Prevalence and Antibiotics Susceptibility Profile of *Helicobacter pylori* Isolated from Outdoor Patients Presenting Signs of Ulcerative Colitis in Three Hospitals in Niger State, Nigeria

***Kuta, F.A., Audu, S.L., Garba, S.A., Abdusalam, R., Adabara, N.U.**

Department of Microbiology, School of Life Sciences, Federal University of Technology, Minna, Nigeria

Received: September 21, 2016; Revised: December 5, 2016; Accepted: December 16, 2016

Abstract

The present study was conducted to determine the prevalence and antibiotic susceptibility profile of *Helicobacter pylori* isolated from patients presenting signs of ulcerative colitis in three hospitals in Minna, Niger State, Nigeria. The study obtained a prevalence of 12%, out of the 350 patients screened for *Helicobacter pylori*, using Enzyme Linked Immunosorbent Assay (ELISA) technique. Patients with the highest rate of infection (3.43%) were within the age range 26-30years and the lowest prevalence of 0.29% was recorded among patients within the age range 1-5 years. Male patients recorded more prevalence (7.71%) compared to their female counterparts (4.29%). Patients from rural areas had more infection rate (10%) than patients from urban areas (2%). Patients that use water from streams recorded more infection rate (6%) compared to those that use water from the well (4%). The prevalence was more in Minna (5.43%), followed by Paiko (3.71%) and lastly Kuta (2.86%). *H. Pylori* was isolated from the gastric duodenal biopsies, using blood agar. Further identification of the isolates was through biochemical tests. Antibiotics susceptibility pattern indicated that all the antibiotics used were active against the *H. pylori* at high concentration but the organism demonstrated resistance on almost all the drugs used at low concentrations. Extensive awareness on *H. Pylori* infection among rural dwellers should be encouraged.

Keywords: Antibiotic, Susceptibility. *H. Pylori*, Prevalence, Ulcerative colitis

1.0 Introduction

Helicobacter pylori is a gram negative, micro-aerophilic spiral shaped, flagellated, bacillus which colonize the mucus layer of the gastric epithelium [1]. The organism tests positive for oxidase, catalase and urease. *H. Pylori* infection is common worldwide with prevalence rates ranging 30 to 40% in the United States, 80 to 90% in South America and 70 to 90% in Africa [2]. It is more common in developing countries, and its prevalence increases with age from 20% among teenagers to 50 and 60% of subjects in the 6th and 7th decades of life respectively. This agent is now regarded as the most important risk factor for developing peptic ulcer disease (PUD). To a large extent, the epidemiology of PUD reflects that *H. Pylori* infection increases dramatically with age [3]. An estimate of annual incidence of PUD in *H. Pylori* infected individual is about 6 to 10 fold higher than that for uninfected individuals. In Europe, Australia and the United States, 95% of duodenal ulcers and 60 to 70% of gastric ulcers are associated with *H. Pylori* infection [4]. In Nigeria, almost 100% of duodenal ulcer and 82% of gastric ulcer patients are *H. Pylori* positive [3]. Further evidence that links *H. Pylori* to the development of PUD is the low recurrence rate of peptic ulcers, (less than 20%) [5].

H. pylori is found in half the population of the world. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors— high in developing countries and lowest in the developed world. In general, however, there has been a decreasing trend in the prevalence of *H. Pylori* in many parts of the world in recent years. Direct epidemiologic comparisons of PUD between developing and developed countries are complex, as peptic ulcers may be asymptomatic and the availability and accessibility of the tests required for diagnosis vary widely.

***Corresponding Author: Tel: +234(0)7032738427, E-mail: farukkuta@gmail.com**

Methods available for diagnosis of *H. pylori* include invasive (via endoscopic biopsy specimens) and non-invasive tests. The most reliable non-invasive test is the urea breath test (UBT) with specificity and sensitivity approaching 95 to 100% [6]. It is however costly and not readily available in most developing countries. Other non-invasive tests like serological detection of serum antibodies to *H. Pylori* infection and the stool antigen test are also not widely available. Histology of endoscopically taken gastric biopsy has a very high sensitivity (96%) and specificity (98.8%) and also cheap, but it requires expertise. Other invasive methods of detecting *H. Pylori* are Gram stain and culture with sensitivities of 92.2 and 98.4% respectively [7].

