

Prevalence and Microbiological diagnosis of *Helicobacter pylori* infection and it's antibiotic resistance pattern in the patients suffering from "Acid-peptic Diseases"

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INTRODUCTION

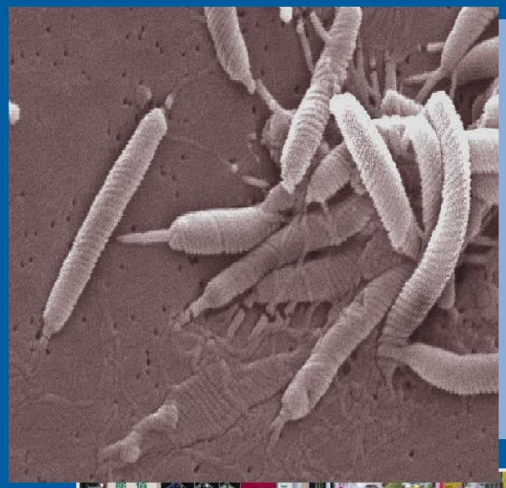
Warren and Marshall

- Discovered *H.pylori in* 1982
- Transform our concept of gastro duodenal pathology, diverted the world wide attention from pH to Hp. They Contended that most stomach ulcers and gastritis were caused by this bacterium and not by stress or spicy food.
- *H.pylori* is a spiral shape bacteria, etiological agent causing Gastritis, Peptic ulcers and Gastric adenocarcinoma



Awarded Nobel prize in medicine in 2005

Epidemiology of *H. pylori* Infection



Person to person1

Fecal-oral, oral-oral routes most likely1,2

Acquired predominantly in childhood; becomes chronic if not cleared1,3

Risk Factors



Infected family members, increased number of siblings2,3

Crowded living conditions1,2

Poor sanitation and hygiene1,2

1. Vale FF, Vitor JMB. *Int J Food Microbiol*. 2010;138(1-2):1-12; 2. Goh K-L, et al. *Helicobacter.* 2011;16 (suppl 1):1-9;
3. Garg PK, et al. *Epidemiol Infect*. 2006;134(3):450-459;

AIMS AND OBJECTIVES

1. **To find the prevalence rate of *H.pylori* infection in ANAND District of CENTRAL GUJARAT, INDIA**
2. **To evaluate various invasive and non-invasive microbiological diagnostic methods for the identification of the *H.pylori*.**
3. **To assess the drug susceptibility pattern and the prevalence of Metronidazole, Clarithromycin, Erythromycin, Tetracycline, Amoxicillin , Levofloxacin, Ciprofloxacin and Furazolidone resistant isolates of *H. pylori* .**

MATERIALS AND METHODS

- **Total 855 symptomatic cases of gastro intestinal disorders were investigated for association with *H.pylori* infection.**

- **Exclusion criteria:**

1) **Previous therapy to eradicate *H. pylori***
2) **Patients taking aspirin or non-steroidal anti-inflammatory drugs (NSAIDS) in the past 4 weeks or are on PPI.**

Samples

4 antral biopsies and

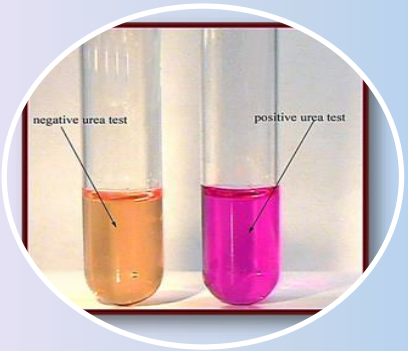
blood sample for antibody

2 biopsy samples in BHI broth

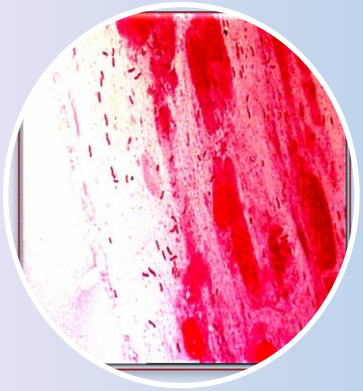
1 in 10% formal saline

1 in urea broth

PROCESSING OF SAMPLE



Rapid urease test: **Time taken for color changes from yellow to pink was noted within 4 hrs.**



Gram's staining **was done on the smears made from crushed biopsy material**



Culture **was done on Brucella blood agar and Columbia blood agar with skirrow's antibiotic supplement, for at least 7 days in an anaerobic jar with Gas pak kit**

❖ Serology

ELISA (IgG) : Antigens: Highly purified proteins associated with *CagA* genes (120 KD), *VacA* genes (87 KD) as well as urease-antigens.

