Original Article

Detection of Helicobacter Pylori from Stomach Biopsies: A Rapid Culture Method

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ABSTRACT

Objective: To identify a rapid culture method capable of isolating and detecting H. pylori from stomach biopsies.
Study Design: Descriptive / cross-sectional study
Place and Duration of Study: This study was conducted at the Omdurman Islamic University, Sudan from 1st November 2021 to 30th August 2022.
Materials and Methods: The stomach biopsy specimens from 40 patients were collected and centrifugation was done in phosphate buffer saline at 3000 rpm for two minutes. The culture was performed on Modified Brain Heart Urea Agar (MBHUA) media by placing two to three drops of sediment. The cultures were incubated at 37°C in microaerophilic conditions using a candle jar for up to 5 days and observed for growth and change in the color of the medium daily.
Results: Modified Brain Heart Urea Agar media allowed for rapid detection of urease activity in H. pylori within 24 hours after its addition. The bacterial growth was observed three days following the incubation of cultured plates under microaerophilic conditions provided by a candle jar. These results develop rapid detection of H. pylori with clear growth.
Conclusion: H. pylori can be detected more rapidly in modified brain Heart urea agar than the other H. pylori growth media.
Key Words: Helicobacter pylori, Modified Brain Heart Urea Agar, Culture technique

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INTRODUCTION

It has been determined that Helicobacter pylori (H. pylori) are frequently responsible for gastrointestinal infections worldwide. This bacterium is a spiral, Gram-negative rod that contributes to the pathogenesis of several disorders.[1] Most infections are acquired during childhood and remain with the individual for the rest of their lives. Within the protective mucus layer attached to the epithelium, H. pylori may live as a planktonic population. There is a patchy distribution of H. pylori across the entire stomach surface with a substantial spread over the whole area.[2] The mucosal lymphoid tissue lymphoma and gastric adenocarcinoma are caused by these bacteria and have been linked to gastritis and gastroduodenal ulcers in the stomach leading to inflammation in the stomach.[3]

H. pylori transmission appears to be most commonly transmitted from one person to another via oral-oral contact. Therefore, it can be explained why the infection is so prevalent in closely associated family members, like mother, father, and offspring, as they share a common environment. Water contamination, mainly from unsatisfactory sanitation conditions, plays a major role in fecal-oral transmission.[4] WHO classified this bacterium as a group I carcinogen in 1994 for its association with duodenal ulcers.[5] When an acute

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infection occurs, nausea, vomiting, and fever may accompany the upper gastrointestinal illness. Symptoms of H. pylori may resolve in less than seven to fourteen days; however, they may persist for years, perhaps decades, or even a lifetime after colonization.[6] This bacterial infection can also develop other pathological conditions such as cardiovascular, neurologic problems, metabolic disorders, autoimmunity, and skin diseases. Some dermatological diseases are associated with H. pylori, but it is unclear whether the bacterium is a trigger or the underlying cause.[7-9]

The infection of H. pylori in the gastrointestinal mucosa is directly linked to mucosal inflammation due to neutrophil infiltration into the mucosa and monocyte infiltration. Several diagnostic methods have been developed since H. pylori were discovered to diagnose this infection. H. pylori can be detected both invasively and non-invasively and can be treated in a number of ways.[9-11] Due to its unique characteristics, culture continues to depict an important part in a wide-ranging evaluation of pathogen characteristics and antibiotic resistance in the diagnostic spectrum.[12] To eradicate this bacterium, a triple therapy regimen has been widely used, including a proton pump inhibitor and two antibacterial drugs, such as amoxicillin, clarithromycin, and metronidazole. The latest therapeutic regimens, as well as probiotics, have been implemented to enhance the efficacy of the treatment.[13]

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted from October 2021 to September 2022 at the Omdurman Islamic University, Sudan. The faculty of Medical Laboratory Science, Omdurman Islamic University Ethical Committee, approved the study and its procedures. Informed consent was obtained from the participants. The biopsy specimens were collected from Military Teaching Hospital, Almotatoir Center, and Fedail Hospital in Khartoum, Sudan. As part of the present study, 40 patients with endoscopic gastric biopsies were included; samples were taken from each patient who underwent endoscopic gastric biopsies. Stomach biopsy specimens were collected and transported in a plain container containing 2 ml of normal saline or phosphate buffer saline and transported at 4°C.

