Seroprevalence of *Helicobacter pylori* IgG Antibodies and associated Risk Factors among Asymptomatic Vegetable Sellers in Uyo, South South Nigeria

Abstract

Background: Vegetables are essential part of people's diet and are sometimes consumed raw and often without heat treatment or thorough washing, hence serve as vehicle for the transmission of pathogenic microorganisms like *Helicobacter pylori*. The source of contamination has also been traced to sellers of vegetables who are involved in unhygienic practices by vegetable sellers contribute to dissemination of *H. pylori*. The aim of this study was to provide information on the seroprevalence of *H. pylori* IgG antibodies and associated risk factors among asymptomatic vegetable sellers in Uyo, Akwa Ibom State, Nigeria. **Materials and Methods:** Blood and stool samples from 142 participants were included in the study. Blood samples were analyzed using the rapid diagnostic method while the stool samples were analyzed using Enzyme linked immunosorbent Assays (ELISA). **Results:** A total of 85 (59.9%) were positive by rapid diagnostic test (RDT) while 53 (37.3%) were positive by stool ELISA test. Risk factors like age, marital status, residency family size, years in vegetable business, source of vegetable, wash before stalling, eating veg. while cleaning, use cleaned instrument, convenience type, hand washing after convenience, how often do you eat meat, spicy food intake, fried food intake, number per room, waste disposal type of house floor soil contact were all statistically associated with ($p<0.05$) with *H. pylori* positivity. **Conclusion:** This study revealed that unhygienic behaviors by vegetable sellers expose vegetable buyers to risk of *H. pylori* infection.

Keywords: Vegetables, Enzyme linked immunosorbent Assays (ELISA), *Helicobacter pylori*, seroprevalence, Antibodies

Background of the Study

Vegetables are the edible parts of plants which could be leaves, stems, roots, flowers, seeds, fruits, bulbs, tubers and fungi that are consumed wholly or in parts, raw or cooked as part of main dish (1). In recent years, vegetables gain consumer's attraction due to their reputation of being healthy in combination with low energy density. However, since fresh produce is often eaten raw, it may also be a source for food borne illness (2). Vegetables that usually make up this recipe include cucumber, carrots, green chilli, cabbage and lettuce. They are sold in almost every market and can be seen hawked around by traders (3).

The tropical and sub-tropical countries of the world are blessed with varieties of vegetables and Nigeria is known worldwide for its great biodiversity of plants used in several ways such as culinary, medicinal, therapeutic and nutritional purposes (4,5). In many parts of Nigeria, green leafy vegetables have gained widespread acceptance as dietary constituents, generally forming a substantial portion of the diet in the preparation of soups and stews (6).

Vegetables come in a close contact with soil manure, human stool and water through irrigation (7). They can be source of *Helicobacter pylori (H. pylori)* infection when consumed raw without heat treatment or thorough washing (3). *Helicobacter pylori* are microaerophilic gram negative bacteria known to be the causative agent of mucosa associated lymphoid tissue lymphoma (8). *Helicobacter pylori* infection is one of the most prevalent chronic gastric infections affecting more than 60.3% of the world population (9). Epidemiological studies have demonstrated that *H. pylori* infection is most prevalent in developing countries and among populations with low socioeconomic background (9). According to reports, the main routes of infection remain unproven. However, its transmission occurs by person to person either by faecal-oral or oral-oral routes (10) with education level, living standards such as sanitation and hygiene, crowding index and source of drinking water being part of *H. pylori* risk factor (11). A study in Iran by Yahaghi *et al*. (12) reported Helicobacter seropositivity to be associated with consumption of uncooked vegetables, perhaps this was related to contaminated water used on the vegetables. Therefore, this research aimed at providing information on the seroprevalence and risk factor of *Helicobacter pylori* IgG antibodies among asymptomatic vegetable sellers in Uyo, Akwa Ibom State.

Materials and Methods

Study Area and Design

This study was conducted in Uyo which is the capital of Akwa Ibom State, South-south part of Nigeria. Uyo lies between latitude 5.5^{o}N and 6.0^{o}N and longitude 6.0^{o}E and 6.5^{o}E of the Greenwich meridian. A cross sectional study design with a random sampling technique was used.

