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# Which Gastric Microbiota Associated with *H. pylori* in Dyspeptic Patients with Gastritis

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## Abstract

**Introduction:** *H. pylori* is the comment infection worldwide. Its relation to dyspepsia has long been investigated. However, the association between *H. pylori* and other microbiota in the context of dyspepsia is less understood. The aim of this study was to determine different bacterial species isolated from the stomachs of patients with *H. pylori* infection and dyspepsia. **Methods:** A total of 81 patients were included and divided into: group I (N = 50) patients complaining of chronic dyspepsia and group II (N = 31) are patients with non-dyspeptic symptoms endoscoped for reasons other than dyspepsia. All patients were endoscoped and 4 gastric biopsies were obtained from each patient. All patients were examined initially by rapid urease test then histopathology to confirm *H. pylori* infection and determine the degree of gastric inflammation and finally tissue cultures for *H. pylori* and other bacterial species using matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy (MALDI-TOF MS). **Results:** Regarding the demographics; patients of dyspepsia in group I were more consumers of spicy food and smoking than non-dyspeptic patients. Almost, all dyspeptic patients (98%) who had underlying gastric pathology with active gastritis and erosions were the most frequent reported pathological findings. Culture results showed significant association of *Staphyloccus* and *Lactobacillus* with dyspepsia while *Streptoccous* and *Klebsilla* were more frequent among non-dyspeptic patients. **Conclusion:** Dyspeptic patients in this study had different grades of gastric pathology and different species of microbiota were isolated, which seems to have concomitant interaction with *H. pylori* in pathogenicity of gastric mucosa and cause symptoms of chronic dyspepsia.

## Keywords

Dyspepsia, *H. pylori*, Microbiota, Histopathology

## 1. Introduction

*H. pylori* bacteria is the most common infection worldwide and induces different gastrodudenal diseases like peptic ulcer, MALT lymphoma, and gastric carcinoma. However, most individuals infected with *H. pylori* are asymptomatic [1]. Microbiota within the gut occurs in a balance. Dysbiosis occurs if this balance becomes disturbed and consequently disturbance of proinflamatory immune response with susceptibility to invading pathogens and initiation of diseases [2].

The acidic gastric environment is expected to hinder growth of bacteria but the growth of *H. pylori* in the gastric mucosa means that the gastric environment is not absolutely sterile. In fact, different species have been isolated from the stomach and these included protebacteria, firmicutes, actinobacteria and fusobacterium phyla. But the most common non *H. pylori* bacteria in the stomach were *Streptococcus* and *Staphylcoccus* [3] [4].

An important question is why not all patients of *H. pylori* infection develop diseases and the answer is that several pathogenic factors have role in progression to disease which include host factors [4] [5] *H. pylori* factors, environmental factors and probably non *H. pylori* gastric organisms may have a role.

The primary aim of this study is to determine the gastric microbiota in patients with *H. pylori* and chronic dyspepsia and to use culturalable gastric mucosa by use matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MAIDI-TGF MS) [6].

## 2. Patients and Methods

During 12 months period from (July 2017 to July 2018) this study was carried out and included 81 patients (59 Males/22 Females) in Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt. All patients were examined by upper endoscopy and 4 gastric biopsies were taken (from antrum and corpus) for purpose of this study one sample for rapid urease test to determine patients positive for *H. pylori* (and consequently inclusion in this study) and 2$^{nd}$ sample for histopathology and confirming *H. pylori* status, while the 3$^{rd}$ and 4$^{th}$ samples taken for microbiological examination including gram stain (and when needed other stains) and tissue culture to confirm infection with *H. pylori* and checking other non *H. pylori* bacterial flora.

All patients in this study were positive *H. pylori* infection initially by rapid urease test and confirmation was achieved by histologic confirmation by staining and/or positive culture isolates.

**Exclusion criteria:** All patients who received antibiotics, acid suppression or on long term use of NSAID during 4 weeks before endoscopy were excluded from the study. Also, patients complaining from organic disease that can cause dyspepsia were excluded.

A total of 81 patients were included and divided into two groups. Group I: were 50 patients complaining of chronic dyspepsia (more than 6 months dura-

tion) as an indication for endoscopy, (dyspeptic n = 50, median age 40 range 19 to 65 years) and 2nd group non-dyspeptic patients (n = 31, median age 40 range 19 to 65 years). All patients were endoscoped; as indication for dyspepsia in group I and for other indications e.g. dysphagia, obscure anaemia in group II.

