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PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN ULCER PATIENTS ATTENDING NNEWI TEACHING HOSPITALS: BLOOD AND STOOL SAMPLE ANALYSIS

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ABSTRACT

Helicobacter pylori is primarily associated with gastrointestinal diseases. In Nigeria, the prevalence of *H. pylori* infection remains a public health challenge, with varying rates reported across different regions. The study targeted the prevalence of *H. pylori* (HP) infection in the blood and stool samples of patients attending Nnewi Teaching Hospital. A total of 186 each of stool and blood samples were collected and screened for HP using Columbia agar supplemented with minor nutrients. The isolates were characterized using their morphological, biochemical and molecular properties. The prevalence of the isolates was determine using cross sectional study. The data obtained were analyzed Analysis of Variance (ANOVA), and postdoc test using Turkeys test in excel package. *H. pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain K115 (HPK115) were mostly encountered in the study, and HPBS07 was mostly encountered. The culture medium significantly ($p \leq 0.05$) detected

H. pylori in blood samples (17.20 %) than stool samples (2.29%), and HPBS07 was mostly seen both in blood and stool samples. Therefore, this study has shown the occurrences varying strains of H. pylori in the samples, blood samples recorded the highest occurrence of the organism, and HPBS07 was detected most

Key words: *Helicobacter, pylori*, Prevalence, Blood, Ulcer

INTRODUCTION

Helicobacter pylori is a Gram-negative, microaerophilic, spiral-shaped bacterium belonging to the family Helicobacteraceae. Members of the *Helicobacter* genus comprise several species, some of which colonize the upper gastrointestinal tract and liver of various mammals and birds. *H. pylori* is uniquely adapted to survive in the harsh, acidic environment of the human stomach. One of its key characteristics is motility, which is facilitated by the presence of multiple unipolar flagella, allowing it to penetrate the gastric mucus layer and establish colonization within the host mucosa.

Research had revealed that *H. pylori* is a highly pathogenic species of the genus, which infects mostly mammals especially man (Goderska *et al*., 2018). The infection that occurs due to the presence of the bacterium in the gastrointestinal tract has been recognized globally as a threat because high level of disorderliness of the system is experienced by the infected individuals. The most debilitating aspect of the infection is that all age groups, occupation, and gender are vulnerable (Garrido-Trevino *et al*., 2022).

Several researchers had reported that the ability of the bacterium to cause severe infection with acute clinical manifestations could be attributed to the presence of virulent factors such adhesins, which enable the organism to attach firmly to the mucosa of the stomach and urease, which enables it to breakdown urea, releasing ammonia and carbon dioxide (Avala *et al*., 2014; Azadi *et al*., 2019).

Some researchers had purported that the ability of the pathogen to produce ammonia from urea provides conducive environment for proliferation (Spinu *et al*., 2016; Mintah *et al*., 2019). The attachment of the organism in the mucosa enables it to destroy the epithelial cells in the tissue, thereby leading to bleeding in severe cases. Some of the infected patients had excreted the organism in faeces, which also provides relevant diagnostic information (Bouhenni *et al*., 2019). The wound caused by the organism is capable of depriving an infected person of several foods, especially when prepared using pepper, as it aggravates pain.

Several efforts had been made by health practitioners, especially those in pharmaceutical industries to ensuring that an ideal conventional antimicrobial

agent is produced, which would curtail the menace (Ngan *et al*., 2021). It is appalling that for decades, most of the drugs and antibacterial agents had not yielded the desired goal. To an extent, it was speculated that stomach ulcer caused by *H. pylori* has no cure but can only be checked. As a result of this, it has been difficult to have access to a drug that can totally cure ulcer infection, especially in developing countries, where the prevalent rate remains almost constant (Alibi *et al*., 2020).

MATERIALS AND METHODS

Study area: Nnewi North Local Government metropolitan commercial city and Uli in Ihiala local government Area both in Anambra State, Nigeria are towns of historic importance. 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 centre of commercial activities. The city spans over 1,076.9 square miles (2,789 km^2) in Anambra State with four autonomous quarters: Otolo, Uruagu, Umudim, and Nnewichi. It has a land mass area of 60.0 $km^{2,}$ density of 3, 428 /km^2 as of (2016). According to World Bank, United Nations Census (2019), population in urban areas as at 2016 was 1,004706, and 193,987 in city area, though bearing in mind that all population figures for Nigeria show high error rates since census results are disputed. Nnewi metropolitan area and its satellite towns is home to nearly 2.5 million residents as of 2005 and Nnewi–North Local Government Area, with a land mass of 128 Km sq. an estimated number population of 121,063 and a population density of 946 (Anambra State Statistical Year Book, 2010). The average monthly maximum temperature is 27.46^{o}C and a minimum of 23.65 ^{0}C in 2010, and the annual monthly relative humidity is 79.66 in 2010. Nnewi is bounded in the west by Ekwusigo, northwest by Idemili, northeast by Aniocha, east by Aguata, and southwest by Ihiala local government areas. The occupation of the inhabitants includes trading. Uli is situated at the extreme southeast corner of Ihiala local government area of Anambra state in Nigeria.

