Evaluation of Culture and PCR for detection of *H. pylori* isolated from gastric biopsies

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Abstract-

Objective: To evaluate the reliability of the different available diagnostic tests like culture (microscopy) and PCR and to compare and understand the accuracy of these diagnostic tests for *H. pylori* detection in gastric biopsies samples. **Design:** Experimental. **Methodology:** Biopsy specimens was collected from a total of 78 suspected patients with gastrointestinal complications and who visited endoscopy section of Hayatabad Medical Complex Peshawar. The gastric biopsy samples were cultured and then subjected to microscopy and various biochemical assays. Genomic DNA was extracted from urease positive samples and PCR was performed for the amplification of species specific 16SrRNA gene of *H. pylori*. Kappa coefficient and McNemar's test was applied for the evaluation of the agreement between the results of both assays. **Result:** Infection with *H. pylori* was confirmed by both assays i.e., culture (microscopy) and PCR in 10 (13%) infected individuals. In a total of 10 infected individuals, 10 patients were confirmed by PCR and 3 patients by culture (microscopy). The agreement between two test results was 70.5% and disagreed by 29.5% considering the p value > 0.05 as significant. **Conclusion:** PCR assay was found to be more sensitive than culture (microscopy) for the detection of *H. pylori* infection. However, due to unavailability of the gold standard assay for *H. pylori* detection, combination of both assays were recommended to boost the diagnostic accuracy.

Index Terms- *H. pylori*; Culture; PCR; Endoscopy; Biopsy

I. Introduction

H. pylori, previously known as Campylobacter pylori, is a Gram negative, flagellated, microaerophilic spiral bacterium (1). It is an ancient microorganism dating back to 10,0000 years (2) with geographical variations believed to have coexisted with humans (3). Globally, the bacterium is reported to infect 50% of the population with relatively higher prevalence of 80% in developing countries. In addition to geographical variations, differences in the incidence have been observed in age, gender, and ethnic groups (4). Despite high prevalence of *H. pylori*, only small proportion of the population develops symptomatic gastrointestinal diseases (5). Only 3% of *H. pylori* infected patients will develop gastric cancer with 3-6-fold increase as compared to non-infected patients (6). *H. pylori* infection, if untreated can persist for lifetime either asymptomatically or would cause chronic diseases (7). Therefore, timely and accurate diagnosis and treatment is deemed important. Currently, many invasive and non-invasive diagnostic tests are in use for detection of *H. pylori* infection. The non-invasive tests employed for detection are urea breath test (UBT), pathogen specific antigen in stool and serological tests (8). The invasive techniques are those requiring endoscopic examination and biopsy specimen such as culture test, histological examination, and PCR (9). But the most reliable test is the one with high specificity and high sensitivity subject to accessibility and viability of cases (10).

In resource-limited settings, the molecular technique such as PCR has proven to be successful test assisting traditional techniques like culture (microscopy) and rapid urease testing for *H. pylori* infection diagnosis and treatment. However, due to lack of gold standard test for the rapid diagnosis of the *H. pylori*, the current study is designed to detect *H. pylori* obtained from upper gastro-intestinal endoscopic biopsy specimens using cultural (microscopic), biochemical, and molecular techniques in order to evaluate the accuracy of *H. pylori* detection methods in patients.

II. Methodology

2.1 Study setup, Ethical approval, and Informed consent

This research study was conducted at the Department of Microbiology, Abdul Wali Khan University, Mardan. The Institutional Ethical Committee of HMCP has evaluated all the protocols and procedures and approved the study (reference number: 370/HEC/B&PSC/2020). Written informed consent was taken from the enrolled subjects.

2.2 Inclusion and exclusion criteria

Biopsy samples were obtained from patients with vomiting, abdominal pain, and nausea, while HCV, HBV, HIV-positive patients, and patients who have taken immunosuppressive drugs were excluded from the study.

2.3 Study Subjects and Data Collection

A total of 78 patients were included in the study and data was collected through designed questionnaire.

