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BACTERIOLOGICAL STUDIES ON THE TRANSMISSION OF HELICOBACTER PYLORI

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ABSTRACT

The present study aims to detect Helicobacter pylori by isolation of samples (stool, saliva, dental plaque) from patients suffering from gastric disorders and to study the transmission of Helicobacter pylori by isolation from other samples (water, milk, luncheon, sausage, frankfurt, and beaf). This study has been carried out on 50 cases including 35 sputum samples, 29 dental plaque samples, and 35 stool samples. All of samples were collected from patients suffering from gastroduodenal disorders (gastritis, duodenitis, gastric ulcer, duodenal ulcer). Patients were 16 males and 34 females, with ages ranging from 16 -75 years. Twenty water samples (10 samples before water station, 10 samples after water station), 10 milk samples, 10 luncheon samples, 5 beaf samples, 10 frankfurt samples, and 10 sausage samples were collected from the environment surrounding the patients. Two types of antibiotic sensitivity test were done for the tested isolate of H.pylori. The first was plant extract sensitivity test in which equal volumes of plant extract were put into wells cut by sterile cork borer and incubated at 37°C for 24 hours. The second was the antibiotic sensitivity test in which the antibiotic discs were placed on surface of chocolate agar plates heavily streaked with young culture of H. pylori and incubated at 37°C for 24 hours.

he study showed that there is a significant correlation between age of patients and H.pylori infection (P= 0.013) as well as a correlation between the pathogenicity and the prevalence of H.pylori. The study shows a significant correlatin between the samples of foodstuffs and positive H.pylori and no significant correlatin between water samples and H.pylori.

It is found that H.pylori is highly sensitive to white pepper, Cinnamomum zeylanicum, Syzygium aromaticum, and followed by black pepper, Apium graveolens, Cuminum cyminum. Helicobacter pylori showed less sensitivity to Martricaria chamomilla, Elettaria cardamomum, Solenostemna arghel, and highly resistant to Nigella sativa, Foeniculum vulgare, Zinigber officinalis, Rosmarinus officinalis, and Allium sativum.

H. pylori is highly susceptible to Cefotaxime , and Ertapenem ,followed by Ceftriaxone, Imipenem, and Ceftazidime while H.pylori showed less sensitivity to Levofloxacin, Ciprofloxacin, and E-Moxaclav.

Keywords: *Helicobacter pylori, Antibiotic sensitivity test, H.pylori transmission.*

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Keywords: *Helicobacter pylori, Antibiotic sensitivity test, H.pylori transmission.*

INTRODUCTION

Helicobacter pylori, previously known as *Campylobacter pylori*, is a gram negative spiral microaerophilic bacterium that can inhibit various areas of the stomach and duodenum. It was first isolated from gastric mucosa by Warren and Marshall in 1983. Its presence is associated with gastritis, peptic ulcers, duodenal ulcers, and chronic gastritis and has been recognized as class I gastric carcinogen (Labgine and de Resue H., 1996; McColl KEL., 1997; Riegg *et al.*, 1995). The prevalence of *H.pylori* in the developed countries is about 50% of adults and is about 90 % in the developing countries (Farthing Mjg., 1998; Dunn BE *et al.*, 1997; Bardhan PK., 1997). Over 80% of individuals infected with the bacterium are asymptomatic.

The surface of the human stomach mucosa is the only known habitat of *H.pylori*, but the organism has occasionally been detected in gastric juices, saliva, dental plaques, bile and faeces (Owen, 1995). The bacteria colonize on the surface of the mucosa, especially of the antrum, and the overlying layer of mucus.

Childhood is the critical period for *H.pylori* infection and transmission most probably occurs from person to person. The person- to-person mode of transmission is supported by the higher incidence of infection among institutionalized children and adults and the clustering of *H. pylori* infection within families. Also lending support to this concept is the detection of *H. pylori* DNA in vomitus, saliva, dental plaque, gastric juice, and feces (Brown LM., 2000). There are three possible routes of transmission: Iatrogenic route through endoscope, faecal- oral route through transmission of faeces to drinking water or to irrigation water then to food, and oral – oral route through saliva or vomitus (Dunn et al., 1997; Akmatsu T *et al.*, 1996; Neale KR, and logan RPH., 1995). *H.pylori* has been found in the faeces, and survival and transmission can occure via faeces-contaminated water. Transmission can also occur via uncooked vegetables that had been irrigated with water contaminated with sewage.