Study Population and Sample Size

This study involved 142 individuals from Uyo metropolis consisting of male and female sellers of vegetables and salads. This sample size was obtained using Godden's (13) formula from a prevalence rate of 89.7% in a study at Agbor in Delta state, Nigeria by Ophori *et al.* (14).

Inclusion and Exclusion criteria

Eligible participants for this study were sellers of vegetables within Uyo metropolis, Akwa Ibom State.

Those excluded were people with known peptic ulcer condition or those with history of peptic ulcer and those on proton pump inhibitors, antibiotics, steroids or non-steroid anti-inflammatory drugs for the past one month.

Ethical consideration

Ethical approval was sought and obtained from the Akwa Ibom State Health Research Ethics Committee through their letter referenced AKHREC/15/7/21/011. Informed consent forms were issued to the participant before sample collections.

Specimen Collection and Laboratory Analysis

Blood Analysis

Five (5) millilitres of venous blood samples were collected into plain bottles and allowed to clot to get the sera following blood clotting, the serum would be separated by aspiration using Pasteur pipette. The blood samples were quantitatively examined serologically for *Helicobacter pylori* IgG antibodies using PROMED Rapid Diagnostic test, Xinghu Avenue, Nantong226010, China. The analysis was carried out according to manufacturer's description. Two distinct red lines (one on the control C and the other on the test T) indicate positive result. One red line only on the control C indicates negative result. One red line only on the test T or no line at all on both control C and test T indicate invalid result and the test repeated with new test kit.

Stool Specimen Analysis

Stool specimen was collected from each participant using a sterile leak proof single universal bottle with a screw-capped lid. The stool specimen was qualitatively screened for *Helicobacter pylori* IgG antibodies using Accu-Diag TM ELISA kit Cat 1506 -11, USA. The analysis was carried out according to manufacturer's description. The minimum detection concentration was 0.5ng/ml, and results > 20ng/ml were considered positive.

Statistics

Data obtained was analyzed using chi square test through the Statistical package for social science (SPSS) version 25.0. A p-value of ≤ 0.05 was considered statistically significant.

Results

Detection of H. pylori antibody based on Socio-demographic variables

Table 1 shows the positivity of *H. pylori* antibody across Socio-demographic variables and also the association between *H. pylori* and Socio-demographic variables. Variables that were statistically significant ($p<0.05$) with positivity of *H. pylori* antibody were age, marital status, place of residence and family size. Variables that were not statistically significant ($p>0.05$) with positivity of *H. pylori* antibody were gender and education.

Detection and association of H. pylori positivity across vegetable related risk factors

The distribution of *H. pylori* antibody positivity across vegetable-related risk factors are shown in Table 2. Variables that were statistically significant ($p<0.05$) with positivity of *H. pylori* antibody were years in vegetable business, source of vegetable, wash before stalling, eating vegetables while cleaning, use of cleaned instrument and frequency of vegetable consumption.

Distribution and association of H. pylori positivity across behavioral risk factors

The distribution of *H. pylori* antibody across behavioral risk factors is listed in Table 3. Variables that were statistically significant ($p<0.05$) with positivity of *H. pylori* antibody were convenience type, hand washing after convenience, spicy food intake and fried food intake. Hand washing before eating was not statistically significant ($p>0.05$) with *H. pylori* antibody positivity.

Distribution and association of *H. pylori* positivity across related risk factors

Table 4 shows the distribution of *H. pylori* positivity across *H. pylori* related risk factors. Variables that were statistically significant ($p<0.05$) with positivity of *H. pylori* antibody were Number per room, Waste disposal. Variables that were not statistically significant ($p>0.05$) with *H. pylori* antibody positivity were pet ownership and water source.

Discussion

Helicobacter pylori is a common bacterium found in various sources including soil, animal manure, and human stool and as a result, cross-contamination of vegetables with *H. pylori* is unavoidable (15). Consumption of contaminated materials, including fruits and vegetables, is a significant means of transmission for this infection, with a reported prevalence of 90% among regular consumers of these foods (16).

