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COMPARISON OF STOOL ANTIGEN TEST OVER UREA BREATH TEST IN DETECTION OF HELICOBACTER PYLORI INFECTION AMONG PATIENTS ATTENDING NSAMBYA HOSPITAL IN KAMPALA DISTRICT.A CROSS-SECTIONAL STUDY.

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ABSTRACT

Background
H. pylori infection rates are higher in resource-poor settings and developing -countries such as Uganda. Even though most health facilities in Uganda including government health facilities have put in place several measures including diagnostic methods for H. pylori, there is still a rise in the prevalence of H. pylori infection.

Aim
The study aimed to compare stool antigen test over urea breath test in the detection of helicobacter pylori infection among patients attending Nsambya Hospital in Kampala district.

Methods
The study employed a cross-sectional design and a questionnaire guide to gather information from 375 individuals at the Outpatient department at Nsambya Hospital in Kampala district and these were selected using simple random sampling.

Results
The study results indicate that the prevalence of *H. pylori* was 48.0% by UBT and that of SAT was 46.9%, with most of the positive cases being in females 30.4% for UBT and 29.6% for SAT. On comparing SAT with UBT, SAT had a sensitivity of 97.8% and a specificity of 99.5%
.

Conclusion
In this study, UBT performance showed a slightly higher prevalence of detecting H. pylori infection compared to SAT thus UBT remains the gold standard method in diagnosing *H. pylori infection*. In addition, UBT also detects *H. pylori* bacteria in low parasitemia.

Recommendations
The SAT, which detects present but not previous infection of *H. pylori* would be applicable in mass survey.UBT should be done for every patient suspected of having *H. pylori infection* with negative SAT in order not to miss out on people with the disease as it remains the gold standard for *H.* P diagnosis. The Health authorities and other stakeholders should encourage using UBT on all patients suspected of having H. pylori infection.

Keywords: *Stool, Antigen Test, Urea Breath, Detection, Helicobacter Pylori Infection, Patients.*
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Background of the study
H. pylori is a gram-negative, non-spore-forming, spirally shaped bacterium, the bacteria usually colonizes the epithelium of the human stomach, in particular the gastric antrum. (Alzoubi *et al*., 2020). *H. Pylori* is a gram-negative microaerophilic bacterium that causes an infection that is generally acquired during childhood and persists lifelong in the absence of treatment with antibiotics (Khoder *et al*., 2019). *H. Pylori* bacterium is found on the luminal surface of the gastric Epithelium and was discovered by Marshall and Warren in 1983(Ochung'o *et al*., 2015)

H. pylori is capable of producing a powerful urease enzyme that can hydrolyze gastric urea to liberate ammonia and carbon dioxide, neutralizing the gastric acid and increasing the periplasmic pH to an alkaline medium, leading to peptic ulcer diseases, (alzoubi *et al*., 2020) and is associated with gastric adenocarcinoma and gastric high-grade B cell lymphoma (Chang *et al*.,2018). *Helicobacter pylori* is transmitted through the fecal-oral route and oral route. They have tried to isolate HP from feces, saliva, and dental plaque (Mladenova, I., & Durazzo, M 2018).

According to Jambi, (2022), Helicobacter pylori infection affects two-thirds of the world's population, and more than 90% of the duodenal ulcers and 80% of the stomach ulcers are due to H. pylori bacteria. Africa has a prevalence of *Helicobacter pylori* infection of 70.1% (Hooi *et al*., 2017) and the prevalence in Uganda was 45.2% but varies via topographical location from 18.2% in Apac District to 60.5% at Kawempe Health Centre (Baingana *et al*., 2014).In Kampala, the prevalence of *Helicobacter Pylori* was 68% (Angol et al., 2017). With this high prevalence, an accurate

and timely diagnosis is required for early management of this infection.

The currently used diagnostic method includes both invasive and non-invasive test methods. Among the invasive, rapid urease test (RUT), microbiology, histology (Chang, *et al*., 2018), and endoscopy are the gold standard but it is expensive. So, non-invasive methods have been adopted and these include *the Helicobacter Pylori* antibody test, SAT, and the UBT (Batts *et al*., 2013). However, a few studies are reporting which non-invasive test is superior in performance over the other. Collective guidelines presently endorse the use of UBT as a non-invasive test to detect *Helicobacter pylori* infection and to confirm the eradication of the infection after treatment (Sheu *et al*., 2017). To compare the stool antigen test over the urea breath test in detection of *Helicobacter pylori* infection among patients attending Nsambya Hospital in Kampala district.

