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ANTIBIOTIC RESISTANCE STUDY OF *HELICOBACTER PYLORI* ISOLATED FROM STOMACH BIOPSY SAMPLES FROM QUETTA

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Abstract

Helicobactor pylori is a main contributor in the increase of gastric problems. Many antibiotic regimens are available for the treatment but none of them could achieve the peak of eradication. In this study patients with gastric troubles were recruited from Bolan Medical Complex Quetta and about 60 isolates of H. pylori from biopsy samles were subjected to 5 common antimicrobial agents; Metronidazole, Amoxicillin, Clarithromycin, Ciprofloxacin and Tetracycline by disk diffusion method. Majority of the isolates showed resistance to Metronidazole 50% while the isolates showed 28.33 % resistance to Clarithromycin, and the other three antibiotics exhibited the various resistant Tetracycline 21.66%, Amoxicillin 18.33% and Ciprofloxacin 11.66%, respectively. It is concluded that Mertronidazole showed high level of resistance because of the drug used in the treatment of other infectious disease. Such a high frequency of resistance to the choice of antibiotics for the control and eradication of H.pylori stress on the need of discovery of new antibacterial agents against this pathogen.

Keywords: *Biopsy, Antibiogram, Quetta, H. pylori, Resistance*

INTRODUCTION

H. pylori is gram negative spiral flagellate bacterium live in the gastric mucosa of the stomach of human and other primates. The infection with the *H. pylori* related to chronic gastritis, peptic ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma, and was classified type 1 cancer causing agent (1). The bacterium first introduced by Marshall and warren in 1982 (2). The bacterium *H.pylori* infects the 50% of world population (3). The bacterium was at first named *Campylobacter pyloridis*, and later the name changed to *Helicobacter* (4).The colonization of bacterium in stomach persist infection, if the infection left untreated it lasts for lifetime (5), primarily

the infection spread from person to person by fecal-oral route. Mostly the infection of *H. pylori* is considered to be acquired in childhood, and the infection with this bacterium in young age increases the risk of associated complications later in life (6)

The *H. pylori* have unique features that enable the bacterium to survive in the harsh environment of the stomach and also evade the host immune response (3). The precise way of transmission of *H. pylori* is unknown, some studies showed that the reservoir of *H. pylori* is outside the gastrointestinal niche of human and consider the ground water, drinking water and surface water (1) or domestic animals are also considered as a source of transmission (7). The infection of *H. pylori* is high in the developing countries than in developed world, in developed countries the low prevalence rate of infection is due to education, good industrialization and public health management.

The diagnostic tests of *H. pylori* infection can be divided into invasive and non-invasive methods which involve the use of techniques either directly or indirectly. Culture and microscopic detection of the bacterium *H. pylori* is a direct method whereas the determination of urease production and an antibody or detection of stool antigen is measured as an indirect method. Improvement in molecular methods is now used as a dependable method for diagnosis of infectious disease (8).

In vitro H. pylori is sensitive to a broad range of antibiotics, but in vivo they fail as monotherapy. Clarithromycin is more effective as single drug given twice daily for 10 to 14 days to infected patients. Dual therapies combine twice-daily-dose of Proton Pump Inhibitor (PPI) with an Amoxicillin. The dual therapies have been replaced by triple therapies. The triple therapies are the combination of two antibiotics with a PPI or a Bismuth compound. An alternative is by quadruple therapies, the therapy is combining the two antibiotics with the bismuth compound and PPI (9 - 11).

Amoxicillin, Tetracycline, Imidazoles (Metronidazole, Tinidazole), and some selected macrolides (Clarithromycin, sometimes Azithromycin) are those drugs which are most extensively used for the eradication therapy of *H. pylori* (12). Recently, Furazolidone and Rifabutin has also documented. But the effectiveness of these two drugs is limited and mostly patients have not able to tolerate the drug Furazolidone (12).

Herbs, spices and many plants have remained source medicines from thousands of years (13). Nature has embedded and provided a source of medicinal plants from the past and the modern drugs have also been isolated from natural sources (14). Isolation and biochemical characterization of pharmacologically active compounds from medicinal plants are continued (15).

