Article

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# Characterization of Metronidazole, Clarithromycin and Amoxicillin Resistance Genes in *Helicobacter pylori* Isolated from Gastroenteritis Patients

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**Background: *Helicobacter pylori* (*H. pylori*)is a Gram-negative, microaerophilic, and spiral shape bacterium that resides inside the human stomach. The human stomach serves as its primary reservoir. Complaints about stomach complication due to *H. pylori* infections are reported in the majority of populations around the globe. Chronic gastritis and intestinal metaplasia of the gastric mucosa are major complications of a long-term *H. pylori* infections that can lead to gastric cancer in severe cases. This study aims to characterize *H. pylori* isolates from gastroenteritis patients and to determine the resistance of *H. pylori* to various antibiotics.**

**Methods: In the current study, a total of (n = 80) gastric biopsy samples were randomly collected from gastroenteritis patients in brain-heart infusion broth. These were inoculated on Columbia blood agar supplemented with *Helicobacter pylori* selective supplement (DENT). After culturing, Microscopy and biochemical tests were performed. The susceptibility profile of *H. pylori* isolates was evaluated using the Kirby Bauer disk diffusion method. On the basis of the drug resistance profile, a total of (n = 20) isolates including (n = 10) from females and (n = 10) from males were selected for the detection and characterization of resistant genes. After confirmation of *H. pylori* using *16s rRNA*, polymerase chain reaction (PCR) was done for the detection of resistance genes including Metronidazole resistance (*rdxA* gene), Clarithromycin resistance (*23s rRNA* gene) and Amoxicillin resistance (Penicillin-binding protein A1 (*pbpA1*) gene).**

**Results: In a total of (n = 80) samples, *H. pylori* was isolated from 72.5% (n = 58) samples. Among the positive patients, there were 62% (n = 36) of female positive patients while in males, its ratio was 38% (n = 22). It was more common in the age between 30–50 years 55.17% (n = 32). It has shown the highest resistance towards Metronidazole 90% (n = 52), and the lowest toward Levofloxacin 65% (n = 38). Metronidazole resistance gene (*rdxA* gene) was detected in (n = 13) isolates including (n = 9) isolates from females and (n = 4) from males. In the case of, the Clarithromycin resistance gene (*23s rRNA*) (n = 10) was positive for *H. pylori* including (n = 6) isolates from females and (n = 7) were positive for Amoxicillin (*pbpA1* gene) including (n = 2) in female and (n = 5) from male patients.**

**Conclusion: This study highlights the increasing incidence of *H. pylori* infections in both male and female patients. It also revealed the current status of antibiotic resistance and its resistance genes in patients facing gastrointestinal issues. Continuous surveillance of resistant clones will help in formulating strategies that can help in combating of resistant clone. It will also help clinician in proper prescription and management of *H. pylori* infections.**

**Keywords:** *Helicobacter pylori*; intestinal metaplasia; gastric cancer; gastric biopsy; chronic gastritis

## Introduction

*Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerophilic, spiral shape bacterium reported in the human gastrointestinal mucosa. It has been labeled as microaerophilic since it grows best in environments with 5–15% oxygen. Its primary cultures often have lower oxygen tolerance, having a growth threshold of 3–7% oxygen [1]. Its subcultures can be quickly modified to grow aerobically in an incubator with a typical $CO_2$ combination (18% $O_2$, 10% $CO_2$). Its principal reservoir is the human stomach, with the potential to initiate infection. Constant stomach infection results in inflammation that can either subside or worsen into more serious problems [2]. Chronic gastritis and intestinal metaplasia of the stomach mucosa are substantial risk factors for gastric cancer brought on by long-term *H. pylori* infection [3]. It possesses a number of virulence characteristics that allow it to invade and infect stomach mucosa. These include the activity of urease, flagella, adhesin, acid inhibitory protein, catalase, vacuolating cytotoxin gene A (*vacA*), and cytotoxin-associated gene A (*cagA*) [4]. The *vacA* causes increasing vacuolation and

**Table 1. Antibiotics used for antibiogram assay.**

| Antibiotic | Concentration | Batch number | Manufacturer |
|---|---|---|---|
| Metronidazole | MTZ 5 µg | 1537293 | Thermo Scientific Oxoid, Karachi 74200, Pakistan |
| Clarithromycin | CLR 1 µg | 1764658 | Thermo Scientific Oxoid, Karachi 74200, Pakistan |
| Amoxicillin | AML 5 µg | 1989392 | Thermo Scientific Oxoid, Karachi 74200, Pakistan |
| Levofloxacin | LEV 5 µg | 2288856 | Thermo Scientific Oxoid, Karachi 74200, Pakistan |
| Doxycycline | DO 30 µg | 2295558 | Thermo Scientific Oxoid, Karachi 74200, Pakistan |

damage to the stomach epithelium, while the *cagA* is linked to adenocarcinoma and peptic ulcer disease [5].