2.4 Culturing and identification

A 4mm of fresh biopsies samples collected from the antrum of the stomach, were crushed in to pieces and seeded on Columbia blood agar base (Oxoid, Hampshire, UK), supplemented with 5% sheep blood, DENT antibiotic supplements (Oxoid, Hampshire, UK) and by using CampyGen sachet (Thermo Fisher Scientific, Waltham, USA), the cultured plates were observed for bacterial growth after 72 hours. After Phenotypic and morphological identification, bacterial culture was subjected to different biochemical tests like oxidase, catalase, and urease for the identification.

2.5 Molecular identification of *H. pylori*

Urease positive samples were subjected to genomic DNA extraction by using Gene Jet Genomic DNA purification kit (Thermofisher scientific, USA) and by using the oligonucleotide primers F (5′- GCGACCTGCTGGAACATTAC-3′) R (5′- CGTTAGCTGCATTACTGGAGA- 3′), PCR was performed for the amplification of the species specific 16S rRNA gene.

2.6 Data Analysis

Data from the questionnaires and results from experiments were entered in Microsoft Excel 365 and analyzed with R package (version 1.4.1). Descriptive statistics was applied on socio-demographic data and chi-square was used for association of categorical data. Disagreement between the two detection methods was evaluated by McNemar's test.

III. Results

3.1 Demographs of the study

The biopsy tissue samples obtained from 78 infected individuals were processed for culturing. Median age of the subjects were recorded to be 44 years. Out of 78 enrolled subjects, 41 (52.6%) were male and 37 (47.4%) were females and majority of the subjects (73.1%) uses tube well as source of drinking water and 84.6% were non-smokers. 50% of the subjects have never been to school followed by 24.4% of matriculate subjects (Table 1).

Table 1: Demographic details of *H. pylori* infected individuals

Factors		Sample Distribution (*n* %)
Age	**[Median (IQR)]**	44 (39)
Gender	Female	37 (47.4)
	Male	41 (52.6)
Education	Graduation	7 (9)
	Intermediate	13 (16.6)
	Matric	19 (24.4)
	Nil	39 (50)
Smoking	Yes	12 (15.4)
	No	66 (84.6)
Source of water	Tube well	57 (73.1)
	Tank water	21 (27)
Family History	Yes	8 (10.2)

	No	70 (89.8)
PPI Intake	Yes	36 (46.2)
	No	42 (53.8)
Antibiotic Intake	Yes	22 (28.2)
	No	56 (71.8)
Endoscopic Findings		
Culture	Negative	45 (57.7)
	Positive	33 (42.3)
PCR	Negative	68 (87.2)
	Positive	10 (12.8)

3.2 Culturing and Molecular identification

Among the endoscopically isolated 78 samples, 33 (42.3%) were culture positive, 29 (37.1%) samples showed spiral or curved shaped morphology, 25 (32.1%) samples exhibited positive urease activity, while 10 (12.8%) samples were found positive by PCR (Table 1; Figure 1).

