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Prevalence of *Helicobacter pylory* and its associated factors among out patients visiting Wolkite University Specialized Hospital, Wolkite,Southern Ethiopia

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Abbreviations and Acronym

ELISA	Enzyme Linked Immune Sorbent Assay
H.pylori	*Helicobacter pylori*
WKUSH	Wolkite University Specialized Hospital
IBT	Immune Blotting Technique.
MALT	Mucosa associated lymphoid tissue
NSAIDS	Nonsteroidal Anti-Inflamatory Drugs
NUD	Non Ulcer Dyspepsia.
PAF	Platelet Activating Factors
PCR	Polymerase Chain Reaction
SD	Standard Deviation
SOP	Standard Operational Procedure.
SNNP	South Nation Nationality and People

Contents

ABSTRACT

Back ground*:Globally, H. pylori is one of the public health important bacteria that affect a great proportion of human population.* It causes *of chronic gastritis, duodenal ulcers and subsequentely gastric carcinoma. Approximately,50% and 90% of adults are infected in developed and developing countries, respectively. Hence, risk of infection is high in the developing world.*

Objective: *This study is aimed to determine the prevalence of H. pylori infection and associated factors among out patients visiting Wolkite University Specialized Hospital from September to January.*

Methodology*: A hospital based cross-sectional study was conducted from September to January, 2020. Totally, 304 samples were collected from conveniently selected outpatients visiting the hospital during the study period.* Majority (73.1%) of them had secondary and above formal education while 82(26.9%) were illiterate. *Stool specimens were collected and diagnosed using H.pylori stool antigen test method. Socio-demographic data and associated risk factors were collected using pre-structured questionnaire and the quantitative datas were analyised using IBM SPSS v. 23. Moreover, analysis of association between variables were performed using chi-square, at p-value <0.05.*

Results: *A total of 304 individuals were participated in the study, of which, 172(56.6%) were males.* In our study, the overall prevalence of *H.pylori* infection was 35.9%. *The current finding shows, human habits, i.e. drinking of alcohol and coffee, smoking, and chewing chat were stastically correlated with the prevalence of H.pylori however, sociodemographic of participants, i.e. sex, education,and number of families, were not shown statistically associated.*

Conclusion and Recommendation: *We concluded that, the human habits were correlated with H.pylori infection. The prevalence of H. pylori infection detected by fecal H.pylori antigen among outpatients at WUSH was 35.9%. Hence, increasing awareness of the communities on the H.pylori and assessed risk factors possibly reduce prevalence of the disease.*

Key words*: Helicobacter pylori, Prevalence, Wolkite University Specialized Hospital*

CHAPTER-ONE

1. INTRODUCTION

1.1.Back ground

Helicobacter pylori is a gram-negative micro-aerophilic bacterium that infects the epithelial lining of the stomach (1). It is a helix-shaped and the principal cause of chronic gastritis and peptic ulcer disease and a major contributor to gastric carcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. Although the route of transmission is, person to person transmission by either oral to oral or faeco-oral is the most likely transmission routes (2). The bacteria have been implicated for acid peptic disease and today, it is regarded as essential factors and also causative agent of gastritis and peptic ulcer disease (3). The organism classified as a class one carcinogen because of its causal relationship to gastric adenocarcinoma, one of the world's dead least cancers (4, 5). Endoscopic removal of gastrointestinal stromal tumors (GISTs) is recently recognized, but less is known about its efficacy and safety (6). *H.pylori* cuases gastric inflammation. The bacterium result in peptic ulcer by eroding the epithelial tissue of the stomach and the upper part of the small intestine, which makes the stomach acid to get through to the sensitive lining layer. The motile *H.Pylory* compasses through the gastric mucosa and adhere to epithelial cell. Initial colonization of stomach is facilitated by blockage of acid production by a bacterial acids inhibitors protein and neutralization of gastric acids by ammonia produced by bacterial urease activity (7). *H.pylori* also produces factor that stimulate secretion of interleukin -8 and production of platelet activating factor(PAF) that cause hyper secretion of gastric acid and programmed death of gastric. *H.pylori* can be diagnosed by using serological, urea breath test (UBT), culture, PCR and endoscopy where the latter one are highly sensitive and has a specific >90 %(8, 9). Serological tests are limited in areas of high prevalence because of non-distinction between previous and current infection (9).

1.2 Statement of the problem

H.pylori infection has become the most chronic bacteria in the world. This pathogen colonizes more than half of the World's inhabitants (6). Approximately 50% of the world populations are infected making it the most wide spread infection in the world population (10). However, only about 10-20% of infected persons become symptomatic (11). Although infection with *H.pylori* occurs worldwide prevalence varies greatly among nations and among populations groups in the same nation. It is more in developing countries where the prevalence is over 80% among aged adults as compared to 20-50% in developed countries (2). The overall prevalence of *H.pylori* is strongly correlated with socio-economic conditions and prevalence tends to increase with age.The lower rate infection in developed countries is largely attributed to higher hygiene standards and wide spread use of antibiotics. Nearly 50% of adults in the developed and 70-90% of adults in the developing countries were infected (10).The prevalence of *H.pylori* inUnited States, in Europe and North America are 35-40%, 20-50% respectively. Africa is the third world developing continent, facing high infection of *H.pylori*. Forinstance, the study conducted in 2010 shows 79.1% and 85.6% of the population were infected with H.pylori in Nigeria and Northern Ehiopian, respectively(12).Therefore, *H.pylori*mostly infect the peoples of developing than developed countries as indicated by the above magnitude of prevalence (10).The causes of H. pylori in Ethiopia are lack of clean supply of water, unsanitary living condition and poor hygiene's, overcrowding of population and etc. The peoples living in and around Wolkite city are facing the same problems and expected to be vulnerable to *H. pylori* infection.Prevously, ther was no documented research articles regarding the prevalence of *H.pylori* infection in and around Wolkite town. Therefore, this research proposal will intended to fill the gap on the prevalence, distribution and associated risk factors with *H.pylori* infection in the specified area.

