

Original Research Article

Molecular characterization and prevalence study of stomach ulcer using standard techniques at Nnewi north local government area, Anambra state

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ABSTRACT

Background: Abnormality in one part of the body is capable of generating pandemonium in all body parts, which interferes with the normal body physiology and standard of living. This study was undertaken to evaluate the prevalence of stomach ulcer using standard techniques at Nnewi North Local Government Area, Anambra State.

Methods: Four hundred (400) samples were aseptically collected from participants using a well-capped sterile container and analyzed for the presence of *Helicobacter pylori* (*H. pylori*) using immunochromatographic assay for Stool Antigen Test (SAT) and Blood Antibody Test (BAT), Enzyme-linked Immunosorbent Assay ELISA for gamma Immunoglobulin (IgG) and mu immunoglobulin (IgM), conventional culture technique on selective Columbia blood agar base plus antibiotic and Vitalex growth supplement (Oxoid, England) was used for isolation of *H. pylori*. The bacterial isolate was characterized based on cultural, morphological, biochemical, and molecular characteristics [Polymerase Chain Reaction (PCR)].

Results: The highest number of *H. pylori* was detected using ELISA technique at Otolo community 48 (24.0 %) followed by culture technique (CT) at Umudim community [32(16.0 %), while the least was SAT at Otolo community 2 (1.0 %). Molecular characterization revealed certain strains of *H. pylori* such as *H. pylori* strain K154 (HPK154) complete genome, *Helicobacter pylori* strain BS07 (HPBS07) complete genome, *Helicobacter pylori* strain K93 (HPK93) complete genome, and *Helicobacter pylori* strain K115 (HPK115) complete genome.

Conclusions: Therefore, the prevalence of stomach ulcer in the sampled communities is high, especially at Otolo community, while ELISA proved to be the best diagnosing technique for accurate detection of *Helicobacter pylori*.

Keywords: Diagnosis, *Helicobacter pylori*, Prevalence, Stomach ulcer, Stool antigen test, Blood antibody test, ELISA, Culture, Molecular characterization

INTRODUCTION

Ensuring optimum health of mankind has been prioritized in biomedical sciences because, health is wealth indeed. Humans for decades had experienced several abnormalities in their physiology, which greatly lowered standard of living. These abnormalities had been attributed to infectious and non-infectious diseases. Infectious diseases had been described by the World Health Organization as the major source of ill health globally. One of the infectious diseases is stomach ulcer caused by *Helicobacter pylori*. *Helicobacter pylori* is a Gram-negative, microaerophilic, and spiral-shaped bacterium that affects more than half of the human population worldwide, and is more predominant in developing countries.^{1,2}

Research had revealed that *H. pylori* is the major bacterium responsible for stomach ulcer, which produces unbearable clinical manifestations to the infected individuals.^{3,4} The infection is generally acquired during childhood, but can remain asymptomatic with long-term clinical signs and symptoms such as gastric reflux, abdominal pain, intestinal bleeding, occasional fevers, and loss of weight which if not treated well can result to ulceration and perforation.^{5,6} The mode of transmission of *H. pylori* has not been fully elucidated, but studies had revealed that person-to-person transmission, fecal-oral and oral-to-oral routes can lead to the transmission of this infection.⁴

Moreover, several researchers had reported that stomach ulcer caused by *H. pylori* has predisposing or risk factors such as age, occupation, personal hygiene, educational background, nutrition, and location.^{3,4} The global report had shown that people in developing countries are more susceptible to the disease compared to people in developed countries, and this report is similar to the observation made by Liang et al on high level of the disease among rural dwellers who are tender and poorly educated.⁷

It is worthy to note that accurate detection of *H. pylori* in samples enhances effective treatment, though using an accurate detecting technique and experienced technicians had been a major challenge in several diagnostic laboratories. Several patients had been misdiagnosed of stomach ulcer resulting to abuse of antibiotics and increase in severity of the disease.