MATERIALS AND METHODS

This study has been carried out on 50 cases including 35 sputum samples, 29 dental plaque samples, and 35 stool samples. All of samples were collected from patients suffering from gastroduodenal disorders (gastritis, duodenitis, gastric ulcer, duodenal ulcer). Patients were 16 males and 34 females, with ages ranging from 16 -75 years.

Each sample, by using sterile glass rod, was inoculated onto chocolate agar selective media which prepared as follow (39 g Columbia Agar Base + *H.pylori* selective supplement+ Human blood or heamoglobin 10%). The samples were incubated in Co_2 incubator at 37 °C with microaerophilic condition of 5% O_2, 10% Co_2, 85% N_2 and 99% relative humidity for 5-7 days. The identification of *H. pylori* was done (morphology, Gram's stain, urease test, catalase test and oxidase test).

Two types of sensitivity tests were done. First, plant extract sensitivity test in which Mentha longifolia, Cyperus alopecuroides, Withania somnifera, Datura stramonium, Silybum marianum, Plantago major, Hyoscyamus muticus, Pulicaria undulata, Achillea fragrantissima and Artemisia judaica were

dried at room temperature (20 – 23 °C) and ground into a powder using a blender . The dried plant powders were macerated with methanol (80%) with continuous shaking for two days at room temperature. The extract was filtered and the filtrate was evaporated to dryness. A stock solution of extract was reconstituted in dimethyle sulfoxide (DMSO) and preserved under refrigeration until using (Mehraban et al., 2005). Four solvents varied in their polarity were used for extraction (petroleum ether 60-80 °C, chloroform, ethyl acetate and ethanol 95%). Sample of 150g powder was soaked in 750ml of the first solvent (petroleum ether 60-80 °C) for 3 days in brown coloured bottles (1.5 litters), which used as containers and was provided with tight stoppers. In addition, the extraction solution in the brown coloured bottles was intermittently shaked by an electric shaker for 2 hours daily. After that, the combined extract was filtered. The solvent was evaporated under reduced pressure using a rotary evaporator at temperatures not exceeding 50 °C. The marc was subsequently subjected to extraction with the second solvent (chloroform). As for the third solvent (ethyl acetate) and the last solvent (ethanol 95%), the same procedure was used. By using sterile cork borer, wells were cut from the plates. Equal volumes of plant extract were put into each well. After incubation at 37°C for 24 hours, plate is examined, and the complete inhibition zone is indicated. The antibacterial activity was recorded as the diameter (cm) of the clear inhibition zone surrounding the disks.

Second, antibiotic sensitivity test in which Cefotaxime (CTX), Sulfamethoxazole/ Trimethoprim (SXT), Azithromycin (AZM), Flomoquine (UB), Levofloxacin (LEV), Vancomycin (VA), Ciprofloxacin (CIP), Imipenem (IPM), E-Moxaclav (AMC), Ampcillin / Sulbactam (SAM), Ceftriaxone (CRO), Cefoprazone (CFP), Ceftazidime (CAZ), Ertapenem (ETP) were used. The antibiotic discs were placed on the surface of chocolate agar plates heavily streaked with a young culture of *H. pylori* and incubated at 37°C for 24 hours.

RESULTS

After incubation period, colonies appear as a small pin point (1-2 mm) trans-lucent and non hemolytic. Microbiological identification of *H.pylori* includes Gram stain, urease test, catalase test and oxidase test (Figs. 1-4). *Helicobacter pylori* was identified as gram–negative curved or S-shaped bacilli (Fig. 1), positive urease test (Fig. 2), positive Catalase test (Fig. 3), and positive oxidase test (Fig. 4).