In developing countries, *H. Pylori* infection is a public-health issue and the high prevalence of the infection implies that public-health interventions may be required. Therapeutic vaccination is probably the only strategy that would make a decisive difference in the prevalence and incidence of *H. Pylori* throughout the world. This study therefore attempt to determine the prevalence and antibiotic susceptibility profile of *H. Pylori* isolated from patients presenting signs of ulcerative colitis in three hospitals in Niger State, Nigeria.

2.0 Materials and Methods

2.1 Study Population

A total of 350 outpatients visiting three general hospitals (Minna, Kuta and Paiko) were recruited for the study. Ethical clearance and informed consent were sought for from the Ethical Committee of the University (provide clearance number). A structured questionnaire was administered in order to obtain demographic information about the study population, before the collection of the blood samples. The sample size was determined by the following equation [8].

$$n = \frac{t^2 p(1-p)}{m^2}$$

Where: n = Sample size
t = Standard normal deviate at 1.96
p = prevalence of the disease (National prevalence)
m = marginal tolerable error at 0.05%

2.2 Blood sample collection and preparation for screening

The vein puncture method was employed and 2 ml of blood sample each from the 350 patients was aseptically collected, using a disposable sterile needle and syringe. Blood samples were collected and transferred into labeled plastic microtitre tubes coated with ethylene diamine tetraacetic acid (EDTA) and transported to the Microbiology Laboratory, Federal University of Technology, Minna for analysis. The blood samples were transferred into centrifuge tubes and spun at 10,000 revolutions per minute (rpm) for 10 minutes. The plasma cells were collected in each case separately and stored at 4°C for further analysis [9].

2.3 Screening of Blood Sample

Acume® test strip (with an accuracy of 98.5%), a rapid chromatographic immunoassay for the qualitative detection of *H. pylori* in plasma was used for the screening of plasma cells from the patients. The test was carried out and interpreted according to the manufacturer's instructions. Briefly, the test strips, plasma and control were allowed to equilibrate to room temperature (15-30◦C) prior testing. The test strip was removed from the sealed pouch and used immediately. The test device was then placed on a clean and level surface. For Plasma specimen: The dropper was held vertically and 4 drops of plasma were transferred (approximately 100 µl) to the specimen well (S) of the test device, then the timer was set and the result was read after 20 minutes. The one step test *H. pylori* strip (with accuracy of 98.5%), has a control band (C) and a test band (T).When two distinct bands appear, the result is said to be positive. However, the intensity of the red colour in the test band region (T) will vary depending on the concentration of *H. pylori* present in the specimen. Therefore, any shade on the test band region (T) is considered positive. The absence of apparent red bands on the test band region (T) indicates a negative result and the result is said to be invalid when the control band fails to appear [10].

2.4 Isolation of Helicobacter pylori

The gastric/duodenal biopsies were collected from 350 outdoor patients presenting signs of ulcerative colitis at the three general hospitals (Kuta, Minna and Paiko). The specimens were placed in sterile normal saline and were transported to the Microbiology Laboratory, Federal University of Technology, Minna for further analysis [10].

2.5 Inoculation of the Isolates

The homogenized specimens were inoculated on the blood agar. The inoculated media were incubated at 37^0C for 24 hours, under microaerophilic condition [10].

2.6 Identification of the Isolates

Biochemical tests were performed on the isolates for identification purpose. The tests conducted include Gram stain, oxidase test, ureases test and catalase test. These tests were conducted to complement the result obtained from Enzyme Linked Immunosorbent Assay [10].

2.7 Antibiotic Susceptibility Test

The disc diffusion technique was used to determine the antibiotic susceptibility profile of the isolates. The procedures involved dispensing 400ml of nutrient agar in 20 plates (i.e. 20 ml in each plate) and the media were allowed to stay for 5 minutes. The plates were sterilized by autoclave at 121^oC for 15 minutes before inoculation with the test organisms and then incubated at 37^0C for 24 hours further impregnated with discs of different antibiotics [10].