Several researchers had worked on detection of *H. pylori* in clinical specimen using different methods but there had been little research considering different diagnostic techniques in prevalence study of stomach ulcer in rural and urban community. Hence, the aim of this study is to evaluate the prevalence of stomach ulcer using standard techniques at Nnewi North Local Government Area, Anambra State. The findings in this study would provide vital information on the prevalence of stomach ulcer in the sampled region and the best diagnosing technique to arresting the menace.^{2,8,9}

METHODS

Study place

The study was carried out at Nnewi North Local Government metropolitan commercial city in Anambra State, Nigeria. Nnewi is located in the South-East zone in Anambra state, Nigeria. It is the second largest commercial city in Anambra State in south-eastern Nigeria with two local government areas, Nnewi North and Nnewi South. Nnewi North is commonly referred to as Nnewi central and is the center of commercial activities.

Study design

This is cross-sectional research to evaluate some immunological profile of *Helicobacter pylori* at Nnewi North Local Government Area Anambra State Nigeria. It is a hospital/school/Laboratory-based study.

Sample size

The sample size was calculated using the formula described in the study by Chukwuma et al.⁶

$$n = (z^2 pq) / d^2$$

Where n= minimum sample size.

P= proportion in target population using a prevalence of (51.4%) previous research on *H. pylori* in Nnewi according to Chukwuma et al.⁶

Z= Standard normal confidence interval, usually set at 1.96.

d = degree of accuracy, usually set at 0.05

q = 1.0 – p (that is, 1 - 0.514)

$$\text{Therefore:} = (1.96^2 \times 0.514 \times 0.48) / (0.05)^2 = (3.8416 \times 0.514 \times 0.48) / 0.0025 = 0.9477 / 0.0025$$

$$\text{Sample Size} = 379$$

However, for attrition, 400 samples were used in this study.

Study period

This study was conducted between June to September 2023.

Ethical Approval

This was obtained from the ethical committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka, Anambra State, Nigeria (COOUTH/CMAC/ETH.C/Vol.1/FN:04/220).

Inclusion criteria

All willing subjects within age range of 2 and above. Those not on antibiotics or herbal therapy one week prior to sample collection. Those attending clinic in the selected hospitals, Schools and Laboratory in Nnewi metropolis. Male and Female. Patients not on active immune suppressive therapy, coagulation drugs or showing coagulation effect, and malignant diseases (cancer) or are allergic to drugs used were included in inclusion criteria.

Exclusion criteria

Unwilling patients. Children below 2 years of age. Those on antibiotics or herbal therapy three weeks before sample collection. Those with a history of underlying diseases like diabetes, asthma, physical or mental impairment, pregnant or breastfeeding women. Patients on active immune suppressive therapy, proton pump inhibitors or Pepto-Bismol for at least 2 weeks, coagulation drugs or showing coagulation effect, and malignant diseases (cancer) or signs of allergy to drugs used were excluded.

Sampling technique

Random sampling technique was used to recruit participants in the study, comprising those who presented with or without the symptoms and signs that were suggestive of ulcers or gastritis. They were educated about the study and those willing to participate gave their consent in writing, and that of their parents/guardian until the required sample size was attained.

Sample collection

Four hundred (400) samples comprising of 200 blood and 200 stool samples were aseptically collected from different age groups in the sampled communities (Umudim, Uruagu, Otolu, and Nnewichi) using a well-capped sterile container. Before the collection, oral and written consent were obtained from the participants. The children that participated in the study were from private and public schools within Nnewi North metropolis while the adults that participated were drawn randomly from the area and private diagnostic centres.

Sterile Stericon wide open-mouth plastic stool container with a screw cap spoon without preservatives were given to each subject, and they were instructed to collect stool specimens following preclusive measures as described by.¹⁰

Freshly voided stool specimens were used. Blood samples were collected by vein-puncture method from the antecubital fossa of the hand in EDTA vacutainer and plain vacutainer tubes. 7 ml of blood were drawn from each participant, 2 ml of the blood dispensed into EDTA container were used for hematological parameter. The other 5ml in plain container were allowed to retract, serum separated from cells after centrifugation. Some of the sera were used for *H. pylori* rapid test.

Characterization of the isolates

The purified colonies were characterized using cultural, morphology, biochemical and molecular characterization.

