CHAPTER IV

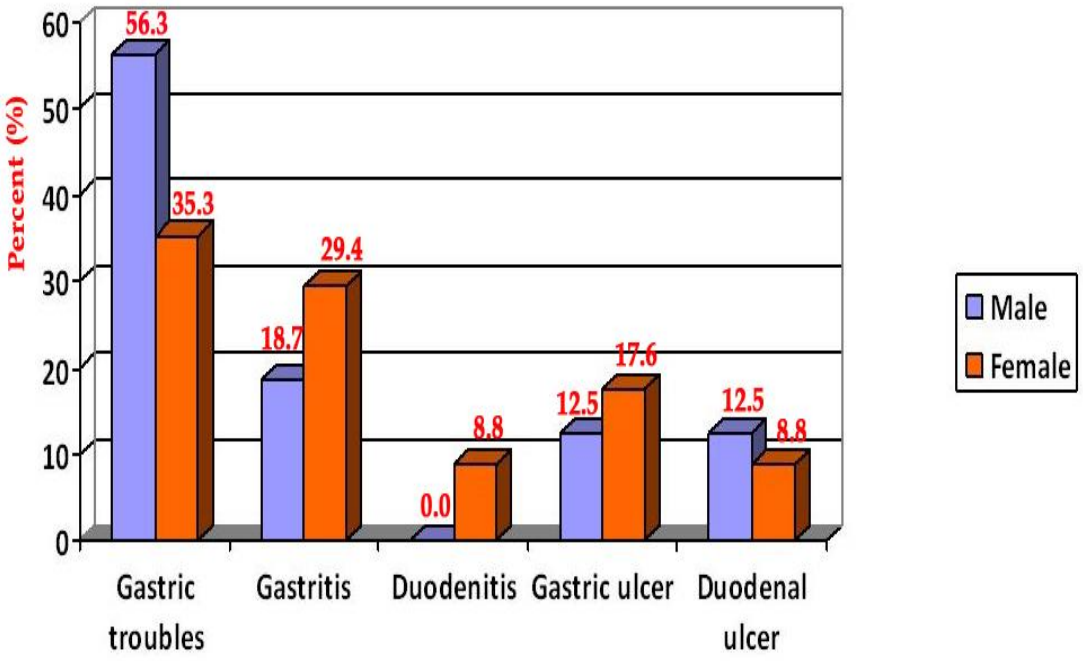
RESULTS

This study has been carried out on 50 patients including 34 females and 16 males. The patients ages ranged from 25 to 75 years. They were suffering from gastric troubles (42%), gastritis (26%), duodenitis (6%), gastric ulcer (16%) and duodenal ulcer (10%) **(Table 2)**.

Table (2): Pathogenicity of 50 patients in relation to sex of patient:

Sex	Pathogenicity					Total
	Gastric Troubles	Gastritis	Duodenitis	Gastric Ulcer	Duodenal Ulcer	
Male	9	3	0	2	2	16
Percent (%)	**56.3%**	**18.7%**	**0.0%**	**12.5%**	**12.5%**	**100%**
Female	12	10	3	6	3	34
Percent (%)	**35.3%**	**29.4%**	**8.8**	**17.6%**	**8.8%**	**100%**
Total	21	13	3	8	5	50
Percent (%)	**42%**	**26%**	**6%**	**16%**	**10%**	**100%**

X^2 Chi square (Test of significance) = 3.352, P= 0.5



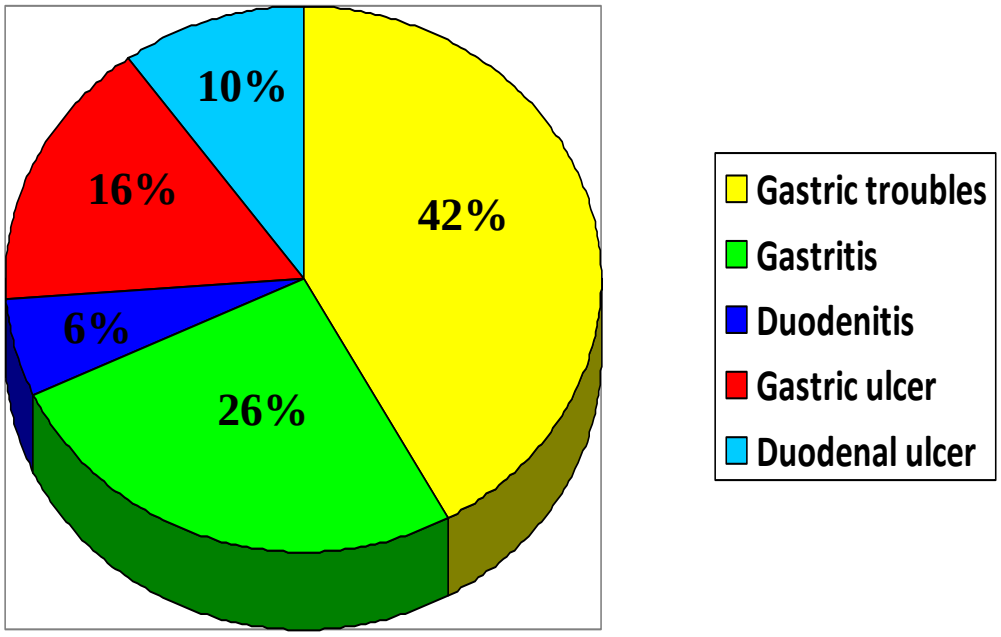


Figure (4): Pathogenicity of 50 patients in relation to sex of patients and pathogenicity total percents of 50 patients .

Table (3): Relationship between positive *H. pylori* and sex of patients:

Sex of Patient	No. of Patient	Positive *H. pylori*	
		No. of Patient	Percent (%)
Male	16	7	**43.8**
Female	34	23	**67.6**
Total	50	30	**60**

X^2 Chi square (Test of significance) = 2.589, P= 0.108, Risk value= 2.688

This table shows the correlation between patients sex and positive *H. pylori.* It is apparent that 16 patients are male, there were seven (43.8%) cases positive and 34 patients are female, there were 23 (67.6%) cases positive. From this table, it has been found that positive *H. pylori* increased in females than in males.