❖ Histological Staining Techniques

1.Giemsa stain 2. Warthin-Starry stain

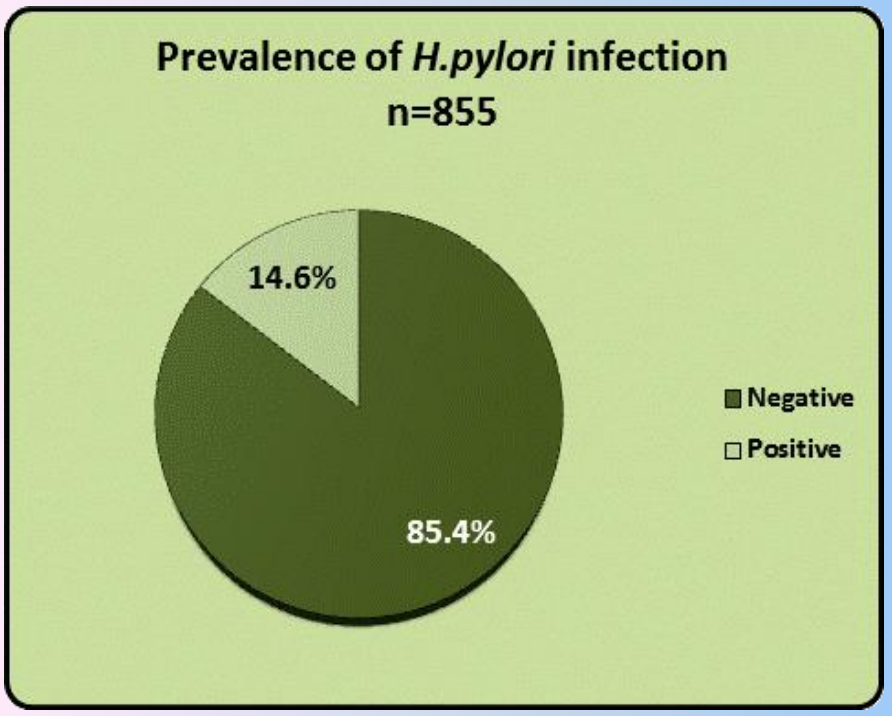
❖ Antimicrobial susceptibility testing:

Kirby-Bauer disk diffusion method:

- ***H.pylori*** **pure culture (McFarland's 3) was suspended on Brain-Heart infusion broth**
- **Antibiotics used: Metronidazole (5µg), Clarithromycin (15µg), Ciprofloxacin (5µg), Amoxicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Levofloxacin (5µg), and Furazolidone (50µg).**
- **Resistance was determined by zone of growth inhibition ≤ 16mm for metronidazole, ≤ 25mm for amoxicillin and a zone diameter ≤ 30mm for clarithromycin, ciprofloxacin, tetracycline, furazolidone and erythromycin**
- ***H.pylori*** **(NCTC 11637), used as control strain.**