Geographical differences in infection rates exist, and it has been supported by some additional factors, including lower socioeconomic level, patient age, ethnicity, exhibition to contaminated water, smoking, and having an infected parent, which can also make an infection more likely. Person-to-person transmission through oral, fecal, or gastro-oral exposure is the most typical form of infection [6]. The mainstay for eliminating *H. pylori* is antibiotic therapy, although this strategy is hindered by the rise of antibiotic resistance [7]. The main factor in treatment failure or insufficient elimination in patients who have received treatment is antibiotic resistance. It is mostly caused by genetic mutation [8].

Most frequently, first-line conventional triple therapy is employed to treat *H. pylori* infection which may contain two of the antibiotics including, Metronidazole, levofloxacin, clarithromycin, amoxicillin in composition with proton-pump inhibitor (ppi) [9]. However, if first-line medications prove ineffective, the bismuth-based quadruple therapy is also recommended for successful treatment. The process of antibiotic resistance for *H. pylori* isolates has undergone numerous sorts of mutations in prior research, which can result in treatment failure. The development of biofilms, modifications in membrane permeability, and efflux mechanisms are the causes of antibiotic resistance. The less virulent strains are less resistant to antibiotics than the more virulent ones because they produce less biofilm or have less blood supply to the stomach than more virulent strains [10].

The incidence of *H. pylori* eradication failure and antibiotic resistance has been erupted and documented worldwide. In Pakistan, the high prevalence of *H. pylori* is caused by improper dyspepsia diagnosis. In our population of District Kohat in the province of Khyber Pakhtunkhwa, Pakistan, the rate of *H. pylori* infection is rising daily, and the causes of the emergence of resistance are yet unknown [11,12]. Therefore the study has been designed to determine the resistance of *H. pylori* to various antibiotics and to characterize the resistance genes in *H. pylori* isolates. This study will provide updated knowledge about antibiotic resistance in the populations and will help clinicians in more prudent use of antibiotics to lessen the emergence and spread of antibiotic resistance.

## Materials and Methods

This study has been approved by Kohat University of Science and Technology Ethical Committee Ref No: 590/KUST/Ethical Committee. Before beginning any procedure, all participants have been informed verbally and have signed a written consent form following the ethical guidelines of the 1964 Helsinki Declaration and its implementing legislation. Stomach biopsy samples of *H. pylori* patients were obtained during endoscopy from DHQ Hospital Kohat, Pakistan. All of the patients were also asked about any gastrointestinal issues they had, such as nausea, vomiting, epigastric pain, or ballottement.

### *Inclusion and Exclusion Criteria*

We included all the patients with history of gastric ulcer, gastrointestinal disease and complications while the patients having no gastric ulcer are excluded in this study.

### *Endoscopy*

Those patients were subjected to endoscopy having burning pain in the upper abdomen, frequent nausea, recurrent nausea, frequent bloating and severe pain in the epigastrium and immediate heaviness in the epigastrium after meal. Gastric biopsy samples were obtained by fiber optic endoscopes (1587213, Xuzhou AKF Electronic Science and Technology Co., Ltd, Xuzhou, China) after the patients had fasted 6–8 hours. Before starting the endoscopy, the patients were anesthetized with 4% Xylocaine for 10–15 min. After 15 min, the stomach was accessed by fiber optic endoscope. The doctor can observe the esophagus, stomach, and upper section of the small intestine by passing an endoscope through the mouth, throat and into the esophagus. Samples were then taken with the help of sterile forceps.

### *Isolation of H. pylori from Gastric Biopsy Samples*

In this study, a total of (n = 80) gastric biopsy samples were collected from the antral region of the patient's stomach. To prevent desiccation and bacterial violability, gastric biopsy samples were obtained from the antral area of the stomach and collected in sterile falcon tubes containing 10 mL of brain-heart infusion (BHI) media. Within a day after being collected, all of the samples were brought to the Department of Microbiology, Kohat University of Science and Technology, Kohat for processing.

**Table 2. Primer and PCR conditions.**

| Genes | Primer sequence (5–3) | Size (bp) | Ref | PCR conditions |
|---|---|---|---|---|
| *16s rRNA* | F: 5′-GGTTACCTTGTTACGACTT-3′<br>R: 5′-AGAGTTTGATCCTGGCTCAG-3′ | 1492 bp | [16] | 95 °C, 5 min denaturation 95 °C 1 min, 55 °C annealing, 1 min, 72 °C 1 min extension (35 cycles) |
| *23s rRNA* | F: 5′-CCACAGCGATGTGGTCTCAGCAAA-3′<br>R: 5′-ATGACTCCATAAGAGCCAAAGCCCT-3′ | 429 bp | [17] | 95 °C, 5 min 95 °C denaturation, 30 s, 54 °C annealing, 30 s, 72 °C, 30 s extension (35 cycles) |
| *rdxA* | F: 5′-AATTTGAGCATGGGGCAGA-3′<br>R: 5′GAAACGCTTGAAAACACCCCT-3′ | 850 bp | [17] | 94 °C, 5 min 94 °C denaturation, 45 s, 58 °C, 1 min anealing, 72 °C extension, 30 s (35 cycles) |
| *pbpA1* | F: 5′-CGATAGATTTGGATTACCAACGC-3′<br>R: 5′-ACGATTTCTTTACGCAAGCC-3′ | 1035 bp | [18] | 95 °C, 15 min 95 °C denaturation, 30 s, 60 °C, 30 s annealing, 72 °C, 1 min extension (40 cycles) |

PCR, polymerase chain reaction; *pbpA1*, Penicillin-binding protein A1.

