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Research article

Helicobacter pylori infection in Angola: comparative epidemiological patterns identified by serology and stool antigen testing

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Abstract

Helicobacter pylori (*H. pylori*) is common in low- and middle-income countries, but recent data from Angola are scarce, and positivity estimates and associated factors may vary by diagnostic test used. This prospective cross-sectional sub-study, part of a five-year project on blood group polymorphisms and malaria susceptibility, compared seropositivity and stool antigen positivity to assess whether both tests identify similar epidemiological patterns. Data were collected at two Angolan healthcare facilities between July and September 2025. Of 357 individuals assessed, 172 were eligible and had complete results; no a priori sample size was calculated. *H. pylori* status was determined by rapid anti-*H. pylori* IgG serology and stool antigen testing. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for sociodemographic, clinical, behavioral, environmental, and ABO/Rh factors. Overall, 37.8% of participants were seropositive, and 30.8% were stool antigen-positive. Age ≥ 21 years was associated with seropositivity (OR = 2.17; 95% CI: 1.07–4.42; $p = 0.032$) and with stool antigen positivity (OR = 2.52; 95% CI: 1.15–5.52; $p = 0.021$). Living outside Sequele was associated with both outcomes (OR = 4.82; 95% CI: 2.27–10.21 and OR = 5.00; 95% CI: 2.17–11.51, respectively; both $p < 0.001$). Gastrointestinal symptoms showed strong associations (OR = 193.2; 95% CI: 43.2–864.4 and OR = 86.8; 95% CI: 19.8–380.8; both $p < 0.001$), but the wide CIs reflect sparse data. Eating outside the home and treating water by methods other than boiling were also associated with positivity. Sex, education, produce washing, ABO group, and Rh factor showed no significant associations. Both tests showed broadly similar patterns, although positivity rates and effect sizes differed. Because antibodies can persist after past infection, whereas stool antigen reflects current infection, the two tests are not equivalent measures of active infection. These facility-based findings do not represent national prevalence; larger, representative studies are needed across Angola.

Keywords: Angola, Associated factors, Diagnostic methods, Epidemiology, *Helicobacter pylori*, Serology, Stool antigen test

Introduction

Helicobacter pylori (H. pylori) is among the world's most common bacterial infections and remains a major public health concern because of its association with chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer [1, 2]. Despite a decline in global prevalence, infection remains highly prevalent in low- and middle-income countries. A recent meta-analysis estimated the global prevalence at 43.1% from 2011 to 2022, with substantial geographic variation and higher estimates from serological methods than from non-serological approaches [1].

Africa bears a particularly high burden of *H. pylori* infection, and prevalence varies widely across countries and populations. A recent meta-analysis estimated a pooled prevalence of 50.98% in East Africa, with marked differences by diagnostic method: 60.8% by serology versus 34.6% by stool antigen testing [3, 4]. This variation underscores the importance of diagnostic methods when interpreting and comparing epidemiological estimates. In addition, *H. pylori* transmission in African settings has been linked to socioeconomic and environmental conditions, including household crowding, inadequate sanitation, limited access to safe drinking water, and other living conditions [3, 4, 5].

In Angola, evidence on *H. pylori* remains limited. A previous study of community populations in Angola reported a positivity rate of 69.6%, with substantial geographic variation [6]. Although these findings suggest a considerable burden of infection, they cannot be interpreted as a national prevalence estimate. More recent, locally generated evidence is therefore needed to better characterize *H. pylori* infection and its associated factors in Angolan populations.

The choice of diagnostic method is especially important in resource-limited settings. Serology is widely used because it is accessible and relatively simple, but antibodies may persist after bacterial clearance, making it unreliable for distinguishing current from previous infection [2, 7]. In contrast, stool antigen testing detects bacterial antigens and more accurately reflects active infection. Validated monoclonal stool antigen assays are

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recommended as practical, noninvasive tools for diagnosis and for assessing eradication [7, 8]. The 2024 First Lagos Consensus Statement emphasized the need to adapt *H. pylori* diagnostic strategies to the realities of African healthcare systems, where access to endoscopy, histopathology, and other specialized methods remains limited [8].

