Journal of Medical Case Reports and Reviews

Received 15 Nov 2023 | Revised 16 Nov 2023 | Accepted 14 Jan 2024 | Published Online 17 Jan 2024

DOI:https://doi.org/10.52845/JMCRR/2024/7-1-1

JMCRR 07 (1), 1370-1376

ISSN (O) 2589-8655 | (P) 2589-8647 IF: 2.964

Original research Articles

Detection of Helicobacter Pylori and Genotyping Its Virulence Genes (Caga, And Vaca) Among Patients with Gastroduodenal Diseases in Wad Medani City, Sudan 2022

Weam S. Shashoug†(1), Bakri Y. Nour(2), Mohammed Bushra(3), Alaa Faroug(4)

1. Department of Microbiology, Faculty of Medical laboratory Sciences, University of Gezira, Sudan

2. Associate Professor, Department of Parasitology Faculty of Medical laboratory Sciences, University of Gezira, Sudan

3. Assistant professor, Faculty of Medicine, University of Gezira, Sudan

4. Lecturer, Department of Microbiology, Faculty of Medical laboratory Sciences, University of Gezira, Sudan

Abstract:- Background: Helicobacter pylori (*H. pylori)* linked with the etiopathogenesis of numerous digestive disorders. A wide variety of virulence factors influence the development of *H. pylori*-associated illnesses, and the most well-known virulence genes are the Cytotoxin-associated gene A (cagA) and vacuolating cytotoxin genes (vacA)

Aimes: The purpose of this study was to determine the frequency of *H. pylori* infections and virulence genes (cagA, vacA), as well as the association between virulence factors and gastroduodenal disorders.

Materials and methods: Our study contained 100 adult patients with dyspeptic symptoms. Stool samples were collected for stool antigen examination (SAT), and molecular methods were used to identify *H.pylori* and characterize the cagA and vacA genes associated with different diseases presented by patients.

Results: Out of a total of 100, 35 samples tested were positive for *H. pylori* (35%). The CagA virulence gene was found in 48.5% (17/35) and vacA in 82.8% (29/35). VacA genotype s1 m1 was the most prevalent [58.6%] followed by s2 m2 [34.5%] and s1 m2 [10.3%].**Conclusion:** The findings emphasize the need of performing regional research and characterizing *H.pylori* virulence genes linked with various diseases. The study revealed a significant association between virulence genes and the development of certain forms of gastric infections.

Keywords: Helicobacter pylori. Virulence genes. Polymerase chain reaction. Stool antigen test

Copyright : © 2024 The Authors. Published by Publisher. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

Corresponding Author: Weam S. Shashoug†, Department of Microbiology, Faculty of Medical laboratory Sciences, University of Gezira, Sudan

Introduction:

A spiral-shaped, gram-negative, microaerophilic bacillus. Helicobacter pylori was first identified in 1983, it has been linked to the etiopathogenesis of various digestive system illnesses[1]. Despite the fact that *H. pylori* infection might go undetected, it is thought to be the key factor in the development of gastric illnesses, like chronic gastritis, peptic

ulcers, also *H. pylori* infection can predispose to the development of adenocarcinoma, gastric cancer, and gastric mucosa-associated lymphoid tissue lymphoma[2][3].

In relation to race, ethnicity, and geographic region, the incidence of infection varies among various groups of individuals and within nations[4]. Variations in incidence rates have been linked to variables including poor socioeconomic standing, unhygienic living conditions, and crowded housing[5].

Several virulence variables affect how *H. pylori*-associated diseases develop and progress[6], among such, the virulence markers cytotoxin-associated gene A (cagA) and vacuolating cytotoxin gene A (vacA) have received a great deal of attention[7].

The first virulence factor identified in H. pylori strains was the cagA gene, this gene produces a protein that is linked to worse clinical outcomes and an increase in the severity of stomach inflammation[8]. The *H.pylori* pathogenicity island (PAI) was initially known as cytotoxin- associated gene and was thought to be connected to the production of the vacuolating toxin (vacA). Nevertheless, it was later shown that both components, the PAI and vacA, are independent of one another, despite the fact that cag-negative strains frequently do not express vacA[9]. CagA has been reported as the first bacterial oncoprotein and is most likely the virulence factor of *H. pylori* with the greatest potential[10].