**Table 3. Demographic characteristics of positive patients (n = 58).**

| Distribution | Number of patients | Percentage (%) |
|---|---|---|
| Age | | |
| Below 30 years | 19 | 32.76% |
| 30–50 years | 32 | 55.17% |
| Above 50 years | 7 | 12.07% |
| Gender | | |
| Male | 22 | 38% |
| Female | 36 | 62% |
| Associated disease | | |
| Diabetes | 18 | 31.03% |
| Gastric cancer | 7 | 12.07% |
| Living area | | |
| Urban | 23 | 40% |
| Rural | 35 | 60% |

### *Cultural Identification*

All the biopsy samples were inoculated in Columbia agar and Columbia blood agar both supplemented with selective antibiotic supplement known as DENT (*Helicobacter pylori* selective supplement) (product number SR0147E, Thermo Scientific Oxoid, Karachi 74200, Pakistan). Typically, the plates were incubated for 3–4 days at 37 °C in a microaerobic environment (including 5% oxygen, 10% carbon dioxide, 85% nitrogen) using Camy Gensachet (Catalog No.OXCN0025A, Thermo Scientific Oxoid, Karachi 74200, Pakistan) and were then observed for bacterial growth. Phenotypic properties of *H. pylori*, such as tiny, translucent, non-hemolytic colonies on Columbia blood agar, were used to identify it [13].

Identification involves microscopy which was done by the Gram staining technique for which a smear was prepared on the clean class slide. After Gram staining procedure, the slide was examined under oil immersion (100×) microscopic (lens, Ay13074, Pomona, CA, USA). Several biochemical tests were also performed. This includes catalase, oxidase and urease test were performed as per protocol [14].

### *Antibiotic Resistance Profile of H. pylori*

Antibiotic susceptibility tests of all the isolates were determined by the Kirby Bauer disk diffusion method. Before the assay, the turbidity of broth was made equal to 0.5 M McFarland standard. The broth was prepared by growing *H. pylori* on a separate agar plate and incubated at 37 °C for 3–4 days. 2–3 overnight culture colonies were inoculated in 8 mL of BHI, which was then incubated at 37 °C for 48 hours along with CampyGen until the turbidity equaled the 0.5 M McFarland standard. A sterile swab was dipped into the inoculums, pressed against the test tube wall to remove extra fluid, and then streaked over the Mueller Hinton agar plates while still wet. The antibiotic discs were placed on plates with aseptic techniques using sterile forceps. The plates were then incubated microaerophically at 37 °C for 48 hours [15]. The antibiotic disc that is used in this study is shown in Table 1.

### *Molecular Characterization*

DNA of positive samples was isolated by using the standard phenol-chloroform method. The polymerase chain reaction (PCR) was done for molecular identification and detection of resistance genes. For detection purposes, the total reaction mixture was 10 µL comprised 5 µL Thermo Scientific green master mix solution (Made in Lithuania,