The developing of new anti-*H. Pylori* candidates, it vast and open the new doors of research in treatment. The scientists took interest equally in both fields in the medicinal chemistry field and in the field of creation a new synthetic drug compounds (16 - 18). Complementary and alternative routes of treatment, mainly natural, non-toxic and

inexpensive products are attractive. There are numerous studies on the antibacterial properties of plant and vegetables extracts.

Herbs and spices having a good potency to protect against infection, reduce inflammation help to detoxify the liver, cleans the organs and also defend from cell damage that can lead to, osteoporosis, heart disease, rheumatoid arthritis and other degenerative diseases Evaluation of new compounds of curcumin for their anti-microbial effect is interesting for researchers (19).

Curcumin is an important component of turmeric it's yellow in color and a rich source of bioactive pigment and is the important component of turmeric, and a rich source of valuable phenolic compounds (20). It has been shown that it has a broad range of biological actions and also has a broad spectrum of pharmacological uses (21, 22). Curcumin has also a long history of therapeutic use (23). The wide range of biological activity of curcumin including anti-inflammatory, anti-carcinogenic, antiviral activities, anti-diabetic, antioxidant property and also a antifungal activity (24, 25). Clove oil is a phenolic compound, the main component of E. cryophilic and has the ability against many pathogenic microbes, bacteria, fungi, viruses (26 - 28).

A study reported that clove oil totally inhibited all the strains, both resistant and sensitive at a concentration of 2mg/mL. Furthermore, the organisms did not show any resistance towards this compound (29).

In last few decades research on spices has been directed to investigate their medicinal, antimicrobial and anti-carcinogenic activities. Thus, spices like turmeric can protect the human body against bacterial infections and other metabolism related disorders.

This study was aimed to analyze the resistance pattern of local isolates of *H. pylori* from stomach biopsy samples against commonly used antibiotics.

MATERIAL AND METHODS

SAMPLING

A total of 100 biopsy samples were analyzed during this study. The patients who visited gastro-enterology Department of Bolan Medical University Hospital Quetta with complications of acid reflux, abdominal cramp, heart burn, bloating, vomiting were subjected to endoscopy. Three biopsy samples were taken from each patient, from antrum, fundus and insoseria. The sample were placed in a sterile tube containing 5mL NS (Normal saline) and brought to Bacteriology Laboratory, CASVAB, University of Balochistan Quetta for analysis.

ISOLATION AND CULTURE

The biopsy samples from NS were poured in to a sterile petri plates and chopped well into smaller pieces with a help of sterile scalpel blade. The contents then were inoculated on Columbia blood broth and agar (Oxide, UK), with 10% laked horse blood and supplemented with vancomycin, trimethoprim, amphotricine-B. The plates and tubes were incubated for 48hrs at temperature 37^{o}C by providing micro-aerophilic environment.

After 48hrs the plates were checked for suspected *H. pylori* colonies. The positive growth plates with small rounded translucent colonies were further sub-cultured on new plates to obtain pure colonies. The identification of the *H. pylori* was done through Gram-staining, microscopic morphology and presumptive biochemcial tests like oxidase test, urease test, and catalase test. For confirmation the PCR was also performed by targeting the genes of interests (ureC and cagA) through use of specific primers.

ANTIBODY SENSTIVITY TESTING

In this study the antibiotic susceptibility test were performed by using a standard Kirby-Bauer disk diffusion method as defined by Bauer *et al*., 1966 (30), all *H. pylori* isolates were exposed to five antibiotics Clarithromycin, Metronidazole, Amoxicillin, Tetracycline and Ciprofloxacin. The activated *H. pylori* isolates were suspended in normal saline (NS) at 10^6 CFU/ml and the turbidity of bacterial suspension was compared with 0.5 McFarland, and then the suspension was spread on the surface of Muller Hinton agar plates supplemented with 10% laked horse blood and placed the five commercially antibiotic discs (Oxoid, UK) on the agar surface, then the plates were incubated for 24hrs at 37^{o}C by providing a microaerophilic environment. After the completion of incubation period, the diameters of the clear growth zone of inhibition around the antibiotic discs were measured in millimeters.

