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ORIGINAL ARTICLE

Detection *H. pylori* Infection by *BabA* Gene From Clinical Isolate in Karbala City, Iraq

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ABSTRACT

Objectives: The goal of this study is focused on the effect of *BabA* was investigated as being responsible for the adhesion of bacteria to epithelial tissue to make colonization and using this gene is effective methods to diagnosis this bacteria.

Methods: The clinical samples was taken during the period from August 2018 to March 2019 for 58 men and 54 women, their ages between 10-80 years old. Detection of the *H. pylori* infection by demonstration of *BabA* gene. Out of 112 patients suffering from dyspepsia attending Al- Husain medical city / digestive system diseases center, Karbala city**.** Detection of *H. pylori* infection from gastric biopsies by many methods culturing bacteria from the clinical samples on selective media Columbia agar, morphological test likes gram's stain, biochemical including urease, oxidase, catalase, Oxoid biochemical identification system (D-O.B.I.S) and API CAMPY identification test and molecular identification.

Results: It was found high infection rates of *H. pylori* in Karbala city 87 (71.3%) patients were positive *H. pylori* infection, the females had more frequent of *H. pylori* infection than males, and the infection was more in younger ages. The distribution of *H. pylori* infection according to age group and gender revealed that the majority of infected patients found in younger ages and then the curve declined after forties. The total males/ females ratio was 0.89/ 1 of the infected patients. The current study showed that the *H. pylori* patients with various pathologies cases as follow: gastritis in 48 case, peptic ulcer 19 case, gastric hemorrhagic 8 case, gastric pain 5 case and gastric cancer 3 case. There are many tests using to detect *H. pylori* infection. The highest percentage of these was PCR, especially when using more than one gene multiplex PCR, then urease test as rapid local test , then culturing that used especial requirements to grow bacteria which was considered fastidious and then gram's stain. Also this study showed a very important relation between *H. pylori* infection and iron deficiency anemia due to the iron was utilized by bacterial colonization during infection leading to malabsorption of iron.

Conclusion: The most frequent medical cases associated with *H. pylori* infection are gastritis , peptic ulcer, gastric hemorrhagic and then gastric cancer respectively. The *H. pylori* had high rates in Karbala city population and female patient are more frequently infection, and the infection was more in younger ages. There were many methods used to diagnosis *H. pylori* infection, PCR sensitive one and specialy when used more one gene duplex PCR. There are important relations between *H. pylori* infection and iron deficiency anemia.

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INTRODUCTION

Helicobacter pylori (*H.pylori*) Bacteria is a microaerophilic gram negative spiral shaped flagellated bacteria that localized in stomach mucosa specially pyloric part for nearly the entire lifetime of the host [1,2]. *H. pylori* contagion is an endemic worldwide health problem that infects more than half of the world population and reasonable of gastrointestinal disturbance. The infection source variety of gastrointestinal symptoms such as gastritis, peptic ulcer, gastric hemorrhage and gastric carcinoma [3]. *H. pylori* correlating inflammation is observed by mucosal infiltration of polymorphonuclear leukocytes (PMN),macrophages and T cells [4].

In developing countries, record 80% of the population is *H. pylori* positive . There have been some reports showing appear a decrease in global prevalence of *H. pylori* infection and peptic diseases inclusive many Asian countries. There are many virulence factor in *H. pylori* bacteria like *veca*, *cog*, *Bab* and *sab* genes . Bacterial adherence is considered necessary for role of colonizing in gastric epithelial by *Helicobacter*. The concerned sialyl Lewis x/a antigens and fucosylated ABO blood group antigens are intellect render as one collection of function receptors to *Helicobacter* adherence [5,6]. The ABO antigens were famous through blood group antigen binding adhesion *BabA* [3,4] and sialyl-Lewis x/a antigens are known through *Helicobacter* sialic acid binding adhesion [7].

Many of researches had showed a related between *BabA*-positive *H. pylori* and rising inflammations of cellular mucosa and rising risk of development clinical result [8].

