Isolation, Diagnosis And Extraction Of Outer Membrane Proteins Of Helicobacter Pylori Bacteria

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INTRODUCTION

The discovery of Helicobacter pylori as a gastroduodenal pathogen has affected the management of many gastrological diseases and since its isolation and associated with inflammation of the gastroduodenal mucosa [1, 2]. It was generally believed that following acquisition of H. pylori and in the absence of treatment, infection world persist throughout life [3]. H. pylori is one of the most common chronic bacterial infections world–wide, and it is currently estimated that approximately half of the world’s population is infected with bacterium. However, the prevalence of H. pylori is not homogeneous world-wide [4]. The duration of incubation for isolation of H. pylori has been recommended to be 2 to 7 days [5]. H. pylori serotypes for optimum growth requires complex basal medium with supplements such as serum, whole blood of horse or sheep, charcoal, egg yolk
emulsion or corn starch. These supplements may detoxify medium or protect *H. pylori* growth or serve as nutritional substrates [6]. *H. pylori* can transfer from mother to baby either during pregnancy or horizontally through breast-milk in the postnatal period. Various pathways, such as person-to-person, fecal-oral and oral-oral transmission play a role in transmission of the infection. Feces, saliva or vomit can potentially transmit the organism [7]. Several studies refer that there is a relationship between *H. pylori* and other disease non related to digestive system, for example, Coronary Heart disease, Myocardial Infection, Insulin dependant diapets, and other disease [8]. *H. pylori* in its 95 paralogous gene families there is a large outer membrane protein (Hop) family. It includes 32 members, such as adhesion protein, proinflammatory protein, and micropore protein. Although some functions of these OMP have still been indefinite, the scholars at home and abroad have paid attention to them on diagnosis, protective immunity, pathogenicity and so on. The documents show that Hop is significantly associated with high *H. pylori* colonization, the damage of gastric mucosa, high mucosal IL-8 levels, and neutrophil infiltration [9]. The outer membrane profile of *H. pylori* on sodium dodecyl sulfate-polyacrylamide gels differs from that of other gram-negative bacteria, as the highly abundant nonselective porins are absent and a number of less abundant species of proteins are observed [10].

**MATERIALS AND METHODS**

**Collection and diagnosis of Bacterial isolates.**

Ninety two patient with dyspepsia, gastritis, duodenal ulcer, and gastric cancer were tested Rapid Anti-*H. pylori* test at AL-Kadhymia teaching hospital in Baghdad during a period between December 2011 and April 2012. One ml of blood was taken from each patient and placed in heparinized test tube then take one drop from heparinized blood and applied to the sample well and add 2 drops from provided sample diluent immediately, wait for 15-20 minutes for reading the test result as follow:

1-Positive: Both purplish red test band and purplish red control band appear on the membrane. The lower the antibody concentration, the weaker the test band.

2- Negative: Only the purplish red control band appears on the membrane. The absence of a test band indicates a negative.

According to this result 55 biopsy were took from patients who gave positive result, the biopsy was taken from antrum then tested by rapid urease test using urea broth then cultured on chocolate brain heart
agar supplemented with supplement material [11] and they incubated at 37°C in candle jar with a pad of cotton which was soaked in water, which was placed at the bottom, to provide a humidified microaerophilic environment [12]. The plate would be examined after 2-7 days. Bacterial diagnoses including morphological and biochemical tests which included: Gram stain, catalase, oxidase and urease test [13].

*H. pylori* on chocolate brain heart agar

**Outer membrain proteins (OMP) extraction.**

Outer membrain proteins extracted according to Murpy *et al.*, (1983) which include the use of DNase, RNase, lysozyme and SDS detergent after refract the cells using sonicater, all these steps occurs after several steps of centrifugation and washing the bacterial growth, the final step was dialysis against distilled water [6] and polyethelenglycol 10000 [15].

**Evaluation of protein concentration in the OMP extraction**

According to Essa, (1986 ) the concentration of OMP in the extraction evaluated by using the equation [16]:

Concentration of protein (mg/cm³)

\[ = 280 \text{ absorption} \times 1.55 - 260 \text{ absorption} \times 0.76 \]

**Protein detection by Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (Lammili, 1970)**

Resolving gel (10%) was poured in electrophoresis tubes, and these tubes were then left for 30 minutes to ensure complet solidification. Then staking gel (3%) was added to resolving gel in tubes, the tubes were then left for 15 min for complete polymerization. After 24 hr, they were placed in electrophoresis unit. Finally the electrophoresis system was connected to the power supply with current density 2mA/tube for 30 min to remove positive ions for free protein movement [17].
RESULTS AND DISCUSSION

Collection and diagnosis of Bacterial isolates.

Rapid anti *H. pylori* test is easy and rapid for use to detection Anti *H. pylori* in patients serum. Fifty five patients gave positive result to rapid anti *H. pylori* test and 37 patients gave negative result figure (1).

![Rapid Anti H. pylori test](image)

Figure-1: Rapid Anti *H. pylori* test

Culture is considered the important step for detection of bacteria, but the method is not sensitive, and specific only if additional testing is performed on the isolates. Three isolates was obtained from 55 biopsys, growing bacteria was identified as *Helicobacter pylori* based on the morphology of the colony, gram staining, oxidase, catalase and urease production. The identified colonies were small translucent like water spray. The organism was gram negative, curved or bacill and it was catalase, oxidase and urease positive and this result is agree with the result of Al-Dhaher, (2001). This low rate of isolation may be because of the fastidious nature of *H. pylori* or because of the patient had taken antibiotic and because of a number of other factors like the patchy distribution of the organism, inadequate mincing of the biopsy material, the presence of oropharyngeal flora, the loss of viability of the specimen during transportation, etc. These factors are difficult to control. All these factors together, result in low sensitivity and a low negative predictive value [12].

Outer memran proteins (OMP) extraction.

One isolate was selected for outer membrain proteins extraction which was made by using sonicater which refract most of the cell
according to Murpy et al., (1983). the addition of DNase, RNase enzyme was to decrease viscosity of solution through refraction of DNA, RNA molecules, the Lsozyme split Murine and weaken linkage between peptidoglycan layer and outer membrain proteins and then make the exposure to SDS detergent is greater [15].

**Evaluation of protein concentration in the OMP extraction**

According to Essa, (1986 ) the solution absorption evaluated at 260nm and 280nm befor and after dialysis, according to the previous equation the final proteins concentration was (2.76028)mg/ml.

**Protein detection by Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (Lammili , 1970)**

*H. pylori* outer membrane proteins were analyzed by 10% SDS-PAGE [17] and the lane of proteins band obtained were compared with six marker proteins (Esterase MW=230KDa, γ-globulin MW=150KDa, Transferrin MW=80KDa, Bovine serum albumen MW=67KDa, Trypsin MW= 23KDa, Lysozyme MW=14KDa ) table(1), Results of protein profile by SDS-PAGE revealed that ten bands with M.W range between 340.476 – 16.914 kDa figure(2)and table(2).

![Image](image_url)

**Figure-2:** a/ protein profile analysis of *Helicobacter pylori* outer membrane proteins by 10% SDS – PAGE. b/standard protein profile analysis.
Table-1: Molecular weight of standard proteins

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Table-2: Molecular weight of *Helicobacter pylori* proteins

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*Helicobacter pylori* proteins curve
CONCLUSIONS
1. The combination of multiple tests is very accurate to detect *H. pylori* bacteria.
2. Appearance of ten protein bands with different molecular weight ranged between 340.476 kDa – 16.914 kDa analysis of *H. pylori* outer membrane proteins by 10% SDS-PAGE.

REFERENCES