Review

An overview of antimicrobial resistance and the future of medicinal plants in the treatment of *Helicobacter pylori* infections

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Helicobacter pylori, **a gram negative helical bacillus that inhabits the human stomach is now recognised as the causative agent of chronic gastritis, peptic ulcer, duodenal ulcer and mucosa associated lymphoid tissue (MALT) lymphoma. The treatment of *H. pylori* infection typically employs a triple drug regimen using two antibiotics and a proton pump inhibitor (PPI) or bismuth with a success rate of 80 - 90%. However, resistance to the most commonly used antibiotics is a growing global concern. With an alarming *H. pylori* prevalence of 90% reported in some African countries and 90 - 100% metronidazole (MTZ)-resistance, one of the drugs used in the treatment regimen, the need for alternative treatment regimens is imperative. Medicinal plants seem to provide an alternative source of treatment and are among the attractive sources of new drugs shown to produce promising results against *H. pylori in-vitro* . Researchers have been testing crude extracts of a wide variety of plants used in folklore medicine for the identification of potential anti- *H. pylori* agents. This review appraises the current state of *H . pylori* antimicrobial resistance, medicinal plant constituents ranging from extracts commonly in use, by the lay community, to substances being prospected and tested by researchers as possible substitutes to treat *H. pylori* infection. There is a need to continue to monitor resistance and revolutionize the search for alternative treatment regimens against *H. pylori* particularly as the life span of any drug is limited.**

Key words: *Helicobacter pylori*, drug resistance, antibiotics, alternative treatment, medicinal plants.

INTRODUCTION

Helicobacter pylori, a pleomorphic, microaerophilic slightly curved gram negative motile rod that inhabits various areas of the stomach is now recognized as the ethiologic agent of chronic gastritis, gastric mucosa-associated lymphoid tissue lymphoma, gastric and duodenal ulcer (Ndip et al., 2003; Mégraud and Lehours,

2007; Ndip et al., 2008b). It is classified by the World Health Organization and the International Agency for Research on cancer as a class 1 carcinogen (Lu et al., 2004; Romero-Gallo et al., 2008). Eradication of *H. pylori* results in significant remission from the above diseases (Inatsu et al., 2006; Adeniyi et al., 2009).

In affluent societies, *H. pylori* infection is increasingly treated with potent combination therapies (a proton pump inhibitor) and two of the following antibiotics; amoxicillin, tetracycline, clarithromycin and metronidazole. These have a success rate of 80 to 90% (Peitz et al., 1998), but problems, including undesirable side effects (nausea, vomiting, epigastric pain, abdominal discomfort and diarrhoea) and poor patient compliance, are associated with significant levels of treatment failure and contraindications for some patients. In addition, the cost of combination therapy is high.

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Abbreviations: MALT, mucosa associated lymphoid tissue; **PPI,** proton pump inhibitor; **MTZ,** metronidazole; **CLSI,** clinical laboratory standard institute; **PCR,** polymerase chain reaction; **RT,** reverse transcription; **PBP,** penicillin binding proteins.

With prevalences of more than 90% reported in developing countries (Feldman et al., 1998), coupled with the fact that around half of the world's population is infected (Ndip et al., 2004; Adrienne et al., 2007; Tanih et al., 2008a), there is a need to explore alternatively cheap and readily available means of treating, suppressing, or preventing *H. pylori* infection. The eradication of *H. pylori* is generally considered poor since failure rate remains as high as 5 – 20% along with frequent relapses of gastric ulcers (Yang et al., 2005). In addition, it is important to seek new therapies because the widespread use of antibiotics or drug pressure in combination therapies is associated with increasing drug resistance problems (Kalach et al., 2001; Cameron et al., 2004). Auto medication or the purchase of street drugs (over the counter) with suboptimal concentrations of the active component, a phenomenon common in the developing world is also a crucial issue as such drugs, when taken, will produce sub-inhibitory antibiotic blood concentrations that predispose to the selection of resistant bacterial strains. This means that people seeking medical attention for infections caused by this organism may have the problem recurring a few weeks after treatment or fail to respond to treatment. The success of any antimicrobial regimen for *H. pylori* eradication depends on patient compliance and lack of antimicrobial resistance (Tanih et al., 2009a). The use of regimens with minimal side effects may be the first step in encouraging patient compliance, cost and availability.

Plant extracts are among the attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers and other infections (Afolayan and Meyer, 1997; O'Gara et al., 2000; Afolayan and Lewu, 2009). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties. Medicinal plants seem to provide an alternative source of treatment for the moment, particularly in Africa, where their usage for the treatment of diseases is common, since most of the inhabitants cannot afford hospital treatment (Kambizi and Afolayan, 2008; Afolayan and Lewu, 2009). Plant based substances might provide a suitable basis for new anti-*H. pylori* therapies because they possess well-established antimicrobial actions (Eloff et al., 2008; Atapour et al., 2009); the chemical complexity of these substances and the broad-spectrum effectiveness of some of them suggest that acquired antibiotic resistance would be unlikely (O'Gara et al., 2000).

The extracts of *Garcinia kola* Heckel seeds have been reported to show potentials of synergy in combination with some antibiotics against reference strains of pathogenic organisms often presenting with problems of drug resistance (Sibanda and Okoh, 2008). The detection of synergy between crude extract of *G. kola* and antibiotics demonstrates the potential of this plant as a source of antibiotic resistance -modifying compounds. Direct intra-gastric effects are feasible with plant extracts because some plant antimicrobials, e.g, garlic, are unaffected by acid environments and also because gastric juice may enhance the antimicrobial activity of some of the plant constituents (Cellini et al., 1996; Sivam, 2001). The search for alternative anti *H. pylori* compounds from medicinal plants is very important as the effective life span of any antibiotic is limited.

