FULL LENGTH RESEARCH ARTICLE

# PREVALENCE AND MOLECULAR CHARACTERIZATION OF *Helicobacter pylori* ISOLATED FROM SUBJECTS ATTENDING SELECTED HOSPITALS IN NASARAWA STATE

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## ABSTRACT

*Helicobacter pylori is a Gram negative, slow growing, resilient, helical shaped, microaerophilic bacillus whose normal habitat is the epithelial tissues of the human stomach's mucosa. This study determined the prevalence and molecular characteristics of H. pylori in Akwanga and Keffi both in Nasarawa State. The presence of H. pylori in stool samples collected from 200 subjects (100 each from General Hospital Akwanga and Federal Medical Centre, Keffi) was detected using rapid test kit while the bacterial DNA was extracted using standard methods. This was followed by DNA amplification using PCR, gel electrophoresis of the amplicons, sequence analysis and multiple sequence alignment. Eighteen (9.00 %) out of the 200 samples analysed were found to be positive for H. pylori. The highest prevalence (3.00 %) was recorded among the 26-30 years age group while the age group 6 - 10 years had no infection at all. The males had higher prevalence of infection (5.50 %) compared to the females (3.50%). The rate of infection was found to be higher among rural dwellers (5.50%), those that take stream/river (4.50 %) and those that use pit latrine toilet facility (5.50 %) compared with those using water closet (3.5 %). The molecular result showed that H. pylori strain G-Mx-2003-108 was detected in the selected hospital each having 500 base pair. The result of this study has shown that the circulating strain of H. pylori in Nasarawa State is H. pylori G-Mx-2003-108. The carriage rate of H. pylori in the study area underscores the need for public enlightenment and the provision of public sanitary facilities.*

**Keywords:** *Helicobacter pylori* G-Mx-2003-108, Nasarawa State, stool, prevalence, molecular characteristics

## INTRODUCTION

*Helicobacter pylori* initially known as *Campylobacter pylori* is a Gram negative, slow growing, resilient, helical shaped, microaerophilic bacillus that uses bipolar flagella to move around in the epithelial tissues of the stomach's mucosa, which serve as its normal habitat in its amphibiotic relationship with humans (Bani-Hani *et al.*, 2020; Ruiz-Rico *et al.*, 2020).

The stomach of humans is essentially an inhospitable environment for the survival and growth of disease causing agents because of its low pH which is usually between 1.5 and 3.5 (Meyer and Morey, 2020). However, *H. pylori* has evolved to adapt to the high acidic nature of the gastric mucosa (Alsakee *et al.*, 2019). Through the production of urease enzyme that catalyses the hydrolysis of urea to ammonia in the stomach thereby raising the pH of the immediate surroundings of the microorganism (Ruiz-Rico *et al.*, 2020). It also possesses proton pump inhibitors (PPIs), an anti-secretory agent, which are sufficient in inhibiting acid secretion by the stomach (Bani-Hani *et al.*, 2020). The ability of *H. pylori* to withstand the unfavourably harsh acidic pH of the stomach as well as the evasion of the human immune system is thought to be the result of over 100,000 years of adaptations through a process of coevolution with humans (Meyer and Morey, 2020).

Since its discovery by Barry Marshall and Robin Warren in 1983 from a person suffering from chronic ulcer and chronic gastritis, conditions initially believed to have a non-microbiological etiology (Alsakee *et al.*, 2019; Meyer and Morey, 2020; Muhsen *et al.*, 2020; Outliouaa *et al.*, 2020; Zheng *et al.*, 2020), efforts have been made to eradicate this bacterium through endoscopic intervention, which led to a reduction in *H. pylori* infection from 66.9 % in 1998 to 51.0 % in 2015 (Jekarl *et al.*, 2020).

Despite continuing global preventive efforts, approximately 52.1 % - 58 % of the world's population are currently living with *H. pylori* infections (Papaefthymiou *et al.*, 2020) with clinical manifestations such as gastritis, peptic ulcer disease, gastric atrophy, adenocarcinoma, gastric mucosal associated lymphoid tissue lymphoma, iron deficiency anemia, gastric cancer (Bani-Hani *et al.*, 2020; Jekarl *et al.*, 2020; Papaefthymiou *et al.*, 2020; Ruiz-Rico *et al.*, 2020) and glaucoma (Qian, 2020). The country prevalence of *H. pylori* is believed to currently range between 20% in developed countries of North America, Oceania and Europe to 80 % in developing countries mostly in Africa (Papaefthymiou *et al.*, 2020).

The high prevalence of *H. pylori* infection in Africa (~70%) is a direct result of the poor standard of living among the majority of African people due mainly to poverty and ignorance (Šterbenc *et al.*, 2019). Though, high prevalence rates of *H. pylori* carriage have also been reported in some developed nations such as between 30-40 % in USA (Goit *et al.*, 2019), 78.5% in Russia (Zhestkova *et al.*, 2019) and 43 % in West Iran (Mbang *et al.*, 2019). A prevalence rate of between 70 to 90 % for *H. pylori* was also reported among South Americans (Mbang *et al.*, 2019). According to Wawro *et al.* (2019), Nigeria has the highest prevalence (87.7 %) of *H. pylori* infections in Africa. The routes of transmission of *H. pylori* remains unclear, however, the fecal-oral route (i.e. consumption of food materials contaminated with faeces of infected individual) and oral-oral routes (i.e. sharing of kitchen utensils such as spoons and cups) appears to be the most significant means of transmission (Alsakee *et al.*, 2020; Ruiz-Rico *et al.*, 2020).

