Detectionof*H. pylori*from Stool Samples of Patients Attending a GovernmentHospital in Port-Harcourt using Antigenic Screening and Culture-Based Techniques

ABSTRACT

Background:*Helicobacter pylori* is well-known among other bacteria causing ulcers, stomach cancer and other forms of gastrointestinal infections in developing countries.
Aim: This research was intended to evaluate the prevalence of *H. pylori* infection among patients attending a Government Hospital in Port-Harcourt, Rivers State Nigeria.
Methodology: One hundred and five (105) stool samples of individuals comprising of 48 males and 57 females were studied. The samples were investigated by antigenic screeningusing *H. pylori* stool antigen (HpSA) test kit. In addition, culture-based isolation was done using Columbia agar plus 10% horse blood, supplemented with antibiotics (amphotericin B, vancomycin, trimethoprim and ceftazidime).
Results: Out of 105 samples screened, 56 (53.3%) tested positive for both antigenic screening and culture-based techniques while 49 (46.7%) tested negative for both. Positive isolates were phenotypically characterized by colony morphology, Gram stain, and biochemical reactions and a total of 19 isolates were suspected to be *H. pylori.* The result indicated that *H. pylori*detection was relatively low in male (21.9%) compared to female (31.4%) using antigenic screening. However, the culture-based technique yielded low recovery in male (2.9%) compared to female (4.7%), making the detection of infection high among the female than the male. Age group prevalence increased with age, recording infections within age group ≥31 (20.0%) followed by 26-30, (12.3%) and lowest in age group 21-25 (10.5%) and below using antigenic screening. while in culture-based technique, age group 26-30, (3.8%) followed by ≥31 (3.8%) yieldinglow recovery, while age group 21-25 and below had 0 recovery.
Conclusion: This study revealed an infection rateof *H. pylori* infection among the female than the male population sampled. To reduce the detection, regular screening, treatment and public health awareness campaign should be developed for the control, elimination and prevention of *H. pylori* infection.

Key word:Helicobacter pylori, Stool Samples, Indigenous microbes, ecology of human diseases, Molecular Genetics, Gastric Cancer

1. Introduction

"*Helicobacter pylori* previously known as *Campylobacter pylori*, is a Gram-negative, microaerophilic, spiral (helical) bacterium usually found in the stomach"[1]. "Its helical body (from which the genus name, *Helicobacter* derives) is thought to have evolved in order to penetrate the mucous lining of the stomach, helped by its flagella, and thereby establish infection"[2; 3]."*H. pylori* infection usually has no symptoms however typically causes gastritis (stomach inflammation) or ulcers of the stomach or first part of the small intestine. The infection is also associated with development of certain cancers occurring in less than 20% of cases"[4].

"In 2015, it was estimated that over 50% of the world's population had *H. pylori* in their upper gastrointestinal tracts with this infection (or colonization) being more common in developing countries. In recent decades, however, the prevalence of *H. pylori* colonization of the gastrointestinal tract has declined in many countries"[5]. "Up to 90% of people infected with *H. pylori* never experience symptoms or complications. However, individuals infected with *H. pylori* have a 10% to 20% lifetime risk of developing peptic ulcers"[6]. "Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: Stomach pains, nausea, bloating, belching, and sometimes vomiting. Pain typically occurs when the stomach is empty, between meals, and in the early morning hours, but it can also occur at other times. Less common ulcer symptoms include nausea, vomiting, and loss of appetite"[7]. "Bleeding in the stomach can also occur as evidenced by the passage of black stools; prolonged bleeding may cause anemia leading to weakness and fatigue. If bleeding is heavy, hematemesis, hematochezia, or melena may occur. Inflammation of the pyloric antrum, which connects the stomach to the duodenum, is more likely to lead to duodenal ulcers, while inflammation of the corpus (i.e. body of the stomach) is more likely to lead to gastric ulcers". [8]. "Individuals with chronic *H. pylori* infection have an increased risk of acquiring a cancer that is directly related to this infection such as stomach cancer which is not common". [9].