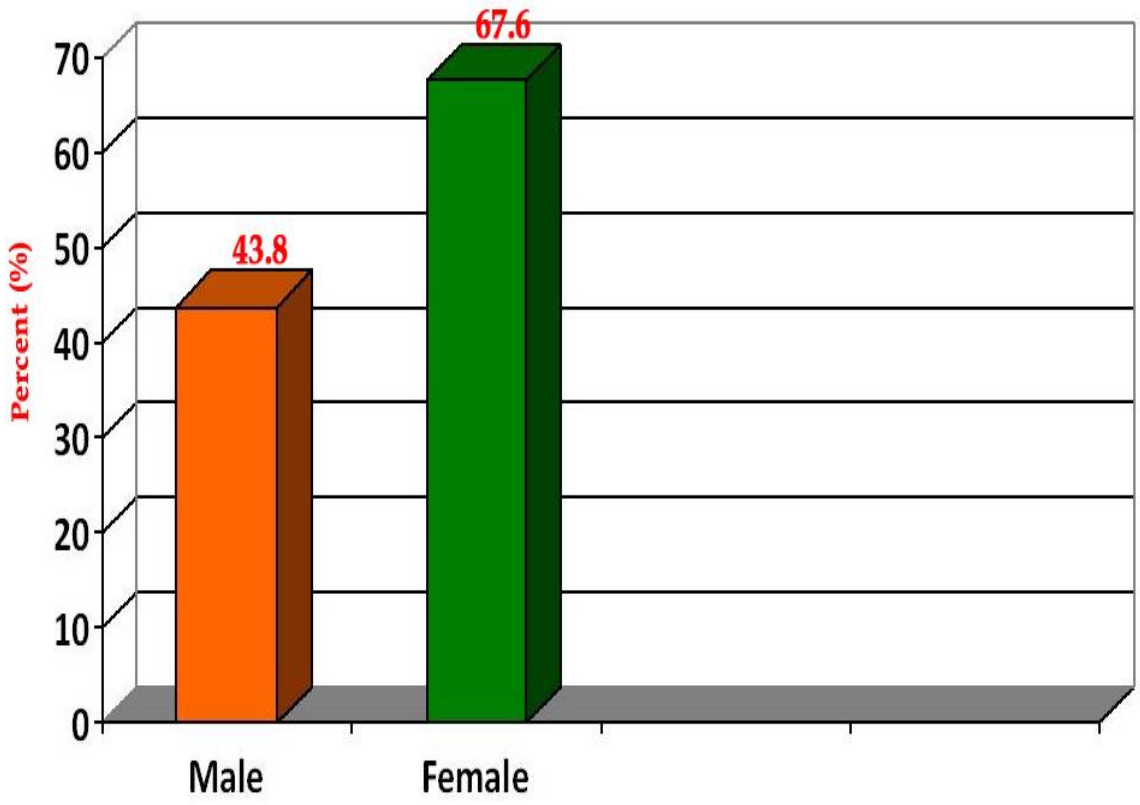


Figure (5): Relationship between positive *H. pylori* and sex of patients.

Table (4): Relationship between positive *H. pylori* and age of patients:

Age of Patient	No. of Patient	Positive *H. pylori*	
		No. of Patient	Percent (%)
25 – 35	12	9	**66.6**
36 – 45	15	10	**60**
46 – 55	13	9	**61**
56 – 65	5	1	**20**
66 – 75	5	1	**20**
Total	50	30	**60**

This table shows the relationship between age of patients and positive *H. pylori*. It is apparent that 12 patients were age 25 – 35 years, there were 9 (66.6%) positive cases, 15 patients were age 36 – 45 years, there were 10 (60%) positive cases, 13 patients were age 46 – 55 years, there were 9 (61%) positive cases, 5 patients were age 56 – 65 years, there were 1 (20%) positive case and 5 patients were age 66 – 75 years, there were 1 (20%) positive case. From this table, it has been found that the positive *H. pylori* cases increased in age between 25 – 55 years.

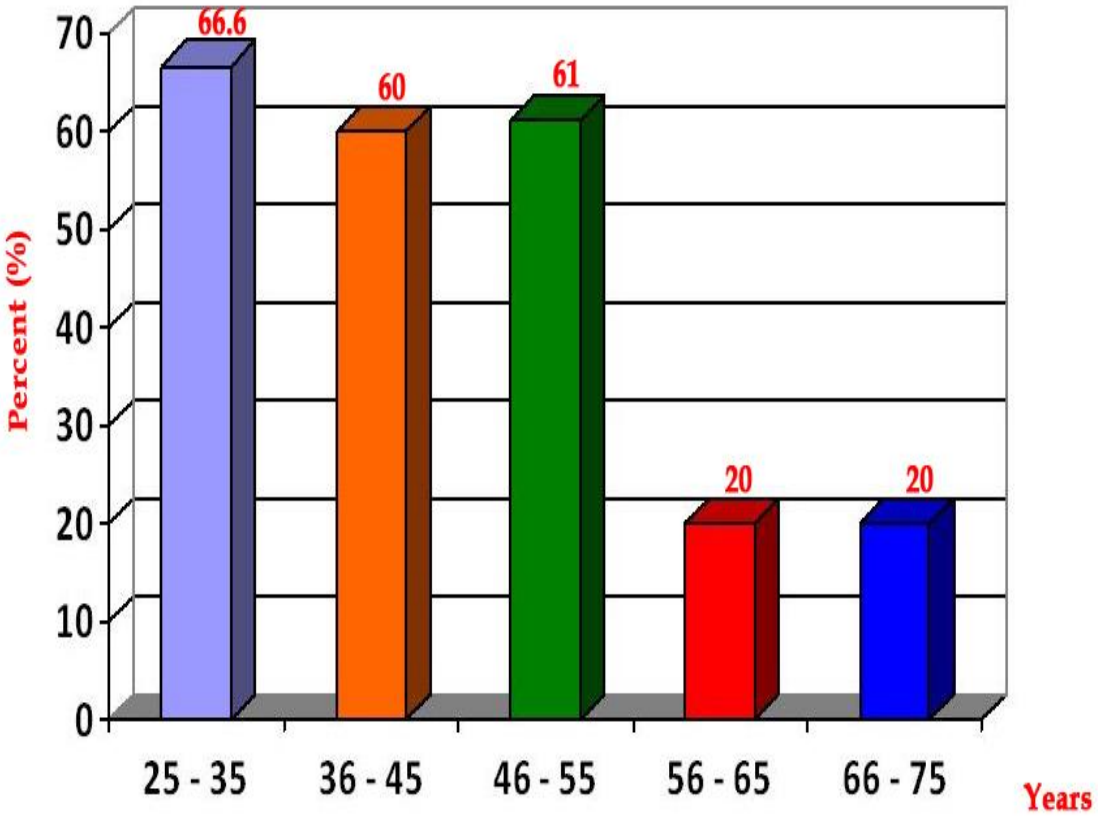


Figure (6): Relationship between positive *H. pylori* and age of patients.

Table (5): Relationship between sex of patients and sputum samples:

Sex of Patient	Sputum Samples				Total	
	Negative	Percent (%)	Positive	Percent (%)	No. of patients	Percent (%)
Male	14	**87.5**	2	**12.5**	16	**100**
Female	27	**79.4**	7	**20.6**	34	**100**
Count	41	**82**	9	**18**	50	**100**

X^2 Chi square (Test of significance) = 0.482, P= 0.487, Risk value= 1.815

The result in this table shows the relationship between patients sex and sputum samples. It is apparent that 16 patients are male, there were 14 (87.5%) cases are negative and 2 (12.5%) cases are positive and 34 patients are female, there were 27 (79.4%) cases are negative and 7 (20.6%) cases are positive. Total positive of sputum is 9 (18%) cases. From this table, it has been found that positive *H. pylori* cases are low in sputum samples whether in relation to the males and females.