Although colonization with *H. pylori* is not in itself a disease, it is however a risk factor for the development of various clinical disorders of the upper gastrointestinal tract, and possibly the hepatobiliary tract (Kusters *et al.*, 2006). It has been reported that the result of colonization as well as its severity depend on the colonizing strain, host factors as well as environmental factors (Blaser and Berg, 2001). This observation which gave rise to two concepts namely the "Asian Enigma" and the "African Enigma" on account of the clear differences in the clinical outcome of bacterial colonization in both continents which further reinforces the earlier claims that virulence is a function of genotype (Wu *et al.*, 2012). It is therefore imperative to identify the strains circulating in communities as part of control efforts hence the current attempt in this study.

## MATERIALS AND METHODS

### Ethical Consideration

Ethical clearance was obtained from the authorities of General Hospital, Akwanga and Federal Medical Centre, Keffi both in Nasarawa State, Nigeria while the administration of questionnaire and the collection of samples from subjects was based on informed consent.

### Collection of Stool Samples

Stool samples 100 each from General Hospital, Akwanga and Federal Medical Centre, Keffi were collected in clean, dry, screw-top container and labelled appropriately.

### Screening of Stool Samples

The screening of stool samples for *H. pylori* was carried out using the Aria *H. pylori* Ag rapid test kit (U.S.A) which is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human stool. Two drops (70-90 µl) of the homogeneous liquid suspension of the stool sample and the reagent in the stool collection device was dispensed into the sample well of the test kit cassette without overloading the well and left for 10 minutes before taking the reading. Appearance of a single red line on the Control (C) indicates a negative result while double lines on the Test (T) and Control (C) indicate positive sample as earlier reported by Fashner and Gitu (2015).

### DNA Extraction

Exactly 980 mL phosphate buffer and 180 mL 7.5 M guanidine solution containing 5 % sarcosine was added to 100 mg of stool in a 3 mL microtube and mixed gently using a 200ml pipette tip to homogenize. The mixture was centrifuged at 16,000 ×g for 30s and the approximately 750 mL supernatant was transferred to 1.5 mL Eppendorf tube and 250 mL of 3.5 M sodium acetate (pH 5.2) was added and mixed gently. The resulting mixture was centrifuged at 16,000 ×g for 5 min. The supernatant was transferred to a fresh Eppendorf tube to which 700 mL of membrane binding solution (Promega) was add and mixed gently by pipetting. The 700 mL mixture was pipetted into SV Minicolumn in a Collection Tube (Promega) and incubated at room temperature for 1 min after which it was centrifuged at 16,000 ×g for 1 min. The flow through was discarded and the process was repeated for the remaining 700 mL mix. Exactly 700 µL of membrane wash solution (ethanol added as per the instructions) was added to the column and centrifuged at 16,000 ×g for 1 min. The flow through was discarded and a collection tube was reinserted into minicolumn. This was repeated with 500 µL of Membrane Wash Solution and centrifuged at 16,000×g for 5 min. The collection tube was then emptied and the column assembly was centrifuged for 1 min with the microcentrifuge lid open to allow evaporation of any residual ethanol. The column was transferred to a 1.5 mL Eppendorf tube and 58 mL of nuclease-free water added to the column followed by incubating at room temperature for 2 min and then centrifuged at 16,000 ×g for 1 min. DNA solution was stored at −20°C until use for PCR (Laith and Abeer, 2017).

### PCR Amplification, Integrity Check and Purification of Amplified Product

PCR amplification was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 47°C for 30s and 72°C for 40s; and a final termination at 72°C for 10 min and chilled at 4°C.GEL (2, 3) as described by Laith and Abeer (2017). The integrity of the amplified PCR product of about 500bp gene fragment was checked on a 1.5 % Agarose gel to confirm amplification as described by Laith and Abeer (2017). After the integrity check, the amplified fragments were ethanol purified in order to remove the PCR reagents as described by Laith and Abeer (2017).

### Sequencing Analysis of the Amplicons

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (Sanger *et al.*, 2002).

### Analysing Gene Sequence Results with BLAST

Highly similar sequences were retrieved from National Centre of Biotechnology Information (NCBI) and subjected to multiple sequence alignment using the BioEdit software (Hall *et al.*, 1999). Genetic distance was estimated using molecular evolutionary genetic analysis (MEGA) version 4.0 MUSCLE (Edgar, 2004; Tamura *et al.*, 2007). *H. pylori* genotypes and subtypes were classified based on the NS5b sequence and by comparing each sequence with published reference sequences from Genbank. An initial evolutionary history was inferred using neighbour- joining method, with sequence distances calculated using the maximum composite likelihood method (Saitu and Nei, 1987). The percentage of replicate trees in which taxa clustered together was calculated using a bootstrap test of 1000 replicate, with value above 70 % as cut-off for defining clusters and a maximum genetic distance threshold between 0.025-0.065 to determine the effect on the


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identification of factors associated with clustering (Felsenstein, 1985).

