African Helicobacter & Microbiota Study Group

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Culturing of *Helicobacter pylori*

Presented

By

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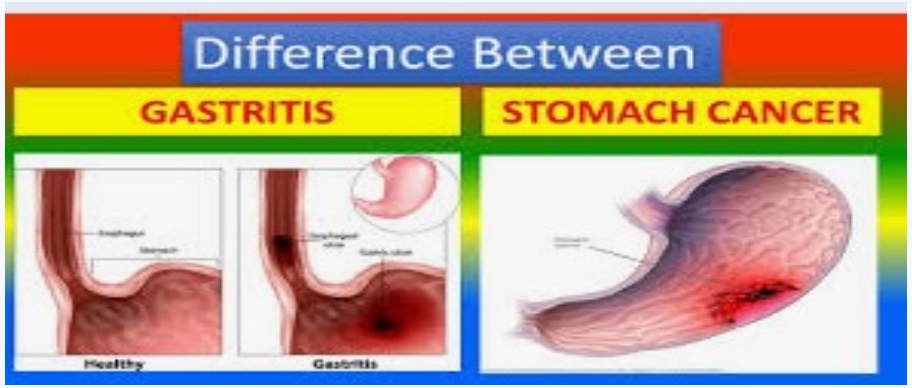
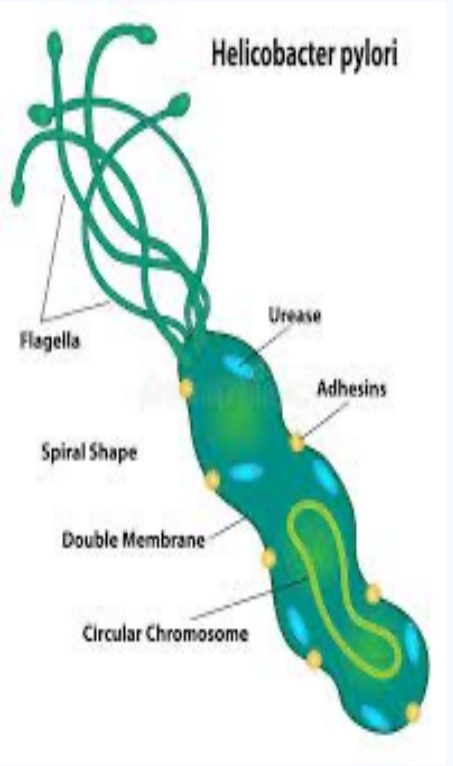
Introduction

Helicobacter pylori is a flagellated, microaerophilic, gram-negative, spiral-shaped, fastidious bacterium associated with gastritis, peptic ulcers, and gastric cancer (Ng *et al*., 2019).





It has also been implicated in extra-gastrointestinal diseases such as vitamin B12 deficiency, immune thrombocytopenia, Iron deficiency anaemia, irritable bowel syndrome and hyperemesis gravidarum (Youssefi *et al*., 2020).

Introduction

Culturing *Helicobacter pylori* is essential for diagnosis, antimicrobial susceptibility testing, eradication therapy monitoring, epidemiological studies, understanding antibiotic resistance, personalized treatment, research, and quality control and assurance (Zullo *et al*., 2022).

Figure 4: *H. pylori* growth and MIC test on blood agar
Source: Pokhrel, 2015

Table 1: Clinical Specimens for *H. pylori* Culture

Specimen types	Collection procedures	Transportation Guidelines	References
Gastric biopsy (antrum and corpus)	Collect during endoscopic procedure using sterile forceps and embedded into the transport medium e.g Stuart's medium or portagerm pylori transported within 2hrs but still grows at 24hr. For optimal result at least 4 biopsies should be culture	Transport at 4°C, isolation decreases at Room temperature within 2hrs	Palamides *et al.* (2020)
Stool	Collect fresh with no preservation into sterile container, transport to the lab. within 2hrs	Transport at room temperature in 2hrs	Fabricio *et al.,* (2018).
Saliva	Collect early morning saliva into sterile universal container, transport to the lab within 2hrs	Transport at room temperature in 2hrs	Tongtawe *et al.* (2011)
Blood (rarely used)	Collect venous blood in sterile anticoagulated tube containing like EDTA	Transport at room temperature in 2hrs	Andersen and Waddstrom, 2001; CLSI, (2019);

Endoscopic Procedure for Biopsy Sample

Suitable Culture Media

Portagerm pylori transport medium (Biomerieux, SA) within two hours.