H. pylori strains carrying the Vacuolating cytotoxin A (vacA) gene vary in their vacuolating ability, due to five vacA regions variations: s-region (s1 and s2), i-region (i1, i2, i3), m-region (m1 and m2), d-region (d1 and d2), and c-region (c1 and c2)[11]. vacA promotes chronic infection by inhibiting immune cells, stimulation of protein kinases, and autophagy modulation[12]. Also the induction of stomach cancer and immunological regulation are significantly influenced by the vacA toxin[13].

Information on *H. pylori* virulence factors and their roles in the development of gastroduodenal disorders needs to be updated. Therefore, the objectives of this study were to assess the incidence of *H. pylori,* identify the existence of cagA and vacA virulence genes in patients at Wad Medani Sudan, and examine the association between the emergence of gastroduodenal illness and these virulence genes. Therefore, the choice of virulence indicators is critical when utilizing them to predict disease risk.

To identify or confirm *H. pylori* in the current investigation, we employed a stool antigen test (SAT) and a polymerase chain reaction (PCR) assay, respectively. The two diagnostic techniques are frequently referred to as "gold standards" and have been reported to be both sensitive and specific[14].

Materials and methods:

Patient recruitment: This study included patients attended at private clinics in Wad Medani city, Sudan between July-october 2022. The present study was submitted to the Ethics Committee of University of Gezira and ministry of health-Sudan, for approval and the participating patients signed a free informed consent form prior to sample collection.

This cross-sectional descriptive study was conducted with a total of 100 dyspeptic patients, 46 (46%) of which were male and 54 (54%) female. Patients with previous gastric surgery were excluded and patients recently on antibiotics and other eradication therapies in the last 3 months were also excluded, research questionnaires were administered to volunteer participants.

Stool antigen test (SAT): In the present study, stool samples were collected and participants obtained their own stool samples. The samples prepared immediately for (SAT) as follows; the stick of the stool container was removed, and the feces were scraped to fill the groove at the tip of the fecal bite. Then, the feces were returned to the stool container and vigorously shaken several times to uniformly suspend the feces. The dropper of the stool container was uncapped, and the first one of the prepared samples were squeezed out. Three drops (approximately 110 µl) of the sample were then added onto the evaluation reagent test plate and allowed to stand at 15°C to 30°C for 3 to 10 min; lines were visible in the judgment and confirmation line parts. The remaining samples were stored immediately at -20°C for further PCR test.

DNA extraction: Frozen residual samples were returned to room temperature and were used as samples for PCR. DNA was extracted from approximately 200 grams of each sample using the QIAamp Fast DNA Stool Mini kit

(Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Briefly, samples were lysed in 1 ml inhibitEX buffer and vortex for 1 min, centrifuge samples for 1 min, 25 µl of proteinase K and 200 supernatant samples and 200 µl of buffer AL added and vortex for 15 seconds, then incubated for 10 min at 70°C. Two hundred microliter of ethanol (96%) was added to the lysate and mix by vortex, lysates were purified over a QIAamp column as specified by the manufacturer. The column was washed stepwisely with 500 µl buffer AW1 and buffer AW2, after which an ultra-pure DNA product was eluted for PCR assay.

Molecular confirmation of H. pylori: PCR was performed on extracted DNA from stool samples using primers specific for *H. pylori* ureC (table 1), under the following conditions: Initial denaturation of 94 °C for 3 mins and 35 cycles of 94 °C for 45s, 55 °C for 50s and 72 °C for 50s and a final extension time of 72 °C for 7 min. The PCR amplification was performed using a thermocycler system (Geneamp 9700 PCR Thermal Cycler). Each 25 µl PCR reaction mixture contained 5 µl PCR master mix (Maxime PCR premix, iNtRON® Korea), 1 µl each of primer (Macrogen, Europe), 3 µl of template DNA and 15 µl of PCR grade water. For each PCR experiment, appropriate positive and negative controls were included.