METHODOLOGY

Study design

The study adopted a cross-sectional study on outpatients being treated at Nsambya Hospital. A cross-sectional study is a study where the researcher measures outcomes and exposures in the population at a point in time (Kermodel, U.S. 2018). This type of study allowed a researcher to compare many different variables at the same time.

Study area

The study was conducted at Nsambya Hospital which is located on Nsambya Hill in Makindye Division along the old Ggaba road about 6km from Kampala city center. St. Francis Hospital is a catholic mission hospital private not-for-profit, owned by the Archdiocese of Kampala and managed and founded by the Little Sisters of St. Francis of Assisi offering laboratory, maternity, pharmacy, inpatient, and outpatient services with 300 outpatients every day. The study was conducted between August 2023 to November 2023.

Inclusion criteria

Individuals who presented with gastrointestinal problems, as well as signs and symptoms of *H. pylori* infection. All patients of 18 years and above who attended Nsambya Hospital and also those who consented will be included.

Exclusion criteria

Individuals below 18 years and patients on treatment for Ulcers using drugs like proton pump inhibitors were excluded from the study.

Sample size determination

A statistical formula by Kish and Leslie (1965) was used to calculate the sample size.

Where:

N= sample size

Z= is the standard normal variable estimated at 1.96 (adopted from the Z distribution table) at a 95 % confidence level

P= P is the estimated proportion; considering the prevalence of *H. pylori* in Kiryandongo General Hospital, at 42.4% =0.424(Asiimwe *et al*., 2023).

q = 1- p, (1-0.424) = 0.576

d = 0.05, Represents the error allowed

$$n = \frac{1.96 \times 1.96 \times 0.424 \times 0.576}{0.05 \times 0.05}$$

=375 respondents

375 respondents will be considered for the study.

Sampling technique

The study employed simple random sampling which is defined as; a type of probability sampling in which each participant included in the sample has an equal chance of inclusion in the sample (Singh *et al*., 2014). This method was chosen because it lacks bias and it allowed a researcher to make generalizations about the population.

Sampling procedure

The sample size (375) was distributed by the number of days for data collection to ascertain the number of respondents required per day. Afterward, the selection of respondents will be done daily as follows based on the previous number of patients with GIT symptoms; the study will be conducted for 25 consecutive working days; therefore;

$$K = \frac{N1}{n1}$$ where; K is the required sample for each day

N1 is the sampling interval

n1 number of days

K = 375 / 25

= 15

Therefore, 15 respondents were sampled on each day for 25 consecutive days.

Data collection methods

The interview method was used to obtain the social demographic characteristics of participants.

Data collection tools

Data of participants on the prevalence *of H. pylori* infection using SAT and UBT was collected using a self-administered structured questionnaire that contained closed-ended questions. The prevalence of *H. pylori* was determined using *H. pylori* Rapid SAT cassettes and UBT kits. SAT specifically identified current infection by detecting antigen(s) that were produced by live *H. pylori* bacteria and shed into the stool while UBT specifically identified current infection by detecting the concentration of carbon 13/14 obtained from the breakdown of urea. Stool samples were used for SAT and breath samples containing carbon 13/14 were used for UBT

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Data collection procedures

Data was collected after obtaining an introductory letter from the school then approval to conduct the research from the St Francis hospital was sought after which the respondents were met at the health facility and then the researcher explained the purpose of the study to the respondents and those who were willing to participate in the study were consented. The researcher worked on 15 consented respondents a day for 25 days who were asked questions from the questionnaire about the study and those who knew how to read and write filled the questionnaires themselves while those who did not know how to read and write were verbally asked questions from the questionnaire and assisted to fill it by the researcher after which a stool and breath sample was collected and analyzed.