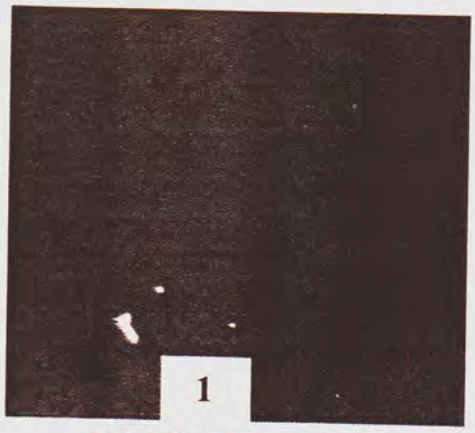


Fig. (1) : Gram–negative Curved bacilli

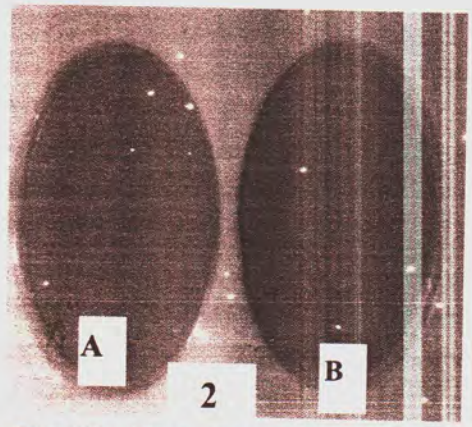


Fig. (2) : Christensen urea agar test
(A) Negative urease .
(B) positive urease.

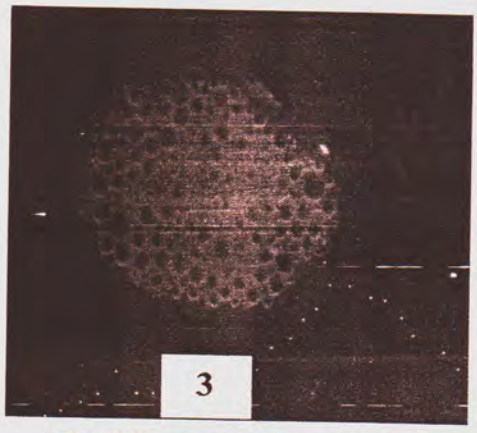


Fig. (3) : Positive Catalase test

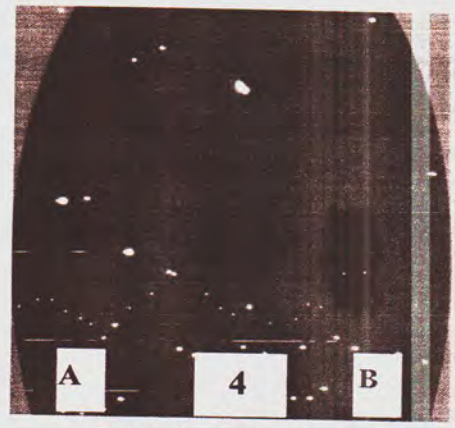


Fig. (4) : Oxidase test
A : negative oxidase test
B : positive oxidase test

Table 1 and Fig. 5 show the correlation between different patients age and positive *H.pylori* indicating that there is a significant correlation between age of patients and *H.pylori* infection (P =0.013) .

Table (1) : Correlation between patients age and positive H.pylori

Age	No. of patients	Positive *H.pylori*	
		No. of patients	%
25-35	12	9	66.6
36-45	15	10	60.0
46-55	13	9	61.0
56-65	5	1	20.0
66-75	5	1	20.0
Total	50	30	60.0