Selective media are GC agar, Columbia agar, Muller Hinton agar, Trypticase soy agar, Casman agar, Brain-heart infusion agar , Brucella agar, Skirrow's medium, Dents medium, (Andersen and Waddstrom, 2001; Abadi *et al.*, 2018).

H. pylori can grow on solid media containing blood or serum about 7-10%

Figure 5: Portagerm pylori transport mediu
Source: *https://www.biomerieux. com*

Figure 6: GC Agar base
Source: *https://www.oxoid.com*

Suitable Culture Media

Non-selective media: Blood agar and Chocolate agar (Marshall and Gilman, 2011).

- The biopsies can be cultured on GC agar plates (Oxoid CM0367) containing Dent antibiotic selective supplement (Dent, SR0147E Oxoid, Basingstoke, United Kingdom) at pH 6.8 -7.0 (Oxoid, 2024).

Figure 7: Dent medium and horse serum
Source: *https://www.oxoid. com*

- Vitamin mix (1 % Isovitale-X) enrichment provides V factor (nicotinamide adenine dinucleotide, NAD), vitamins, amino acids, coenzymes, dextrose, ferric ions and other factors which improve the growth of pathogenic and horse serum (SR0035) (7 %).

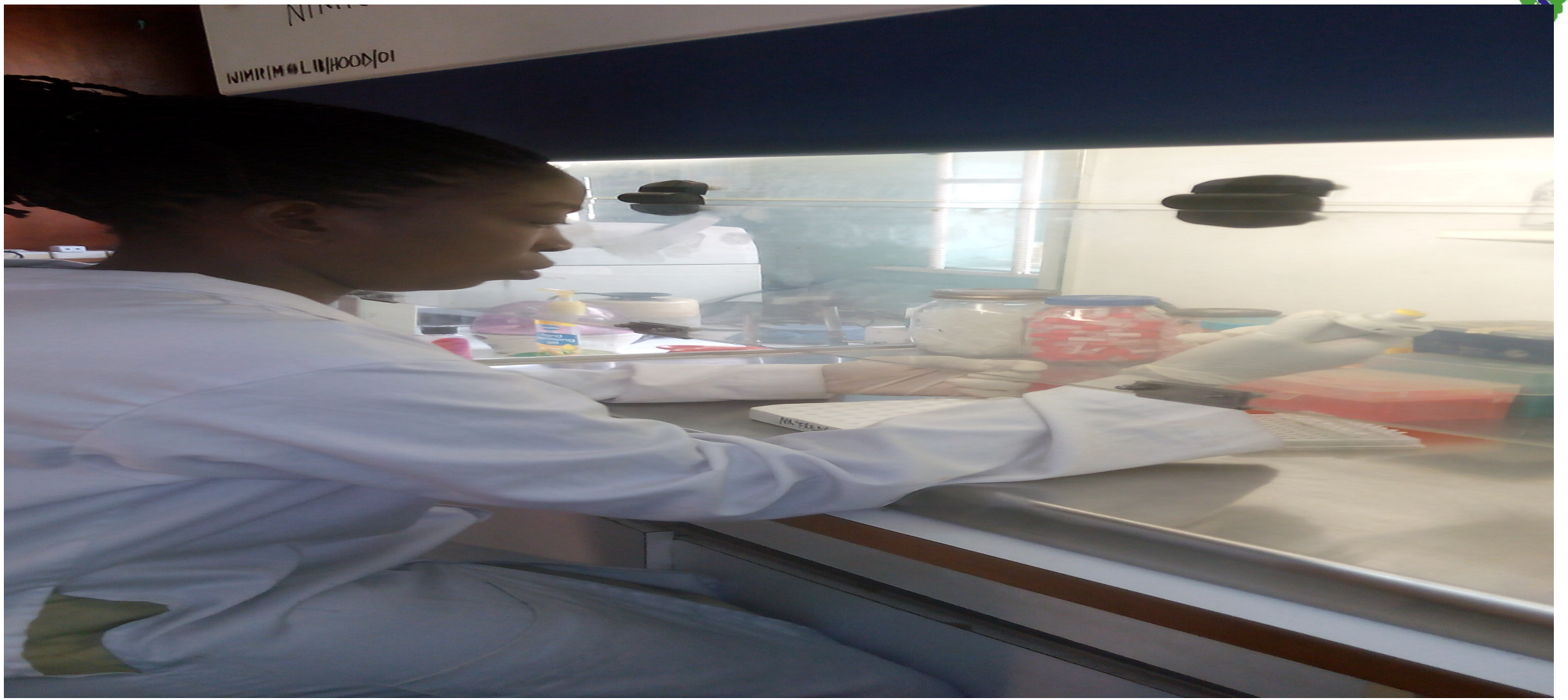


Figure 8: Processing samples in Biosafety Cabinet 2

Incubation Conditions

Figure 9: Gas jars and gas pack
Source: *https://www.oxoid. com*

Microaerophilic Condition: Most studies have used 2-5% O_2, 5-10%CO_2, 0-10% H_2, 75-85% N_2.

