SUMMARY

AND

CONCLUSIONS
1) **Prevalence of \textit{H. pylori} infection:**

- Overall Prevalence rate of \textit{H. pylori} infection was found to be 14.6% (125/855).
- Highest percent prevalence of \textit{H. pylori} was found in gastric ulcer (33%) cases, followed by duodenal ulcer cases (25%), then in duodenitis cases (21%) and reflux esophagitis cases (15%) and lastly in gastritis cases (13%).
- Pain in the upper abdomen was the most frequent symptom seen in 94/125 (75%) of \textit{H. pylori} positive patients with hematemesis (26%), regurgitation (21%) and vomiting (16%) the second, third and the fourth most complain, while rest of the clinical features like melena, hiccups, sore throat, anemia were not related to presence of \textit{H. pylori} infection.
- Prevalence of \textit{H. pylori} infection was utmost in older age group (61-70) yrs. i.e. 24.7% and (81-90) yrs. i.e. 20%, and was lower most in younger age group (11-20) yrs. i.e. 5%. ($p$ value- 0.065, NS)
- Gender wise we found that the prevalence of \textit{H. pylori} positive rate was almost the same i.e. 15.8% in males and 12.8% in females. Statistically there is no significant association between \textit{H. pylori} infection and gender of the patient. ($p$ value- 0.214, NS).

2) **Comparison of various diagnostic methods (invasive versus non-invasive methods):**

- Out of all five diagnostic techniques \textbf{Major Sensitivity} was achieved by \textit{Serology} (98.8%) and \textit{Gram’s staining} (96.8%) for detecting \textit{H. pylori} infection as well as was having high
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Negative Predictive Values (NPV), 99.7% and 99.4% respectively.

- **Culture (100%) and Rapid Urease Test (99.9%)** was found to be most **Specific** test with high **Positive Predictive Values (PPV)**, 96.4% and 96.9% respectively.

- Analyzing the **Agreement** between all methods we observed that major agreement occurred between **Culture and RUT (99.06%)**, and **Culture with Gram’s staining (95.67%)**, (*p* value=0.000, S).followed by **RUT and Gram’s staining (95.44%)** and finally **Histopathology and RUT (87.6%)**. Other methods are weakly agreed with each other.

- **Gram’s staining, RUT and Culture** showed the **Accuracy** of 98.25%, 96.96% and 96.49% respectively.

- **Detection rate of H.pylori infection by RUT** was 11.8%, **culture** had a rate of 11.1%, **Gram stain** had a rate of 11.3%, and **Serology** had a rate of 57.4%.

- Among the invasive methods our result suggested that association of **RUT with Gram’s staining** constitute the best choice for confirming the diagnosis, due to its high concordance rate, the high sensitivity of Gram’s staining and high specificity of RUT.

- Our result suggests that **Culture** undoubtfully the most specific test but alone cannot be used as a “gold standard” test due to its low sensitivity.

- **Gram’s staining** is very simple, highly sensitive and specific method, and is appropriate for the routine clinical use.

- High negative predictive value of Serology makes it a useful screening test to exclude *H.pylori* infection.

- No single test can be relied upon to detect *H.pylori* infection and a combination of the tests is recommended as a “Gold standard”.

*H.pylori*
3) Comparison between EIA- IgG and EIA- IgA Serology for *H. pylori* detection:

- EIA-G showed 100% sensitivity and negative predictive values (NPV).
- Specificity of EIA-G was very low (48.6%) compared to EIA-A (61.4%).
- Accuracy of EIA-A was high (63.16%) compared to EIA-G (52.63%)
- There is a significant association between detection of antibody and *H. pylori* infection. When both the IgG & IgA are negative *H. pylori* infection is also negative and the proportion of *H. pylori* infection is higher in patients who had both IgG and IgA positive (p value < 0.01).
- EIA-G update is reliable and accurate test and because of its 100% sensitivity and negative predictive values, makes it useful screening test and thus serve as an alternative to endoscopy.
- For the purpose of excluding infection with *H. pylori*, the performance of IgG is moderate (low specificity) but can be improved by conjunctional IgA testing which will offer some additional diagnostic value.
- Our data showed that frequency of IgA positive IgG-negative patients with gastrointestinal disorders and suggestive of *H. pylori* infection was 7% (16 patients out of 228) therefore, a positive finding of IgA antibody may be of significant clinical value in supporting diagnosis of infection especially if IgG serology is negative.
- IgG response was more common in reflux esophagitis patients (OR 1.451, 95%CI-0.850-2.477) and then in gastritis (OR 0.962, 95%CI-0.570-1.621) and duodenitis (OR-0.806,
95\%CI-0.112-5.827) and last in duodenal ulcer (OR 0.271, 95\% CI-0.084-0.879). This data states that IgG response is associated with increase (1.451 times) risk of reflux esophagitis, followed by gastritis and duodenitis.

- IgA response was more common in duodenitis patients(OR-1.383, 95\%CI-0.191-9.995) and reflux esophagitis patients(OR 1.289, 95\% CI-0.756-2.197) and least in duodenal ulcer patients( OR 0.670, 95\%CI-0.222-2.029). This data shows that IgA response is associated with the increase risk of duodenitis and reflux esophagitis followed by duodenal ulcer and least risk for gastritis.

4) Histopathological diagnosis (Warthin- starry, H&E, Giemsa) of \textit{H.pylori} and its comparison with PCR.