Importantly, differences between diagnostic methods may extend beyond overall positivity and may influence the epidemiological patterns observed within populations. Comparing serology and stool antigen testing in the same population can therefore provide valuable information on whether both approaches identify similar demographic, clinical, behavioral, and environmental patterns of *H. pylori* positivity. This is particularly relevant in Angola, where contemporary epidemiological data remain scarce.

In this study, we compared serology and stool antigen detection of *H. pylori* in an Angolan population, assessing positivity and its associations with sociodemographic, clinical, behavioral, environmental, and ABO/Rh blood-group characteristics. We aimed to determine whether the two diagnostic approaches identify similar epidemiological patterns and to characterize the factors associated with positivity detected by each method.

Materials and methods

Study design and setting

A cross-sectional sub-study was conducted from July to September 2025 as the second component of a broader five-year research project investigating blood group polymorphisms and susceptibility to malaria. This sub-study focused on detecting *H. pylori* infection among eligible participants recruited through the parent project. All data and biological specimens used in this analysis were collected during this period. The study was conducted at two healthcare facilities in Angola: the LAICISA Health Unit in the urban district of Calemba 2, Luanda Province, and Saúde com S Medical Center in Sequele, Icolo e Bengo Province.

Study population and participant selection

A total of 357 individuals were screened for eligibility against predefined selection criteria. Data on medical history, gastrointestinal conditions, previous treatments, and other relevant criteria were collected directly from adult participants and from the parents or legal guardians of participating children. Individuals were enrolled if they met these criteria and provided both required specimens: a blood sample and an appropriately collected stool sample, both obtained by the research team. Exclusion criteria included a current or past history of gastrointestinal disease; reported use of treatments that could interfere with *H. pylori* detection or alter gastrointestinal status; failure to provide either specimen; and incomplete information or missing laboratory results needed for the analysis.

Of the 357 individuals assessed for eligibility, 185 were excluded for the following reasons: current gastrointestinal disease (n = 18); a history of gastrointestinal disease (n = 48); use of treatments that could affect *H. pylori* detection or gastrointestinal status (n = 6); failure to provide a stool specimen (n = 22); refusal or inability to provide a blood specimen (n = 28); incomplete clinical, epidemiological, or laboratory information (n = 19); missing serological or stool antigen test results (n = 12); and failure to meet other eligibility criteria (n = 32). The final analytical sample comprised 172 participants with available results for both serological and stool antigen testing. Participants were stratified into two age groups: 1–20 years and ≥ 21 years.

Data collection and study variables

Clinical and epidemiological data were gathered from all participants during the study period. The variables examined were sex, age, educational level, place of residence, healthcare facility attended, gastrointestinal symptoms, frequency of eating outside the home, drinking-water treatment, fruit and vegetable washing practices, ABO blood group, and Rh(D) status. For analysis, educational level was dichotomized as no formal or primary education versus secondary or higher education. Place of residence was grouped as Sequele versus Camama or other areas, and healthcare facility as Saúde com S Medical Center versus LAICISA Health Unit. Gastrointestinal symptoms were coded as present or absent, and eating outside the home as rarely versus sometimes or always. Drinking-water treatment was classified as boiling versus other methods (chlorination or filtration) or no treatment, and produce washing as bleach or vinegar versus water alone. ABO blood group was dichotomized as O versus non-O, and Rh(D) status as positive versus negative. The primary outcomes were *H. pylori* serology and stool antigen results, each analyzed separately as positive or negative.

Blood and stool specimen collection

Blood samples were drawn by the research team at the participating healthcare facilities. Each participant was then given a stool collection container and asked to collect a specimen the next morning and return it to the research team by 12:00 noon that same day. Stool specimens were processed promptly upon receipt. Only participants who provided both a blood and a stool specimen meeting the study's quality requirements were included in the final analysis.

Detection of *H. pylori*

H. pylori status was assessed using two rapid diagnostic tests applied to every participant: a serological test for

anti-*H. pylori* IgG antibodies and a stool antigen test. Both assays were performed and interpreted according to the manufacturers' instructions, and results were recorded qualitatively as positive or negative.