RESULTS AND DISCUSSION

Overall prevalence rate of *H.pylori* infection



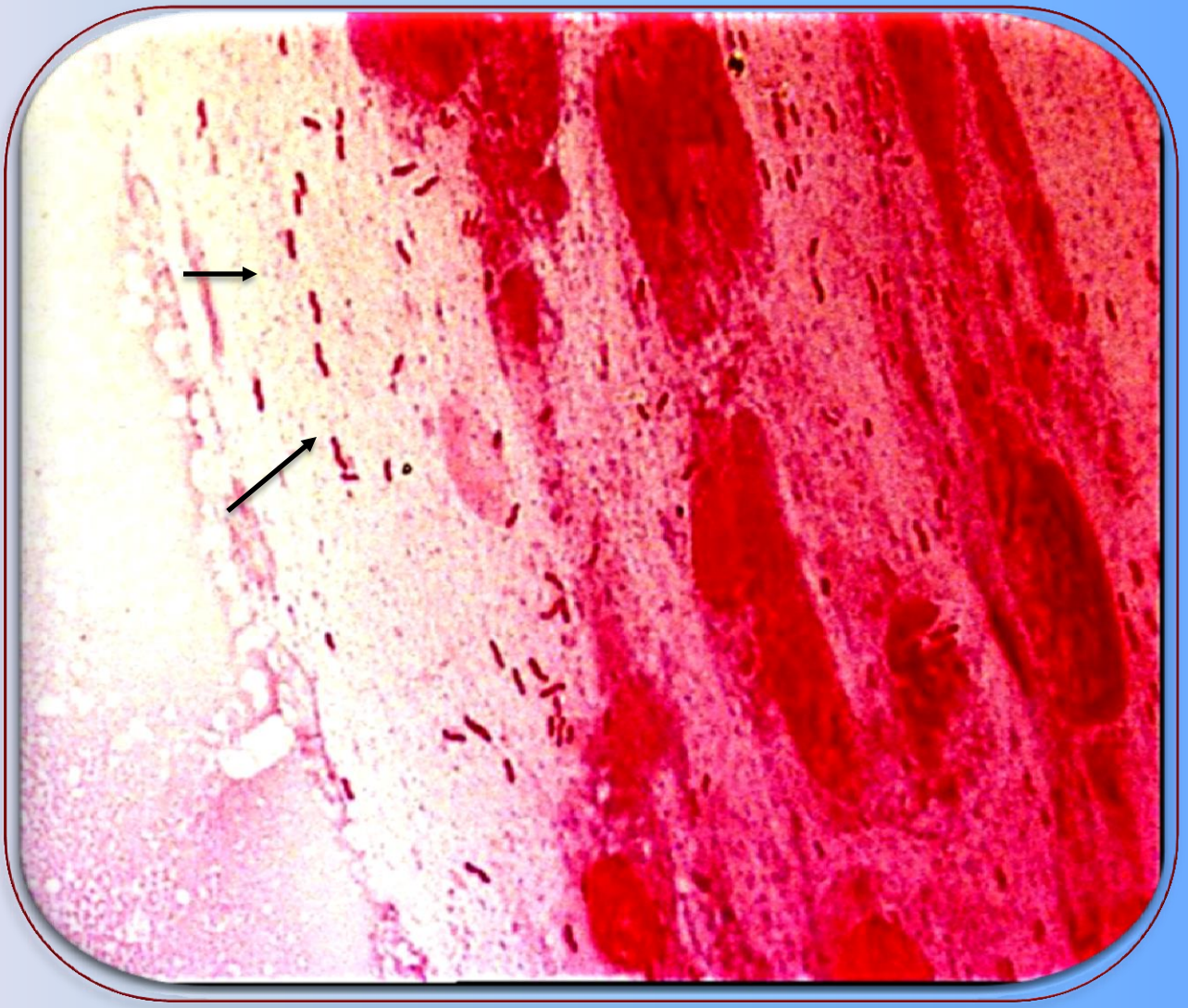
Mahmood Reza Hashemi et al.	2006	67.1%
Vandana Berry et al.	2006	10.9%
A.Al-Sulami et al.	2008	67.8%
S. Sasidharan et al.	2012	35.6%
Vijaya D. et al.	2012	62.7%

1. **Seroprevalence: 387/855 (45%), males: 237(61%), females: 150 (39%), higher in 30-39 years age group (91/387) and lowest in old age group of 80-89 years (10/387)**
2. **We can say that the Positive rate is affected by sampling errors , processing and transportation of specimen. It also varies widely depending on the environment, host and laboratory detection method.**

Prevalence of *H.pylori* infection in various gastro -duodenal diseases in correlation with gender

Diseases	Gender		No. of HP infected
	Female	Male	
Duodenal Ulcer	3(37%)	5(63%)	8 (6.4%)
Duodenitis	2(25%)	6(75%)	8(6.4%)
Gastric Ulcer	-	1(100%)	1 (0.8%)
Gastritis	17(31%)	38(69%)	55 (44%)
Reflux esophagitis	21(40%)	32(60%)	53(42.4%)
Grand Total	43(34%)	82(66%)	125

Gram negative “ seagull shape” bacilli in a crushed biopsy smear (magnification 1000X)