MATERIALS AND METHODS

Patients and samples: A total of 112 patients who received endoscopy examination in Al- Hussany general hospital G.I.T center, patient had symptoms of gastrointestinal disorders and dyspepsia for 58 men and 54 women and their ages between 10-80 years old .The infection of *H. pylori* was diagnosis by rapid urease test (RUT),Bacterial culturing polymerase chain reaction (PCR).

Culturing of bacteria and condition of growth: Bacteria was cultured on Columbia agar and brain heart infusion media (Oxiod prepare condition) adding *H. pylori* supplement (vancomycin, 2.5 units/mL polymyxin B 5 µ g/mL trimethoprim, 10 µ g/mL and 2.5 µ g/mL amphotericin B) before sterilize and add 5-7% lysed blood horse after cooling media then culturing bacteria and incubation in condition nitrogen 90%, carbon dioxide7.6%, oxygen5% [16].

Biochemical test: The biochemical test had done after bacterial culturing like urease test , oxidase, catalase and iron deficiency to detection *H. pylori* infection [10,11].

Molecular diagnosis: RNA was extracted from all specimen by RNA extraction kit (Geneaid, Sengapure) . Specific primers for virulence factors *BabA* and *16S rRNA* of *H. pylori* PCR and its PCR cycler condition initial denaturation (95 °C for 3 min.), denaturation (95 °C for 15 s), annealing (50 °C for 30 s, extension at 72 °C for 30 s), and final hold (10 °C for 1 min) [15].

RESULTS AND DISCUSSION

Patients of study: A total of 112 patients who received endoscopy examination in al- Hussany general hospital (G.I.T) center in period from August 2018 to March 2019 for 58 men and 54 women and their ages between (10-80) years old as show in Figure 1. In this study we included the randomly cases. This study showed gastritis as highest medical case in (36%), peptic ulcer as second in (18%), Gastric pain (16%), Normal (14%), Gastric hemorrhagic (10%), and Gastric cancer (6%) as show in Table 1.

Table 1: **Medical cases diagnosis by endoscope.**

NO.	Medical cases	No. of cases	Percentage
1	**Gastritis**	41	36%
2	**Peptic ulcer**	19	18%
3	**Gastric hemorrhagic**	11	10%
4	**Gastric cancer**	6	6%
6	**Gastric pain**	18	16%
7	**Normal**	17	14%
Total		**112**	**100%**

This results arrangement local studies [24], [20] and [18], while disarrangement with [23,25,27]. [26] (2017) mention the gastritis percentage (83.4%) and peptic ulcer (13.6%) in Bhutan, Myanmar, Nepal and Bangladesh. [22] (2007) referred to many studies in the world for gastritis in Taiwan , Japan, China , Brazil, Germany, France, Finland, Portugal and United state. [22] Confirmed gastric endoscopy surgery is necessary to diagnose gastritis. The area from which the sample is taken may suffer. Therefore, more than one sample has been advised to determine the presence of gastric atrophy or intestinal metaplasia, Which are in the form of a spotted.

In Figure 1 which showed distribution age groups of *H. pylori* infection . this study discovered the majority of infection group were found in younger ages between (21-30) , (31-40) , (41-50) years old in arrangement, this shown clearing in the histogram after forty years old.

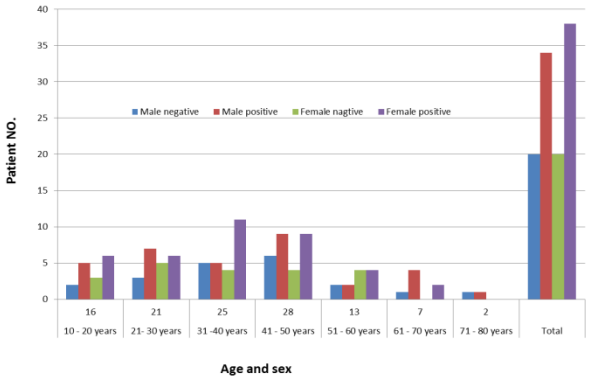


Figure 1. **Distribution of *Helicobacter pylori* infected patients according to age groups and gender distribution.**

The distribution agrees with Matsuhisa and Aftab (2012). It is also explicit that females face to be infected with *H. pylori* in younger ages, while the males resort to to be comparatively steady. This manner found by one other research in Kuwait [27]. This may be occur due to the difference in patients' race and dominant strains obtainable in different parts of the world. Environmental differences may play an substantial role in *H. pylori* infection apportionment in addition to socioeconomic factors.