1.3 SIGNIFICANCE OF THE STUDY

Thestudy will provide scientific finding evidence and can be used a basic information for a researcher concerningthe prevalence of *H.pylori* infection and its associated risk factors among patients visiting the Hospital.Moreover, this study will helpa a Wolkite town and Health office administrators in formulating the controlling mechanism of the infection and improving the overall health of the communities.

CHAPTER TWO
2. LITERATURE REVIEW
2.1. Prevalence of H.pylori infection

The prevalence of H.pylori is high in developin countries due to lack of clean drinking water, poor sanitation and hygiene. Forinstance, the study conducted in Yemen on 83 patients (43 males and 40 females) with age range of 15-80 years shows, 53 %(44) were tested *H.pylori* positive while 47 %(39) detected negative (13). In addition, in Kuwait, Urea Breathing Test (UBT) resultedin 49.7% positive*H.pylori* among 362 peoples suspected diagnosized. The study conducted in Cape-Province show that 66.1%(168 out of 254) were H.pylori positive, and of the 168 positive subjects,*H.pylori* prevalence was highest in patient with non-ulcer dyspepsia(NUD) (32.7%, 55/168). The study from University of Sydney Nepean Hospital on both symptomatic and asymptomatic patients with *H.pylori*shows the prevalence of *H.pylori* infection washigher in symptomatic patients(14). Several studies have compared the prevalence of *H.pylori* in symptomatic and asymptomatic individuals while some investigators have reported higher prevalence of *H. pylori* in dyspepsia than in controls, other have found no difference in the controls (14). Study on 309 subjects with dyspepsia and 310 controls in all elegant Norwegian population found that, overall 48% dyspeptic subjects had *H.pylori* compared with 36% the controls, which was a significant difference and the prevalence was 53% and 35% respectively, in dyspeptic subjects and control with normal endoscopic findings(15).

Moreover, the study in Nigeria found that, about 45(52.35%) and 52 (60.5%) patients were *H.pylori* positive with histology and RUT test, respectively. In addition, about 55(64%) patients were found positive when results of both tests were combined. The Gastric ulcer (GU) wasrecorded in 8(9.3%) patients, 7(8.1%) patients had oesophagitis, while 3(3.5%) and 2(2.3%) patients had gastric cancer and duodenal ulcer (DU) respectively. Serious gastero-duodenal pathologies (GU, DUandgastric cancer) were documented in only 12(14%) patients.

The report, among 203 tested patients using ELISA sera shows, 148(72.9%) were positive while 55(27.1%) shown negative to *H.pylori*infection (16). They was an agreement between SDBioline*H.Pylori* and ELISA results in 193 of 203 sera (0verall accuracy 95.1%)

(17).According to the study done in North Gondar North West Ethiopia , among all the study subjects 912(65.7%) were found to be sera positive. The prevalence in male was 449/679(66.1%) and in females it was 463/709(65.3%). The sera prevalence was 86.5% in 2009 and it decreased to 51.8% in 2010. But the sero-prevalence increased to 61.3% in 2011 (18).

2.2 Factor Associated H.Pylory

A Met analysis conducted in 2009 concluded that the eradication of *H.pylori* reduces gastric cancer risk in previously infected individuals suggesting that the continual of presence of *H.pylori* constitutes a relative factors of 65% for gastric cancers-in terms of absolute factor the increase was from 1.1-1.7%(19) and the percent increased with age (35.8 at 20-29 years , 95% CI=25.4%-47.2%, 59.3% at 30-39 years (95%CI=48.5-69.5%) (P=0.013)(19). The prevalence of *H.pylori* infection was significantly higher (77%) in patients whose age were greater than 60 years and the lowest positive age group was between 0-20 in which only 59.1% were positive.

The study conducted 2004 in Cape-Province(south Africa), race show that the overall prevalence of *H.pylori* was 66.1%(168/254) of the 168 positive subjects, *H.pylori* prevalence was highest in patient with non-ulcer dyspepsia(NUD) (32.7%, 55/168), and lowest (0%,0/168) in those with atypically oesophagal reflux disease and gagster duodnitis ,respectively. The prevalence of infection was and gastro duodnitis respectively. The prevalence of infection was highest among colored (68.4%, 89/30) and lowest in whites (59.5%,25/42). The prevalence increased with age dyspeptic (20). Several studies have compared the prevalence of *H.pylori* in symptomatic and asymptomatic individuals while some investigators have reported higher prevalence of *H.pylori* in dyspepsia than in controls, other have found no difference in the controls.Bernersevetalendoscoped 309 subjects with dyspepsia and 310 controls in all elegant Norwegian population based study they found that, overall 48% dyspeptic subjects had *H. pylori* compared with 36% the controls, which was a significant difference, the prevalence was 53% and 35% respectively, in dyspeptic subjects and control with normal endoscopic findings(15).