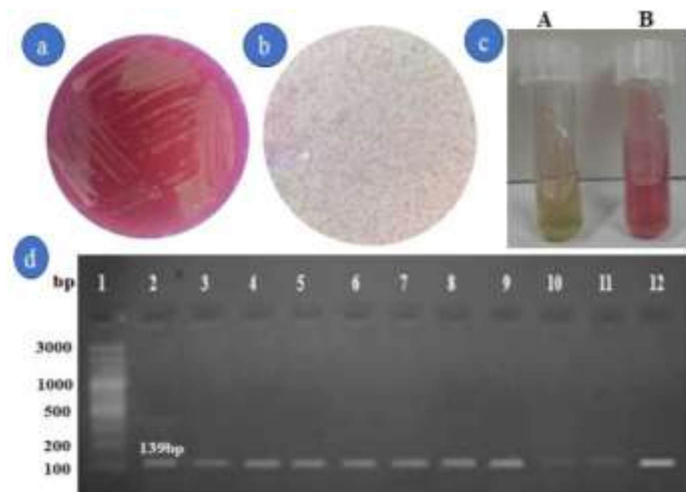

Figure 1:Identification of *H. pylori*: a) Identification of *H. pylori* isolates by Culture; small, non-hemolytic, translucent colonies on Columbia agar plate, were observed. **b)** Gram stain of a single isolated colony of *H. pylori* showed curved rods at 100X microscopy. **c)** Urease assay; The yellow color signals for negative result whereas, positive reaction is indicated by bright pink color. A: negative urease test ; B representing urease positive assay. **d)** Molecular Identification of *H. pylori*; The band at 139bp showed species specific 16SrRNA gene. Lane 1: Marker 100bp; Lane 2: *H. pylori* positive control; Lane 3-12 represents amplifications of the samples at 139bp.

Comparing the PPI intake in culture and PCR positive patients, 10 (27.8%) of the samples were found positive by culture and 4 (11.1%) of the samples were identified by PCR. The culture was found to be more significant ($p < 0.016$) as compared to PCR ($p < 0.676$). Similarly, both in culture positive and PCR positive cases, only 4.54% with positive history of antibiotic intake were detected (Table 2).

Table 2: Culture and PCR results of the patients using PPI and Antibiotics

History of PPI Intake	Culture Positive	PCR Positive
Yes ($n = 36$)	10 (27.8)	4 (11.1)
No ($n = 42$)	23 (54.8)	6 (14.3)
p- value	0.016	0.676
History of Antibiotic Intake		
Yes ($n = 22$)	1 (4.54)	1 (4.54)
No ($n = 56$)	2 (3.6)	9 (16.1)
p- value	0.840	0.171

Endoscopic findings revealed 46.2% of the positive patients have gastritis while 34.6% have ulcer and 19.2% have gastric cancer (Table 3).

Table 3: Comparison of Endoscopic Findings and positive *H. pylori* tests

Endoscopic Findings ($n = 78$)	Culture		PCR	
	Negative	Positive	Negative	Positive
Gastritis ($n = 36$)	26	10	33	3
Cancer ($n = 15$)	9	6	13	2
Ulcer ($n = 27$)	10	17	22	5
p – value	0.019		0.487	
Total	45	33	68	10

3.3 Comparative Analysis of Culture and PCR

Comparing the detection methods, only 3 cultured samples were identified by microscopy, which were also confirmed by PCR. All the 10 positive samples were identified directly by PCR. Comparing the diagnostic precision of the cultured samples by microscopy and PCR using McNemar's test and kappa test resulted in a disagreement of 29.5% between both tests and a significant value of 0.001 in Kappa test and 0.016 in McNemar test confirmed a significant difference in the diagnostic efficacy of both tests (Table 4).

Table 4: Comparison of Culture and PCR test

Culture	PCR	Frequency	Agreement	Disagreement	Chi Square	McNemar Test
Positive	Positive	10	70.5%	29.5%	15.642 (0.000)	.000
Positive	Negative	23				
Negative	Positive	0				
Negative	Negative	45				

IV. Discussion

The current study pursued the comparative analysis of the diagnostic ability of *H. pylori* using culture (microscopy) and PCR assays. Polymerase chain reaction (PCR) is a cost-effective molecular diagnostic test and has higher efficacy as compared to traditional techniques. In the current study, only 33 (42.3%) positive cases have been detected by culture subjected to microscopy indicating its low precision. Similar studies conducted by Al-Saad et al., in 2020 on ulcerative patients reported a detection rate of 35% by culture (11) while Hossein et al., in 2021 reported a detection rate of 47.8% of *H. pylori* by culture assay (10), and Nga etal., in 2023 reported a prevalence of 21.8% by culture assay (12), however; a relatively lower detection rate of only 6% by culture was also reported by Bhandari et al., in 2022 (13). The reason underlying the contrasting rate might be the handling techniques, staining procedures, and sample population.

