

Antibiotic Resistance Profile of Helicobacter Pylori Isolates from Dyspeptic Patients of Civil Hospital, Khairpur

Helicobacter
Pylori Isolates
from Dyspeptic
Patients

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ABSTRACT

Objective: To examine the frequency of H. pylori in patients with dyspepsia and also to test the antibiotic susceptibility profile of isolates.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Department of Microbiology, Shah Abdul Latif University, Khairpur for one year from 01-Jan-2019 to 31-12-2019.

Materials and Methods: Biopsy specimen from 100 patients with dyspepsia from both sexes was collected from civil hospital, Khairpur. After culturing the samples in Microbiology Institute, Shah Abdul Latif University, Khairpur, the identification of the samples was carried out (gram staining, catalase, oxidase and urease). The susceptibility profile to ten common antibiotics was tested by Disk diffusion method.

Results: From total 100 subjects, 100 biopsy samples from were collected. Mean age of the patients in years were 40±15.4. Out of 100 biopsies, 30 were positive for H. pylori. From these positive samples, majority of 56.7% were isolated from males and 43.3% from females. Out of 30 isolates, 11.7% isolates were sensitive and 88.3% showed intermediate resistance. From 30 isolates, 4.7% isolates were sensitive to amoxicillin, 2.46% to metronidazole, 18.94% isolates to amikacin, 1.07% to novobiocin, 21.57% to ciprofloxacin, 31.88% isolates to levofloxacin, 31.88% to lincomycin and 22.90% isolates were sensitive to clarithromycin.

Conclusion: The results showed that clarithromycin, levofloxacin, ciprofloxacin and moxifloxacin were sensitive while Amikacin and clavulanic acid/ amoxicillin showed intermediate resistance and novobiocin, amoxil and metronidazole, were resistant to H. pylori.

Key Words: H. pylori, Gram negative bacterium, Antibiotic susceptibility test, Dyspepsia.

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INTRODUCTION

H. pylori belong to Helicobacteraceae family and is a Gram negative bacterium¹. Globally, it is one of the most prevalent bacterial infections with 4.4 billion people infected with H. pylori.

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Global H. pylori infection prevalence is 44.3%. Prevalence rate (50.8%) is higher in developing countries and Africa (70.1%) when compared with other regions. Prevalence rate differ widely in different regions, the rate is high in developing countries². It show well-established association with socio-economic status and sanitation³. H. pylori is foremost source of gastro-intestinal illnesses and most significant risk factors for gastric cancer as well as mucosa-associated lymphoid tissue (MALT) lymphoma⁴. For elimination of H. pylori, PPI and antibiotics are first-line treatment. Successful eradication of H. pylori primarily depends on the selection of suitable antibiotics. But universally, the anti-microbial susceptibility testing for H. pylori is nearly absent. It is suggested to select an effective empirical eradication therapy is built on region and antibiotic resistance patterns specific to that population⁵.

Unfortunately, over last decade, rate of eradication by above-mentioned treatment has declined to less than 90% mostly because of rapid antibiotic resistance, specifically against clarithromycin and metronidazole⁶. H. pylori antibiotic resistance differs considerably in

different countries and among regions of same country⁷. So, surveillance of antibiotic resistance at local level is essential.

Antimicrobial resistance of *H. pylori* is a severe problem leading to eradication failure. For selection of a proper treatment regimen, knowledge of antimicrobial susceptibility is significant. Before the selection of an optimal treatment regimen, it is essential to have information regarding the frequency of resistance to a specific antimicrobial agent. The reports show that resistance rate vary from 10 to 90% for metronidazole, 5-59% for tetracycline, 0-45% for clarithromycin and 0-33% for amoxicillin⁸. Worldwide increase in resistance of *H. pylori* to metronidazole, clarithromycin and amoxicillin is reported⁹. The increase has serious implications as, not only for patient compliance, but also for determining the results of antibiotic treatment¹⁰. Antibiotic resistance might fluctuate over the years and not stable. It fluctuate in different regions¹¹. Knowledge of the patterns of drug resistance is key to formulate the strategies to minimize the development of resistance and to help clinicians to improve treatment guidelines¹². The objective of current study was to assess the *H. pylori* resistance rate to the different antibiotics used in local setting.

MATERIALS AND METHODS

This cross sectional study was conducted at department of microbiology, Shah Abdul Latif University, Khairpur. Duration of study was one year from 01-Jan-2019 to 31-12-2019 and non probability consecutive sampling technique was used for collection of samples. Sample size was calculated by openepi.com at 10% margin of error, 95% confidence level and taking *H. pylori* prevalence in functional dyspepsia as 64.4%. Patients of either gender, with age range of 15 to 60 years who were admitted with signs and symptoms of dyspepsia included in this study.