**Gastric Biopsies:** One sample was placed in a commercial urea-based strip (Halifax Research laboratory, Kolkata, India), for the bedside rapid urease test for *H. pylori* while 2nd sample was fixed in formalin for histopathology examination [7], whereas the 3rd and 4th samples were used for tissue culture.

**Tissue culture:** Samples were dispersed using a homogenizer then inoculated into sterile tryptic soy broth. All samples were cultured using Muller Hinton agar plates then incubated at 37˚C under aerobic conditions (for 24 - 48 hours) and anaerobic conditions (for 3 - 5 days). Samples that yielded positive results on RUT were cultured using Skirrows medium plates, incubated at 37˚C under microaerophilic conditions for 3 - 7 days [8].

### 2.1. Identification by MALDI-TOF MS

A fraction of a single colony was smeared with a plastic loop onto a 48-well steel plate and dried. One µL of matrix solution (saturated solution of cyano-4-hydroxycinnamic acid in 50% acetonitrile + 2.5% trifluoroacetic acid) was added to each well and allowed to air dry at room temperature [9].

Measurement was done using VITEK MS MALDI-TOF Mass Spectrometer for Microorganism Identification (BioMérieux; USA). Each run was validated with an *Escherichia coli* (*E. coli*) control sample to ensure that the spectrometer was set properly. Raw spectra of the strains were analyzed by bioMérieux platform Myla™ v2.0.

### 2.2. Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance; difference and association of qualitative variable by Chi square test ($X^2$). Differences between quantitative independent groups were evaluated by t-test. P value was set at <0.05 for significant results & <0.001 for high significant result.

### 2.3. Ethical Considerations

All patients gave a written informed consent for participation in the study, for performing the endoscopy with biopsy. And they were offered to cancel their participation in the study at any time point without any obstacle.

## 3. Results

### 3.1. Study Patients

This study comprised a total of 81 patients undergoing upper GIT endoscopy; of

them 59 were males (73%) and 22 (27%) were females, their age ranged between 19 and 65 years, mean age was 42.69 ± 10.83 years. They were divided into; Group I dyspeptic patients: included 50 patients (61.7% of the study patients) and group II: non dyspeptic patient and included 31 patients (38.3% of the study patients). There was no statistically significant difference between dyspepsia (group I) and non-dyspepsia patients (group II) concerning the age and gender. However, group I dyspeptic patients were more smokers and consumers of spicy foods when compared with non-dyspeptic patient group in this study (Table 1).

### 3.2. Gastric Histology

Histopathological examination of the gastric mucosal biopsies using different stains showed that histological abnormalities noticed in both groups included chronic active gastritis, gastric erosions and chronic inactive gastritis respectively in order of frequency with active chronic gastritis and gastric erosions showed significant association with dyspepsia (Table 2). A total of 38.7% of non-dyspeptic patients showed chronic inactive gastritis in their mucosal examination. However, there was no gastric mucosal pathology seen in 61.3% and 2% of non-dyspeptic and dyspeptic patients respectively denoting that almost always patients with dyspepsia had underlying gastric mucosal pathology.

### 3.3. Microbiological Examination

All patients in this study were *H. pylori* positive. Regarding the distribution of microbial species isolated by culture of the gastric mucosal biopsies there was isolation of the following species *Staphylococci*, *Streptococci*, *Lactobacilli*, *Klebsilla*, *E coli*, *Pseudomonas*, *Enterococci*, *Aspergillus*in order of frequency (Table 3). In this study 16 patients had at least one bacterial species isolated besides

Table 1. Patient characters in both groups.

| | | | Dyspepsia | | Total (N = 81) | P |
|---|---|---|---|---|---|---|
| | | | No (N = 31) | Yes (N = 50) | | |
| **Age** (years, mean ± SD) | | | 42.48 ± 12.5 | 42.82 ± 9.7 | | 0.89 |
| **Sex** | 1.00 | N | 20 | 39 | 59 | 0.18 |
| | | % | 64.5% | 78.0% | 72.8% | |
| | 2.00 | N | 11 | 11 | 22 | |
| | | % | 35.5% | 22.0% | 27.2% | |
| **Smoking** | 0.00 | N | 23 | 19 | 42 | 0.002* |
| | | % | 74.2% | 38.0% | 51.9% | |
| | 1.00 | N | 8 | 31 | 39 | |
| | | % | 25.8% | 62.0% | 48.1% | |
| **Spicy food** | 0.00 | N | 28 | 12 | 40 | <0.01** |
| | | % | 90.3% | 24.0% | 49.4% | |
| | 1.00 | N | 3 | 38 | 41 | |
| | | % | 9.7% | 76.0% | 50.6% | |

*Significant, **Highly significant.