2.8 Data analysis

Data were entered and analyzed using Statistical Package for Social Science (SPSS) version 20 computer software. Comparison of categorical variable was done using the Chi- square test, non- parametric test, whereas p value of 0.05 was considered significant level.

3.0 Results

3.1 Distribution of Infection Rate According to Sex of the Patients

Out of the 350 blood samples screened from the three hospitals used as case study, 42 were found positive representing 12% prevalence. The isolates were found to be Gram negative, Oxidase positive, catalase positive and urease positive. Patients within the age range 26–30 years had more infection rate of 12(3.43%), compared to the other age groups (Table 1).

Table 1: Distribution of Infection According to Age of the Patients

Age group	Number of samples screened	Number of positive samples	Prevalence (%)
1-5	25	1	0.29
6-10	31	3	0.86
11-15	30	3	0.86
16-20	39	6	1.71
21-25	35	2	0.57
26-30	42	12	3.43
31-35	63	5	1.43
36-40	30	6	1.71
41 and above	55	4	1.14
Total	**350**	**42**	**12.00**

χ^2 Calculated = 18.4086, $\chi^2_{0.05}$, 8 =15.507

The distribution of infection rate according to sex is presented in Table 2. From the 350 samples screened, male patients had more prevalence rate of 27(7.71%) compared to their female counterpart 15(4.29%). The distribution of infection rate, according to location of residences is given in Table 3. Patients from rural areas recorded 35(10%) prevalence compared to patients from urban areas 7(2%).

Table 2: Distribution of Infection According to Sex of the Patients

Sex	Number of samples screened	Number of positive samples	Prevalence (%)
Male	240	27	7.71
Female	110	15	4.29
Total	**350**	**42**	**12.00**

χ^2 Calculated = 0.4066, $\chi^2_{0.05}$, 1 = 3.84

Table 3: Distribution of Infection According to Location of Residence of the patients

Location	Number of samples screened	Number of positive samples	Prevalence (%)
Rural	250	35	10.0
Urban	100	7	2.0
Total	**350**	**42**	**12.0**

$\chi^{2\ Calculated}$ = 3.3143, $\chi^2_{0.05}$, 1 = 3.84.

The distribution of infection according to water sources is given in Table 4. Patients that drink water from streams (untreated surface water) recorded more prevalence (6%) and the least (2%) prevalence was recorded among patients that drink water from the tap (pipe borne water). The distribution of infection according to the three hospitals used as case study is presented given in Table 5. The prevalence of infection was more in Minna, the state capital with a prevalence of 5.43%, followed by Paiko (3.71%) and Kuta (2.86%).

Table 4: Distribution of infection according to water source

Source of water	Number of samples screened	Number of positive sample	Prevalence (%)
Pipe bone	100	7	2.0
Stream	180	21	6.0
Well	70	14	4.0
Total	**350**	**42**	**12.0**

χ^2Calculated = 6.6285, $\chi^2_{0.05}$, 2 = 5.991

Table 5: Prevalence of *H pylori* according to hospitals

Hospital location	Number of samples screened	Number of positive samples	Prevalence (%)
Minna	120	19	5.43
Kuta	115	10	2.86
Paiko	115	13	3.71
Total	350	42	12.00

The data presented in Table 6 show the relationship between demographic information about the patients and infection rate. The results revealed that there is a significance difference between age and water source while sex and location of residence of the patients are not significantly linked to infection (P>0.05).

Table 6: Relationship between demographic information of patients and infection rates

Factor	Number of positive samples	P-value
Age		
1-5	1	
6-10	3	
11-15	3	
16-20	6	
21-25	2	18.4086
26-30	12	
31-35	5	
36-40	6	
41 and above	4	
Sex		
Male	28	
Female	14	0.4066
Location		
Rural	35	
Urban	7	3.3143
Source of water		
Pipe Bone	7	
Stream	21	6.6285
Well	14	

Antibiotic susceptibility pattern of *H. pylori* isolated from the patients attending the three hospitals revealed that the activity of the drugs on the organism was concentration dependent i.e. as the concentration increases the better the activity (Table 7).