This study found the prevalence of *H. pylori* IgG antibodies by rapid diagnostic test (RDT) to be higher (59.9%) than stool ELISA test (37.3%). This may be due to the fact that serology tests detect IgG regardless of current infection, previous exposure, or treatment with proton pump inhibitors. However, the low rate by stool test may be due to the influence of relatively low bacterial load, use of antibiotics, and proton-pump inhibitors. The study's findings are supported by previous studies by Jemilohun and Otegbayo (17) and Sabbagh *et al*. (18). A study conducted in Owerri, Nigeria by Emerenini *et al*. (19) reported a lower RDT rate of 20%.

H. pylori cases were more prevalent in females than male, which could be attributed to the fact that the study focused on vegetable sellers who are mostly women. This finding is consistent with other studies by Goto *et al*. (20) and Zawaya *et al*. (21) where the majority of *H. pylori* cases were found in females.

The prevalence of *H. pylori* was 56.6% for those between the ages of 36-45 years and this was found to be highest among the participants. This may be because people in this age range are more involved in agriculture or vegetable industries. However, this rate is consistent with previous studies by Okezie *et al*. (22) and Zawaya *et al*. (21), where the highest rates of infection were found among individuals aged 31-40 years (27.9%) and 35-44 years (27.8%), respectively.

The prevalence of *H. pylori* did not show any significant correlation with an individual's level of education or knowledge about the infection. This suggests that regardless of education level, disregarding proper hygiene can increase the risk of infection. However, other studies by Mbang *et al*. (23) and Okoroiwu *et al*. (24) reported a high prevalence of *H. pylori* among individuals with a tertiary level of education or those belonging to the literate class, possibly because they assume immunity and overlook simple hygiene procedures for handling vegetables.

There was a statistical correlation between marital status and *H. pylori* infection, with higher rates observed among singles compared to those who are married. This could be due to behavioral differences, as single individuals may lack a sense of responsibility in ensuring healthy meals are consumed regularly. This finding is consistent with previous studies by Enitan *et al*. (25) and Okezie *et al*. (22), which also reported a higher prevalence of *H. pylori* among singles.

The length of time an individual has been involved in the vegetable business was found to have a statistically significant association (p<0.05) with *H. pylori* infection. The study found that the rate of infection decreased as the number of years in business increased. It is possible that individuals who have been in the vegetable business for a longer period of time have been previously exposed to *H. pylori*, which may have created immunologic memory. This immunologic memory could help the individual respond more effectively and rapidly in subsequent exposures.

The source of vegetables, washing before stalling, eating vegetables while cleaning, use of cleaned instruments, hand washing before eating, hand washing after convenience, waste disposal, and consumption of vegetables were all found to have a statistically significant association (p<0.05) with *H. pylori* infection. The reason for this association may be due to the behavioral and hygienic conditions which promotes infections as reported by Edem *et al*. (26). Krueger *et al*. (27) reported soil contact to be statistically associated with *H. pylori* prevalence, which agrees with our observations. Poor fecal disposal or management by vegetable sellers can also pose a risk to vegetable consumers, in line with the fecal-oral route of transmission. Etukudo *et al*. (28) found waste disposal and hand washing after convenience, respectively, to be associated with *H. pylori* prevalence. However, the habit of hand washing before eating was not found to be statistically associated (p>0.05) with *H. pylori* infection in this study.

Participants who used pit toilets had a 50.9% prevalence of *H. pylori* infection, and there was a statistically significant association (p<0.05) between the type of toilet and *H. pylori* infection. This finding supports the theory that *H. pylori* can be transmitted through the fecal-oral route and that environmental sources can play a role in the spread of this infection.

The prevalence of *H. pylori* infection was not statistically associated with pet ownership. While Papiez *et al*. (29) proposed that pets may serve as a source of *H. pylori* transmission, this study found that individuals who had never owned a pet had the highest prevalence of *H. pylori* infection. However, our results are consistent with the findings of Mnena *et al*. (30), which also found no association between owning a pet and *H. pylori* infection.