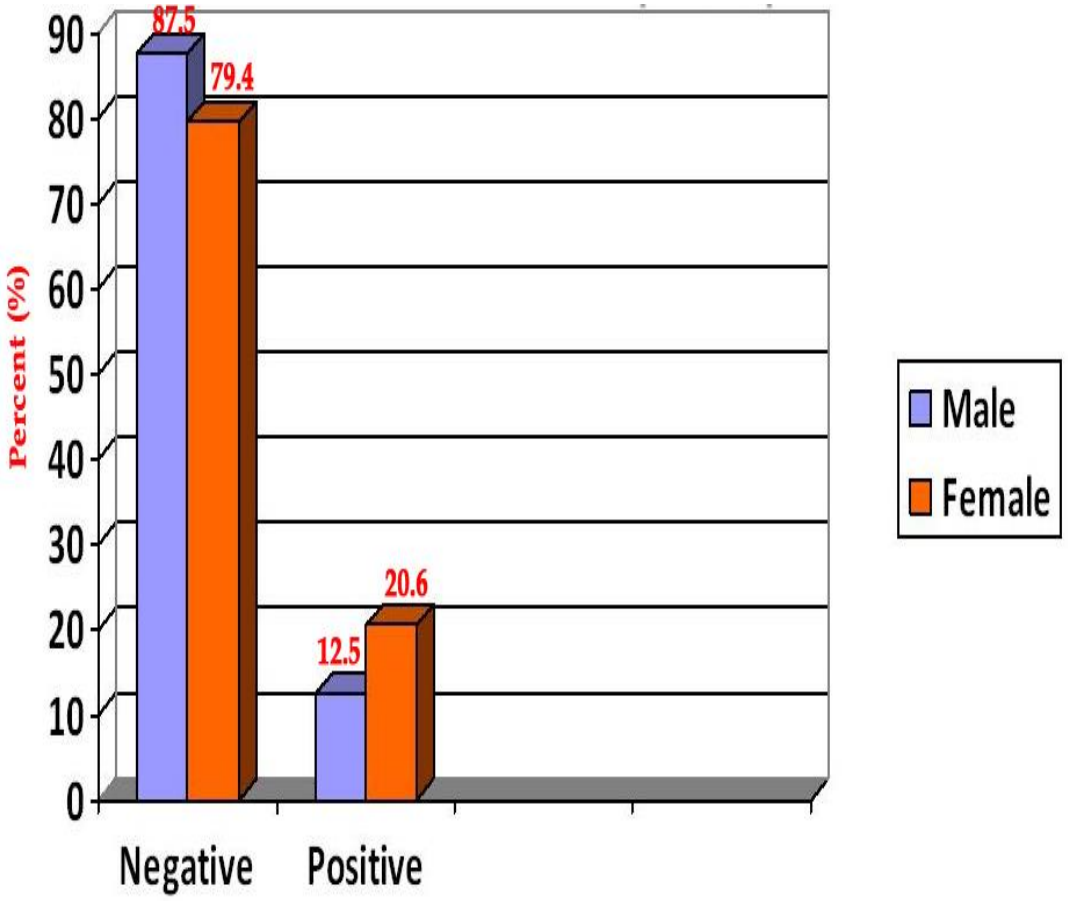


Figure (7): Relationship between sex of patients and sputum samples.

Table (6): Relationship between sex of patients and stool samples:

Sex of Patient	Stool Samples				Total	
	Negative	Percent (%)	Positive	Percent (%)	No. of patients	Percent (%)
Male	9	**56.3**	7	**43.8**	16	**100**
Female	11	**32.4**	23	**67.6**	34	**100**
Count	20	**40**	30	**60**	50	**100**

X^2 Chi square (Test of significance) = 2.589, P= 0.108, Risk value= 2.688

The result in this table shows the relationship between patients sex and stool samples. It is apparent that 16 patients are male, there were 9 (56.3%) cases are negative and 7 (43.8%) cases are positive and 34 patients are female, there were 11 (32.4%) cases are negative and 23 (67.6%) cases are positive. Total positive of stool is 30 (60%) cases. From this table, it has been found that positive *H. pylori* cases are high in females stool samples and moderate in males stool samples.

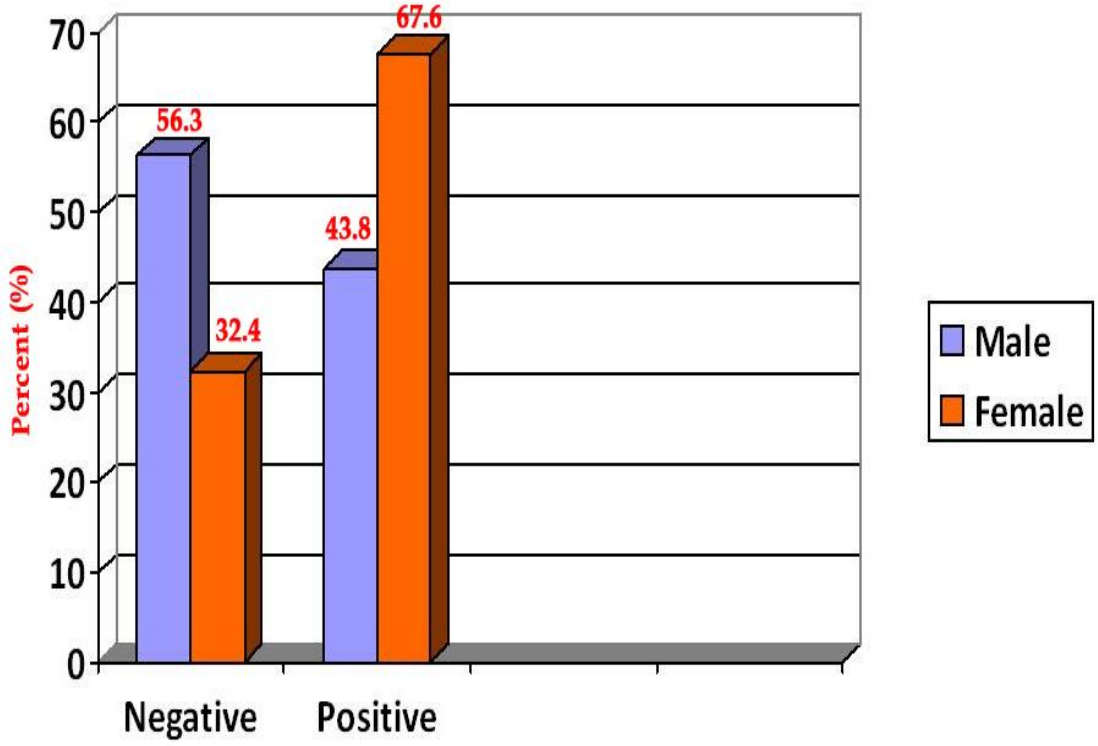


Figure (8): Relationship between sex of patients stool samples.