This review appraises the current state of *H. pylori* antimicrobial resistance, medicinal plant constituents ranging from extracts commonly in use, by the lay community, to substances being prospected and tested by researchers as possible substitutes to treat *H. pylori* infection.

Current treatment of *H. pylori* infection

The current recommended treatment for *H. pylori* eradication includes two antibiotics and an antisecretory drug, such as a proton pump inhibitor (PPI), to which a bismuth salt can be added (subcitrate or subsalicylate), H_2 antagonists; ranitidine, ebrotidine, etc (Alarcón et al., 1999; Romano and Cuomo, 2004; Mégraud and Lehours, 2007). The most commonly used association worldwide is a double dose of PPI (omeprazole, lansoprazole, pantoprazole, rabeprazole, or esomeprazole) plus clarithromycin (500 mg twice a day [b.i.d.]) and amoxicillin (1 g b.i.d.) for 7 days (Treatment 1). Other 7-day regimens include a double dose of PPI plus clarithromycin (500 mg b.i.d.) and metronidazole (500 mg b.i.d.) (Treatment 2) or a double dose of PPI plus amoxicillin (1 g b.i.d.) and metronidazole (500 mg b.i.d.) (Treatment 3), with the latter being mostly used as a second choice treatment for 14 days in the case of failure of Treatment 1 (Choung et al., 2006; Malfertheiner et al., 2007; Fuccio et al., 2007; Stasi and Provan, 2008; Eisig et al., 2009; Bakir et al., 2009).

Like any infectious agent, *H. pylori* can acquire resistance to the antimicrobial agents used to treat the infection and therefore, susceptibility testing is important in the management of the infection. It is important to understand the mechanisms by which *H. pylori* develops resistance to antibiotics, in other to manage patients with treatment failure better.

Susceptibility testing methods

The usual phenotypic methods of susceptibility testing can be applied to *H. pylori*, but because resistance is essentially due to point mutations, genotypic methods are also used, especially for clarithromycin (Alarcon et al., 2001). Among the phenotypic methods (Disk diffusion, E-test, broth dilution and agar dilution), the agar dilution method is usually considered the reference method and has been proposed by the Clinical Laboratory Standard

Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) as the method to be used for *H. pylori* clarithromycin susceptibility testing (NCCLS, 1998). The disk diffusion method is the simplest and most economic for routine susceptibility testing. However, it is generally not recommended for slow-growing bacteria. The E-test method has the advantage of being a quantitative method with a direct expression of MICs and furthermore, it is adapted to slow-growing bacteria like *H. pylori*. A good correlation has been found between this method and the agar dilution method (Mégraud and Lehours, 2007) . Genotypically, *H. pylori* resistance is essentially due to chromosomal mutations and for the most part, a limited number of punctual mutations are present which can be easily detected with molecular tools (Wang et al., 1998; Vega et al., 2007).

The Polymerase Chain Reaction (PCR) has been applied in the field of *H. pylori* not only in detection of the bacterium but also its quantification and detection of specific genes relevant to pathogenesis (*cagA*) and specific mutations associated with antimicrobial resistance (Monteiro et al., 2001; Dzierzanowska et al., 2005). Gastric juice, faeces, serum and biopsy specimens have been used to carry out standard PCR for the detection of *H. pylori* (Monteiro et al., 2001; Mégraud, 2004). The choice of the target is important in designing the primers which must be specific for *H. pylori* but conserved in all strains of the species. It is therefore necessary to know the DNA sequence of the target in as many strains of *H. pylori* as possible. Commonly used targets include 23S rRNA, *gyrA*, *rdxA*/*frxA* genes for clarithromycin, ciprofloxacin and metronidazole resistance studies, respectively (Posteraro et al., 2005; Moder et al., 2007; Kim et al., 2009). PCR has its disadvantages; it is technically demanding and expensive, requiring special laboratory conditions and facilities at various stages. Its high sensitivity could give false positive results as a result of contamination from exogenous *H. pylori* DNA or the presence of dead organisms.

Apart from standard PCR, other forms; nested or seminested PCR, reverse transcription-PCR (RT- PCR), PCR- RFLP and real-time PCR have been used in *H. pylori* studies (Mégraud, 2004; Dzierzanowska et al., 2005; Kim et al., 2009). A method to detect Clarithro-mycin resistance without performing PCR, fluorescence in situ hybridisation (FISH) is one of those methods reported to produce accurate results in short time intervals. It is applied directly on fresh shock frozen tissue or formalin fixed paraffin-embedded biopsies. This method has been applied to the detection of *H . pylori* and its resistance to clarithromycin by a number of authors (Elitsur et al., 2006; Caristo et al., 2008) and is highly recommended for its specificity and sensitivity compared with standard methods of culture and susceptibility testing. It consists of an rRNA based whole cell *in situ* hybridisation using a set of fluorescent labelled

oligonucleotide probes. Labeling of intact single bacteria is monitored by fluorescence microscopy. This method allows detection of *H. pylori* with a 16S rRNA probe labelled with the fluorochrome Cy3 (red) and of resistant mutants with a 23S rRNA probe labelled with fluorescein (green) simultaneously.

Equally important is the use of amino acid sequencing of *rdxA* and *frxA* to detect MTZ- sensitive and resistant *H. pylori* isolates (Albert et al., 2001; Moder et al., 2007; Kim et al., 2009). After sequencing and cloning experiments of mutated and non mutated *rdxA* and *frxA*, Kim et al. (2009) demonstrated that other factors are responsible for MTZ-resistance and not just the alterations of the above genes.