Cultural characterization of the isolates

The colonies were characterized considering features such as colonial colour, elevation, size, surface appearance, consistency, margin, and optical nature according to Cheesbrough.¹⁰

Morphological characterization of the isolates

Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75 mm×25 mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2 %) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01 %) was then applied, and allowed for 60 seconds. This was rinsed with cleaned water.

This was followed by decolourizing the slide content with 95 %w/v ethyl alcohol for 10 seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025 %) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens.¹⁰

Motility test

A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml portion in different test tubes.

The test tubes were allowed to set in vertical positions, and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35±2°C for 24 h.

Biochemical characterization of the isolates

The capability of the isolates to produce catalase, indole, oxidase, and urease was done using the methods described in the study published by Iheukwumere et al.¹¹

Molecular characterization of the isolates

Extraction and purification of DNA

All strains were plated on nutrient agar (Biotech) and incubated at 37°C for 24 h. By means of the procedures of Zymo Research (ZR) DNA miniprep™ kit, bacterial

genomic DNA was then extracted and purified (Category No. D6005; Irvine, California, USA) as described in the study published by Iheukwumere et al.¹¹

Determination of the quality of the extracted DNA

Using mass spectrophotometer (Nanodrop), 1µl was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280 nm as described in the study published by Iheukwumere et al.¹¹

Amplification of DNA and gel electrophoresis of PCR product

This was analyzed using master cycler nexus gradient (Eppendorf). A mixture of primer (20 µl), template DNA (20 µl), water (72 µl) and master mix (108 µl), which comprises taq polymerase, dimethyl sulfoxide (DMSO), magnesium chloride (MgCl₂) and nucleotides triphosphates (NdTPs), was made in 1.5 ml tube and homogenized using vortex mixer (Eppendorf).

This was then positioned in the block chamber of the master cycler and then programmed. The amplified products were electrophoresed in 1.0 % agarose gel and a 1 kb DNA ladder was used as a size reference. After staining with 3 µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus as described in the study published by Iheukwumere et al.¹¹

DNA sequencing of 16s rRNA fragment

The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) as described in the study published by Iheukwumere et al.¹¹

The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using pair wise alignment tool.

The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the basic local alignment search tool (BLAST) using national centre for biotechnology information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted.¹¹

Catalase test

A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30 % hydrogen peroxide (H₂O₂) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test

The test involved two drops of freshly prepared oxidase reagent that were dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolates was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds for positive results.

Urease test

The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at 37°C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

Indole test

Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 ml of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized.

The medium was then inoculated with the isolates and kept in an incubator at 37°C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Statistical analysis

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 25. Chi-square test was used to check for the relationship between groups. P value<0.05 was considered statistically significant.

RESULTS

The prevalence of the isolates in the sampled villages at Nnewi North is presented in Table 1. The results revealed that *H. pylori* diagnostic test method with highest prevalence was observed with IgM ELISA antibody test kit method (81.5%), while lowest prevalence was observed with SAT (18%).

More so, across all villages sampled, highest positive prevalence for *H. pylori* using SAT was observed at Umudim village (44.4%).

At Uruagu village with BAT method (34.7%; 31.0%) with positive results was observed in IgG method and (27%) in IgM method. Prevalence at Otolu village (29.5%); Umudim village subjects sampled had the highest prevalence used culture method (32.7%); while highest positive samples using PCR method was observed at

Umudim village subjects (46.7%). There was a significant difference across the villages among positive and negative samples using; SAT method ($X^2=13.709$; $p=0.003$); BAT

method ($X^2=18.869$; $p=0.000$); IgM ab Elisa method ($X^2=14.695$; $p=0.023$); and culture method ($X^2=25.385$; $p=0.000$).

Table 1: Prevalence of the isolates in the sampled villages at Nnewi North.