Temperature: 37°C

Incubation Period: 3-7 days

Hussain, 2005

Cultural Characteristics of *H. pylori*

They are micro-aerophilic, require 5-10% CO_2 , and high humidity.

They are fastidious organism.

They grow best at 37°C but not at 43°C and below 30°C.

Growth is best on blood agar and chocolate agar after incubation for 2-5 days. Colonies are circular, convex and translucent and grow bigger than 2 mm in diameter.

On Columbia blood agar they give small, dome shaped translucent and sometime weakly haemolytic colonies.

On modified Columbia urea agar (MCUA) – give small-middle size rounded and creamy colour colony. Change in the colour of the slant MCUA tube from orange to pink.

On Marshall's Brain Heart Infusion medium along with Vancomycin, Nalidixic acid and Amphotericin – give discrete dome shaped colonies.

On Egg Yolk Emulsion Agar – give large (~ 3mm) and red colour colony against yellow background (Pokhrel, 2015).

Isolation and Identification

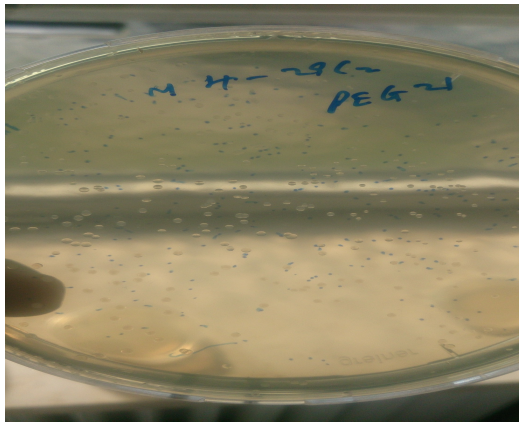
Helicobacter pylori colonies are small (0.5 to 2 mm), translucent to yellowish colonies on horse blood agar.

Gram Reaction: Gram negative spiral shaped bacterium

Biochemical Tests: Urease, Catalase, Oxidase positive.

Molecular Methods: PCR and Sequencing

(Pokhrel, 2015).



Contamination, Inhibition and Quality Control

Common contaminants:

Bacteria, Fungi and yeast (Kusters *et al.*, 2006; Abadi, 2018).

Inhibition of growth by antibiotics and antimicrobial agents (Megraud and Lehours, 2007).

Quality Control: P12, J99, G27 etc, *H. pylori* strains

Troubleshooting

- Failure to isolate
- Inhibition
- Long transportation decreases *H. pylori* viability
- If number of *H. pylori* is low, culture becomes negative, but yield can be improved by prolonging incubation up to 12days
- Contamination
- Monitoring of culture media and reagents
- (Abadi, 2018)

Results of Culture from Nigeria

492 Subjects

204 Males 288 Females (2015-2018)

184 (37.4%) UBT positive

125 isolates were recovered from culture (urease positive).

Isolation rate was 67.9%

Susceptibility Result of *H. pylori* Isolates from Nigeria

The results from E-test on GC agar serum/blood plate produced a clear and easily readable zone of inhibition for metronidazole (M), tetracycline (T), amoxicillin (A) and clarithromycin (C).

The highest concentration recorded on E-strip for the four antibiotics was 256 µg/ml while the lowest was 0.016 µg/ml.

Table 2: Susceptibility Result of *H. pylori* Isolates from Nigeria

Antimicrobial Agent	Susceptibility Result %
Metronidazole	93 % (97/104)
Amoxicillin	42 % (44/104)
Clarithromycin	39 % (41/104)
Tetracycline	27 % (28/104)

E-test Results

Conclusion

Despite the long use of culture, it is still challenging to culture *H. pylori* because its fastidious, there are limited access to resources, shortage of trained personnel, high cost of equipment and reagents, limited awareness of *H. pylori* infection and priority are given to other diseases (Smith *et al.*, 2019).

Culturing *Helicobacter pylori* is crucial for diagnosis, antimicrobial susceptibility testing, eradication therapy monitoring, epidemiological studies, understanding antibiotic resistance, personalized treatment, research, understanding the culture of *H. pylori* is essential for effective management of H. pylori-related diseases.

References

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THANK YOU