Amplification of virulence genes by Polymerase Chain Reaction (PCR): The cagA and vacA (alleles s1, s2, m1, m2) virulence genes of the clinical isolates were amplified by the polymerase chain reaction in a final volume of 25 µL containing 2 µL genomic DNA, 1 µL of each primer, Multiplex PCR was used to identify vacA and cagA genes in samples positive for ureC gene of h.pylori, using primers for vacA s1/vacA s2, vacA m1/vacA m2, and cagA (Table 1). Agarose gel (1.5%) with ethidium bromide was used for separation of the PCR product, using a 100 bp ladder as DNA molecular weight standard.

Statistical Analysis: Statistical analysis was done using IBM Statistical Package for the Social Sciences (SPSS) software, version 20.0, for Windows. Data was presented as mean ± standard deviation or percentages. Chi-squared test was used for categorical variables. P value was significant at < 0.05.

Table 1: Primer sequences used for the amplification of vacA alleles and cagA genes

Target gene	Primer Pairs (5'-3')	con length (bp)	References
gA	F:ACCGCTCGAGAACCCTAGTCGGTAATGGG R:CAGGTACCGCGGCCGCTTAAGATTTTTGGAAACCA C	981	(15)
vacA s1/s2	F: ATGGAAATACAACAAACACAC R: CTGCTTGAATGCGCCAAAC	259/286	(16)
vacA m1	F: GGTCAAAATGCGGTCATGG R: CCATTGGTACCTGTAGAAAC	290	(16)
vacA m2	F: CATAACTAGCGCCTTGCAC R: GGAGCCCCAGGAAACATTG	352	(16)
ureC	F: AAGCTTTTAGGGGTGTTAGGGGTTT R: -AAGC TTACTTTCTAACACTAACGC	300	(17)

Results:

Result: Recurrent abdominal pain was the most common presentation followed by heartburn, dyspepsia, bloating, acidity, nausea and vomiting (82%, 79%, 67%, 62%, 54% and 23%) respectively.

35 samples (35%) were positive for *H. pylori* ureC gene. Three participants who tested negative for (SAT) were confirmed positive for *H. pylori* using PCR, giving an overall prevalence of (35%).

Participant gender and *H. pylori* status: Of the 100 participants, 54 (54%) were females and 46 (46%) males. Of the 54 females, 19 (35.1%) were H. pylori positive; while in males, 16 (34.7%) of 46 were positive. Regarding the patients' gender (p=0.493), there was no statistically significant difference**.**

The age range was between 18 and 77 years, median age was 47.5 years. The prevalence of H. pylori in five age subgroups 20-29; 30-39; 40-49; 50-59; and 60-80 years (Table 2) show that prevalence variable with age up to 55 years and then slightly increase, but without reaching statistical significance (p=0.153).

Table 2: Characteristics of the population studied by gender and age.

Variable	Total of Patients	Prevalence
Male	46	16 (34.7%)
Female	54	19 (35.1%)
Group 1 (18-27)	13	2
Group 2 (28-37)	18	2
Group 3 (38-47)	24	6
Group 4 (48-57)	21	9
Group 5 (58-67)	13	7
Group 6 (68-77)	11	9

Molecular detection of *H. pylori*: Helicobacter pylori was detected by PCR technique (figure1). Where 35 samples (35%) were positive for *H. pylori* ureC gene. Three participants who tested negative for (SAT) were confirmed positive for *H. pylori* using PCR, giving an overall prevalence of (35%).