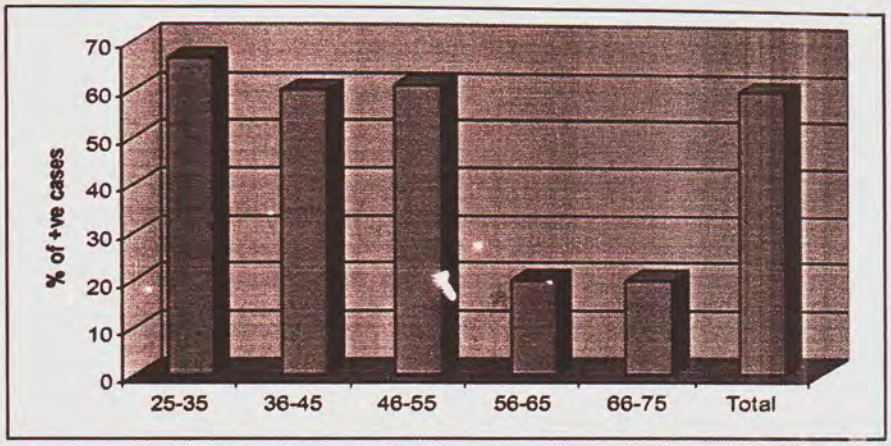


Fig. (5) : Shows the correlation between different patients age and positive *H.pylori*

Correlation between the pathogenicity and positive *H. pylori* was given in Table (2) and shown in Fig (6) .

Table (2) : shows the correlation between the pathogenicity and positive *H.pylori.*

