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Prevalence of Clarithromycin-Resistant *Helicobacter pylori* Strains in Zambia: A Sub-Saharan African Country

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Keywords
Helicobacter pylori · Clarithromycin · Resistance · Africa · Zambia

Abstract
Introduction: *Helicobacter pylori* (*H. pylori*) is one of the most important infections globally, affecting more than 50% of the human population. Clarithromycin (CLA)-containing regimens are recommended for empirical eradication of *H. pylori* in populations with less than 15% resistance. The aim of this study was to estimate the prevalence of CLA resistance in samples collected from Zambian patients to determine if CLA is suitable for first-line *H. pylori* empirical treatment. ***Methodology:*** We used archival biopsy samples collected from dyspeptic patients undergoing endoscopy. The samples had been snap-frozen immediately after collection and stored at −80°C. We performed multiplex real-time PCR using Bosphore *Helicobacter pylori* Genotyping Kits v1, Istanbul, Turkey, to determine the presence of wild-type *H. pylori* and three mutations, A2142G, A2142C, and A2143G, of domain V in 23s rRNA gene. ***Results:*** We tested 259 gastric biopsy samples from patients with dyspepsia, of which 136 (53%) were from females. The median age was 48 years (IQR 40–61 years). Endoscopically, most of the patients, 164 (63%), had a normal gastric mucosa. CLA resistance was found in 48 (28%) samples, with A2142G mutation in 23 (13%), A2143G mutation in 32 (18%), and double mutations A2142C and A2143G in 6 (3%). ***Conclusions:*** The presence of significant levels of CLA resistance in Zambia suggests that it should not be used as first-line empirical treatment for *H. pylori* infection. However, with a limitation of suitable alternatives, there is an urgent need to formulate new treatment approaches.

Introduction

Helicobacter pylori (*H. pylori*) is considered one of the most successful human pathogens. About half of the world's population has the infection, all of whom would have gastritis, with the majority having no clinical symptoms [1]. The prevalence of *H. pylori* is highest in developing countries with more than 70% of the African population having the infection [2]. It is believed that most of the infection is acquired in childhood, and it is closely associated with unsanitary and overcrowded living conditions [1].

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H. pylori colonizes the stomach using various mechanisms that protect it from clearance by the immune system and acidic environment in the stomach [3]. Eradication of the infection is therefore done pharmacologically with several regimes that have been developed over the years. These regimes consist of antibiotics, typically two, and acid suppression medication, particularly proton pump inhibitors. However, *H. pylori* antibiotic resistance is now emerging as a significant health problem, occurring at different rates around the world [4].

Clarithromycin (CLA), a macrolide, is one of the first-line antibiotics used to treat *H. pylori* infection. The Maastricht VI/Florence consensus report recommends that CLA-based first-line antibiotics should only be used empirically if the prevalence of resistance is less than 15% [5]. The alternative should be a bismuth-based regimen. Continued use of CLA-based regimen in the presence of high resistance is associated with significant failure rates [6]. Different CLA prevalence rates have been reported from various countries, but such reports from Africa are limited.

H. pylori resistance to CLA is largely due to structural change associated with the single-nucleotide polymorphism of 23S rRNA gene [7]. Most of the CLA-resistant *H. pylori* strains contain one of the three-point mutations A2142G, A2142C, and/or A2143G [8]. Mutations have the ability to interrupt the peptidyl transferase loop conformation and prevent the binding of CLA to 23S rRNA, decreasing its efficiency and causing a resistance phenotype [9]. These mutations in 23S rRNA genes are thought to be enough to confer CLA resistance as their presence corresponds well to resistance phenotypes [10]. There are other point mutations in the region (such as A2115G, T2117C, G2141A, A2144T, T2182C, G2223A, T2288C, and T2711C) which have also been associated with CLA resistance. However, their importance is still unclear [11]. Interest in *H. pylori* resistance is increasing with most contributions arising from Europe, the USA, and East Asia [12].

The prevalence of *H. pylori* infection in Zambia is more than 80%, with 2% peptic ulceration [13–15]. Treatment for *H. pylori* in Zambia is done empirically using CLA-based regimen. No studies have been done to demonstrate the efficacy of this approach, and treatment success is seldom checked for. To the authors' knowledge, this is the first study evaluating antibiotic *H. pylori* resistance in Zambia. The aim of this study was to determine the prevalence of CLA-resistant *H. pylori* in Zambian isolates in order to demonstrate the appropriateness of CLA-based empirical treatment.