### Phylogenetic Analysis

Phylogenetic tree of 16s rRNA genes and their evolutionary relationship with those obtained from database was done online by Clustal W 2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Atschul *et al.*, 1997) and phylogenetic tree was constructed based on the sequences of fragments, using One click mode from Phylogeny.fr.home (http://www.phylogeny.fr.home) (Castresana, 2000).

### Data Analysis

Data was analyzed using Statistical Package for Social Science (SPSS) version 23 software, 2018. The *H. pylori* DNA levels and other clinical/ laboratory parameters was expressed as the median value and rang. 16.0 for windows was used. Comparison of prevalence was done using chi-square test while *P* values less than 0.05 was considered significant. Cluster analysis was used to determine the genetic relationships among the identified strains.

## RESULTS

Distribution of Infection Rates of *H. pylori* General Hospital Akwanga and Federal Medical Centre, Keffi

Of the 200 stool samples collected from both hospitals, 18 (9.00 %) samples were found to be positive for *H. pylori*. The highest prevalence was recorded within age group 26-30 years with 3.00 % prevalence rate followed by age group 16-20 with prevalence rate of 2 %. Age group 6-10 had 0 % prevalence rate. *Helicobacter pylori* infection was not significantly higher in subject within the age group 26-30 years ($P < 0.05$) than in other age group as observed (Table 1).

**Table 1.** Distribution of infection rate according to age of the subjects attending Akwanga and Keffi General hospitals in Nasarawa State.

| Age group | NSS | NPS | NNS | P-value |
|---|---|---|---|---|
| 1-5 | 8 | 1(0.5) | 7(3.5) | |
| 6-10 | 4 | 0(0.0) | 4(2) | |
| 11-15 | 9 | 1(0.5) | 8(4) | |
| 16-20 | 43 | 4(2) | 43(21.5) | |
| 21-25 | 42 | 2(1) | 40(20) | 0.951 |
| 26-30 | 46 | 6(3) | 40(20) | |
| 31-35 | 18 | 2(1) | 16(8) | |
| 36-40 | 14 | 1(0.5) | 13(6.5) | |
| 41 and above | 16 | 1(0.5) | 11(5.5) | |
| **Total** | **200** | **18(9)** | **182(91)** | |

Keys: NSS = Number of samples screened, NPS = Number of positive samples, NNS = Number of negative samples

The distribution of *Helicobacter pylori* according to gender of the subjects in General Hospital Akwanga and Federal Medical Centre, Keffi is presented in Table 2 and the male gender has the highest prevalence rate with 5.5 % while the least prevalence rate was recorded among the female with 3.5 %.

**Table 2.** Distribution of infection according to the gender of subjects

| Gender | NSS | NPS | NNS | P-value |
|---|---|---|---|---|
| Male | 97 | 11(5.5) | 86(43) | |
| Female | 103 | 77(3.5) | 96(48) | 0.191 |
| Total | 200 | 18 (9) | 182 (91) | |

Keys: NSS = Number of samples screened, NPS = Number of positive samples, NNS = Number of negative samples
$P > 0.05$ shows that *Helicobacter pylori* infection was not significantly higher in male subject.

The distribution of *Helicobacter pylori* prevalence rate according to the location of patient's residence is presented in Table 3. The rural resident had the highest prevalence rate of 6.5 % while the least prevalence was recorded among the urban dwellers.

**Table 3.** Distribution of infection according to the geographical location of the subjects

| Location | NSS | NPS | NNS | P-value |
|---|---|---|---|---|
| Urban | 66 | 5(2.5) | 61(30.5) | |
| Rural | 154 | 13(6.5) | 121(60.5) | 0.418 |
| **Total** | **200** | **18(8)** | **182(91)** | |

Keys: NSS = Number of samples screened, NPS = Number of positive samples, NNS = Number of negative samples
$P > 0.05$ shows that *Helicobacter pylori* infection was not significantly higher in subject
living in the rural areas

Table 4 presents the prevalence rate of *Helicobacter pylori* according to the water sources of subjects, with highest prevalence rate record among those that take well water (2.5 %), stream or river water recorded 4.5 % while the least was among those that take pipe borne water with 2% prevalence


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rate. P > 0.05 shows that *Helicobacter pylori* infection was significantly higher in subject using the well water source in subjects attending General Hospital, Akwanga and Federal Medical Centre, Keffi.

Table 4. Distribution of infection rate according to water sources of the subjects attending General Hospital, Akwanga and Federal Medical Centre, Keffi

| Source of water | NSS | NPS | NNS | P-value |
|---|---|---|---|---|
| Pipe borne | 74 | 4(2) | 70(35) | |
| Stream/ River | 97 | 9(4.5) | 88(44) | 0.167 |
| Well | 29 | 5(2.5) | 24(12) | |
| **Total** | **200** | **18(9)** | **182(91)** | |

Keys: NSS = Number of samples screened, NPS = Number of positive samples, NNS = Number of negative samples
P > 0.05 shows that *Helicobacter pylori* infection was not significantly higher in subject using the well water source

The distribution of the infection rate of *Helicobacter pylori* according to the patient's type of toilet facility is presented in Table 5 The subjects using pit latrine toilet facility had a prevalence rate of 5.5 % when compared with those using water closet (3.5 %).

