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Review

Helicobacter pylori infection in Africa: Pathology and microbiological diagnosis

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Helicobacter pylori **is a microaerophilic motile curve rod that inhabits the gastric mucosa of the human stomach. The organism chronically infects billions of people worldwide and is one of the most genetically diverse of bacterial species. Infection with the bacterium which leads to chronic gastritis, peptic ulceration, gastric cancers and gastric MALT lymphoma has been reported to follow a pattern linked to geographic and socio-demographic factors. However; the infection rate in various populations does not parallel the incidence of morbidity caused by the infection. This has been termed by a number of authors as the 'African enigma' based on an apparently low incidence of gastric carcinoma and other *H. pylori*-associated morbidities in the continent of Africa. There are various techniques employed to detect *H. pylori* from specimens. These tests may be invasive or non-invasive. Endoscopy and gastric mucosal biopsy, microscopic examination of histological sections, PCR and rapid urease test are forms of invasive test that could be used. Non-invasive tests such as Urea Breath Test (UBT) make use of the ability of the organism to produce urease; enzyme linked immunosorbent Assay (ELISA), *H. pylori* stool antigen test, and latex agglutination tests are important non-invasive serological approaches employed to detect the presence of antibody or antigen from a specimen. *H. pylori* is a very fastidious bacterium. Restraint should therefore be exercised to allow for efficient performance of some of these techniques.**

Key words: *Helicobacter pylori*, prevalence, Africa, pathology, microbiological diagnosis.

INTRODUCTION

Helicobacter pylori is a helical shaped, gram-negative, microaerophilic bacterium (2 – 4 µm long with diameter of 0.5 µm) that infects the stomach and duodenum of humans (Sasaki et al., 1999); and has been recognized as a class I carcinogen (Sasaki et al., 1999). They were initially noticed as clinically important by Robin Warren and Barry Marshall from the human gastric mucosa in 1982; and the demonstration of its involvement in gastro-duodenal pathologies has radically changed peoples' perception of these diseases (Marshall and Warren, 1983). *Helicobacter* species infect the gastrointestinal tracts of several animals, including humans. Two *species* known to infect humans are *Helicobacter felis* and *H. pylori* (Fritz et al., 2006).

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Infection with the organism causes peptic ulcers, gastritis, duodenitis, and gastric cancers (Figueroa et al., 2002; Ahmed et al., 2007). More than half of the world's population in both developed and developing countries are infected with this organism (Ahmed et al., 2007). Infection usually occurs without overt clinical symptoms, particularly in poor communities (Figueroa et al., 2002). If untreated, the infection can last for decades without causing symptoms. It is not known how *H. pylori* initially enters the stomach; however it is suspected to enter by being ingested with food and contaminated water which physically shield it from stomach acid (Tran et al., 2008).

Infection with the organism has been shown to follow geographic and socio-demographic distribution (Ndip et al., 2004; Asrat et al., 2004). Interestingly however; the infection rate in various populations does not parallel the incidence of morbidity caused by the infection. This has been termed by a number of authors as the 'African enigma' based on an apparently low incidence of gastric

carcinoma and other *H*. *pylori*-associated morbidities in the continent of Africa (Ahmed et al., 2007).

The organism attaches on the surface of the gastric epithelium and produces urease that converts urea into ammonia and bicarbonate (Urea + $H_2O \rightarrow CO_2 + NH_3$) (McNamara and El-Omar, 2008). These end products form a protective acid resistance cloud around the bacterium allowing *H*. *pylori* to flourish. Also as a result of bacterial infection, the epithelial cell releases cytokines which recruit inflammatory cells to the mucosa. These inflammatory cells consist of leukocytes that gather in the Lumina propia causing gastritis (Konturek et al., 2000). Environmental factors are not unique in determining the clinical impact of *H*. *pylori* infection in a given population, as the host's immune status and some virulence characteristics of the infecting strains appear to influence the severity of clinical symptoms (Figueroa et al., 2002).

H. *pylori* transmission remains poorly understood, however it is believed to spread from person to person through oral-to oral or feacal-to-oral routes. Such observations suggest that these infections may be associated with low socioeconomic status and overcrowded living conditions (Delport et al., 2006). Studies have documented a higher prevalence in Africa (Asrat et al., 2004; Ndip et al., 2004; Ndip et al., 2008). The organism is the main cause of at least 90% of duodenal ulcers and 70% of gastric ulcers (Ndip et al., 2008). Palmer et al. (1994) and Ndip et al. (2008) documented the involvement of *H*. *pylori* in gastric patients in Cameroon, similar to that recorded by Baako and Darko (1996) in Ghanian patients with dyspeptic symptoms.