Methods

We used archival biopsies from patients collected during a previous case-control study in Lusaka, Zambia [16]. Enrolled in that study were consenting adults above 18 years. Upon collection, the gastric biopsies were snap-frozen in liquid nitrogen and stored at −80°C.

To test for CLA resistance, we used the Bosphore *Helicobacter pylori* Genotyping Kit v1, Istanbul, Turkey, of the gastric biopsies. Briefly, primers bearing point mutations A2142G, A2142C, and A2143G of domain V within the 23S ribosomal RNA gene were used for PCR amplification and fluorescence detection accomplished using the FAM and Cy5 labels. An internal control as integrated into the kit was used to check for PCR inhibition. Amplification data of the internal control were detected with the HEX filter whose analytic detection limit was 43 copies/reaction. Sample processing for DNA isolation was done strictly adhering to the manufacturer's instructions. PCR Master Mix 1, 2, and 3, internal controls, dH_2O, and three positive controls were employed in accordance with the instructions. Outflow analysis was done by Rotor-Gene Q real-time PCR machine, which automatically calculated the baseline cycles and the threshold. Results were interpreted as instructed in the manufacturer's manual and reported as the presence of wild-type mutations 2142 A- > G, 2143 A- > G, 2142 A- > C, double mutation (2142 A- > G and 2143 A- > G), or double mutation (2142 A- > C and 2143 A- > G). Samples without any amplification were considered to be negative for *H. pylori* infection.

H. pylori *Testing Using Serology and Histology*

Previously, we tested the patients included in this study for *H. pylori* antibodies in serum using commercial antibody ELISA kits and *H. pylori* multiplex serology testing assay [15]. This assay demonstrated the presence of *H. pylori* antibodies, including chaperonin HS60, urease alpha subunit, hypothetical proteins, neutrophil-activating protein A, neuraminylactose-binding hemagglutinin homolog, cytotoxin-associated gene A, hydantoin utilization protein A, catalase, vacuolating cytotoxin A, Helicobacter cysteine-rich protein C, cinnamyl alcohol dehydrogenase, and outer membrane protein or hypothetical protein HP1564. The presence of four or more of these proteins was considered positive. In addition, we had examined the gastric biopsies histologically for the presence of *H. pylori* using hematoxylin and eosin staining. In this study, we compared these findings with the real-time PCR results.

Data Analysis

Data were entered in structured forms coded and exported into StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX, USA: StataCorp LLC, for analysis. Categorical variables were presented in frequencies, proportions, and percentages. For association of CLA resistance and risk factors, logistic regression was used. A 95% confidence interval with a p value of <0.05 was considered statistically significant in all cases.

Results

We tested gastric biopsy samples from 259 patients. Included were samples from 136 (53%) females and 123 (47%) males. The median age was 48 years (IQR 40–61