Table 5. Distribution of infection rate according to the type of toilet facilities of the subjects Attending General Hospital, Akwanga and Federal Medical Centre, Keffi

| Toilet Facilities | NSS | NPS | NNS | |
|---|---|---|---|---|
| Pit latrine | 106 | 11(5.5) | 95(47.5) | |
| Water closet | 94 | 7(3.5) | 87(43.5) | 0.319 |
| **Total** | **200** | **18(9)** | **182(91)** | |

Keys: NSS = Number of samples screened, NPS = Number of positive samples, NNS = Number of negative samples
P > 0.05 shows that *Helicobacter pylori* infection was not significantly higher in subject
using the pit latrine

**Relationship Between Demographic Information of the Subjects and the Infection Rates**

The relationship between demographic information of the subjects and the infection rates attending General Hospital, Akwanga and Federal Medical Centre, Keffi in Nasarawa State is presented in Table 6. In general, there was no significant difference (P>0.05) between the age, sex, occupation, location, source of water and toilet facilities of subjects attending General Hospital, Akwanga and Federal Medical Centre, Keffi.

Table 6: Relationship between demographic information of the subjects and the infection rates attending General Hospital, Akwanga and Federal Medical Centre, Keffi

| Factors | | Number of Positive | P-value |
|---|---|---|---|
| Age | 1-5 | 1 | |
| | 6-10 | 0 | |
| | 11-15 | 1 | |
| | 16-20 | 4 | 0.951 |
| | 21-25 | 2 | |
| | 26-30 | 6 | |
| | 31-35 | 2 | |
| | 36-40 | 1 | |
| | 41 and above | 1 | |
| Sex | Male | 11 | 0.191 |
| | Female | 7 | |
| Occupation | Non-working class | 13 | |
| | Working class | 5 | 0.475 |
| Location | Rural | 5 | |
| | Urban | 13 | 0.418 |
| Source of Water | Pipe Bone | 4 | |
| | Stream | 9 | |
| | Well | 5 | 0.167 |
| Toilet Facility | Pit latrine | 11 | |
| | Water closet | 7 | 0.319 |

**Molecular Identity of the *H. pylori* Positive Samples**

The *gel electrophoresis micrograph of amplified product for* General Hospital Akwanga (NaGHA) *(Plate 1) and* Federal Medical Centre, Keffi (NaFMCKe) *(Plate 2) has 500 base pair. Eighteen (18) strains of H. pylori strain were detected in* General Hospital Akwanga and Federal Medical Centre, Keffi. *The strains identified was* G-Mx-2003-108 (18). *The % identity is presented in Table 7. The phylogenetic tree that shows relationship between the strains of H. pylori is presented in Figure 1. This is followed by the nucleotide sequences of the H. pylori strains.*


Plate 1: *Gel electrophoresis micrograph of amplified product for* NaGHA


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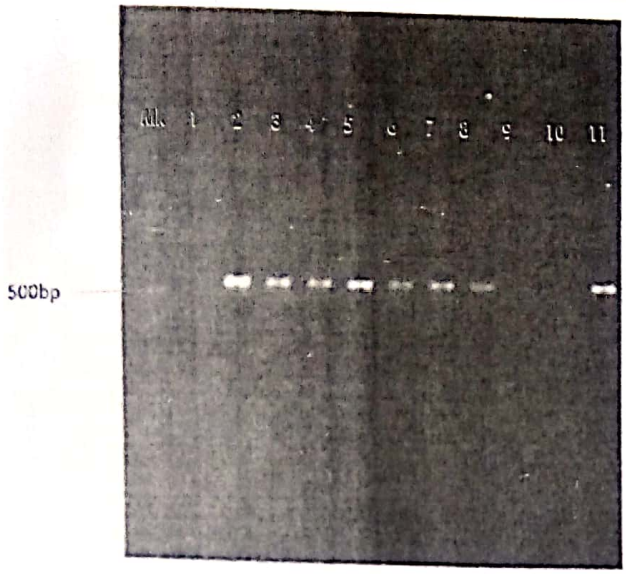


Plate 2: *Gel electrophoresis micrograph of amplified product for* NaFMCKe

Table 7: Molecular identity of *Helicobacter pylori* positive samples in Nassarawa State.

| Samples | Description | % identity | Accession |
|---|---|---|---|
| AudS_NaGHA_1 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_2 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_3 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_4 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_5 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_6 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaGHA_7 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_1 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_2 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_3 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_4 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_5 | *H. pylori* strain G-Mx-2003-108 chromosome | 99.80% | CP032044.1 |
| AudS_NaFMCKe_6 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_7 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_8 | *H. pylori* strain G-Mx-2003-108 chromosome | 99.81% | CP032044.1 |
| AudS_NaFMCKe_9 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_10 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |
| AudS_NaFMCKe_11 | *H. pylori* strain G-Mx-2003-108 chromosome | 100.00% | CP032044.1 |