Patients age less than 15 years and greater than 60 years, females who were pregnant and lactating, patients using PPI, receiving *H. pylori* treatment and patients not suitable for gastroscopy were excluded from study.

Ethical approval of the study was taken from hospital ethical committee. After giving details of study, informed consent (written) was obtained from patients. To collect samples of patients, endoscopy was done in gastroenterology department in patients with gastritis (acute or chronic) and peptic ulcer problem. Stuarts transport medium was used to transport biopsy samples to histopathology lab. In case of identification of *H. pylori* infection by gram staining method report was considered positive. Disk diffusion method was used to test anti-microbial sensitivity. Commercially available antimicrobial discs were purchased. Bacterial isolates were cultured overnight in Muller Hinton agar (MHT). To see number of bacteria, turbidity was matched with 0.5 McFarland assay. Lawn of bacterial isolates was

spread on MHT plates and inoculation disks were dispensed on plates after 15 minutes of. Plates were incubated at 37°C, plates for 24 hours. Test was performed in triplicate with both controls (positive & negative). Zone of inhibition was measured after 24 hours of incubation. Antibiotic were labeled as resistant when inhibition zone was less than 9 mm, intermediate diameter when zone range from 10-35 mm and sensitive at diameter of greater than 36 mm. (Figure 1 & 2).

Data was analyzed by SPSS version 25. For quantitative data like age mean and SD was calculated. While for qualitative data, frequency percentages were calculated. Chi-square test was applied to see association between study variables. p-value ≤ 0.05 was taken as significant statistically.

RESULTS

100 samples were collected from 100 subjects from which 35% were females and 65% were males (figure 3). Mean age of the patients in years were 40 ± 15.4 years. Majority of patients with *H. pylori* positive were from age group between 40-60 years, from which 56.7% of them were *H. pylori* positive; while 30% of patients were from age between 20-40 years of age and only 13.3% were from age below 20 years of age.

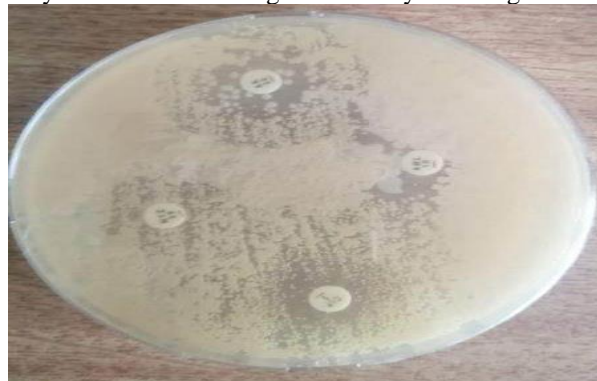


Figure No. 1: Inhibitions zone for amoxicillin



Fig 2: Inhibition zone of metronidazole

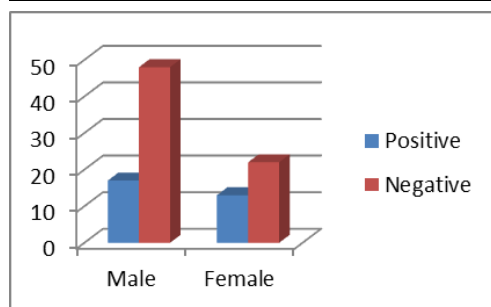


Figure No.3: Gender-wise distribution of Patients - H. pylori prevalence according to gender

Biopsies of 30 subjects were positive for H. pylori out of 17 (56.7) were males and 13 (43.3) females. So, positivity rate was more in males compared to females. This study shows that a range of multidrug resistant H. pylori isolates were presented in untreated patients posing risk for nearby population. From 30 isolates, 11.7% isolates were sensitive and 88.3% showed intermediate resistance. From 30 isolates, 4.7% isolates were sensitive to amoxicillin, 2.46% to metronidazole, 18.94% isolates to amikacin, 1.07% to novobiocin, 21.57% to ciprofloxacin, 31.88% isolates to levofloxacin, 31.88% to lincomycin and 22.90% isolates were sensitive to clarithromycin.