Sample collection: Clinical samples of blood and stool were used for the analysis. Before the collection oral consent was obtained from participants. Blood samples were collected by vein-

puncture method from the anti-cubital fossa of the hand. Four milliliters (4 mL) of blood was drawn from each participant, dispensed into a non-anticoagulated container and allowed to clot. Sterile plastic stool containers without preservatives were given to each subject and they were instructed to collect stool specimens following preclusive measures as described by (Cheesbrough, 2010; Ekesiobi *et al*., 2025a; Ekesiobi *et al*., 2025b; Ekesiobi *et al*., 2025c; Ekesiobi *et al*., 2025d). The collected samples were kept inside the cooler containing an ice pack, and the samples were transported to the laboratory for immediate analysis.

Culture and Isolation of *H. pylori:* *H. pylori* bacteria were isolated from stool sample according to method described by Umeaku C.N. using pre-enrichment in Columbian Agar broth (Oxoid, England), with selective antibiotic (Trimethoprim, sigma, St Louis, MO, H77883), Amphotericin B (Amresco Inc., Solar, OH, HO414), dissolved in Dimethyl- sulphoxide (DMSO) (sigma, HD5879). The stool sample was emulsified in phosphate-buffered saline and 1g of Chlorestyramine in suspension to dissolve and nullify the effect of bile in the stool as described by Ndip et al (2003), Ekesiobi *et al*. (2025e), Ekesiobi *et al*. (2025f). The emulsion was filtered using a sterile Muslin cloth to remove the stool debris and further filtered with a membrane filter of pore size 0.45um to retain the H. *pylori* present in the stool.

Step 1 (primary culture): as recommended by (Shahamat et al 1991);

Culture broth 1; Columbia agar-based broth (oxoid–England) was prepared according to the manufacturer's instruction, together with the following antibiotics supplements: vencomycin (10mg), Trimethoprim (4mg), and Nystatin (2.5mg). 5ml aliquot was dispensed in sterile bijou bottles. The deposit on the membrane filter was cultured on the broth and incubated at the microaerophilic condition for 3-5 days using an anaerobic gas park (oxoid-England) at 37˚C. This was checking immediately for the presence of visible growth (turbidity) after the first 3 days through the 12th day before discarding as no growth.

Step 2. Selective plating:

As soon as turbidity was noted, it was sub cultured on *H. pylori* selective media (liophilchem, Italy) by a conventional surface-streaking technique using a sterile standard (0.02 ul) wire loop. Plates were incubated at 37˚C at microaerophilic condition for 3

to 7 days checking intermediately for growth.

Purification of the isolates: The plates that showed discrete colonies were selected and aseptically streaked each colony on sterile plates (90mm×15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions and incubated at 35 ± 2^0C for 24 h for bacteria as described in Cheesbrough (2010).

Characterization of the pure bacterial isolates: The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described in the study published by Iheukwumere *et al.* (2018), Iheukwumere *et al*. (2025a), Iheukwumere *et al.* (2025b).

Determination of Prevalence of the Isolates in the Studied Samples

The occurrences of different strains of *H. pylori* that were encountered in blood and stool samples were counted and recorded according to the method of Iheukwumere *et al.* (2018). The strains of H. pylori encountered in the positive samples were countered. And their percentages were determined.

Statistical Analysis

The results of the data generated were expressed in percentages, tables and figures. The significance of the prevalence and susceptibility study were determined using Analysis of variance (ANOVA) at 95% confidence level. Pairwise comparison was carried out in an Excel sheet using the student "t" test (Iheukwumere *et al*., 2020; Iheukwumere *et al*., 2025c; Iheukwumere *et al*., 2025d; Iheukwumere *et al.,*2025e).

RESULTS

Characterization of the Bacterial Isolates

The four predominant isolates (M, N, O and P) exhibited similar cultural and morphological characteristics but differed slightly in their appearances on Columbia blood Agar and in sizes as shown in Table 1. Isolates M and P were pale grayish whereas isolates N and O were light grayish on Columbia blood Agar. The isolates were all catalase, oxidase, urease and hydrogen production positive. They fermented glucose but were negative to arabinose, lactose and maltose. They showed varied slight reactions to xylose, inositol, sorbitol and mannitol and these formed the basis of their strain variations.