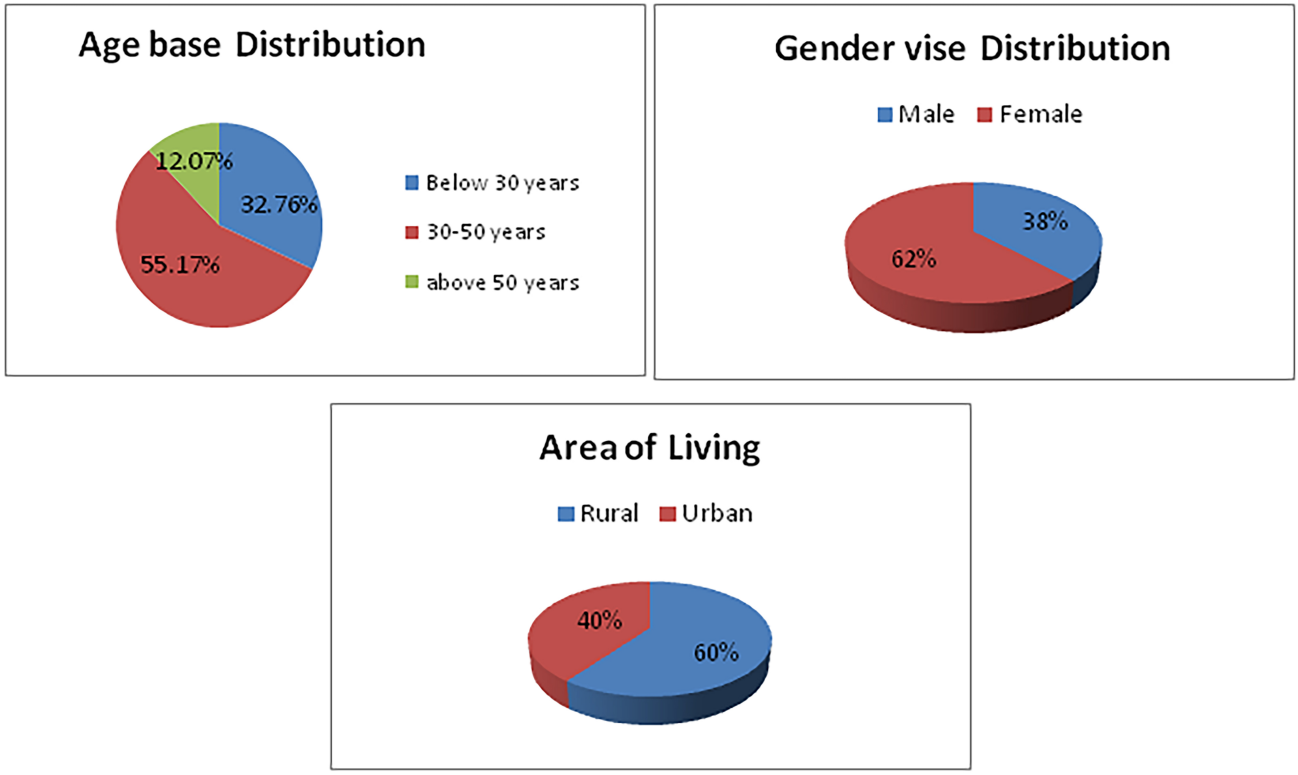


**Fig. 1. Graphical representation of demographic data of positive patients on the basis of age, gender and living status.**

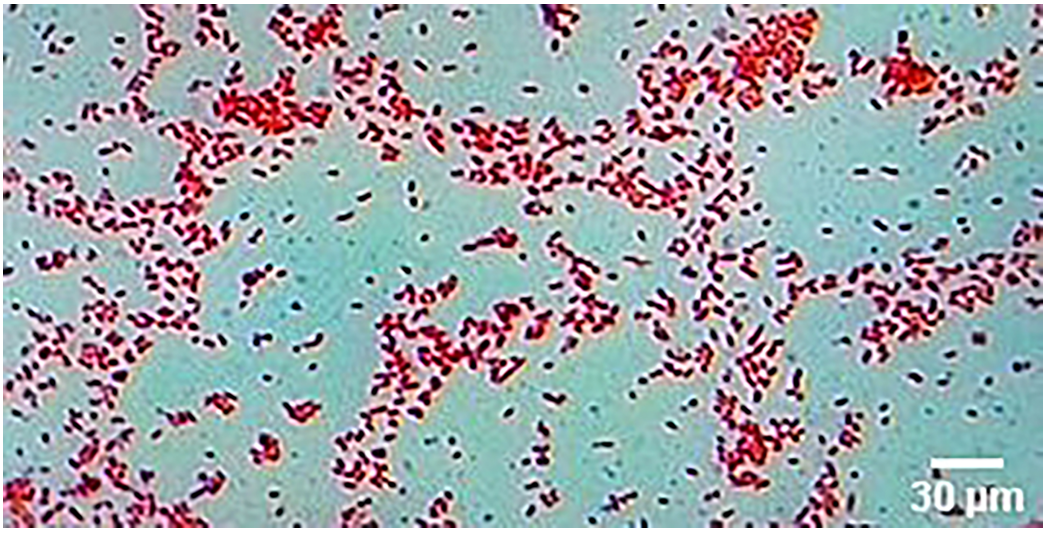


**Fig. 2. Microscopy of *Helicobacter pylori* (*H. pylori*): *H. pylori* showed up as a Gram-negative rod.**

Catalog No: 01034649, Thermo Scientific, Graiciuno g.8, Lithuania), 1 µL of reverse and 1 µL of forward primers, 2 µL DNA and 1 µL of PCR water. For sequence, the total reaction mixture of 25 µL was used containing 12.5 µL green master mix solution, 2.5 µL of reverse and 2.5 µL of forward primers, 5 µL DNA and 2.5 µL of PCR water. The 4 µL of the final PCR product was visualized through gel electrophoresis on 2% agarose gel (Catalog No: 00699250, Ref No: R0492, Thermo Scientific Oxoid, Karachi 74200, Pakistan)

### *H. pylori Confirmation Using 16s rRNA*

The identification of *H. pylori* was performed by the amplification of *16s rRNA* using PCR. The reaction conditions for *16s rRNA* using both *16s*-F and *16s*-R (Table 1) primers were initial denaturation at 96 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min [16].