1. pylori Rapid Antigen test

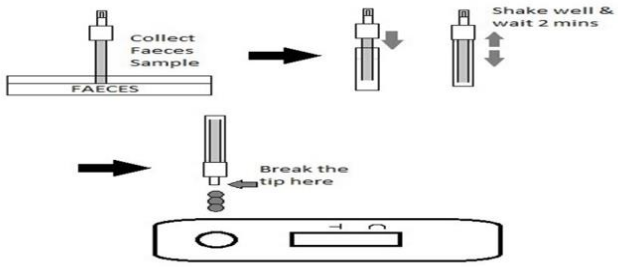
Principle

The *H. pylori* SAT Cassette is a rapid qualitative, lateral flow immunoassay for the detection of *H. pylori* antigens in human feces specimens. In this test, the membrane is coated with anti-H *pylori* on the test line region. During testing, the specimen reacts with the particle and the mixture migrates upward on the membrane hence reacting with anti-H-pylori antibodies on the membrane to form a coloured line (positive results). The sample continues up to the control region indicating negative results

Test Procedure

1. Using an applicator, a small portion of stool specimen (50mg) was collected from the sample container.
2. The applicator was placed into the dilution buffer and mixed well by shaking. The buffer tube was left for 2 minutes.
3. The test cassette was removed from its foil pouch and placed on a flat table surface.
4. The tip of the tube was carefully broken off, and 2 drops (~80 µl) of the sample solution were placed in the specimen well of the cassette while taking care not to trap air bubbles
5. Results were read at 10 minutes.
6. Results after 20 minutes were considered invalid.
7. The sample collection container and the test cassette were disposed of in the infectious waste bin after reading and recording the results.

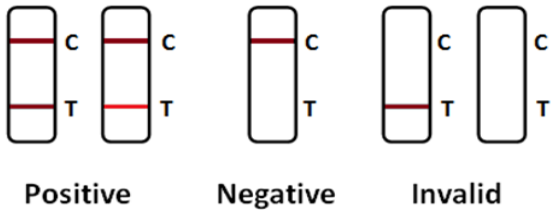
Figure 1: A figure illustrating how the SAT is performed



Interpretation of results

Two lines on both the control and test region indicated positive results, one line on only the control region indicated negative results and no line on the control region indicated invalid results.

Figure 2: A figure illustrating how positive, negative, and invalid results appear on a test casette



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Principle

The UBT is a simple, non-invasive test used for the quantitative detection of active *Helicobacter pylori* infection in humans. The test uses a urea tablet which is swallowed by the patient who has fasted; a patient is then left to settle without walking for 15 minutes for proper breakdown of urea to ammonia and CO2. A collection card is provided for the patient to blow into (CO2) for 3-5 minutes until the card turns from orange to yellow.

Patient preparation

1. Do not take any antibiotics for at least 4 weeks before the test
2. Do not eat anything for four hours before the test procedure
3. Respondent should inform the medical personnel whether she is pregnant or has allergies to any medication and taking PPI.

Test procedure

1. The patient took a 14C-Urea Capsule with water and then sat for 15 minutes
2. He or She blew into the collection card for 3minutes
3. The collection card was tested using an analyzer to obtain CO2 concentration
4. The collection card was disposed of into the infectious waste bin after reading and recording results obtained within 4 minutes from the UBT machine.

Figure 3: A figure illustrating how UBT is performed

Interpretation of results

Negative: when CO2 concentration was ≤ 40
Positive: when CO2 concentration was >50
Study variables

Dependent variable

The dependent variable is the outcome variable. In this study, *H. pylori* status was the dependent variable and it was measured as positive for samples that showed a positive result for both SAT and UBT and negative for those that showed a negative result.

Independent variables

The independent variable included the administration of SAT and the administration of UBT.

Data Quality control

Clinical data including demographic information were collected by the clinicians. Before the commencement of the study, standardized questionnaires involving structured questions on patient demographics were developed, reviewed, and approved by the supervisor and then pretested in a similar population to ensure the validity of designed questions.

During the administration of the questionnaires, research assistants who had been previously trained checked for consistency and completeness of information obtained from the study participants to ensure the reliability of the collected information. Before closure, all questionnaires were double-checked for completeness and approved for storage by the researcher. The completed questionnaires were stored in lockers that could only be accessible by the researcher.

Laboratory SOPs were followed for sample collection and analysis. Two tests were conducted for each specimen to establish consistency of results and all materials were checked for expiry dates.

Data analysis and presentation

Data was entered in a computer analyzed using Microsoft version and then presented using frequency tables, pie charts, and graphs.

Ethical consideration

After obtaining an introductory letter from the dean of students of Mildmay Institute of Health Sciences, I sought ethical approval from the St. Francis Hospital Research and Ethics Committee and thereafter sought permission from the administration of St. Francis Hospital Nsambya.

Confidentiality of the patient's results was highly considered by making sure that respondent identifiers were not used during data collection such as names instead study identification numbers were used, and only the investigator and clinician attending to the patients had access to the participant's records. The results of the study were communicated to the attending doctor for immediate attention to the participant.