- Sensitivity and Negative Predictive Values of all the three stains was found 100%.
- Specificity of H & E was little better (87.9\%) then Warthin-starry (84.5\%) and Giemsa (84.5\%).
- H&E was found most accurate (90.14\%) method for detecting \textit{H.pylori} infection compare to other two methods.
- Warthin-starry showed highest agreement with Giemsa (100\%, \textit{p value}-0.000) and then with H&E (97.18\%, \textit{p value}-0.047). H&E showed good agreement with Giemsa (97 \%, \textit{p value}-0.047).
- Sensitivity and specificity of PCR was low compare to histopathological stains, but accuracy was almost equivalent.
- In the patients with duodenal ulcer detection rate of histopathology was higher (77\%) than PCR (44\%). In reflux patients also the detection rate of histopathology was
higher (46.2%) than PCR (30.8%), but in gastritis patients the difference was not that significant.

- In a mediocre laboratory where PCR facility is not available histological stain can be a better substitute for the diagnosis of *H. pylori*.

5) **Prevalence and clinical relevance and of cagA, vacA, ice A, ureC genotypes (n=71):**

- Out of 71 clinical samples, 22 samples were positive by PCR, prevalence of *H. pylori* infection by PCR was 30.99%.

- The PCR results showed high proportion of cagA positive (17/71 specimen; 23.94%), vac A positive (1/71 specimen; 1.4%), ureC positive (4/71 specimen; 5.63%), iceA positive (0/71 specimen; 0%).

- Out of 22 strains, prevalence of cagA (17/22) strain was 77.27%, vacA (1/22) was 4.54%, ureC (4/22) was 18.18% and ice A (0/22) was 0%.

- PCR positivity in different age group Irrespective of any genotypes showed highest positivity in the 31-40 years age group and lowest rate in 61-70 years age group.

- PCR based method to detect *H. pylori* and the virulent gene cagA directly from the gastric biopsy specimen appears to be promising and can curtail the lengthy process of culture based method.

- cagA genotype is strongly associated with Gastritis compare to other three genotypes.

- High incidence of cagA positive *H. pylori* strains found in adults, may suggest that this population may be at risk of developing more serious pathology at the gastric mucosa.

- High prevalence of cagA strains were found in the individuals with Gastritis (52.94%), followed by reflux esophagitis (29.41%) and duodenal ulcer (17.65%)
- *cagA* strains were not found to be associated with duodenitis (0%).

- Prevalence of *ureC* genotype was found to be very low, 5.8% in gastritis patient and 7.6% in reflux esophagitis patients.

- We found significant association between *cagA* and gender of the patient, prevalence was higher in females (10/17), compare to males (7/17), *p value*-0.042 (< 0.05).

- Prevalence of *cagA* was higher in age group 21-40 yrs. (52.94%), followed by 41-60 yrs. (35.29%) and 61-80 yrs.(11.76%).

6) **Prevalence and association of *H. pylori* in patients with gastro esophageal reflux disease (GERD):**

- Prevalence rate of GERD was found to be 40.8% (349/855)

- Persistent abdominal pain was significantly associated with the development of GERD.

- Prevalence rate of *H.pylori* infection was higher in GERD negative patients (81.6%) compared to GERD positive patients (18.3%).

- We found no significant correlation or outcome between habits (Acute smoking, alcohol, and tobacco) and development of GERD.

- Patients having Hiatus hernia had 22 times higher risk in developing GERD, while there was no association found between *H.pylori* and hernia status.

- Prevalence rate of *H.pylori* infection was significantly higher in Grade C & D Esophagitis (25.8%) compare to Grade A & B esophagitis (9.9%).

- Prevalence of *H. pylori* in GERD patients is lower than that in general population and its presence is associated with the milder form of GERD. Based on these findings it seems
that role of *H. pylori* in the development of GERD is protective and high prevalence of *cagA* positive *H. pylori* may contribute to lower prevalence and protect against the complications of GERD.

7) **Antimicrobial resistance pattern:**

- In our geographical area we documented a high rate of resistance to **metronidazole** and a rising resistance to **amoxicillin and clarithromycin**. It is of concern to us that multidrug resistance to these antimicrobial agents is also rising significantly.
- We could not demonstrate higher metronidazole resistance rate in females despite the fact that gender has been a significant risk factor for resistance.
- There is a need for continuous monitoring of antimicrobial susceptibility pattern for *H.pylori* for determination of optimal treatment regimen.
- We did not find any marked difference in metronidazole susceptibility of *H.pylori* strains isolated from patients with gastritis compare to patients with peptic ulcer disease. Therefore patients with gastritis and PUD can be colonized both with resistant and sensitive *H.pylori* isolates.
- Metronidazole resistance was found higher in patients suffering from reflux esophagitis (89%, *p value*-0.018) and Duodenal ulcer (80%, *p value*-0.00)
- Clarithromycin resistance was found higher in patients with Duodenitis (80%, *p value*-0.018) and found least in patients with Duodenal ulcer (40%, *p value*-0.5)
- Amoxicillin resistance rate was found 100% in Duodenitis patients (*p value*-0.00) and 80% in duodenal ulcer patients (*p value*-0.018).
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- We did not find any significant correlation between Ciprofloxacin resistance and gastro duodenal diseases.
- Tetracycline resistance rate was also found higher in patients with Duodenitis patients (80\%, \( p \text{ value}-0.018 \)).
- The encouraging finding in our study was 86% sensitivity of \( H.\text{pylori} \) strains to furazolidone, erythromycin and levofloxacin.
- There was a significant difference in rate of resistance to any of the antibiotic tested when we compare male versus female, the rate was higher in males compare to females.
- There was a remarkable difference in antimicrobial resistance according to the age-group. Clarithromycin, tetracycline, ciprofloxacin resistance was higher in the age group of 31-40 yrs. whereas metronidazole resistance is higher in the age group 21-30 yrs.
- The most common resistance antibiogram were those combinations containing MET, TET, CLR (85\%, high frequency of multiple antibiotic resistance observed in this study most probably reflects the ease of access and extensive use of antibiotics in Anand.
- 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.