Seroprevalence of *Helicobacter pylori* Infection and Risk Factors among Asymptomatic Subjects in Delta State, Nigeria

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**Abstract**

**Purpose:** The study aimed at evaluating the seroprevalence of *H. pylori* infection and its associated risk factors in a cross-section of asymptomatic adult population in Niger-Delta, Nigeria. **Methodology:** 408 apparently healthy volunteers, aged between 18 - 87 years were recruited for this study. Blood samples were collected from participants and analyzed for *H. pylori* antibody (IgG) qualitatively with Combo rapid kits and quantitatively with Accu-Bind ELISA Kits. **Results:** The overall prevalence of *Helicobacter pylori* colonization in 408 asymptomatic adults was 52.5% (n = 214) and 48.3% (n = 197) by qualitative (Combo rapid kits) and quantitative (Accu-Bind ELISA Kits) serological test methods respectively. *H. pylori* infection did not differ statistically between genders (p = 0.962) and among age groups (p = 0.185). In addition, multivariate logistic regression indicated that sex and age were not associated with risk of *H. pylori*. However, participants from Delta Central were at greater risk (OR = 1.89; p = 0.014) of *H. pylori* infection compared with those from Delta South, but those from Delta North were not at greater risk of infection compared with those from Delta South (p = 0.476). **Conclusion:** This study indicated an intermediate seroprevalence of *H. pylori* among asymptomatic adult population.
matic adults in Delta state, Nigeria. The prevalence of \textit{H. pylori} infection was linked to geographical regions but not with sex and age.

**Keywords**

\textit{Helicobacter pylori}, Infection, Risk Factors, Delta State, Nigeria

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1. Introduction

\textit{Helicobacter pylori}, a Gram-negative bacterium that colonizes human gastric mucosa is one of the most common chronic infections which occurs worldwide, with 50% of the world’s population estimated to be carriers of this organism [1] [2]. It is a major aetiological factor in chronic gastritis, peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue lymphoma [3] [4] [5] [6] [7]. The mode of transmission still remains unclear, but literature data suggest different modalities of transmission of the infection to include, person-to-person, faeco-oral, oro-oral, gastro-oral and gastro-gastric transmissions [1] [8].

It has been established that \textit{Helicobacter pylori} infection is commonly acquired during childhood [9] [10] [11], thus, any differences in prevalence of the infection within or among populations likely result from factors that were in effect during childhood. Previous reports show that age, race, living in rural area, overcrowding, socioeconomic status, poor sanitary conditions, mothers with lower educational level, poor diet and poor water supply are some of the important risk factors for transmission of \textit{H. pylori} [1] [2] [12] [13] [14]. Prevalence of \textit{H. pylori} infection in developing countries is higher compared with developed countries [15]. The decline in prevalence in developed countries is a reflection of higher socio-economic status and improved hygiene and sanitation and the active elimination of carriership via antimicrobial treatment [16] [17] [18]. In Nigeria, one of the developing countries, a high prevalence of \textit{H. pylori} has been reported among adult populations with gastric and duodenal ulcers [19] [20] [21].

Diagnostic methods for \textit{Helicobacter pylori} infection are classified as invasive and non-invasive. Invasive methods include culture, immunohistochemistry, rapid urease tests, or the polymerase chain reaction, which require upper gastrointestinal endoscopy for obtaining the diagnostic sample. On the other hand, non-invasive detection methods include the urea breath test, serological and stool antigen methods. It is not clear which method should be used as gold standard for the detection of \textit{H. pylori} infection. However, of all the available tests, invasive tests are considered the most accurate. Serological methods are based on the detection of \textit{Helicobacter pylori} specific antibodies in serum, saliva, or urine and are used for initial screening. The stool antigen test is particularly used when the urea breath test is not available.
Previous studies on the seroprevalence of *H. pylori* have focused mainly on children and symptomatic adult populations. Limited studies exist on the prevalence of *H. pylori* among asymptomatic adult population in Nigeria. In the light of the rising incidents of gastritis and duodenal ulcers in Nigeria, as well as the importance of *H. pylori* infection in the pathogenesis of gastroduodenal diseases, there is therefore a need to re-appraise its seroprevalence rate in a cross-section of asymptomatic adult population. This study therefore was aimed at evaluating the seroprevalence of *H. pylori* infection and its associated risk factors in asymptomatic adult population in a Niger-Delta region of Nigeria. It is believed that the result of the present study will be helpful to plan a future large-scale population survey in different sex, age and socioeconomic groups.

