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DETECTION OF *HELICOBACTER PYLORI* IN LEAFY VEGETABLES BY PCR AND DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF GREEN TEA AND GARLIC EXTRACTS AGAINST ISOLATED BACTERIA

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Abstract

The present study was carried out to detecting *Helicobacter pylori* in local leafy vegetable samples which collected from different sources in Erbil city. Out of 50 samples; the presences of *H. pylori* were 5 (10%); isolated from spinach and parsley, but negative results seen in celery. The results appeared the media BHI supplemented with 7% HRBC and Vancomycin 5 mg + Trimethoprim lactate 25 mg, was the most effective medium for isolation with a positivity rate was (4%), other media which used in the isolation was Columbia agar with7% HRBC and Vancomycin 5 mg + Trimethoprim lactate 25 mg the percent of positive results were (4)%, while Brucella agar; the third media which used in the isolation, the rate were (2) %. The bacteria were identified according to cultural, morphological, biochemical characteristics (Urease, oxidase, catalase, motility), most of the isolates had positive reaction and gave 100% positive results except the motility which only 40% could motile, the identification of the isolates confirmed by polymerase chain reaction (PCR), The general bacterial gene *16SrRNA* and virulence *ureA* and *ureC* /Hp, the results appeared that from the total 5 isolates; 100 % were confirmed as *H. pylori* by the amplification of 16*sRna,* as well as 100% of the isolates possessed the gene *ureA* which responsible for producing urease enzyme; the main factors in pathogenicity, and 60 % of the isolates varied in the presences of *ureC* genes. All *H. pylori* isolates were screened for 12 antimicrobial agents using disc diffusion method, resistance was observed against the groups of Gentamycin, Erythromycin, Linomycin, Triemethprim, Vancomycin none of isolates were resistance to (Amoxicillin, Ampicillin, Ciprofloxacin, Oxacillin Tetracycline). The results shown the ethanol extracts of green tea had the ability to inhibit the growth of the bacteria, the inhibition zone ranging between(21-22mm), but the range of inhibition zone was (20-22mm) for the watery green tea extract against the isolated bacteria and the inhibition zone (23-29mm) in watery solution and (20-40mm) in ethanol solution, then garlic effected on the *Helicobacter pylori* shown the inhibition zone (28mm) in water solution and (20mm) in ethanol extract. The activity of lactic acid bacteria a bio controlling test had the ability to inhibit the growth of *Helicobacter pylori* shown the inhibition zone (20-22mm) from the total of 18 isolated of different sources.

Key words : *H. pylori*, Vegetable, PCR, *UreC, UreA*, 16S*rRNA* gene.

Introduction

Vegetables are elevated as wide-ranging foods. Their high values for minerals and vitamins are irrefutable and, in a day, a lot of people use the vegetables and its salads in their main food. Therefore, sanitary quality of vegetables and salad has a high importance in public health but sometimes it will be converted and several infections and illnesses will occur. Vegetables are in close contact with soil, animal manure, and even human stool. They are usually irrigated with polluted water. Previous studies showed that soil (P´erez *et al.,* 2010), water (Lu *et al.,* 2002), animal manure (Tabatabaei 2012; Safaei *et al.,* 2011), and human stool (Safaei *et al.,* 2007) are the main possessions for *Helicobacter pylori* (*H. pylori*). Therefore, vegetables can simply be contaminated with *H. pylori*. In addition, their cross contamination in processing stages is indisputable. Some of the most