It is not worthy however, that there was a considerable overlap in the endoscopic findings. About 68(79.1%) patients had endoscopic ally identifiable cause for their dyspepsia while the remaining 18(20.9%) had normal endoscopic findings.Seroprevalence studies conducted in region showed prevalence rates as high as 88% to 94.5% (12). According to the study done in Benghazi at Libiya showed in 2007,that the main endoscopic findings were gastritis (34.3%) hiatalhemia (14.5%), reflux esophagitis (12.5%) erosive gastritis (12.5%) and duodena ulcer (8.5%). The main symptom for all patients was abdominal pain (97.6%). The frequency of *H.pylori* infection was high among studied 122 patients 84(67.2%) out of 125 patients (14)

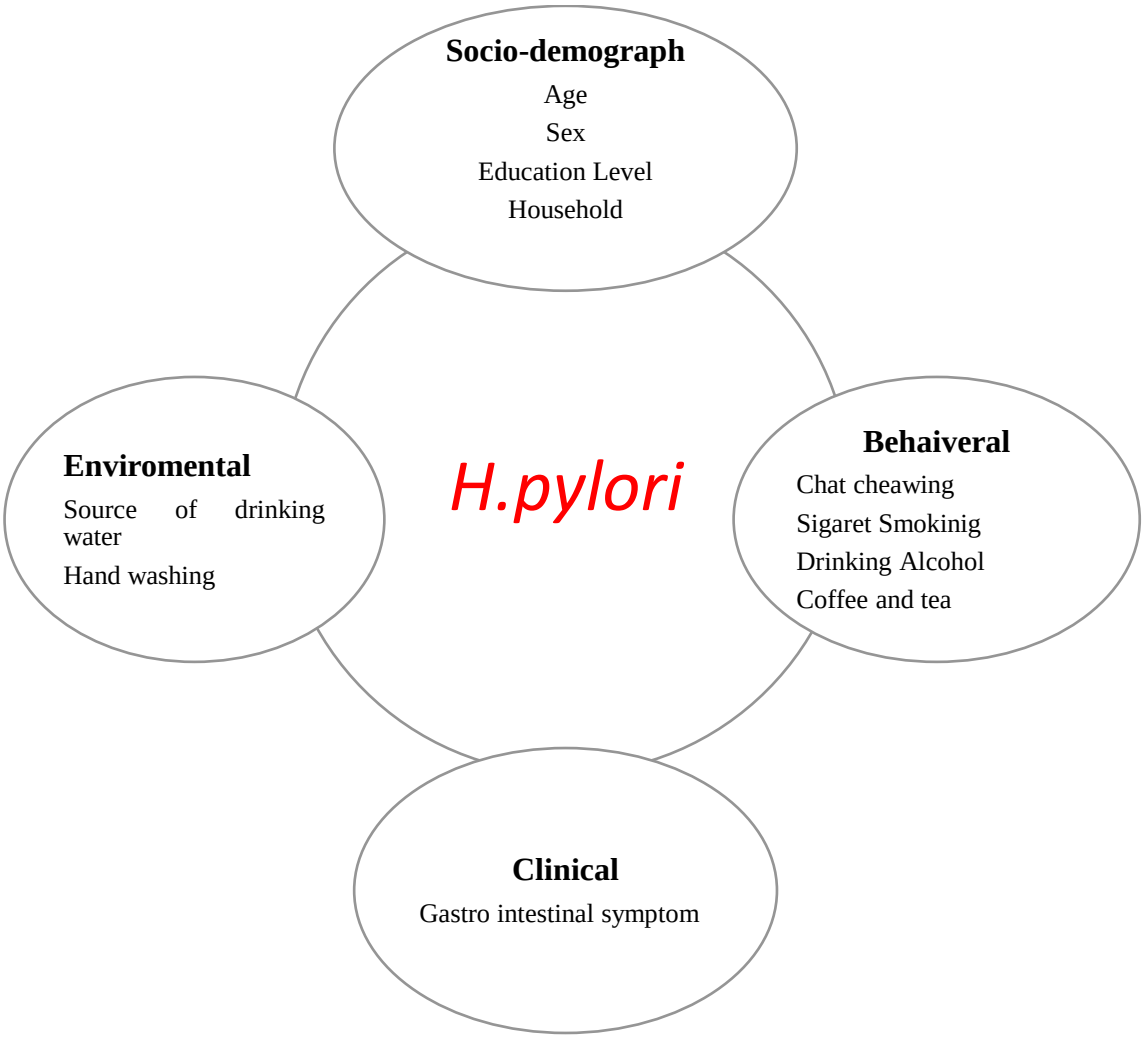

Figure 1 : Conceptual frame work about the prevalence of H.pylori infection and its associated factors

CHAPTER-THREE

OBJECTIVES

3.1 General objective

- To determine the prevalence of *H.pylori* infection and associated factors among outpatients at Wolkite University Specialized Hospital.

3.2 Specific objectives

- To determine the prevalence of *H.pylori* infection among out patients of Wolkite University specialized hospital.
- To assess the associated factors for *H.pylori* infection among out patients of Wolkite University specialized Hospital.

CHAPTER FOUR

4. METHODS AND MATERIALS

4.1. Study Design, Sampling, Area and Period

Hospital-based cross-sectional study design was employed from Sep, to Janu, 2021 at Wolkite University Specialized Hospital (WUSH), South Nation Nationality and Peoples Regional State (SNNPR), Ethiopia among convienantly selected 304 patients who were diagnosed and confirmed to be positive for *H.pylori*. WUSH is situated at Gubriye sub-city, 13km far away from the zonal town, Wolkite which is located at 158km from the capital, Addis Ababa. At present, WUSH has 127 beds of six wards with no functional intensive care unit (ICU), serving for more than1.2 million people living in the zone (based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA). The Hospital counted 275 healthcare workers (HCWs) consisting of 18 specialists, 45 general practitioners, and 212 other health professional (public officers, emergence surgeons, nurses, Midwives, pharmacists, laboratory technicians, radiologists, anesthesia professionals, and psychiatric nurses)[21].