Modified Brain heart Urea Agar (MBHUA) was prepared by adding 52 gm of brain heart agar (Hi media - India), 20 gm of urea base (Hi media - India), and 0.0012 gm of phenol red as an indicator (Hi media - India) to 740 ml of distilled water and sterilized by autoclaving at 121ºC for 15minutes, then cooled to 50-55ºC. 10 ml of antibiotic solution was added (1 mg of vancomycin, 5 mg of trimethoprim, and 5mg of amphotericin B in 10 ml sterile distilled water), and 3gm of urea crystal (Hi media - India) was added and then mixed thoroughly and poured into sterile disposable Petri dishes (Fig. 1).

In addition to 52 g brain heart agar (Hi Media - India), add 940ml distilled water and autoclave the media at 121ºC for 15 minutes, cool to 50-55ºC, aseptically add 50ml of horse blood and 10ml of the antibiotic solution, mix thoroughly, and place in disposable sterile Petri dishes. In normal saline or phosphate buffer saline, 40 stomach biopsy specimens were centrifuged for two minutes at 3000 rpm. We discarded the supernatant from the tubes, and a few drops of sediment were cultured using sterile wire loops under septic conditions. The cultures on MBHUA mediawere incubated at 37°C in microaerophilic conditions using a candle jar for up to 5 days and observed for growth. We observed media daily to see if there was any color change. Colonies showed a change in the color of the medium to pink (indicating a rise in the pH of the medium due to urease production with subsequent breakdown of urea into ammonia and carbon dioxide) and were subcultured on Modified Brain Heart Urea Agar. The isolated H. pylori colonies were used to perform the Gram staining and confirmed biochemically using oxidase, catalase, and urease tests.

Genomic DNA was extracted manually from endoscopic biopsies using Guanidine hydrochloride protocols following the collection of the samples with 2 ml of phosphate-buffered saline (PBS).[14] A high-speed centrifuge was used to spin the samples at 3000 rpm for 10 minutes, and the pallet was collected and transferred to a falcon tube. Afterward, 2ml of lysis buffer, 1ml of guanidine hydrochloride, 300 ml of NH_4 acetate, and 10 ml of proteinase K were added to the pallet. Incubation at 65°C for two hours was followed by cooling at room temperature before adding 2ml of pre-chilled chloroform. A centrifuge was then run at 6000 rpm for 5 minutes to centrifuge the samples. We added 2 ml of absolute cold ethanol to the upper layer of samples; they were then stored overnight at -20°C. After washing the pellet with 2 ml of 70% ethanol, it was centrifugedat 6000 rpm for 10 minutes. The pallet was washed with 2ml of 70% ethanol and then centrifuged for 10minutes at 6000 rpm. The supernatant was poured off, and the pallet was allowed to dry and then dissolved in 50µl by adding H_2O.[14,15].Samples were amplified using a PCR machine (classic K960, China). PCR for the ure A gene of H. pylori was performed using a Maxime PCR PreMix kit (iNtRON, Biotechnology, Korea) and primers ureA_197F (5′AACCGGATGATGTGATGGAT3′) and ureA_413R (5′GGTCTGTCGCCAACATTTTT-3′).

The amplification and detection of genes were performed as described in an earlier study.[16]

RESULTS

We found that four out of 40 patients (10%) were culture-positive for H.pylori. Bacterial contamination of

the Modified Brain heart Urea Agar (MBHUA) was not observed because of the addition of the antibiotic to the medium. H. pylori grew slowly on classic brain heart agar for five to seven days (mean was six days, and the standard deviation was 0.82).Moreover, the colonies appear as very small translucent, tiny colonies.