Beta-lactam resistance mechanisms of *in vitro*-selected amoxicillin resistant *H. pylori* are also studied with gene probes. In one such study, Co and Schiller (2006) demonstrated that resistance to amoxicillin is due to a combination of amino acid substitutions in the penicillin binding proteins. This corroborates with the reports earlier made by Van-Zwet et al. (1999) in which it was indicated that amoxicillin-resistant *H. pylori* strains harbour mutations on the *pbp-1a* gene.

Commonly observed phenotypes of resistant *H. pylori* strains

H. pylori is intrinsically resistant to glycopeptides, cefsulodin, polymyxins, nalidixic acid, trimethoprim, sulfonamides, cycloheximide and a few antifungal drugs some of which are used as selective agents in isolation media (Mégraud and Lehours, 2007). These exposures particularly in suboptimal concentrations give the bacteria a chance to develop resistance against the drugs (Cowan, 1999). Wild-type strains are susceptible to -lactams (except cefsulodin), fosfomycin, macrolides, aminoglycosides, tetracyclines, chloramphenicol, rifampins, fluoroquinolones, 5-nitroimidazoles and nitrofurans (Megraud, 1998). With the exception of chloramphenicol (because of toxicity) and aminoglycosides (because of a lack of diffusion), they have all been used in *H. pylori* eradication regimens (Choung et al., 2006; Malfertheiner et al., 2007; Fuccio et al., 2007; Tanih et al., 2008).

The development of new anti-*H. pylori* therapies presents enormous challenges to clinical pharmacolo-gists, not only in the identification of novel targets, but also in ensuring adequate drug delivery to the unique gastric mucus niche of *H. pylori* (Goddard and Logan, 2003). Table 1, adopted from Mégraud and Lehours (2007), shows the MIC of current drugs determined at an acidic pH (e.g., 5.5 instead of 7.2), MICs increased markedly indicating that some of these drugs may not attain their maximum activity *in vivo*.

Looking at the table, it is obvious that *H. pylori* survives very well in acidic milieu and so antimicrobial agents to *H.*

Table 1. Distribution of MIC_{90} of various antibiotics against wild-type *H. pylori* at various pH.

Agent	MIC_{90} (mg/l) at pH:		
	7.5	6.0	5.5
Penicillin	0.03	0.5	0.5
Ampicillin	0.06	0.25	0.5
Cephalexin	2	16	32
Erythromycin	0.06	2	8
Clarithromycin	0.03	0.06	0.25
Ciprofloxacin	0.12	0.5	2
Tetracycline	0.12	0.25	0.5
Nitrofurantoin	1	2	2
Metronidazole	2	2	2
Bismuth subcitrate	16	8	-

pylori must be very potent in these conditions. This is important in the development of new drugs which should be delivered to the unique gastric mucus niche of *H. pylori* without a decrease in their antimicrobial activity.

Prevalence of *H. pylori* resistance to antibiotics

Numerous studies have been performed to determine the prevalence of *H. pylori* resistance to antibiotics (Megraud, 2004; Cameron et al., 2004; Moore and Salama, 2005; Boyanova et al., 2008; Ndip et al., 2008a; Boyanova, 2009). However, many of them have pitfalls, especially concerning the number and the types of the strains tested. Most of the studies were performed in medical centers for *H. pylori* treatment where patients who experienced previous treatment failures were recruited, increasing the probability of finding resistant strains. These cases are not representative of the patients as a whole and because these studies are monocentric, the number of patients may be low, leading to wide confidence intervals of the prevalence obtained. Ideal studies involving patients who are representative of a given region by being selected at random are few. They however give an indication of the situation. Table 2 shows the percentage resistance of *H. pylori* to four commonly used antibiotics from different parts of the world.

Looking at the table, it is obvious that *H. pylori* is most resistant to Metronidazole with some developing countries recording 100% resistance. This could be attributed to its multiple uses and the abuse of pharmaceutical regulations. Weather mutations in the nitroreductase, *rdxA* or flavin oxidoreductase, *frxA* genes are responsible for this resistance is still a debatable issue.

Prevalence of *H. pylori* resistance to clarithromycin

The most attractive compound in the treatment of *H.*

pylori infection is clarithromycin because it has the best efficacy *in vitro,* it is moderately affected by a decrease in pH and good concentrations can be obtained in gastric mucosa (Megraud, 1998). Resistance to clarithromycin has been the most widely studied so far (Wang et al., 1998; Osato et al., 2001; Gu et al., 2006; Mégraud and Lehours, 2007; Caristo et al., 2008; Bakir et al., 2009). It ranges from close to 0 to 25%. In the United States, a prevalence of 10 to 15% was found based on strains isolated during clinical trials, regardless of the region studied (Osato et al., 2001).

It is known that clarithromycin resistance in *H. pylori* has sharply decreased the success of eradication by 40 to 50%. The prevalence of clarithromycin resistance may be higher in children as reported by Kalach et al. (2001) where a resistance rate of 29% was reported in Europe. Consumption of macrolides is a risk factor for clarithromycin resistance. The high level of clarithromycin resistance among *H. pylori* strains from children compared to that among strains from adults suggests the importance of macrolide use in children especially in respiratory infections (Kalach et al., 2001). The use of amoxycillin and metronidazole as first-line eradication therapy in combination with a proton pump inhibitor is good as it will help to reduce the spread of clarithromycin resistance (Cameron et al., 2004) and is considerably cheaper.