Table (7): Relationship between sex of patients and positive stool, sputum and dental plaque samples:

Sample / Sex	No. of Patients	Positive Stool		Positive Sputum		Positive Dental Plaque	
		No. of Patients	Percent (%)	No. of Patients	Percent (%)	No. of Patients	Percent (%)
Male	16	7	**43.8**	2	**12.5**	0	**0**
Female	34	23	**67.6**	7	**20.5**	0	**0**

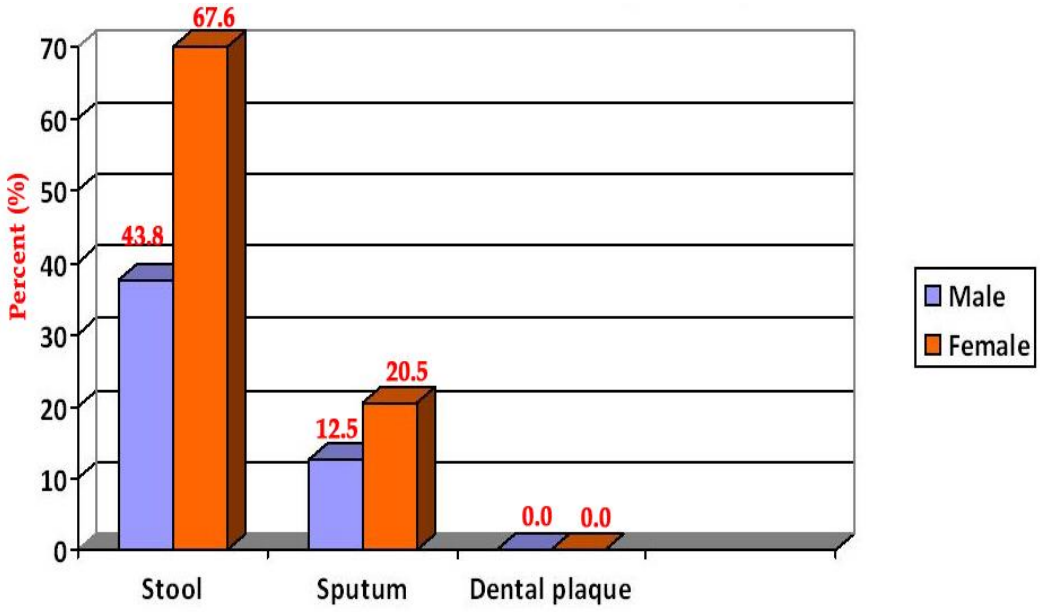


Figure (9): Relationship between sex of patients and positive stool, sputum and dental plaque samples.

The correlation between age of patients and positive samples (stool, sputum and dental plaque) was depicted in table (7) and Fig. (9). It is apparent that 12 patients were age 25 – 35 years, there were 9 (66.6%) stool positive cases, 2 (16.6%) sputum positive cases and 0 (0.0%) dental plaque positive cases, 15 patients were age 36 – 45 years, there were 10 (60%) stool positive cases, 4 (26.6%) sputum positive cases and 0 (0.0%) dental plaque positive cases, 13 patients were age 46 – 55 years, there were 9 (61%) stool positive cases, 3 (23.7%) sputum positive cases and 0 (0.0%) dental plaque positive cases, 5 patients were age 56 – 65 years, there were 1 (20%) stool positive case, 0 (0.0%) sputum positive cases and 0 (0.0%) dental plaque positive cases and 5 patients were age 66 – 75 years, there were 1 (20%) positive case, 0 (0.0%) sputum positive cases and 0 (0.0%) dental plaque positive cases. From this table, it has been found that the positive stool and sputum samples are increased in age between 25 – 55 years.

Table (8): Relationship between age of patients and positive stool, sputum and dental plaque samples:

Age	No. of Patient	Positive Stool		Positive Sputum		Positive Dental Plaque	
		No. of Patient	Percent (%)	No. of Patient	Percent (%)	No. of Patient	Percent (%)
25 – 35	12	9	**66.6**	2	**16.6**	0	**0**
36 – 45	15	10	**60**	4	**26.6**	0	**0**
46 – 55	13	9	**61**	3	**23.7**	0	**0**
56 – 65	5	1	**20**	0	**0**	0	**0**
66 – 75	5	1	**20**	0	**0**	0	**0**
Total	50	30	**60**	9	**18**	0	**0**

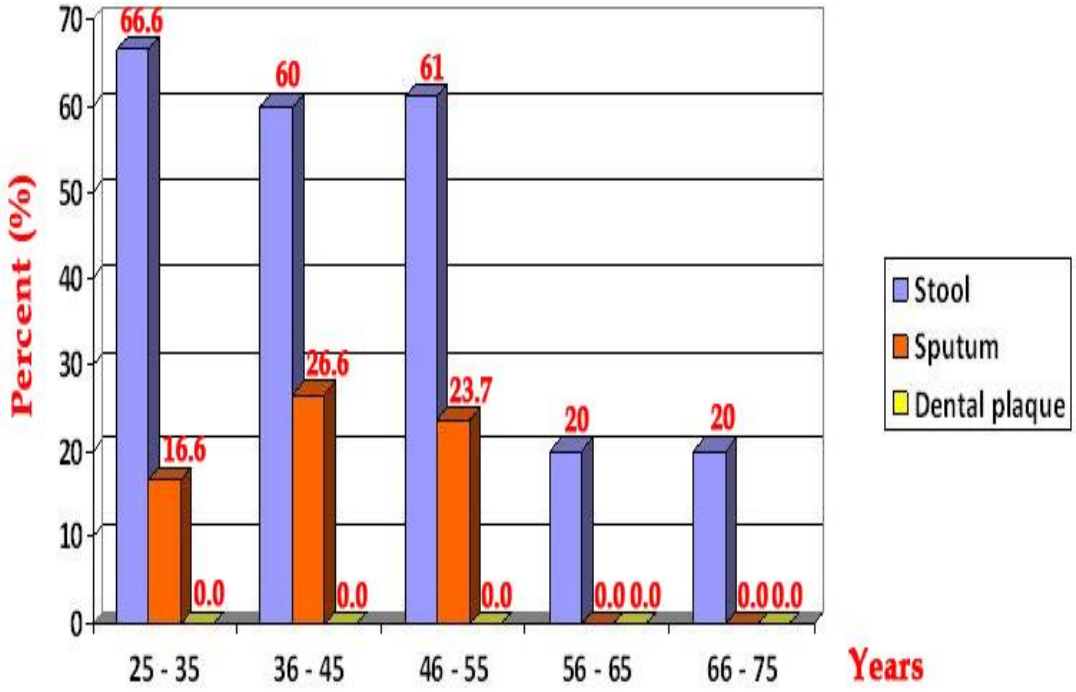


Figure (10): Relationship between age of patients and positive stool, sputum and dental plaque samples.

1- Identification and Confirmation of Bacterial Isolates:

The suspected bacteria isolated from stool, sputum and dental plaque of 50 Egyptian patients with symptoms was cultural on chocolate agar plates and incubated for 6 days at 37˚C under microaerophilic condition (5 – 10% CO_2) . After incubation period colonies appear as a small, pinpoint (1 - 2 mm), translucent, whitish-gray and non-hemolytic (Fig. 11).