There was no significant association between receiving the *H. pylori* vaccine, having a history of peptic ulcer, or having an underlying condition, and the prevalence of *H. pylori* infection. For participants who received the *H. pylori* vaccine, this lack of association may be due to the fact that the majority of those who received the vaccine still had a prevalence rate of 3.7%, which could be because the vaccine's weaning period may have elapsed. This finding is supported by Schacher *et al*. (31), who also reported a non-significant association between *H. pylori* vaccination and prevalence. The lack of association with a history of peptic ulcer can be explained by the fact that those with no history of peptic ulcer had the highest *H. pylori* prevalence rate in this study. However, Suzuki *et al*. (32) reported a significant association between *H. pylori* infection and peptic ulcer history, which disagrees with our observation.

There was a statistically significant association (p< 0.05) between a history of using proton pump inhibitors (PPIs) and *H. pylori* infection. This association may be due to the fact that long-term use of PPIs can induce a provocative reaction in the stomach mucosa, which can aid in *H. pylori* proliferation, as reported by Poly *et al*. (33). However, it is possible that respondents who were unaware of PPIs may have been taking them without knowing, which could contribute to the high prevalence observed in this study. Interestingly, studies by Cheung *et al*. (34) and Poly *et al*. (33) supports our observation that there is a statistically significant association ($p < 0.05$) between a history of using PPIs and *H. pylori* infection.

Conclusion

In conclusion, *H. pylori* infection is more common in developing countries and can be spread from vegetables through person-to-person, oral-oral and fecal-oral routes of transmission. The prevalence of the infection was associated with various factors such as age, marital status, residency, family size, years in vegetable business, source of vegetables, hand washing habits, eating habits, and food intake. The data from this study could have some limitations which include small sample size or measurement error, which could have affected the results.

Reference

1. Nnamani, C.V., Oselebe, H.O. and Okporie, E.O. Aspect of ethnobotany of traditional leafy vegetables utilized as human food in rural tropical communities. *Animal Research International*, 2010; 7(1):1110-1115.
2. Hölzel, C. S., Tetens, J. L., Schwaiger, K. Unraveling the Role of Vegetables in Spreading Antimicrobial-Resistant Bacteria: A Need for Quantitative Risk Assessment. *Foodborne pathogens and disease*, 2018; 15(11), 671–688.
3. Itohan, A.M., Peters, O. and Kolo, I. Bacterial contaminants of salad vegetables in Abuja municipal area council, Nigeria. *Malaysian journal of microbiology*, 2011; 7(2):111-114.
4. Chubike, N. E., Okaka, JC., Okoli, EC. Evaluation of vegetable consumption in South Eastern Nigeria. *Int. J. Nutr. Metab*, 2013; 5 (4): 57–60.
5. Arowosegbe, S. Preliminary domestication and cultivation efforts on some medicinally importance wild vegetables in Ado-Ekiti, Nigeria. *Bio-Science Research Bulletin*, 2013; 29(2): 101-107.
6. Arowosegbe, S. Olanipekun, M.K., Adeloye, I.A. Ethnobotanical Survey of Indigenous Leafy Vegetables Consumed in Ekiti State, *Nigeria. European Journal of Biology and Medical Science Research*, 2018; 6(1):7-14
7. Atapoor, S., Dehkordi, F.S., Rahimi, E. Detection of *Helicobacter pylori* in Various Types of Vegetables and Salads. *Jundishapur Journal of Microbiology*. 2014; 7(5): e10013.
8. Krzyżek, P and Gościniak, G. Morphology of *Helicobacter pylori* as a result of peptidoglycan and cytoskeleton rearrangements. *Przeglad gastroenterologiczny*, 2018; 13(3), 182–195.
9. Sjomina, O., Pavlova, J., Niv, Y. and Leja, M. Epidemiology of *Helicobacter pylori* infection. *Helicobacter*, 2018; 23:e12514.
10. Mladeova, I. and Durazzo M. Transmission of *Helicobacter pylori*. *Minerva Gastroenterologica e Dietologica*; 2018; 64(3):251-254.
11. Goh, K. L., Chan, W. K., Shiota, S., Yamaoka, Y. Epidemiology of *Helicobacter pylori* infection and public health implications. *Helicobacter*, 2011; 16 Suppl 1(01), 1–9.
12. Yahaghi, E., Khamesipour, F., Mashayekhi, F., Safarpoor Dehkordi, F., Sakhaei, M. H., Masoudimanesh, M., Khameneie, M. K. (2014). *Helicobacter pylori* in vegetables

and salads: genotyping and antimicrobial resistance properties. *BioMed research international*, *2014*, 757941.