Diagnosis of *H*. *pylori* can be made by both invasive and noninvasive tests. Invasive tests, including histology, culture, polymerase chain reaction (PCR) and rapid urease test, require endoscopy to obtain biopsies of the gastric mucosa.

This review highlights *H*. *pylori* infection in Africa and approaches currently employed for laboratory diagnosis.

PATHOGENESIS

The bacteria are genetically versatile, diverse and adapt readily to stress conditions such as gastric acid, which kills most other bacteria entering the stomach with food or contaminated water (Konturek et al., 2000). *H*. *pylori* are specifically adapted to colonise and survive in the hostile acidic gastric environment. Their ability to adhere, invade, evade host defences and cause tissue damage is largely due to their production of colonization and virulence factors. It has been suggested that up to 95% of duodenal and 70% of gastric ulcers are attributable to infection by this pathogen and most cases occur in middle aged subjects (Rothenbacher, 2007). The increased risk of infection is especially high among those living in the developing world due to precarious hygiene standards, crowded households and deficient sanitation associated with this part of the world (Ahmed et al., 2007).

The organism possesses several virulence factors which are cytotoxin, flagella, minicase, lipopolysaccharides, adhesin, urease, *vacA* and *cagA* (Horiuchi et al., 2001). The ability of many strains to adhere to sialylated glycoconjugates expressed during chronic inflammation contributes to virulence and the extraordinary chronicity of infection (Tahereh et al., 2005). However, the clinical outcome of long-term infection is variable and is considered to relate both to bacterial virulence factors (Wang et al., 2003; Datta et al., 2003; Monica et al., 2006) and host genotype. The vacuolating cytotoxin *vacA* (Ye et al., 2000) and the *cag* pathogenicity island (Atherton, 1997; Segal et al., 2001: Bravo et al., 2003) are two identified virulence factors that are considered to have an important role in the pathogenesis of *H*. *pylori* infection. *VacA* gene comprises two variable regions, the s region which exists as an s1a, s1b, s1c, or s2 allele, and the m region, which occurs as an m1, m2a, or m2b allele (Figueiredo et al., 2000). *H*. *pylori vacA* type s1 strains appear to be more virulent than type s2 strains and are associated with higher risks for peptic ulcer disease, gastric atrophy, and gastric carcinoma (Figueiredo et al., 2000). The *vacA* s1 and *vacA* m1 strains are also strongly associated with a higher degree of inflammation and epithelial damage in the gastric mucosa. The intragastric distribution and severity of the chronic inflammatory process depends on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production (Kusters et al., 2006).

Although the relation between *H*. *pylori* infection and gastric cancer has been well-established, some studies have shown a negative correlation of *H*. *pylori* infection and development of gastric cancer in Africa. The high prevalence of infection, contrary with low rate of development into gastric cancer, well expressed as 'African enigma', is an ambiguity because the headway to atrophic gastritis in the African population does not differ from that reported in other regions (Segal et al., 2001). The variants of the interferon-c gene (IFNGR1), which are more prevalent in Africans appear to play a significant role in the infection of human host contributing to a high prevalence even-though there is relatively low pathogenecity in Africa (Thye et al., 2003; Ndip et al., 2004). In an Ethiopian population, molecular analysis demonstrated that more than 80% of Ethiopian *H*. *pylori* strains harboured both *vacA* and *cagA* genes (Asrat et al., 2004). In a Sowetan study of asymptomatic children aged 6 - 15, 86.5% were infected with a *vacA* positive strain and 87% with a *cagA*-positive strain. The majority of strains carried *vacA* s1 allele while most *cag*-negative carried the *vacA* s2 allele (Alley et al., 1999). In a sepa-rate study, *vacA* diversity was demonstrated among South African *H*. *pylori* strains, interestingly, no strains

with the *vacA* s1a genotype were found among *H*. *pylori* isolates from black or mixed-race South Africans (Letley et al., 1999).

Flagella and urease allow the organism to escape the

stomach defence mechanism. Its helicoidal shape and the action of flagella allow it to cross the thick layer of mucus lining of the stomach. It then binds to lewis antigens present on host gastric cells, and secrete factors that attract and stimulate inflammatory cells, as well as the multifunctional toxin *vacA* (Tamer et al., 2007). The urease enzyme is an important factor because it helps the bacterium to escape the gastric acidic pH of the stomach by forming an external cloud that protects it (Bravo et al., 2003).