Identification and Incidence of *Helicobacter pylori*

Infection percentage: This study show infection percentage (71.3%) ,the positive case 87 from 112 total case and negative case 25, the diagnosis done by different methods biochemical tests (urease test and catalase %76, oxidase (%75.70), culture test and molecular diagnosis. The effect of age groups pathologies distribution of *H. pylori* positive patients infection from Karbala governorate is appear in Figure2.

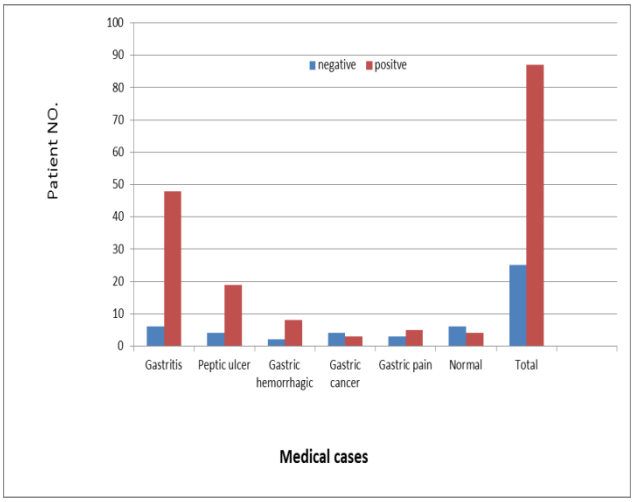


Figure 2. **Relation between medical cases diagnosis by endoscope and *H. pylori* infection.**

In case of gastritis, the infected patients is 48 Case more than other medical case. The result agrees with another study worked in Baghdad by [29]. As for the other medical case including, Peptic ulcer, Gastric hemorrhagic, Gastric cancer, Gastric pain, arrangement on their distribution.

And this also agreement with another local study [24,20,17,18]. These studies showed also gastritis the first medical case in *H. pylori* infection then other medical case including, Peptic ulcer Gastric hemorrhagic, Gastric cancer, Gastric pain, while [23,25] dominant of peptic ulcer, then gastritis.

The result show in the Figure 3 show relation *H. pylori* infection with Iron deficiency anemia, during this study GIT center received forty patient with iron deficiency anemia 28 male (26 positive, 2 negative) percentage of male infection by *H. pylori* was 92.8% and 12 female (positive 11, negative 1) percentage of female infection by *H. pylori* was 91.6%. So there is closely relation between iron deficiency anemia and *H. pylori* infection, this result agree with [21].

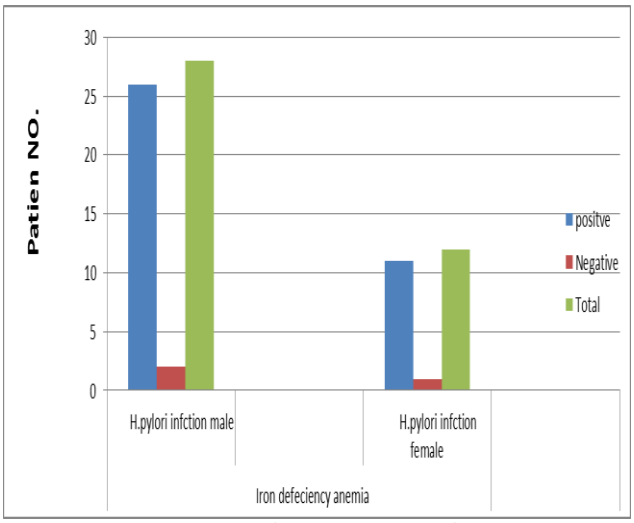


Figure 3. **Relation *H. pylori* infection with Iron deficiency anemia.**

The possession of different haem-utilization systems could greatly contribute to the virulence of *H. pylori* since the ability of pathogenic microorganisms to scavenge iron and/or haem compounds from their host environment is fundamental for survival in the host and thus production of disease. Any free haem or free haemoglobin is bound by protein carriers: haptoglobin and haemopexin [30].