4.2. Population

4.2.1. Source Population

All patients attended outpatient ward of Wolkite University Specialized Hospital during the study period.

4.2.2. Study Population

Patients who are suspected for *H.pylori* infection among outpatients of Wolkite University Specialized Hospital during the study period

4.3. Study Unit

Our study participants were 304 selected individuals who are suspected of *H.pylori* infection among patient of Wolkite University Specialized Hospital.

4.4. Eligibility Criteria

4.3.1. Inclusion Criteria

All *H.pylori* suspected patients whose stool specimen was sent to the laboratory with full laboratory record formats for stool antigen examination.

4.4.2.Exclusion Criteria

Severely illed patients who were unable to give information and those who were involutatry to participate in the study were excluded.

4.5. Sample Size and Sampling Technique

4.5.1. Sample Size Determination

The sample size for the study was determined by using a single population proportion formula by assuming the prevalence of *H. pylori* from the study conducted in Hawassa (72.9%) at 95% level of confidence and 0.05 margin of error [17]. Accordingly, a total of 304 samples were taken for the study.

$$n=\frac{(Z)^2 xp(q)}{d^2}$$

$$=\frac{(1.96)^2 x(0.729)(0.271)}{(0.05)^2}=304$$

Where n=Sample size

Z= Confidence level is 95%

P= estimation of population proportion is 72.9%

q= 1-p and N=200

d= Margin of error 5%

Thus, the final sample size calculated was 304 individuals.

4.5.2. Sampling Technique

To collect the required number of data, convenience-sampling technique was be used.

4.6. Variables

4.6.1. Independent variables

-Age -Hand washing habit

-Sex - Utilization of the latrine

-Marital Status -Source of Water

-Educational Status - Water treatment Type

-Smoking - Habit of alcohol drinking and chewing Khat

- Habit of coffee and tea utilization -GIT history

4.6.2. Dependent Variable

- Prevalence of *H.pylori infection.*

4.7. Operational definition

Dyspepsia: poor digestion with heart burn and regurgitation of stomach acid

Gastric: pertaining to the stomach where Gastritis-inflammation of the stomach lining with either congested and boggy or inflamed membrane

Gastric Ulcer: a usually chronic condition, started by irritation, with congestion in time, leading to edema, blistering and the formation of an ulcer.

Outpatient: patients who come to the OPD unit after referral or for primary diagnosis for the first time.

4.8. Data collection and laboratory methods

A total of 304 stool specimens were collected by using a clean, dry and leak proof container with the sterile applicator stick. Data regarding socio-demographic factors of the patient and associated factors with *H.pylori* infection was collected through face to face interview using pre-structured questionnaire. About two to three drop of stool samples were taken and processed. A piece of tissue was used to prevent solution from splashing. Timer was used to read the result with in time. The samples were collected in containers that do not contain media, preservative, animal serum or detergent as any of this additive may interfere *H.pylori* stool antigen test.

4.9.Specimen collection, transportation, processing and pathogen identification

4.9.1. Specimen Collection

Stool specimen was collected in clean wide mouth and screw caped containers

4.9.2. Specimen Transportation

The collected Spacemen were transported to diagnostic laboratory of Wolkite university specialized hospital by using stool cup.

4.9.3. Specimen Processing

To evaluate the status of *H. pylori*, small amount of stool specimen was taken by applicator stick and *Helicobacteri pylori* stool antigen (HpSA) test was performed. After that about two to three drop of stool samples were taken and processed

4.9.4. Pathogen identification

Stool antigen test is a qualitative immune chromatography assay for the determination of *H.pylori* antigen in fecal sample. The membrane is pre-coated with monoclonal antibodies on the test band region against *H.pylori*.