Prevalence of *H. pylori* resistance to metronidazole

A high prevalence (>90%) of metronidazole (MTZ) resistance in *H. pylori* has been reported in developing countries (Alarcon et al., 1999; Lwai-lume et al., 2005; Aboderine et al., 2007; Ndip et al., 2008a). Banatvala et al. (1994) reported that among those born in the United Kingdom, women were more likely to harbour MTZ-resistant *H. pylori* strains than men (54 versus 18%, respectively) and more likely to have a history of previous nitroimidazole ingestion (41 versus 11%, respectively) and patients previously exposed to either metronidazole

Table 2. Global resistance of *Helicobacter pylori* to metronidazole, clarithromycin, amoxillin and tetracycline.

Countries. Percentage resistance of *H. pylori* to different antimicrobial agents					
	Mtz	**Clr**	**Amx**	**Te**	**Reference(s)**
Africa					
Cameroon	93.2	44.7	85.6	44	Ndip et al., 2008a
Nigeria	100	100	100	87.1	Aboderin et al., 2007
Kenya	100	6.4	4.6	1.9	Lwai-lume et al., 2005
DRC, Burkina Faso	80-90	ND	ND	ND	Alarcón et al., 1999
Egypt	60-80	4	ND	ND	Sherif et al., 2004
South Africa	95.5	20	2.5	32.5	Tanih et al., 2009b
Europe					
France	43	21	0	ND	Kalach et al., 2001
Croatia	15.8	21	0	ND	Tonki et al., 2005
Bulgaria	14.5	11.5	0	3.3	Boyanova et al., 2004
Bulgaria	31.2	27.5	1.1	5.6	Boyanova, 2009
England	24	7	0	0.3	Elviss et al., 2004
Spain	23.5	12.9	0	0.7	Mégraud, 2004
Portugal	34.1	22	0	0	Mégraud, 2004
Netherlands	21.2	1.7	0	0	Mégraud, 2004
Belgium	27	10.5	ND	ND	Alarcón et al., 1999
Finland	26	ND	ND	ND	Alarcón et al., 1999
Sweden	26.1	2.9	0	ND	Mégraud. 2004
Australia	17	ND	ND	ND	Alarcón et al., 1999
Poland	46	28	0	0	Dzierzanowska et al., 2005
Italy	29.4	16.9	0	ND	Pilotto et al., 2000; Zullo et al., 2007
Germany	21	5.3	0	0	Cameron et al., 2004; Mégraud, 2004
Asia					
China	39.2	7.8	0	ND	Gu et al., 2006
Eastern Taiwan	51.9	13.5	36.1	0	Hu et al., 2006
Japan	9-12.4	27.7	16.3	ND	Mégraud, 2004; Kobayashi et al., 2007
Hong Kong	29	4.5	0.3	0.5	Mégraud, 2004
Korea	40.6-41.9	5.4-5.9	0	5.3	Mégraud, 2004
Singapore	31.7-62.7	ND	ND	ND	Mégraud, 2004
Malaysia	10	0	ND	ND	Alarcón et al., 1999
North America					
Canada	18-22	<4	ND	ND	Fallone, 2000
USA	21.6-33.9	10.6-12.2	0-0.8	ND	Mégraud, 2004
South America					
Mexico	80	24	18	ND	Torres et al., 2001
Brazil	53	9.8	ND	ND	Mégraud, 2004
Peru	61	50	ND	ND	Alarcón et al., 1999
Middle East					
Israel	38.2	8.2	0.9	0	Mégraud, 2004
Lebanon	29.5	4	0	2	Sharara et al., 2002
Iran	ND	17	ND	ND	Mégraud, 2004

Mtz, Metronidazole; Clr, Clarithromycin; Amx, Amoxicillin; Te, Tetracycline; ND, Not Defined.

or tinidazole were more likely to harbour resistant strains (84 versus 41%). Metronidazole consumption appears to be an important risk factor for this resistance.

A marked difference has been found between the rate of resistance to nitroimidazoles in developed and developing countries. This difference may be linked to the high level of general use of metronidazole in developing countries, because these countries are mostly tropical and this inexpensive drug also sold in the street corners is commonly used to treat parasitic infections such as amoebiasis (Alarcon et al., 1999). The use of metronidazole and tetracycline has been abused over the years owing to illegal practices and poor compliance with pharmaceutical regulations (Ani et al., 1999). Resistance rates can be as high as 100% in developing countries, as reported in Kenya and Nigeria (Lwai-lume et al., 2005; Aboderine et al., 2007). Ndip et al. (2008a) reported a 93.2% resistance in Cameroon and in their study, more than 60% of the isolates exhibited multi-drug resistance to three or four antibiotics. They concluded that multi-drug resistance is common against the current treatment regimen in Cameroon and called for urgent studies involving newer and broad spectrum antibiotics to address the problem. Sherif et al. (2004) also recorded a high MTZ-resistance (60 - 80%) in Egyptian children and recommended that the use of metronidazole for the treatment of *H. pylori* in Egypt be avoided. The cause of this resistance may also be linked to the use of these compounds for genital infections, especially trichomonasis and therefore, strains isolated from women are more likely to be resistant than strains isolated from men (Cameron et al., 2004). Another possible cause may be the use of these compounds to treat dental infections (Me´graud, 1997).