**Table 4. Microscopy and biochemical characteristics of *H. pylori*.**

| Characteristic test | *H. pylori* reaction | Percentage |
|---|---|---|
| Columbia blood agar | Small, grayish translucent | 100% |
| Gram staining | Gram-negative s-shape | 100% |
| Catalase test | +ive | 100% |
| Oxidase test | +ive | 100% |
| Urease test | +ive | 100% |

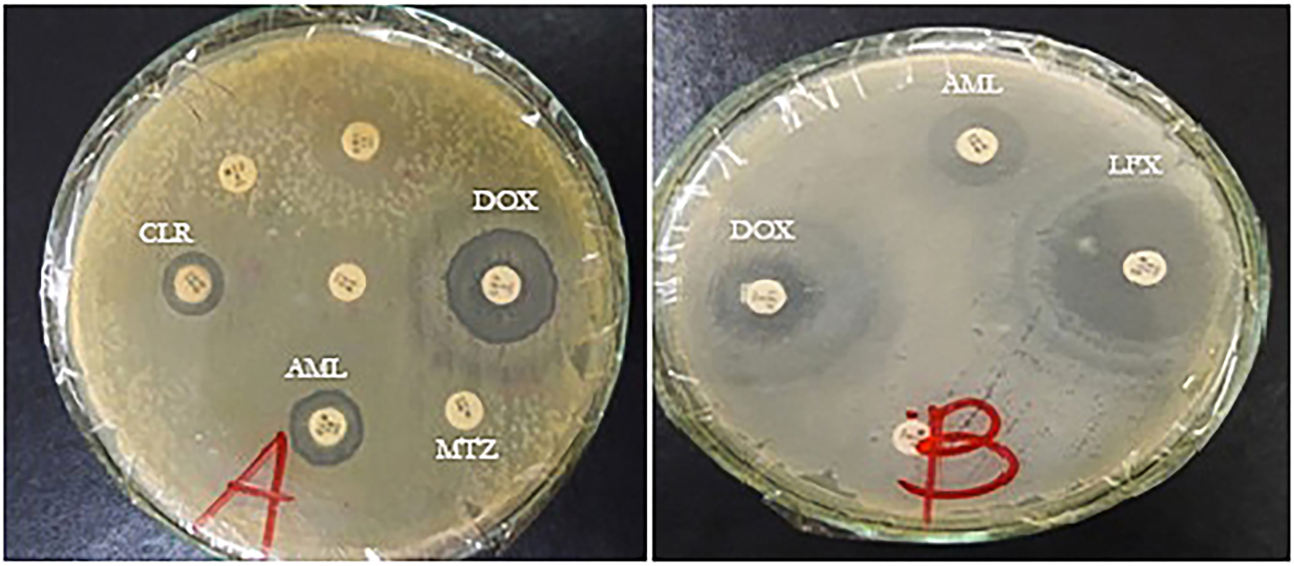


**Fig. 3. Showing antibiotic susceptibility profile of *H. pylori* (A = isolate 1 and B = isolate 2).**

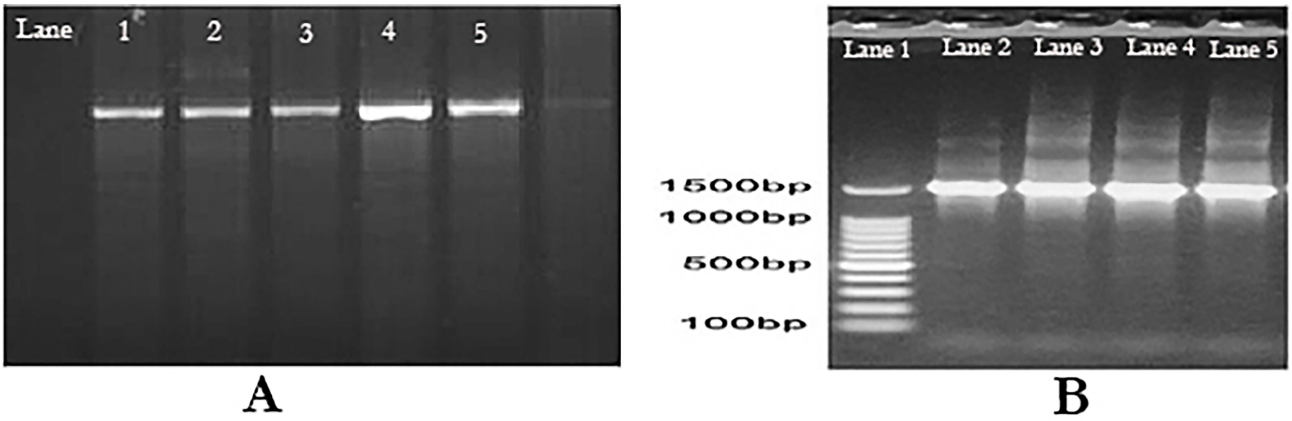


**Fig. 4. (A) DNA bands; lanes 1–5 indicating extracted DNA of *H. pylori*. (B) Showing *16s rRNA* bands; lane 1 indicating Ladder, lanes 2–5 showing the amplified *16s rRNA* bands.**