RESULTS AND DISCUSSION

Antibiotic resistance of *H.pylori* isolates to Metronidazole (MTZ5), Ciprofloxain (CIP5), Clarithromycin (CLR15), Amoxicillin (AML10) and Tetracycline (TE10) were observed in this study. Out of 60 samples 30 (50%) were resistant to Metronidazole, 17 (28.33%) to Clarithromycin, 13 (21.66%) to Tetracycline, 11 (18.33%) to Amoxycillin and 7 (11.66%) to Ciprofloxacin (Table I). The highest resistance were observed towards Metronidazole while the lowest to Ciprofloxacin.

Table I. Resistant Pattern of *H pylori* isolates

Antibiotics	Total no. of resistant *H. pylori* isolates Number (%)
Metronidazole	30 (50%)
Clarithromycin	17 (28.33%)
Tetracycline	13 (21.66%)
Amoxicillin	11 (18.33%)
Ciprofloxacin	7 (11.66%)

This study was conducted to analyze the antibiotic resistance pattern in *H. pylori*, isolated from stomach biopsies from infected patients in Balochistan (Pakistan). In developing country the rate of *H. pylori* infection is high and rising antibiotic resistance is a hostile crisis. In this study we seen the high prevalence resistant of antibiotic, metronidazole the result of this study is high then the study reported by Tarnum in Pakistan, 2016 (31). Same results were cited by Khan *et al* . (2012) From India (32), showed

the high prevalence resistant rate. The low resistant rate of *H. pylori* isolates towards the metronidazole noted by a Turkish study in 2015 (35.5%) (33).

The antibiotic clarithromycin had shown the resistant rate of 28.57 % in the study conducted in Turkey Resistant rate 36.5% (33), and another study from Indonesia reported 27.8% (34) results same to this findings. The Resistance is because, the clarithromycin, which is used in upper and lower respiratory system and also because of the mutation in gene of the *H. pylori* 23S rRNA and A2143G gene (35, 36).

Fig. 1. Antibiotic sensitivity test results (Disc diffusion)

The sensitivity of *H. pylori* to antibiotic Amoxicillin is acceptable 18.57%, the study from India noted resistant rate of antibiotic Amoxicillin 32.8 % (37), and the study from South Korea reported resistant rate of Amoxicillin 18.5% (38). The difference in the result of this study may be the strain and the different region, the medium and incubation period.

Resistant to Tetracycline in current study is 20.38%.Study from Pakistan (Karachi) has been reported the resistant rate of tetracycline is 48. 4% (31) their findings is high then the finding of this study. The higher result has been reported in Iran (39). However resistant to tetracycline is the main cause is mutation in the 16SrRNA (40).

Resistant rate of ciprofloxacin in this study is 8.1%. The study carried out in Indonesia reported the resistant rate 6.9% (34). The prevalence rate of resistant is low than this current study. The study Vilaichone *et al* (2013) from Thailand reported the resistant rate 7.7% (41) which come and matched with the result of this study. Resistance shown by the ciprofloxacin is may be the reason that the antibiotic ciprofloxacin used in respiratory and urinary tract infection (42).

CONCLUSION

High resistance of this pathogen to the important antibiotics making it difficult or rather impossible to eradicate. A further research and discovery of new antibiotics needed to control the problem of *H. pylori.*

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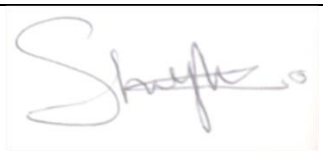
1	Shughla	03350292338 Shughlapanezai@gmail.com		Participated in research design, Experimentation, data analysis and manuscript writing
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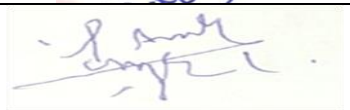
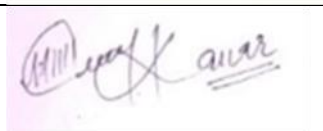
5	Dr. Zahoor Ahmed Kakar	03003850122 Zahoorkakar92@gmail.com		Data analysis
6	Dr. Hayat Muhammad Kakar	03337802602 Dr.hayatsiut@gmail.com		Sample analysis and drafted the initial manuscript
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