In addition, surface-bound urease that catalyzes the hydrolysis of urea to ammonia and carbon dioxide allows the microbe to 'neutralize' its microenvironment. The amount of urease produced by the bacterium varies with culture conditions and may reach as much as 10% of total bacterial protein. The essential role of urease as a virulence factor is shown by the fact that urease-defective *H*. *pylori* mutants cannot colonize the stomach. However, urea is toxic to the bacterium at neutral pH because an unfavourable alkaline environment is generated. Therefore, the urea channel is regulated positively by protons, opening at acidic pH values to allow more urea in to buffer cytosolic and surface pH, and closing at neutral pH to avoid over-alkalinization (McNamara and El-Omar, 2008). The role of urease in the pathogenesis of *H*. *pylori* associated diseases is not limited to colonization as ammonia produced by the urease enters the *H*. *pylori* nitrogen metabolism and is eventually incorporated into proteins. Urease might also help to recruit neutrophils and monocytes in the inflamed mucosa and to activate production of proinflamatory cytokines. Moreover, urease is one of the main antigens recognized by the human immune response to *H*. *pylori* although the extent and nature of this immune response after infection is not fully clear (Cardenas et al., 2006).

CLINICAL MANIFESTATIONS

It is well known that gastric mucosa is the main target of *H*. *pylori*, which colonizes and damages surface epithelium and induces a chronic inflammatory response in the lamina propria. Gland atrophy and intestinal metaplasia are among the long-term consequences of this process. In some cases, this organism elicits antibodies cross-reacting with epithelial components of the gastric mucosa, periglandular T-cell infiltrates, and increased glandular cell apoptosis, which may cause diffuse, corpus-fundus restricted, atrophic gastritis of autoimmune type (Larizza et al., 2005). Among the clinical consequences of this *H*. *pylori*-initiated autoimmune process are achlorhydria with secondary hypergastrinemia, with or without pernicious anemia, and increased risk for gastric enterochromaffin-like cell carcinoids and cancer (Larizza et al., 2005).

The organism is considered as an important human pathogen causing gastritis, duodenal ulceration, proved to be associated with gastric lymphoma and seems to be a risk factor for adenocarcinoma of the stomach (Sommer et al., 1998). Acute infection with *H*. *pylori* may cause a transient clinical illness, characterized by nausea and abdominal pain that may last for several days. After these symptoms resolve, the majority of people progress to chronic infection. However there is no recognizable symptom complex or syndrome that can be ascribed to chronic gastritis, whether or not due to *H*. *pylori*. Peptic ulcers often present with dyspepsia. There may be pain at night; some patients report relief of pain with food or antacids and a recurrence of pain in two to four hours (Graham and Shiotani, 2005).

Once a person is infected, it can persist in the stomach for decades despite a systemic immune response. The reasons for the failure of the immune system to control infection may be attributed to the fact that *H*. *pylori* produces chemical components in their cell walls that are very much like molecules made by the stomach cells of the host. This creates a problem for the immune system, because it is designed to ignore molecules made by the host (self) and to recognize molecules produced by infectious agents (non-self). Once the immune system recognizes foreign molecules, it directs a vigorous attack that ordinarily destroys the foreign cells. To a certain extent, this mimicry disguises *Helicobacter* from the immune system so that the immune response is attenuated. This is thought as a probable reason why most people infected with *H*. *pylori* can't get rid of it, but instead remain infected for life unless they are treated with antibiotics. However, the disguise is not perfect (Lynch, 2007).

Respective immunological studies of systemic and local T- and B-cell responses in patients chronically infected with *H*. *pylori* have shown that the numbers of $CD4^+$ and $CD8^+$ T cells are increased in the gastric mucosa of patients with gastritis and that T cells isolated from the antrum region are able to produce gamma interferon (Appelmelk et al., 1997; Haeberle et al., 1997). Studies have revealed that infection by more virulent *cagA*-positive strain is significantly associated with ischemic heart disease. These findings strongly suggest that the association between *H*. *pylori* and ischemic heart disease is related to the virulence of this bacterium (Pasceri et al., 1998).

TRANSMISSION AND PREVALENCE IN AFRICA

H. *pylori*, the principal species of the genus *Helicobacter*, is a common human pathogen, which is responsible for a variety of gastroduodenal pathologies in the developing world. Infections have been reported to be higher in the developing countries, especially in Africa.

Epidemiologic studies have addressed a variety of factors such as bacterial host, genetic and environmental factors to determine the causative links to *H*. *pylori* infection, but knowledge of reservoirs and transmission still remains elusive (Thomas et al., 1992; Asrat et al.,

2004; Ndip et al., 2004). There appears to be a substantial reservoir of the organism aside from the human stomach. Other animals, e.g. cats, harbour organisms that resemble *H*. *pylori* (Dubois et al., 1999) but under particular circumstance (Fox et al., 1995). Thus the major question of transmission is how *H*. *pylori* is transmitted from the stomach of one person to that of another.