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important virulence factors such as vacuolating cytotoxin A (*vacA*), cytotoxin associated gene (*cag*), induced by contact with the epithelium antigen (*iceA*), outer inflammatory protein (*oipA*), and urease (*ureC*) play a major role in pathogenicity of *H. pylori* infection (BenMansour *et al.,*2010). These genes are usually induced by bond to and attack of the gastric epithelial cells (Blanke 2005; Cover, *et al.,* 2003; Yamaoka,*et al.,* 2010). Genotyping using these well-known virulence indicator genes is measured as one of the best approaches for study of correlations between *H. pylori* isolates from different samples (Scholte and van Doorn 2002; Wang *et al.,* 2002). Studies on the isolation of *H. pylori* in vegetable products are rather little, it has been defined that persons who consume raw vegetables are more likely to get *H. pylori* (Vale and V´ýtor, 2010). The relationship of the infection with consumption of raw vegetables is an additional indirect evidence of the presence of *H. pylori* in water used for irrigation of these vegetables, (Mazari-Hiriart *et al.,*2008; Gomes and De Martinis, 2004). Likewise, the isolation of *H. pylori* from food products is extremely difficult due to the presence of accompanying microflora and to the presumably very low *H. pylori* load (Quaglia *et al.,* 2009). The contamination of vegetable within the industrial facilities or in households by poor hygiene management of infected personnel that cannot exclude the transmission of *H. pylori* by these foods. Proof of the ability of *H. pylori* to survive in common foods supports the hypothesis that primary contamination of a food product (animal reservoir) or secondary contamination due to inappropriate handling (human reservoir) can be a vehicle for *H. pylori* transmission (Quaglia *et al.,* 2008). Suggesting that food may act as a vehicle rather than a reservoir, although it is unlikely that *H. pylori* grows on food, it may survive as VNBC (van Duynhoven and de Jonge, 2001). The exact mode by which *H. pylori* gains access to the human stomach is unknown, DNA and VBNC forms may enter as VBNC under conditions. Several studies address the role of food in the transmission of *H. pylori*. Food products analyzed are mainly milk, meat and vegetables. Bacterial urease neutralizes the gastric pH, enabling the colonization of gastric epithelial cells by the bacteria and their motility in the mucus layer (Yamaoka 2012, Yamaoka *et al.,* 2000). Treatment of diseases caused by *H. pylori* often requires antimicrobial therapy; however, antibiotic-resistant strains of bacteria cause more severe diseases for longer periods of time than their antibiotic-susceptible counterparts. Several studies have shown that antibiotic resistance in *H. pylori* has improved over time (Boyanova and Mitov 2010, Megraud 2004). Data on the spreading of genotypes and antibiotic resistance pattern of *H. pylori* strains isolated from vegetable samples are rare. Therefore, the aim of the current study was isolation and identification of *H. pylori* from vegetable samples and examining their susceptibility to commonly used antibiotics, as well as investigating the presences of virulence genes *ureC and ureA* as well as identification of isolated bacteria by 16S *rRNA* gene of *H. pylori* by PCR.

Material and Methods

Pretreatment of Leafy vegetables samples

Ten gm. of different leafy vegetable (celery, spinach, parsley) were weighted, cut to small pieces in septic conditions mixed well with 90ml of distilled water, grinded very well till formation of homogenized mixture then filtered with sterilized cotton cloth, Subsamples (1000 µl) of each final concentrate of all 50 vegetables samples were cultured on three culture media: the first, Brain Heart Infusion Agar plus 7% HRBC plus antibiotic supplement contains (Vancomycin 5 mg, Trimethoprim lactate 25 mg, Polymyxin B 0.05 mg.), the second media was, Columbia Agar plus 7% HRBC plus growth supplement contains: a. Sodium pyruvate 0.125 g, b. Sodium metabisulphate 0.125 g and c. Ferrous sulphate 0.125 g. and Brucella agar. Small smooth colonies resembling *H. pylori* were sub-cultured, and were subsequently identified by Gram-stain and tests for rapid urease, cytochrome b oxidase and catalase activity (Salih, 2009). The plates were incubated under microaerophilic conditions at 37^0C, using an anaerobic jar and anaerobic Gas Pak kit without catalyst to generate a microaerophilic environment. Plates were inspected first on day 3 and then after one-day interval for a total of ten days. Gas Pak was changed whenever the jar was opened. *H. pylori* were identified by the following criteria: 1. Colonial morphology, Motility test, Catalase, oxidase and Urease Tests.