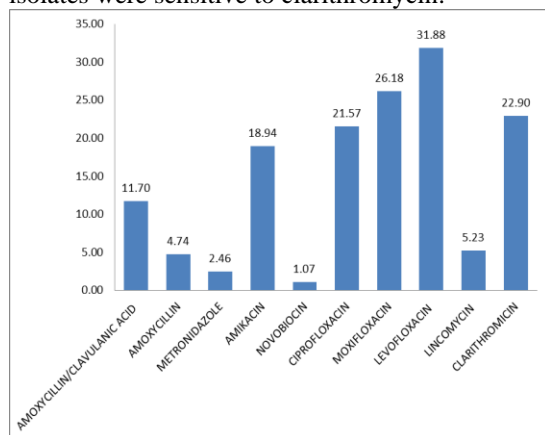


Figure No.4: Antibiotics susceptibility of H. pylori isolates

DISCUSSION

Helicobacter pylori belongs to Helicobacteraceae family and is a Gram negative bacterium. H. pylori is etiological agent of peptic and gastritis ulcer. For gastrointestinal pathologies is antibiotics are the principle treatment. Globally, resistance of antibiotics is a public health problem comprising of diverse bacteria. In 2017, H. pylori is categorized as a pathogen of high priority by WHO because of increased number of clarithromycin resistance¹³. For establishing the finest treatments with maximum efficacy and minimum adverse effects, knowledge of actual antibiotic resistance in each patient is necessary.

This is the first study to the best of our knowledge that reports the antibiotic susceptibility profile in dyspeptic patients in Interior Sindh. From total 100 subjects, 100

biopsy samples from were collected. From 100 biopsies, 30 were positive for H. pylori. From 30 positive samples, 56.7% were isolated from males and 43.3% from females. Our results are comparable to the study conducted by Faisal Rasheed et al which showed prevalence of H. pylori as 32.7%¹⁰. Our results are noticeably lesser as compared to old studies. A study by Mujataba et reported the prevalence of H. pylori as 41.6%.¹⁴ Results by Tahir et al displayed the frequency of H. pylori as 56.4% from a study carried out at in Mansehra's King Abdullah Teaching Hospital¹⁵. A study from Nilore, Islamabad conducted by Qureshi et al in dyspeptic patients displayed very high prevalence of 66.5% of H. pylori infection¹⁶. Another study from at Liaquat National Hospital, Karachi by Tooba et al et reported 64.4% prevalence of H. pylori infection¹⁷.

Current study investigated the anti-microbial sensitivity of isolates from dyspeptic patients. Antibiotic susceptibility of H. pylori isolates was tested for 10 antibiotics. Sensitivity to amoxicillin was 4.7%, 2.46% to metronidazole, 18.94% isolates to amikacin, 1.07% to novobiocin, 21.57% to ciprofloxacin, 31.88% isolates to levofloxacin, 31.88% to lincomycin and 22.90% isolates were sensitive to clarithromycin. Similar findings are stated in other studies carried out in different countries as well as in Pakistan. A meta-analysis showed antibiotics resistance to Clarithromycin (37%), Levofloxacin (19%), Amoxicillin (37%)¹⁸. In last 10 years, the increase in resistance to clarithromycin from 2.2 to 7.5% is of substantial health importance, because resistance to clarithromycin has decreased the effectiveness of standard therapy bases on clarithromycin up-to 70%. But Amikacin and clavulanic acid/ amoxicillin showed intermediary resistance. Whereas novobiocin, amoxil, metronidazole showed resistant to H. pylori. In Asian pacific region, amplified macrolides consumption might be responsible for clarithromycin resistance from 7% before 2000 to 21% in 2011–15. Resistance to metronidazole resistance is greater in developing countries as compared to developed countries. In developing countries, practice of economical antibiotic for other infections added to this difference¹⁸.

The range antibiotics use among diverse groups of community is related to the geo-graphical differences in the antimicrobial resistance of H. pylori. There is indiscriminating usage of antibiotics for treatment of various infections for treatment of various infections in Pakistan, particularly that of amoxicillin, erythromycin, metronidazole, tetracycline and clarithromycin. Additionally, free access of medicines due to unobstructed sale of antibiotics encourages the self-prescription. This is contributing cause of the augmented anti-microbial resistance in H. pylori isolates to amoxicillin clarithromycin, tetracycline and metronidazole.

Current study displays that H. pylori from untreated patients are multidrug resistant. This poses a severe risk to neighboring population. Easier access to antibiotics

lacking proper prescription and use for respiratory and skin infections may explain these findings.

CONCLUSION

The results showed that clarithromycin, levofloxacin, ciprofloxacin and moxifloxacin were sensitive while Amikacin and clavulanic acid/ amoxicillin showed intermediate resistance and novobiocin, amoxicil and metronidazole, were resistant to *H. pylori*. This study shows that *H. pylori* from untreated patients grasp a variety of multi-drug resistance which carries serious risk to nearby population.

Author's Contribution:

Concept & Design of Study: Shehzad Tariq
 Drafting: Farukh Imtiaz, Tabish Akbar
 Data Analysis: Bakhtiar Ahmed, Sarmad Saeed, Bakhat Ali Ansari
 Revisiting Critically: Shehzad Tariq, Farukh Imtiaz
 Final Approval of version: Shehzad Tariq

Conflict of Interest: The study has no conflict of interest to declare by any author.

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