"Studies have revealed that the most common causes of ulcer disease are the bacteria *H. pylori* andNon-steroidalAnti-inflammatory Drugs (NSAIDs)", [10]. "The infections caused by this bacterium mostly progress from childhood and remain chronically (lifelong) without any

symptoms. Acute gastric infections, however, may be developed in some patients". [11]."*H. pylori* is a fastidious microorganism and requires complex growth media. Often these media are supplemented with blood or serum mixed with anti-microbial agents". [12].

"The mode of transmission of *H. pylori* is not known exactly, but the fecal–oral or oral–oral routes via water or food consumption are thought to be a very common cause". [13]. "As an example, mother-to-child transmission occurs if the mother's saliva is contaminated or due to poor hand hygiene. The pathogen is transmitted by direct means, e.g., kissing or sharing utensils, or by indirect means, such as drinking water, air, animals, flies, and food"[14, 15]. while other microbes have been reported as causative agents of gastritis, there is need to ascertain the actual cause of these infections and risk factors associated with gastroenteritis related to *H. pylori* infections which include, living in crowded conditions, fecal contamination of food, water and utensils, lack of clean water and poor sanitary conditions etc.*Helicobacter pylori* infection can be diagnosed using polymerase chain reaction (PCR), stool antigen test, culture, urea breath test (UBT), serology, histology etc. However, this research was intended to evaluate thedetection of *Helicobacter pylori* infections among patients attending a Government Hospital in Port-Harcourt Rivers State Nigeria.

2. Materialsand Methods

2.1.Study design

A cross-sectional study was carried out using simple random sampling technique to group the subjects from August 2023 to February 2024

2.2. Study Area

This study was conducted in a Government Hospital in Port-Harcourt, Rivers State Nigeria with GPS coordinates of 4°49'27"N 7°2'1"E.

2.3.Sample Collection

In this study, clinical data and information of patients were recorded using a well-structured questionnaire.A total of one hundred and five (105) stool samples were collected from patients (48 males and 57 females) aged 10 - ≥31 in a sterile screw-cap bottle (universal bottle) labeled with the specimens I.D number.The sample size was calculated using the formula: $n = Z^2 P (1 - P) /d^2$[16] and a confidence level of 95%.

2.4. *H. pylori* Screening

2.4.1. *H. pylori* Antigenic Screening

*H. pylori*antibody test is a qualitative membrane-based immunoassay for the identification of *H. pylori* antibodies in blood, serum or plasma[17]. Stool samples were collected and tested using the *H. pylori* test strip kit (OnSite *H. pylori* Ag Rapid Test (cassette) CTK Biotech USA).Approx. 10g of stool samples was collected in a stool collection device containing 1ml sample extraction bufferand shaked firmly to ensure a homogenous liquid suspension. Few drops of the solution were dispensed into the sample well of the cassette and timed for 10minutes, if only the control line (C line) develops, the test indicates that no detectable *H. pylori* antigen is present in the specimen. The result is negative or non-reactive. If both C and Test line (T line) develops, the test indicates the presence of detectable *H. pylori* antigen in the specimen. The result is positive or reactive. If no C line develops, the test is invalid regardless of any color development in the T line as described by the manufacturer.

2.4.2. Culture-based Technique

Primary isolation was carried out on Columbia agar with Dent selective supplement containing amphotericin B (25µl), vancomycin (50µl), trimethoprim (156.25µl) and ceftazidime (25µl) replaces cefsulodine[18], plus 10% horse blood. Using a sterile inoculating loop, inoculum of stool sample was aseptically streaked on the surface of the agar plates. Inverted, the plates were incubated in an anaerobic jar undisturbed witha micro-aerophilic kit GasPak (CO_2) AnaeroGen™, Oxoid, (Basingstoke, United Kingdom) for 3 days at 37°C in an anaerobic atmosphere (80% - 90% N_2, 5%-10% CO_2 and 5%-10% O_2. After 3 days, the plates were removed and visually inspected. When the colonies appeared too small, a new GasPak (CO_2) AnaeroGen™, Oxoid, kit was placed in the anaerobic jar and the plates re-incubated for a further 2 days. Pure cultures were harvested using a sterile inoculating loop to transfer discrete colonies of *H. pylori* into cryotubes containing 5ml brain heart infusion (BHI) broth enriched with 20% glycerol and stored at -80°C for further analysis. [19].Results was obtained through morphological characterization of *H. pylori*.Discrete colonies were picked from each culture plates and classified as*H. pylori*on the basis of typical colonial morphology (small and translucent colonies), the presence of curved gram- negative cells on Gramstain, urease,