Table 1. Basic characteristics of the study participants

Characteristic	Total samples tested, *n* (%)	Positive *H. pylori* amplification, *N* (%)	Wild-type strains, *N* (%)	CLA-resistant strains, *N* (%)	OR; 95% CI for resistant strains	*p* value
Total number	259	174 (67)	126 (72)	48 (28)		
Sex						
Female	136 (53)	94 (54)	65 (52)	29 (60)	1.5; 0.7–3	0.31
Male	123 (47)	80 (46)	61 (48)	19 (40)	Reference	
Age						
<30 years	10 (4)	9 (5)	5 (4)	4 (8)	–	0.23
30–45 years	90 (35)	63 (36)	52 (41)	11 (23)		
45–60 years	87 (33)	55 (32)	35 (28)	20 (42)		
>60 years	72 (28)	47 (27)	34 (27)	13 (27)		
Residence						
Rural	50 (19)	36 (21)	29 (33)	7 (15)	0.6; 0.2–1.5	0.30
Urban	209 (81)	138 (79)	97 (76)	41 (85)	Reference	
Body mass index						
<18.5 kg/m^2	40 (17)	25 (14)	21 (17)	4 (8)	0.5; 0.1–1.6	0.32
≥18.5 kg/m^2	193 (83)	128 (74)	92 (73)	36 (75)	Reference	
Missing	26 (10)	21 (12)	13 (10)	(17)		
Gastric pH						
<4	138 (53)	91 (52)	62 (49)	29 (70)	1.7; 0.7–1.4	0.19
≥4	87 (34)	56 (32)	44 (35)	12 (30)	Reference	
Missing	34 (13)	27 (16)	20 (16)			
PPI use						
Yes	166 (64)	114 (66)	84 (67)	29 (60)	0.7; 0.3–1.5	0.32
No	59 (23)	43 (25)	28 (22)	12 (25)	Reference	
Missing	34 (13)	17 (9)	14 (11)	7 (15)		
Endoscopic diagnosis						
Normal	164 (63)	111 (64)	77 (61)	34 (71)	–	0.13
Gastric ulcer	46 (18)	32 (18)	22 (17)	10 (21)		
Duodenal ulcer	34 (13)	23 (13)	20 (16)	3 (6)		
Other diagnoses*	15 (6)	8 (5)	7 (6)	1 (2)		
Histological diagnosis						
Normal	3 (1)	2 (1)	2 (2)	0 (0)	–	0.92
NAG	220 (85)	144 (85)	102 (81)	42 (88)		
CAG	8 (3)	6 (4)	5 (4)	1 (2)		
GIM	22 (9)	18 (11)	13 (10)	0 (0)		
Missing	6 (2)	4 (2)	4 (3)	5 (10)		
HIV						
Positive	42 (16)	30 (17)	23 (18)	8 (17)	0.7; 0.2–2	0.65
Negative	192 (74)	131 (75)	93 (74)	36 (75)	Reference	
Missing	25 (10)	13 (8)	10 (8)	4 (8)		
Alcohol consumption						
Yes	58 (58)	36 (21)	26 (21)	10 (21)	1.1; 0.4–2.6	0.83
No	186 (72)	126 (72)	93 (74)	33 (69)	Reference	
Missing	15 (15)	12 (7)	7 (5)	5 (10)		
Cigarette smoking						
Yes	22 (9)	8 (5)	8 (6)	0 (0)	–	0.11
No	205 (59)	145 (83)	100 (80)	45 (94)		
Missing	32 (12)	21 (12)	18 (14)	3 (6)		

None of the histology sections had dysplasia or malignancy. NAG, non-atrophic gastritis; CAG, chronic atrophic gastritis; GIM, gastric intestinal metaplasia; HIV, human immunodeficiency virus; PPI, proton pump inhibitor. *Other diagnoses include gastritis, erosions, polyps, reflux esophagitis, esophageal ulcer, and esophageal candidiasis.

Table 2. Presence of mutations that confer resistance to CLA evaluated in 174 samples that showed positive *H. pylori* DNA amplification

Real-time PCR result	Number	Proportion
A2142G mutation	23	13%
A2143G mutation	32	18%
A2142C mutation	0	0%
A2142C and A2143G double mutation	6	3%
A2142G and A2143G double mutation	0	0%
Any mutation	48	28%

years). Fifty-nine (23%) of the patients had history of proton pump inhibitor use prior to sample collection. The most common presenting symptom was abdominal pain in 192 (74%) followed by hematemesis in 30 (12%). The remaining patients presented with vomiting (n = 8, 3%), unexplained anemia (n = 7, 3%), or dysphagia (n = 2, 1%) with the rest (n = 20, 8%) having either non-specific or non-gastrointestinal symptoms. Endoscopically, most of the patients, 164 (63%), had a normal gastric mucosa (Table 1). Histologically, 220 (85%) had non-atrophic gastritis and 30 (12%) had chronic atrophic gastritis. Of those with chronic atrophic gastritis, 22 (9%) had gastric intestinal metaplasia while 8 (3%) did not have gastric intestinal metaplasia. There were no statistically significant differences of characteristics between patients with CLA resistance and those without (Table 1).

Real-Time PCR for H. pylori *in Gastric Biopsies*

Of the 256 samples tested, 174 (67%) had positive amplification for *H. pylori* DNA. Wild-type *H. pylori* was present in 126 (72%) of the samples, with the remaining 48 (28%) having mutations that confer CLA resistance. A2142G mutation was present in 23 (13%) and A2143G mutation in 32 (18%). Double mutations A2142C and A2143G were demonstrated in 6 (3%) of the samples (Table 2).

