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An intermittent culturing protocol for *Helicobacter pylori* from clinical biopsies

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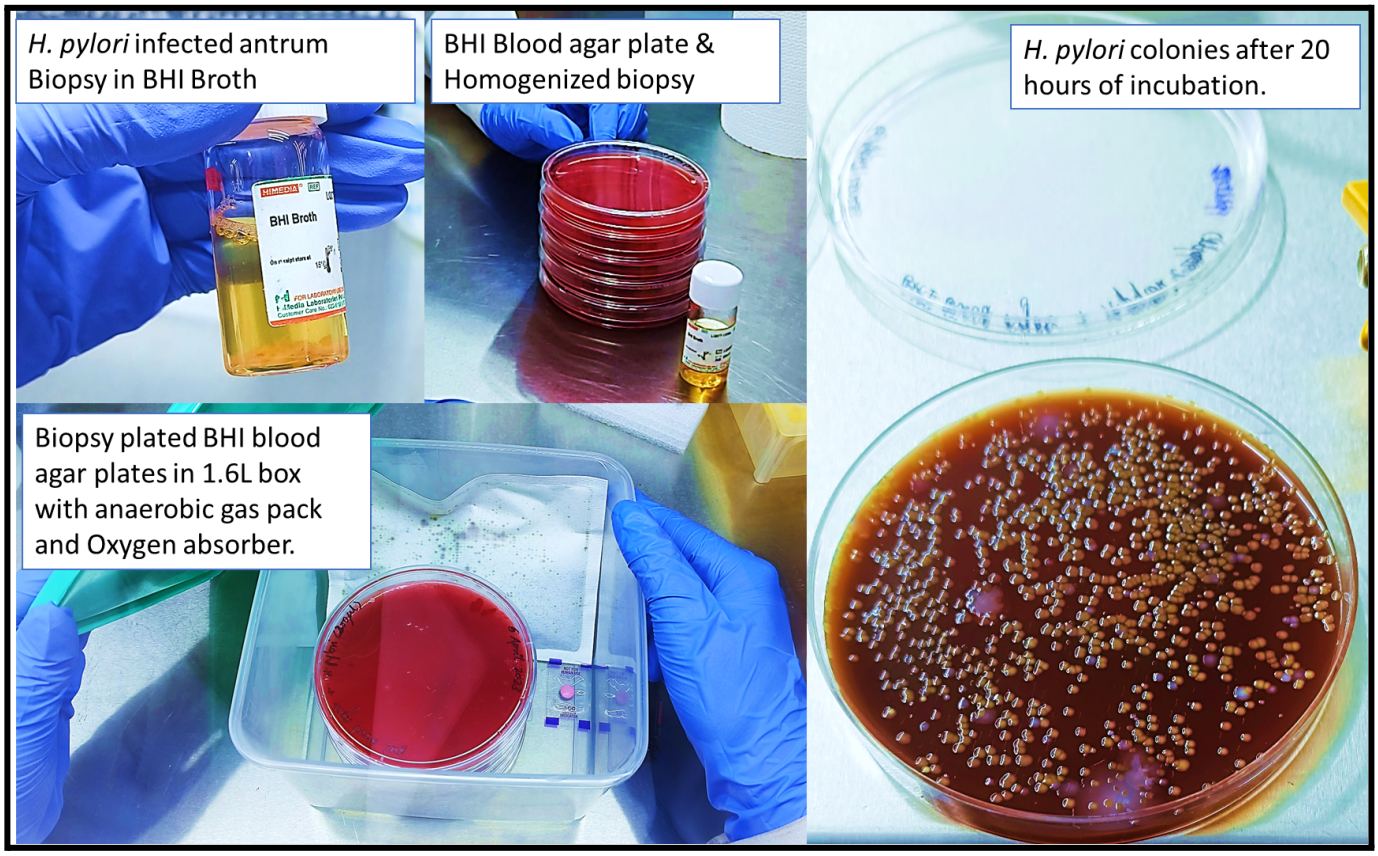

Figure 1. Obtaining *H. pylori* colonies from the antrum biopsy in the BHI blood agar plates. Biopsies were directly collected into the BHI broth (top left panel) and were plated on the BHI Blood agar plates (top middle panel). Plates were incubated in microaerobic conditions using a gas pack (bottom left panel) for 20 hours to obtain the bacterial colonies (right panel).