To confirm resistance genes, PCR was done for the detection of resistance genes including Metronidazole resistance (*rdxA* gene), Clarithromycin resistance (*23s rRNA*) and Amoxicillin resistance (Penicillin-binding protein A1 gene (*pbpA1*) gene). On the basis of its drug resistance profile, total of (n = 20) isolates including (n = 10) from females and (n = 10) from males were selected for the detection and characterization of resistant genes.

### *Detection of Clarithromycin, Metronidazole and Amoxicillin Resistance Gene*

Resistance to clarithromycin isolates was selected for the detection of *23s rRNA* using an automated thermocycler with primers *23s*-F and *23s*-R (Table 2, Ref. [16–18]). The PCR was used as follows; denaturation at 95 °C for 5 minutes was after by 35 rounds of denaturation at 95 °C for 30 seconds, 35 cycles of annealing at 54 °C for 30 seconds, extension at 72 °C for 30 seconds, and finally extension at 72 °C for 10 minutes [17].

**Table 5. Antibiotic susceptibility pattern of *H. pylori*.**

| S.No | Antibiotic | Code | Susceptible isolates (%) | Resistance isolates (%) |
|---|---|---|---|---|
| 1. | Metronidazole | MTZ | 6 (10%) | 52 (90%) |
| 2. | Clarithromycin | CLR | 24 (41.27%) | 34 (58.62%) |
| 3. | Amoxicillin | AML | 21 (53.5%) | 27 (46.5%) |
| 4. | Levofloxacin | LFX | 38 (65%) | 20 (35%) |
| 5. | Doxycycline | DOX | 35 (60%) | 23 (40%) |

**Table 6. Prevalence of resistance gene in *H. pylori* (n = 20).**

| Resistance gene | PCR detection | Male (n = 10) | Female (n = 10) |
|---|---|---|---|
| Metranadizol (*rdxA*) | 13 (65%) | 4 | 9 |
| Clarithromycin (*23s rRNA*) | 10 (50%) | 4 | 6 |
| Amoxicillin (*pbpA1*) | 7 (35%) | 5 | 2 |

To determine resistance to Metronidazole, the PCR was done for the detection of *rdxA* gene with both primers *rdxA-F* and *rdxA-R* (Table 2). The PCR conditions contain 94 °C denaturation for 5 minutes, then 35 cycles of annealing at 58 °C for 1 minute, extension at 72 °C for 30 seconds, and a final extension at 72 °C for 10 minutes [17].

The amoxicillin resistance gene with both primers *pbpA1*-F and *pbpA1*-R (Table 2) was amplified by denaturing it at 95 °C for 15 minutes, 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 seconds extending it at 72 °C for 1 minute, and finally extending it at 72 °C for 10 minutes [18].

### *Statistical Analysis*

Qualitative and quantitative variables are shown as percentages. The phylogenetic tree of *16s rRNA* was constructed with the help of MEGA-11 software. Mutation analysis of resistance genes was done with the help of "BioEdit 7.2" software.

## Results

A total of (n = 80) samples were taken from patients who had stomach disturbances. These samples were delivered and processed at the Department of Microbiology, KUST within 24 hours of collection. *H. pylori* was isolated from 72.5% (n = 58) of patients with stomach disturbance. Among the positive patients, there were 62% (n = 36) of female patients and 38% (n = 22) were male positive patient. It was more prevalent in the age group between 30–50 years 55.17% (n = 32). Similarly, it was more common in rural population as compared to urban populations as shown in Table 3 and Fig. 1.

### *Characteristics of H. pylori*

Colonies of *H. pylori* were observed under 100× microscope lens and all the colonies appeared pink in color, whereas Gram-positive bacteria were thought to be purplein color. As seen in Fig. 2, the *H. pylori* was a little, Gram-negative, s-shaped bacteria. All the characteristics of *H. pylori* are been shown in Table 4.

### *Antibiotic Susceptibility Pattern*

The zone of inhibition of the antibiotic susceptibility test was measured and compared with the standard already provided by the CLSI 2021. Among the antibiotics examined, most *H. pylori* isolates showed high resistance to Metronidazole 90% (n = 52), intermediate resistance to Clarithromycin 58.62% (n = 34) and Amoxicillin 46.5% (n = 27). *H. pylori* was susceptible to Levofloxacin 65% (n = 38). Table 5 and Fig. 3 show the antibiotic susceptibility pattern of *H. pylori* to different antibiotics.