The nucleic acid extracted from the bacterial isolates revealed that the nucleic acids were all DNA (1.80 - 1.90) as shown in Table 2. The sequence analysis of the bacterial isolates showed 100% identifies for all the four isolates and the identified isolates were: *Helicobacter pylori* strain K154 (HPK154, *Helicobacter pylori* strain BS07 (HPBS07) ,*Helicobacter pylori* strain K93 (HPK93) and *Helicobacter pylori* strain K115 (HPK115) as shown in Table 3

The number and percentages of blood and stool samples from the participants that showed positive cultural growth are shown in Table 4. The study revealed that 32 and 5 samples were positive compared to 59 and 31 positive samples reavealed for BAT and SAT. Hence the percentage false positive were 31.70% and 16.71% respectively for blood and stool samples.

The study also revealed that out of 32 isolates generated from the blood samples, HPBS07 significantly ($p<0.05$) recorded the highest number of occurrences, followed by HPK154, HPK93 and HPK115 was the least. Similarly HPBS07 recorded the highest occurrences in the stool samples whereas HPK154 was only detected once, HPK93 and HPK115 were not detected.

Table 1: Morphological and biochemical characteristics of the isolates

Parameter	M	N	O	P
Appearance on Columbia blood agar	Pale greyish	Light greyish	Light greyish	Pale greyish
Size (mm)	1.00	0.80	0.90	1.10
Optical Nature	Translucent	Translucent	Translucent	Translucent
Edge	Smooth	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth	Smooth
Gram reaction	-	-	-	-
Shape	Curved-spiral	Curved-spiral	Curved-spiral	Curved-spiral
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease	+	+	+	+
Hydrogen sulfide production	+	+	+	+
Glucose	+	+	+	+
Arabinose	-	-	+/-	-
Lactose	-	-	-	-
Maltose	-	-	-	-
Xylose	-	-	-	+/-
Inositol	+/-	-	+/-	-
Sorbitol	+/-	-	-	-
Mannitol	+/-	-	+/-	-

Table 2: Purity of nucleic acids extracted from the isolates

Isolate	Conc (ng/ul)	ABS_{260}	ABS_{280}	$^{260}/_{280}$
M	122.30	3.118	1.705	1.83
N	125.70	3.212	1.736	1.85
O	118.20	3.108	1.698	1.83
P	128.60	3.226	1.773	1.82

Table 3: Molecular identities of the bacterial isolates

Isolate	Maximum score	Total score	Query Cover	E-value	Identity (%)	Accession Number	Description
M	23555	23555	100	0.0	100.00	CP091771.1	*Helicobacter pylori* strain K154 (HPK154) complete genome
N	12770	12770	100	0.0	100.00	CP122947.1	*Helicobacter pylori* strain BS07 (HPBS07) complete genome
O	47493	47493	100	0.0	100.00	CP091769.1	*Helicobacter pylori* strain K93 (HPK93) complete genome
P	29676	29676	100	0.0	100.00	CP091770.1	*Helicobacter pylori* strain K115 (HPK115) complete genome

Table 4: Culture positive samples

	N=186	
Parameter	Blood (%)	Stool (%)
Positive	32 (17.20)	5 (2.69)
Negative	154 (82.80)	181 (97.31)
FP	59 (31.70)	31 (16.71)

% FP – False positive

Blood / stool ≤ 0.05

Table 5: Occurrences of the characterized *H. pylori* in the blood and stool samples of the participants

Isolate	Blood N = 32	Stool N = 5
HPK154	9 (28.13)	1 (25.00)
HPBS07	16 (50.00)	4 (80.00)
HPK93	5 (15.63)	0 (0.00)
HPK115	2 (6.25)	0 (0.00)
Total	32 (86.49)	5 (13.51)

DISCUSSION

The characteristics and identities of different strains of *H. pylori* encountered in both stool and blood samples are in line with the reports of many researchers (Egwu and Chukwubike, 2014; Lopes *et al*., 2014; El-Shabrawi *et al*., 2018; Nwachukwu *et al*., 2020; Hussein *et al*., 2021). *H. pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain K115 (HPK115) were encountered in the studied samples. Man researchers (El-Shabrawi *et al*., 2018; Nwachukwu *et al*., 2020; Hussein *et al*., 2021) encountered *H. pylori* from their studied samples but with varied strains.

The highest occurrence of HPBS07 in the studied samples (stool and samples) could be attributed to its diversity and adaptation to different environments particularly within the study area, and this corroborates with the detection from the report of Nwachukwu *et al*. (2021).

Several researchers (Graham, 2014; Sugano, *et al*., 2015; Pizzorno JE *et al.,* 2016). have reported that about 70%-80% of all gastric ulcers and 90%-100% of duodenal ulcers (DU) are caused by *H. pylori* infection. The distribution of H. *pylori* in the studied samples agrees with the findings of many researchers (Graham, 2014; Sugano, *et al*., 2015; Pizzorno JE *et al.,* 2016) who detected the same organism from the same samples

CONCLUSION

The study revealed that *Helicobacter pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain k115 (HPK115) were encountered in the studied stool and blood samples, and HPBS07 recorded the highest occurrence.

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