The first and least common is Iatrogenic, in which tubes, endoscopes or specimens in contact with the gastric mucosa from one person are introduced to another person (Akamatsu et al., 1996). Improved disinfection of endoscopes has reduced the incidence of transmission (Tytgat, 1995). Interestingly, endoscopists, especially those who do not wear gloves during procedures, are at risk of becoming infected (Mitchell et al., 1989). Occupationally acquired infections also have been reported (Sobala et al., 1991).

Faecal-oral transmission is perhaps the most important. Although *H*. *pylori* has been isolated from the faeces of young children infected with the organism (Thomas et al., 1992), faecal isolation is not common; this could indicate that shedding is intermittent. Faecally contaminated water maybe a source of infection (Klein et al., 1991), but the organism has not been isolated from water. Food borne transmission has not been substantiated. Transmission probably occurs mostly by the faecal- oral and oral- oral routes and via recently contaminated food and water and unclean hands (Kersulyte et al., 1999). Oral-oral transmission has been identified in the case of African women who premasticate foods given to their infants (Mégraud, 1995). There is no identified association of infection with sexual transmission (Perez-perez et al., 1991); therefore, if it occurs, it is uncommon. Transmission via aspiration of the organism from vomitus is another possibility but has not been documented (Williams et al., 1999).

Serological studies conducted in different parts in Africa have shown that a majority of subjects are infected with the organism (61 - 100%), having antibodies for the most of their lives (Holcombe, 1992). However, seropositivity increases with age at a rate of 0.3 - 1% per year. The prevalence of infection varies both among countries and within different racial groups present within the same country. The organism is ubiquitous with acquisition in childhood being the rule. Generally, more than 50% of children are infected by the age of 10 years, the prevalence rising to more than 80% in adults (Segal et al., 2001). A high prevalence of the infection was reported by Nabwera et al. *(*2000) among Kenyan school children aged 3 – 5 years, suggesting that most acquisition occur before the age of 3 years. In Tunisia, 21% of children less than 5 years of age are infected, with an increase to 69% in children above 6 years of age (Maherzi et al., 2003). In another study in Tunisia, high colonization rates were recorded among asymptomatic

individuals (Ben-Ammar et al., 2003). In Ivory Coast, 55% of children aged less than 10 years have been reported to be infected. In Cameroon, Nigeria and the Gambia, 50% of children less than 5 years are infected (Segal et al., 2001; Ndip et al., 2004). In South Africa, Pelsar et al. (1997) documented a high prevalence (67 - 84%) of *H*. *pylori* antibodies in children in Bloemfontein, while Mosane et al. (2004) also documented *H*. *pylori* IgG antibodies in South African mothers and their children. Mbengue et al. (1997) reported a high prevalence (82.8%) of infection among Senegalese people. Recently, Samie et al. (2007) reported an *H*. *pylori* prevalence of 50.6% in their study in Venda, North of South Africa. In the Democratic Republic of Congo, 62.4% of participants tested positive for *H*. *pylori* antibody (Longo-Mbenza et al., 2007) while a prevalence of 93% was detected in Ethiopia (Henriksen et al., 1999). These are in support to the evidence of intrafamilial transmission and suggest that improvement of living conditions would protect against infections (Aguemon et al., 2005). This difference in the rate of childhood acquisition of infection is probably responsible for the differences in prevalence of infection observed between developed and developing countries (Segal et al., 2001).

The high prevalence is an indication that public health intervention needs to be developed. Variation in the prevalence of infection between and among populations suggest that parameters such as age, cultural background, genetic predisposition, socio-economic status and environmental factors all play a role in the acquisition and transmission of the organism. Within countries, there may be a similarly wide variation in the prevalence between more affluent urban populations and rural population. A lack of proper sensitization, drinking water and basic hygiene as well as poor diet and overcrowding all play a role in determining the overall prevalence of infection (Ndip et al., 2004).

MICROBIOLOGICAL DIAGNOSIS

There are various techniques of detecting *H*. *pylori* from specimens. These tests may be invasive or non-invasive (Shepherd et al., 2000). Endoscopy and gastric mucosal biopsy, microscopic examination of histological sections and rapid urease test are forms of invasive test that could be used (Stromar et al., 2008). Non-invasive tests such as Urea Breath Test (UBT) make use of the ability of the organism to produce urease; enzyme linked immunosorbent assay (ELISA), *H*. *pylori* stool antigen test (HpSTAR and HpSA), and latex agglutination tests are important non-invasive serological approaches employed to detect the presence of antibody or antigen from a specimen (Krogfelt et al., 2005). Detection of the antigen however gives a more precise result considering the waning nature of antibodies especially after an infection.