Detection of H. pylori *Infection Using Different Methods*

A total of 202 patients had results for real-time PCR, histology, and serology. Serological antibody detection was done using a commercial ELISA kit and a multiplex assay. Positive results for *H. pylori* were found in 200/202 (99%) by commercial ELISA antibody testing, 177/202 (88%) by the multiplex, 138/202 by PCR, and 35/202 (17%) on histology. There was considerable overlap among all the tests, with histology detecting the least infections (Fig. 1).

Factors Associated with CLA Resistance

We ran a stepwise logistic regression to evaluate factors that could be associated with CLA resistance among the patients enrolled in the study. Included in the model were age, sex, residence, body mass index, gastric pH, endoscopic diagnosis, histological diagnosis, human immunodeficiency virus infection, alcohol consumption, and cigarette smoking. None of the other factors were associated with CLA resistance.

Discussion

This study has provided evidence against empirical use of CLA as a first-line treatment for *H. pylori* infection in Zambia. We found that 28% of the samples had mutations that confer *H. pylori* resistance to CLA. Current guidelines are that CLA should not be used empirically in settings with more than 15% resistance [5].

The World Health Organization has included CLA-resistant *H. pylori* on the high-priority pathogen list of antibiotic-resistant bacteria (WHO) [17]. *H. pylori* eradication is becoming more challenging due to increasing antimicrobial resistance, but most data are drawn from outside the African continent. The prevalence of *H. pylori* resistance differs worldwide, and it is increasing in several countries [18]. Some African countries such as Egypt have reported rates of CLA resistance as high as 59% [19]. In Tunisia, the resistance rate was 32% [20], 36% in Sudan [21], 29% in Tanzania [22], 30% in Algeria [23], and 20% in South Africa [24]. These reports from other African countries demonstrate that our findings are not unique, but rather conform to the fact that CLA should not be used empirically for treatment of *H. pylori* infection in Africa. The outlook is not very different outside the African continent. For example, a meta-analysis on CLA in China involving 38,804 *H. pylori* samples from 22 trials reported a resistance rate of 24% [25]. And in Europe, a large international multicenter prospective non-interventional registry reported a pre-treatment CLA resistance rate of 23% [26].

This information poses a challenge on appropriate recommendations for *H. pylori* eradication in Africa. *H. pylori* is a common infection on the continent, but the exact burden-associated disease conditions such as peptic ulceration and gastric cancer remain unclear. This lack of clarity led to the African enigma being proposed, stating that despite a high prevalence of *H. pylori* infection in Africa, related gastrointestinal conditions were uncommon [27]. The African enigma has however been contested by other scientists, who believe that it is just a

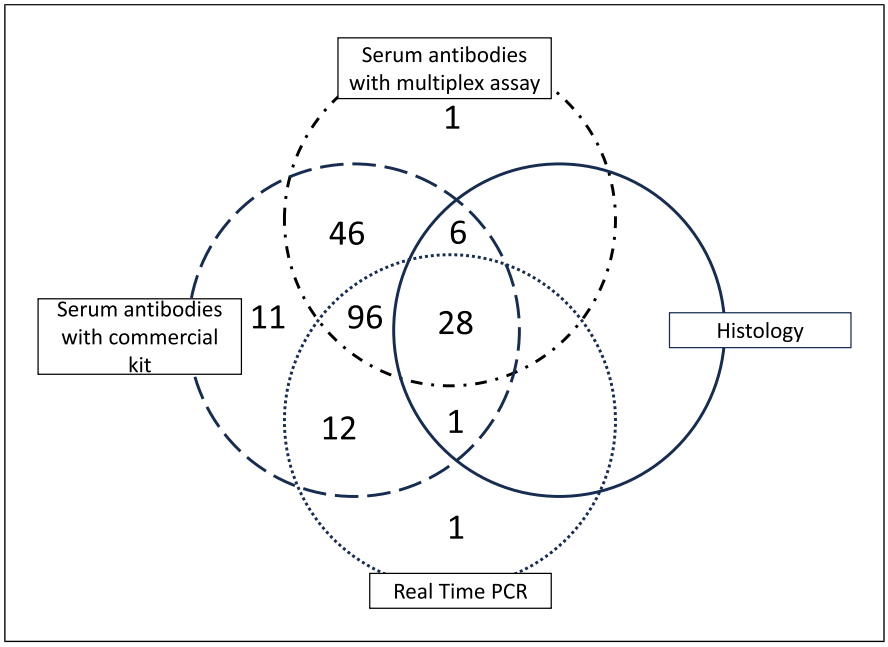

Fig. 1. A comparison of different *H. pylori* detection techniques including antibody detection in serum, histology, and real-time PCR.