Growth of *H.pylori*

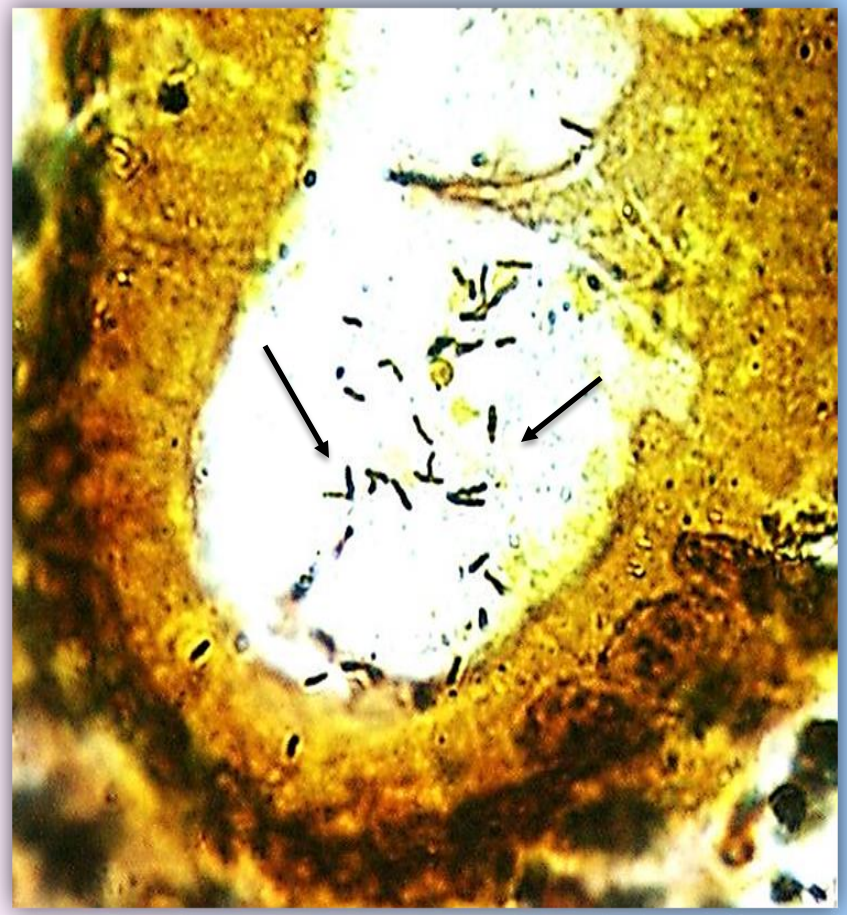


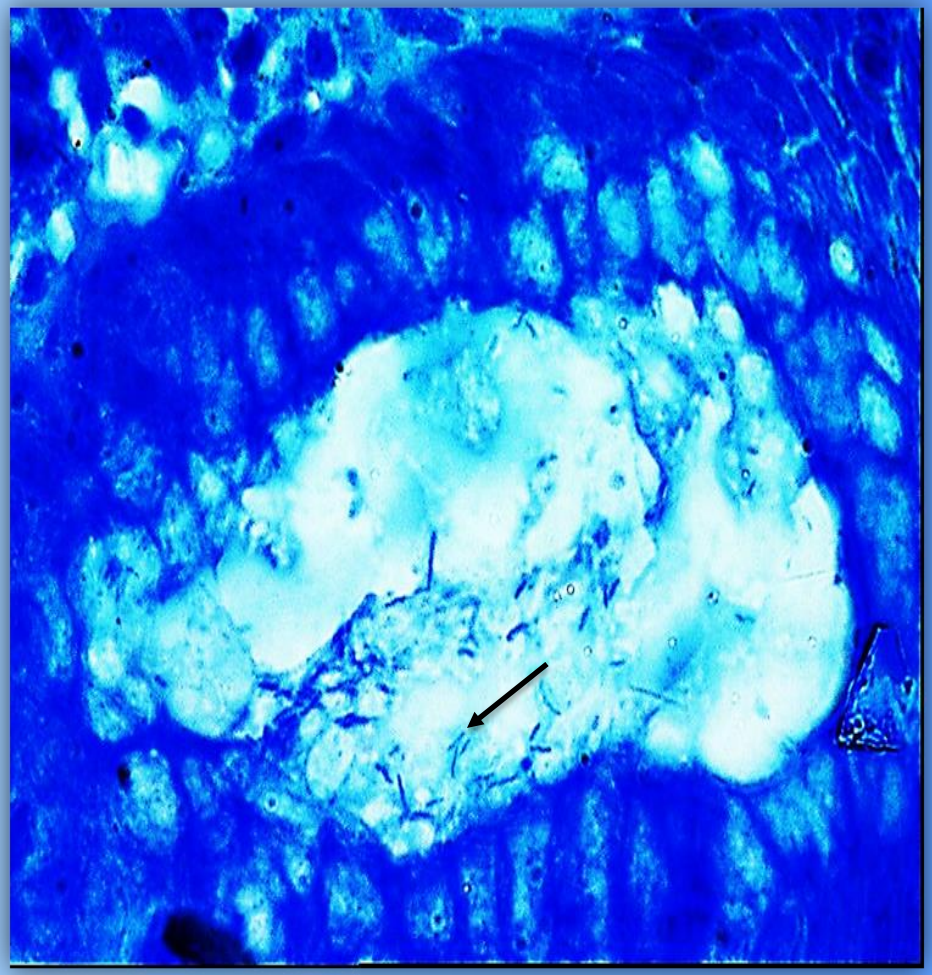
Brucella blood agar:
Colonies are minute, translucent and round.