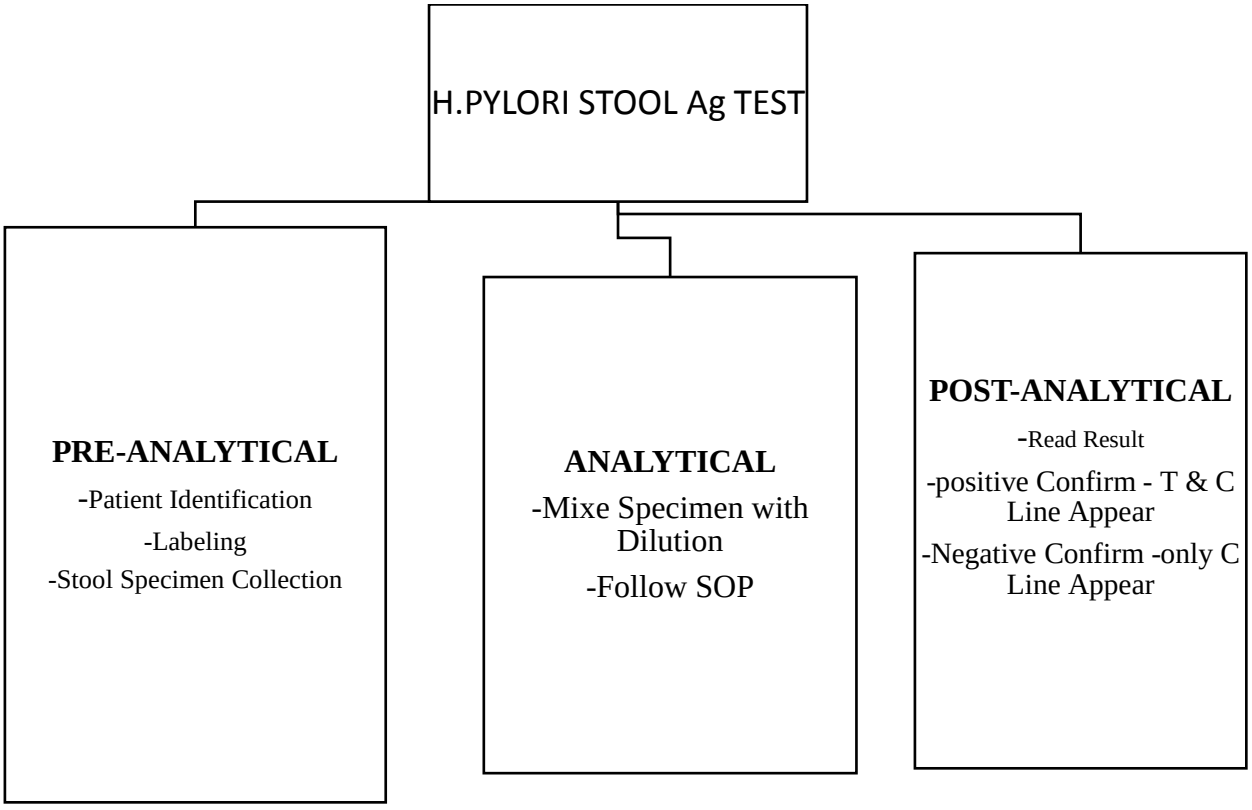

Figure 2: Laboratory Flow Chart of H .Pylori Test

4.10. Data processing and analysis

The data was analyzed using descriptive statistics using SPSS v. 23. The results were interpreted with frequency tables and percentage. Association factor analysis was be performed using chi-squire, where p-value (<0.05) was statistically significant.

4.11. Data quality control

To ensure the reliability and validity of the study, all the three phase of quality assurance (pre analytical phase, analytical phase, and post analytical phase) was followed. Manufacturer instruction standard operational procedures(SOP) for casset examination was used while examining *H. pylori*, pretesting, and completeness of questionnaire was used

4. 12. Ethical Issue

Ethical clearance was obtained from Wolkite University ethical review board. Written informed consent was obtained from each study participants and every information of the patients was kept confidential and their result was also communicated with their respective physician.

4.13. Plan for dissemination of the findings

The result of the study was appropriately recommended and communicated with the hospital community and representative of the department. Our result was disseminated to Wolkite University college of Medicine and Health Science, department of medical laboratory sciences for further investigation by senior researcher.

CHAPTER-FIVE

5. Results

5.1. Socio-demographic distribution of study participants

A total of 304 individuals were participated in the study, of which, 172(56.6%) ,and 132(43.4%) were males and females, respectively. Among the total participants, 61(35.5%) males and 48(36.4%) females were found to be positive for *H. pylori* stool antigen. The age of participants were ranged between 7-80 years.

Among 304 study participants, 109(35.9%) were positive for fecal H. pylori antigen. There was an increase in the prevalence of *H. pylori* infection was recorded among participants aged ≥ 30 years (44.3%). Majority (73.1%) of them had secondary and above formal education while 82(26.9%) were illiterate. The prevalence of *H. pylori* was assessed for any association with the socio-demographic data (sex, age, marital status, educational level and number of household) were summarized in table below.

Table 1: The analysis of sociodemographic factors associated with H.Pylory infect

Variable	Frequency	Percent (%)	*H. pylori*		Pearson Chi-Square	Df	P-value
			Positive	Negative			
Sex							
Male	172	56.6	61	111	0.026[a]	1	0.871
Female	132	43.4	48	84			
Total	304	100%	109	195			
Age							
0-14	14	4.6	2	12	24.842[a]	4	.000
15-29	101	33.2	22	79			
30-44	105	34.5	46	59			
45-59	64	21.1	25	39			
>/=60	20	6.6	14	6			
M. Status							
Single	97	31.9	22	75	12.213	3	.007
Widowed	16	5.3	7	9			
Divorced	29	9.5	15	14			
Marriage	162	53.3	65	97			
Education							
Illiterate	82	27.0	36	46	4.284	3	0.232
Primary	65	21.4	21	44			
Secondary	72	23.7	27	45			
College/Uni.	85	28.0	25	60			
Household							
Two	16	5.3	3	15	6.080	3	0.108

Three	30	9.9	11	19
Four &>	73	24.0	20	53

5.2. Human habits and *H. pylori* infection

An assessment on alcohol ,smoking ,chat ,coffee, and tea habits of the study participants revealed that 53.9% ,23.7%, 47.0%, 79.6%,and 69.4% had a habit of alcohol, smoking, chat, coffee and tea consumption respectively. Out of this number of 46.0% and 76.3% had never drank alcohol and smoking cigarate in their life. Among personal habits: alcohol drinking, coffee drinking, cigarette smoking and chat chewing have significant association with H. pylori infection ($p<0.05$) (Table 2). However, the drinking tea with p-value of (0.227) has no association with *H.pylor i* prevalence at p-value of 0.05.

