

Antibacterial Activity of Garlic (*Allium sativum L.*) on Multi-Drug Resistant *Helicobacter pylori* Isolated From Gastric Biopsies

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Background: Garlic (*Allium sativum L.*) exhibit a broad-spectrum of antimicrobial activity against both gram negative and gram-positive bacteria. *Helicobacter pylori* (*H. pylori*) is the main factor in peptic and duodenal ulcer diseases. Some strains of *H. pylori* have become resistant to the current antibiotics.

Objectives: The purpose of this study was to determine the antibacterial effects of garlic on *H. pylori*.

Materials and Methods: The gastric biopsies were inoculated on Brucella agar base (Conda Pronadisa, Spain) supplemented with 5% sterile sheep blood, 7% fetal calf serum, vancomycin (5 mg/L), trimethoprim (5 mg/L), and polymyxin B (2500 U/L). Antibiotic susceptibility to garlic and commercial antibiotic disc was determined by agar disc diffusion method.

Results: In this study, ten strains of *H. pylori* out of 120 samples were isolated. The minimum inhibitory concentration of garlic extract for all isolates ranged between 25- 400 mg/mL. Resistance rates of 10 strains of *H. pylori* to amoxicillin, ciprofloxacin, clarithromycin, metronidazole, and tetracycline were 20%, 20%, 30%, 60%, and 30%, respectively.

Conclusions: In this study, the antibacterial effect of aqueous extract of garlic on all *H. pylori* clinical isolates was confirmed. These findings might help us to use a new strategy for treatment of peptic ulcer.

Keywords: Anti-Bacterial Agents; Garlic; *Helicobacter pylori*

1. Background

Helicobacter pylori (*H. Pylori*) is a spiral-shaped and microaerophilic gram-negative bacterium (1). This bacterium is usually colonized in childhood and perhaps for the rest of life (2). There is a close relationship between *H. pylori* associated-infections with social and economic situations (3). Human is the main reservoir of this bacterium. *H. pylori* associated-infections are higher in developing countries rather than developed countries as 80-90% of population of developing countries and less than 40% of adults in developed countries are affected (4). This bacterium is the main cause peptic ulcer and increases gastric cancer risk (5). Treatment regimens involves two antibiotics and a proton pump inhibitor (PPI). The most common antibiotics for treatment of *H. pylori* are amoxicillin, clarithromycin, and metronidazole (6). Antibiotic resistant, in particular macrolides, is a major cause of treatment failure (7). Louis Pasteur described the antibacterial effects of juice of garlic (*Allium Sativum L.*) for the first time and found that allicin had antimicrobial activity (8). It is

responsible for the pungent odor and for a variety of biological effects attributed to garlic preparations including antimicrobial, anticancer, and antiatherogenic activities (9). Allicin is a water-soluble compound composed of diallyl thiosulphinate and has strong inhibitory effects on gram-negative enteric bacteria and *Staphylococcus aureus* (10). Allicin is also very effective on antibiotic-resistant bacteria and no resistance to it has been reported yet (11). Jezowa had shown that antibiotic activity of 1mg of allicin has been equated to 15 IU of penicillin (12). The antibiotic qualities of garlic appear to be a direct result of the allicin produced from raw, crushed garlic. This is destroyed by age and cooking; cooked garlic has virtually no antibiotic value although it still retains other benefits (13).

2. Objectives

The purpose of this study was to determine the antibacterial effects of garlic on multi-drug resistance *H. pylori* isolates from gastric biopsies.

Implication for health policy/practice/research/medical education:

In this study antimicrobial effect of the garlic extract on multi-drug resistant *Helicobacter pylori* isolated from gastric biopsy of patients referred to Imam Khomeini hospital in Ahvaz city- Iran was evaluated.

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3. Materials and Methods

3.1. Preparation of Garlic Juice

The cloves of garlic was collected from Ramhormoz city, Khuzestan province, in southwest of Iran. After washing the garlic cloves with sterile distilled water, they were put in juice extractor. Filtration was done through Whatman filter paper No. 1. The dry weight of garlic juice was measured by pycnometer (Erichsen, model 290/II). The garlic juice was kept at 4 °C for the next step of test.

3.2. Bacterial Strains

One hundred twenty gastric biopsies were obtained from the antrum of the patients presenting with gastroduodenal pathologies at Imam Khomeini Hospital, Ahvaz, Iran. The enrolled individuals did not take any antibiotics or proton pump inhibitor (PPI) for at least one week prior to study. The samples were kept in Stuart media (Liofilchem, Italy) culture for transport to laboratory. In the lab the gastric biopsies were inoculated in Brucella agar base (Conda Pronadisa, Spain) supplemented with 5% sterile sheep blood, 7 % fetal calf serum, vancomycin (5 mg/L), trimethoprim (5 mg/L), and polymyxin B (2500 U/L). The Plates were incubated at 37 °C for 3-7 days under microaerophilic conditions (8% carbon dioxide). Identification of *H. pylori* was based on colony morphology microscopy as well as positive urease, catalase, and oxidase activities (14).