Identification of the isolates by PCR:

Genomic DNA Extraction and PCR amplifications

The subjected isolates to the Genomic DNA Extraction was according to the kit manufacturing company information procedures was done as follows; 0.1ml. of activated bacteria in the Luria Brittani broth transferred to sterilized test tube and centrifuged at 8000 rpm for (1) min. added 180 µl of Lysis Buffer (T1) and 25ml Proteinase K solution Vortex to mix, Incubated at $56c^0$ until complete lysis was obtained (at least 1-3h). Added 200µl Buffer B3,vortex vigorously and incubate at 70c for 10 min. Vortex was done briefly, Dissolved Wash Buffer (B5) 7 µl in 210 µl ethanol (96-100%)Added 500 µl Buffer BW, centrifuge for 1 min at 11,000xg. Discard flow-through and place the column

back into the Collection Tube. The second wash added 600 µl Buffer B5 to the column and centrifuge for 1 min at 11,000xg, Discard flow-through and place the column back into the collection tube. Centrifuged the column for 1 min 11, 000 x g. residual ethanol is removed during this step. Placed the Nucleo Spin Tissue Column into a 1.5 µl centrifuge tube and added 100ml pre-warmed Buffer BE (70c^0). Incubate at room temperature for 1 min, Centrifuged 1 min at 11,000xg (MACHERTY NaGeL). Stored the DNA in the freezing at -20c (Falsafi *et al.,* 2009).

The primers sequencing which used for the identification were appeared in (Table 1).

Detection of *16srRNA* gene and *UreA* gene and *Hp/ UreC* gene

A sterile amplification tube was used; the following orders were mixed at final volume 20 µl for each primer (Bioneer kit), as shown the PCR reaction mixture(master mix 20 µl, forward primer 1 µl, reverse primer1 µl, nuclease free water 16 µl and template DAN 2 µl. The master mix composed from the following dNTP, 250µM each: (dATP, dCTP, dGTP, dTTP; Taq DNA Polymerase, 1U/ml; Tris-HCl(pH 9.0), 10µM; KCl,30mM; $Mgcl_2$, 1.5mM. DNA amplification was carried out for 35 cycles in 20ml total reaction mixture as follows: Temperature cycling by using *16S rRNA*, denaturation at 94c^0 for 1 min., annealing at 55c^0 for 30 s extension at 72c^0 for 2 min., final extension at 72c^0 for 7min, hold at 4c^0.

Temperature cycling of *UreA* gene

Denaturation at 94c^0 for 1 min. annealing at 45c^0 for 1min, extension at 72c^0 for 2 min, final extension at 72c^0 for 7min, hold at 4c^0

Temperature cycling of HP *ureC* gene

Denaturation at 94c^0 for 1 min. Annealing at 55c^0 for 1min. extension at 72c^0 for 2 min final extension at 72c^0 for 7min, hold at 4c^0. Aliquots of each PCR product were separated by electrophoresis in a 1% (w/v) agarose gel in TBE buffer (90 mm Tris-HCl, 90 mm boric acid, 2·0 mm EDTA) and stained in ethidium bromide at 0·5 μg ml^{-1}. Assays on all samples were repeated in duplicate. Samples were interpreted as being positive for the presence of *Helicobacter* DNA if one or more of the assays produced a fragment comparable in size to that of the positive control DNA (Al-Sulami *et al.,* 2012).

Table 1: The primers sequencing design.

Target gene & Primer name	Nucleotide sequence	Amplicon Size (bp)	Reference
16 SrRNA		500 BP	Falsafi *et al* ., 2009
16 SrRNAF	5GCAATCAGCGTCAGTAATGTTC3		
16 SrRNAR	5GCTAAGAGATCAGCCTATGTCC3		
ureA		411Bp	Notarnicola, *et al*.,1996
ureAF	5 GCC AAT GGT AAA GCCTTA GTT3		
ureAR	5CTC CTT AAT TGTTTT TAC 3		
Hp /UreC		294Bp	Ahmad *et al*., 2013
HpF	GAATAAGCTTTTAGGGGTGTTAGGGG		
HpR	AAGCTTACTTTCTAACACTAACGCGC		