INVASIVE TESTS

Histology

Histology can reveal the presence of bacteria as well as

the type of inflammation. Many stains can be used to detect the organism, for example Warthine-Starry, Hp silver stain, Dieterle, Giemsa, Giminez, acridine orange, McMullen and immunostaining (Gatta et al., 2003; Ndip et al., 2003). Haematoxylin and eosin are two stains that are normally used to evaluate the inflammatory cells and the Giemsa or Genta stain are used to detect the organism. Giemsa stain is most preferred because of its technical simplicity, high sensitivity and low cost (Gatta et al., 2003).

Biopsy specimens are fixed in formalin embedded in paraffin and stained with hematoxylin–eosin for histological examination under a light microscope (Kel et al., 2002). The organism is recognised by its appearance as a short, curved or spiral bacillus resting on the epithelial surface or in the mucus layer (Gatta et al., 2003). The average time for a histological diagnosis is 2 - 3 days, however, this increases when multiple biopsies are taken, which also increases the processing costs of the biopsies and the overall costs of the diagnosis (Kel et al., 2002).

Culture

Collection and transport of biopsy specimens

Helicobacter pylori can be cultured from gastric biopsies obtained during endoscopy (Kel et al., 2002; Ndip et al., 2003; Ndip et al., 2007a; Ndip et al., 2008). Antibiotics and bismuth, when used in suboptimal therapy, can suppress but not eliminate the organism. Therefore, the patient should not have used these agents for several weeks prior to culture. Following inadequate therapy, the organism may re-grow in a patchy manner and may not be detected by random biopsies. Care must be taken to ensure that patients do not receive antibiotics or anti-secretory drugs, especially proton pump inhibitors (PPI), omeprazole, as this drug has been shown to inhibit growth of the organism. Although PPI have no direct antimicrobial effect at the concentration present in the gastric mucosa (Mégraud et al., 1991), they indirectly interfere with their distribution in the stomach by changing the pH of its niche, leading to its disappearance in the antrum (Mégraud et al., 1991).

The number of biopsies necessary to diagnose infection is a subject of controversy. A single biopsy specimen taken from the antrum (2 cm from the pylorus) gives good sensitivity but is not sufficient for a reliable diagnosis. Indeed, *H*. *pylori* may have a patchy distribution, and the more biopsy specimens analyzed, the higher the chance of detection (Ndip et al., 2007b; Ndip et al., 2008). The recommendation is, therefore, to take two biopsy specimens from the antrum as well as two specimens each from the anterior and posterior corpus. Biopsy specimens for culture must be taken before specimens for histological examination; the latter being introduced in a fixative, otherwise there is a risk of transferring small amounts of fixative to the container for biopsy specimens to be used for culture (Mégraud and Lehours, 2007).

H. *pylori* is a fragile organism. It must be protected from desiccation and contact with oxygen and room temperature. It is mandatory not to expose the biopsy specimens to air and to place them either in a saline solution for short-term transport (4 h maximum) or in a transport medium, usually consisting of semisolid agar, maintained at 4°C (Meunier et al., 1997). A commercially available medium, Portagerm pylori (BioMérieux, Marcy l'Etoile, France), is effective for this purpose and culture may be delayed by 24 h if such transport media are used. Brucella broth (Difco, Detroit, Mich.) supplemented with 5% lysed horse blood (BBLH), phosphate-buffered saline (PBS), 20% glucose (GLUC), Stuart medium (Oxoid, Basingstoke, United Kingdom), and PBS with 10% Fildes enrichment (PBS-F; Oxoid) are mostly used transport media within 24 h (Ndip et al., 2003). Recovery of *H*. *pylori* was 100% when the organism was suspended (10^6 CFU/ml) in brain heart infusion with 10% horse serum and storage at room temperature for 24 h. Generally, it is emphasized that transport at a relatively low temperature is essential for obtaining a satisfactory recovery of the organism from gastric tissue specimens (Ndip et al., 2003). If these transport conditions cannot be used, it is better to freeze the biopsy specimens at –70°C or in liquid nitrogen in a dry tube before transported to the laboratory. Storage at 4°C in a medium containing 20% glycerol also led to *H*. *pylori* recovery in 81% of the biopsy specimens tested (Han et al., 1995; Grove et al., 2000).