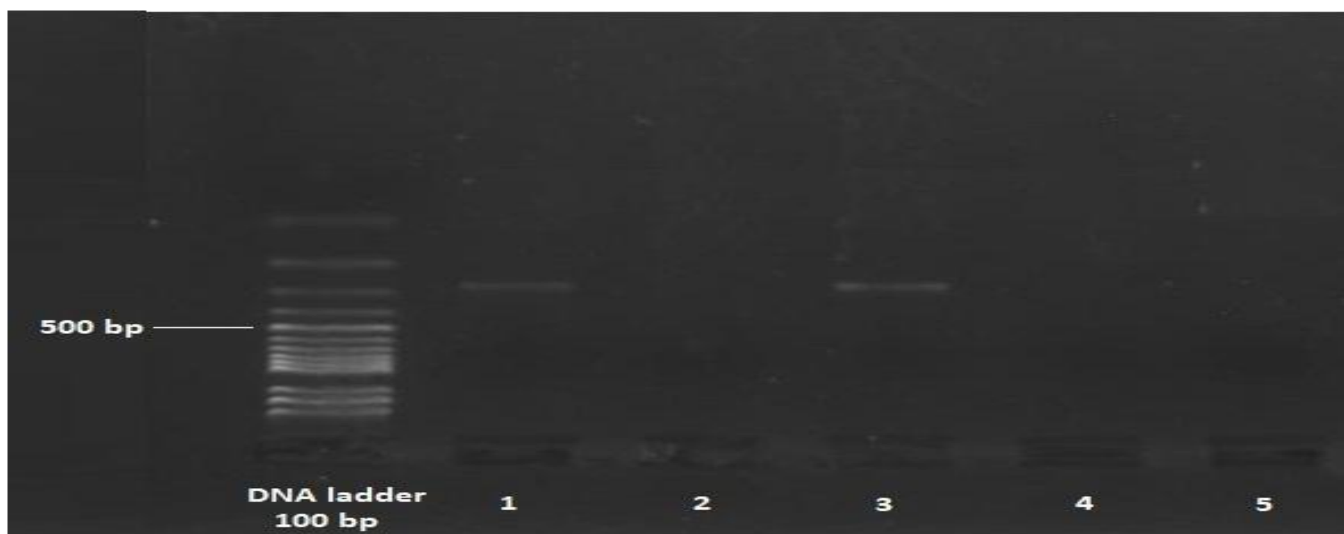

Figure 1: PCR amplification of UreC for H.pylori detection: 100bp DNA ladder, lane 1 positive control (294bp) lane 2 negative control, lane 3 positive sample, lane 5,5 negative samples.

Virulence genes detection and clinical conditions in *H. pylori* positive individuals: vacA and cagA genotypes were identified by multiplex PCR (Figure 2). vacA and cagA genes were identified in 29 (82.8%) and 13 (37.1%) of the 35 *H. pylori* strains, respectively. Out of the 35

H. pylori Positive strains, 80% (28\35) had at least one virulence gene and 20% (7\35) did not show any of cafA and vacA virulence genes.

The cagA virulence gene was compared to the many indications and symptoms in patients who tested positive to *H.pylori* in this study, but no significant correlation between the two was found, indicating

that this gene cannot be used as a particular indicator of severity in our environment.

The multiple alleles of vacA gene's were examined, and it was discovered that 14 samples had several allelic forms present at once. VacA genotype s1/m1 was the most prevalent (57.1%) followed by s2/m2 (42.8%) and s1/m2 (21.4%), but we did not find vac s2/m1 genotype.