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Participation in this study was voluntary and participants had the right to withdraw from it without any penalty.

RESULTS

Introduction

This chapter presents results obtained from 375 respondents at Nsambya Hospital in Kampala district. The researcher gathered data from the respondents using semi-structured questionnaires and performed SAT and UBT on stool and breath samples respectively. The study aimed to compare the performance of SAT against UBT in the diagnosis of *H. pylori infection*.

Socio-demographic characteristics of the respondents

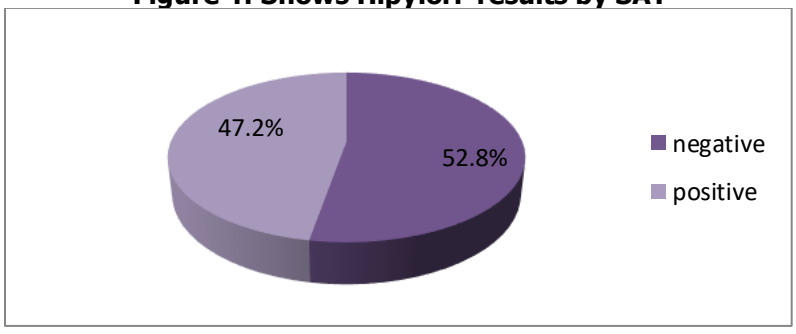
Table 1: shows the demographic distribution of the respondents (n=375)

Variable	Category	Frequency (n = 375)	Percentage (%)
Age	18 to 27 years	153	40.8%
	28 to 37 years	113	30.1%
	38 and above	109	29.1%
Gender	Male	155	41.3%
	Female	220	58.7%
Marital status	Single	100	26.6%
	Married	253	67.5%
	Divorced	16	4.3%
	Window	6	1.6%
Education level	Primary	23	6.1%
	Secondary	130	34.7%
	Tertiary	208	55.5%
	None	14	3.7%
Place of residence	Kampala	265	70.7%
	Masaka	26	6.9%
	Mpigi	30	8.0%
	Wakiso	36	9.6%
	Mityana	18	4.8%

From Table 1, (40.8%, n=153) of respondents were 18 to 27 years and the majority of the respondents (58.7%, n=220) were females. With regards to marital status, more of the respondents were married (67.5%, n=253), and concerning Education level, the majority of respondents had Tertiary education (55.5%,n=208), whereas (70.7%, n=265) were from Kampala district

Prevalence of H. pylori infection using the SAT among patients attending Nsambya Hospital in Kampala district.

Figure 4: Shows H.pylori results by SAT



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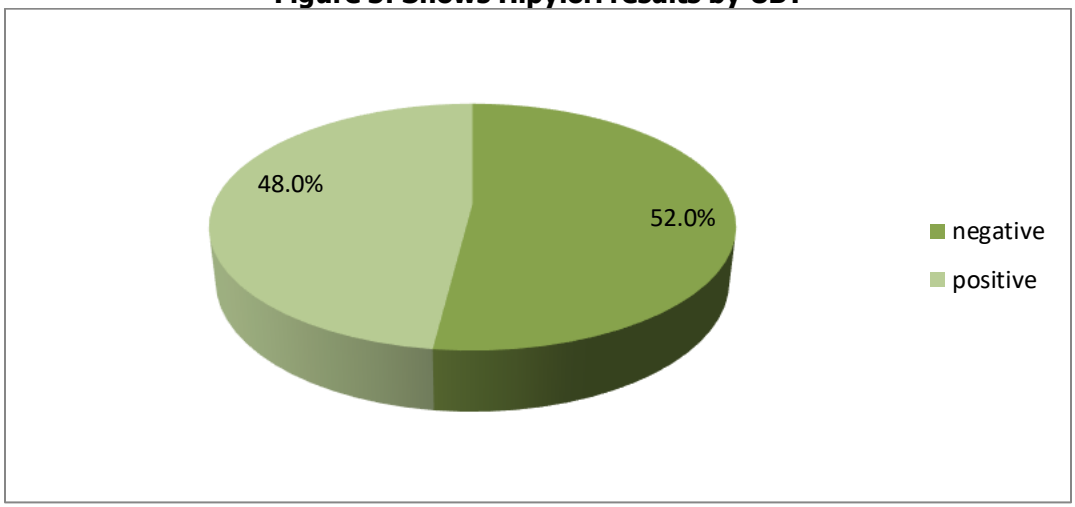
From the figure 4, the prevalence of *H. pylori* infection using SAT was 47.2%

