Antibiotic resistance of *Helicobacter pylori* from patients in Ile-Ife, South-west, Nigeria

Oladiipo A. Aboderin[1], Abdul R. Abdu,[2] Babatunde 'Wumi Odetoyin[1], Iruka N. Okeke, [3], Oladejo O. Lawal[4], Dennis A. Ndububa[5], Augustine E. Agbakwuru[4] and Adebayo Lamikanra[6]

[1]Department of Medical Microbiology & Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health sciences, Niger Delta University, Wilberforce Island, Nigeria
[3]Department of Biology, Haverford College, Haverford, PA, USA.
[4]Department of Surgery, Faculty of Clinical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
[5]Department of Medicine, Faculty of Clinical Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.
[6]Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Abstract

Background: *Helicobacter pylori* has become recognized as a major cause of gastroduodenal diseases in man. Evidence indicates that once acquired, *H. pylori* persists, usually for life unless eradicated by antimicrobial therapy. Over the past few years, we have accumulated some knowledge of the epidemiology of *H. pylori* in Ile-Ife, South-West Nigeria. In one collaborative study, we detected *H. pylori* in 195 (73%) patients referred for endoscopy at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC). Furthermore we have observed a variegated gastric inflammatory response and atrophy including atrophic pangastritis but are yet to demonstrate MALToma in any of our patients. In addition we have demonstrated that dental plaque is a possible source of gastric *H. pylori* infection and such an endogenous source could account for difficulty in eradication leading to re-infection. Presently, infected patients are treated with standard combination therapy made up of amoxycilin and ciprofloxacin with a proton pump inhibitor /bismuth. Reports however have shown that the incidence of antimicrobial resistance in *Helicobacter pylori* is a growing problem and which has been linked with failures in treatment and eradication. Given this situation it has become necessary to have information about the susceptibility of isolates to particular antimicrobial agents before the selection of an appropriate treatment regimen.
Objectives: More recently, we sought to study antimicrobial susceptibility of locally isolated *H. pylori* strains.
Methods: We subjected 32 isolates to antimicrobial susceptibility testing against seven agents.
Results: All the isolates showed multiple acquired antimicrobial resistance as they were all resistant to amoxicillin, clarithromycin, metronidazole, while 29/31, 27/31 showed resistance to rifampicin and tetracycline respectively. Five (15.6%) of these isolates showed resistance to ciprofloxacin.
Conclusions: Our findings suggest that *H. pylori* strains isolated within our study environment have acquired resistance to all the commonly prescribed antibiotics. On the basis of the findings it would be necessary to re-evaluate the eradication treatment regime in our setting.
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Introduction

Helicobacter pylori (H. pylori) is a ubiquitous gram-negative, microaerophilic spiral bacterium infecting half the world's population and causing chronic active gastritis in virtually all infected individuals [1]. Apart from gastritis however, this organism has also been associated with gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (MALToma).[2] Evidence indicates that once acquired, *H. pylori* persists, usually for life unless eradicated by antimicrobial therapy. Treatment regimens for *H. pylori* infection have been evolving since the early 1990s. Antimicrobial therapy for this infection is a complex issue, and the following drugs are currently used in combination regimens: proton-pump inhibitors and or bismuth, metronidazole, clarithromycin, and amoxicillin[3]. Tetracycline is used in rescue therapy[4].

Guidelines on the management of *H. pylori* have been developed in a number of regions around the world. The European Helicobacter Pylori Study Group (EHSG) produced the first Maastricht Concensus Report in 1996 which has undergone two revisions until the current Maastricht 3 − 2005 report[5]. The report gave directions on who to treat, how to treat and the need for *H. pylori*

Correspondence author:
A.O.Aboderin,
Department of Medical Microbiology & Parasitology,
Faculty of Basic Medical Sciences,
College of Health Sciences,
Obafemi Awolowo University,
Ile-Ife, Nigeria.
E-mail: aaboderi@oauife.edu.ng, diipo_aboderin@yahoo.com
Phone: 234 803 6706730

eradication with a view to reducing the risk for gastric cancer development. Although *H. pylori* is sensitive to many antibiotics *in vitro,* the *in vivo* eradication rate is often disappointing a major reason being the quick appearance of resistant strains. Strains resistant to metronidazole[6] and clarithromycin[7] have been well documented, while reports from Asia indicate the prevalence of resistance to amoxicillin and tetracycline.[8] [9] The resistance problem with *H. pylori* shows that treatment regimens will continue to evolve as the search continues for effective treatment eradication protocols. What is required is a simple and more efficacious strategy for the treatment of *H. pylori* infection[10].