2. Methodology

2.1. Participants

A total number of 408 apparently healthy volunteers, aged between 18 - 87 years were recruited for this study from the three senatorial districts of Delta state (Delta South, n = 141; Delta Central, n = 128 and Delta North, n = 139). A well-structured questionnaire was administered to every participant of this study to obtain their demographic information and health history. All volunteers with history of gastric ulcers were excluded from the study. Informed consents of participants were sought and obtained after explaining the purpose of the research. The Ethics committee of Ministry of Health, Delta State approved the study.

2.2. Sample Collection

Five (5 mL) of venous blood sample was collected from each participant and the serum separated immediately into sterile tubes and stored at a temperature of 2˚C - 8˚C for up to 3 days prior to analysis. For a longer storage they were kept at −20˚C until analyzed for anti- *Helicobacter pylori* antibodies—IgG detection. All blood samples obtained from volunteers were screened qualitatively using aria *Helicobacter pylori* antibody combo rapid test kit (serum/plasma/whole blood) which is a sandwich lateral flow chromatographic immunoassay for the qualitative detection of antibodies against *Helicobacter pylori* in human serum/plasma or whole blood [22]. Serum/plasma samples were assayed quantitatively using the Accu-Bind ELISA Micro-wells for the detection of IgG Antibodies to *H. pylori* in human serum (system code, 1425-300; Monobind Inc., Lake Forest, CA 92630, USA).

Stool specimens were collected from the participants. Small piece of stool (~5 mm in diameter; ~150 mg) was transferred into 1 ml of Sample Treatment Solution in a test tube and mixed thoroughly. Stool samples obtained were screened qualitatively using aria *Helicobacter pylori* antigen rapid test kit (fecal specimen) which is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human stool samples [23]. The stool samples were
also assayed quantitatively using the stool antigen Accu-Diag™ H. pylori Antigen ELISA Kit (Cat #1506-11, USA).

All assay procedures were carried out according to manufacturer’s instruction. All samples were analyzed at Shalom Medical Services, Warri-Delta State, Nigeria.

2.3. Data Analysis

Data was expressed as percentages for categorical variables. Comparative analyses to evaluate differences in H. pylori positivity between categorical variables were done using Chi-square test. The association between each potential risk factor and the outcome (H. pylori status) was measured using logistic regression. Statistical significance was set at p < 0.05. All statistics were done using SPSS/IBM statistical software (version 20).

3. Results

Table 1 shows the socio-demographic characteristics of the study population. Data indicated that majority (81.6%) of the participants were aged between 18 - 39 years of age and followed by the age group, 40 - 59 years (13%). Those aged ≥60 years had the least percentage of the population (5.4%). The mean age of the study population was 32.55 ± 11.98 years (range 18 - 87 years). Majority (70.6%) of the study population were females, while the males constituted a smaller percentage (29.4%) of the population. The participants were selected from Delta South (34.6%), Delta North (34.1%) and Delta Central (31.4%) districts.

The overall prevalence of Helicobacter pylori colonization in 408 asymptomatics was 52.5% (n = 214) and 48.3% (n = 197) by qualitative (Combo rapid kits) and quantitative (Accu-Bind ELISA kits) serological test methods respectively (Figure 1).

The prevalence of H. pylori according to sex, age and geographical location of participants is summarized in Tables 2-4 respectively. Among the 408 subjects,
Figure 1. The seroprevalence of *H. pylori* in the study population using two different test methods.

Table 2. The seroprevalence rate of *H. pylori* according to sex of subjects.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Subjects</th>
<th>Number of Positive Tests</th>
<th>Prevalence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>120</td>
<td>66</td>
<td>55.0</td>
</tr>
<tr>
<td>Females</td>
<td>288</td>
<td>148</td>
<td>51.4</td>
</tr>
<tr>
<td>Total</td>
<td>408</td>
<td>214</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Table 3. The seroprevalence rate of *H. pylori* in different age groups.

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Number of Subjects</th>
<th>Number of Positive Tests</th>
<th>Prevalence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (18 - 39 yrs)</td>
<td>333</td>
<td>176</td>
<td>52.9</td>
</tr>
<tr>
<td>Middle-aged (40 - 59 yrs)</td>
<td>53</td>
<td>24</td>
<td>45.3</td>
</tr>
<tr>
<td>Elderly (≥60 yrs)</td>
<td>22</td>
<td>14</td>
<td>63.6</td>
</tr>
<tr>
<td>Total</td>
<td>408</td>
<td>214</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Table 4. The seroprevalence rate of *H. pylori* according to geographical regions of participants.