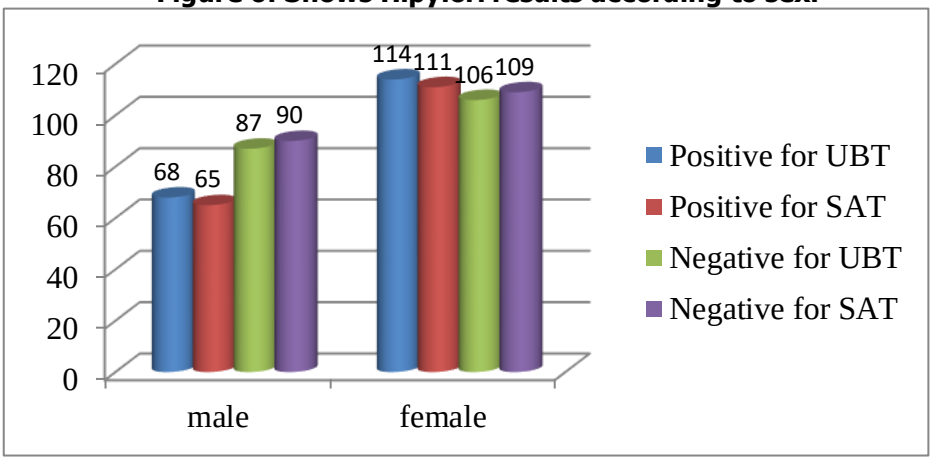
Prevalence of H. pylori infection using the UBT among patients attending Nsambya Hospital in Kampala district.

Figure 5: Shows H.pylori results by UBT



From the figure 5, the prevalence of *H. pylori* infection by UBT was 48.0%. Prevalence of *H. pylori* infection according to sex among patients attending Nsambya Hospital in Kampala district.

Figure 6: Shows H.pylori results according to sex.



From the figure 6, (43.9%, n=68) respondents accounted for positive *H pylori* results for UBT and (41.9%, n=65) for SAT. The overwhelming majority of female respondents were positive; accounting for (51.8%, n=114) for UBT and (50.4%, n=111) for SAT.

Comparison of the SAT over the UBT in the detection of Helicobacter pylori infection among patients attending Nsambya Hospital in Kampala district.

Table 5: Shows comparison between SAT and UBT results

	UBT		
SAT	Positive	Negative	Total
Positive	176	01	177

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Negative	04	194	198
Total	180	195	375

In Table 5 out of 375 respondents, 180 account for *H. pylori* positive by UBT while 195 account for *H. pylori* negative. 177 respondents out of 375 tested *H. pylori* positive by SAT while 198 respondents tested negative. Out of 375 respondents, 176 respondents account for *H. pylori* positive by both UBT and SAT while 194 respondents account for *H. pylori* negative by both SAT and UBT. Out of 180 respondents that were *H. pylori* positive by UBT, 4 respondents account for *H. pylori* negative by SAT while out of the 177 respondents that were positive by SAT, 1 respondent showed *H. pylori* negative by UBT.

The sensitivity and specificity of SAT when using UBT as a gold standard in the diagnosis of H. pylori infection

Table 6: The sensitivity and specificity of SAT when using UBT as a gold standard

Test	True Positive	True Negative	False Positive	False Negative	Sensitivity	Specificity
SAT	176	194	01	04	97.8% (176/176+4*100)	99.5% (194/194+1*100)

In Table 6, 176 test results were true positive, 194 were true negative, 01 were false positive and 04 were false negative giving SAT a sensitivity of 97.8% and specificity of 99.5%.

Discussion

The prevalence of H. pylori infection using the stool antigen test(Gisbert & Pajares, 2004)

The study revealed that the prevalence of H. pylori infection obtained from the sampled respondents was 47.2%, this prevalence is higher than that presented by Aitila *et al.,* (2019) of 24.3% and this could be due to the difference in the target population since the current study was among adults and the previous was among children. However, a study done by Angol *et al.,* (2017) showed a higher prevalence of 68% and this might be because the study involved one health facility while the former study involved three health facilities

The prevalence of H. pylori infection using Urea breath test

From the study findings, the prevalence of *H. pylori* obtained from 375 respondents was 48.0% which is less than the prevalence of 51.2% by Mohammed *et al.,* (2017) and this alteration in prevalence is related to dissimilarity in the sample size which is 86 patients for previous study and 375 patients for the recent one. However, the prevalence obtained was more than that obtained by Yuan *et al.*, (2020) at 35.9% because the aforementioned study was a systematic review and meta-analysis that involved a collection of different studies.