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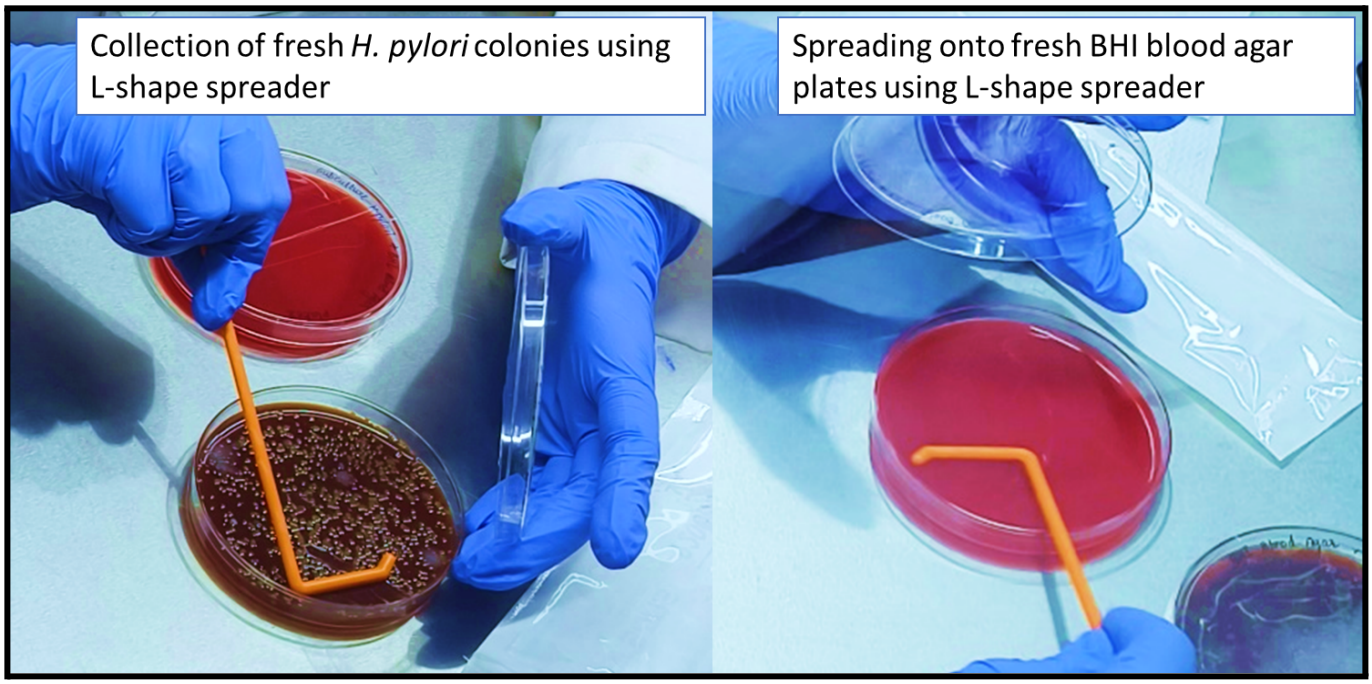

Figure 2: Subculturing procedure of *H. pylori* colonies by spread plate method. Plate full of colonies (left panel) was used to harvest the colonies using a sterile L-shaped disposable spreader and the cells were spread onto the new plate (right panel).