Studies of *H. pylori* antibiotic resistance in South Africa are lacking. This could be a serious problem owing to the fact that susceptibility patterns are changing globally and rapidly too. As a result, eradication failures will be frequent as some of the drugs given to the patients may fail to produce the desired effects and yet left on the shelf. However, a recent study by Tanih et al. (2009b) indicated an MTZ-resistance of 95.5% which seems to corroborate earlier reports made in other African countries, thus, confirming high MTZ-resistance in the developing world.

Prevalence of *H. pylori* resistance to other antibiotics

Increasing resistance of *H. pylori* to ciprofloxacin is being reported in the literature (Cabrita et al., 2000; Bogaerts et al., 2006; Aboderin et al., 2007; Hung et al., 2009). Bogaerts et al. (2006) reported a prevalence of 16.8% in the Belgian population. The prevalence of *H. pylori* resistance to amoxicillin is very low in Europe, North America and the Middle East, (<1%) (Van-Zwet et al., 1999; Sharara et al., 2002; Me´graud and Lehours, 2007), so also is the prevalence of *H. pylori* resistance to

tetracycline (Osato et al., 2001). The prevalence of *H. pylori* resistance to rifampins is virtually absent, given that these antibiotics have a limited use (Me´graud and Lehours, 2007) . In contrast, resistance to the fluoroquinolones, which have shown an increasing consumption over the past few years is coming up; e.g., over 20% in adults in Portugal (Cabrita et al., 2000).

Resistance mechanisms

H. pylori, like a few other bacteria such as *Mycobacterium tuberculosis*, acquires resistance by mutation to all the antibiotics used in the treatment regimens (Me´graud and Lehours, 2007). The mechanism does not involve plasmids which could be transmitted horizontally but point mutations which are transmitted vertically; however, transformation may be possible if two strains are present simultaneously in the stomach. The consequence is a progressive increase in the resistance rate due to the selection pressure.

As in many bacteria, drug efflux proteins can contribute to natural insensitivity to antibiotics and to emerging antibiotic resistance. Bina et al. (2000) evaluated the relevance of three putative efflux systems in *H. pylori* resistance to antibiotics and concluded that, in contrast to what is usually described for gram-negative bacteria such as *Escherichia coli* or *Pseudomonas aeruginosa*, efflux systems did not play a role in the intrinsic resistance to antibiotics.

Resistance to macrolides

Macrolides act by binding to ribosomes at the level of the peptidyl transferase loop of the 23S rRNA gene. Resistance of *H. pylori* to macrolides is a major cause of failure of eradication therapies. *H. pylori* resistance is the consequence of point mutations at two nucleotide positions, 2142 (A2142G and A2142C) and 2143 (A2143G), which lead to a conformational change and a decrease in macrolide binding (Occhialini et al., 1997).

Resistance to amoxicillin

H. pylori resistance to amoxicillin is rare (Van-Zwet et al., 1999; Osato et al., 2001; Sharara et al., 2002; Mégraud, 2004; Dzierzanowska et al., 2005). Amoxicillin acts by interfering with peptidoglycan synthesis, especially by blocking transporters named penicillin binding proteins (PBP). The rare amoxicillin-resistant *H. pylori* strains harbour mutations on the *pbp-1a* gene. Amino acid substitution Ser-414 Arg appears to be involved, leading to a blockage of penicillin transport. Tolerance to amoxicillin has been described and the mechanisms involved include exchange of DNA through natural

transformation and conjugation (Van-Zwet et al., 1999).

Resistance to tetracyclines

Tetracyclines interfere in protein synthesis at the ribosome level by binding to the 30S ribosomal subunit. The change in a nucleotidic triplet (AGA-926 to 928 TTC), cognate of the positions 965 to 967 in *Escherichia coli*, has been associated with resistance to these compounds probably because of a lack of binding to the h_1 loop, which is the binding site of tetracyclines. Tetracycline targets the two *rrn* 16S operons (Catharine et al., 2002). Single or dual mutations at these positions lead to intermediary MICs (Mégraud and Lehours, 2007). The need to have three mutational events can explain the rarity of tetracycline resistance. Tetracycline-resistant strains with no mutation in position 926 to 928 have also been described, and efflux is the mechanism most likely to be involved. Generally, *H . pylori* tetracycline resistance is rare (Osato et al., 2001; Mégraud, 2004; Cameron et al., 2004; Dzierzanowska et al., 2005).

Resistance to fluoroquinolones

Fluoroquinolones inhibit the A subunit of the DNA gyrase, encoded by the *gyrA* gene. Mutations in the quinolone resistance-determining region of *gyrA* are found in *H. pylori* as well as in other bacteria. The amino acid positions concerned are mainly 87 and 91 (Tankovic et al., 2003). In a study in Belgium, Bogaerts et al. (2006) showed that resistance to ciprofloxacin is essentially mediated through a variety of point mutations occurring in a few loci of *gyrA*.

Resistance to rifampins

Rifampins inhibit the B subunit of the DNA- dependent RNA polymerase encoded by the *rpoB* gene. Mutations have been described for the *rpoB* gene of *H. pylori* at positions 524, 525 and 585 (Heep et al., 1999).