Over the past few years, we have accumulated some knowledge of the epidemiology of *H. pylori* in Ile-Ife, South-West Nigeria. Much of this has been in the area of endoscopy in adult patients suffering from dyspepsia. In those studied, a strong association exists between *H. pylori* infection and duodenal ulcer as well as severe erosive gastritis[11]. In a study in which 268 patients at the OAUTHC were investigated for *H. pylori* infection, Ndububa *et al.*[12] reported that 195 (73%) were infected with the organism. Furthermore a variegated gastric inflammatory response and atrophy including atrophic pangastritis have been observed in infected subjects. It is noteworthy however that none of the patients studied had developed MALToma (Unpublished report). In a different study carried out in the same hospital, dental plaque was shown to be a possible source of *H. pylori* infection and presence of this endogenous source of infection in the patients could account for incidences of re-infection[13].

Having established the prevalence of *H. pylori* infection, patients are currently treated (with a view to eradication) with combination therapy (amoxicillin, ciprofloxacin and a proton pump inhibitor/bismuth). The relationship between antimicrobial resistance and the successful treatment of *H. pylori* infection has dictated that a study of the incidence of acquired antimicrobial resistance among *H. pylori* isolates obtained within our environment be determined hence the importance of this study.

Methods

Gastric biopsies were collected from consecutive patients who had oesophagogastroduodenoscopy at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) from July 2002 to January 2003 and from September 2005 to March 2006. These were dyspeptic patients referred from the Surgical and Medical outpatient departments of the OAUTHC. Initially biopsies were transported using Stuart's transport medium

in ice packs. Later specimens were transported to the laboratory in screwtop bottles with 0.1-0.5ml of 0.9% saline[14]. Specimens were inoculated onto Dent's medium and then cultured under a microaerophilic atmosphere in a candle extinction jar. Incorporating $NaHCO_3$ and citric acid or tartaric acid in the environment generated the required CO_2. High humidity was achieved by placing a moistened towel at the bottom of the jar. Plates were incubated at 37^0C for 3-7 days. Bacterial colonies were identified as *H. pylori* on the basis of colonial morphology, positive urease, catalase and oxidase tests and a Gram stain[15].
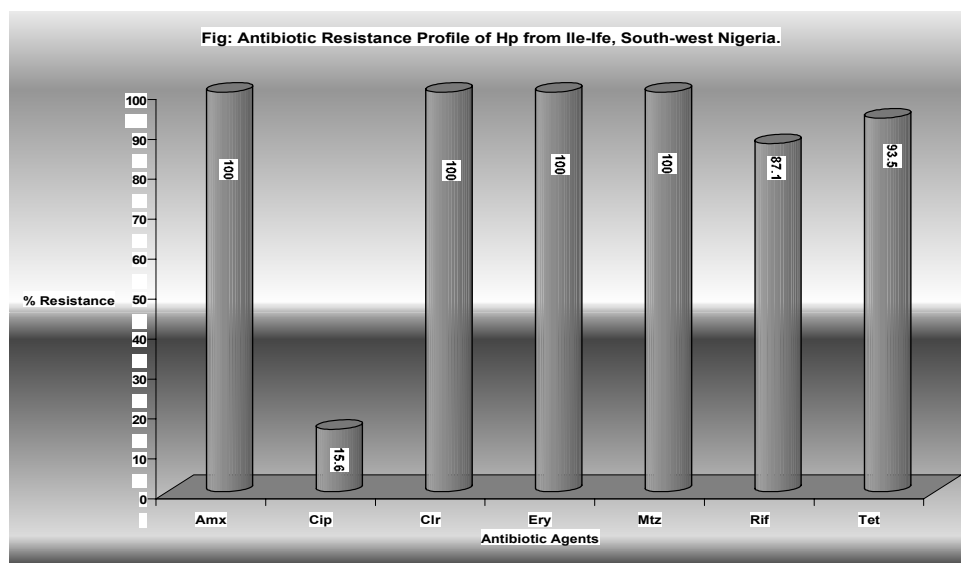
The susceptibility of isolates to antibiotics was tested using the disc diffusion assay, according to the methodology described by Lopez Brea and Alarcon[16]. Frozen vials containing the different strains were inoculated on blood agar and incubated in a microaerophilic environment for five days. The colonies obtained were suspended in 1ml tryptic soy broth ($\sim 10^6$CFU/ml). 0.5ml of the inoculum was flooded to a non-selective blood agar plate and allowed to dry for 5-10minutes. Seven different discs were tested using amoxycillin (25µg), ciprofloxacin (5µg), clarithromycin (15µg), erythromycin (15µg), metronidazole (5µg), rifampicin (5µg) and tetracycline (30µg) (Oxoid, England). They were then incubated under microaerophilic conditions for 72hours. Quality control was performed with *E. coli* K-12 C600. The breakpoint for resistance was as defined by National Committee for Clinical Laboratory Standards[17]. An interpretative correlate (susceptible or resistant) was provided by reference to published guidelines. Intermediate zones between susceptible and resistant were recorded as resistant.