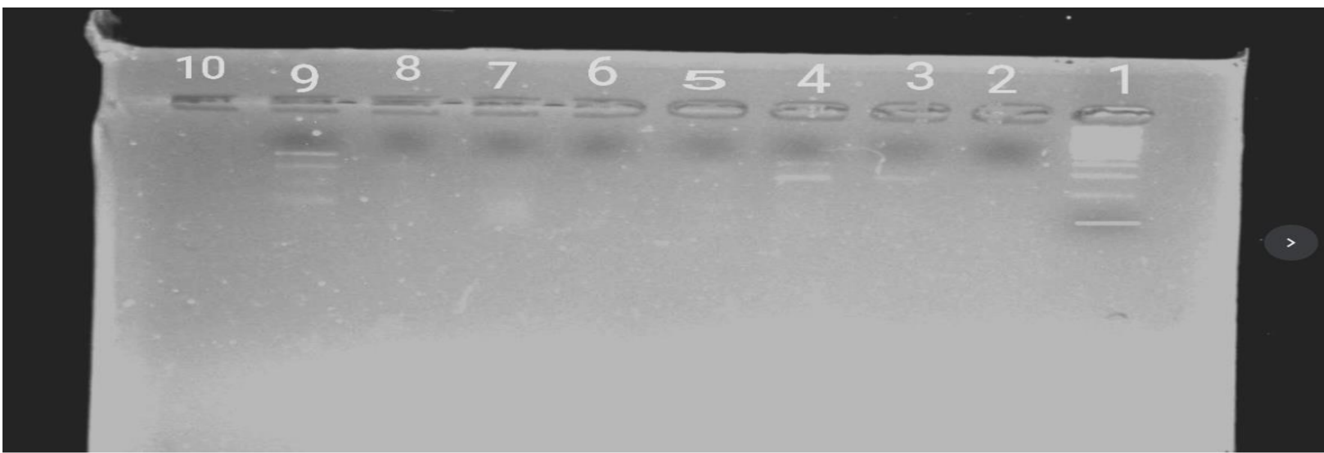

Figure 2: PCR amplification of vacA alleles, lane 1: 100bp DNA ladder, lane 2: negative control, lane 3: s1/s2 genotype, lane 4: s1/m1 genotype, lane 5-8: negative samples, lane 9: s1/m2 genotype

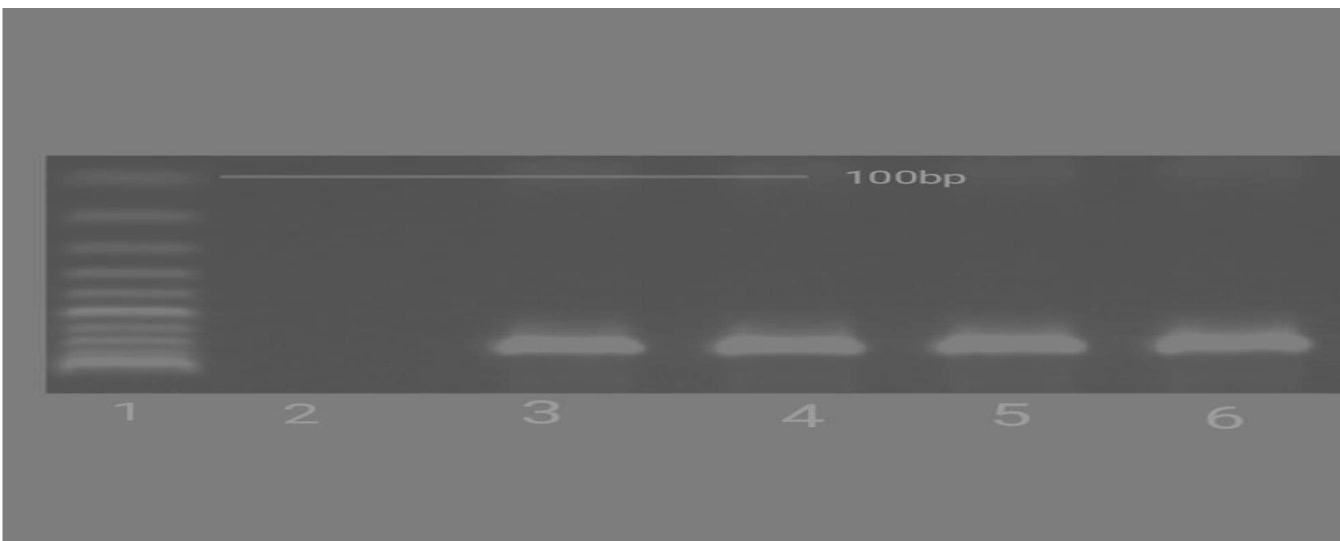

Figure 3: PCR amplification of cagA, lane 1: 100 bp DNA ladder, lane2: negative sample, lane 3-6: cagA+ samples (981bp)

Discussion:

The prevalence of *H. pylori* infection ranged from more than 70% in developing countries such as Bangladesh, India, and Mexico to around 20% in developed countries such as Netherland and Australia[18].