### *Molecular Characterization of H. pylori*

The 3 µL of extracted DNA was visualized under UV gel doc by using 2% agarose gel as shown in Fig. 4A. The molecular identification of *H. pylori* was carried out by amplifying *16s rRNA* gene. The amplified product was run on 1% gel. Positive results are indicated by a clear band on the gel doc as shown in Fig. 4A,B. Fig. 4A shows the bands of extracted DNA in a gel dox system using 1% agarose gel while Fig. 4B shows the bands of *16s rRNA* using 2% gel.

### *Analysis of Phylogenetic Tree*

The phylogenetic tree of positive sequencing of *16s rRNA* was constructed with the help of MEGA-11 software and it was formed for 10 sequences along with positive sequence. The evolutionary history was inferred using the maximum likelihood method (supporting bootstrap values from 100 replicates). The derived phylogenetic tree consists of two clusters. The cluster shows intermingling of *H. pylori* species as shown in Fig. 5.

### *Molecular Detection of rdxA, 23s rRNA and pbpA1 Resistance Genes*

Metronidazole resistance (*rdxA*), Clarithromycin resistance (*23s rRNA*) and Amoxicillin resistance (*pbpA1*) genes were detected by using their specific primers through PCR. A total of (n = 20) isolates were selected for the detection of resistance genes as shown in Table 5. In the selected isolates, only n = 13 (65%) were positive for Metronida-


**Fig. 5. Phylogenetic tree of isolated *H. pylori.***

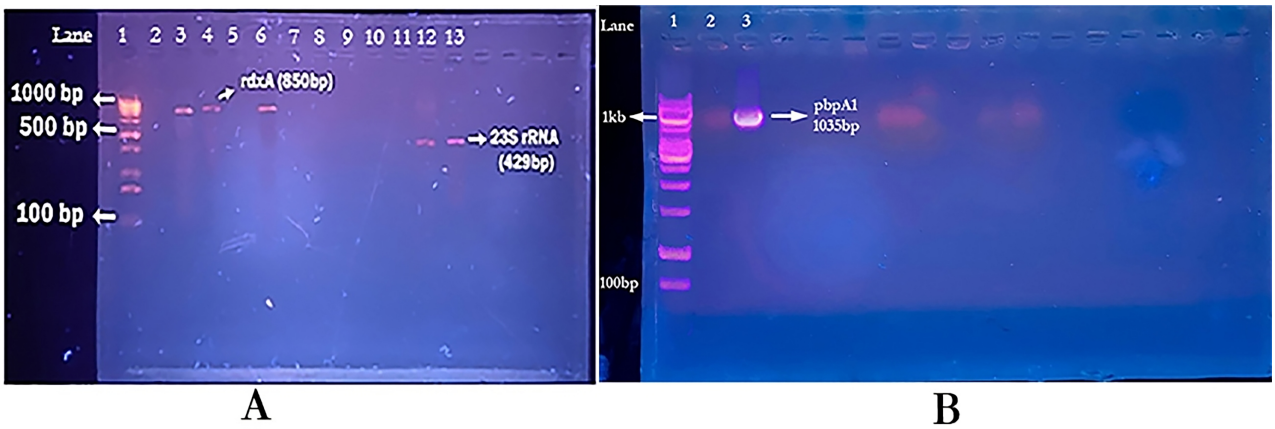


**Fig. 6. (A) Showing the bands of *rdxA* and *23s rRNA* gene; lane 1 indicates ladder, lanes 3, 4 and 6 show *rdxA* gene band and lanes 12–13 show *23s rRNA.* (B) Showing the bands of *pbpA1* gene; lane 1 indicates ladder and lane 3 shows the band of *pbpA1* gene.**

zole resistance gene including (n = 4) from male and (n = 9) from female, in case of Clarithromycin and Amoxicillin resistance gene total of (n = 10) and (n = 7) were detected respectively as shown in Table 6. These resistance genes were visualized under Ultraviolet (UV) gel dox by using 2% agarose gel as shown in Fig. 6A,B.

### *Bioinformatics Analysis of Resistance Genes*

The sequence was first Basic Local Alignment Search Tool (BLAST) and the results show similarity with different sequences. This sequence was then compared with already known sequence (CP122947.1:566761-567612 *Helicobacter pylori* strain BS07 chromosome) using "BioEdit 7.2" software. In BioEdit the sequence was opened with the known sequence in the same window. It was selected


**Fig. 7. Bioinformatics analysis of resistance genes.**

and ClustalW was performed using 500 bootstrap methods. The aligned sequences were then compared and no mutation was detected as shown in the Fig. 7.

## Discussion

*Helicobacter pylori* is Gram-negative, microaerophilic bacteria that is responsible for gastric pathology. Its infection is currently the most prevalent bacterial infection in the world, and the human stomach serves as its primary reservoir. Past research has demonstrated a substantial link between *H. pylori* and peptic ulcer. Stomach illnesses manifest in some way due to *H. pylori* infection. Following the initial gastritis, dyspepsia and intestinal metaplasia might result in stomach cancer.