Nucleotide sequences of *H. pylori* from General Hospital Akwanga are as follows:

MN736557 *H. pylori* strain AudS_NaGHA_1

CTACCCACCAAGCATTGTCCTGCCTGTGGATAACACAG
GCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCA
AGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAG
CCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGT
GCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTT
GCCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTC
ACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGC
CATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCG
CTACCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTC
GGGCTTCAATTCAACGCTTCATCTTGCGACTGACGCATC
CTCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTTA
TACTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGG
TAAACAGTCGGGAGGGACTCTTTGCTGAGACCGCATTG
CTGCGGCACCTT

MN736558 *H. pylori* strain AudS_NaGHA_2

ACCCACCAAGCATTGTCCTGCCTGTGGATAACACAGGC
CAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCAAGG
ATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAGCCTC
CCACCTATCCTGCGCATGATATTCCCATTAGCAGTGCTA
AGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTTGCC
GCGGGTAGGAGGAATTTTCACCTCCACTACAATTTCACT
GAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGCCAT
TCATGCAGGTCGGTATTTAACCGACAAGGAATTTCGCTA
CCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTCGG
GCTTCAATTCAACGCTTCATCTTGCGACTGACGCATCCT
CTTAACCTTCGAGCACCGGGCAGGCGTCACACCTTATA
CTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGGTA
AACAGTCGGGAGGGACTCTTTGCTGAGACCGCATTGCT
GCGGCACCTT

MN736559 *H. pylori* strain AudS_NaGHA_3

CAACTACCCACCAAGCATTGTCCTGCCTGTGGATAACAC
AGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC
AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAG
CCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGT
GCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTT
GCCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTC
ACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGC
CATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCG
CTACCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTC
GGGCTTCAATTCAACGCTTCATCTTGCGACTGACGCATC
CTCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTTA
TACTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGG
TAAACAGTCGGGAGGGACTCTTTGCTGAGACCGCATTG
CTGCGGCACCTT

MN736560 *H. pylori* strain AudS_NaGHA_4

AACTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA
GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCA
AGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAG
CCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGT
GCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTT
GCCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTC
ACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGC
CATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCG
CTACCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTC
GGGCTTCAATTCAACGCTTCATCTTGCGACTGACGCATC


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CTCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTT
ATACTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTT
GGTAAACAGTCGGGAGGGACTCTTTGCTGAGACCGCA
TTGCTGCGGCACCT

MN736561 *H. pylori* strain AudS_NaGHA_5

CACTACCCACCAAGCATTGTCCTGCCTGTGGATAACAC
AGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCT
CAAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAA
AGCCTCCCACCTATCCTGCGCATGATATTCCCATTAGC
AGTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCC
GTCTTGCCGCGGGTAGGAGGAATTTTCACCTCCACTA
CAATTTCACTGAATCTCTGGTTGAGACAGCTCCCATCT
CGTTACGCCATTCATGCAGGTCGGTATTTAACCGACAA
GGAATTTCGCTACCTTAGGACCGTTATAGTTACGGCCG
CCGTTTACTCGGGCTTCAATTCAACGCTTCATCTTGCG
ACTGACGCATCCTCTTAACCTTCGAGCACCGGGCAGG
CGTCACACCTTATACTTCCTCTTACGAGTTGGCAAAGT
GCTGTGTTTTTGGTAAACAGTCGGGAGGGACTCTTTG
CTGAGACCGCATTGCTGCGGCACCTT

MN736562 *H. pylori* strain AudS_NaGHA_6

ACTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA
GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC
AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAA
GCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCA
GTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCG
TCTTGCCGCGGGTAGGAGGAATTTTCACCTCCACTAC
AATTTCACTGAATCTCTGGTTGAGACAGCTCCCATCTC
GTTACGCCATTCATGCAGGTCGGTATTTAACCGACAAG
GAATTTCGCTACCTTAGGACCGTTATAGTTACGGCCGC
CGTTTACTCGGGCTTCAATTCAACGCTTCATCTTGCGA
CTGACGCATCCTCTTAACCTTCGAGCACCGGGCAGGC
GTCACACCTTATACTTCCTCTTACGAGTTGGCAAAGTG
CTGTGTTTTTGGTAAACAGTCGGGAGGGACTCTTTGC
TGAGACCGCATTGCTGCGGCACCTT

MN736563 *H. pylori* strain AudS_NaGHA_7

AAACTACCCACCAAGCATTGTCCTGCCTGTGGATAACA
CAGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTAT
CTCAAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTC
AAAGCCTCCCACCTATCCTGCGCATGATATTCCCATTA
GCAGTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTT
CCGTCTTGCCGCGGGTAGGAGGAATTTTCACCTCCAC
TACAATTTCACTGAATCTCTGGTTGAGACAGCTCCCAT
CTCGTTACGCCATTCATGCAGGTCGGTATTTAACCGAC
AAGGAATTTCGCTACCTTAGGACCGTTATAGTTACGGC
CGCCGTTTACTCGGGCTTCAATTCAACGCTTCATCTTG
CGACTGACGCATCCTCTTAACCTTCGAGCACCGGGCA
GGCGTCACACCTTATACTTCCTCTTACGAGTTGGCAAA
GTGCTGTGTTTTTGGTAAACAGTCGGGAGGGACTCTT
TGCTGAGACCGCATTGCTGCGGCACCTT