Table 2: Human habits associated with H. pylori infection

Variable	**Frequency**	**Present (%)**	***H.pylori***	**Pearson Chi-Square**	**Df**	**P-value**

			Positive	Negative			
Alcohol							
Yes	164	53.9	73	91	11.604[a]	1	0.001
No	140	46.1	36	104			
Total	304	100%	109	195			
Smoking							
Yes	72	23.7	38	34	11.747a	1	0.001
No	232	76.3	71	161			
Chew Chat							
Yes	143	47.0	66	77	12.452[a]	1	0.000
No	161	53.0	43	118			
Coffee							
Yes	242	79.6	97	145	9.220[a]	1	0.002
No	62	20.4	12	50			
Total	304						
Tea							
Yes	211	69.4	71	140	1.459[a]	1	0.227
No	93	30.6	38	55			
GIT							
Yes	135	44.4	77	58	47.373[a]	1	.000
No	169	55.6	32	137			

5.3. Hygienic practice ssociation with *H.pylori infection*

Among 160(52.6%) study subjects who did not have habit of washing their hands after toilet, 67 (41.9%) were positive for *H. pylori* infection and it has statistically significant association with fecal *H. pylori* antigen positivity ($p<0.05$). Hence, the infected by *H. Pylori* bacteria among individuals who never wash their hands after toilet is higher than those wash their hands always after toilet use.

Therefore, this hygienic practice is an independent predictor of H. pylori infection. Generally, when consider life style and hygiene 289(95.1%) and 144(47.4%) has a good hygiene practice

before meal and after toilet visiting. While the remaining 15(4.9%) and 160(52.6%) are does not have any personal hygiene practice ether before meal or after visiting toilet. In this study distribution of *H.pylori* infection and association with different hygienic practices of the participant at WKU specialized hospital the person who have poor personal and environmental hygiene contributed to the transmission of H .pylori infectionas shown in table 3 below.

Table 3: Human habits associated with H. pylori infection

Variables	Frequency	present (%)	*H.pylori*		Pearson Chi-Square	Df	P-value
			Positive	Negative			
Washing hand							
Before meal							
Yes	289	95.1	105	184	0.579[a]	1	0.447
No	15	4.9	4	11			
Total	304						
After toilet							
Yes	144	47.4	42	102	5.322[a]	1	0.021
No	160	52.6	67	93			
Water Sour.							
Tanker	63	20.7	25	38			
Wheel	69	22.7	28	41	1.884[a]	2	.390
Pipe	172	56.6	56	116			
Latrine							
Private	172	56.6	56	116			
Public	91	29.9	39	52	2.805[a]	2	.246
Field	41	13.5	14	27			
Water treat.							
Chlorine	198	65.1	65	133			
Filtration	70	23.0	29	41	2.263[a]	2	.323
Boiling	36	11.8	15	21			

CHAPTER SIX

6.1.Discussion

Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country. In this study, the *H.pylori* stool antigen test was used to detect *H. pylori* antigen at Wolkite University Specialized Hospital, Gurage Zone,

Southern Ethiopia. In our study, the overall prevalence of *H.pylori* infection was 35.9% (109 of 304), which is less than studies done in Addis Ababa (89%)[22], Gondor (85.6%)[23] and Hawassa (72.9)[17]. The possible explanation is that the prevalence of H. pylori infection varies among countries and among population groups within the same country. This is may be due to the above studies were done in different age groups and used different study design and sample. Serological testing was used in the above studies whereas *H. pylori* stool antigen was used in this study [24]. Moreover, the variation may be as a result of different sensitivity of the method of laboratory diagnosis test detects active infection, while serology does not differentiate between current and past infection. Therefore, the use of serology may lead to an overestimation of prevalence by including subjects who had been infected but were cured prior to testing [25]. Although the significance of age to influence H. pylori infection was shown elsewhere [26], this contradicts our and others' findings [27]. There are conflicting reports on the relationship between age of patients and prevalence of H. pylori. studies conducted in Bhutan [28] documented identical prevalence with no statistically significance different among age group.

In our study, there was an increase in the prevalence of *H. pylori* with age and statistically significant association was found between age and prevalence of H. pylori. A similar finding was reported from studies done in developing countries [29]. This increase in prevalence with age is attributed to annual increase in the rate of infection and birth cohort effect[29]. The prevalence H. pylori were associated with marital status of participants ($p< 0.05$) which is in line with other studies in Northwest Ethiopia [30]. Nevertheless different from other study done in Ethiopia[31] and China[32], where marital status was not associated with prevalence of H. pylori. These may be due to life style which is different in developing and developed Country. Lower case there was no statistically significant difference in the prevalence of H. pylori with respect to number of family in the household which is parallel to other studies in Ethiopia[26], Brazil [33] and Benin[34]. Among the socio demographic characteristics of the participants, statistically significant difference was not obtained for educational attainment which is in agreement to studies [33,34] and inconsistent to other studies[35].

There are conflicting reports on the association between alcohol consumption and H. pylori infection. In Ethiopia, reported that alcohol consumption protects against H. pylori infection[36] but showed no significant association in his report[27]. Furthermore, frequent consumption of

alcohol (>once/week) showed no significant association but less frequent consumption (<once/week) was protective [28]. In Germany, an inverse relationship was also documented between amount of alcohol consumption and H. pylori infection [13] as moderate amount of alcohol consumption in the form of wine and beer protects against H. pylori infection [30]. However, the current study is in opposition to previously mentioned studies [17,18] as frequent consumption of alcohol (>once/week) was significantly associated with feco-prevalence of H. pylori ($p = 0.001$). But our result is in line with previous report as large amount of alcohol consumption is positively associated with active H. pylori infection [31]. The reason for these contradictory results might be due to difference in types and amounts of consumed alcoholic beverages. In Ethiopia, common local alcoholic drinks are“ Tella”, “Teji” “Araki” and “beer” which are quite different in alcohol content. The significant association in our study could be explained due to the hypothesis that heavy alcohol consumption facilitates H. pylori infection bydamaging the gastric mucosa. Besides the damaged gastricmucosa, bacterial adherence and host factors may also be involved in the synergistic effect for infection.