Isolation of the organism

Grinding of biopsy is the first and mandatory step in *H*. *pylori* isolation. In most instances, the bacteria are not distributed homogeneously in the biopsy specimens. Therefore, if they are only streaked on plates, several contiguous organisms will lead to one colony, whereas if the same bacteria were dispersed, several colonies would appear. Comparison of culture performed with or without grinding showed a higher number of colonies after grinding, although the number of positive specimens did not change (Goodwin et al., 1985; Ndip et al., 2008).

It is usually recommended to use an electrical/ mechanical grinder and a small volume of broth (Ndip et al., 2003). Care must be taken to thoroughly wash and sterilize the probes. This solution avoids the risk of possible DNA contamination when performing molecular techniques (Mégraud and Lehours, 2007).

The media components should include an agar base, growth supplements, and selective supplements. Most agar bases e.g., brain heart infusion agar, Columbia agar, and Wilkins Chalgren agar are satisfactory for growing *H*. *pylori* (Ndip et al., 2003, 2008). For growth supplement, it is mandatory to add blood or serum, which includes numerous nutrients (vitamins and oligoelements, etc.) that enhances growth. The proportion of blood or serum can be 5, 7, or preferably, 10%. Red blood cells

can be lysed for these growth substances to be more readily available. Animal blood, e.g., sheep and horse blood, can be added. Different selective supplements containing antimicrobial compounds have been proposed: vancomycin or teicoplanin to inhibit gram-positive cocci; polymyxin, nalidixic acid, colistin, trimethoprim, or cefsulodin to inhibit gram-negative rods; and nystatin or amphotericin B to inhibit fungi. The Dent supplement, a modification of Skirrow's formula in which cefsulodin replaces polymyxin and amphotericin B is added, is commercially available (Ndip et al 2003; Mégraud and Lehours, 2007).

Phenotypical growth of small, circular, smooth colonies observed after 3 to 4 days on the selective media plated with gastric biopsy specimens is an important criterion for *H*. *pylori* identification. No hemolytic activity is readily observed but may appear after a few days at 4°C. The typical morphology of *H*. *pylori* in biopsy specimens is a comma or S-shaped bacillus (2.5 to 4 µm long and 0.5 to 1 µm thick) or bacilli which are neither spiral shaped nor motile, but straight or curved (Ndip et al., 2003; Benaissa et al., 1996; Kusters et al., 1997). When nutrients are lacking, *H*. *pylori* loses its spiral shape and becomes progressively coccoidal. Most people believe that these forms are both non-culturable and nonviable. However, others claim that some of them may be viable but non-culturable and constitute a resistant form of the bacterium (Benaissa et al., 1996; Kusters et al., 1997).

There are limits to this technique (cultural use of biopsy). First, it is necessary to obtain good quality biopsy specimens, which is not always possible, and from a series of patients, it is common to have biopsy specimens where few epithelial surfaces can be observed. Second, when there is a low number of bacteria present, and in the event that they do not have a typical morphology, it is difficult to draw a conclusion.

H. *pylori* is difficult to maintain. Colonies can survive on plates for a week provided they are kept in a microaerobic atmosphere at 4°C. For long-term preservation, bacteria must be frozen at a low temperature (–70°C freezer or liquid nitrogen). Different broth media have been used, always with a cryoprotective agent, such as glycerol. The freezing-thawing process is always deadly for the bacteria, and only a small proportion survives. For this reason, it is mandatory to use bacteria in their exponential growth phase, as they are more likely to survive. Frozen *H*. *pylori* specimens can be maintained for decades at –70°C (Spengler et al., 1992; Ndip et al., 2003).

Biochemical characterization

The identification of cultured bacteria consists essentially of testing for the presence of certain enzymes: cytochrome oxidase, catalase, and urease (Konturek, 2004; Samie et al., 2007) and eventually {gamma}-glutamyl transpeptidase, leucine aminopeptidase, and alkaline phosphatase. Aminopeptidases and esterases (C4 to C12) are also present. The family Helicobacteraceae is comprised of the genera *Helicobacter* and *Wolinella*. Helicobacteraceae and Campylobacteraceae are in the Epsilonproteobacteria, according to the latest edition of Bergey's Manual. Cytochrome oxidase is present in all members of the Epsilonproteobacteria. It is usually detected with special reagents on a disk or a strip. Catalase is also present in all Helicobacters and most members of the Campylobacteraceae and is detected by introducing a loopful of bacteria into a drop of hydrogen peroxide and observing a very abundant pro-duction of bubbles. Nevertheless, catalase-negative mutants of *H*. *pylori* have been reported (Westblom et al., 1992).