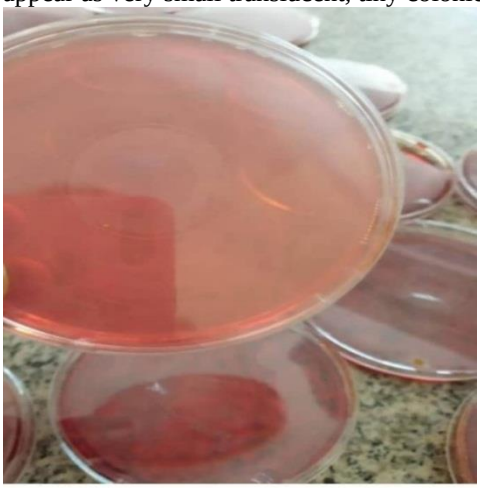

Figure No. 1: Sterile plate of modified brain heart urea agar

H. pylori urease activity was detectable within 24 hours of incubation of MBHUA. Bacterial growth was detected after three days of incubation under microaerophilic conditions provided by a candle jar. H. pylori was detected in the tissue specimens due to the change in color of the media plates caused by a positive urease action. The plates that show no growth after seven days of incubation under a microaerophilic atmosphere also show no color change, indicating that the indicator was not affected by the incubation condition. Compared with the colonies on the classic brain heart agar, the isolated bacterial growth was prominent; however, slow fungal contamination was also observed. In addition, the colonies appear as small translucent, shiny pink colonies **(**Fig. 2a). H. pylori isolates were observed as Gram-negative rods with spiral shapes **(**Fig. 2b). Three further tests, oxidase, catalase, and urease, were also positive to ensure the isolate's identity.

Four (10%) out of 40 patients were found to be positive for H.pylori (ure A gene) by using PCR; the four positive samples for ure A gene detected by PCR are the same samples that give visible colonies in Modified Brain Heart Urea Agar (Fig. 3).

a **b**

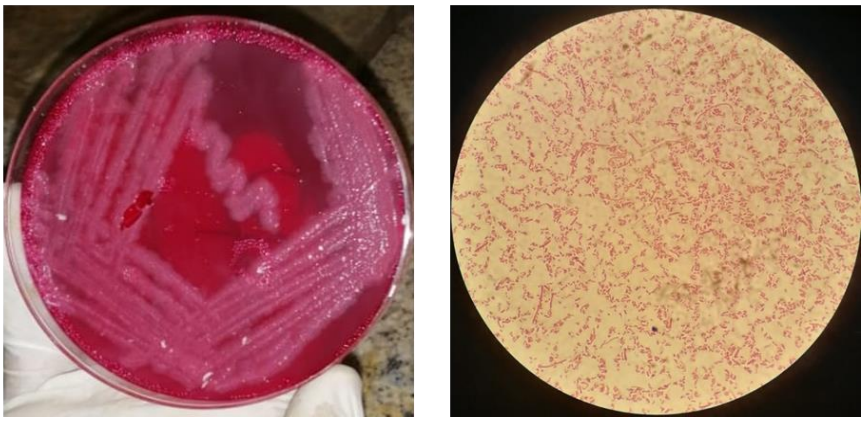

Fig. 2: **a) Colonies of H. pylori showing positive urease activity on Modified Brain (b) Gram stain of H. pylori showing curved gram-negative bacteria**

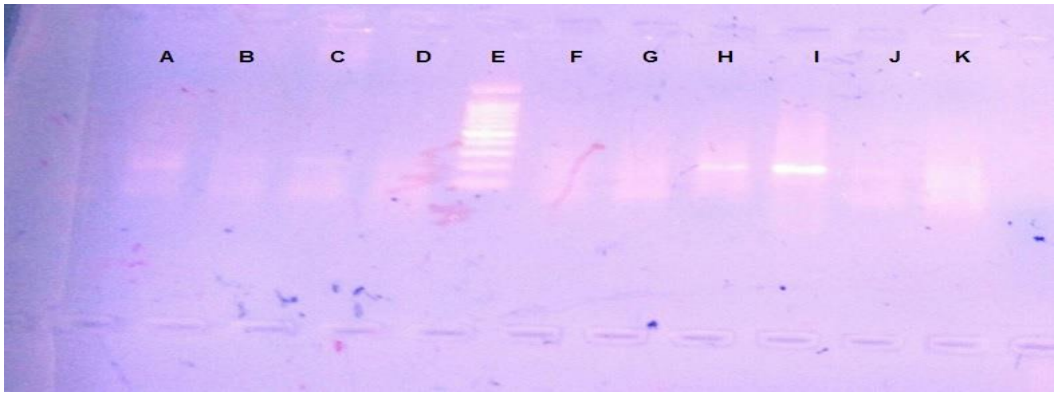

Fig. 3: Lane E DNA Ladder was 100 bp, lanes B, D, F, G, J, and K negative H. pylori samples, lanes A, C, H, and I positive H. pylori samples showed 217 bp fragment