13. Godden, B. Sample Size Formulas. *Journal of Statistics*, 2004; 3: 66.
14. Ophori E, Isibor C, Onemu S, Johnny E. Immunological response to *Helicobacter pylori* among healthy volunteers in Agbor, Nigeria. *Asian Pacific Journal of Tropical Disease*, 2011; 1:38-40
15. Safaei, H. G., Rahimi, E., Zandi, A., Rashidipour, A. Helicobacter pylori as a zoonotic infection: the detection of H. pylori antigens in the milk and faeces of cows. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, 2011; 16(2), 184–187.
16. Oreh, A. C., Onu, A., Moses, A. L. Prevalence and Determinants of Helicobacter Pylori Infection among Adult Dyspeptic Patients in Abuja, Nigeria. *West African journal of medicine*, 2021; 38(8), 775–784.
17. Jemilohun AC, Otegbayo JA. *Helicobacter pylori* infection: past, present and future. *Pan Afr Med J*, 2016; 23(1)
18. Sabbagh, P., Mohammadnia-Afrouzi, M., Javanian, M., Babazadeh, A., Koppolu, V., Vasigala, V. R., Nouri, H. R., Ebrahimpour, S. Diagnostic methods for *Helicobacter pylori* infection: ideals, options, and limitations. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*, 2019; 38(1), 55–66.
19. Emerenini, F. C., Nwolisa, E. C., Iregbu, F. U., Eke, C. B., Ikefuna, A. N. Prevalence and risk factors for helicobacter pylori infection among children in Owerri, Nigeria. *Nigerian journal of clinical practice*, 2021; 24(8), 1188–1193.
20. Goto, Y., Syam, AF., Darnindro, N., Hapsari, FCP. Prevalence and risk factors for *Helicobacter pylori* infection among healthy inhabitants in northern Jakarta, Indonesia. *Asian Pac J Cancer Prev*, 2016; 17, 4747-53.
21. Zawaya, K. P., Dunga, J. A., Adamu, Y., Bathan, S. J., Liman, H. U., Musa, J .J., Yusuf, S. Y., Vakkai, I., Adabe, R., Okeke, E. N., Agabe, E. I. *Helicobacter pylori* Infection a Risk for Upper Gastrointestinal Diseases among Patients in North Central Nigeria. *Nigerian Journal of Medicine*, 2021; 30:607-14.
22. Okezie, G.C., Nwobu, R.A.U., Mgbowula, G.I. Prevalence of *Helicobacter Pylori* among Clients Attending Private Medical Laboratory Diagnostic Center In Karsh, Abuja, Nigeria. *International Journal of Scientific and Research Publications*, 2020; 10(11): 489-493.
23. Mbang, K., Uchenna, O., Emmanuel, U., Aniekan, E., Evaristus, C., Donald, E., Ogbu, N. Prevalence of *Helicobacter pylori* infection among dyspepsia patients in Calabar. *Global Journal of Pure and Applied Sciences*, 2019: 25: 145-151
24. Okoroiwu, G. I. A., Okoroiwu, I. L., Ubosi, N. I., and Sani, N. M. Sero-prevalence of and risk factors associated with *Helicobacter pylori* infections among individuals with peptic ulcer in Owerri, Imo State, Nigeria during 2020-2021. *African Journal of Clinical and Experimental Microbiology*, 2022; 23 (3): 238 – 247
25. Enitan SS, Ochei JO, Akele YR, Faloye TG, Adeniyi LO, *et al*, “Screening for *Helicobacter pylori* Infection among Undergraduate Students of a Tertiary Institution using serum Antibody and Stool Antigen Detection Methods”. Biomedical Journal of Science &Technology Research 3(2)- 2018. BJSTR.MS.ID.000883.
26. Edem EN, Mbong EO, Olaniyan UO. Environmental and human behavioral factors associated with Vulvovaginal Candidiasis among single and married Women in Eket. *Glob J Infect Dis Clin Res*, 2021; 7(1): 037-042.