Pathogenicity	No. of patients	Positive *H.pylori*	
		No. of patients	%
Gastritis	13	10	76.9
Duodenitis	3	1	33.3
Gastric Ulcer	8	6	75
Duodenal Ulcer	5	3	60
Normal (Gastric Troubles)	21	10	52.3

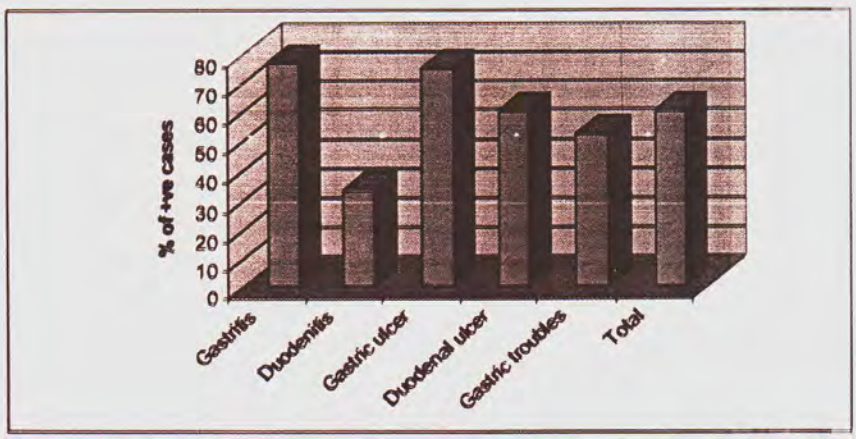
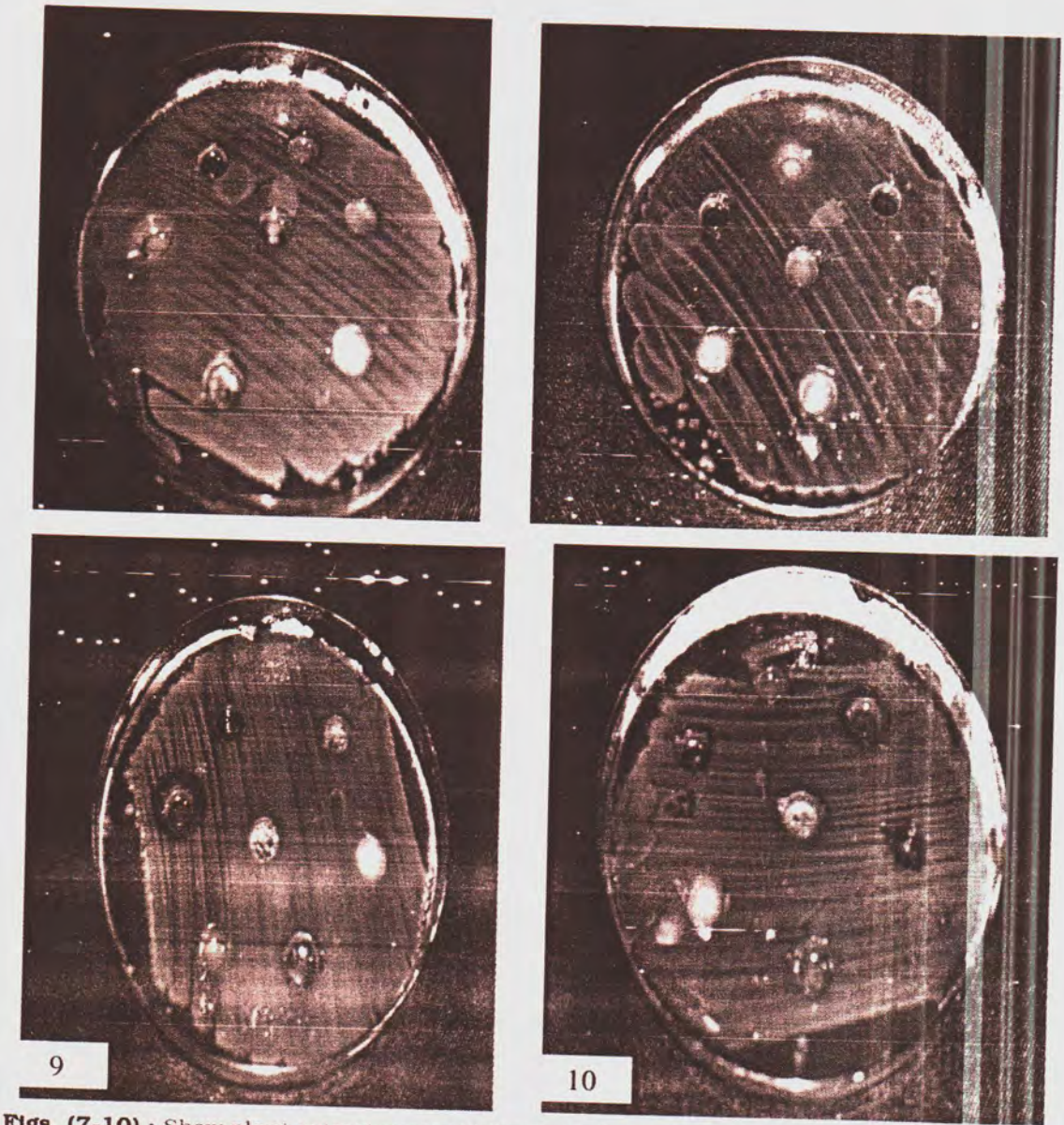