For the confirmation and detection of *H. pylori* at molecular level, the current study amplified the of species specific 16SrRNA gene and confirmed the presence of *H. pylori* infection in 10 (22.2%) of the enrolled study subjects. Different studies while utilizing different PCR techniques like duplex PCR, and by the amplification of different markers like BabA, CagA, UreA and UreC reported different detection rates. The detection rate of *H. pylori* by PCR in the current study is much lower (13%) as compared to previously reported rate of 71.3% using the same species specific 16SrRNA gene along with BabA with duplex PCR strategy (14). By amplifying different markers like CagA, VacA and iceA for detection of *H. pylori*, different studies reported different detection rates of 50% (15), 33.9% and 35.5% respectively (16). This low detection rate in the current study by both assays is attributed to staining procedure, sample population and target gene selection. Genetic flexibility of the strain makes it difficult to select perfect target gene for molecular detection.

Out of 78 biopsy samples obtained, despite the consumption of proton pump inhibitors (PPI), 10 (27.8%) of the samples were Culture positive, while 4 (11.1%) of the samples were found PCR positive, indicating that the culture and PCR might not be affected by consumption of PPI in some infected individuals. Comparing the accuracy of the culture and PCR assays in patients with previous history of using proton pump inhibitors (PPI), the culture assay was found to be more significantly correlated ($p < 0.016$) as compared to PCR assay ($p < 0.676$). This led to the indication that PPI consumption has negative impact on *H. pylori* culture assay. The findings of the current study in-spite of the low enrolled subjects, was (culture positive in PPI users and statistical significance) in-line with the previously reported findings of the Cheung et al., (17) reporting the correlation of PPI with gastric cancer in *H. pylori* infected individuals. In case of Antibiotics intake by the infected individuals, 1 (4.54%) of the samples were found to be positive by both the assays (culture & PCR). There was no statistical significance was observed in patients with previous history of antibiotics intake. A 1.75% of recurrence rate of *H. pylori* after one year was reported by Yan Xue et al., (18) which is almost similar to findings of the current study.

In comparison of the different disease conditions caused by *H. pylori,* 10 (27.7%) samples were culture positive whereas 3 (8.3%) samples were PCR positive for gastritis. In case of gastric cancer condition, 6 (40%) of the samples were found to be culture positive, whereas 2 (13.3%) of the samples were detected positive by PCR. About 17 (62.9%) of the samples were culture and 5 (18.5%) of the samples were found positive in ulcer condition. A strong statistical correlation was observed among the three disease conditions caused by *H. pylori* by culture assay, whereas no statistical significance was observed for PCR assay. No correlation between the disease conditions by PCR assay might be attributed to handling techniques, primers optimization strategies and gene selection.

Comparing the homogeneity of culture (microscopy) and PCR, a total of 3 infected patients were confirmed by culture and 10 patients were confirmed by PCR (13%). A recent study reported a diagnostic sensitivity of 53.3% by culture and 95.9 % by PCR (19). Only 2 infected individuals were confirmed by both the assays. The agreement between the result of both assays were found to be 70.5% and disagreed by 29.5% considering the p value > 0.05 as significant. The results confirmed a difference in the accuracy of both tests with higher diagnostic ability of PCR compared to culture (microscopy). There is no gold standard test for the detection of *H. pylori*, although the efficacy of PCR test has proven better as compared to culture. A better input in the diagnosis of *H. pylori* can be brought by comparing staining procedures and target genes for PCR.

Limitations of the study

This study has employed a small sample size and only single PCR assay and culture procedure has been used. Large population based comparative study and multiple PCR strategies would be better for detection of infection.

Conclusion

PCR and culture have different detection efficacy with PCR being more sensitive assay compared to culture. Molecular detection of *H. pylori* has been proven as a better diagnostic method than traditional culture or staining based methods.

Conflict of interest

The authors declare no conflict of interest.

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Author's Contribution

SARS.: conceptualization, designing, investigation and writing original draft. **MA.:** Validation and writing. **SS.:** formal analysis, data curation and writing. **AT.:** validation, review, and editing. **FM.:** data curation, critical review, and editing. All authors have read and agreed to the published version of the manuscript.

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