<table>
<thead>
<tr>
<th>Geographical Area</th>
<th>Number of Subjects</th>
<th>Number of Positive Tests</th>
<th>Prevalence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta Central</td>
<td>128</td>
<td>79</td>
<td>61.7</td>
</tr>
<tr>
<td>Delta North</td>
<td>139</td>
<td>64</td>
<td>46.0</td>
</tr>
<tr>
<td>Delta South</td>
<td>141</td>
<td>71</td>
<td>50.4</td>
</tr>
<tr>
<td>Total</td>
<td>408</td>
<td>214</td>
<td>52.5</td>
</tr>
</tbody>
</table>

214 (52.5%) tested positive while 194 (47.5%) tested negative for blood antibody test. The highest positive result for blood antibody test (55%) was observed in males, while in female it constituted 51.4%. The percentages of positive cases appear to be higher in males (55%) compared to the females (51.4%). However, the difference was not statistically significant (p = 0.962). The highest positive
result was found in the age group of ≥60 yrs, which indicated 63.6% seropositivity (Table 3). This was followed by seropositivity in age groups 18 - 39 years (52.9%) and 40 - 59 years (45.3%). No significant difference (p = 0.185) was observed in the prevalence of infection among the different age groups. Table 4 indicated that the highest prevalence rate for \textit{H. pylori} was observed in Delta Central (61.7%), followed by Delta North (50.4%), while Delta South had the least percentage of \textit{H. pylori} seropositivity (46.0%). The seropositivity observed in Delta Central varied with Delta South (p = 0.01), but not with Delta North (p = 0.06).

The univariate analysis and multivariate logistic regression analysis for the association between characteristics of participants and \textit{H. pylori} status are shown in Table 5. In the univariate analysis, demographic characteristics such as age (p = 0.330) and gender (p = 0.506) were not associated with \textit{H. pylori} status. Geographical region had a statistically significant association (p = 0.031) with \textit{H. pylori} status. Multivariate logistic regression further indicated that participants aged ≥65 years were not at greater risk of \textit{H. pylori} infection compared with those aged 18 - 39 yrs (OR = 0.64; p = 0.382) or those aged 40 - 59 yrs (OR = 0.47; p = 0.206). Similarly, females did not indicate significantly (p = 0.516) greater risk for \textit{H. pylori} seropositivity compared to the males. Participants from Delta Central were at greater risk (OR = 1.89; p = 0.014) of \textit{H. pylori} infection compared with those from Delta South. On the other hand, participants from Delta North were not at greater risk of infection compared with those from Delta South (p = 0.476).

4. Discussion

Serologic testing represents a good primary screening test used in epidemiologic

\textbf{Table 5.} Univariate analysis and multivariate logistic regression test indicating association and risk of age, sex and geographical regions of participants with \textit{H. pylori}.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>\textit{H. pylori} (n = 408)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (n (%))</td>
<td>Absent (n (%))</td>
<td>Odds Ratio (OR)</td>
</tr>
<tr>
<td>\textbf{AGE}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 - 39</td>
<td>176 (52.9)</td>
<td>157 (47.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>40 - 59</td>
<td>24 (45.3)</td>
<td>29 (54.7)</td>
<td>0.47</td>
</tr>
<tr>
<td>≥60*</td>
<td>14 (63.4)</td>
<td>8 (36.4)</td>
<td>1</td>
</tr>
<tr>
<td>\textbf{SEX}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males*</td>
<td>66 (55.0)</td>
<td>54 (45.0)</td>
<td>0.506</td>
</tr>
<tr>
<td>Females</td>
<td>148 (51.4)</td>
<td>140 (48.6)</td>
<td>0.87</td>
</tr>
<tr>
<td>\textbf{Geographical Area}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta South*</td>
<td>64 (46.0)</td>
<td>75 (54.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>Delta North</td>
<td>71 (50.4)</td>
<td>70 (49.6)</td>
<td>1.19</td>
</tr>
<tr>
<td>Delta Central</td>
<td>79 (61.7)</td>
<td>49 (38.3)</td>
<td>1.89</td>
</tr>
</tbody>
</table>