Gastritis is the most common problem all over the world. Especially in countries like India where people ingest a lot of spicy food, gastritis is more frequently observed. In addition to these complications, effects of *Helicobacter pylori* like severe gastritis, gastric ulcers which later on develop as gastric cancer is observed to increase as the years pass by. Nearly 80% of gastric cancers are *H. pylori* related cancers and there is no specific treatment available to *H. pylori* related infections. Though there are some general antibiotics like amoxicillin, tetracycline, ciprofloxacin, Clarithromycin and proton-pump inhibitors were given and they could only control the infections to some extent. To test any drugs or to develop any treatments towards the *H. pylori* the biggest barrier is to culture them. This communication discusses the protocol of how to culture the *H. pylori* in 20 hours of time with the highest efficient colonies for the subcultures from the clinical samples.

H*elicobacter pylori* is a gram negative bacteria which is able to survive in the acidic conditions of the stomach by releasing the urease enzyme to survive and grow in the epithelial cells of gastric walls. Severe gastritis, gastric ulcers, peptic ulcers & gastric cancers are the manifestations that the *H. pylori* can cause. Gastritis is one of the general problems observed world-wide where 6.3 out of every 1,00,000 people were suffering from this problem as of 2020 out of which the range is differing between 2 to 57 in 1,00,000 people for the cases of gastric cancers (1-5). The clinical tests to identify the infection of ulcers and gastritis related to *H. pylori* are Rapid Urease Tests (RUT), ^{13}C Urea Breath Test (^{13}C UBT) (6). The treatments of *H. pylori* are Proton-pump Inhibitors and the general other antibiotics like Clarithromycin, amoxicillin etc., (2,6). Culturing the *H. pylori* is observed to be a task in the general laboratory methods and requires a lot of keen observations, sterility maintenance as there are theories stating that getting the colonies of *H. pylori* takes nearly 72 hours of time or it is generally stated as 3-7 days at 37℃ in the CO_2 incubator as they require microaerophilic conditions for proper growth (6-8). Other requirements besides CO_2 incubator are the maintenance of 2%-5% O_2, 0%-10% of H_2 and also some hydration (2, 9-13). The complications in the culturing of the *H. pylori* is observed when the bacteria is stored even for a day. The efficiency of the *H. pylori* is observed to be high when the biopsy of the infected area is plated in less than three hours in the procedure that we followed at TCABS-E Laboratory. The biopsy of the *H. pylori* infected antrum is directly collected into the Brain-Heart infusion (BHI) broth (purchased from HiMedia Laboratories) and stored on the ice. This stored biopsy is then further homogenized within 30 minutes after collecting the biopsy sample. The homogenization was performed using the basic laboratory

procedures by using the forceps. The forceps were dipped into the 70% isopropanol and placed inside the laminar air flow chamber under UV light for 20 minutes. The sterile forceps were used to chop the pieces of infected antrum biopsy into further more fine pieces. The mixture of the broth and the fine pieces of the biopsy were briefly vortexed carefully for 1 minute. Two hundred µl of the homogenized sample is spread on the BHI Blood agar plates (purchased from HiMedia Laboratories) and is stored in a box of 1.6 L capacity with the anaerobic gas pack (purchased from HiMedia Laboratories) and an oxygen detector tablet (purchased from HiMedia Laboratories) in it. The box containing plates is placed in the incubator at 37ºC for 20 hours.

There were silver-gold coloured colonies observed of diameter ~2 mm each (Figure 1). These colonies were then carefully collected using a sterile L-shaped spreader and are spreaded on to the fresh BHI blood agar plates for subculturing and the same procedure of placing in the box 1.6L capacity box with the microaerophilic conditions at 37ºC for 20 hours is followed (Figure 2). Bacterial colonies from both the original biopsy spread plates and the sub cultured plates will further be used for their 16S rRNA sequencing to identify and confirm if there is any species level diversity in the obtained patient biopsy samples. With this established protocol, we are going to further evaluate our newly designed small molecule compound, HelicoTAC© (15) that was previously patented (202141058294).

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Conflict of interest: The authors declare no conflict of interest in this study. However, this research article is an ongoing project currently at TCABS-E, Visakhapatnam, India.

Author contributions: M.A. performed all the work under the supervision of R.S.Y., the principal investigator who designed the project, trained M.A. in experiments, secured required material for the project, provided laboratory space, facilities needed and wrote/edited the manuscript.

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