Fig. (6) : Shows the correlation between the pathogenicity and positive *H.pylori*

The results of the well diffusion test Figs. (7- 10) indicated that *H.pylori* is highly sensitive to white pepper, *Cinnamomum zeylanicum, Syzygium aromaticum*, and followed by black pepper, *Apium graveolens. Cuminum cyminum* .

Helicobacter pylori showed less sensitivity to *Martricaria chamomilla, Elettaria cardamomum, Solenostemna arghel*, and highly resistant to *Nigella sativa, Foeniculum vulgare*, Zinigber officinalis, *Rosmarinus officinalis* , *Rosmarinus officinalis, Allium sat-*



Figs. (7-10) : Show plant extract susceptibility test of *H.pylori* against different plant extracts.

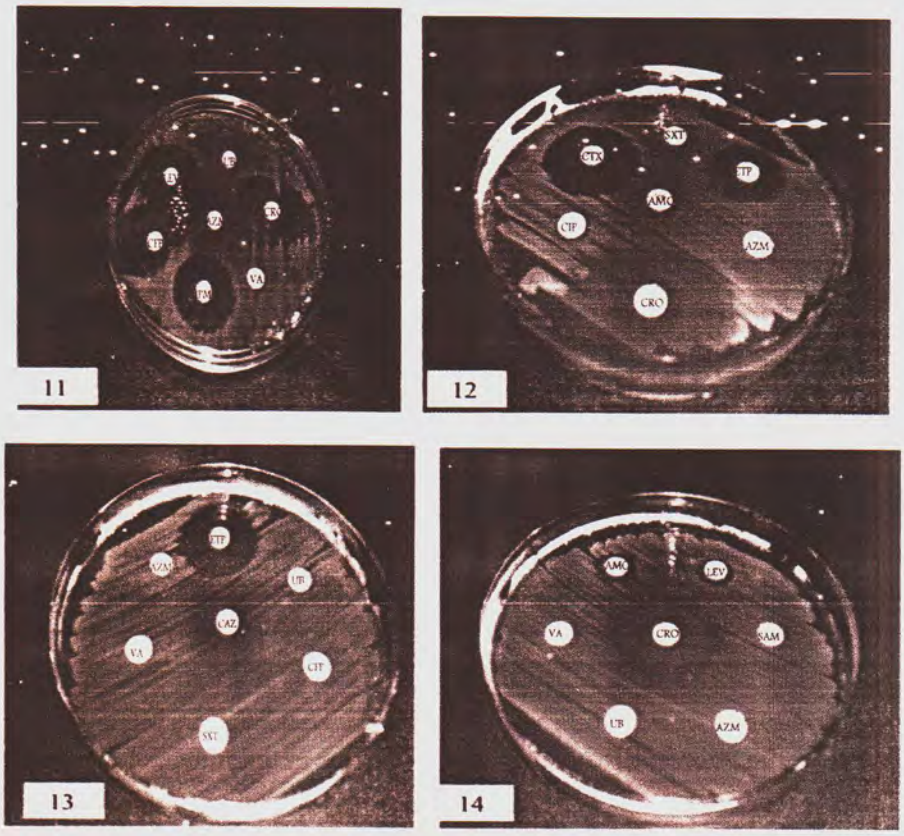
The isolated *H. pylori* was tested for its antibiotic susceptibility using fourteen different antibiotics as shown in Table (3).

Table (3) : Types of antibiotic used, their concentrations and effects.

No	Antibiotic	Abbreviation	Concentration (µg)	Zone of inhibition(mm)
1	Cefotaxime	CTX	30	36
2	Sulfamethoxazole/Trimethoprim	SXT	25	R
3	Azithromycin	AZM	15	R
4	Flomoquine	UB	30	R
5	Levafloxacin	LEV	5	20
6	Vancomycin	VA	30	R
7	Ciprofloxacin	CIP	5	26
8	Imipenem	IPM	10	28
9	E-Moxaclav	AMC	30	18
10	Ampcillin/ Sulbactam	SAM	20	R
11	Ceftriaxone	CRO	30	32
12	Cefoprazone	CFP	75	R
13	Ceftazidime	CAZ	30	34
14	Ertapenem	ETP	10	36

(R = Resistant)

Data in Table 3 revealed that *H. pylori* is highly susceptible to Cefotaxime , and Ertapenem giving rise to maximum inhibition zone (36 mm), followed by Ceftriaxone, Imipenem, and Ceftazidime (32 mm), while showed less sensitivity to Levofloxacin, Ciprofloxacin, and E-Moxaclav giving rise to zone diameters (20 mm) , respectively *H. pylori* was highly resistant to the other six examined antibiotics.



Figs. (11-14) : Antibiotic susceptibility test for isolated *H. pylori* against different antibiotics.

DISCUSSION

Helicobacter pylori is a spiral gram negative microaerophilic bacteria that has been first isolated and collected from gastric mucosa by Warren and Marshall (1983). It was originally classified as *Campylobacter* but was subsequently reclassified as a new genus, Helicobacter. It is adapted to colonize the gastric epithelium which results in gastritis ,peptic ulcers, duodenal ulcers, and chronic gastritis and has been recognized as class I gastric carcinogen (Labgine and de Resue, 1996; McColl KEL,1997, and Riegg *et al.*,1995).There is one non-gastric Helicoacter species , H. hepaticus (mouse liver),has been identified (Fox *et al.*,1994).

In 1995, Edward Arnold showed that the morphology of *H.pylori* is distinct which is short spiral or S-shaped in vivo and curved rod in vitro. *H.pylori* has four to six sheathed flagella which are essential for motility (Malfertheiner P. and Pieramico O., 1992). *Helicobacter pylori*, to grow, needs low oxygen (microaerophilic), Co2 (5-20) %, and high humidity (Edward Arnold, 1995). *Helicobacter pylori* grows at 37ºC for 3-5 days which appear as circular convex translucent.