Antimicrobial susceptibility test

Isolates that were confirmed as *H. pylori* were activated by culturing in Brain Heart Infusion broth for 48hr. at 37C, till it becomes slightly turbid, then the tube was compared with the McFarland standards and was adjusted to the density of test suspension to that of the standard by adding more bacteria or more sterile saline. Sterile cotton swab was dipped into the standardized bacterial test suspension and used to evenly inoculate the entire surface of Mueller –Hinton agar and then spread evenly all over the surface, After the inoculum had been dried, the disc of the following antibiotics: Amoxicillin (30mg), Ampicillin (10mg), Erythromycin (15mg), Gentamycin (30mg), Rifampicin (30mg), Kanamycin (5mg) and Tetracycline (30mg) was applied to the agar with a sterile forceps, the disk were firmly to ensure contact with the agar, within 15 min. of disc placement, incubated at 37c for 48 to 72hr. in anaerobic conditions (CLSI,2012)

Effect of Green tea and Garlic on *H. pylori*

Aqueous and alcohol extraction were done as followed; Ten gram of dry green tea mixed with 100 ml of ethanol 70% and 100ml. of distillated water (the temperature of water was 50 C. Then kept for 2hr in room temperature until the ethanol evaporated then by streaking *H. pylori* bacteria on surface of Muller – Hinton medium with loop and left for fifteen min. Then well were done by making 4 holes and filled with (0.5) ml of the green tea extract in each hole cultured plate incubate for 2-3 days in 37 C in anaerobic conditions (Calin *et al.,* 2009).

Effect of Garlic extract on the growth of *H. pylori*

Three gm. of fresh Garlic crushed in 8ml ethanol 70% left for 1hr the mixture was filtered with sterilized cotton cloth piece . The extract was tested against the growth of isolated

bacteria by well diffusion agar as mentioned above.

Results and Discussion

The presences of *H. pylori* in the total 50samples of vegetables were 5 (10%); isolated from spinach and parsley, but negative results seen in celery, may be the bacteria found in uncultivable, this result comparative with the Adnan and Coworkers (2012) when they reported the presence of a viable but uncultivable (VBNC) form of *H. pylori* in raw vegetables that indicates the possible contribution of water or soil. There were multiple risk factors for *H. pylori* infection; consumption of raw vegetables increased the rates of infection (Buck and Oliver, 2010). Alan and coworkers (2010) indicated that the environmental reservoir and mode of transmission of *Helicobacter pylori* is currently unknown due to difficulties in isolating the bacteria from non-human sources, the ability of *H. pylori* to survive in a viable but non cultivable (VBNC) state association with vegetables, and the cells rapidly became non-detectable by plating, however mRNA transcripts were detected 6 days after the cells were introduced to the vegetables, it was found that exposure to white light rapidly induced the VBNC state in *H. pylori*, suggesting sunlight may be a factor in loss of cultivability of this pathogen which may help explain the lack of cultivability from environmental sources, low socioeconomic status, and consumption of uncooked vegetables by use of a logistic regression analysis. Although multiple modes of transmission of *H. pylori* undoubtedly exist, prior studies have suggested that contamination of irrigation water by raw sewage and the subsequent contamination of vegetables that are eaten uncooked is a key factor in the transmission of enteric pathogens and *H. pylori* may be transmitted by a similar route. Comparison among different culture media for successfully isolation of this bacteria were done by using three different selective and enrichment media to all tested samples. The results appeared as shown in table 1 the media BHI supplemented with 7% HRBC and Vancomycin 5 mg + Trimethoprim lactate 25 mg, was the most effective medium with a positivity rate was (4%) which means that the enrichment medium with blood and antibiotics supplement suppress the contaminants and encouraged the growth of the bacteria, The second media which used in the isolation was Columbia agar with7% HRBC and Vancomycin 5 mg + Trimethoprim lactate 25 mg the percent of positive results were (4)% obtained from the isolation of the bacteria from vegetables samples. Brucella agar was the third media which used in the isolation, the rate were 2 % from vegetables samples. The results when comparative to that founding Farhat and Abdul (2000), they isolated *H. pylori* from patients with (10%) of positive results.