**Table 2.** Histopathology distribution in groups.

| | | | Dyspepsia | | Total | P |
|---|---|---|---|---|---|---|
| | | | No (N = 31) | Yes (N = 50) | | |
| **Histopathology** | Active chronic gastritis | N | 0 | 31 | 31 | <0.01** |
| | | % | 0.0% | 62.0% | 38.3% | |
| | Erosion | N | 0 | 18 | 18 | |
| | | % | 0.0% | 36.0% | 22.2% | |
| | Inactive chronic gastritis | N | 12 | 0 | 12 | |
| | | % | 38.7% | 0.0% | 14.8% | |
| | Normal | N | 19 | 1 | 20 | |
| | | % | 61.3% | 2.0% | 24.7% | |

**Highly significant.

**Table 3.** Culture result distribution between groups.

| | | | Dyspepsia | | Total | P |
|---|---|---|---|---|---|---|
| | | | No (N = 31) | Yes (N = 50) | | |
| ***Staph.*** | Negative | N | 21 | 1 | 22 | 0.00** |
| | | % | 67.7% | 2.0% | 27.2% | |
| | Positive | N | 10 | 49 | 59 | |
| | | % | 32.3% | 98.0% | 72.8% | |
| ***Strept.*** | Negative | N | 10 | 47 | 57 | 0.00** |
| | | % | 32.3% | 94.0% | 70.4% | |
| | Positive | N | 21 | 3 | 24 | |
| | | % | 67.7% | 6.0% | 29.6% | |
| ***Lactobacill*** | Negative | N | 31 | 30 | 61 | 0.00** |
| | | % | 100.0% | 60.0% | 75.3% | |
| | Positive | N | 0 | 20 | 20 | |
| | | % | 0.0% | 40.0% | 24.7% | |
| ***Enter_colitis*** | Negative | N | 28 | 48 | 76 | 0.302 |
| | | % | 90.3% | 96.0% | 93.8% | |
| | Positive | N | 3 | 2 | 5 | |
| | | % | 9.7% | 4.0% | 6.2% | |
| ***Pseudo.*** | Negative | N | 26 | 48 | 74 | 0.059 |
| | | % | 83.9% | 96.0% | 91.4% | |
| | Positive | N | 5 | 2 | 7 | |
| | | % | 16.1% | 4.0% | 8.6% | |
| ***Asper.*** | Negative | N | 30 | 49 | 79 | 0.73 |
| | | % | 96.8% | 98.0% | 97.5% | |
| | Positive | N | 1 | 1 | 2 | |
| | | % | 3.2% | 2.0% | 2.5% | |

**Continued**

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Klebs.*** | Negative | N | 21 | 50 | 71 | 0.00** |
| | | % | 67.7% | 100.0% | 87.7% | |
| | Positive | N | 10 | 0 | 10 | |
| | | % | 32.3% | 0.0% | 12.3% | |
| ***E coli.*** | Negative | N | 28 | 45 | 73 | 0.96 |
| | | % | 90.3% | 90.0% | 90.1% | |
| | Positive | N | 3 | 5 | 8 | |
| | | % | 9.7% | 10.0% | 9.9% | |

**Highly significant.

their *H. pylori* and 43 patients had 2 bacterial species besides *H. pylori* while 22 patients had multiple strains. When both groups are compared there was difference in the distribution between both groups. Both *Staph.* and *Lactobacillus* significantly (P 0.00) associated with dyspepsia, *Streptococcus* and *Klebsilla* are significantly (P 0.00) associated with non-dyspepsia but *Entercocci*, *Pseudomona*, *Aspergillus* and *E. coli* had no significant association with any group.

## 4. Discussion

Due to the high prevalence rates of both dyspepsia and *H. pylori* especially in developing countries like our Egyptian community, we investigated dyspeptic and non-dyspeptic patients for the presence of *H. pylori* and other microbial species inhabiting the gastric mucosa. In fact, the relation of *H. pylori* to dyspepsia have long been investigated and according to the current guidelines it is wise to test and treat for *H. pylori* in high prevalence areas [10]. But, the microbial species other than *H. pylori* concomitantly found with *H. pylori* have not been well investigated particularly in our community and this was the primary aim of this study.

This study shaded the light on several important points. First, the frequency of bacterial species in the gastric mucosa other than *H. pylori*, in contrast to the concept of sterile gastric environment this study showed that patients with and without dyspepsia with underlying gastric mucosal pathology have different species of bacteria probably due to increased gastric PH following different degrees of gastritis with associated hypochlorhydria [11].