Serological testing

Anti-*H. pylori* IgG antibodies were detected using the NewPos *H. pylori* rapid antibody test (bioMérieux, Marcy-l'Étoile, France). Results were interpreted visually according to the kit's criteria; no antibody titers or quantitative cut-off values were determined. IgG antibodies can persist for months to years after the infection clears, so a positive result was taken to indicate past exposure or possible current infection, not confirmed active infection [2].

Stool antigen testing

The *H. pylori* stool antigen was detected using a rapid immunochromatographic test (DAS Diagnostar®, Monocent, Inc., Chatsworth, CA, USA), according to the manufacturer's instructions. This rapid stool antigen test uses an immunochromatographic principle, in which specific antibodies recognize target antigens in the stool sample and the resulting antigen–antibody complexes migrate along a membrane to produce a visible test signal. A positive stool antigen result was considered indicative of current infection. The manufacturer reports a sensitivity of 98.8% and a specificity of 100%; these values were not independently verified in this study.

Interpretation of test results

The two tests measure biologically distinct markers, and their results were therefore analyzed separately. Serology reflects the host antibody response, and results were recorded qualitatively as positive or negative. No quantitative antibody titers or cut-off values were determined. The stool antigen test indicates the presence of the bacterium, and results were likewise recorded as positive or negative. Seropositivity was interpreted as evidence of prior exposure or possible infection, because IgG antibodies may persist after bacterial clearance. By contrast, a positive stool antigen result was considered evidence of current infection. All 172 participants included in the final analysis had results available for both tests. No independent reference standard (e.g., urea breath test, histology, or culture) was used, so the sensitivity, specificity, and predictive values of either test could not be estimated. The findings were therefore interpreted as an epidemiological comparison of two tests measuring related but biologically distinct markers, rather than as an assessment of their relative diagnostic accuracy.

ABO and Rh(D) blood group determination

ABO and Rh(D) blood groups were determined by slide agglutination using monoclonal anti-A, anti-B, anti-AB, and anti-D reagents. Agglutination reactions were interpreted according to the laboratory procedure. For statistical analysis, blood groups were categorized as O or non-O, and Rh(D) status was classified as Rh-positive or Rh-negative.

Statistical analysis

Data were entered and cleaned in Microsoft Excel 2013 and analyzed using IBM SPSS Statistics, version 25 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages, and the proportions of positive serological and stool antigen results were calculated separately. Associations between participant characteristics and each test result were assessed using Pearson's chi-square test or Fisher's exact test when more than 20% of cells had an expected count below five. The strength of each association was expressed as a crude odds ratio (OR) with its 95% confidence interval (CI). Because no multivariable modeling was performed, these ORs are unadjusted. They reflect bivariate associations, not independent effects, and may be confounded by other demographic, clinical, or environmental factors. All tests were two-sided, with statistical significance set at $p < 0.05$.

Ethical considerations

The study was conducted in accordance with ethical approval granted by the Scientific Council of the Institute of Health Sciences at Agostinho Neto University. Written informed consent was obtained from adult participants, and consent procedures were documented. For children and other minor participants, written consent was obtained from their parents or legal guardians in accordance with the approved study protocol. Participant confidentiality was maintained throughout data collection, data management, and analysis.

Results

In this study, we compared serological and stool antigen testing using observed positivity proportions and epidemiological associations rather than a direct comparison of diagnostic accuracy. Overall, 65 participants (37.8%) were seropositive, and 53 (30.8%) had a positive stool antigen test. Although both tests were performed on the same participants, a formal paired analysis of agreement could not be conducted because participant-level cross-tabulation was unavailable. Consequently, overall agreement, Cohen's kappa, and McNemar's test were not reported. The comparison was therefore limited to marginal positivity proportions and associations observed separately for each diagnostic method. These results should not be interpreted as a formal assessment of agreement between the tests.