Table 7: Antibiotic susceptibility profile on *H. pylori*

	Concentration of antibiotics (mg/ml)				
Antibiotic	**25**	**50**	**100**	**125**	**150**
Penicillin	-	-	+	+	+
Tetracycline	-	+	+	+	+
Chloramphenicol	-	-	+	+	+
Erythromycin	-	-	+	+	+
Kanamycin	-	-	+	+	+
Amikacin	-	-	-	+	+
Gentamycin	-	+	+	+	+

Key: (-) Resistance (+) Susceptible

4.0 Discussion

In this study, the prevalence of *H. pylori* among patients visiting three General Hospitals in Niger State (General Hospital Minna, Kuta, and Paiko) for medical care was investigated. Out of the three hundred and fifty (350) blood samples screened, 42(12.0%) were found to be positive. The prevalence obtained in this study appears to be low when compared to the prevalence reported in previous studies [8, 11] and this could be attributed to improvement in good hygienic practices, coupled with awareness on the possible route of transmission.

The prevalence of infection was higher among patients within the age group 26–30years 12(3.43%) and the least prevalence (0.29%) was recorded between 1-5 years age group (Table 1). Studies have reported high prevalence of *H. pylori* among adolescents consistently [8,11,12]. The outcome of this study is therefore comparable with the previous studies. Chi square analysis revealed that the age of patients was a significant factor in the infection rate with *H. pylori* in the study area at $p>0.05$ (Table 6). Male outdoor patients recorded more prevalence compared to their female counterparts (Table 2). Similar trend has been reported by Kuta *et al*. [8] which was attributed to life style and occupational hazards. This can also be used as the possible explanation for the nature of result obtained in this study.

Patients from rural areas had a prevalence of 35(10%) while those from urban areas recorded a low prevalence of 7(2.0%) (Table 3). The result of this study is in agreement with previous work which reported low prevalence of *H. pylori* among urban dwellers as against the rural dwellers [13,14]. Similarly, patients who drink water from streams had a higher prevalence of 21(6.0%) as against those that drinks water from the tap (pipe borne water) 7(2.0%) (Table 4). Several studies have implicated untreated water as a potential source of infection with *H. pylori* in Nigeria [14, 15]. The outcome of this study corroborates previous reports. Chi square analysis further confirmed that the source of water relates to the infection rates with *H. pylori* (Table 6).

The prevalence of *H. pylori* infection was observed to be high in outdoor patients visiting Minna General Hospital (Table 5). It is interesting to note that *H. pylori* infection which is associated with poor hygienic practices is more serious in the urban area as observed in this study. However, similar incidence has been reported by Wang *et* al. [9] and it was attributed to the cosmopolitan nature of most cities in Nigeria coupled with poor refuse disposal system. This may be the possible explanation for the result obtained in this study.

The *H. pylori* isolated from all the patients screened were Gram –ve but oxidase, catalase and urease positive. The result of the biochemical tests obtained from this study agrees with previously documented report [10]. The antibiotic susceptibility pattern observed was concentration dependent (Table 7). The susceptibility was observed at high concentration (50–150 mg/ml). Similar pattern has been documented [3,10]. Therefore, this study is comparable with previous studies.

5.0 Conclusion and Recommendations

The antibiotic susceptibility pattern of *H. pylori* suggests a dangerous trend, in the sense that all the antibiotics used exhibited activities only at high concentration. The implication of this pattern is that with time, the organisms may be resistant to the concentrations of the drugs that showed activities in this study and ultimately lose activity against such isolates. Adequate enlightenment campaigns should be encouraged, particularly in rural areas on the danger of drug abuse, administration of antibiotics not prescribed by trained medical personnel and self-medication.