Person –to-person contact is considered the most likely transmission route of *H. pylori*. No predominant route of transmission, but there are possible routes including iatrogenic route through endoscopes, feacal-oral route through transmission of feaces to drinking water or to irrigation water then to food, oral-oral route through saliva or vomitus, and gastro-oral route through vomitus or gastroesophageal reflux (Thomas *et al.*, 1992; Krajden *et al.*, 1989; Axon, 1995). There are sources of H.pylori infection such feaces –contaminated water, uncooked vegetables, fresh poultry, fresh meats, and some dairy products (Hopkins *et al.*, 1993).

For *H. pylori* identification, the isolates were subjected to microbiological tests such as (Gram stain, urease test, catalase test, and oxidase test).

The study shows the correlation between different patients age and positive *H. pylori* indicating that there is a significant correlation between age of patients and *H. pylori* infection (P =0.013). The results of this study indicated the correlation between the pathogenicity and the prevalence of *H. pylori*. The results revealed that 76.9 % of cases showed gastritis, 33.3% duodenitis, 75% gastric ulcer, 60% duodenal ulcer, and 52.3% gastric troubles. *H.pylori* is found clearly in stool samples than sputum samples. The results revealed that *H.pylori* is found in 76.9% (of stool samples), 23% (of sputum samples) of gastritis cases , 33.3% (stool), 0%(sputum) of duodenitis cases, 75%(stool), 25% (sputum) of gastric ulcer cases, 60% (stool), 0% (sputum) of duodenal ulcer cases, and 52.3% (stool), 19% (sputum) of gastric troubles.

On the other hand, other samples were collected in this study from the environment surrounding the patients suffering from gastric disorders including water, milk, luncheon, sausage, Frankfurt, beaf to study whether the foodstuffs have a role in the transmission of *H.pylori* or not. The results confirmed that there is a significant correlation between the samples of foodstuffs and the prevalence of *H.pylori* (P=0.023) showing that Frankfurt,

luncheon, and sausage have high percentage of *H.pylori* prevalence with (40%, 50%, 60%) respectively. On contrast, beaf, water, milk have low percentage of *H.pylori* prevalence with (0%, 10%, 20%) respectively.

To study the correlation between water samples and the prevalence of *H.pylori*, 10 water samples have been collected before the inlet of water station and 10 samples the inlet of after water station. The results proved that 20% of samples before the station have *H.pylori* and 0% after the station showing no significant correlation between water samples and the prevalence of *H.pylori* (McNemar P=0.50).

The current study showed the effect of 14 plant extracts on *H.pylori* growth. The present study showed that *H.pylori* was highly sensitive to white pepper, *Cinnamomum zeylanicum, Syzygium aromaticum*, and followed by black pepper, *Apium graveolens, Cuminum cyminum* . However, the isolated organism was less sensitive to *Martricaria chamomilla, Elettaria cardamomum, Solenostemna arghel*, and highly resistant to *Nigella sativa, Foeniculum vulgare, Zinigber officinalis, Rosmarinus officinalis, Allium sativum.*

By another way of susceptibility test, the antibiotic sensitivity test was done for the tested isolate of *H.pylori*. This test indiated that the organism was sensitive to Cefotaxime , and Ertapenem followed by Ceftriaxone, Imipenem, and Ceftazidime. However, the isolated organism was resistant to Levofloxacin, Ciprofloxacin, and E-Moxaclav.

Balah (2004) found that *H.pylori* is sensitive to Ceftraxone, Cefamandole, Cefoperazone, Imipenem, Amoxicillin+Clavulanic acid, Tobramycin, Cefotaxime, Cefuroxime, Amikacin, and Ampicillin However, the isolated organism was resistant to Vancomycin, Lincocin-Dalacin, Flumequine, Erythromycin, Methicillin, Metronidazol, Aztreonam, Ampicillin Penbritin, Neomycin, and Vibtamycin .

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