Test method	Status	Villages					X2	P value
		Otolo N (%)	Umudim N (%)	Uruagu N (%)	Nnewichi N (%)	Grand total N (%)		
SAT	Positive	2 (1.00)	16 (8.00)	8 (4.00)	10 (5.00)	36 (18.00)	13.709	0.003*
	Negative	50 (25.00)	36 (18.00)	44 (22.00)	34 (17.00)	164 (82.00)		
	Total	52 (26.00)	52 (26%)	52 (26.00)	44 (22.00)	200 (100)		
BAT	Positive	30 (15.00)	22 (11.00)	33 (16.50)	10 (5.00)	95 (47.50)	18.869	0.000*
	Negative	22 (11.00)	30 (15.00)	19 (9.50)	34 (17.00)	105 (52.5)		
	Total	52 (26.00)	52 (26.00)	52 (26.00)	44 (22.00)	200 (100)		
IgG	Positive	19 (9.50)	26 (13.00)	27 (13.50)	15 (7.50)	87 (43.50)	5.953	0.429
	Negative	30 (15.00)	22 (11.00)	21 (10.50)	26 (13.00)	99 (49.50)		
	Equivocal	3 (1.50)	4 (2.00)	4 (2.00)	3 (1.50)	14 (7.00)		
	Total	52 (26.00)	52 (26.00)	52 (26.00)	44 (22.00)	200 (100)		
IgM	Positive	48 (24.00)	41 (20.50)	44 (22.00)	30 (15.00)	163 (81.50)	14.695	0.023*
	Negative	2 (1.00)	4 (2.00)	2 (1.00)	9 (4.50)	17 (8.50)		
	Equivocal	2 (1.00)	7 (3.50)	6 (3.00)	5 (2.50)	20 (10.00)		
	Total	52 (26.00)	52 (26.00)	52 (26.00)	44 (22.00)	200 (100)		
Culture	Growth	28 (14.00)	32 (16.00)	31 (15.50)	7 (3.5)	98 (49.00)	25.385	0.000*
	No growth	24 (12.00)	20 (10.00)	21 (10.50)	37 (18.50)	102 (51.00)		
	Total	52 (26.00)	52 (26.00)	52 (26.00)	44 (22.00)	200 (100)		
PCR	Positive	10 (5.00)	14 (7.00)	6 (3.00)	0 (0)	30	-	-

SAT: Stool Antigen Test; BAT: Blood Antibody Test; IgG: Immunoglobulin G; IgM: Immunoglobulin M; PCR: Polymerase Chain Reaction. *Statistically significant.

Table 2: Cultural characteristic features of the bacterial isolates.

Parameter	Isolate A	Isolate B
Colour on Columbia agar	Yellowish	Milkish
Size of colony	2 mm	3 mm
Elevation	Convex	Raised
Optical density	Translucent	Translucent
Surface appearance	Mucoid	Mucoid
Shape of colony	Circular	Circular
Margin	Smooth	Smooth
Cell morphology	Rod	Slightly curved
Motility	Motile	Motile
Gram reaction	Negative	Negative
Possible bacterium	<i>Helicobacter pylori</i>	<i>H. pylori</i>

Table 3: Biochemical characteristics of the bacterial isolates and the prevalence in the sampled villages.

Sampled village	No. of stool sample	NI (%)	NCP (%)	NUP (%)	NIP (%)	NOP (%)	Occurrence (%)
Otolo	52	18 (36.73)	18 (36.73)	18 (36.73)	18 (36.7)	18 (36.7)	36.73
Uruagu	52	13 (26.53)	13 (26.53)	13 (26.53)	13 (26.53)	13 (26.53)	26.53
Umudim	52	13 (26.53)	13 (26.53)	13 (26.53)	13 (26.53)	13 (26.53)	26.53
Nnewichi	44	5 (10.20)	5 (10.20)	5 (10.20)	5 (10.20)	5 (10.20)	10.20
Total	200	49 (100)	49 (100)	49 (100)	49 (100)	49 (100)	100

NI=Number of isolates; NCP=Number of catalase positive; NUP=Number urease positive; NIP=Number of indole positive; NOP=Number of oxidase-positive

Table 4: Molecular identities of the bacterial isolates.