**Nucleotide sequences of *H. pylori* from Keffi General Hospital are as follows:**

MN736564 *H. pylori* strain AudS_NaFMCKe_1

CCTACCCACCAAGCATTGTCCTGCCTGTGGATAACACAG
GCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCAA
GGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAGCC
TCCCACCTATCCTGCGCATGATATTCCCATTAGCAGTGCT
AAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTTGCC
GCGGGTAGGAGGAATTTTCACCTCCACTACAATTTCACT
GAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGCCAT
TCATGCAGGTCGGTATTTAACCGACAAGGAATTTCGCTA
CCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTCGGG
CTTCAATTCAACGCTTCATCTTGCGACTGACGCATCCTCT
TAACCTTCGAGCACCGGGCAGGCGTCACACCTTATACTT
CCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGGTAAAC
AGTCGGGAGGGACTCTTTGCTGAGACCGCATTGCTGCG
GCACCTT

MN736565 *H. pylori* strain AudS_NaFMCKe_2

CTACCCACCAAGCATTGTCCTGCCTGTGGATAACACAGG
CCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCAAG
GATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAGCCT
CCCACCTATCCTGCGCATGATATTCCCATTAGCAGTGCTA
AGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTTGCCG
CGGGTAGGAGGAATTTTCACCTCCACTACAATTTCACTG
AATCTCTGGTTGAGACAGCTCCCATCTCGTTACGCCATT
CATGCAGGTCGGTATTTAACCGACAAGGAATTTCGCTAC
CTTAGGACCGTTATAGTTACGGCCGCCGTTTACTCGGGC
TTCAATTCAACGCTTCATCTTGCGACTGACGCATCCTCTT
AACCTTCGAGCACCGGGCAGGCGTCACACCTTATACTTC
CTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGGTAAACA
GTCGGGAGGGACTCTTTGCTGAGACCGCATTGCTGCGG
CACCTT

MN736566 *H. pylori* strain AudS_NaFMCKe_3

CACTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA
GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCA
AGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAGC
CTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGTGC
TAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTTGC
CGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTCAC
TGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGCCA
TTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCGCTA
CCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTCGGG
CTTCAATTCAACGCTTCATCTTGCGACTGACGCATCCTCT
TAACCTTCGAGCACCGGGCAGGCGTCACACCTTATACTT
CCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGGTAAAC
AGTCGGGAGGGACTCTTTGCTGAGACCGCATTGCTGCG
GCACCTT

MN736567 *H. pylori* strain AudS_NaFMCKe_4
AAACTACCCACCAAGCATTGTCCTGCCTGTGGATAACAC
AGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC


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AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAA
GCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAG
TGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTC
TTGCCGCGGGTAGGAGGAATTTTCACCTCCACTACAAT
TTCACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTT
ACGCCATTCATGCAGGTCGGTATTTAACCGACAAGGAA
TTTCGCTACCTTAGGACCGTTATAGTTACGGCCGCCGT
TTACTCGGGCTTCAATTCAACGCTTCATCTTGCGACTG
ACGCATCCTCTTAACCTTCGAGCACCGGGCAGGCGTC
ACACCTTATACTTCCTCTTACGAGTTGGCAAAGTGCTGT
GTTTTTGGTAAACAGTCGGGAGGGACTCTTTGCTGAGA
CCGCATTGCTGCGGCACCTT

MN736568 *H. pylori* strain AudS_NaFMCKe_5

CCTACCCACCCAAGCATTGTCCTGCCTGTGGATAACAC
AGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCT
CAAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAA
AGCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCA
GTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGT
CTTGCCGCGGGTAGGAGGAATTTTCACCTCCACTACAA
TTTCACTGAATCTCTGGTTGAGACAGCTCCCATCTCGT
TACGCCATTCATGCAGGTCGGTATTTAACCGACAAGGA
ATTTCGCTACCTTAGGACCGTTATAGTTACGGCCGCCG
TTTACTCGGGCTTCAATTCAACGCTTCATCTTGCGACT
GACGCATCCTCTTAACCTTCGAGCACCGGGCAGGCGT
CACACCTTATACTTCCTCTTACGAGTTGGCAAAGTGCT
GTGTTTTTGGTAAACAGTCGGGAGGGACTCTTTGCTGA
GACCGCATTGCTGCGGCACCTTA

MN736569 *H. pylori* strain AudS_NaFMCKe_6
AAACTACCCACCAAGCATTGTCCTGCCTGTGGATAACA
CAGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATC
TCAAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCA
AAGCCTCCCACCTATCCTGCGCATGATATTCCCATTAGC
AGTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCG
TCTTGCCGCGGGTAGGAGGAATTTTCACCTCCACTACA
ATTTCACTGAATCTCTGGTTGAGACAGCTCCCATCTCG
TTACGCCATTCATGCAGGTCGGTATTTAACCGACAAGG
AATTTCGCTACCTTAGGACCGTTATAGTTACGGCCGCC
GTTTACTCGGGCTTCAATTCAACGCTTCATCTTGCGAC
TGACGCATCCTCTTAACCTTCGAGCACCGGGCAGGCG
TCACACCTTATACTTCCTCTTACGAGTTGGCAAAGTGCT
GTGTTTTTGGTAAACAGTCGGGAGGGACTCTTTGCTGA
GACCGCATTGCTGCGGCACCTT