medical myth based on limited scientific evidence [28]. Whether the African enigma is true or not, it is clear that African individuals presenting with active *H. pylori*-induced disease have to be treated. Therefore, there is a need to come up with optimal regimens that are not only available, but also affordable. The widely recommended test-and-treat strategy has not been investigated in Africa and is therefore, to the authors' knowledge, not widely implemented. Testing is mostly done on individuals presenting to healthcare centers with symptoms requiring therapy. With the clear benefits of successful eradication [5], there is an urgent need to come up with an appropriate first-line regimen for the African population.

The lack of comprehensive data on *H. pylori* infection in Africa is a significant factor limiting the development of treatment guidelines applicable on the continent. With these new available data, it is clear that international guidelines on *H. pylori* infection need to be modified for application in Africa, or indeed scientists on the continent should formulate appropriate guidelines. According to the latest Maastricht VI/Florence consensus report, in the presence of significant CLA resistance, a bismuth-based regimen should be used [5]. However, for unclear reasons, bismuth is generally not used or is unavailable in most parts of Africa [29]. Pooled resistance rates for a few African countries have shown that *H. pylori* resistance to other antibiotics that could be used as first line is also high. Reported prevalence rates of resistance include 29.2% for CLA, 75.8% for metronidazole, 72.6% for amoxycillin, 48.7% for tetracycline, and 17.4% for quinolones [30]. Mashiko and Smith [29] recently recommended that CLA continues being used as first-line empirical therapy in Africa as it has lower resistance rates when compared to the alternatives.

Using samples from the patients included in this study, we previously made diagnoses of *H. pylori* infection. Our comparisons suggest that histology is of a very low sensitivity for detection of the infection. However, it should be pointed out that none of the special stains such as Giemsa, Gimenez, periodic acid-Schiff, Alcian blue, or Warthin-Starry stains were used. We did not find any scientifically significant factors associated with CLA resistance using our logical regression model. This is an area that requires further investigation, to identify modifiable factors that could limit the spread or emergence of CLA resistance.

This was the first study from Zambia evaluating the prevalence of *H. pylori* CLA resistance. One limitation might be that our assessment was focused on the genotype and not the phenotypic properties of the bacteria. However, we are confident of the reliability of this approach as it has been used by many other researchers, demonstrating the accuracy of mutation testing [10]. In addition, a recent study showed that all specimens with culture-based resistance to CLA had A2143G mutations in 23S rRNA gene [31]. Another limitation was that we did not have information on whether the resistance detected was primary or secondary. It is therefore unknown if Zambian patients are acquiring resistant infections or if it develops as a result of

partial or inappropriate exposure to macrolides. CLA-resistant strains were isolated from food and houseflies in Egypt, and this is, therefore, an area that requires further investigation [32, 33].

Conclusions

New treatment guidelines for *H. pylori* are needed. With a resistance rate of 28% demonstrated in this study, international guidelines advise against using CLA as first line in Zambia. However, in the absence of appropriate alternatives, we may be obliged to continue its use.

Statement of Ethics

This study protocol was reviewed and approved by the University of Zambia Health Sciences Research Ethics Committee, approval number 202211230194. Fully informed and written consent was obtained from the study participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

V.K. and P.K. conceptualized the study. V.K., T.K., and D.M. were involved in writing the proposal and submitting it for ethical approval. V.K. collected the biological samples and wrote the initial version of the manuscript. T.K. and L.B. conducted the laboratory assays. T.K., L.B., D.M., P.K., and V.K. all read, edited, and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are openly available in Dryad (https://doi.org/10.5061/dryad.7sqv9s4zz). Further inquiries can be directed to the corresponding author.

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