The current study showed the species harvesting the inflamed gastric mucosa belongs to both gram positive and negative bacteria. The species isolated from the stomachs of our cohort are not different from the species reported in recent studies [8] recently published. We found *Staphylococcus spp.*, *Lactibacillus* which belonged to phyla firmuicules and protebacteria [12] in our study, the flora in *H. pylori* positive patients with chronic dyspepsia was dominated by *Staphylococcus* and *Lactobacillus* and without dyspepsia showed dominance *Streptoccous* and *Klebsilla*. We cannot explain dominance of these species in relation to dyspepsia because each group harbored gram positive cocci and gram

negative bacilli. Our reports about the significant difference in gastric microbiota in both groups dyspeptic and not dyspeptic is in agreement with Pereira *et al.*, [8] who found qualitative difference in the gastric microbial spectrum between patients harbouring *H. pylori* with and without chronic dyspepsia .The prevalence comprised *Staphylococcus* followed by *Lactobacillus* in patients with *H. pylori*-positive chronic dyspepsia and by *Streptococcus* followed by *Staphylococcus* and *Klebsiella* in those without dyspepsia.

Second, in fact our finding of the association between *Lactobacillus* with dyspepsia and *H. pylori* is not novel because *Lactobacillus* has been incriminated to have a role in progression to gastric cancer in presence of *H. pylori* infection [13].

The inclusion of 100% *H. pylori* infected patients in our study is probably related to the use of different invasive testing methods (rapid urease test, Gram staining, culture). A scenario is not commonly used in the daily medical practice. However, Egypt had high prevalence rates of infection that approaches 70% - 90% in elderly population [14]. We usually rely on non-invasive use of *H. pylori* antigen in stool in daily medical practice [15].

Third, the 98% pathology rate reported in dyspeptic patients. In fact this finding had been reported by one paper from our institution [15] which concluded that all dyspeptic patients had endoscopic and pathologic abnormality even in absence of positive *H. pylori* testing. When both groups of our study are compared there was a significant difference in chronic gastritis with erosive mucosa in dyspeptic patients than non-dyspeptic patients which emphasizes the findings of Emara *et al.*, 2017 [15] who found pathologic gastric mucosa in all patients with dyspepsia, probably due to inclusion of patients from the same locality.

The question here is: How *H. pylori* facilitated the change in gastric microbiome we reported in this study? And the answer can be inferred from the natural history of *H. pylori* infection. *H. pylori* creates a special niches that allow survival and colonization of bacteria in the stomach and *H. pylori* will increase the gastric pH to <4 which change gastric environment and facilities colonization of different bacteria [16]. Also, the underlying gastric inflammation and changes in the local immune system in the stomach induced by *H. pylori* may influence the microbial spectrum [16].

Another question pops up: Is it a role that *H. pylori* will change the gastric microbiome in every patient? And the answer can be concluded from the study of Bik *et al.*, [12] who found that there was no influence of *H. pylori* on the diversity of gastric microbiota. They also showed that there is no difference in microbiota isolated from gastric antrum and corpus.

One controversial issue that should be discussed here is the role of the other bacterial species isolated from the stomach in causing the underlying gastric pathology reported. In our study, although *H. pylori* is isolated from all patients we cannot cancel the role of other species in causing gastric and extra-gastric dis-

eases attributed to *H pylori*. In the other hand, some of gastric microbiota e.g. *Lactobacilli* are known to have probiotic properties including antibacterial activity besides stimulation of the local immune system [17]. In fact some *lactobacillus* species have even been used in treatment regimens of *H. pylori* increasing the eradication rates and lower treatment related side effects [18]. Other types of microbiota isolated in this study are known to induce diseases in other clinical situations [8] and this probably may incriminate these organisms in facilitating the pathology induced by *H. pylori* among our patients however these are not solid conclusions and needs further investigations.

This study has some limitations. First of all, it is a single center study, the small number of patients and lack of follow up and this can be corrected in future studies.

In conclusion, in this study we showed that gastric mucosa harbor other microbiota concomitant with *H. pylori* in both dyspeptic and non-dyspeptic patients. The predominant microbiota in dyspeptic patient in presence of *H. pylori* comprised *Staphylococcus* followed by *Lactobacillus* and *Streptoccous* followed by *Klebsiella* in non dyspeptic patients. The concomitant interaction between *H. pylori* and other gastric microbiota may have an effect on the gastric pathology reported.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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