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Detection of *H. pylori* from Stool Samples of Patients Attending a Government Hospital in Port-Harcourt Using Antigenic Screening and Culture-Based Techniques

Maureen, O. O. [a*], Akani, N. P. [a] and Sampson, T. [a]

[a] Department of Microbiology, Rivers State University, Port-Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 designed to investigate 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 for *H. pylori* using *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).

**Corresponding author: E-mail: maunics2000@yahoo.com;*

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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%) yielding low recovery, while age group 21-25 and below had 0 recovery. There was no significant difference (*P*>0.05) among the different genders and age groups sampled

Conclusion: This study revealed a high level of *H. pylori* infection among the female than the male population sampled. To reduce the detection rates, regular screening, treatment and public health awareness campaign should be developed for the control, elimination and prevention of *H. pylori* infection.

Keywords: 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" (Alfarouk, et al., 2019). "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" (Rust, et al. 2008, Salama, 2020). "*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" (Blaser, 2006).

"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" (Minalyan et al., 2017). "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" (Bytzer et al., 2011). "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" (Ryan, 2010). "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". (Wagner et al., 2017). "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" (Chang and Parsonnet, 2010).

"Studies have revealed that the most common causes of ulcer disease are the bacteria *H. pylori* and Non-steroidal Anti-inflammatory Drugs (NSAIDs)", (Najm, 2011). "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" (Blanchard and Czinn, 2017). "*H. pylori* is a fastidious microorganism and requires complex growth media, often these media are supplemented with blood or serum. These supplements may act as additional sources of nutrients and possibly also protect against the toxic effects of long-chain fatty acids" (Taneera et al., 2002).

"*Helicobacter pylori* appears to have a narrow host range, new infections are thought to occur as a consequence of direct human-to-human transmission or environmental contamination. Person-to-person transmission can be subdivided in two main categories: vertical and horizontal transmission. The vertical mode is infection spread from ascendant to descendent within the same family, while horizontal transmission involves contact with individuals outside the family or environmental contamination" (Kayali et al., 2018). "The exact route of transmission remains unclear, the four major routes of *H. pylori* infection include the fecal–oral route, the oral–oral route, the gastric–oral route, and gastro-gastric route which may occur through the ingestion of contaminated food, water or during endoscopic procedure" (Asghar and Khalid, 2024). 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 includes, 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 the detection of *Helicobacter pylori* infections among patients attending a Government Hospital in Port-Harcourt Rivers State Nigeria.

2. MATERIALS AND 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: $n = Z^2 P (1 - P) /d^2$ (Naing et al., 2006) and a confidence level of 95%.

2.4 *H. pylori* Screening

2.4.1 *H. pylori* antigenic screening

Stool Antigen Tests using enzyme immunoassays or immunochromatography, one can determine whether saliva, blood, or stool contains an antigen induced by *H. pylori* infection. There is no doubt that a SAT is an extremely useful diagnostic tool for detecting and confirming bacterial persistence after treatment, with an overall accuracy of over 90%. (Cardos et al., 2022). Stool samples were collected and tested using the *H. pylori H. pylori* Ag Rapid Test cassette (OnSite CTK Biotech USA). Approx. 1g of stool samples was collected in a stool collection device containing 1ml sample extraction buffer and 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 10 minutes, 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 plus 10% horse blood supplemented with DENT selective supplement containing amphotericin B (25µl), vancomycin (50µl), trimethoprim (156.25µl) and ceftazidime (25µl) replaces cefsulodine (Bayoma and Martin, 2013). Using a sterile inoculating loop, inoculum of stool sample was aseptically spread on the surface of the agar plates. Inverted, the plates were placed in an anaerobic jar, a micro-aerophilic kit GasPak (CO_2) AnaeroGen™, Oxoid, (Basingstoke, United Kingdom) was added, and the jar immediately closed and incubated undisturbed at 37°C for 3 days at 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. If growth was not satisfactory after 5 days, further incubation was done up to 7 days with a new GasPak (CO_2) until the colonies where well

visible. 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 (Kimang'a et al., 2010). 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 Gram stain, 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 and growth on MacConkey agar (Sreenivasan and Abdul, 2009).

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.

3. RESULTS AND DISCUSSION

3.1 Antigenic Screening/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. A sum total of (53.3%) tested positive for antigenic screening for both genders with (21.9%) male and (31.4%) female making *H. pylori* infection in females relatively high with no gender associated significance between the male and female participants (*P*>0.05).

