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Antibiotic Susceptibility of *Helicobacter pylori* From Gastric Biopsy of Patient Attending Benue State University Teaching Hospital, Makurdi, Benue State

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Abstract
Helicobacter pylori is a Gram-negative bacterium that causes chronic infections such as gastritis, peptic ulcer, gastric cancers and gastric malt lymphoma. The aim of the study was to assess the distribution of *Helicobacter pylori* from patients referred for endoscopy at Benue State University Teaching Hospital (BSUTH), Makurdi. Two (2) gastric biopsy samples (n=160) were obtained from the antrum of 80 consenting endoscopy patients at BSUTH, Makurdi, and used for isolation, identification of *H. pylori* using standard microbiological techniques. Antibiotic susceptibility determination was by the Clinical and Laboratory Standards Institute (CLSI) M100-Ed33 standard. Positive culture results were, however, poor as only one (1.25%) *H. pylori* isolate was isolated, which showed a 14 (82. 4%) susceptibility rate to Amoxicilin (20µg), Augmentin (20/10µg), Ampiclox (10/10µg), Zinacef (30µg), Gentamycin (10µg), Nitrofurantoin (300µg), Streptomycin (10µg), Tetracycline (30µg), Erythromycin (15µg), Clarithromycin (15µg), Chloramphenicol (30µg), Moxifloxacin (5µg), Ciprofloxacin (5µg), and Ofloxacin (5µg), and a 3 (17.6%) resistance to Ceftriaxone (30µg), Pefloxacin (5 µg) and Cotrimoxazole 1.3/23.8µg). The susceptibility of the isolated bacterium to most of the antibiotics used showed encouraging prospects for eradication therapies
Key words: Gastric biopsies, *Helicobacter pylori*, Antibiotic, Susceptibility

Introduction

Helicobacter pylori infection causes chronic gastritis, peptic ulceration, gastric cancers and gastric Mucosa Associated Lymphoid Tissue (MALT) Lymphoma **[1]**. *Helicobacter pylori* are rated as a "class one" carcinogen to the gastrointestinal tract by the World Health Organization **[2]**. It is in the same category as cigarette smoke is to lung cancer.

Although *H. pylori* are susceptible to a variety of antibiotics in vitro, few antibiotics are currently being used to treat the infection. The limited choice of antibiotics coupled with the emergence of drug resistance, especially metronidazole, amoxillin and clarythromycin and the capacity for horizontal gene exchange in *H. pylori* has posed a substantial challenge in the success of treatment regimens on a global scale **[3]**.

The prevalence of *H. pylori* and its resistance to different antibiotics have become increasingly widespread with a global prevalence of more than 50%. The prevalence is generally high in developing countries like Nigeria. The principal reasons for these variations may involve socioeconomic differences between populations.

Although extensive research has been carried out on *H. pylori*, the research so far in Nigeria has tended to focus on its prevalence in States other than Benue. Very little literature was found on the antibiotic susceptibility patterns in Benue State. The study was designed to isolate the bacterium in Makurdi, Benue State, and to determine its antibiotic sensitivity patterns so that appropriate therapy can be proposed for clinical practice within the State.

H. pylori is very common in Nigeria as in other developing countries **[5]**. Resistance of *H. pylori* towards different antibiotics is increasing worldwide **[6]**. As such, isolating the bacterium and evaluating the antibiotic sensitivity patterns of *H. pylori* isolates among patients in Benue State University Teaching Hospital, Makurdi, will help provide local information in managing, controlling and planning strategies for improving care and treatment of patients. The results of this work will help provide an effective means of treatment of ulcers caused by the bacterium which will bring a big relief from the burden of this infection on patients.

Materials and Methods

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi. All participants had medical referrals for gastric biopsy at the Department of Gastroenterology of the Benue State University Teaching Hospital, Makurdi. Volunteer participants were informed of the details of the study and consent duly obtained from them.

Sample Size Determination

Sample size was calculated using Raosoft Sample Size Calculator **[7]**. At 0.05 alpha level of significance, 95% confidence level, and a patient population size of 99 and a previous prevalence 50%, a sample size of 80 was obtained.

Sample Collection

Two (2) gastric biopsy samples each were taken from the antrum of consenting patients by a Consultant Gastroenterologist. Pieces of tissue samples were collected into sterile McCartney bottles containing Brain Heart infusion broth with 1.5% glycerol and stored in the freezer at -20°C within 2 hours of collection until transported to the Safety Molecular Pathology Laboratory, Enugu, in ice packs, to maintain the cold chain, for culture.