Urease is definitely the most important enzyme for identification. To survive in its particular ecological niche, *H*. *pylori* produces large amounts of this enzyme to buffer the acidic medium and creates a micro-environment. When a loopful of the organism is put in contact with a few drops of urease medium, a color change occurs instantaneously regardless of the formulation. Other diagnostic tests are indeed either strictly based on urease, like the rapid urease test and urea breath test, or partially based, like serology and PCR, which may target urease genes (Mégraud and Lehours, 2007).

Mobley et al. (1988) reported that in *H*. *pylori*, 6% of the total protein content is urease which also has the highest specific activity (36 ± 28 µmol/min/mg of protein) among bacterial ureases. Other urease-positive bacteria present in the gastric mucosa, that is, streptococci and staphylococci, produce lower amounts of urease, which does not interfere in a short-time detection (<2 h), rendering the method specific to *H*. *pylori*. When a biopsy specimen containing *H*. *pylori* is introduced into a urea-rich medium, the urease breaks the urea down into carbon dioxide and ammonia. The ammonium ion increases the pH, and a pH indicator, e.g., phenol red, changes color, in this case from yellow to red or violet (Butcher et al., 1992; Mégraud and Lehours, 2007).

Different urease media are commonly utilized in bacteriology e.g., Christensen medium and urea-indole medium, but specific media are preferable. Different ways to improve the sensitivity of the tests include:

i.) Incubating at a higher temperature (37°C)
ii.) Suppression of the buffer and
iii.) Increasing the urea concentration from 2 to 10% (Boyanova et al., 1996; Laine et al., 1996; Yousfi et al., 1996).

The agar-based tests exhibit a good sensitivity only after 24 h (90 to 95%), compared to 70 to 80% after 1 h. Many urease tests are commercially available, including gel-based tests (CLOtest, HpFast), paper-based tests (PyloriTek, ProntoDry, HpOne) and liquid-based tests (CPtest, EndoscHp). These tests give results in 1 - 24 h, depending in part on the format of the test and the num-

ber of *Helicobacter* in the biopsy specimen (Kaklikkaya et al., 2006).

Helicobacter heilmannii, which may be present in the stomach, is also a urease-positive bacterium but may not give a quick positive result, as the bacterial load is often limited. Therefore, the distinction between *H*. *heilmannii* and *H*. *pylori* is made by histological examination (Butcher et al., 1992; Mégraud and Lehours, 2007).

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) has increasingly been described as the latest gold standard for detecting some microbes. PCR is the most commonly used nucleic acid amplification technique for the diagnosis of infectious disease, surpassing the probe and signal amplification methods (Mackay et al., 2003; Bresson et al., 2006). PCR has been used extensively for the diagnosis of *H*. *pylori* from gastric biopsy specimens, saliva, faeces and archival specimens (Smith et al., 2002; Kidd et al., 2002; Samie et al., 2007). PCR yields information on the presence of potential virulence markers in the strain, which might have implications for the development of severe disease or efficacy of eradication. Different primers have been utilised and some have been developed into commercial kits. Different loci have been used as target for amplification: 16S rRNA; A-, B- and C-urease; *flaA; cagA; vacA* and heat-shock protein (hsp). Real-time PCR results can be obtained using light-cycle technology (Racci et al., 2007).

The disadvantages of PCR as a routine test are that it is a technically demanding and expensive test compared to culture, histology and the rapid urease tests. It requires special laboratory conditions with separate facilities for each stage of the technique and, as it is highly sensitive, it is subject to false-positive results by contamination. A positive result detected by any of the molecular techniques does not indicate current infection as it can also detect the DNA of dead organisms (Ricci et al., 2007).

NON-INVASIVE TESTS

Non-invasive tests are based on the analysis of samples of breath, blood, or stool. These tests are relatively inexpensive and rapid, all are proxy measurements of infection; thus, validation, typically using histology or culture as the gold standard, must be performed initially (Frenck et al., 2006). These tests can either be active or passive (Oderda et al., 2001). Active tests detect the presence of *H*. *pylori* and provide evidence of a current infection while the passive tests provide evidence of exposure to *H*. *pylori* and do not indicate whether the infection is currently active.

Passive tests are based on the detection of antibodies to *H*. *pylori* and they include: serological tests, near-patient tests and tests on saliva and urine (Oderda et al., 2001). Active tests detect the presence of antigen (*H*. *pylori*) than of antibody. They include: Urea breath and Stool antigen tests.

SEROLOGICAL TESTS

The most common is the enzyme-linked immunosorbent assay (ELISA), which detects the totality of the immunoglobulin in a patients' serum and can detect any of the immunoglobulin isotypes. The patient's body produces antibodies that are specific to antigen (*H*. *pylori*) after exposure. ELISA relies on the fact of the hydrogen bond formed between the antibodies and antigen which is monitored by a colour change when there is a reaction of antigen- antibody (Oderda et al., 2001).