So the significant decrease in haptoglobin may be due to H. pylori scavenge heme compounds from their host. While other study reported that *H. pylori* has haemolytic activity and has been found to adhere to red blood cells in capillaries in the lamina propria. Although *H. pylori* has previously been reported to use hemoglobin for growth, the methods usedpreviously did not permit the workers to prove that growth was not due to free heme present as a contaminant or due to other heme-containing complexes, such as heme-hemopexin or hemoglobin-haptoglobin [30].

Diagnosis of *H. pylori*: To determine *H. pylori* infection in humans, multiple laboratory methods have been reported [12]; however, based on estimated resources. The biopsy after hospital collection subjected RUT to primary diagnosis, then directed transfer to the pharmical collage to make other test to diagnosis samples like gram's stain for histological smear and culture smear, sample culture, biochemical test (urease test, catalase, oxidase), molecular detection by PCR of *BabA* gene.

Gram' stain: The isolates of *H. pylori* undergo to Gram's staining directed histological smear and colony after culture . The feature *Helicobacter* were observation like spiral shaped rods, gram's negative stain, this appears clearly in Figure 4. The diagnosis of gastric bacteria was spiral-shaped, Gram-negative organisms in biopsy stained specimens find in 11 from 87 *H. pylori* illness people.

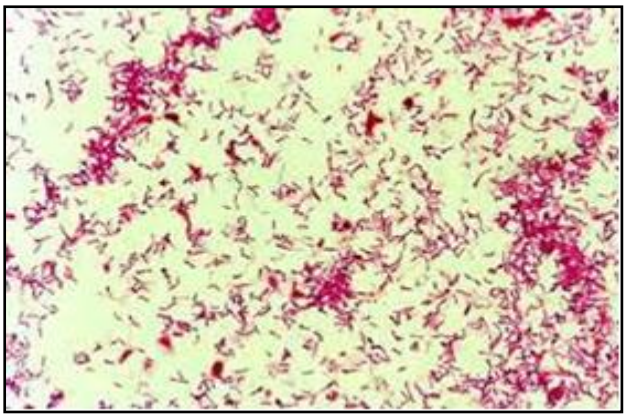


Figure 4. **A- *H. pylori* gram's stain in culture smear (40X)**.

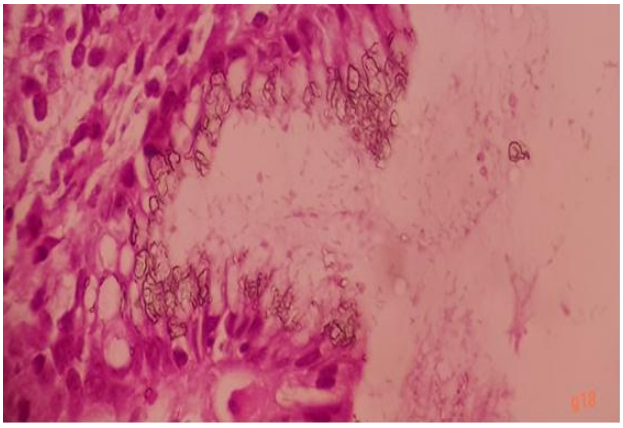


Figure 4. **B- Hametoxilin and Eosin (H and E) stain of biopsy shows *H. pylori* inside tissue (40X).**

The Figure 5 show accuracy different diagnostic methods for *H. pylori* infection, in this figure consider urease test important test for detection *H. pylori* infection and give primary indicate for this infection during time not exceed twenty four hours and consider negative after this time.