The distributions of sociodemographic, behavioral, environmental, and ABO/Rh characteristics by serological

and stool antigen results are shown in Table 1. Among sociodemographic characteristics, no statistically significant association was observed between sex and positivity for either diagnostic method. Compared with females, males had lower odds of seropositivity (OR = 0.84; 95% CI: 0.44–1.58; $p = 0.585$) and higher odds of stool antigen positivity (OR = 1.26; 95% CI: 0.65–2.43; $p = 0.504$). Positivity rates were 35.3% among males for serology and 33.8% for stool antigen testing. Thus, neither method provided statistical evidence of an association between sex and test positivity.

Age was significantly associated with positivity across both diagnostic methods. Among participants aged ≥ 21 years, 51 of 118 (43.2%) were seropositive, and 43 of 118 (36.4%) had a positive stool antigen test. Compared with participants aged 1–20 years, those aged ≥ 21 years had approximately twice the odds of seropositivity (OR = 2.17; 95% CI: 1.07–4.42; $p = 0.032$) and about 2.5 times the odds of a positive stool antigen test (OR = 2.52; 95% CI: 1.15–5.52; $p = 0.021$). Both associations were statistically significant, indicating that older participants had higher odds of positivity with either diagnostic method.

Educational level was not significantly associated with positivity by either diagnostic method. Among participants with secondary or higher education, 52 of 130 (40.0%) were seropositive, and 44 of 130 (33.8%) had a positive stool antigen test. Compared with participants with no formal or primary education, those with secondary or higher education had higher odds of seropositivity (OR = 1.47; 95% CI: 0.70–3.08; $p = 0.310$) and of stool antigen positivity (OR = 1.80; 95% CI: 0.85–4.10; $p = 0.163$). However, neither association was statistically significant, and both confidence intervals included the null value. Therefore, the available data provide insufficient evidence of an association between educational level and *H. pylori* test positivity.

Place of residence showed a consistent, statistically significant association with positivity across both diagnostic methods. Compared with participants in Sequele, those in Camama and other municipalities had positivity rates of 50.0% for serology and 41.7% for stool antigen testing. The odds of seropositivity were approximately 4.8 times higher (OR = 4.82; 95% CI: 2.27–10.21; $p < 0.001$), and the odds of stool antigen positivity were five times higher (OR = 5.00; 95% CI: 2.17–11.51; $p < 0.001$). These findings indicate a strong association between place of residence and *H. pylori* test positivity, although the observational design does not establish causality.

A strong association was also observed between healthcare facility and test positivity. Compared with participants attending Saúde com S, those attending LAICISA had higher positivity rates for both serology (62.8%) and stool antigen testing (52.6%). Attendance at LAICISA was associated with substantially higher odds of seropositivity (OR = 8.24; 95% CI: 4.06–16.71; $p < 0.001$) and stool antigen positivity (OR = 7.57; 95% CI: 3.57–16.05; $p < 0.001$). These findings demonstrate a statistically significant association between healthcare facility and test positivity. However, they should not be interpreted as evidence of a causal effect of the facility itself, as differences in referral patterns, clinical characteristics, socioeconomic profiles, or other population characteristics may contribute to the observed associations.

Among the behavioral and environmental factors examined, gastrointestinal symptoms showed the strongest association with positivity across both diagnostic methods. Among participants reporting gastrointestinal symptoms, 63 of 78 (80.8%) were seropositive, and 51 of 78 (65.4%) had a positive stool antigen test. Gastrointestinal symptoms were strongly associated with seropositivity (OR = 193.2; 95% CI: 43.2–864.4; $p < 0.001$) and with stool antigen positivity (OR = 86.8; 95% CI: 19.8–380.8; $p < 0.001$). These very large ORs should be interpreted cautiously because only two participants without gastrointestinal symptoms tested positive using either method. This sparse distribution produced large point estimates and wide confidence intervals, indicating substantial uncertainty about the magnitude of the associations. The findings therefore support a strong association within this sample but should not be interpreted as precise estimates of the corresponding population effects.

The habit of eating outside the home was significantly associated with positivity by both diagnostic methods. Among participants who reported eating outside the home sometimes or always, 51 of 93 (54.8%) were seropositive, and 39 of 93 (41.9%) were positive by stool antigen test. Compared with participants who rarely ate outside the home, those who sometimes or always ate outside the home had higher odds of seropositivity (OR = 5.64; 95% CI: 2.78–11.44; $p < 0.001$) and of stool antigen positivity (OR = 3.35; 95% CI: 1.64–6.85; $p = 0.001$). Both associations were statistically significant. Although the point estimate was higher for serology, the two tests capture different infection states (cumulative exposure versus current infection), so the OR magnitudes should not be compared directly.