High incidence of H. pylori in uncooked vegetables that had been irrigated with water contaminated with sewage was reported previously (Hopkins and Vial 1993, van Duynhoven and de Jonge, 2001). Frequent consumption of raw vegetables was associated with likelihood of H. pylori infection (Goodman *et al.,*1996). Also, individuals who consume vegetables are more likely to acquire *H. pylori* (Chen *et al.,* 2005). Foods with water activity higher than 0.96 and pH from 4.9 to 9.0 (like vegetables) theoretically provide conditions for the survival of *H. pylori* (Jiang and Doyle 1998).

The studies which carried by AL Sulami *et al.,*(2008) and ALSulami *et al.,*(2010) in Basrah were cultured on modified Columbia urea agar medium, Culture is considered the gold standard for detection of bacteria, but the method is not sensitive, and is specific only if additional testing is performed on the isolating. Cultivability of *H. pylori* was assessed by plating proper dilutions onto Columbia blood agar (CBA). *H. pylori* are a fastidious microorganism that grows very slowly, other species present may easily overgrow it, making it impossible to obtain cultivable data which could give important information. Another study was done by Sujana and Kumar, (2012) in India indicated that there were notable differences in media recipes used for isolating and culturing *H. pylori* and the ideal medium remains to be developed. Great care is needed in the collection, transport and culture of this microorganism. Collection procedure and transport of the samples and biopsy specimens had been found to be a very important factor in the successful growth of this microorganism. In this study, the samples were transported within 4 hours of collection and the rates of isolation from the two media (BHI supplement and Colombia agar supplement) were nearly the same results were recorded.

Identification of isolated *H. pylori*

The characters of isolated bacteria were determined, the microscopic examination was gram negative spiral to coccobacilli, rod shaped. The cultural morphology on Colombia and Brain Heart Infusion supplemented agar; appeared small to middle in size, rounded, and creamy in color, raised and smooth, the morphology characters of isolated *H.pylori* from different sources were comparative to that found by (Wang *et al.,* 2015) refers to the bacterium *Helicobacter pylori* is a gram-negative, microaerophilic, spiral rod with polar flagella, Giao *et al.,* (2008) referred to that morphology of the majority of cells was spiral shaped, as well as Yan *et al.,*(2013) had been demonstrated *in vitro* that *H. pylori* cells can transform from a cultivatable spiral-shaped form to a non-

cultivatable coccoid form, in which the recovery of the bacterium is very difficult by routine culture methods. on the other side (Alikhan *et al.,* 2007) showed that *H. pylori* growth in spiral and coccoid forms in the human gut. Azevedo and coworkers (2007) reported that the shape of *H. pylori* changes from the normal spiral-shaped bacillary form into the coccoid form when it is exposed to water or to other adverse conditions.

H. pylori isolates examined to Urease which was the key for more confirmation test also catalase, oxidase and motility as shown in table 3, most of the isolates were positive reaction and gave 100% positive results except the motility which only 40% could motile, the results were comparative to that found by (Yan *et al.,* 2019) as shown Urease is one of the key enzymes in *H. pylori* pathogenesis also urease is strongly immunogenic and chemotaxic for phagocytes. Otherwise AL Sulam*i* and coworkers (2010); Amieva and omar (2008), indicated that catalase, oxidase and urease; three feature of the bacterium were essential to colonization, including urease, motility and adherence, infection depends up on a combination of these bacterial enzymes, this bacteria possess enzymes like super oxidase dismutase which breaks down superoxide produced in polymer pronuclear leukocytes and macrophages and thereby prevents the killing of these organisms also had Catalase which protects *H. pylori* against the damaging effects of hydrogen peroxide released from phagocytes. Urease and catalase may be excreted from *H. pylori* to the surrounding environment and may protect this pathogen from the humeral immune response (Hawtin *et al.,*2000) about the motility which appeared when the tube white color changed to turbidity and spreads the bacteria, the rate of positivity was less than (urease, oxidase,catalase)

Table 2: Isolation of *H. pylori* by using different Cultures media.