Culture and Identification of H. pylori from Gastric biopsies

The gastric biopsy specimens were transported to Safety Molecular Pathology Laboratory, Enugu, Nigeria in Brain Heart infusion broth containing 1.5% glycerol in ice packs. They were homogenized using an electrical vortexing machine in a small volume of broth [8]. The Oxoid commercially available selective medium (Columbia Blood Agar Base supplemented with lysed horse blood) for *H. pylori* was used to culture the organism at 37°C in a microaerophilic condition (6% Oxygen and 10% Carbon dioxide) by placing the culture plate for 5 days in a closed jar containing GENbox microaer (a generator for culture in jars of microaerophilic bacteria). Small, circular, smooth colonies observed were Gram stained to check for Gram reaction. Colonies were also examined for urease, catalase and oxidase reactions.

A). ***Urease Test for Detection of H. pylori***

Urea Agar Base (Oxoid) was used. Urea agar base (2.4 %) was suspended in 95ml of distilled water, boiled to dissolve completely and was sterilized by autoclaving at 115°C for 20 minutes and cooled to 50°C. One ampule of 40% sterile urea solution was added aseptically, and the suspension was mixed well. A 10ml aliquot of the suspension were distributed into sterile tubes and allowed to set in a sloppy position and the gastric biopsy specimens in brain heart infusion broth were stab inoculated in the urease agar, covered with cotton wool and incubated for 16 hours. Positive samples changed the colour of the agar from yellow to purple pink.

Urease in the presence of water converts urea to ammonia and carbon dioxide. The Ammonia makes the medium alkaline and the phenol red indicator changes to purple red.

B). Catalase test

A drop of three percent hydrogen peroxide was placed on the colonies of *H. pylori* to see if there would be prompt effervescence indicating catalase production.

C). Oxidase test

A fresh solution of 1% tetramethyl-p-phenylenediamine dihydrochloride (oxidase reagent) was prepared by dissolving 0.1g of the reagent into 10ml of sterile distilled water. A drop of the solution was placed on a piece of filter paper. The test colony was picked with a sterile loop and rubbed on the filter paper in the area impregnated with the oxidase reagent. Oxidase-positive organisms would turn the paper deep purple blue in color in a few seconds. This test indicates the ability of organisms to oxidize amines. *H. pylori* are Gram-negative, urease, catalase, and oxidase positive.

Antibiotic Susceptibility of H. pylori

A suspension of a single colony of the test isolates equal to McFarland's turbidity standard 0.3, which matched the bacterial count 9 x 10^8/mL was used to estimate the number of bacteria. The bacterial number adjusted on a spectrophotometer (SCITEK Uv-Vis Model sp-VG722) to 10^6 colony-forming units per ml of suspension (1 × 10^6 cfu/m^{-1}) was inoculated onto Mueller-Hinton agar plates supplemented with red blood cells [9]. Commercially prepared antimicrobial discs for the antibiotics Amoxicillin (10 *µg*), Clarithromycin (15 *µg*), Ciprofloxacin (5 *µg*), Moxifloxacin (5 *µg*), Gentamycin (10 *µg*), Peflacine (5 *µg*), Ceftriaxone (30 *µg*), Augmentin (20/10 *µg*), Ampiclox (10/10 *µg*), Zinnacef/Cefuroxime (30 *µg*), Cotrimoxazole [Septrin] (1.3/23.8 *µg*), Erythromycin (15 *µg*), Ofloxacin (5 *µg*), Streptomycin (10 *µg*), Nitrofurantoin 300 *µg*), and Chloramphenicol (30 *µg*) were aseptically placed on the plates using the Oxoid Antimicrobial Susceptibility Testing Disc Dispenser. The plates were incubated microaerophilically at 37 °C. Zones of inhibition were read after 24-48 hours of incubation and interpreted according to **[10]**.

Results

Out of the eighty (80) biopsy specimen cultured, 1 (1.3 %) was positive for *H. pylori*. (Table 1). The *H. pylori* isolate was sensitive to 14 (82.4%) out of the 17 antibiotics used according to **[10]** diameter interpretative criteria for *H. pylori*, and was resistant to the remaining 3 (17.6%) (Table 2).