Resistance to nitroimidazoles

5-Nitroimidazoles have to be reduced in the cell to alter bacterial DNA. An important gene in this respect is *rdxA*, an oxygen- insensitive nitroreductase. Mutations in *rdxA* can render the protein ineffective (Hoffman et al., 1996). However, it has not been possible to identify a clear panel of point mutations with the *rdxA* gene to explain the phenomenon of resistance (Mégraud, 2004). It is believed that other genes such as *frxA* may also be involved in the reduction process. MTZ-resistance reduces the efficacy of MTZ-containing regimens but

does not make them completely ineffective. There is a discrepancy between *in vitro* MTZ -resistance and treatment outcome which may partially be explained by changes in oxygen pressure in the gastric environment as MTZ-resistant *H. pylori* isolates become MTZ-sensitive under low oxygen conditions *in vitro* (Gerrits et al., 2004). Gerrits et al. (2004) have demonstrated that mutations in *rdxA* and/or *frxA* may not be solely responsible for MTZ-resistance but other environmental factors like oxygen tensions, a finding that seems to corroborate with that of Kim et al. (2009). Gerrits and colleagues (2004) showed that under low oxygen conditions, using *H. pylori* mutated *rdxA* and/or *frxA i*solates, MTZ - resistance was reversed and this did not require *de novo* protein synthesis. However, the debate as to whether changes in these genes are solely responsible for MTZ- resistance in *H. pylori* is an issue of ongoing research.

Medicinal plants as alternatives in the treatment of *H. pylori* infections

The use of medicinal plants in the treatment of diseases is an ancient tradition that has co-existed with human habitation (Bizimenyera et al., 2007). Herbal medicines form a significant part of culture and traditions of rural people in developing countries (Kambizi and Afolayan, 2008; Afolayan and Lewu, 2009). As a result, there is an increasing trend to integrate traditional medicine with primary healthcare. This arises because about 80% of people in the developing world today, especially where modern drugs are not affordable, inaccessible or unacceptable depend on traditional herbal remedies (Cunningham, 1991; Bizimenyera et al., 2007).

Plant cells fundamentally are chemical factories and many possess a rich supply of therapeutically useful constituents (Akinpelu et al., 2009). Many plants contain large varieties of chemical substances with significant biological effects on humans. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. In many cases, these substances serve as plant defence mechanisms against predation by insects, herbivores and infection by microorganisms. Some, such as terpenoids, give plants their odours; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavour (e.g., the terpenoid capsaicin from chili peppers) and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Cowan, 1999).

Just like in many other continents, African plants have long been the source of important products with nutri-tional and therapeutic properties (Akinpelu et al., 2009). Traditional healers prepare a wide range of healing juices, crude extracts, paste and tincture from various herbs by using water extracts which are usually given to their patients orally. Almost every part of the plant (roots,

leaves, stalks, stem back, wood, flowers and seeds) is used. The neem (*Azadirachta indica*), Verasingam pattai (*Zanthoxylum limonella*), Indian babool (*Acacia nilotica*) stick are widely used as tooth brushes by various tribes throughout India, Nigeria and other parts of Africa (Samy and Gopalakrishnakone, 2008). Table 3 shows a list of some medicinal plants employed in the treatment of infections symptomatic of *H. pylori* infections in Africa. Some of the plants are reported to contain substances that could be useful in the development of lead molecules against *H. pylori* infections (Ndip et al., 2007b), but data on their toxic effects and therapeutic potentials are lacking, an aspect which is currently being studied in our group. Methanol, ethanol, chloroform, ethylacetate, hexane, aqueous and many other solvent extracts and fractions of a wide variety of plants used in folklore medicine are being investigated for lead molecules for potential antimicrobial agents. Plants with antibacterial effects are rich in polyphenolic substances such as tannins, catechins, alkaloids, steroids, essential oils and polyphenolic acids in their right concentrations (Samy and Gopalakrishnakone, 2008).

Solvents employed in the study of plant antimicrobials

The insolubility of essential oils and non-polar extracts make it very difficult for them to be used in an aqueous medium during the study of anti-microbial activity (Pellecure et al., 1976). Water or alcohol (methanol/ethanol) are mainly used for a large number of crude extract/library preparations (dry powder soaking or suspension, mechanical shaker, distillation of essential oils), sequential grinding (alkaloids, steroids, triterpenoids), gradial centrifugation (lectins and polypeptides) and acid hydrolysis (phenols) for a specific time frame (Eloff et al., 2008). A variety of extractants are used for their ability to solubilize anti-microbials and also other factors from plants. The type of solvent used may have an effect on the nature of the compounds extracted and the resulting bioactivity of the extract (Eloff, 1998b; Eloff et al., 2008). To ascertain the value of each extractant therefore, several parameters, including the rate of extraction, the quantity extracted, the diversity of compounds extracted, the diversity of inhibitory compounds extracted, the ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and the potential health hazard of the extractants have to be evaluated. The efficiency of extraction has to be optimized to ensure that as many of the potentially active constituents as possible are extracted.

A series of solvents of varying polarity (hexane, carbon tetrachloride, di-isopropyl ether, ethyl ether, methylene dichloride, tetrahydrofuran, chloroform, acetone, ethanol, ethyl acetate, methanol, water or mixtures of different solvents) are used on the plant material (Eloff et al.,

2008). In many reports, methanol or ethanol are used for alkaloid extraction; acetone for flavonoids and steroids, hexane, diethyl ether and chloroform for fat soluble oils, wax, lipids and esters; dichloromethane for terpenoids, ethyl acetate for esters, ethanol may also be used for sterols, polyphenols, tannins and water for the water soluble components like glycosides, polysaccharides, polypeptides and lectins, which are very effective against pathogens probably because of their ability to intercalate with DNA and/or cell membranes (Büssing, 1996).

The crude extracts or mixtures of compound- rich residues are used for the initial screening of plants for anti-microbial activities. Thin Layer Chromatography (TLC), other chromatography separations and several solvent systems are used for the elution of enormous water and organic solvent soluble anti-microbial compounds (Eloff, 1998b; Eloff et al., 2008). The *in vitro* and at times *in vivo* activities of many antimicrobial compounds from plants have been reported. However, very few of these studies have reported the *in vivo* anti- *H. pylori* activity of these compounds. This is very important particularly as there is need to know whether these compounds will still maintain their maximum activity in the gastric mucus niche of *H. pylori*.