Sources of Samples	No. of sample	Brain heart fusion agar + 7% blood Horse RBC + Antibiotic	Columbia agar +7%Horse RBC + Antibiotic	Brucella agar
Vegetables	50	2 (4%)	2 (4%)	1 (2%)

Table 3: Biochemical tests of *H. pylori* isolates.

Sources	Total Samples	No. of Isolates	Urease %	Oxidase %	Catalase%	Motility%
Vegetables	50	5	5(100%)	5(100%)	5(100%)	2(40%)

Table 4: Detection of *H. pylori* gene by PCR.

Type of Sources	No. Isolates	PCR+ve and (%)of 16srRNA	PCR +ve and (%)of*UreaA*	PCR+ve and (%) of*ureC HP*
Vegetable	5	5 (100)	5 (100)	3(60)

because of the environmental condition which is changing the bacteria growth from bacilli to cocci that's mean the bacteria lost the capability to move, Catalase, Urease, oxidase and Motility, enabling the bacteria to survive, proliferate and cause ulcer (Farhat and Abdul,2000; Sujana and Kumar, 2012).

Identification of *H. pylori* isolates by amplification *16SrRNA*, *ureA* and *ureC* / HP genes

The general bacterial gene *16SrRNA* and virulence *ureA* and *ureC* /Hp 4 were done to confirming the identification of isolated bacteria by PCR, the result as appeared that all the 5 isolates as shown in table 4; were confirmed as *H. pylori* by the amplification of 16*sRna,* as well as (100%) of the isolates possessed the gene *ureA* which responsible for producing urease enzyme; the main factors in pathogenicity as appeared in figure 1and 2, while 3(60 %) of the isolates varied in the presences of *ureC* genes as cleared in figure 3. Similar result found by AL Sulami *et al.,* (2012), when they used Polymerase chain reaction (PCR) methods to detect *H. pylori 16SrRNA* and *ureA* Gene for *H. pylori* and PCR Amplification.

16SrRNA based primers gave bands on agarose gel corresponding to a 500 base pair product when compared to the molecular ladder, this is the first report on using *16SrRNA* amplification and confirmation of *H. pylori* isolates from vegetable samples in Iraq as cleared in fig. 1. The urea genotype was expected to be present in all *Helicobacter* positive isolates. However, our study was able to detect the *ureA* gene in the isolates Fig. 2 already confirmed by*16SrRNA.* It is concluded from the study that the *ure*A gene is more sensitive for the detection of *H. pylori* than ureC gene

Controlling the growth of *H. pylori* isolates by Garlic and Green tea Extracts

The isolates appeared variability of the sensitivity to different antibiotics, the result shown in table 5 refers to those antibiotics were *H. pylori* resistant such as noroflaxacin, erythromycin and gentamycin also resist to trimethoprim and vancomycin as well as lincomycin. Concerning to another antibiotic they had the ability to inhibit the growth of these bacteria such as: Amoxicillin, Ampicillin, Ciprofloxacin, Oxacillin, Tetracyclin and the diameter of inhibition zone ranging between 20-33mm.

The present results when compared with the study for (AL Sulami *et al.,* 2010) in Basrah shown that antibiotic susceptibility tests and referred to 80% of *H. pylori* isolates were

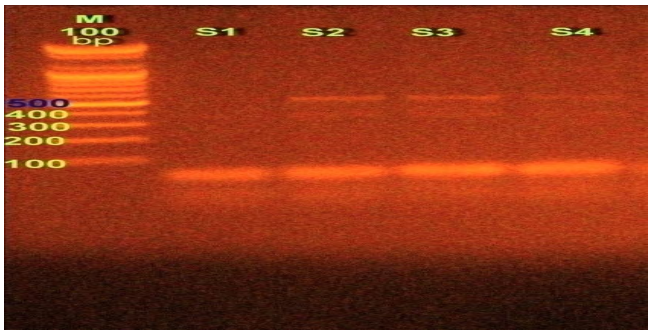

Fig. 1: Gel electrophoresis for amplification of 16s rRNA gene (product size 500 bp. Electrophoresis was performed on 1% agarose gel. Lane M is (100bp) ladder. S1 are negative control, and S2 shows positive isolates, S3 and S4 *H. pylori* isolates.