Table 1: Isolation of *Helicobacter pylori* by Culture (n=80)

Test Culture	Frequency No. (%)
Positive	1 (1.3)
Negative	79 (98.8)

Table 2: Antibiotics Susceptibility of *Helicobacter pylori* Isolate

S/no.	Class/Antibiotic	Disc content (*µg*)	Zone Diameter Interpretive Criteria *(mm)*			Zones of inhibition *(mm)*	Remarks
			Sensitive	Intermediate	Resistant		
1	**Penicillins**						
i	Amoxicillin	20	≥18	14-17	≤13	20	Sensitive
ii	Augmentin	20/10	≥18	14-17	≤13	20	Sensitive
iii	Ampiclox	10/10	≥15	12-14	≤11	18	Sensitive
2	**Cephalosporins**						
i	Ceftriaxone	30	≥23	20-22	≤19	6	Resistant
ii	Zinacef/Cefuroxime	30	≥18	15-17	≤14	20	Sensitive
3	**Aminoglycosides**						
i	Gentamycin	10	≥15	13-14	≤12	16	Sensitive
ii	Streptomycin	10	≥15	12-14	≤11	22	Sensitive
4	**Nitrofurans**						
i	Nitrofurantoin	300	≥17	15-16	≤14	20	Sensitive
5	**Tetracyclines**						
i	Tetracycline	30	≥15	12-14	≤11	21	Sensitive
6	**Macrolides**						
i	Erythromycin	15	≥23	14-22	≤13	24	Sensitive
ii	Clarythromycin	15	≥18	14-17	≤13	20	Sensitive
7	**Chloramphenicols**						
i	Chloramphenicol	30	≥18	13-17	≤12	20	Sensitive
8	**Fluoroquinolones**						
i	Moxifloxacin	5	≥24	21-23	≤20	20	Sensitive
ii	Ciprofloxacin	5	≥21	16-20	≤15	22	Sensitive
iii	Pefloxacin	5	≥24	-	≤23	20	Resistant
iv	Ofloxacin	5	≥16	13-15	≤12	20	Sensitive
9	**Sulfonamide/Trimethoprim**						
i	Co-trimoxazole	1.3/23.8	≥ 16	11-15	≤10	6	Resistant

Discussion

The low positivity with culture isolation method may be because it took a lengthy period for biopsy sample collection. Storage and transportation may have caused the organism to lose viability despite all the preservative measures taken. **[11]** also reported that low positivity rate of cultures in *H. pylori* diagnosis may be due to loss of viability during transport, low number of organisms, absence of organisms in the gastric biopsies, fastidious growth requirements, and presence of non-culturable coccoid forms. Any or all these factors may have come into play, resulting in the low isolation rate recorded.

Antibiotic susceptibility results show that the *H. pylori* isolate was susceptible to numerous antibiotics. In a review by **[12]**, high susceptibility of *H. pylori* to antibiotics was reported in different parts of the world, such as Africa, America, Asia, and Europe. However, these findings do not agree with previous studies in South-West Nigeria, where *H. pylori* isolates were reported to be resistant to Amoxicillin, Clarithromycin, Rifampicin, Tetracyclines, and Ciprofloxacin **[13, 14]**. The resistance shown by the isolate to ceftriaxone, a β-lactam antibiotic is reported to be due to a mutation in the *rdxA* gene which leads to increased resistance due to the inactivation of nitro-reductase **[15]**. Similarly, *H. pylori* typically show resistance to pefloxacin, a fluoroquinolone, driven by the widespread use of these drugs for other infections such as urinary or respiratory tract infections. Resistance to this drug is caused by specific point mutations in the *gyrA* and *gyrB* genes encoding DNA gyrase, the bacterial enzyme that is the target of fluoroquinolone

antibiotics. Mutations for instance, in codons N87 and D91 of *gyrA*, reduce the antibiotic's binding affinity to the enzyme, allowing the bacterium to continue DNA replication **[16]**.

While specific resistance genes were not investigated in this study, the antibiotic resistance observed could be because of differences in the bacteria's genotypes, which significantly play a role in the resistance or susceptibility to specific antimicrobials. Also, resistance in *H. pylori* is reported to be influenced by geographical considerations. Strains prevalent or dominant in the Southwest may have accounted for the observed susceptibility.

Conclusion

The susceptibility to currently used antibiotics showed that out of the seventeen antibiotics used, only three (Co-trimoxazole, Ceftriaxone and Pefloxacin) were ineffective. The remaining 14, including Amoxicillin, Augmentin, Ampiclox, Zinacef, Gentamycin, Nitrofurantoin, Streptomycin, Tetracycline, Erythromycin, Clarythromycin, Chloramphenicol, Maxifloxacin, Ciprofloxacin, and Ofloxacin, were all effective. This suggests that with proper diagnosis, effective treatment of *Helicobacter pylori* infection is possible within Makurdi and Benue.

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