It is crucial to precisely identify *H. pylori* and antibiotic resistance in both adults and children, whether they are exhibiting symptoms or not, because of the high frequency of *H. pylori* infection, the age-related increase in infection rate, and the potential pathogenicity.

Many different diagnostic techniques to detect *H. pylori* have been proposed. The decision is often made based on the materials that are available, the population being sampled, the state of health of the patients, and the investigator's qualifications or expertise, and in this study we choose two techniques.

Using Stool specimens to detect *h.pylori* we should be aware of contamination because it can interfere with the normal results. We should also be concerned about the quality and quantity of DNA extracted when using PCR-based techniques and the consistency of the feces, and the time between sample collection and detection should be carefully considered regarding SAT.

Comparing both results from two techniques, we observed a higher prevalence rate (35%) in the PCR method than SAT (32%), the difference between the two methodologies' finding might have resulted from low colonization of bacteria in stomach leads to low concentration of *H. pylori* antigen in the sample and in some situations, the sensitivity of SAT may decrease, such as those for patients with gastrointestinal bleeding.

Compared to certain other earlier studies in developing countries where greater prevalence statistics were observed[19], the current study's total prevalence of 35% is rather low. The prevalence of 37.1% cagA positive strains obtained in our study lower than researches conducted in other region of Africa[17][20][21], and the outcome is also lower compared to a higher prevalence cagA previously reported Wad Medani city, Sudan[22]. Also cagA+ was found in our study as a single genotype, and linked to vacA in 5 cases.

Conclusion:

The findings of PCR amplification of H. pylori DNA extracted from feces often show variations in the intensity of the bands generated, which is normal given that it is frequently thought that *H. pylori c*olonizes the stomach mucosa in patches rather than consistently.

We hope to include histological examination in the future studies to enable comparison with gastroenterology diagnosis; and Studies of *H. pylori* virulence factors might prove crucial for an epidemiological and clinical study to become more familiar with the pathogenesis of the disease in Sudan.

Acknowledgments:

We thank Dr. Adel Mergani for providing technical assistance and I especially thank our colleague Salma saoud, who provided me with assistance during the collection of samples. Authors Thanks to all patients who volunteered to participate in our study. We are grateful to the medical laboratory college of Gaziera university for facilities provided during sampling and processing.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Microbiology.

References:

1. Kusters, J.G.; Van Vliet, A.H.; Kuipers, E.J. (2006). Pathogenesis of Helicobacter pylori infection. Clin. Microbiol., 19: 449-490.

2. McClain MS, Beckett AC and Cover TL (2017). Helicobacter pylori vacuolating toxin and gastric cancer. Toxins. 9: 316-332.

3. Bandipalliam P. MALT lymphomas--a closer look in the genomics era. South Med J. 2006;99(12):1322–4.

4. Tanih NF, Ndip RN. A South African perspective on Helicobacter pylori: prevalence, epidemiology and antimicrobial chemotherapy. African J Microbiol Res. 2013;7(21):2430–7. Available from https://pdfs. semanticscholar.org/b982/f504624a804b09cde7fea49468e27c44e4c7

5. Dube C, Tanih NF, Clarke AM, Mkwetshana N, Green E, Ndip RN. Helicobacter pylori infection and transmission in Africa: household hygiene and water sources are plausible factors exacerbating spread. African J Biotechnol. 2009; 8(22):6028–35. Available from http://citeseerx.ist.psu.edu/viewdoc/ download?doi=10.1.1.474.4239&rep rep1&type pdf

6. Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. Nat Rev Gastroenterol Hepatol. 2011;7(11):629–41 Available from: https://doi.org/ 10.1038/nrgastro.2010.154

7. Roesler BM. Clinical medicine insights : gastroenterology virulence factors of Helicobacter pylori : a review. Clin Med Insights Gastroenterol. 2014;7:9–17.