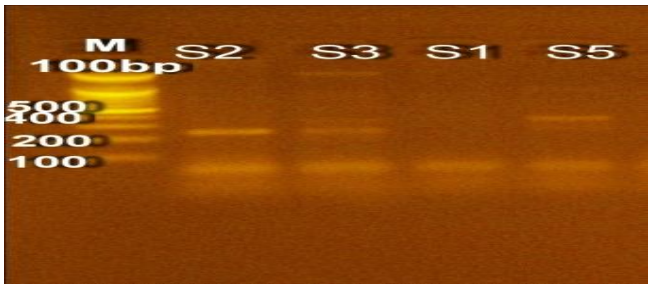

Fig. 2: Gel electrophoresis for amplification of *ureA* Gene for *H. pylori* (product size (411 bp). Electrophoresis was performed on 1% agarose gel. Lane M is (100 bp) ladder , S2 S3and S5 the isolates and S1 are negative control.

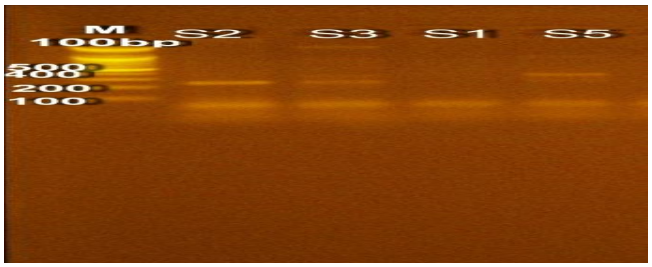

Fig. 3: Gel electrophoresis for amplification of *ureC* Gene for *H. pylori* (product size (294 bp). Electrophoresis was performed on 1% agarose gel. Lane M is (100 bp) ladder, S1 are negative control and S5 is positive control, S2 and S3 *H. pylori* isolates.

susceptible to tetracycline, 50% to ampicillin and amoxicillin, 40% to kanamycin, and rifampicin and 30% to erythromycin. The same results obtained by (Shohreh *et al.,* 2010) in Iran, they referred to the susceptibility of *H. pylori* strains were resistant to metronidazole, amoxicillin, clarithromycin, and tetracycline, also they indicated that the prevalence of resistance varies geographically and that there is a broad range of resistance variability depending on the drug used, on the other hand Torres and coworkers (2009) and Malfertheinerb and coworker, 2017, they been shown that resistance to different antibiotics develops in *H. pylori* strains by acquiring chromosomal mutations at the site where the drug acts.

The results shown as appeared in table 7; ethanol extracts of green tea had the ability to inhibit the growth of the bacteria, the inhibition zone ranging between(21-22mm), but the range of inhibition zone was(20-22mm) for the watery green tea extract against the isolated bacteria, this result refers to different size of inhibition zone between ethanol and water extract to inhibiting the growth of the bacteria *H. pylori.* This result comparative with (Calin *et al.,* 2009) they found that the Components of green tea have been shown to inhibit bacterial growth of *Helicobacter* spp. as well as they assessed the bactericidal and/or bacteriostatic effect of green tea against *Helicobacter felis* and *H. pylori* in *vitro* and evaluated the effects of green tea on the development of *Helicobacter*-induced gastritis in an animal model. The studies demonstrate profound growth effects of green tea against *Helicobacter* and, importantly, demonstrate that green tea consumption can prevent gastric mucosal inflammation if ingested prior to exposure to *Helicobacter* infection. Research in the area of natural food compounds and their effects on various pathogenic bacteria has gained increased acceptance in the past several years. Components within natural remedies such as green tea could be further used for prevention and treatment of *Helicobacter*-induced gastritis in humans (Harada *et al.,* 2004), the green tea catechins may inhibit the *H. pylori* urease (Shibata *et al.,*2005), The importance of green tea and its components has been reviewed by (Hamilton,1995) he evaluated the bactericidal and/or bacteriostatic effect of green tea on *H. pylori* and showed that green tea, in an amount that could be clinically consumed, has bactericidal and bacteriostatic effects *in vitro*. *In vivo* studies demonstrated that consumption of green tea when taken before infection prevents gastric mucosal inflammation and when taken after infection is established diminishes the magnitude of gastritis. The greatest impact on decreasing bacterial counts and limiting inflammation is in those mice that received green tea prior to infection. It is interesting to note, however, that even with an established infection green tea decreased the number of bacteria and the inflammatory score. Some epidemiological studies have suggested that green tea offers protection against gastric cancer (Yu *et al.,* 1995).