However, for culture, the total positive cases were (7.6%) for both genders with (2.9%) male and (4.7%) female (*P*>0.05). The prevalence of *H. pylori* infections was relatively low according to gender in male compared to that recovered from the female according to this study.

3.2 Age Group Prevalence using Antigenic Screening/Culture-Based Detection Technique

The ratio of prevalence among the different age groups for both antigenic screening and culture ranged between 10 - ≥31 years. *H. pylori* infection increased generally with age between (4.8%) to (20.0%) for antigenic screening (*P*>0.05).

Culture reported low frequency of (3.8%) between age group 26-30 and ≥31 respectively, while the least recovery was observed within age group 21-25 years and below (*P*>0.05), lowest than other age groups as provided in Fig. 2.

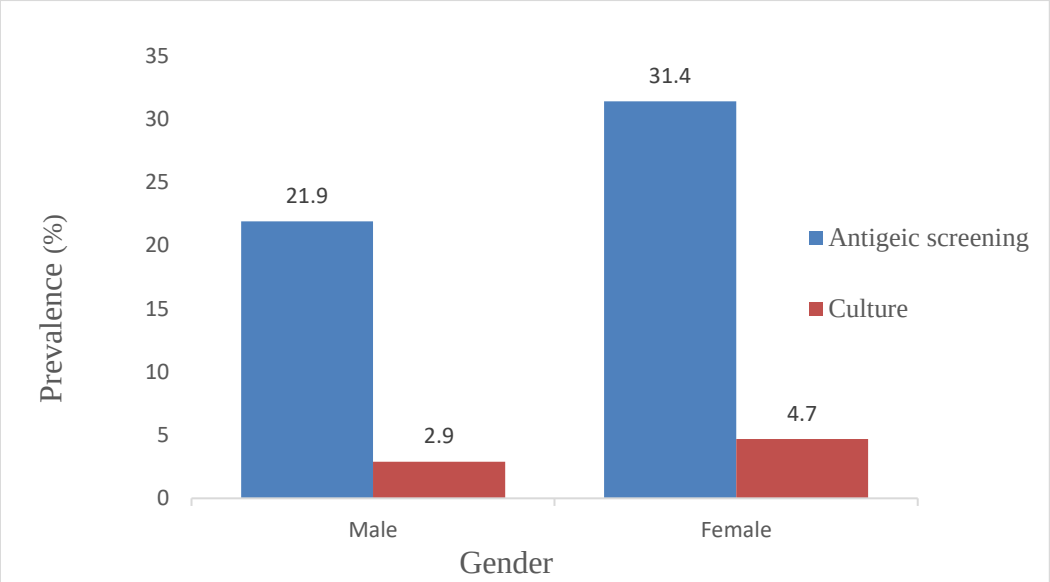

Fig. 1. Prevalence of *H. pylori* using antigenic screening and culture-based technique among the population gender in the study

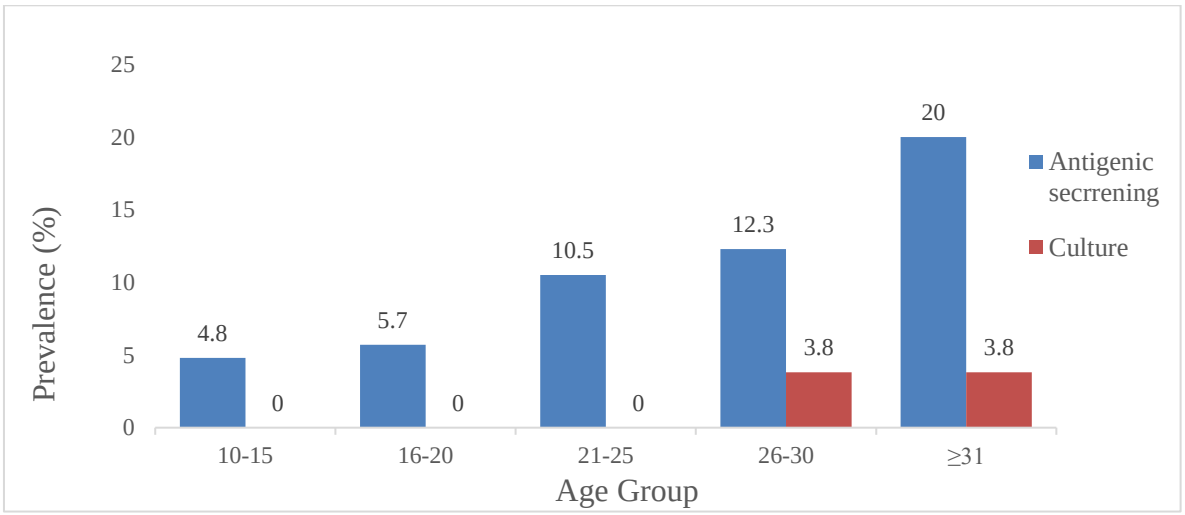

Fig. 2. Prevalence of *H. pylori* among the different age group (years) in the study using antigenic screening and culture-based technique