Isolate	Maximum score	Total score	Query cover	E value	Identity (%)	Accession No.	Description
M	23555	23555	100	0	100	CP091771.1	<i>Helicobacter pylori</i> strain K154 (HPK154) complete genome
N	12770	12770	100	0	100	CP122947.1	<i>Helicobacter pylori</i> strain BS07 (HPBS07) complete genome
O	47493	47493	100	0	100	CP091769.1	<i>Helicobacter pylori</i> strain K93 (HPK93) complete genome
P	29676	29676	100	0	100	CP091770.1	<i>Helicobacter pylori</i> strain K115 (HPK115) complete genome

M, N, O and P. M= *Helicobacter pylori* strain K154 (HPK154) complete genome; N= *Helicobacter pylori* strain BS07 (HPBS07) complete genome; O= *Helicobacter pylori* strain K93 (HPK93) complete genome and P= *Helicobacter pylori* strain K115 (HPK115) complete genome.

The characteristics of the bacterial isolates using convectional culture methods are presented in Table 2. The colonies appeared as small round (2-3 mm), convex (domed-shaped), translucent, non-hemolytic colonies within an average of 72 h after sub-culturing from positive Columbia broth, thus indicating presumptive identification of *Helicobacter pylori*.

The biochemical characteristics of the bacterial isolates are presented in Table 3. All the isolates were catalase positive, urease positive, oxidase positive, and indole positive. The sequence result revealed the presence of *Helicobacter pylori* strain K154 (HPK154) complete genome, *Helicobacter pylori* strain BS07 (HPBS07) complete genome, *Helicobacter pylori* strain K93 (HPK93) complete genome, and *Helicobacter pylori* strain K115 (HPK115) complete genome. (Table 4).

DISCUSSION

Diagnosis of infectious diseases has been considered as a key factor in disease prevention and control, especially in rural areas where poor attention had been paid on health-related issues. The study conducted at the four villages within Nnewi North metropolis revealed a high prevalence of the infection in the samples evaluated, especially blood samples. This observation agrees with the findings of other researchers though this study recorded highest prevalence. The high prevalence in the area could be attributed to some risk factors peculiar to the researched location.^{6,12,13}

These risk factors include poor personal hygiene, poor food hygiene, poor environmental sanitation, overcrowding and poor-quality water supply. This is in line with the findings of other researchers who reported high prevalence of the infection is often attributed to certain risk factors.^{6,12} The ability of ELISA test to record the highest positive value in the sampled villages could be attributed to high sensitivity and specificity. Similar observation was made by other researchers who reported that serological method is sensitive to detecting pathogens in serum.^{13,14}

The cultural, microscopic, and biochemical features of the isolates portray the features of *Helicobacter pylori*. Similar features were reported by other researchers who isolated the bacterium using blood and stool samples. This shows

that the bacterium isolated in this study is responsible for the stomach ulcer in the subjects investigated.^{13,15} Despite the relevance and scientific significance of this research, several limitations were encountered that may have impacted the study's overall outcomes. First, recruiting participants with confirmed cases of stomach ulcer proved challenging, largely due to patients' reluctance to undergo invasive diagnostic procedures such as endoscopy. This limited the positive sample size and may have affected the statistical power and generalizability of the findings. Secondly, while molecular techniques were employed, some initial diagnoses were based on non-invasive or symptom-based assessments, which may not be entirely accurate.

This could have introduced some level of diagnostic bias in the prevalence data. Thirdly, poor road networks and infrastructural challenges within certain areas of Nnewi North limited access to more remote communities. This restricted the geographical spread of samples and reduced our study population. Finally, the study was conducted within a relatively short time frame, limiting opportunities for long-term follow-up or seasonal variation analysis. Limited funding also restricted the number of samples processed and the range of molecular markers analyzed.