Prevalence of H. pylori infection according to sex

The study discovered that the prevalence of *H. pylori* by UBT and SAT in females was 51.8% and 50.4% respectively. However, the prevalence of *H. pylori* by UBT and SAT in males shown by the study was 43.9% and 41.9% respectively. This prevalence obtained was lower than that showed by Min *et al*., (2021) of 66.7% in males and 75% in females. This decrease in prevalence was due to a difference in the target population since the existing study was among both symptomatic and asymptomatic patients while the latter was among asymptomatic patients only. Additionally, the current prevalence was higher than that found by Awuku *et al*., (2017) with 16.8% in females and 10.7% in males, this alteration in prevalence is due to differing areas of study as the prior study was carried out within rural communities in Ghana while the existing was in urban area in Uganda.

The sensitivity and specificity of SAT when using UBT as a gold standard

The study discovered that on comparing SAT with UBT as a gold standard, SAT had a sensitivity of 97.8% and specificity of 99.5% in the diagnosis of *H. pylori infection*. The sensitivity of SAT of 97.8% discovered in this

study was slightly higher compared to the sensitivity of 76.5% that was reported by Alzoubi *et al*., (2020). This means that the probability of the SAT to detect positive cases among those that are positive with UBT is as high as 97.8%. This implies that the sensitivity of SAT is used only to detect *H. pylori* bacteria in high bacteremia leaving out *H pylori* bacteria in low bacteremia.

The specificity of SAT of 99.5% revealed in this study was lower than the specificity of 100% indicated in a study done by Hassan *et al*., (2021) This means that the probability of the SAT detecting negative cases among those that are negative with UBT is as high as 99.5%. This indicates that high specificity was due to some patients getting treated with antibiotics before coming to the hospital.

Conclusion

This study sought to compare SAT and UBT used in the diagnosis of *H. pylori infection* at Nsambya Hospital in Kampala district. The study discovered that *H. pylori* prevalence was 48.0%, the majority of the respondents being females 58.7% and many having an age range of 18-27 years 40.8%. Some SATs were negative (n=4) and yet the UBT of the same patients were positive while 1 SAT was positive as well as negative for UBT which implies that if SATs are used alone to diagnose *H. pylori infection*, they give rise to both false positive and false negative thus UBT remains the gold standard method in diagnosing *H. pylori infection*. In addition, UBT also detects *H. pylori* bacteria in low bacteremia.

Limitations of the study

The researcher encountered
financial constraints while gathering information.

Recommendations

The SAT, which detects present but not previous infection of *H. pylori* would be applicable in mass survey. In addition, if possible both tests should be performed together since SAT may not detect *H. pylori* bacteria in low bacteremia.

UBT should be done for every patient suspected of having *H. pylori infection* with negative SAT in order not to miss out on people with the disease as it remains the gold standard for *H.* P diagnosis. The health authorities and other stakeholders should consider and encourage the use of UBT to improve *H. pylori* diagnostic services for effective case management.

The study should be done with an equal number of participants for each sex to establish gender-related prevalence in all sexes.

Acknowledgment

I sincerely acknowledge the efforts of those who offered guidance in developing, conducting the study, analyzing, compiling, and reporting information on this research especially the administration and staff of St. Francis Hospital Nsambya. I also highly appreciate my supervisor, Mr. Ssekitoleko Anthony Isaiah for the support, guidance, and supervision rendered throughout the research study.

Finally, I would like to express my sincere gratitude to my family members, who provided both financial and moral support during my studies.

Abbreviations And Acronyms

AAR: Africa Air Rescue
CO2 Carbon dioxide
13C: Carbon thirteen
14C: Carbon fourteen
FDA: Food Drug Administration
GIT: Gastrointestinal tract
H: Helicobacter
HP: Helicobacter pylori
PH: Potential of hydrogen
PPI: Proton Pump Inhibitors
SAT: Stool Antigen test
UAHEB: Uganda Allied Health Examinations Board
UBT: Urea Breath Test.
USD: United States dollar
SOPs: Standard Operating Procedures

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