Results

We subjected 32 isolates to antimicrobial susceptibility testing. The 32 isolates were from 16 females and 16 males with an age range of 20-73 years and mean of 48.6±16.23 years. The resistance profile to 7 different antibiotics as determined by disc diffusion test is as shown

Figure 1

All the isolates were resistant to amoxicillin, clarithromycin, metronidazole, while 29/31, 27/31 were resistant to rifampicin and tetracycline respectively. Five (15.6%) of the isolates were resistant to ciprofloxacin.

Fig: Antibiotic Resistance Profile of Hp from Ile-Ife, South-west Nigeria.

A total of 5 distinct antibiograms were encountered in all the *H. pylori* strains and the patterns varied from resistance to 4 antimicrobial agents to 6 antimicrobial agents (Table 1). Most frequently encountered antibiograms were ampicillin, clarithromycin, erythromycin, tetracycline, metronidazole and rifampicin.

Table 1 Resistance antibiograms of *Helicobacter pylori* isolates

Resistance antibiogram	Number
AML, CLR, ERM, RFC	1
AML, CLR, ERM, MTZ	1
AML, CLR, ERM, MTZ, TET	2
AML, CLR, ERM, TET, CIP, RFC	3
AML, CLR, ERM, TET, MET, RFC	25
	32

AML-ampicillin, CLR-clarithromycin, ERM-erythromycin, TET-tetracycline, MET-metronidazole, CIP-ciprofloxacin, RFC-rifampicin

Discussion

The geographical variation in the resistance of *H. pylori* to antimicrobial agents is thought to be related to the level of use of the agents in different communities[18]. In Nigeria, evidence has shown that antimicrobials especially ampicillin, tetracycline, metronidazole and erythromycin are in wide use[19]. In addition antibiotics self-medication is encouraged by free access and over the counter purchase and by ineffective drug control policy[20]. This could be a contributing factor for the very high level of resistance of *H. pylori* to amoxycillin (100%), clarithromycin (100%), metronidazole (100%), and tetracycline (93.5%) observed in this report.

Previous use of any macrolides and metronidazole has been correlated for resistance to clarithromycin and metronidazole respectively[21]. The prevalence of *H. pylori* resistance to metronidazole varies from 20% to 40% in Europe and the USA, with one exception in Northern Italy[22,23]. It is well known that the prevalence is much higher in developing countries (50-90%) for example in Mexico[7]. It must be emphasized that different resistance testing methods may be discrepant in 10-20% when testing for metronidazole. Furthermore, reproducibility using a given method is also not good[24]. Nevertheless, although the exact prevalence rate obtained must be interpreted with caution, the trends of high, medium, or low resistance seem real.

When risk factors are studied, past use of metronidazole, which is very common in tropical countries for parasitic diseases is involved. In developed countries, most studies have reported a higher resistance

rate in women than in men, probably due to the use of nitroimidazole drugs to treat gynaecological infections[25]. The use of metronidazole for dental infections may also add to selection pressure.