catalase,oxidase production, growth at 35 and 40°C, growth at (1.00 and 1.25% NaCl, growth at 0.5 and 0.75% NaCl) glucose fermentation, Hydrogen Sulfide (H_2S), motility test, selenite reduction andgrowthon MacConkey agar [20].

2.5. Statistical Analysis

Data analysis was performed using IBM (SPSS) version 22.0. The Chi-Square test was utilized to summarize all data obtained to determine if*P*-value < 0.05 is statistically significant. While the frequency and percentage of occurrence was used to show prevalence.

Results and Discussion

3.1 AntigenicScreening/Culture-Based Detection Technique

Results of *H. pylori* gender prevalence for both antigenic screening and culture are presented in Fig 1. A total of 105 stool specimens were analyzed for this study,(45.7%) males and (54.3%) females. (21.9%)male and (31.4%) female turned out positive forantigenic screening making*H. pylori* infection in females relatively highwith no gender associated significance between the male and female participants.

However, for culture, the total positive male was (2.9%)and (4.7%) for females.The prevalence of *H. pylori* infections wasrelatively low according to gender in malecompared to that recovered fromthefemale according to this study.

3.2 Age Group Prevalenceusing Antigenic Screening/Culture-BasedDetection Technique

The ratio of prevalence among the different age groups for both antigenic and culturerangedbetween 10 - ≥31 years. *H. pylori* infection increased generally with age between (4.8%) to (20.0%) for antigenic screening.

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Culture reported low frequency of (3.8%) between age group 26 -≥31 respectively, while the least recovery was observed within age group 21-25 years and below, lowest than other age groups as provided in Fig.2.

3.3 Overall Prevalence According to Antigenic Screening/Culture-based Technique

Overall prevalence of *H. pylori* in Port-Harcourt varied from (53.3%) to (7.6%) according to antigenic screeningandculture as shown in Fig 3, while (46.7%)tested negative for antigenic

screeningand (0.0%) were classified as negative by culture.*H. pylori* infections was observed to be relatively high for antigenic screening than that obtained by culture.