The occurring colonies were stained using Gram's stain (Fig. 12) and subjected to further confirmatory biochemical tests as shows a smear of Gram negative curved and spiral rods typical with *H. pylori.*

The bacterial isolates were streaked on surface of christensen urea agar plates and were incubated for 24 hours at 37˚C. A confluent growth appeared accompanied with change of color of medium to pink indicating positive urease test (Fig. 13).

Figure (14) shows rapid and strong effervescence occurring up on addition of H_2O_2 of fresh bacterial culture indicating O_2 production and the catalase test. Thus, the bacterial isolate was suggested to be *H. pylori*.

Figure (15) shows positive oxidase test which recognized by a dark purple color.

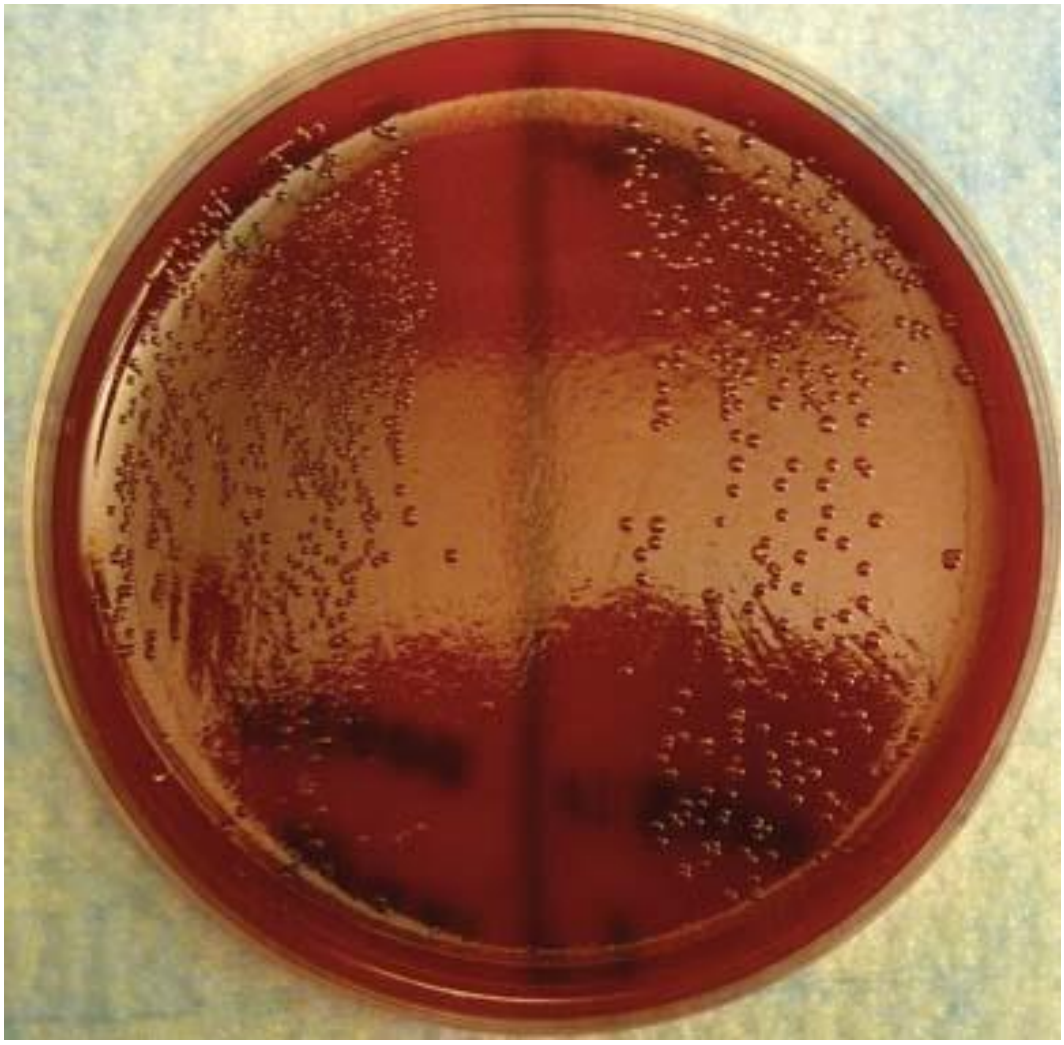


Figure (11): *H. pylori* colonies on chocolate agar after 6 days incubation under microaerophilic conditions (10% CO_2, by tube bottle).

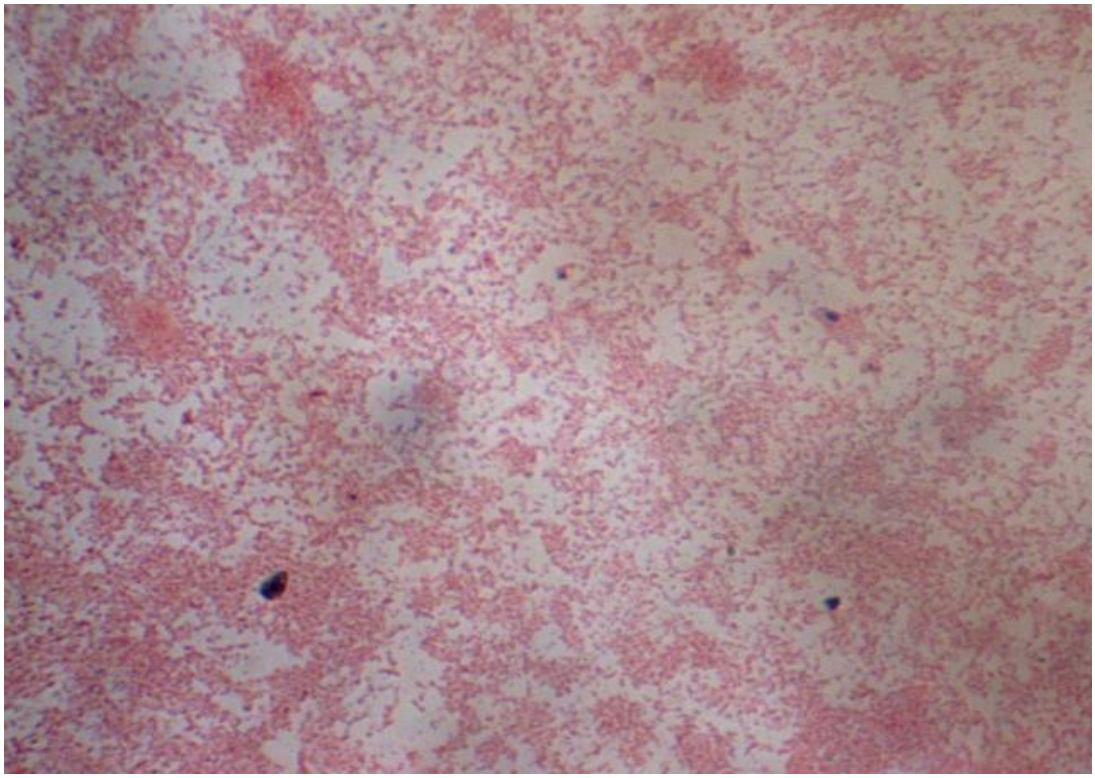


Figure (12): Gram's stain film of *H. pylori* in culture showing gram negative curved and spiral rods as seen with oil immersion lens. (Gram's stain 100x).