27. Krueger, W. S., Hilborn, E. D., Converse, R. R., Wade, T. J. Environmental risk factors associated with *Helicobacter pylori* seroprevalence in the United States: a cross-sectional analysis of NHANES data. *Epidemiology and infection*, 2015; 143(12), 2520–2531.
28. Etukudo, O. M., Ikpeme, E. E and Ekanem, E. E. Seroepidemiology of *Helicobacter pylori* infection among children seen in a tertiary hospital in Uyo, southern Nigeria. *The Pan African medical journal*, 2012; 12, 39.
29. Papiez, D., Konturek, P. C., Bielanski, W., Plonka, M., Dobrzanska, M., Kaminska, A., Szczyrk, U., Bochenek, A., & Wierzchos, E. Prevalence of *Helicobacter pylori* infection in Polish shepherds and their families. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, 2003; 35(1), 10–15.
30. Mnena EY, Ebele U, Emmanuel N. Risk Factors Associated with *Helicobacter Pylori* Infections in Makurdi Northcentral Nigeria. *J Infect Dis Ther*, 2017; 5: 325.
31. Schacher, K., Spotts, H., Correia, C., Walelign, S., Tesfaye, M., Desta, K., Tsegaye, A and Taye, B. Individual and household correlates of *Helicobacter pylori* infection among Young Ethiopian children in Ziway, Central Ethiopia. *BMC infectious diseases*, 2020; 20(1), 310.
32. Suzuki, T., Matsuo, K., Ito, H., Hirose, K., Wakai, K., Saito, T., Sato, S., Morishima, Y., Nakamura, S., Ueda, R., & Tajima, K. A past history of gastric ulcers and *Helicobacter pylori* infection increase the risk of gastric malignant lymphoma. *Carcinogenesis*, 2006; 27(7), 1391–1397.
33. Poly, T. N., Lin, M. C., Syed-Abdul, S., Huang, C. W., Yang, H. C., Li, Y. J. Proton Pump Inhibitor Use and Risk of Gastric Cancer: Current Evidence from Epidemiological Studies and Critical Appraisal. *Cancers*, 2022; 14(13), 3052.
34. Cheung, K. S., Chan, E. W., Wong, A., Chen, L., Wong, I., Leung, W. K. Long-term proton pump inhibitors and risk of gastric cancer development after treatment for *Helicobacter pylori*: a population-based study. *Gut*, 2018; 67(1), 28–35

Table 1: Detection of *H. pylori* antibody across Socio-demographic variables

Variables	Group	No. Screened (n=142)	Blood RDT Positive	p-value	Stool ELISA Positive (%)	p-value
Gender	Male	9	4 (44.4)	0.26	3 (33.3)	0.07
	Female	133	81 (60.9)		50 (37.6)	
Age-group	18-25	10	6 (60.0)	0.000	4 (40)	0.001
	26-35	52	25 (50.0)		10 (19.2)	
	36-45	49	43 (87.8)		30 (66.7)	
	>45	31	11 (35.5)		9 (29.0)	
Education	None	5	2 (40.0)	0.77	1 (20.0)	0.41
	Primary	24	14 (58.3)		6 (25.0)	
	Secondary	102	63 (61.8)		41 (40.2)	
	Tertiary	11	6 (54.5)		5 (45.5)	
Marital Status	Married	95	46 (48.4)	0.000	28 (29.5)	0.006
	Not Married	47	39 (83.0)		25 (53.2)	
Residency	Rural	29	11 (37.9)	0.007	4 (13.8)	0.003
	Urban	113	74 (65.5)		49 (43.4)	
Family Size	<5	96	41 (42.7)	0.000	20 (20.8)	0.001
	>5	46	44 (95.7)		33 (71.7)	
	Total	**142**	**85 (59.9)**		**53 (37.3)**	