Antibody tests play an important role in studies of pathogenesis and virulence. Although these tests are of limited value in the clinical setting, they have increased our understanding of the pathogenesis of disease caused by *H*. *pylori*. Antibodies against the important proteins of *H*. *pylori*, *cagA* and *vacA*, can be detected using different immunological techniques. After eradication of *H*. *pylori*, the antibodies disappear at different times and some, such as the anti-*cagA* antibodies, may persist for years (Oderda et al., 2001). Although the antibody response to *cagA* can be detected using an ELISA technique, immunoblots have a particular advantage in that they are more sensitive and can provide evidence of immunological reactions to several antigens all at once, which might have clinical relevance (Kaklikka et al., 2006).

The sensitivity and specificity of serological tests depends on the antigen used, the clinical context, the gold standard used as a comparator and the prevalence of *H*. *pylori* in the community (Roggero et al., 2002). The disadvantage of this test is that it does not distinguish between active infection and a previous exposure to *H*. *pylori*. Antibody levels can persist in the blood of individuals cured of *H*. *pylori* infection for long periods of time. Therefore, as the numbers of patients successfully treated for *H*. *pylori* increase in a population, the prevalence of false-positive tests with serology increases (Oderda et al., 2001).

NEAR-PATIENT TEST

Near-patient tests provide a rapid diagnosis of *H*. *pylori* infection in clinics or physician offices; they are also called office-based serology tests (Roggero et al., 2002). The test is simple to perform and most convenient because it uses a drop of whole blood obtained by finger-prick. However the results of finger-prick tests can vary significantly when the flow of blood is poor and there is difficulty in obtaining a drop of blood because squeezing the finger can express tissue fluid into the blood sample, thereby changing the concentration of antibody sample being studied (Gatta et al., 2003).

TESTS ON SALIVA AND URINE

Tests on saliva and urine are attractive because samples are easily obtained. They also share the same probability as other antibody tests using serum and have an additional problem in that the concentration of the antibody is lower than in serum, making detection more difficult (Oderda et al., 2001).

UREA BREATH TEST

The test relies on the fact that *H*. *pylori* produces the urease enzyme that splits urea into ammonia and carbon dioxide. The test is based on the principle that urease activity is present in the stomach of individuals infected with *H*. *pylori* (Oderda et al., 2001). Patients ingest urea labelled with either ^{13}C or ^{14}C. Hydrolysis of urea occurs within the mucus layer and results in the production of labelled CO_2. The CO_2 diffuses into the epithelial blood vessels and, within a few minutes, the isotopic CO_2 appears in the subject's breath.

Labelled urea is given to the patient with a test meal to delay gastric emptying and increase contact time with the mucosa. After ingestion of the urea, breath samples are collected for up to 20 min by exhaling into a CO_2-trapping agent (hyamine). Urea breath test has very high sensitivity and specificity (Oderda et al., 2001).

STOOL ANTIGEN TEST

An enzymatic immunoassay (EIA), detects the presence of *H*. *pylori* antigen in stool or faecal specimen. The test is used for diagnosis of antigen infection and for confirmation of eradication after treatment. The most widely used test in the assay uses polyclonal anti-*H*. *pylori*-capture antibodies absorbed to microcells (Ndip et al., 2004; Ricci et al., 2007). Stool antigen test are now commercially available as kits and are performed according to the manufacturer's instruction (Kaklikka et al., 2006). The kit should be stored at 2 – 8°C before testing. The test is highly specific and sensitive like the urea breath test (Oderda et al., 2001).

CONCLUSION

H. *pylori* Infection causes peptic ulcers, gastritis, duodenitis, and gastric cancers. Infection with the organism has been shown to follow geographic and socio-demographic distribution. The increased risk of infection is especially high among those living in Africa due to precarious hygiene standards, crowded households and deficient sanitation associated with this part of the world. Interestingly however; the infection rate in various populations does not parallel the incidence of morbidity caused by the infection. This has been termed by a number of authors as the 'African enigma' based on an apparently low incidence of gastric carcinoma and other *H*. *pylori*-associated morbidities in the continent of Africa.

In Africa, facilities to perform the UBT may be limited due to expense and the technical demand of the procedure. Microbiological culture of gastric biopsy specimen is arguably the best method to detect *H*. *pylori* in clinical specimen. This however, is not easy to perform in Africa where medical infrastructure is very limited for specialized procedures like endoscopy which makes provision for biopsy sample collection. Another problem with *H*. *pylori* culture is the very fastidious nature of the bacterium. Restraint should therefore be exercised to allow for efficient performance of culture.

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