MN736570 *H. pylori* strain AudS_NaFMCKe_7

CCTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA
GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC
AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAA
GCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAG
TGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTC
TTGCCGCGGGTAGGAGGAATTTTCACCTCCACTACAAT
TTCACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTT
ACGCCATTCATGCAGGTCGGTATTTAACCGACAAGGAA
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TTACTCGGGCTTCAATTCAACGCTTCATCTTGCGACTGA
CGCATCCTCTTAACCTTCGAGCACCGGGCAGGCGTCAC
ACCTTATACTTCCTCTTACGAGTTGGCAAAGTGCTGTGT
TTTTGGTAAACAGTCGGGAGGGACTCTTTGCTGAGACC
GCATTGCTGCGGCACCT

MN736571 *H. pylori* strain AudS_NaFMCKe_8
AAACCTACCCACCAAGCATTGTCCTGCCTGTGGATAACA
CAGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCT
CAAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAA
GCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAG
TGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCT
TGCCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTT
CACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACG
CCATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTC
GCTACCTTAGGACCGTTATAGTTACGGCCGCCGTTTACT
CGGGCTTCAATTCAACGCTTCATCTTGCGACTGACGCAT
CCTCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTT
ATACTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTG
GTAAACAGTCGGGAGGGACTCTTTGCTGAGACCGCATT
GCTGCGGCACCTT

MN736572 *H. pylori* strain AudS_NaFMCKe_9

AACTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA
GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTCA
AGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAGC
CTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGTG
CTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTTG
CCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTCA
CTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGCC
ATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCGC
TACCTTAGGACCGTTATAGTTACGGCCGCCGTTTACTCG
GGCTTCAATTCAACGCTTCATCTTGCGACTGACGCATCC
TCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTTATA
CTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGGTA
AACAGTCGGGAGGGACTCTTTGCTGAGACCGCATTGCT
GCGGCACCT

MN736573 *H. pylori* strain AudS_NaFMCKe_10

AAACTACCCACCAAGCATTGTCCTGCCTGTGGATAACAC
AGGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC
AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAAG
CCTCCCACCTATCCTGCGCATGATATTCCCATTAGCAGT
GCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCGTCTT
GCCGCGGGTAGGAGGAATTTTCACCTCCACTACAATTTC
ACTGAATCTCTGGTTGAGACAGCTCCCATCTCGTTACGC
CATTCATGCAGGTCGGTATTTAACCGACAAGGAATTTCG
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GGGCTTCAATTCAACGCTTCATCTTGCGACTGACGCATC
CTCTTAACCTTCGAGCACCGGGCAGGCGTCACACCTTA
TACTTCCTCTTACGAGTTGGCAAAGTGCTGTGTTTTTGG
TAAACAGTCGGGAGGGACTCTTTGCTGAGACCGCATTG
CTGCGGCACCTT

Mn736574 *H. pylori* strain AudS_NaFMCKe_11
AACTACCCACCAAGCATTGTCCTGCCTGTGGATAACACA

GGCCAGTTAGCTAACAGAAACATCAAGGGTGGTATCTC
AAGGATGGCTCCATAAGAGCCAAAGCCCTTACTTCAAA
GCCTCCCACCTATCCTGCGCATGATATTCCCATTAGCA
GTGCTAAGTTGTAGTAAAGGTCCACGGGGTCTTTCCG
TCTTGCCGCGGGTAGGAGGAATTTTCACCTCCACTAC
AATTTCACTGAATCTCTGGTTGAGACAGCTCCCATCTC
GTTACGCCATTCATGCAGGTCGGTATTTAACCGACAAG
GAATTTCGCTACCTTAGGACCGTTATAGTTACGGCCGC
CGTTTACTCGGGCTTCAATTCAACGCTTCATCTTGCGA
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TGAGACCGCATTGCTGCGGCACT