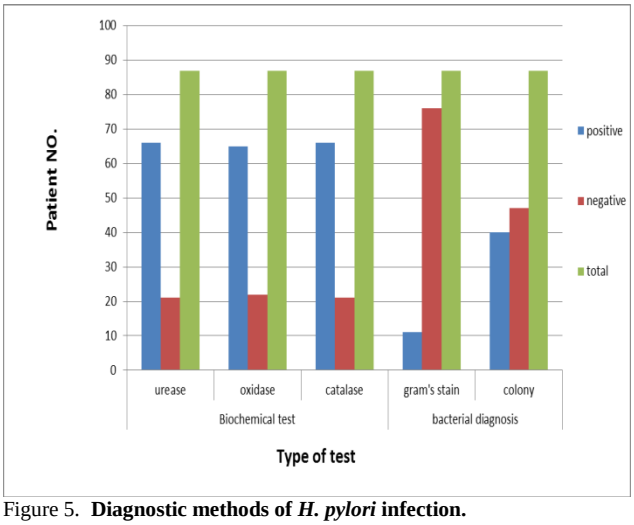


Figure 5. **Diagnostic methods of *H. pylori* infection.**

Some strain needed half hour to change in color , other needed two hours and the other needed four hours these differentiation in time due to presently enzyme in the biopsy number of bacteria in the sample and number, size of biopsy , if there is more one biopsy give urease positive in short time [14].

Other biochemical test oxidase and catalase all so give same result of urease but these widely use after culture and there is overlap with the other bacteria may be give false positive 18. While the biopsy not give change in color because of low number of bacteria and size of the biopsy [17].

Cultural methods give more emphasis for infection but show in figure low level compression with biochemical test because of effected by many factor like patient taken antibiotic or protein pump inhibitor before taken the biopsy , these lead to reduce number in addition to these bacteria very slowly in growth on enrichment media and necessary addition antibiotic for inhibition other normal flora [18] in addition difficulty remain these germ live in room temperature during biopsy collection and electricity power off during culture. All so result show the gram's stain methods is very effective methods after culture and gram's stain show low accuracy with biopsy because of Low number of bacterial in the sample [19].

Biochemical test: Biochemical tests used to diagnosis *H. pylori* (oxidase, catalase and urease) accomplished for assure the identification for *Helicobacter* for all 112 specimen [19].

Urease test: The results show the affectivity of urease test during this study 66 sample from 87 positive sample appear change in color from yellow to red during time between 30 minute to the one day and the percentage of affectivity of this test 76%.

Rapid urease test (RUT), is fast diagnostic exam for identification of gastric bacteria. The principle of the was the capability for *Helicobacter* to secrete the urease enzyme, which stimulate the transformation urea to ammonia and carbon dioxide [20].

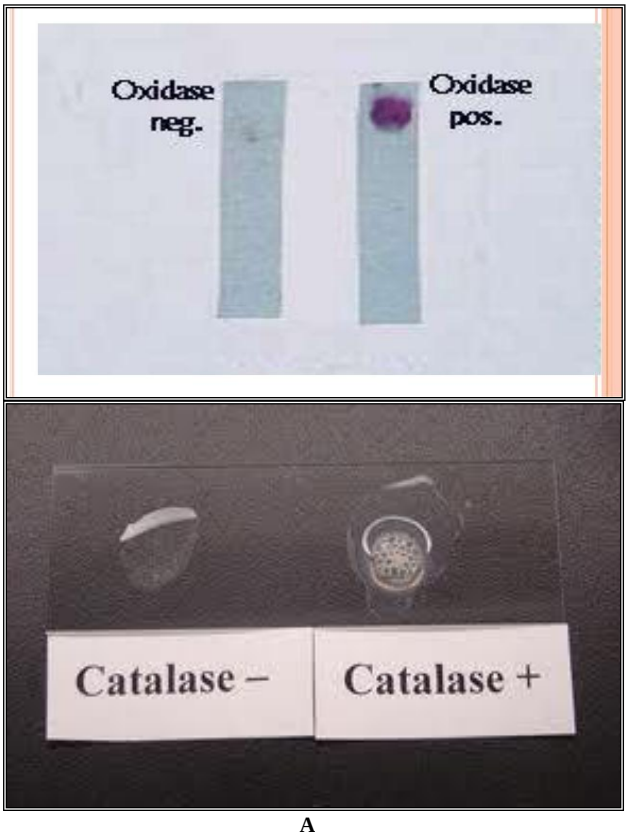
At the time of gastroscopy the exam is formed. From stomach antrum biopsy of mucosa was taken, and put in the media contain an indicator like phenol red and urea. The *Helicobacter* hydrolyses urea to ammonia to produce urease, which increased pH for media, and modification color of the sample "from yellow negative to red positive" [21].