3.3. Minimum Inhibitory Concentration of Garlic Juice

Antibacterial susceptibility was determined by disc diffusion method (15). Two-fold dilutions of garlic juice were prepared with sterilized water. The final concentrations ranged from 6.25 to 800 mg/mL. Bacterial suspensions were prepared in sterile normal saline with the turbidity adjusted to No.2 McFarland standard tube. Surface of Brucella blood agar plates were inoculated with 150

μL of bacterial suspensions by a glass applicator. Before applying the discs, the plates were dried at ambient temperature for 15 minutes. Eight sterile discs (diameter of 6 mm) were kept on the agar surface in a line. Ten microliter of each dilution was separately used to impregnate the disc. The plates were incubated for 2-3 days at 37 °C under microaerobic condition. The minimum inhibitory concentration (MIC) values were read as the antimicrobial concentration at the point where dense colonial growth intersected the disc. All the tests were done twice and their average value was recorded.

3.4. Antibiotic Susceptibility Test

Antibiotic susceptibility was determined by disc diffusion method. Bacterial suspensions were prepared in sterile normal saline with the turbidity adjusted to No.2 McFarland standard tube. The surfaces of Brucella blood agar plates supplemented with 5% sterile sheep blood were inoculated with 150 μL of bacterial suspensions by glass applicator. Antibiotic discs (Neo sensitabs, Denmark) including clarithromycin (15 μg), ciprofloxacin (5 μg), amoxicillin (25 μg), tetracycline (30 μg), and metronidazole (5 μg) were deposited on the plates. After 2-3 days incubation under microaerobic, diameter of the growth inhibition zones was measured. The zone of growth inhibition of antibiotic disks was interpreted according to study of Mishra (16).

4. Results

Ten strains of *H. pylori* out of 120 biopsy samples were isolated in this study. The resistance rates of the ten *H. pylori* isolates to amoxicillin, ciprofloxacin, clarithromycin, metronidazole, and tetracycline were 20%, 20%, 30%, 60%, and 30%, respectively (Table 1). In this study, five isolates of *H. pylori* were resistant to more than one antibiotic. In addition, the juice of garlic inhibited the growth of all *H. pylori* isolates. Out of 10 *H. pylori* isolates, the MIC was 25 mg/mL in two isolates, 50 mg/mL in four isolates, 100 mg/mL in three isolates, and 400 mg/mL in one isolate (Table 1).

Table 1. Pattern of Resistance of the *Helicobacter pylori* Isolates to Different Antibiotics and Minimum Inhibitory Concentration of Garlic^a

	AMX	CIP	CLA	MET	TET	MIC of garlic, mg/mL
Isolates 1	S	S	S	S	R	25
Isolates 2	S	S	S	R	S	50
Isolates 3	R	S	S	R	S	25
Isolates 4	S	S	R	R	S	100
Isolates 5	S	R	S	S	S	400
Isolates 6	R	S	R	R	S	50
Isolates 7	S	R	S	R	R	100
Isolates 8	S	S	S	S	S	50
Isolates 9	S	S	S	R	R	50
Isolates 10	S	S	R	S	S	100

^a Abbreviations: AMX, Amoxicillin; CIP, Ciprofloxacin; CLA, Clarithromycin; MET, Metronidazole; MIC, minimum inhibitory concentration, TET, Tetracycline; S, Sensitive; R, Resistance.

5. Discussion

Phytochemical components of garlic include quercetin, anthocyanin, polyphenol, and diallial sulphate. Allicin is the main component of garlic and causes antibacterial effects (17). *H. pylori* associated-infection is very prevalent in Iran. On the other hand, antibiotic resistance of bacteria to common antibiotics is the main cause of treatment failure (18). For this reason, infections relapse in most patients within a few years (19). The use of medicinal herbs is very effective to treat infections. Garlic is one of the most effective herbs in traditional medicine that belongs to the *Alliaceae* family. Garlic has high antibacterial properties on a wide spectrum of gram-positive and gram-negative bacteria (9). In India, Yousefi had showed that chloroform extract of garlic is more effective than the other extracts of garlic against *Escherichia coli* (20). In England, O' Gara et al. found that the essential oil of garlic had more antibacterial properties than garlic powder against *H. pylori* (13). Our study revealed the effectiveness of the garlic juice antibacterial properties on *H. pylori*. Similar to our study results, Saravanan et al. in India concluded that the aqueous extract of garlic had a great effect on the target bacteria as the growth of bacteria was inhibited within 14 hours (9). Adeniyi et al. in Nigeria reported the inhibitory effects of methanolic extract of garlic on *H. pylori* (21). The main mode of action of allicin in bacteria is through bacteria RNA. It is mainly due to inhibition of RNA synthesis, especially protein and DNA synthesis in bacteria (22). The permeability of cell wall to allicin is also important. Since the amount of lipids in the cell wall of gram-negative bacteria is greater than gram-positive bacteria, entry of allicin is easier in gram-negative bacteria (23). On the other hand, combination of the garlic and ciprofloxacin had a synergistic inhibition effect on bacteria. Ciprofloxacin inhibits DNA gyrase and can interfere with the process of translation (24). Jonkers et al. showed that the combination of the garlic and omeprazole had a synergistic effect on *H. pylori*, which is dependent to concentration (25). In this study, five isolates of *H. pylori* were resistant to more than one antibiotic, but they were inhibited very well with the garlic juice. We can conclude that the combination of garlic and commercial antibiotics might increase the chance of treatment of peptic ulcer disease.

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Authors' Contribution

All authors participated equally.

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There is no disclosure.

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