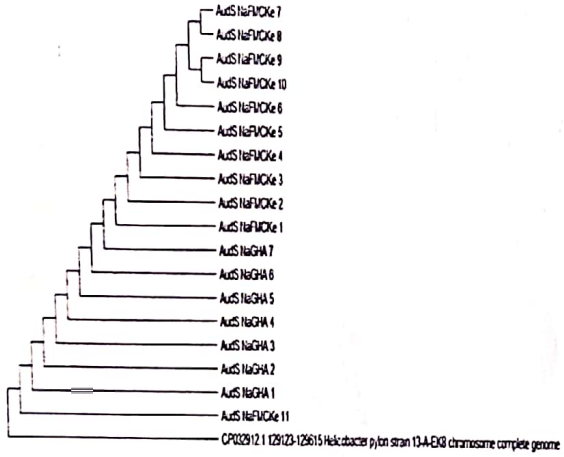


**Figure 1:** Phylogenetic tree of *Helicobacter pylori* in Nasarawa State

## DISCUSSION

All the identified *Helicobacter pylori* in this study had the same amplified fragment length polymorphism (AFLP) fingerprints belonging to *H. pylori* strain G-Mx-2003-108. This indicates that each of the patients tested were colonized by a single strain of *H. pylori*. The 9.00 % positive samples obtained for *H. pylori* in this study indicated a low prevalence of *H. pylori*. This is contrary to what Obiageli and Ivan (2015) obtained in their research on the prevalence of *H. pylori* and its associated peptic ulcer infection among adult residents of Aba, Southern, Nigeria. Of the 300 samples used in the study of Obiageli and Ivan (2015), a total of 119 (39.7%) tested positive to *H. pylori*.

Bello *et al.* (2020), also recorded similar high prevalence of *H. pylori* in the northern Nigeria. In their study, 306 patients were subjected to test, of which, 250 (81.7 %) of them tested positive for *H. pylori*. The sharp decline in the prevalence of *H. pylori* obtained maybe due to better awareness and enlightenment of the masses and improved level of personal hygiene.

The distribution of *H. pylori* infection according to age of subjects in General Hospital, Akwanga and Federal Medical Centre, Keffi, Nasarawa State was recorded to be highest between the age group of 26-30 (3 %) which is similar to the result obtained by Oti *et al.* (2017) that reported the highest prevalence within this age group. The least prevalence of this study recorded between age group of 6-10 (0 %) is contrary to the result obtained by Olufemi *et al.* (2015), which recorded their least prevalence within the age group of 41-60 years. The prevalence rate of *H. pylori* reported by Smith *et al.* (2019), Olufemi *et al.* (2015) and Ayodele *et al.* (2015) was different from the results obtained in this study. The disparity of result based on age group may be as a result of different technique or methods utilized in the different studies. The highest prevalence recorded within age 26-30 years could be due to the lifestyle of the subjects while the lowest prevalence rate recorded within age group 6 - 10 years may also be due to proper hygiene and care for these age group that may have been provided by the parents of the subjects.

In the distribution of *H. pylori* according to gender of the subjects in General Hospital, Akwanga and Federal Medical Centre, Keffi, Nasarawa State, the male gender had the higher prevalence (5.5 %) rate while the less prevalence (3.5 %) rate was recorded among the female. The higher prevalence rate among the male compared to the female may be due to stress and untidy life style of the male sampled. This result is in concordance with the one obtained by Ayodele *et al.* (2018) and Bello *et al.* (2020). However, contrary reports were made by David and Ihiabe (2010), Kumurya (2015), Bojuwoye *et al.* (2016) and Oti *et al.* (2017).

The highest prevalence rate (6.5 %) of *H. pylori* recorded among the non-working class could be due to the sedentary lifestyle and lack of proper hygiene compared to the working class subjects. This is in concordance with the result obtained by Oti *et al.* (2017), which reported nonworking class wives having a high prevalence rate of 76.2 % followed by prevalence of 63.3 % recorded for civil servants.

The distribution of *H. pylori* prevalence rate according to the location of subjects showed the rural residence had the higher prevalence (6.5 %) rate while the less prevalence (2.5 %) rate was recorded among the urban dwellers. The highest prevalence rates of recorded among rural residents and among those using pit latrine may be due to lack of proper hygiene and provision of basic amenities, which pose the subjects to infections from *H. pylori*. The result obtained in this study is contrary to the one obtained by Oti *et al.* (2017), which reported urban residents to have the highest prevalence (58.5 %) rate compared to the least (55.4 %) of the rural residents.

High prevalence rate in terms of sources of drinking water was recorded for subjects that take well water (2.5 %), followed by stream/river water (4.5 %), the least prevalence (2 %) rate was reported to be pipe borne water. The highest prevalence rate recorded among those that take stream/river (4.5 %), is similar to the report of Aitila *et al.* (2019) who attributed lack of safe drinking water as risk factor of *H. pylori* infection. However, reports from Bello *et al.* (2020) reported high prevalence (93.3 %) rate among subjects that take in river/stream water followed by well water (81.7 %) and the least (23.7 %) being pipe borne

water. The low prevalence in terms of source of drinking water reported in this study and that of Bello *et al.* (2020) may be due to the fact that pipe borne water are always treated to eradicate or reduce the microbial load before supplying to households. Access to proper sewage system was a major contributor to high prevalence of *H. pylori* infections as seen in this study. Those subjects that uses pit latrines toilet had the highest prevalence (5.5 %) rate whereas those that uses the water closet recorded a least prevalence (3.5 %) rate. This shows that individuals with unhygienic practices promotes the proliferation of *H. pylori* infections.

## Conclusions

*The result of this study showed that* General Hospital, Akwanga and Federal Medical Centre, Keffi in Nasarawa State had a high prevalence of *H. pylori* G-Mx-2003-108. The high prevalence was recorded among age group 26-30 years, male, rural dwellers, as well as those that use stream/well as source of drinking water and pit latrines toilet facility. The non-working class had a significantly higher prevalence compared with working class subjects.

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