The successful culture of an infectious agent is the precise method for the detection of infectious diseases. The usual method for diagnosis and a need for drug susceptibility testing is the culture of *H. pylori* from a stomach antral biopsy. Although it is sometimes thought of as a time-consuming approach, the culture technique is the standard method employed in many laboratories to detect *H. pylori*. For many years, Columbia blood agar along with specific supplement has been used to cultivate *H. pylori* from antral biopsy samples [19]. When utilizing this medium by itself, the success rate of isolating *H. pylori* varies. In this study Columbia blood agar was also used to culture a total of 80 gastric biopsy samples in the current investigation, *H. pylori* was isolated 72.5% using Columbia blood agar; the colonies were tiny, transparent, and non-hemolytic. Majority of other culture media was contaminated by bacteria. *Pseudomonas spp.* was the contaminating bacteria, and their sources could have included infected biopsy forceps and contamination during the collection, transportation, and preparation of the blood added to the traditional Columbia agar.

Out of 80 samples, 58 samples were positive for *H. pylori.* Compared to industrialized countries, developing countries have a higher frequency of *H. pylori*. Poor hygienic conditions, social level, and environmental variables could be the root cause. Additionally, according to Zamani *et al*., 2018 [20], isolation rates were 50.8% in underdeveloped nations as compared to the developed world (34.7%). Age distribution of infection in this study showed a decrease toward age from young (below 30 years = 55.17%) to the elderly age of 30–50 years (32.75%) and above 50 years (12.6%). This was consistent with the previous study in Nigeria that found a prevalence rate of 52.9% in the 18–39 years and it was decreased in the elderly age patients [21]. In analysis, high numbers of patients were from rural population as compared to the urban population. This may be due to the poor socioeconomic status of the people living in the rural population. Our finding have been supported by another study conducted in Romania have revealed a higher incidence of *H. pylori* infection in rural population [22].

The narrower lower portion of the stomach, the antrum, and the duodenum all contain Gram-negative microaerophilic bacteria when the typical type of duodenal ep-

ithelium is replaced with antral type mucosa. This kind of antral mucosa will become infected by *Helicobacter* [23]. This idea is further supported by the findings of our investigation, for instance, *H. pylori* positive isolates were found in antral biopsy samples. Identification of the resistance pattern of the bacterial agents of infection is necessary since antibiotic resistance is the primary cause of treatment failure. The data gathered will assist in the more logical and precise usage of antibiotics.

Globally, *H. pylori* resistance to levofloxacin is low. Zhang *et al*., 2020 [24] reported 15.2% resistance to levofloxacin in China, in this study, the low was also found in levofloxacin but the widespread use of this antibiotic to treat various infectious diseases may have aided in the emergence of resistant strains. Similarly, almost complete resistance to Metronidazole has been reported in this study (90%) which may be due to higher prescriptions to treat gastrointestinal infections. Similar study was conducted in Rawalpindi, Pakistan reported the highest resistance in metronidazole (73.9%) in gastroenteritis patients [25]. However, because it is used to treat gastrointestinal infections, rising clarithromycin resistance is to be anticipated. Comparing the prevalence of resistance genes, Metronidazole (*rdxA* gene) was found in higher proportion (65%) as compared to Clarithromycin (*23s rRNA*) (50%) and Amoxicillin (*pbpA1*) (35%) resistance gene which is in line with previous study being conducted by Hashemi *et al*., 2019 [26], who revealed that Metronidazole had the highest rate of resistance among the resistant strains.

## Conclusion

The rank of *H. pylori* resistance to antibiotics in district Kohat is alarming. Variation in *H. pylori* was observed in different geographic areas; gender and socioeconomic status suggest that prevalence is influenced by living conditions. This study highlights the molecular resistance and provides tips on how gastroenterologists might design a better treatment plan for this continual bacterium. Due to high resistance to Metronidazole, alone prescription may not useful, while in combination with levofloxacin may be successful in eliminating of *H. pylori*. It will also support clinicians in the management of patients by providing a better treatment plan. Continuous surveillance will be needed to halt the wide spread of resistant clone the environment.

## Availability of Data and Materials

All the data analyzed during this study are available in this published article.

## Author Contributions

SI performed the experiments, conducted statistical analysis, and wrote the manuscript. AG helped in experiments, AB helped in sequence analysis, WU and SFS designed the research project and analyzed the results. Additionally, all authors contributed significantly to editorial changes of important content. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study has been approved from Kohat University of Science and Technology Ethical Committee Ref No: 590/KUST/Ethical Committee. Before beginning any procedure, all participants have been informed verbally and have signed a written consent form following the ethical guidelines of the 1964 Helsinki Declaration and its implementing legislation.

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## Conflict of Interest

The authors declare no conflict of interest.

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