Catalase and oxidase test: The catalase existence in a bacteria or sample tissue can be explain through added hydrogen peroxide and watch reaction. The oxygen produced could observed through bubbles formation. This is simple experiment, can be observe by naked eye seen, without the added any equipment's, was conceivable because of very highly specific action of catalase, which production a reveal response, an addition the one fact that production a gas [20].

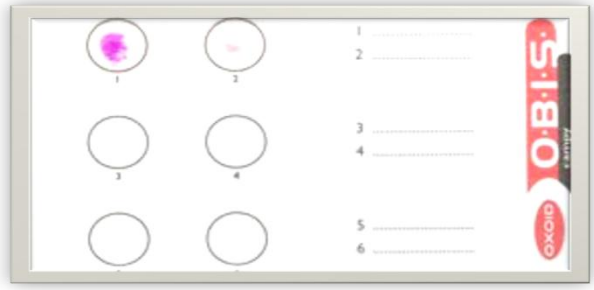
Oxidase test in microbiology was used a phenotypic character to diagnosis of bacteria strains; "it determines whether a given bacterium produces cytochrome oxidases", therefore; used oxygen together with electrons transfer series). The experiment define if bacteria anaerobe or aerobe is oxidase test. color change from light-pink or absence of coloration violet to purple, within 10–30 seconds as show in Figure 6- a.

Oxoid Biochemical Identification System (O.B.I.S) and CAMPI Api system: In Figure 6-b shows also CAMPI Api system that used to the comfermed diagnostic of the *H. pylori* bacteria after used bacterial suspension diluted with normal salin and glucose taken from brain heart infusion culture. The Campy API strip consist of 20 microtubes containing dehydrated substrates. It made up of two parts. The first part of the strip (enzymatic and conventional test). During inoculation, metabolism produces cause color changes that are either spontaneous or revealed by addition of reagents.

The second part of the strip (assimilation or inhibition tests) is inoculated with a culture growth medium for 16 isolates and inoculated in microaerophilic condition. Also the result shown in Figure 6-c Oxoid Biochemical Identification System (O.B.I.S) for *H. pylori* identification system included two part using gram's lysis test, followed by enzymatic detection. The test give positive result with the for KOH test (Gram lysis) test, and same test give Negative result for L-alanylaminopeptied (L-ALA) test and this result agrees with [21].



A



B

C

Figure 6. **Catalase and oxidase tests CAMPI Api system, (a) Oxidase and Catalase tests, (b) CAMPI Api system for characterization of *Helicobacter pylori* showing the results of reactions including the system, (c) Result of Oxoid Biochemical Identification System (O.B.I.S) for *Helicobacter pylori.***
1-Positive result for KOH test (Gram lysis) test.
2- Negative result for L-alanylaminopeptied (L-ALA) test.

Culture findings: Isolation for *H. pylori* range about 45.9% through used classic Columbia agar, 40 positive infections out of 87 patients positive tested it diagnostic. *H. pylori* growth on the medium was scanty, few colonies in number, diaphanous and tiny in size. This agree with the result of the [19,24].

Figure 7. **Bacterial culture in Colombia lysed horse blood agar**.

The growth rate of gastric bacteria on Columbia agar medium slowly; with all cases five to ten days had

colonies needed for the appear. This appears in Figure 7. different media, selective and non-selective ,or incorporation of two types, had suggested in the primary isolation for utilized of *H. pylori*, however, the best way for diagnosis yet remnant to be determined [23]. The nonselective medium columbia blood agar is utilized for several years lonely or incorporation with other selective media and non-selective to growth gastric bacteria taken of biopsy from antral region samples found in peptic ulceration illness people through endoscopy for upper gastrointestinal [19].