Although macrolides have been found to be useful therapeutic agents in treating *H. pylori* infections, our findings negate this as all our strains were shown to be resistant to erythromycin and clarithromycin. This observation corroborates findings of 92.6% erythromycin resistance rates reported by Quintana-Guzmam *et al.*[26]. We expected clarithromycin which has been reported to be acid stable and clinically more effective than erythromycin in the treatment of *H.pylori*[27] to be highly inhibitory, but it is remarkable that our strains were not inhibited by this agent. The finding is surprising because, relative to erythromycin, clarithromycin is new in the Nigerian market. One possible explanation for the finding is that acquired resistance to clarithromycin[28] could be as a result of previous exposure to erythromycin as documented by some workers[23, 29]. Kato *et al.*[28] have also demonstrated similar cross-resistance with azithromycin in clarithromycin resistant strains. The cross-resistance may explain resistance to clarithromycin even when it is introduced newly as observed among our strains.

Midolo and colleagues were first to report tetracycline resistance in *H. pylori* in 1996[30], while in the following year Piccolomini *et al.*[31] reported that 6% of strains were resistant in 1997 in Italy. The prevalence of tetracycline resistance was 58.8% in China[8]. The finding showing very poor inhibitory effect of tetracyclines to *H. pylori* in the present study was in keeping with experience of others[26]. In the Costa Rican[26] report 80.4% of strains are resistant to tetracycline. In fact, the pattern of multiresistance seen in our study is similar to that from Costa Rica where resistance to erythromycin, metronidazole and amoxycilin was 92.6%, 95.1% and 52.2% respectively. On the other hand, resistance to ciprofloxacin and another common agent nitrofurantoin was only 7.3% and 9.8% respectively similar to our report (though we did not include nitrofurantion). With tetracycline, as with other antibiotics, resistance increases with the use of the drugs due to selection pressure.

Amoxycillin resistance was not considered important until when amoxycillin resistance in *H. pylori* isolates were identified in the USA, Canada and Italy. Resistance rates of 31%[32] and 45%[33] have been reported from Italy. Wu *et al.*[8] reported a rate of 71.9%. All our isolates were resistant in vitro to amoxicillin. In the China study reported by Wu *et al.*[8], though the MICs for many resistant strains were high, no strain produced β-lactamase. It was assumed that amoxycillin resistance was probably a result of alterations in penicillin-binding proteins (PBP).

15.6% of our isolates were resistant to the fluoroquinolone, ciprofloxacin. The prevalence of fluoroquinolone has been determined only in a limited number of studies[23]. However, Portugal has reported a high resistance

rate of 20.9% in 110 adult patients[34]. Resistance to fluoroquinolones mirrors the use of these drugs. Ciprofloxacin is relatively new in Nigeria hence the comparatively low resistance to it by the isolates. Studies[23] that have examined resistant *H. pylori* isolates from various geographic locations have identified point mutations in different genes which confer resistance. However the resistance mechanisms of our isolates remain to be investigated.

The most common resistance antibiograms were those combinations containing amoxicillin, clarithromycin, erythromycin, tetracycline, metronidazole and rifampicin. *H. pylori* isolates resistant to multiple drugs have been reported elsewhere[8]. The high frequency of multiple antibiotic resistant *H.pylori* isolates observed in this study most probably reflects the ease of access and extensive use of antibiotics in Nigeria[20].

In the present report, the widespread resistance to the antimicrobial agents documented justified a critical need to monitor local variations in *H. pylori* sensitivity patterns, and resistance rates in order to devise therapeutic guidelines. While the principles of treatment should be based on simplicity, effectiveness, affordability, drug safety and efficacy, the *in vitro* resistance of *H. pylori* isolates to commonly used antimicrobials in this report has resulted in limited therapeutic options. On the basis of the findings it would be necessary to investigate ciprofloxacin in any eradication treatment regime in our setting, since it appears to be the only active antibiotic in eradicating *H. pylori* in this environment.

Furthermore, there is the need to continue the evaluation of new treatment agents such as NE-2001[10], older agents such as nitrofurantoin[26] or introduction of herbal management (we are investigating the promising effects of herbal drugs in the management of *H. pylori* infection) in order to eradicate *Helicobacter pylori*.

Future case-control studies employing larger sample size are needed to demonstrate the effectiveness or otherwise of therapy in the different patient groups as has been stated in the EHSG Maastricht guidelines[5]. A long-term follow-up of our patients will also contribute to the development of guidelines on the issues of referral, diagnostic methods and treatment of *H. pylori*.

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