Belo-Horizonte blood agar:
colonies are round, opaque, with golden yellow pigmentation

Warthin-Starry stain and Giemsa stains showing thick, spiral bacilli in the gastric lumen (magnification 1000X)

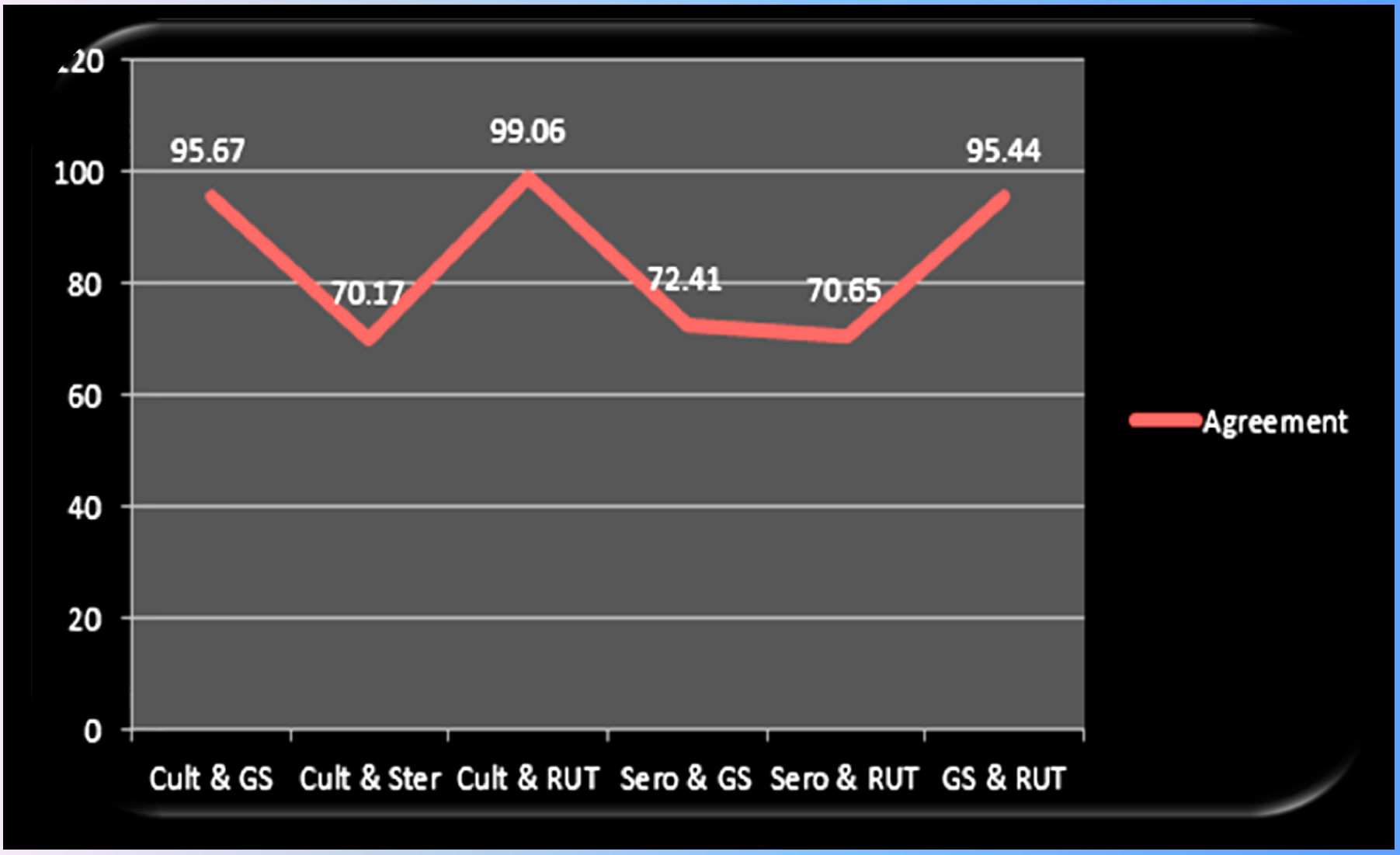




Sensitivity, Specificity, Positive and Negative predictive value of all diagnostic tests