Bacterial contamination of the medium was frequent. The contaminant bacteria like *Klebsiella spp., Pseudomonas spp., and Proteus spp.* the origin for which can been contaminated through taken and contamination biopsy forceps, transmiting and preparation of the lysis horse blood added to the classic Columbia agar. The gold standard for the found in many contagious sickness was success to cultured of the microbiota [31]. In this time, culturing of gastric bacteria from stomach antral biopsy samples is indication mechanisms for bacterial , necessary to antibiotic sensitivity test and analytic for proposed virulence agent [25]. As well as, it was commonly deem a tiresome, time-consumption and expenses mechanisms, culturing on agar medium which standard mechanisms utilized in many laboratories to the detection of gastric bacteria of gastric biopsy samples [26].

H. pylori primary detection of biopsy samples was risque methods, a perfect successful range to culturing microorganism were recorded to be for rate %70 into %80 together %90 into %95 suspitability and %100 specific [27]. Many agent, were complicated to dominance, result quandary to gather with culture tothe microbe: inchoate apportionment for microbes in the mucosa layer for stomach, contamination for forceps biopsy, found in oro pharangyeal microbes, lack for viable microbes through transport, etc. these reason accountable to destitute negative oracular range coleration to culture of gastric bacteria [22].

Diagnosis *H. pylori* Clinical Samples by Duplex PCR technique: Gastric biopsy spicemen (n= 112) were conducted by PCR assay to detecting the present of *H. pylori* specific *16S rRNA* and *Bab* genes. The study revealed that the number of positive isolates for *16S rRNA* or *Bab* genes was 87, Table 2. According to that, all these result primarily confirmed as positive to *H. pylori.* The total number of patient enrolled in the current research was 112, from each patient one gastric biopsy taken, and the positive patient for *H. pylori* were 87 (71.3%) involving 34 (30.3%) males, 38 (33.9%) females.

Results in Table 2 revealed that utilizing more than one gene in detecting of such bacteria from clinical sample by PCR will increased dramatically test sensitivity to 100%. And all isolates that give the positive outcome for *16S rRNA* and/or *Bab* were positive with all *Bab* variable area. This data propose that this combination is suitable to be utilized in a commercial kit for effective *H. pylori* detected. Although PCR test considered as a very sensitive test for pathogens diagnosis, like *H. pylori* [17], the changeable between sequences of the same genes from many strains of the same genus and species of the microbes may influence in result.

Result show in Table 2 that utilizing more than one gene to diagnosis of such bacterium from clinical specimen by PCR will raised dramatically method sensitivity to 100%. And accuracy of diagnosis raised from 82.7% ,72 specimen with 16S rRNA gene and 79.3% ,69 specimen with *BabA* gene to the 100% when utilizing duplex RT PCR to diagnosis same bacterium.

Table 2: **Diagnosis of *Helicobacter pylori* using *16S rRNA* and *BabA* genes.**

Gene	No. of Positive	Sensitivity %
16S rRNA	72	82.7
Bab	69	79.3
Duplex 16S rRNA and Bab	87	100
(RT-PCR)	87	100

For PCR application in bacterial diagnosis, it's substantial to choice such bacterium genes present in most strain, in addition to evading mismatches present in primers attachment locations. From that, the chosen of *16S rRNA* and *BabA* genes for duplex PCR diagnosis of *H. pylori* is found the fact that there are fundamental genes for bacterium life (Housekeeping genes) and must be present in any strain, and possible mismatch in every primer pair will be recompense by the other one, duplex PCR is appeared in Figure 8 [32].

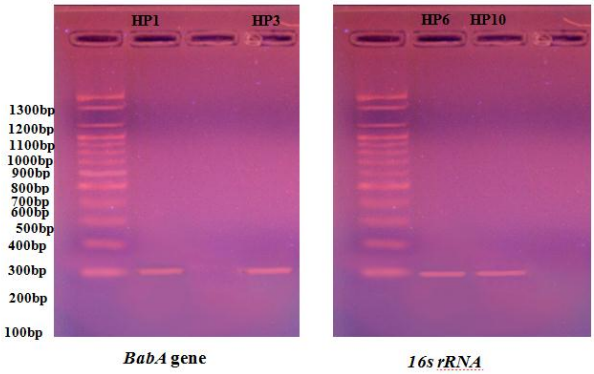


Figure 8. **Agarose gel electrophoresis for *BabA* gene, *BabA* (95 bp), 100 bp ladder.**

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