CONCLUSION

The present study has evaluated the prevalence of stomach ulcer using standard techniques at Nnewi North Local Government Area, Anambra State. The study has revealed that there is a high prevalence of stomach ulcer at Newi North (especially at Otolo village) as shown in the number of positive samples from the subjects. The study also revealed that Enzyme-linked Immunosorbent Assay (ELISA) is the most sensitive diagnosing technique while the least sensitive technique was Stool Antigen Test (SAT). ELISA is the most sensitive standard technique to be employed in diagnosing *H. pylori* in serum. However, further research is needed to confirm various predisposing factors in the sampled villages.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Alfaroouk KO, Bashir AHH, Aljarbou AN, Ramadan AM, Muddathir AK, AlHoufie STS, et al. The possible role of *Helicobacter pylori* in gastric cancer and its management. *Front Oncol*. 2019;9:75.
2. Cardos AI, Maghiar A, Zaha DC, Pop O, Fritea L, Miere Groza F, et al. Evolution of diagnostic methods for *Helicobacter pylori* infections: from traditional tests to high technology, advanced sensitivity and discrimination tools. *Diagnostics (Basel)*. 2022;12(2):508.
3. Borka Balas R, Meliț LE, Mărginean CO. Worldwide Prevalence and Risk Factors of *Helicobacter pylori* Infection in Children. *Children (Basel)*. 2022;9(9):1359.
4. Che TH, Nguyen TC, Vu VNT, Nguyen HT, Hoang DTP, Ngo XM, et al. Factors associated with *Helicobacter Pylori* infection among school-aged children from a high prevalence area in Vietnam. *Int J Public Health*. 2023;68:1605908.
5. Narayanan M, Reddy KM, Marsicano E. Peptic ulcer disease and *Helicobacter pylori* infection. *Mo Med*. 2018;115(3):219-24.
6. Chukwuma OM, Chukwuma GO, Manafa PO, Akulue JC, Jeremiah ZA. Prevalence and possible risk factors for *helicobacter Pylori* seropositivity among peptic ulcerative individuals in Nnewi Nigeria. *Biomedical Res J*. 2020;4(1):166–72.
7. Liang B, Yuan Y, Peng XJ, Liu XL, Hu XK, Xing DM. Current and future perspectives for *Helicobacter pylori* treatment and management: From antibiotics to probiotics. *Front Cell Infect Microbiol*. 2022;12:1042070.
8. Abadi TB. Diagnosis of *Helicobacter pylori* Using Invasive and Noninvasive Approaches. *J Pathog*. 2018;2:9064952.
9. Aitila P, Mutyaba M, Okeny S, Ndawula Kasule M, Kasule R, Ssedwabane F, et al. Prevalence and Risk Factors of *Helicobacter pylori* Infection among Children Aged 1 to 15 Years at Holy Innocents Children's Hospital, Mbarara, South Western Uganda. *J Trop Med*. 2019;1:9303072.
10. Cheesbrough M. *District Laboratory Practice in Tropical Countries* (2nd ed.). Cambridge University Press: Cambridge, England. 2010: 45–70.
11. Iheukwumere IH, Chude C, Unaeze BC. Toxicological study and antibacterial activities of effectively validated medicinal plants against enteric bacteria isolated from chicken Feeds. *J Health Med. Nurs*. 2018;7:19–34.
12. Chukwuma O, Chukwuma G, Manafa P, Ibeh N, Jeremiah Z. Prevalence and possible risk factors for *Helicobacter pylori* seropositivity among peptic ulcerative individuals in Nnewi Nigeria. *J Current Biomed. Res*. 2021;1(1):39–45.
13. Elbehiry A, Marzouk E, Aldubaib M, Abalkhail A, Anagreyah S, Anajirih N, et al. *Helicobacter pylori* Infection: current status and future prospects on diagnostic, therapeutic and control challenges. *Antibiotics (Basel)*. 2023;12(2):191.
14. Nwachukwu EP, Onwurah OW, Amilo GI, Onwuasoanya UF, Ezeugwunne IP. Prevalence of *Helicobacter pylori* among patients with gastritis attending Nnamdi Azikiwe university teaching Hospital, Nnewi, Anambra State, Nigeria. *Ann Current Gastroenterol Report*. 2020;1(1):1003.
15. Peleteiro B, Bastos A, Ferro A, Lunet N. Prevalence of *Helicobacter pylori* infection worldwide: a systematic review of studies with national coverage. *Dig Dis Sci*. 2014;59(8):1698-709.

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