Effect of Garlic extract on the growth of *H. pylori*

The results shown in table 8 that garlic extract had the ability to inhibit the growth of the bacteria, the inhibition zone ranging between (18-20mm), while the range of

inhibition zone was (21-24mm) for the watery garlic extract against the isolated bacteria, this result refers to different size of inhibition zone between ethanol and water extract to inhibiting the growth of the bacteria. This result comparative with (Cellini *et al.,* 1996), they found that the antibacterial effect of aqueous garlic extract (AGE) was investigated against *Helicobacter pylori*. Heat treatment of extracts reduced the inhibitory or bactericidal activity, Fozieh and coworkers (2014) determined the antibacterial effects of garlic on multi-drug resistance *H. pylori* isolates from gastric biopsies. Allicin is a water-soluble compound composed of diallylthiosulphonate and has strong inhibitory effects on gram-negative enteric bacteria and *Staphylococcus aureus* (Shokrzadeh and Ebadi,2006), allicin is also very effective on antibiotic-resistant bacteria and no resistance to it has been recorded (Han *et al.,* 1995), the antibiotic qualities of garlic appear to be a direct result of the allicin produced from raw, crushed garlic. This is destroyed by age and cooking, cooked garlic has virtually no antibiotic value although it still retains other benefits (O'Gara *et al.,* 2000).Garlic has high antibacterial properties on a wide spectrum of gram-positive and gram-negative bacteria. The main mode of action of allicin in bacteria is through bacteria RNA; It is mainly due to inhibition of RNA synthesis, especially protein and DNA synthesis in bacteria (Feldberg *et al.,* 1988) and the permeability of cell wall to allicin is also important. Since the amount of lipids in the cell wall of gram-negative bacteria is greater than gram-positive bacteria, entry of allicin is easier in gram-negative bacteria (Sivam *et al.,* 1997).

Table 7: Effect of Ethanol and Watery Green tea Extracts on the growth of *H. pylori.*

The Isolates	Ethanol Extract of green tea inhibition zone (mm)	Water Extract of Green tea inhibition zone (mm)
SV1	22	22
SV2	21	20
SV3	23	21
SV4	20	20
SV5	21	20

Table 8: Effect of garlic extract on the growth of *H.pylori.*

Sources of Isolates	No. of Isolates	Ethanol garlic extract inhibition zone (mm)	Water garlic extract inhibition zone (mm)
Vegetables	SV1	18	24
	SV2	19	22
	SV3	20	22
	SV4	20	24
	SV5	18	21

In conclusion, leafy vegetable samples harbor *H. pylori* Which identified depending on physiological characteristic and confirmed by 16SrRNA gene as well as detecting some virulence *UreC* and *ureA* genes. Also, there was similarity in the genotyping pattern of *H. pylori* DNA between vegetable samples, contaminated vegetables with *H. pylori* strains may be a threat to human health. Our findings should raise awareness about antibiotic resistance in *H. pylori* in Erbil city. Finally antibacterial activity of green tea and garlic as a bio controlling with different concentration effected on the *Helicobacter pylori* in watery solution and in ethanol solution.

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