Water treatment practices were also significantly associated with positivity by both methods. Among participants who did not treat their drinking water or used chlorination or filtration, 46 of 74 (62.2%) were seropositive and 37 of 74 (50.0%) were positive by stool antigen test. Compared with participants who boiled their drinking water, this group had higher odds of

Table 1: Sociodemographic, clinical, behavioral and environmental factors, and ABO/Rh characteristics according to *H. pylori* serology and stool antigen test results

Variable	Category	Serology positive (%)	Serology negative (%)	OR (95% CI)	*p*-value	Stool antigen positive (%)	Stool antigen negative (%)	OR (95% CI)	*p*-value
Demographic characteristics	Total	65 (37.8)	107 (62.2)	—	—	53 (30.8)	119 (69.2)	—	—
Sex	Female (Ref.)	41 (39.4)	63 (60.6)	1.00	—	30 (28.8)	74 (71.2)	1.00	—
	Male	24 (35.3)	44 (64.7)	0.84 (0.44–1.58)	0.585	23 (33.8)	45 (66.2)	1.26 (0.65–2.43)	0.504
Age group	1–20 years (Ref.)	14 (25.9)	40 (74.1)	1.00	—	10 (18.5)	44 (81.5)	1.00	—
	≥ 21 years	51 (43.2)	67 (56.8)	2.17 (1.07–4.42)	0.032	43 (36.4)	75 (63.6)	2.52 (1.15–5.52)	0.021
Educational level	No formal and primary education (Ref.)	13 (31.0)	29 (69.0)	1.00	—	9 (21.4)	33 (78.6)	1.00	—
	Secondary and higher education	52 (40.0)	78 (60.0)	1.47 (0.70–3.08)	0.310	44 (33.8)	86 (66.2)	1.80 (0.85–4.10)	0.163
Place of residence	Sequele (Ref.)	11 (17.2)	53 (82.8)	1.00	—	8 (12.5)	56 (87.5)	1.00	—
	Camama + other municipalities	54 (50.0)	54 (50.0)	4.82 (2.27–10.21)	< 0.001	45 (41.7)	63 (58.3)	5.00 (2.17–11.51)	< 0.001
Healthcare unit	Saúde com S (Ref.)	16 (17.0)	78 (83.0)	1.00	—	12 (12.8)	82 (87.2)	1.00	—
	LAICISA	49 (62.8)	29 (37.2)	8.24 (4.06–16.71)	< 0.001	41 (52.6)	37 (47.4)	7.57 (3.57–16.05)	< 0.001
Behavioral and environmental factors									
Gastrointestinal symptoms	No symptoms (Ref.)	2 (2.1)	92 (97.9)	1.00	—	2 (2.1)	92 (97.9)	1.00	—
	Any gastrointestinal symptoms	63 (80.8)	15 (19.2)	193.2 (43.2–864.4)	< 0.001	51 (65.4)	27 (34.6)	86.8 (19.8–380.8)	< 0.001
Eating outside the home	Rarely (Ref.)	14 (17.7)	65 (82.3)	1.00	—	14 (17.7)	65 (82.3)	1.00	—
	Sometimes/always	51 (54.8)	42 (45.2)	5.64 (2.78–11.44)	< 0.001	39 (41.9)	54 (58.1)	3.35 (1.64–6.85)	0.001
Water treatment	Boiling (Ref.)	19 (19.4)	79 (80.6)	1.00	—	16 (16.3)	82 (83.7)	1.00	—
	No treatment/chlorine/filter	46 (62.2)	28 (37.8)	6.83 (3.44–13.60)	< 0.001	37 (50.0)	37 (50.0)	5.13 (2.47–10.65)	< 0.001
Fruit and vegetable hygiene	Bleach/vinegar (Ref.)	27 (31.4)	59 (68.6)	1.00	—	24 (27.9)	62 (72.1)	1.00	—
	Water only	38 (44.2)	48 (55.8)	1.73 (0.92–3.26)	0.085	29 (33.7)	57 (66.3)	1.32 (0.68–2.55)	0.409
ABO/Rh blood groups									
ABO blood group	Non-O (A, B, AB) (Ref.)	26 (33.3)	52 (66.7)	1.00	—	24 (30.8)	54 (69.2)	1.00	—
	O	39 (41.5)	55 (58.5)	1.42 (0.76–2.65)	0.273	29 (30.9)	65 (69.1)	1.00 (0.53–1.89)	0.998
Rh factor	Rh-negative (Ref.)	4 (50.0)	4 (50.0)	1.00	—	2 (25.0)	6 (75.0)	1.00	—
	Rh-positive	61 (37.2)	103 (62.8)	0.59 (0.14–2.45)	0.470	51 (31.1)	113 (68.9)	1.35 (0.25–7.39)	0.726