Table 2: Prevalence and association of *H. pylori* across vegetable related risk factors

Variables	Group	No. Screened (n=142)	Blood RDT Positive (n=85) (%)	p-value	Stool ELISA Positive (n=53) (%)	p-value
Year in Veg. Business	≤1	56	48 (85.7)		33 (58.9)	
	2-5	53	26 (49.1)	0.000	13 (24.5)	0.001
	>5	33	11 (33.3)		7 (18.9)	
Source of Vegetable	Bush Market	56	48 (85.7)	0.000	32 (57.1)	0.001
	Open Market	86	37 (43.0)		21 (24.4)	
Wash before Stalling	Yes	118	65 (55.1)	0.010	45 (38.1)	0.001
	No	24	20 (83.3)		8 (33.3)	
Eating Veg. while cleaning	Yes	68	52 (76.5)	0.000	34 (50.0)	0.003
	No	74	33 (44.6)		19 (25.7)	
Use Cleaned Instrument	Yes	61	54 (88.5)	0.000	31 (50.8)	0.004
	No	81	31 (38.3)		22 (27.2)	
How often do you consume Vegetables	Never	10	6 (60.0)		2 (20.0)	
	sometimes	75	36 (48.0)	0.006	20 (26.7)	0.001
	Often	29	19 (65.5)		8 (27.6)	
	Always	28	24 (85.7)		23 (82.1)	

Table 3: Distribution and association of *H. pylori* positivity across behavioral risk factors

Variables	Group	No. Screened (n=142)	Blood RDT Positive (n=85) (%)	p-value	Stool ELISA Positive (n=53) (%)	p-value
Hand washing before eating	Never	5	2 (2.4)	0.76	2 (4.0)	0.24
	Sometimes	6	3 (3.5)		2 (4.0)	
	Often	35	21 (24.7)		9 (17.0)	
	Always	96	59 (69.4)		40 (75.0)	
Convenience Type	Pit Toilet	51	42 (49.4)	0.000	27 (50.9)	0.007
	Bucket	8	4 (4.7)		1 (1.9)	
	Water Closet	76	38(44.7)		25 (47.2)	
	Others	7	1 (1.2)		0 (0)	
Hand washing after convenience	Sometimes	5	3 (3.5)	0.01	3 (5.7)	0.001
	Often	24	8 (9.4)		2 (3.8)	
	Always	113	74 (87.1)		48 (90.6)	
Spicy food intake	Never	32	14 (16.5)	0.02	9 (17.0)	0.001
	Sometimes	41	22 (25.9)		10 (18.9)	
	Often	41	26 (30.6)		12 (22.6)	
	Always	28	23 (27.1)		22 (41.5)	
Fried food intake	Never	12	7 (8.2)	0.01	4 (7.5)	0.001
	Sometimes	70	38 (44.7)		23 (43.4)	
	Often	25	11 (12.9)		4 (7.5)	
	Always	35	29 (34.1)		22 (41.5)	

Table 4: Distribution and association of *H. pylori* positivity across related risk factors

Variables	Group	No. Screened (n=142)	Blood RDT Positive (n=85) (%)	p-value	Stool ELISA Positive (n=53) (%)	p-value
Number per room	1	70	22 (25.9)	0.000	9 (17.0)	0.001
	2-3	33	26 (30.6)		14 (26.4)	
	>3	39	37 (43.5)		30 (56.6)	
Pet	Yes	7	4 (4.7)	0.88	2 (3.8)	0.62
	No	135	81 (95.3)		51 (96.2)	
Water Sources	Tap water	132	77(90.6)	0.18	50 (94.3)	0.80
	non-tap water	10	8 (9.4)		3 (5.7)	
Waste disposal	Sewage	52	49 (57.6)	0.000	30 (56.6)	0.002
	Non sewage	90	36 (42.4)		23 (43.4)	