In this study *H. pylori* stool antigen detection was statistically associated with coffee consumption which is similar to previous reports from Germany where coffee consumption showed a positive dose response relation with active *H.pylori* [19]. But differsfrom Ethiopia [17] and England [30],This contradictory result might bedue to variation in type, frequency as well as amount ofdaily consumed coffee which needs further study. In ourstudy, smoking also show significantly associationwith H. pylori feco -prevalence which is differs from other studies in Ethiopia and elsewhere [17,18]. This may be due the habit of person to consume cigarate smoking per day which may cause peptic ulcer disease and dyspeptic symptoms by impairing immune system.which facilitates the adherence of *H.pylori* to the mucosa. This study also assessed the association of chat chewing and H. pylori infection. There was statistically significant relationship among H. pylori infection and the predictor variable chat chewing in bivariate analysis which is differs in Brazil [37]. The presence of association in this study might be due to high number of chewers that cause easily to compute the association. No significant association was observed between source of drinking water and H. pylori infection which is in line with previousstudies in Ethiopia [17,18]. In the current study, the observed lower contribution of source of water to *H.pylori* infection is probably due to type of study

participantsin which majority of them used pipe water for their daily consumption. No statistically significant association was also observedbetween tea consumption and H. pylori infection. Lack of association of above factors might be due to difference in types and amount of consumed tea.In this study, Participants who did not wash their hands after toilet were significantly associated with occurrence of H. pylori infection which is supported by other report in Kazakhstan (38), but it is contradicted to previous reports that is prevalence of H. pylori was higher in those who washed their hands after toilet (10, 13). Nevertheless, in our study no significant association was observed between source of drinking water and prevalence of H. pylori which is in line with previous study in China (32). This lower contribution of source of water to occurrence of H. pylori is due to most of the participants (56.6%) used pipe water. In this study, type of latrine was not significantly associated with presence of H. pylori which is inconsistent to other studies in Benin (24) and Kazakhstan (38). This is due to more of our study participant uses private types of latrine (56.6%).

6.2. Conclusion and recommmedation

The prevalence of H. pylori infection detected by fecal H.pylori antigen among symptomatic patients at WKUSTH was 35.9%. Among the toatal male study participants, 61(35.5%) of them were found to be positive for H. pylori stool antigen as compared to females 48(36.4%). Statistically significant association was obtained between *H. pylori* infection and associated

factors such as age, marital status, alcohol, chat, smoking, coffee, GIT history and washing of hand after toilet . However, no statistically significant difference was observed with sex, educational level, household, tea, water source, type of water treatment and type of latrinewithpylori infection. Therefore, good hygienic practices may reduce infection with H. pylori bacteria.Moreover, it is needed to design and implement intervention measures that could reduce risk factor and thus lessen the clinical consequences of infection.Further large scale community based studies are needed to better characterize the role of these potential sources of associated factor of *H. pylori* infection.

6.3.Limitations of the study

There are a few limitations of this study. The study population was only outpatients presented to the hospital, which limits the actual prevalence of infections and do not totally reflect the number of infected individuals in the community. Besides cross-sectional, all information on life style factors were collected by a self-administered questionnaire without determination of amount, types and biological markers. Moreover, the small sample size may have an impact on the observed association variables, and thus, interpretations should be made cautiously.

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Annexe

Annex-1Participants information sheet and informed voluntary consent

We are candidate graduate students from WKU, CMHS, and MLS. We are going to collect data for the study being conducted in this community by ……..s who are studying for their bachelor's degree. We kindly request you to giveus your attention to explain you about the study and being selected as a study participant.

The study/project title:

The prevalence of *Helicobacter pylori* and associated factor among outpatients attending at Wolkite University Specialized Hospital in Gurage Zone, SNNP Region, Ethiopia

Purpose/ aim of the study:

To determine the prevalence of *H.pylori* among out patients and also to identify factors associated with *H. pylori infection* among outpatients.

Procedure and duration:

We will be interviewing you using a questionnaire to provide us with pertinent data that is helpful for the study. There are 15 questions to answer where we will fill the questionnaire by interviewing you. The interview will take about 20 minutes, so I kindly request you to spare me this time for the interview.

Risks and benefits:

The risk of being participating in this study is minimal, but only taking few minutes from your time. There would not be any direct payment for participating in this study. But the findings from this research may reveal important information for the local health planners.

Confidentiality:

The information you will provide us will be confidential. There will be no information that will identify you in particular. The findings of the study will be general for the study community and will not reflect anything particular of the individual persons or students. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the research.

Rights:

Participation for this study is fully voluntary. You have the right to declarer to participate or not in this study. If you decide to participate, you have the right to withdraw from the study at any time and this will not label you for any loss of benefits which you otherwise are entitled. You do not have to answer any question that you do not want to answer.

Contact address:

If there are any questions or enquires any time about the study or the procedure, please contact: the principal investigators at

Tel: 0920446931, 0922171185

email:seenaamercy28@gmail.com

P.O.Box 08, Wolkite.

Declaration of informed voluntary consent:

I have read the participant information sheet. I have clearly understood the purpose of the research, the procedures, the risks and benefits, issue of confidentiality, the rights of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to withdraw from the study at any time or not to answer any question that I do not want. Therefore, I declare my voluntary consent to participate in this study with my initials (…….)