Data are presented as numbers (%) within each category; percentages represent the proportion of participants in the corresponding category who were positive or negative by the respective diagnostic method, not the proportion of all positive participants in that category. OR = odds ratio; CI = confidence interval. ORs are crude, unadjusted estimates calculated using the reference categories indicated in the table. Statistically significant associations are represented by *p*-values < 0.05. Associations between participant characteristics and test positivity were assessed using odds ratios (ORs) with 95% confidence intervals (95% CIs), with the reference categories indicated in the table.

seropositivity (OR = 6.83; 95% CI: 3.44–13.60; $p < 0.001$) and of stool antigen positivity (OR = 5.13; 95% CI: 2.47–10.65; $p < 0.001$). However, this exposure category combines heterogeneous practices, and given the cross-sectional design, these findings do not show that any specific water treatment method caused the observed differences.

In contrast, the method used to wash fruits and vegetables was not significantly associated with positivity for either diagnostic method. Among participants who washed fruits and vegetables with water only, 38 of 86 (44.2%) were seropositive, and 29 of 86 (33.7%) were positive to the stool antigen test. Compared with participants who used bleach or vinegar, those who used water only had higher estimated odds of seropositivity (OR = 1.73; 95% CI: 0.92–3.26; $p = 0.085$) and of stool antigen positivity (OR = 1.32; 95% CI: 0.68–2.55; $p = 0.409$). Neither association reached statistical significance. Although the point estimates were above 1.00, the available evidence was insufficient to establish a statistically significant association between this hygiene practice and positivity by either method.

Regarding ABO blood groups, no statistically significant associations were observed with either diagnostic outcome. Among participants with blood group O, 39 of 94 (41.5%) were seropositive, while 29 of 94 (30.9%) had a positive stool antigen test. Compared with participants with non-O blood groups (A, B, or AB), those with blood group O had higher estimated odds of seropositivity (OR = 1.42; 95% CI: 0.76–2.65; $p = 0.273$). For stool antigen positivity, the estimated odds were identical between the two groups (OR = 1.00; 95% CI: 0.53–1.89; $p = 0.998$). Neither association was statistically significant, providing insufficient evidence of an association between blood group O and *H. pylori* positivity according to either diagnostic method.

Similarly, Rh factor was not significantly associated with positivity for either diagnostic method. Among Rh-positive participants, 61 of 164 (37.2%) were seropositive, and 51 of 164 (31.1%) had a positive stool antigen test. Compared with Rh-negative participants, Rh-positive participants had lower estimated odds of seropositivity (OR = 0.59; 95% CI: 0.14–2.45; $p = 0.470$) and higher estimated odds of stool antigen positivity (OR = 1.35; 95% CI: 0.25–7.39; $p = 0.726$). Neither association reached statistical significance. However, only eight participants were Rh-negative, resulting in wide confidence intervals and limited precision. Therefore, the absence of statistical significance should not be interpreted as evidence that no association exists; rather, the findings indicate that the available sample provides limited evidence to determine whether the Rh factor is associated with *H. pylori* test positivity.