Anti -*H. pylori* compounds from

plants Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being coloured, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Herrling et al., 2007). Quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function (Cowan, 1999). For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism.

A number of quinone extracts from plants have been reported to inhibit the growth of *H. pylori in vitro*. Inatsu et al. (2006) reported the inhibitory activity of quinones; idebenone, duroquinone, menadione, juglone and coenzyme Q_1 at low concentrations of 0.8 to 3.2 g/ml against *H. pylori* isolates and indicated that their findings could be useful in developing new quinone analogues to eradicate *H. pylori*. Idebenone [6-(10-hydroxydecyl ubiquinone)] specifically inhibited *H. pylori* growth by inhibiting respiration and decreasing the cellular ATP level (Inatsu et al., 2006) and has no known adverse effects in humans. However, they also indicated that

Table 3. Plants used in the treatment of stomach related morbidities in Africa.

Scientific name	Family	Part(s) used	Traditional uses	Reference
Sclerocarya birrea	Anacardiaceae	Stem back	Powdered back is dissolved in water and swallowed for treatment of gastritis, dysentery and diarrhoea.	Ojewole et al., 2003
Lippia javanica	Verbenaceae	Leaves	Used in the treatment of diarrhoea and dysentery	Samie et al., 2005
Peltophorum africanum	Fabaceae	Roots, leaves and stem bark	Parts are mixed and used to clear intestinal parasites diarrhoea and dysentery.	Bizimenyera et al., 2007
Bridelia micrantha	Euphorbiaceae	Stem bark	Treatment of stomach aches, tapeworms and diarrhoea in HIV infected patients	Bessong et al., 2005
Combretum molle	Combretaceae	Stem bark	Treatment of stomach pains, dysentery, gastric ulcers and abdominal disorders.	Eloff et al., 2008
Carissa edulis	Apocynaceae	Leaves	Treatment of chest and stomach complains	Nedi et al., 2004
Ficus syncomorus	Moraceae	Stem bark	Used by traditional healers to treat diarrhoea in Tanzania.	Maregesi et al., 2008
Garcinia kola Heckel	Cluciaceae	Seeds	Masticated and swallowed with water to treat gastritis and diarrhoea.	Akinpelu et al., 2008
Ageratum conyzoides	Asteraceae	Whole plant	Leaves are used for abdominal pains and flowers alone are used for dysentery.	Ndip et al., 2007b
Acanthus montanus	Acanthaceae	Leaves and stalk	Used for chronic ulcers. The flowerless tops are used for gastritis.	Ndip et al., 2007b Okoli et al., 2008
Eryngium foetidium	Apiaceae	Whole plant	Used in the treatment of poisoning and gastritis, improve digestion, combat colic and soothe stomach pains	Ndip et al., 2007b
Emilia coccinea	Asteraceae	Whole plant	The leaves are used to treat abdominal pains, gastritis. The leafy twig is used for treating gastritis.	Edeoga et al., 2005; Ndip et al., 2007b
Euphorbia hirta	Euphorbiaceae	Whole plant	The leafy twig is used in the treatment of gastritis and diarrhoea. In Nigeria, the leaves are used in traditional medicine for the control of diarrhoea and dysentery	Ogweke et al., 2007

Table 3. Contd.

Lycopodium cernua	Lycopodiaceae	Whole plant	The infusion is used for dysentery and diarrhoea. Plants boiled with water are used as purgatives.	Ndip et al., 2008b
Scleria striatinux	Cyperaceae	Roots	Treatment of abdominal pains	Ndip et al., 2007b
Scleria verrucossa	Cyperaceae	Roots	Used in the treatment of diarrhoea, abdominal pains, gastritis and dyspepsia.	Ndip et al., 2007b
Aulotandria kamerunensis	Zingiberaceae	Rhizomes	Used in the treatment of gastritis and abdominal pains	Ndip et al., 2007b
Tapeinachilus ananassae	Zingiberaceae	Rhizomes	Used in the treatment of abdominal discomfort.	Ndip et al., 2007b
Alepidea amatymbica	Apiaceae	Roots, rhizome, leaf and stem.	Used in the treatment of diarrhoea and other stomach related illnesses by the indigenes of the Eastern cape province of South Africa	Afolayan and Lewu, 2009

precise identification of the inhibitory target of idebenone in the respiratory chain of *H. pylori* will require further investigation using a cell-free system of *H. pylori*. As has always been the case with all plant-derived antimicrobials, the possible toxic effects of quinones must be thoroughly examined.

Flavones, flavonoids and flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol. Flavonoids are also hydroxylated phenolic substances but occur as a C_3- C_6 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection (Hernández et al., 2002), it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. The chloroform extract of *Cistus*

laurifolius flower buds used traditionally in folk medicine in Turkey to treat gastric ailments have been shown to possess significant anti-*H. pylori* activity with flavonoid as the active component (Ustün et al., 2006). The activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. Catechins, the most reduced form of the C_3 unit in flavonoid compounds, deserve special mention as tea catechins have been shown to have anti- *H. pylori* activity both *in vitro* and *in vivo* (Mabe et al., 1999).