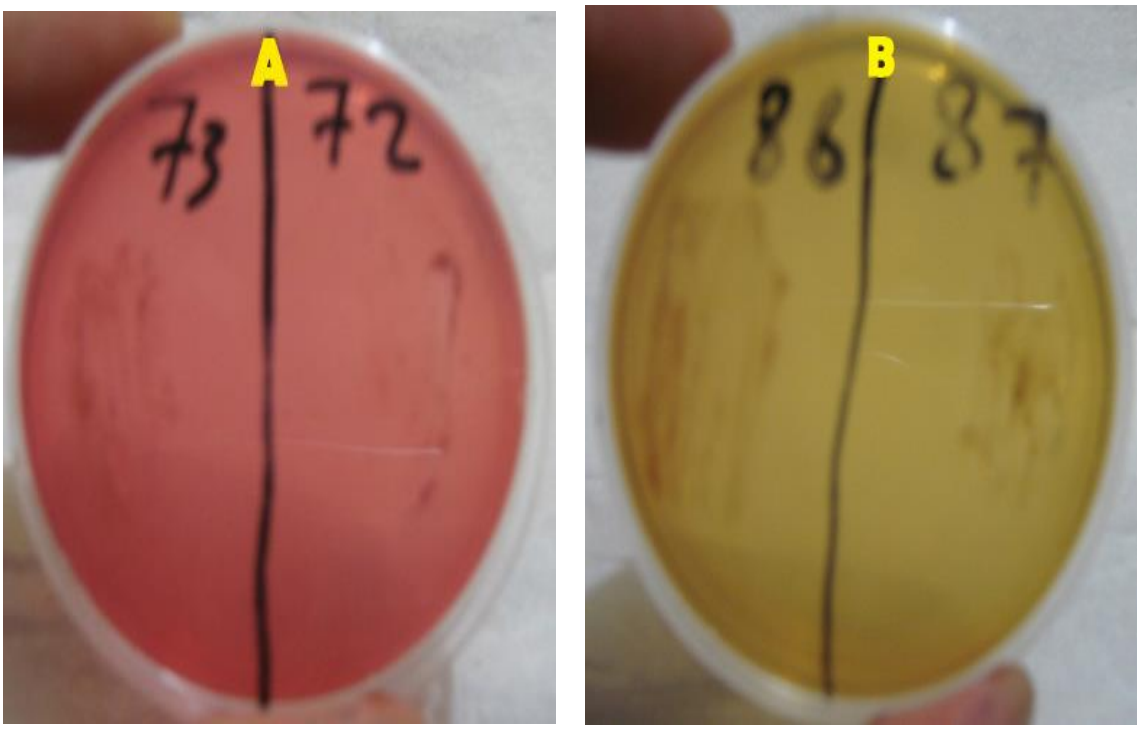


Figure (13): Christensen urea agar test for isolated *H. pylori*.

(A) Positive urease test.

(B) Negative urease test.

* **Note:** Numbers 72,73, 86 and 87 are specimens code.