Discussion

Helicobacter pylori infection remains a public health concern in Low- and Middle-income Countries (LMICs) [1], particularly in settings with limited epidemiological evidence, such as Angola. In this study, we compared seropositivity and stool antigen positivity and their associated epidemiological patterns in the same population in Angola. The study found *H. pylori* positivity rates of 37.8% by serology and 30.8% by stool antigen testing. The higher seropositivity may reflect differences in the biological markers detected by the two methods. Serology detects anti-*H. pylori* antibodies, which may persist after bacterial clearance, whereas stool antigen testing more directly indicates current infection [2]. Differences in diagnostic methods may therefore contribute to variation in reported prevalence estimates across populations [1, 9].

Recent studies support the clinical relevance of stool antigen testing. Silva Luz et al. reported pooled sensitivities and specificities of 92.19% and 92.93%, respectively, for conventional stool antigen tests [10]. Kubo et al. reported high diagnostic performance for a novel stool antigen assay [11], and Chong et al. compared two commercially available stool antigen assays [12]. Abdelmalek et al. also highlighted the potential of stool antigen lateral-flow testing as a rapid, non-invasive approach [13]. These findings support using stool antigen testing to investigate current infection, although performance may vary by assay and study setting. However, the results of the present study should not be interpreted as demonstrating the superiority of either method, because no independent reference standard was used to establish diagnostic accuracy [2, 10, 11, 12, 13].

Age was significantly associated with positivity by both methods, with higher positivity among adults 21 years or older. This pattern may reflect cumulative exposure over time and is consistent with evidence that *H. pylori* prevalence varies with age [14]. However, the cross-sectional design does not establish when infection occurred or distinguish prior exposure from current infection among seropositive participants.

Place of residence and healthcare facility were also strongly associated with positivity by both methods. These associations may reflect differences in socioeconomic conditions, sanitation, water supply, healthcare-seeking behavior, referral patterns, or the characteristics of the populations attending each facility. Because these factors were not assessed independently and the analyses were not adjusted, the observed associations should be interpreted as exploratory, not as evidence of causal relationships.

Gastrointestinal symptoms showed the strongest association with *H. pylori* test positivity in the present study. This finding is clinically relevant, as *H. pylori*

infection is associated with chronic gastritis and dyspeptic manifestations, although a substantial proportion of infected individuals remain asymptomatic. Similarly, a community-based study conducted in Ethiopia [15] reported an association between epigastric symptoms and *H. pylori* seropositivity (adjusted OR = 1.93; 95% CI: 1.28–2.91), providing evidence of a relationship between gastrointestinal manifestations and infection markers in an African population. However, differences in diagnostic methods and study populations should be considered when comparing these findings [16, 17, 18, 19].

Both eating outside the home and the method of drinking-water treatment showed significant associations with *H. pylori* positivity, whether infection was assessed by serology or by stool antigen testing. Plausible explanations include differences in food-handling hygiene, the microbiological quality of drinking water, household sanitation, and wider socioeconomic conditions. These observations agree with reports from several countries that have linked *H. pylori* infection to frequent consumption of food prepared outside the home [20], use of well water [21], and poor drinking-water quality [22]. Taken together, the evidence points to behavioral and environmental factors as useful targets for understanding *H. pylori* transmission and designing prevention measures. Studies in other regions and settings in Angola, ideally with longitudinal designs, are now needed to confirm these determinants and clarify how they operate locally.

No statistically significant associations were observed between test positivity and sex, educational level, fruit and vegetable washing practices, ABO blood group, or Rh factor. These findings indicate insufficient evidence of associations in this study, not proof that such relationships do not exist. The relatively small sample size and the limited number of participants in some categories, particularly the Rh-negative group, may have reduced the precision of the estimates. In addition, combining blood groups A, B, and AB into a single non-O category may have obscured differences among individual blood groups.

The epidemiological patterns identified by serology and stool antigen testing were broadly similar, particularly for age, residence, healthcare facility, gastrointestinal symptoms, eating outside the home, and water-treatment practices. However, similarity in group-level associations does not establish agreement between the tests at the individual level. Al Ofairi et al. evaluated IgG serology and stool antigen testing in Yemen and highlighted the importance of interpreting these methods in light of their different biological targets [16]. In the present study, individual-level paired results were unavailable for agreement analysis; consequently, Cohen's kappa and McNemar's test could not be calculated. Likewise, sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy could not be established without an independent reference standard [2, 10, 11, 12, 13].