8. Martins, L.C.; Corvelo, T.C.O.:, Oti, H.T.; Barile, K.A.S. (2002). Soroprevalência de anticorpos contra o antígeno cagA do Helicobacter pylori em pacientes com úlcera gástrica na região Norte do Brasil. Revista da Sociedade Brasileira de Medicina Tropical 35:307-310

9. M. K. Tummuru, T. L. Cover, and M. J. Blaser, “Mutation of the cytotoxin-associated cagA gene does not affect the vacuolating cytotoxin activity of Helicobacter pylori,” Infection and Immunity, vol. 62, no. 6, pp. 2609–2613, 1994

10. Hatakeyama M. Review Helicobacter pylori CagA and Gastric Cancer. Cell Host Microbe. 2014;15(3):306–16 Available from: https://doi.org/10.1016/j. chom.2014.02.008

11. N. J. Foegeding, R. R. Caston, M. S. McClain, M. D. Ohi, and T. L. Cover, "An overview of Helicobacter pylori vacA toxin biology," Toxins, vol. 8, no. 6, 2016

12. K. R. Jones, J. M. Whitmire, and D. S. Merrell, "A tale of two toxins: Helicobacter pylori cagA and vacA modulate host pathways that impact disease," Frontiers in Microbiology, vol. 1, p. 115, 2010.

13. Utsch C, Haas R. VacA's induction of VacA-containing vacuoles (VCVs) and their immunomodulatory activities on human T cells. Toxins (Basel). 2016; 8(6):190.

14. Kato M, Ota H, Okuda M, Kikuchi S, Satoh K, Shimoyama T, Suzuki H, Handa O, Furuta T, Mabe K, Murakami K, Sugiyama T, Uemura N, Takahashi S (2019) Guidelines for the management of Helicobacter pylori infection in Japan: 2016 Revised Edition. Helicobacter 24 (4):e12597.

15. Harrison U, Fowora MA, Seriki AT, Loell E, Mueller S, Ugo-Ijeh M, et al. Helicobacter pylori strains from a Nigerian cohort show divergent antibiotic resistance rates and a uniform pathogenicity profile. PLoS One. 2017;12(5):1–16.

16. Y. Ito, T. Azuma, S. Ito et al., "Analysis and typing of the vacA gene from cagA-positive strains of Helicobacter pylori isolated in Japan," Journal of Clinical Microbiology, vol. 35, no. 7, pp. 1710–1714, 1997

17. Kishk RM, Soliman NM, Anani MM, Nemr N, Salem A, Attia F, Allithy ANA, Fouad M. Genotyping of *Helicobacter pylori* Virulence Genes *cagA* and *vacA*: Regional and National Study. Int J Microbiol. 2021 Jun 29;2021:5540560. doi: 10.1155/2021/5540560. PMID: 34306090; PMCID: PMC8263242.

18. Khalifa M, Sharaf R, Aziz R. Helicobacter pylori: a poor man's gut pathogen? Gut Pathogens 2010;2(1):2.

19. Tanih NF, Ndip RN. A South African perspective on Helicobacter pylori: prevalence, epidemiology and antimicrobial chemotherapy. African J Microbiol Res. 2013;7(21):2430–7.

20. Ben Mansour K, Fendri C, Zribi M, Masmoudi A, Labbene M, Fillali A, et al. Prevalence of Helicobacter pylori vacA, cagA, iceA and oipA genotypes in Tunisian patients. Ann Clin Microbiol Antimicrob. 2010;9(m):10.

21. El Khadir M, Alaoui Boukhris S, Benajah DA, El Rhazi K, Adil Ibrahimi S, El Abkari M, et al. VacA and CagA status as a biomarker of two opposite end outcomes of Helicobacter pylori infection (gastric cancer and duodenal ulcer) in a Moroccan population. PLoS One. 2017;12(1):1– 14

22. Elsiddig, Yosria & Ibrahim, Alaa & Koko, Roa & Arabi, Omer & Ibrahim, Mohamed & Abakar, Adam & Mohamedahmed, Khalid. (2019). Molecular Detection of H. Pylori from Gastric Biopsies of Dyspeptic Patients Attending Endoscopy Center, Gezira State, Sudan. European Academic Research. 7. 3467-3477.