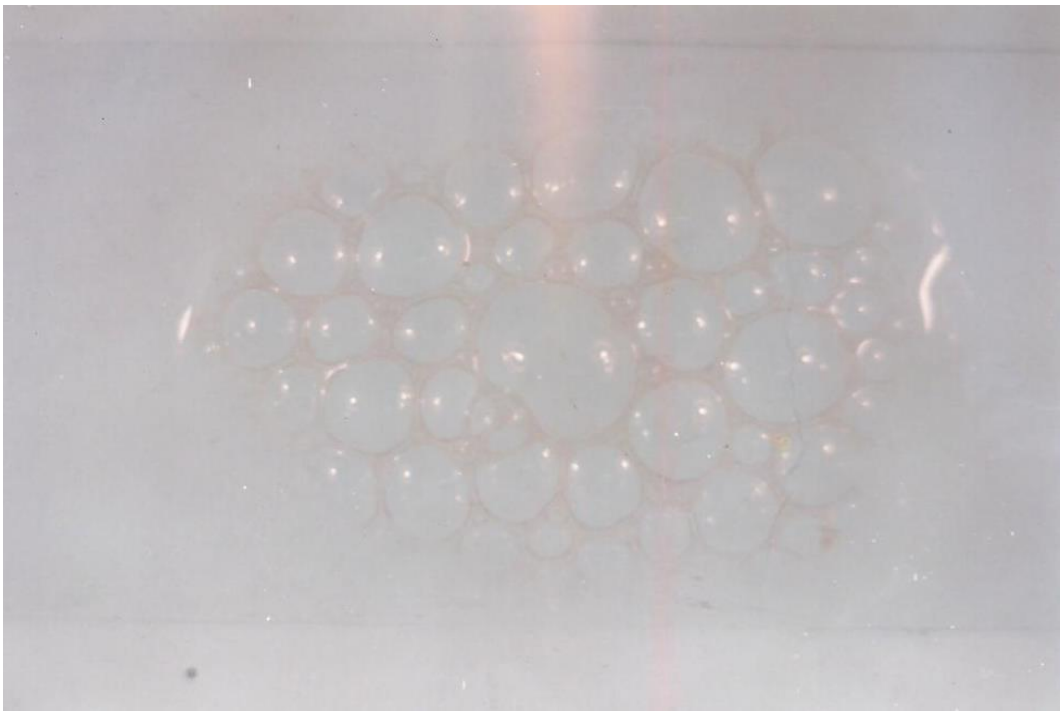


Figure (14): Positive catalase test for isolated *H. pylori.*

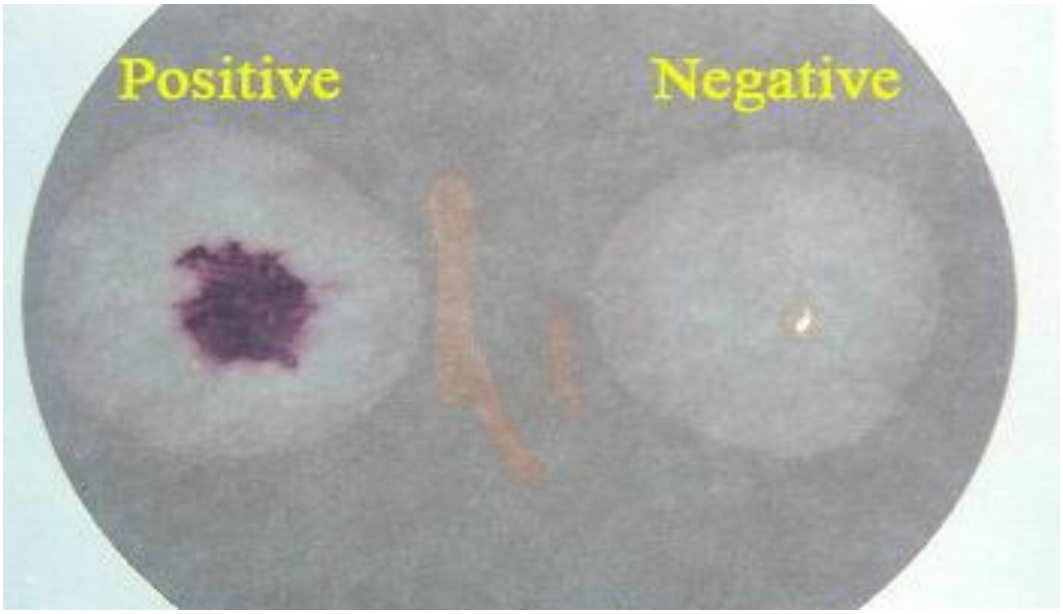


Figure (15): Positive and negative oxidase test for isolated *H. pylori*.

2- Antibiotic Sensitivity Test:-

Identified *H. pylori* isolates were tested for its antibiotic susceptibility using fourteen different antibiotics (Ceftriaxone, Imipenem, Amoxicillin+Clavulanic acid, Cefotaxime sodium, Vancomycin, Ceftazidime, Ciprofloxacin, Trimethoprim, Levofloxacin, Azithromycin, Cefoperazone, Ertapenem, Flumequine, Sulbactam + Ampicillin) in table (9) and illustrated by (Fig. 16).

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.

Table (9): Antibiotics and its abbreviations and concentrations which used in sensitivity test:

Name of Antibiotics	Abbreviations and Concentrations
Ceftazidime	(Oxoid, CAZ 30, 30 µg)
Amoxicillin + Clavulinic acid	(Oxoid, AMC 30, 20+10 µg)
Ceftriaxone	(Oxoid, CRO 30, 30 µg)
Ciprofloxacin	(Oxoid, CIP 5, 5 µg)
Imipenem	(Oxoid, IPM 10, 10 µg)
Cefotaxime sodium	(Oxoid, CTX 30, 30 µg)
Vancomycin	(Oxoid, VA 30, 30 µg)
Trimethoprim	(Oxoid, SXT 25, 25 µg)
Levofloxacin	(Oxoid, LEV 10, 10 µg)
Azithromycin	(Oxoid, AZM 15, 15 µg)
Cefoperazone	(Oxoid, CFP 30, 30 µg)
Ertapenem	(Oxoid, ETP 10, 10 µg)
Flumequine	(Oxoid, UB 30, 30 µg)
Sulbactam + Ampicillin	(Oxoid, SAM 20, 10+10 µg)

Table (10): Percentage of antibiotic sensitivity test and positive *H. pylori* of 30 patients:

Name of Antibiotics	Sensitive		Intermediate		Resistance	
	No. of patients	%	No. of patients	%	No. of patients	%
Imipenem	30	**100**	0.0	**0.0**	0.0	**0.0**
Ertapenem	30	**100**	0.0	**0.0**	0.0	**0.0**
Levofloxacin	26	**86**	2	**7**	2	**7**
Ceftriaxone	24	**80**	0.0	**0.0**	6	**20**
Ciprofloxacin	24	**80**	0.0	**0.0**	6	**20**
Ceftazidime	22	**73**	2	**7**	6	**20**
Cefotaxime sodium	16	**53**	10	**33**	4	**14**
Flumequine	16	**53**	0.0	**0.0**	14	**47**
Sulbactam + Ampicillin	12	**40**	0.0	**0.0**	18	**60**
Trimethoprim	8	**27**	0.0	**0.0**	22	**73**
Cefoperazone	8	**27**	6	**20**	16	**53**
Azithromycin	6	**20**	6	**20**	18	**60**
Amoxicillin + Clavulinic acid	2	**7**	8	**27**	20	**66**
Vancomycin	0.0	**0.0**	0.0	**0.0**	30	**100**

Antibiotics sensitivity test of identified culture of *H. pylori* was depicted in table (10). This test indicated that the organism was sensitive to Imipenem (100%), Ertapenem (100%), Levofloxacin (86%), Ceftriaxone (80%), Ciprofloxacin (80%) and Ceftazidime (73%), While, the isolated organism was resistant to Vancomycin (100%), Trimethoprim (73%), Amoxicillin+Clavulanic acid (66%), Azithromycin (60%) and Sulbactam + Ampicillin (60%).