*Reference group.
studies for the evaluation of \textit{H pylori} status in patients not immediately requiring esophagogastroduodenal endoscopic studies [24]. It is relatively inexpensive, convenient and enables large numbers of subjects to be screened quickly. However the major limitations include the difficulty in defining the cutoff value that divides positive from negative subjects; its sensitivity to changes in reagents and laboratory conditions; its ability to detect and return false negative results up to 60 days after infection and remain positive for a considerable time after eradication [25]. These therefore call for appropriate equipment and techniques and validation of methods for each region, population and age. In this study we used and evaluated the performance of two different commercial serological test methods for the detection of immunoglobulin G (IgG) antibody to \textit{H. pylori}. Our data revealed that antibodies were detected in 52.5\% (\(n = 214\)) of the 408 volunteers who participated in the study, by using qualitative (Combo rapid kits) and 48.3\% (\(n = 197\)) by using quantitative (Accu-Bind ELISA kits) serological test methods. Seventeen (17) of the 214 seropositive samples (tested by qualitative test method) gave negative results when tested by the quantitative ELISA test method. This shows that the qualitative test method is more sensitive than the quantitative method in detecting \textit{H. pylori} antibodies.

The present finding based on the qualitative serology test therefore shows that the overall seroprevalence of \textit{H. pylori} among asymptomatic adults aged 18 - 87 years is 52.5\%. This result is in contrast with previous studies conducted in Nigeria, which showed prevalence of 12.7\%, 38.3\%, 89.7\% and 64\% in Warri, Ethiope, Agbor, and Ibadan respectively [26] [27] [28] [29], but in agreement with a previous study in Sokoto Nigeria, which showed prevalence of 54.8\% [30]. Compared with results from other countries, our finding is in agreement with a study in Hong Kong, which reported seroprevalence of 58\% in healthy adult volunteers [31]. In contrast, \textit{H. pylori} prevalence in this study was lower than those obtained in healthy adult populations of some countries such as China (80\%), Korea (75\%) [31] [32], but higher than other findings found in Netherlands (27\%), Australia (23\%) and United Kingdom (41\%) [33] [34] [35]. It is usually difficult to compare directly the prevalence rates from different studies due to variations in age and type of population. Nevertheless, the seroprevalence variations observed between our study and others may be attributed to differences in methodology and technical factors, variations in age, ethnicity as well as level of sanitation and social economic status of individual subjects screened.

In this study, the prevalence of \textit{H. pylori} infection appeared higher in males 55\% than females 51.4\%, but the difference was not statistically significant. This finding is in agreement with previous studies [27] [30]. In contrast, another study [26] indicated higher prevalence of \textit{H. pylori} in females compared with males, while Kaore \textit{et al.} [36] reported higher prevalence in male gender. Age distribution of \textit{H. pylori} infection showed a trend towards decrease in infection with age from young (52.9\%) to middle-aged (45.3\%), then rises in the elderly (63.6\%). However, there was no statistically significant difference in \textit{H. pylori}
prevalence among age groups. This was consistent with a previous study by Dooley et al. [37]. In contrast, significant difference in H. pylori infection has been observed in age [38]. Studies conducted in the past decade have reported a high prevalence of H. pylori infection within the oldest population [39] [40]. However, a marked reduction in the prevalence of infection is previously reported in elderly people [41] [42]. The higher prevalence in the elderly may be explained by the mode of transmission of H. pylori (oro-fecal or oro-oral), taking into account the living and sanitary conditions of the elderly who are often neglected and uncared for due to high rate of poverty in developing countries. H. pylori prevalence rates are reported to vary widely between different geographical regions and ethnic groups [43]. Our finding which showed geographical variation between Delta Central and Delta South is in agreement with a previous study by Dorji et al. [44].

Our finding showed that H. pylori infection was associated with geographical region, but not with age and sex. The geographical areas or residences of participants were categorized into three senatorial zones of Delta state, Nigeria. Participants from Delta Central were at greater risk of H. pylori infection compared with those from Delta South. The reason behind this geographical variation is not known. However, it is thought that the risk of H. pylori prevalence by geographical areas may reflect differences in social and/or hygiene factors [45] and also related to differences in cultural background, social, dietary and environmental factors [46] [47]. A previous study [48] has also shown no relationship between H. pylori and gender, age in adults. In contrast, another study, [49] reported association between H. pylori infection and age and sex.

5. Conclusion

In conclusion the present study indicated an intermediate seroprevalence (52.5%) of H. pylori among asymptomatic adults in Delta state, Nigeria. The prevalence of H. pylori infection was linked to geographical regions but not with sex and age.

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