_____/_____date_____/____/_____

Name and signature of participant

_____/_____date_____/____/_____

Name and signature of data collector

N:B

- This is signed face to face in the presence of data collector
- Please provide a copy of this signed consent to the participant
- If the participant is allay person and cannot sign initials, can put his/her thumb print in front of a competent witness; and the witness has to sign alongside (with his/her name and address

Annex-2: Data Collection Questionnaire

Wolkite University
College of Medicien And Health Science
Department of Medical Laboratory Science

This questionnaire is prepared by Wolkite university B.Sc. graduating students in Medical Laboratory Sciences to determine the prevalence of Helicobacter pylori and its associated factors among outpatients visiting WolkiteUniversity Specialized Hospital from January 2020. So, we invited you to give us valuable information and we also guaranteed all information you give us will be kept confidential. Finally, we ask your cooperation and patience until we finish our questionnaire.

Data collectors name _____date_____Sign_____

Part-I Socio-demographic information (tick)of study participant

Participant code _____

1.1Sex. Male_____ Female_____

1.2. Age_____

1.3Marital status

1. Single2.Widowed 3.Divorced 4.Marriage

1.4. Whatis your level of education (circle one)

1. Not read and write. 2.Primary school 3.Secondary school 4.College/ University and above

1.5. Numbersof peoples in household?

1.Two 2.Three 3.Four 4. Greater than four

Part –II Associaterisk assessment for *H.pylori* infection

2.1Have you been experienced for Consumption of alcohol?

1. Yes 2. No

2.2. Have you been experienced for SmokingHabits?

1. Yes 2. No

2.3. Have you been experienced for chewing chat?

1. Yes B. No

2.4. Have you been experienced for Consumption coffee?

1. Yes 2. No

2.5. Have you been experienced for consumption of tea?

1 yes 2 No

2.6. Did you have possible history of Gastrointestinal?

1. Yes 2. No

Part-III.Some hygienic habits

3.1. Do you wash your hands before meals?

1. Yes 2. No

3.2. Do you wash your hands after toilet?

1. Yes 2. No

3.3. What types of water source you are using for drink (circle one)?

1. Tanker water 2. Wheel water 3. Pipe water

3.4. What type oflatrine do you use?

1. Private 2. Public 3.Field

3.5. What types of treatment you are using to treat your drinking water?

1. Chlorination 2.Filtration by clothing 3.Boiling

Annex- 3 Test Principle and Procrdure

Helicobacter pylori antigen test kit

Laboquick helicobacter pylori antigen test is used as a diagnostic tool to detect qualitative immunochromatographic helicobacter pylori (HPag) in stool.One step ,quick test to determine helicobacter pylori in stool.product code:LHAG.01.
Manufacturer:korogl tibbi Mals. San.ve tic. Ltd.Sti.1776/23 sokNo:4/A Mevlana Mah. B0rno-iznir-turkey Tel:+90(232)388 73 74 Faks:+90(232) 388 40 43
www.koroglugroup.com
e-mail:medical@koroglogroup.com

PRINCIPLE OF THE TEST

*H.pylori*Ag rapid test a sandwich solid phase immunochromato graphic assay.

TO perform the test an aliquot of diluted stool sample is added to the sample pad of the strip.

The sample flows through a label pad containing *H.pylori* Abs (antibody) coupled to Red – colored colloidal gold.

If the sample contains *H.pylori* Ag (antigen), the antigen will bind to the antibody coated on the colloidal gold particles, to form antigen- antibody-gold complexes.

This complex moves on the nitrocellulose membrane by action toward the test line region on which *H.pylori* specific antibody are immobilized. As the complex reach the test line, they will bind to the antibody on the membrane in the form of line.

A second red control will all ways appear in the result window to indicate that the test has been correctly performed and the device function properly.

If *H.Pylori* antigen is not present or lower than the detection limit of the test, only the control will be appear. If the control line does not developed, the test is invalid.

Laboratory procedure

1. Test device, patient sample, and controls should be brought to room temperature (15-30c) ̊prior to testing. Do not open pouches until ready to perform the assay.

2. The test strip will be removed from its protective pounce. Put it on a clean and Float surface. Be sure to label the strip with patient or specimen number.

3 .The sample prod will be remove from preparation device and coat liberally with fecal sample. Replace probe in vial and sake to disperse solid material. For liquid or semi- liquid stools.100Ul of stool may be added using an appropriate pipette.

4. The mixture will be standing for 1 – 2 minutes.

5. Shape top from preparation device and invert.3 or 4 drops will be added to the sample pad of the test stripe. The timer will be started.Result will be read in 5 - 15 minutes.

6. Full 15 minutes may be required for results of weak and negative samples.

7. The result will be read after 15 minutes is invalid

Annex-4: Materials and reagent

- Stool cup
- Applicator stick
- Test strips. Each strip is packed in a foil punch with package of desiccant
- Diluent(1.5ml/bottle)
- Cotton
- Alcohol
- gloves

Annex-5: Laboratory Report Form

Wolkite University SpecializedHospital

Laboratory request form

DATE_____

Name_____AGE_____SEX_____CARD.NO_____

PHYSICTIAN_____

Test Required

Serology	RESULT
H .pylori Ag	

Declaration

We, the undersigned, declare that this research paper is our original work, has not been presented for a degree in any other universities. We also declare that all sources of materials used for the research paper had been duly acknowledged.

Name of the PI	**Signature**
Esmael Abera	_____
Endashawu Mulugeta	_____
Meheret Mamo	_____
Beruktait Goitem	_____

Date of submission: …….

Advisors: Temesgen Abera (MSC) Signature _____

Co-advisor: Admasu Haile (MSC) Signature _____

NAME OF EXAMIANER

1. Kasahun Haile (MSC) Signature _____
2. Rebi(MSC) Signature _____