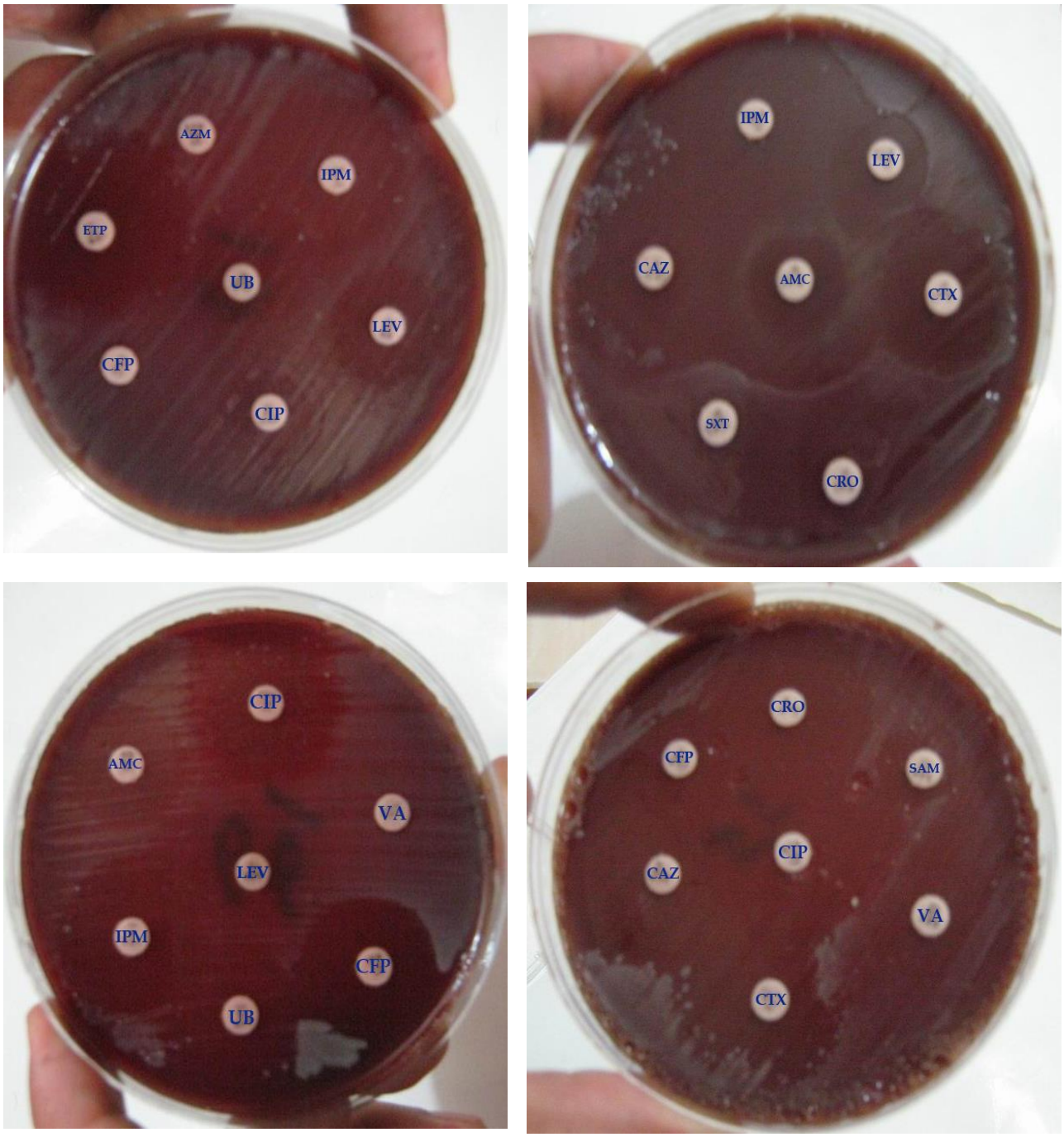


Figure (16): Antibiotic susceptibility test of *H. pylori* against different antibiotics.

3- SDS-PAGE protein pattern of cell lysate:

Fifteen major protein bands were resolved on the gel. The resolved protein bands were located within the high and low molecular weight regions as shown in (Fig. 16). The molecular as weights of different resolved protein bands SDS-PAGE were estimated to their RF values compared to marker proteins bands in (table 10)**.** The calculated molecular weights of the 15 patients bands are 270, 156, 128, 86, 72, 69, 67, 63, 55, 49, 47, 32, 26, 15 and 10 KDa as show in (table 11).

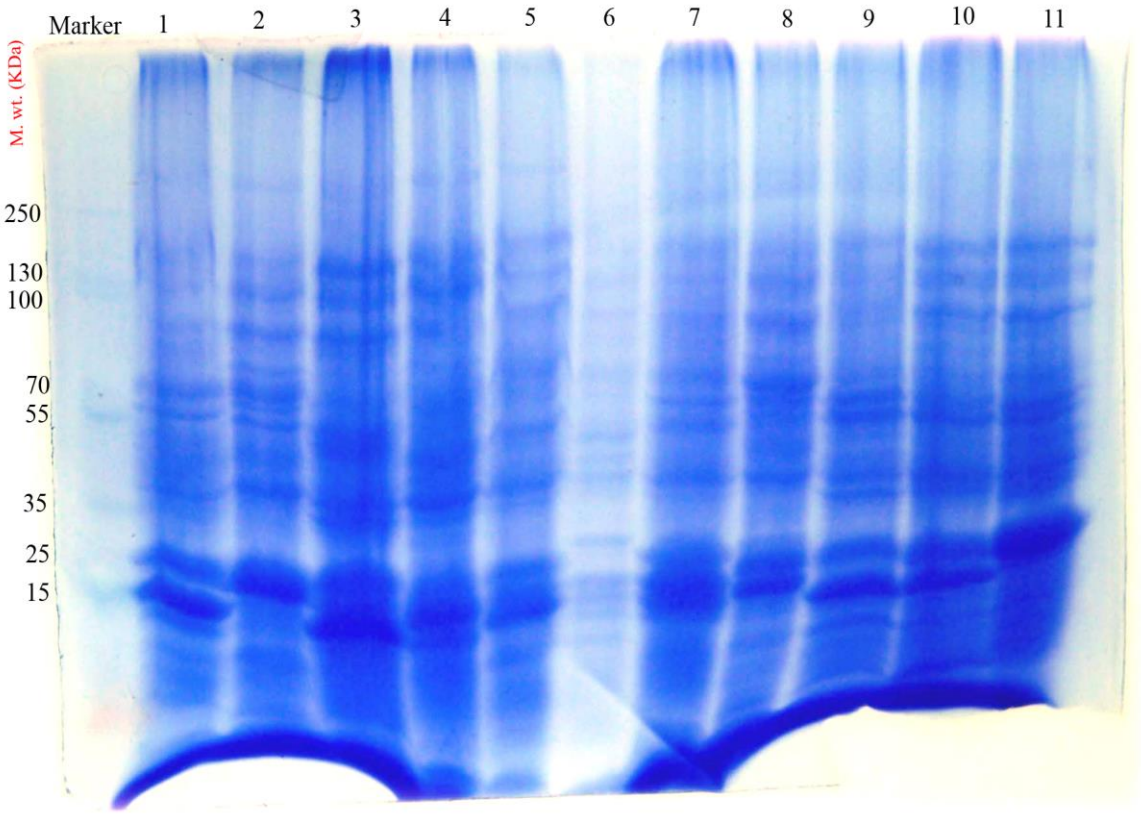


Figure (17): SDS-PAGE showing *H. pylori* protein bands.

Table (11): Molecular weight of standard protein markers:

No. of Bands	M. wt. (KDa)	Rf
1	250	0.22
2	130	0.315
3	100	0.35
4	70	0.46
5	55	0.49
6	35	0.6
7	25	0.65
8	15	0.72

Table (12): Major protein bands present in crude extract of *H. pylori* and their Rf as shown in SDS-PAGE:

Band No.	M. wt. (KDa)	Rf
1	270	0.202
2	156	0.28
3	128	0.32
4	86	0.39
5	72	0.43
6	69	0.47
7	67	0.48
8	63	0.5
9	55	0.55
10	49	0.58
11	47	0.59
12	32	0.65
13	26	0.67
14	15	0.73
15	10	0.78

Notes: Protein bands serial numbers are arranged according to their molecular weight in descending order.

Table (13): Showing the differences and similarities between six strains of *H. pylori*:

Lane / No. of Bands	A	B	C	D	E	F
1	N	Y	Y	N	Y	N
2	Y	Y	Y	Y	Y	Y
3	Y	Y	Y	Y	Y	Y
4	Y	Y	Y	Y	Y	Y
5	N	Y	Y	Y	N	Y
6	Y	Y	N	N	N	Y
7	N	Y	N	N	N	N
8	Y	Y	Y	Y	Y	Y
9	N	N	N	Y	N	N
10	Y	Y	Y	Y	Y	Y
11	Y	N	N	N	Y	N
12	N	N	N	Y	N	N
13	Y	Y	Y	N	Y	Y
14	Y	N	Y	Y	Y	Y
15	Y	N	Y	Y	N	Y

* A= Lane 1, 9, 10, 11. * B= Lane 2. * C= Lane 5. * D= Lane 6.

* E= Lane 3, 4. * F= Lane 7, 8.