its ability to acquire genes enhancing virulence is evident. This adaptation is effective for its survival, genetic variability and persistence. The characteristics of *H. pylori* provide evidence of survival in the environment and therefore acquisition is heightened. It is well known that *H. pylori* in stressful environments convert from the virulent infectious spiral phenotype to that of the less virulent VBNC coccoid state. It is within this VBNC coccoid state that *H. pylori* is thought to reside within biofilms. Biofilms have been associated with persistent infections and increased resistance to antimicrobial action. Thus, the ability of *H. pylori* to resuscitate and revert from the coccoid to spiral form is a mechanism that requires attention in terms understanding the factors that may lead to infection recurrence both in the host and the external environment.

The dissemination of *H. pylori* is significant in its acquisition by the host. Person-to-person transmission is a strong risk factor. However, there is more evidence growing following an initial report in early 2000, that contaminated water may be an important conduit for dissemination and acquisition. However the lack of evidence relating to the presence of *H. pylori*, particularly in biofilm form, in the environment is apparent and may be due to the transformation of *H. pylori* from culturable spiral form to the VBNC coccoid form. The detection methods used to identify *H. pylori*, particularly in the VBNC coccoid state, need to be refined if successful identification of this microorganism is to be made.

The biofilm-forming potential of *H. pylori* means that eradication both within the host and the environment, is significantly reduced, which justifies the need to refine and develop treatment regimes and strategies that are more appropriate and effective than traditional therapies that have high failures rates in eradicating *H. pylori*. In the environment, present evidence suggests that traditionally used disinfectants are effective on planktonic *H.*

pylori but little evidence exists on the effectiveness of antimicrobials on *H. pylori* in environmental biofilms. This environment, be it potable water biofilms or biofilms in hot water systems in domestic houses, may be a possible reservoir for *H. pylori* and aid in its transmission and dissemination.

Appropriate anti-biofilm agents are therefore required to ensure that in the host, *H. pylori* can be eradicated fully and continuing dissemination does not occur.

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