This study did not include bacterial culture, PCR confirmation, or genotyping of *H. pylori* virulence genes. As a result, we could not characterize the circulating strains or assess whether specific virulence determinants, such as *cagA* and *vacA*, contribute to gastrointestinal symptoms. The need for better diagnostic approaches to *H. pylori* infection in African settings has been emphasized previously [15], and PCR-based methods have proved useful for detecting *H. pylori* in patients with gastric ulcers and for profiling its virulence genes [18]. More recently, a multicenter clinical study compared the diagnostic accuracy of available *H. pylori* detection methods [19]. Conventional diagnostic methods remain essential for identifying active *H. pylori* infection. PCR-based assays allow rapid detection of *H. pylori* and of specific resistance-associated mutations. Culture, however, offers an advantage for antimicrobial resistance: phenotypic susceptibility testing can detect resistance that targeted molecular assays may fail to predict. Combining molecular and culture-based approaches therefore provides complementary information for accurate diagnosis, antimicrobial resistance surveillance, and appropriate treatment selection [23, 24]. Future work in Angola should therefore combine non-invasive testing with molecular characterization and clinical evaluation, to clarify how active infection, bacterial virulence factors, and gastrointestinal disease are related.

Several limitations should be considered. This study was a secondary sub-study of a broader project on blood group polymorphisms and malaria susceptibility, rather than a population-based investigation specifically designed to estimate *H. pylori* prevalence. No a priori sample size calculation was performed for this sub-study, and participant inclusion depended on the availability of eligible specimens and associated data. These factors may limit statistical power and generalizability, and the results should not be considered representative of Angola as a whole. In addition, the odds ratios were crude and unadjusted for potential confounders. Relationships among residence, education, water-treatment practices, and healthcare facility may therefore partly explain the observed associations. Future studies with larger samples should use multivariable analyses and consider exact or penalized regression methods when sparse data affect estimation.

Overall, the findings suggest that several demographic, clinical, behavioral, and environmental characteristics were associated with *H. pylori* test positivity in the study population. The consistency of some associations across the two methods is epidemiologically informative, but differences in positivity rates and odds ratios should not

be interpreted as evidence of diagnostic superiority. Larger, specifically designed studies that incorporate paired individual-level results, appropriate adjustment for confounding, and an independent reference standard are needed to clarify these associations and to assess diagnostic agreement and accuracy.

Conclusion

This study provides epidemiological evidence on *H. pylori* test positivity in a selected population recruited from two healthcare facilities in Angola. Seropositivity was 37.8%, whereas stool antigen positivity was 30.8%. The two diagnostic approaches identified broadly similar epidemiological patterns, including associations with age, place of residence, healthcare facility, gastrointestinal symptoms, eating outside the home, and water-treatment practices. However, these findings should be interpreted in light of the different biological indicators assessed by each method and the absence of an independent reference standard. The study was a secondary sub-investigation of a broader research project, and its analytical sample comprised 172 eligible participants, without an a priori sample size calculation specifically for *H. pylori*. The convenience-based recruitment framework, inclusion of participants from only two healthcare facilities, and lack of multivariable adjustment limit the generalizability and interpretation of the observed associations. Therefore, the findings should not be regarded as a national prevalence estimate or as evidence of the diagnostic superiority of either method. Larger studies involving geographically diverse and more representative populations, with appropriate sample size calculations and independent reference standards where diagnostic accuracy is the objective, are needed to further characterize *H. pylori* epidemiology and evaluate diagnostic approaches in Angola.

Declarations and supplementary information

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Ethics statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Scientific Council of the Institute of Health Sciences of Agostinho Neto University (Approval No. 118/GD/ICISA/UAN/2021, February 25, 2021). The study was authorized by the administrations of the LAICISA Health Unit (N° 32DG/LAICISA/2025, 22.03.2025) and Saúde com S Medical Center (N°03DG/CMSS/2025, 05.08.2025).

Data availability: The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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