Phenolics and polyphenols *Simple*

phenols and phenolic acids

Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include

enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 1987). Catechol has two −OH groups and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Biradar et al., 2008). Virgin olive oil is an unrefined vegetable oil that contains a significant amount of phenolic compounds. Romero et al. (2007) under simulated conditions, demonstrated that these substances can diffuse from the oil into the gastric juice and be stable for hours in this acidic environment. This is very important as studies concerning the stability and maximum activity of anti-*H. pylori* medicinal plant extracts in acidic environment are lacking. Biradar et al. (2008) also showed that, these compounds exerted at strong *in vitro* bactericidal activity against eight strains of *H. pylori*, three of them resistant to some antibiotics.

Tannins

"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3,000 and they are found in almost every plant part: bark, wood, leaves, fruits and roots and may be produced in different seasons (Hoste et al., 2006). One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Scalbert, 1991). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. Hydrolyzable tannins have been shown to have potential as new and safe therapeutic regimens against *H. pylori* infection *in vitro* (Funatogawa et al., 2004). These compounds from a group of medicinal plants used in many countries in Asia did not affect the viability of MKN-28 cells derived from human gastric epithelium indicating that they could provide good treatment alternatives with minimal effects on the host.

Coumarins

Coumarins are phenolic substances made of fused benzene and -pyrone rings. They are responsible for the characteristic odour of hay (Cowan, 1999). In a study carried out by Kawase et al. (2003), it was found that 7-hydroxy-4-methylcoumarin,6,7-dihydroxy-4-thylcoumarin, 6-hydroxy-7-methoxy-4-methylcoumarin and 5,7-dihydroxycyclopentanocoumarin showed comparable anti-*H. pylori* activity with metronidazole. This is very important particularly as *H. pylori* resistance to metronidazole is reportedly increasing particularly in the developing world (Banatvala et al., 1994; Moore and Salama, 2005; Aboderin et al., 2007; Ndip et al., 2008a). A recent study has also documented the antimicrobial activity of coumarins isolated from the roots of *Ferulago campestris* against *H. pylori* isolates in Italy (Basile et al., 2009). Generally, data about specific antibiotic properties of coumarins against *H. pylori* are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals.

Terpenoids and essential oils

The fragrance of plants is carried in the so called *quinta essentia*, or essential oil fraction. They are called terpenes, their general chemical structure is $C_{10}H_{16}$ and they occur as diterpenes, triterpenes and tetraterpenes (C_{20}, C_{30} and C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). When the compounds contain additional elements, usually oxygen, they are termed

terpenoids. Anti-*H . pylori* activity of essential oils are being studied with the hope that people with asymptomatic gastritis would certainly benefit from a nutritional approach to help them manage the infection and therefore decrease the risk of development of associated pathologies. Even though these compounds have shown good activity *in vitro*, essential oils are unlikely to be efficient anti-*Helicobacter* agents *in vivo* (Bergonzelli et al., 2003) . However, their effects may not be irrelevant if one plans to use them as food additives to complement present therapies. More research is needed to clarify the use of terpenoids and essential oils as potential anti-*H. pylori* agents.

Alkaloids

Heterocyclic nitrogen compounds called alkaloids are often found in methanolic and ethanolic plant crude extracts. Crude extracts of the fruits of *Evodia rutaecarpa* , used in Chinese medicine has been reported to contain two quinolone alkaloids; 1-methyl-2-[(Z)-8-tridecenyl]-4-(1H)-quinolone and 1-methyl-2-[(Z))-7-tridecenyl]-4-(1H)-quinolone. The minimum inhibitory concentration (MIC) of these compounds against reference strains and clinically isolated *H. pylori* strains were less than 0.05 g/ml, which was similar to the breakpoint MIC values of amoxicillin and clarithromycin that are used worldwide for the eradication of *H. pylori*, clinically (Hamasaki et al., 2000). Furthermore, these investigators noted that the antimicrobial activity of these compounds was highly selective against *H. pylori* and almost non-active against other intestinal pathogens. The results showed that these alkyl methyl quinolone alkaloids could be useful for the eradication of *H. pylori* without affecting other intestinal flora.

Lectins and polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942 (Samy and Gopalakrishnakone, 2008). They are often positively charged and contain disulfide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors (Zhang and Lewis, 1997) . It is worth emphasizing that molecules and compounds such as these whose mode of action may be to inhibit adhesion will not be detected by using most general plant antimicrobial screening protocols, even with the bioassay-guided fractionation procedures used by natural-products chemists. It is an area of ethnopharma-cology which deserves attention, so that initial screens of potentially pharmacologically active plants may be made more useful. Studies on anti-*H*. pylori activity of phytolectins and polypeptides are lacking.

Conclusion

The antimicrobial resistance of *H. pylori* is a global problem. Emergence of multidrug resistance has limited the therapeutic options; hence monitoring resistance is of paramount importance. Antimicrobial resistance monitoring will help to review the current status of antimicrobial resistance locally, nationally and globally and will be helpful in minimizing the consequence of drug resistance and limit the emergence and spread of drug resistant *H. pylori*. The management of *H. pylori* infections requires more than just drugs. Control measures to limit the spread of the infections need to be developed due to the fact that most of the time; it is the resistant organisms that are propagated to the next individual. The problem with *H. pylori* is that the mode of transmission has not been fully elucidated (Bonacorsi et al., 2009). In the near future, antibiotic resistance will be the greatest obstacle in the treatment of *H. pylori* infections. This reiterates the need to revolutionize the search for alternative treatment regimens which seem to lie in medicinal plants. Despite progress in conventional chemistry and pharmacology in producing effective drugs, medicinal plants might provide a useful source of new anti-*H. pylori* compounds for the development of novel pharmaceutical entities or, alternatively, as simple dietary adjuncts to existing therapies.

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