Diagnostic Tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Gram's staining	96.8	98.5	91.7	99.4	98
Serology	98.8	69.8	40.3	99.7	75
Culture	76.0	100	100	96.1	96
RUT	80.0	99.9	99.0	96.7	97

Agreement between various diagnostic methods.



Primary and combined antimicrobial resistance pattern of *H.pylori* (n=80)

Agents	Resistant %	Sensitive %
Metronidazole	83.8	16.2
Clarithromycin	58.8	41.2
Amoxicillin	72.5	27.5
Ciprofloxacin	50.0	50.0
Tetracycline	53.8	46.2
Furazolidone/levofloxacin	13.8	86.2
Metronidazole+ Clarithromycin	57.5	15.0
Metronidazole+ Ciprofloxacin	50.0	16.2
Ciprofloxacin+ Clarithromycin	48.8	40.0
Metronidazole+ tetracycline	53.8	16.2

Prevalence of *H.pylori* isolates resistant to Metronidazole (MTZ) by different researchers.

Study	Resistance %
D.R.Saha *et al.*, 2005	85%
Widyasari kumala *et al.*, 2006	100%
Irfan ali mirza *et al.*, 2008	63%
Varsha singh *et al.*, 2009	100%
M Bruce *et al*., 2010	76%
K.S.Ahmed *et al.*,2012	100%
Ogata SK et al; 2013	40%
Present study	83.8%

we hypothesize that the widespread use of metronidazole in Anand district for the treatment of numerous infectious disease like parasitic, genital and dental infections, led to the development of resistance

Prevalence of H.pylori isolates resistant to Clarithromycin (CLA) by different researchers.

Study	Resistance %
Surg capt. Mishra *et al.*, 2006	71.4%
Kobayashi *et al.*, 2007	3.1%
Panthong kulsuntnoong *et al.*, 2008	5%
Varsha singh *et al.*, 2009	4.7%
M Bruce *et al.*, 2010	30%
K.S.Ahmed *et al.*, 2012	76%
Ogata SK et al; 2013	20%
Present study	58.8%

Clarithromycin is widely used in patients with duodenal ulcer, non-ulcer dyspepsia, and abdominal discomfort without diagnosis of *H. pylori* infection. Another factor contributing to resistance is mutation of the *H. pylori* 23s rRNA gene.

Prevalence of H.pylori isolates resistant to Amoxicillin (AMX) by different researchers.

Study	Resistance %
Irfan ali mirza *et al.*, 2007	0%
Panthong kulsuntnoong *et al.*, 2008	2.4%
Varsha singh *et al.*, 2009	65%
M Bruce *et al.*, 2010	2%
K.S.Ahmed *et al.*, 2012	80%
Ogata SK et al; 2013	11%
Present study	72.5%

Frequent use of this antibiotic for the treatment of respiratory and skin infections in our country may contribute to bacterial resistance.

***H. pylori pbpl* gene mutation makes it resistant to amoxicillin**

CONCLUSIONS

- **Overall Prevalence rate of *H.pylori* infection in Anand District was found to be low and majority of *H.pylori* strains were causing Gastritis. Seroprevalence was high.**
- **The association of Gram staining and the Rapid urease test, most accurate method, allows quick evaluation of the infectious status of patient due to its high concordance rate, the high sensitivity of Gram's staining and high specificity of RUT.**
- **In our geographical area , high rate of resistance to metronidazole and a rising resistance to amoxicillin and clarithromycin.**

  The encouraging finding in our study was 86% sensitivity of *H.pylori* strains to furazolilone, and levofloxacin. Furazolidone has been used to as an alternative to overcome MTZ resistance.
- **Susceptibility test using disk diffusion method is cost effective in the screening of antimicrobial resistance and testing for metronidazole and clarithromycin resistance will certainly facilitate the more rational use of these antibiotics to treat infections.**
- **Our results recommends the need of sensitivity profile for *H.pylori* regionally and periodically before the general use of an eradication schedule.**


Thank You