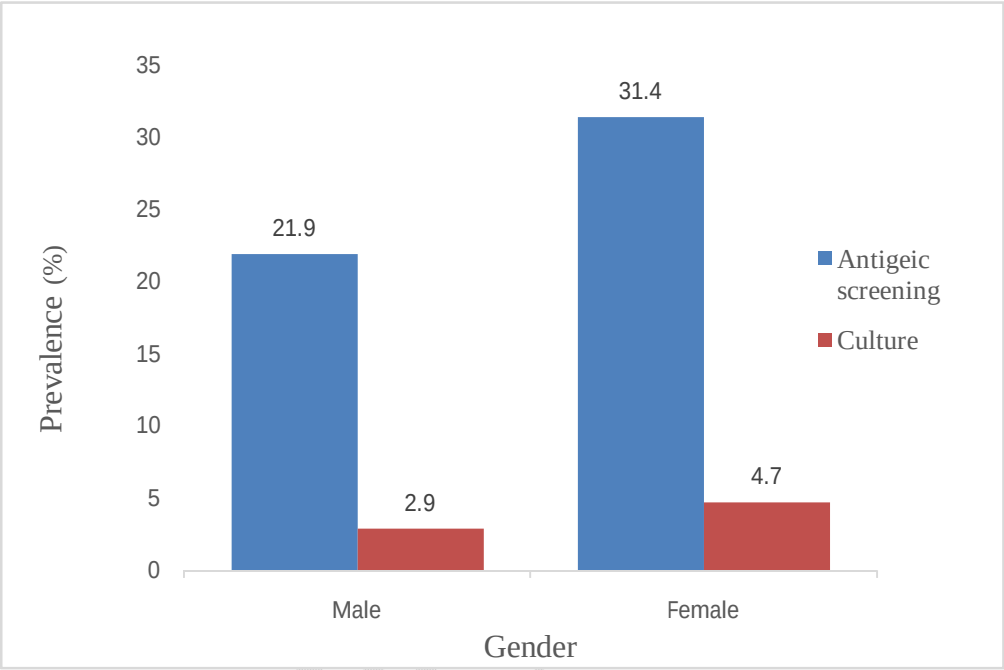

Fig1: Prevalence of *H. pylori* using antigenic screening and culture-basedtechnique among the population gender in the study.

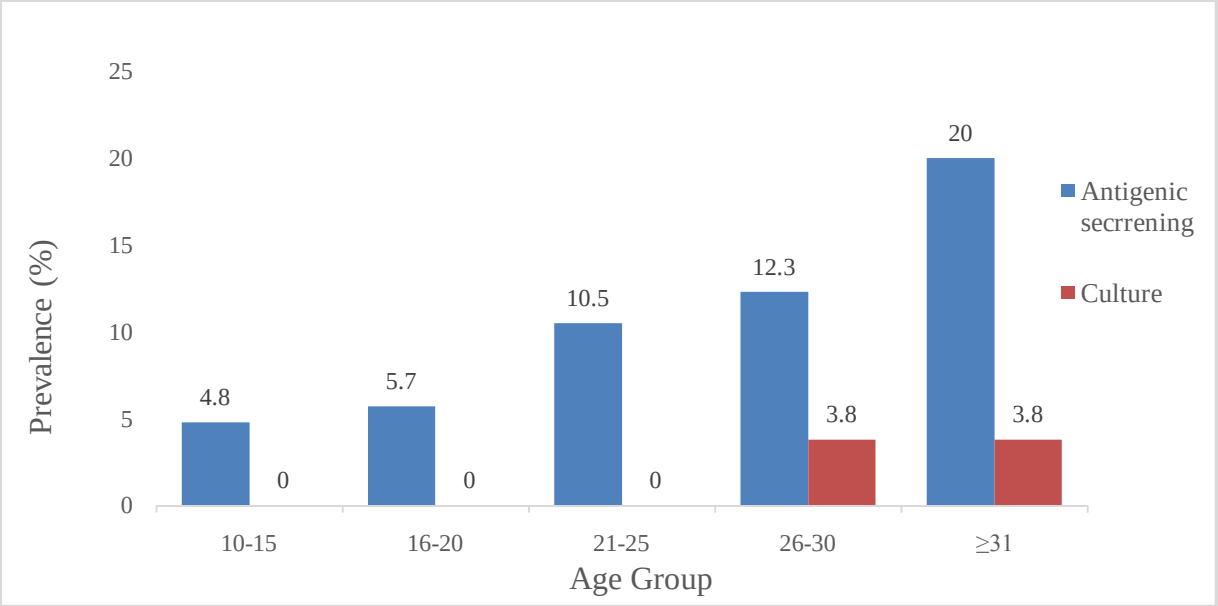

Fig 2: Prevalence of *H. pylori* among the different age group (years) in the study using antigenic screening and culture-based technique.