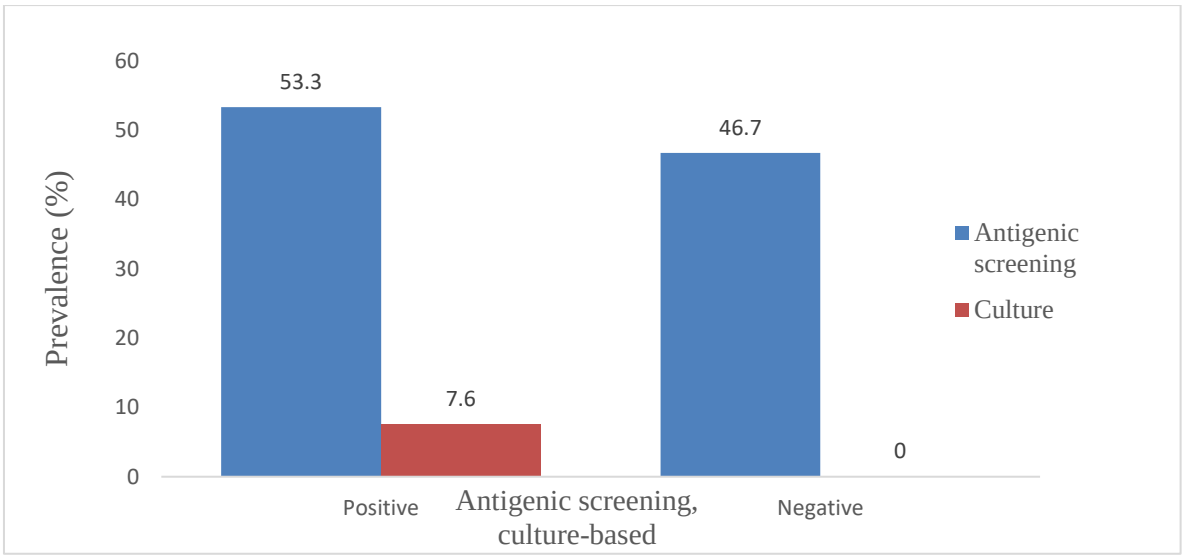

Fig. 3. Overall prevalence of *H. pylori* among the population using antigenic screening and culture-based technique

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

Overall prevalence of *H. pylori* infection in Port-Harcourt out of the 105 patients screened varied from (53.3%) to (7.6%) according to antigenic screening and culture as shown in Fig. 3, while (46.7%) was classified as negative for antigenic screening and (0.0%) negative by culture. *H. pylori* infections was observed to be relatively high for antigenic screening than that obtained by culture with significant difference (P<0.05).

4. 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 antigenic

screening revealed high percentage of prevalence than that recovered by culture respectively. Jhoan *et al.,* (2018) 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

Population detection varied relatively among 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 are in agreement with a study conducted by (Dhary et al., 2023) on gender-based prevalence of *H. pylori* with relatively low infection in the male as compared to the female.

Age group detection generally increased with age ranging from 10-≥31 years for antigenic screening. However, age group for culture ranging from 10-25 years recorded the least recovery, while 26-30 and ≥31 age group revealed low results respectively. These observations were in contrast with (Ghalia, et al., 2019) who reported a higher percentage of prevalence in adult participants aged 16 and ≥30. There was no significant difference in the gender and age group prevalence of *H. pylori* among the studied population. This could probably be due to poor living conditions, fecal contamination of food, water or utensils, lack of clean water and poor sanitary conditions etc. (Kotilea, et al., 2019). Females should 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 (Miftahussurur and Yamaoka, 2016).

5. CONCLUSION

The diagnosis of *H. pylori* infection by antigenic screening has proven to be highly sensitive in the detection of *H. pylori* infection than that obtain by 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.

DISCLAIMER (ARTIFICIAL INTELIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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