DISCUSSION

A variety of diagnostic methods have been proposed to detect H. pylori.[17] Many factors influence the choice, including the availability of materials, the sampling population, the condition of patients, and the investigator's ability or experience. We chose the culture technique based on the availability and ability of isolates to be used in antibiotic susceptibility testing and bacterial studies. We detected H. pylori in the specimens that showed visible growth, but not in the other specimens, using PCR, which is highly sensitive and has been reported by many authors to be very effective.[18] Cultures of most bacteria and microorganisms in clinical practice are the gold standard for diagnosing infectious diseases. In bacteriology, H. pylori culture from stomach biopsies is a gold standard technique fundamental to AMR testing. Moreover, culture plays a vital role in identifying new drugs, developing vaccines, and analyzing putative virulence factors. Instead, the problem lies in H. pylori growth, which is time-consuming and unavailable in every diagnostic laboratory.[12]

It is challenging to isolate H. pylori from biopsy specimens; culture success rates are typically 70% to 80%, with 90% to 95% specificity and 90% to 95% sensitivity. It is difficult to culture the organism due to several factors, some of which are hard to control: patchily distributed on the gastric mucosa, biopsy forceps are contaminated, oropharyngeal flora are present, and the organism loses its viability during transportation. All of these factors may contribute to the poor negative predictive value associated with H. pylori cultures. H. pylori is often isolated using a combination of selective and nonselective media. However, the most effective method of recovery has yet to be determined. As a non-selective medium, brain heart agar has been used for many years to cultivate H. pylori from antral biopsy specimens acquired during upper gastrointestinal endoscopy from patients suffering from peptic ulcers.[19-21]

Using this medium alone for H. pylori isolation can be very variable since the colonies are small and few. During the acquisition of samples, the medium frequently became contaminated with bacteria from contaminated biopsy forceps. It took 5 to 7 days for colonies of H. pylori to appear on this medium, showing a slow growth rate. Efforts have been invested; scientists supplement the agar plates with 5 to 7% horse blood to improve the reliability of brain heart agar.[20] The molecular detection of the samples gave the same result asa culture on modified brain heart urea agar, giving more value to the selectivity and specificity of the modified media.

The frequency of H. pylori growth among gender is equal. We found no relationship between age, gender, or culture in our study; this is consistent with a study done by Uszczynska et al[22] in Poland, Akbar and Eltahawy[23] in Saudi Arabia and Petrovic et al[24] in Serbia. Moreover, in a Saudi Arabian study on upper gastrointestinal symptoms, the prevalence of H. pylori was assessed through histology, rapid urease, and enzyme immunoassay. Study results showed that H. pylori infection and age were significantly correlated.[25] Several studies have described the mechanisms underlying AMR in bacterial strains originating from diverse sources.[26-29] Optimizing reliable culture techniques and microscopyare crucial to understanding antimicrobial resistance mechanisms.[30,31] It is equally important to implement reliable cultural techniques to detect H. pylori efficiently, just as they are used to detect other bacteria. Several antibiotics have been studied in the AMR of H. pylori, including metronidazole, tetracycline, clarithromycin, amoxicillin, furazolidone, ciprofloxacin, and rifampin.[32] There is a possibility that AMR is one of the significant reasons for the failure to eradicate these infections. There is a significant need for continuous prospective surveillance of H. pylori infections. Culture and AMR tests play a pivotal role in cases that have failed to respond to the first attempt at therapy.[33] This may be due to the use of more than one test for the detection of H. pylori, which is more sensitive than culture, increasing the chance of detection of the organism. The study has some limitations in samples from a different body side that may contain H. pylori and need to be cultured in MBHUA media

CONCLUSION

By accurately diagnosing H. pylori infections, the diseases can be managed more effectively. Our study concludes that H. pylori can be detected more rapidly in modified brain Heart urea agar than the other H. pylori growth media. In order to establish a method for recovering H. pylori from antral biopsy specimens in patients with peptic ulcers, we modified the brain heart agar medium to make it selective and differential for H. pylori culturing. When H. pylori-specific primers are used to target at least two conserved genes, PCR can be considered the best choice for diagnosis.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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