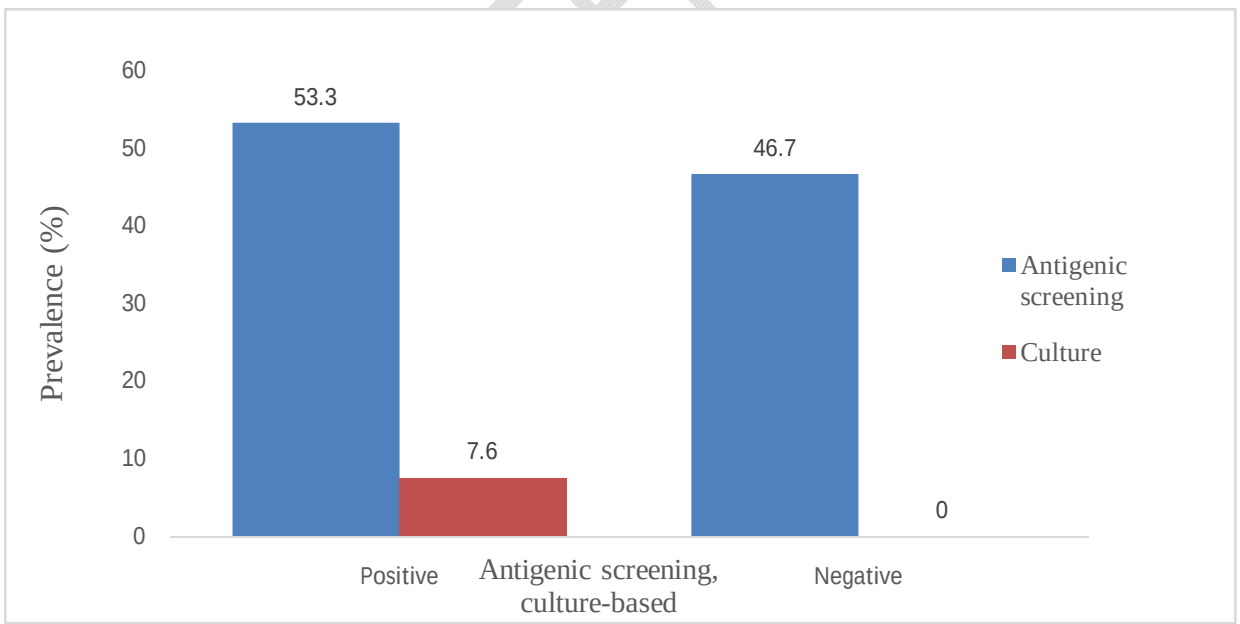

Fig 3. Overall prevalence of *H. pylori* among the population using antigenic screening and culture-based technique.

3. Discussion

The detection of *H. pylori* from stool using antigenic screening and culture-based technique in a government hospital in Port-Harcourt, Rivers State Nigeria revealed that antigenic screening was highly sensitive in the detection of *H. pylori*with culture recording the least recovery.

The current study identified variations in the detection of *H. pylori* using antigenic and culture-based technique.The percentage of total positive cases identified using antigenicscreeningrevealed high percentage of prevalence than that recovered by culturerespectively. [21] reported that detection rate by antigenic screening were higher than that obtained by culture. This report is in agreement with the present study where a greater percentage of detection was observed using antigenic screening than that obtained by culture. This could possibly be due to lack of viable cells or the difficulty of adapting appropriate isolation techniques

Populationdetectionvaried relatively in both genders and age groups (years). Although it is unclear if gender and age group are risk factors in the heightened prevalence observed among the females than in the males for both antigenic screening and culture. These observations is in dispute with the current worldwide *H. pylori* prevalence study which revealed no differences in *H. pylori* infection among male and female genders. [22].

Age group detection generally increased withage ranging from10-≥31 years for antigenic screening. However, culturefor age group between 10-25 age group recordedthe least recovery while 26-≥31 age group revealedlow resultsrespectively. These observations were in contrast with [23] who reported a higher prevalence of adult participants aged 16 and ≥30 years. There was no significant difference in the gender and age group prevalence of *H. pylori* among the studied population. Thiscould be probably due to living in crowded conditions, fecal contamination of food, water and utensils, lack of clean water and poor sanitary conditions etc.[24;25].Femalesshould often be educated on the importance of personal hygiene practices as they are majorly involved in taking care of the family as this could play an important role in the transmission of infection.

However, these diagnostic techniques have their advantages and limitations but none of them can be considered gold standard as a single test for the diagnosis of *H. pylori* infection according to reports[26].

4. Conclusion

The diagnosis of *H. pylori* infection by antigenic screening has proven to behighly sensitive in the detection of *H. pylori* infection than culture in this study.

Findings of this study revealed a high percentage of *H. pylori* infection among the female than the male gender, also age-group prevalence was found to be high among age-groups 26-30 and ≥31 age-groups compared to other age group of 25 years and below for both antigenic screening and culture-based technique in this study.

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