Reference

[1] Genta, R.M. (2015). After gastritis – An imaginary journey into a *Helicobacter*-free world. Alimentary Pharmacology Therapy, Vol. 16, No. 4**,** pp. 89-94.

[2] Perez-Perez, G.I., Olivares, A. Z., Foo, F. Y., Foo, S., Neusy, A. J., Ng, C., et al. (2005). Seroprevalence of *Helicobacter pylori* in New York City populations originating in East Asia. Journal of Urban Health, Vol. 82, pp. 510-516.

[3] Ndububa, D.A., Agbakwuru, A.E. and Adebayo, R.A. (2001). Upper gastrointestinal findings and incidence of *H. pylori* infection among Nigerian patients with dyspepsia. West African Journal of Medicine, Vol. 20 No.2, pp. 140-145.

[4] McLoughlin, R.M., O'Morain, C.A. and O'Connor, H.J. (2005). Eradication of *Helicobacter pylori*: Recent advances in treatment. Fundamentals of Clinical Pharmacology, Vol. 19, pp. 421-427.

[5] Fischbach, W., Malfertheiner, P., Hoffmann, J.C., Bolten, W., Kist, M. and Koletzko, S. (2009). *Helicobacter pylori* and gastroduodenal ulcer disease. Dtsch. Arztebl. Intl., Vol. 106, No. 49, pp. 801-808.

[6] Michel, A., Waterboer, T., Kist, M. and Pawlita, M. (2009). *Helicobacter pylori* multiplex serology. Helicobacter, Vol. 14, No. 6, pp. 525-535.

[7] Oyedeji, K.S., Smith, S.I., Arigbabu, A.O., Coker, A.O., Ndububa, D.A., Agbakwuru, E.A. *et al*. (2002). Use of direct Gram stain of stomach biopsy as a rapid screening method for detection of *Helicobacter pylori* from peptic ulcer and gastritis patients. Journal of Basic Microbiology, Vol. 42, No. 2, pp.121-125.

[8] Kuta, F.A., Ijalana, O.M., Damisa, D. and Abdulrahman, A.A. (2014). Epidemiology of *Helicobacter pylori* among out Patients Visiting Ibrahim Badamasi Babangida (IBB) Specialized Hospital, Minna, Niger State, Nigeria. Scientia Research Library Journal of Applied Science and Research, Vol. 2 No. 4, pp.11-13.

[9] Wang, D., Chiu, T. and Chiu, K.W. (2014). Clinical implication of immunoglobulin G levels in the management of patients with *Helicobacter pylori* infection. Journal of American Board of Family Medicine, Vol. *27*, pp.682–689.

[10] Blaser, M.J. and Atherton, J.C. (2014). *Helicobacter pylori* persistence, Biology and Disease. Journal of Clinical Investigation, Vol. 113, pp. 321-333.

[11] Ochei, J. and Kolhatkar, A. (2007). Medical Laboratory Science: Theory and Practice. 5th Edn., McGram-Hill, New Delhi, India, pp: 788-812.

[12] Linz, B., Balloux, F., Moodley, Y., Manica, A., Liu, H., Roumagnac, P. and Achtman, M. (2007). An African origin for the intimate association between humans and *Helicobacter pylori*. Nature, Vol. 445, No.7130, pp. 915-918.

[13] Malaty, H. M. (2007). Epidemiology of *Helicobacter pylori* infection. Best Practice and Research Clinical Gastroenterology, Vol. 21, No.2, pp. 205-214.

[14] Hong, C., Fulian, H., Lian, Z., Guibin, Y., Junling, M., Jichun, H., Weihong, W., Wen, G. and Xinhong, D. (2009). Prevalence of *Helicobacter pylori* Infection and Identification of Risk Factors in Rural and Urban Beijing, China. Wiley, Vol. 14, No.2, pp. 128 – 133.

[15] Timothy, N. A. A., Richard, H. A., Edwin, K. W., Richard, K. G., Kofi, N. N. and Kumar, R. (2015). Epidemiology of *Helicobacter pylori* infection in dyspeptic Ghanaian patients. The Pan African Medical Journal, Vol. 20, pp. 178 – 184.