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# Garlic, a Functional Food Used in Treatment of Gastritis Involving *Helicobacter pylori* Infection: A Therapeutic Approach

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## Abstract

*Helicobacter pylori* is the most widespread chronic bacterial agent in humans, and is well recognized for its association with peptic ulcer disease and gastric cancer with both representing major global health and socioeconomic issues. Garlic (*Allium sativum* L.) has an important dietary and medicinal role for centuries. It is mostly used as a compulsory condiment in most of the south Asian cuisines. The research focused on two varieties of garlic extract to carry out *in vitro* study for its antibacterial activity against *Helicobacter pylori*. Along with this, the study also focused on the antimicrobial activities of garlic among different pathogens. The study was carried out among 30 samples of Nepalese variety and 30 samples of Chinese variety of garlic. Among Nepalese and Chinese varieties, Nepalese variety showed higher zone of inhibition as compared to Chinese variety of garlic. The maximum zone of inhibition of Nepalese variety of garlic was found to be 16 mm and minimum zone of inhibition was 4 mm. While the zone of inhibition by Chinese variety of garlic ranges from 4 mm to 11 mm. So, it was obvious that in respect to antibacterial property of garlic, Nepalese variety of garlic than Chinese variety seems to be more susceptible to *Helicobacter pylori*. Along with antibacterial effect against *H. pylori*, other pathogens were also tested against garlic extract. Among four pathogens, viz, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhimurium*, *L. monocytogenes* were also slightly inhibited by garlic extract. So, this study has also opened the door to study antibacterial activity of garlic with *L. monocytogenes* along with *H. pylori*. Thus, the traditional use of garlic in treating gastritis caused by *H. pylori* appears to be justified.

***Keywords:*** *Garlic (Allium sativum L.); Gastritis; Helicobacter pylori Infection*

## Introduction

*Allium sativum*, commonly known as garlic is a bulbous plant which belongs to Amaryllidaceae family. With a history of over 7000 years of human consumption, it has been used both as food flavoring and traditional medicine. The garlic plant's bulb is the most commonly used part of the plant for its pungent flavor as a seasoning or condiment. Because of its strong odor, garlic is sometimes called the "stinking rose". However, it is the fundamental component in many dishes of various regions of Asia, Africa, and America. There are different varieties or subspecies of garlic, most notably hard neck garlic and soft neck garlic. It is grown globally, but China is by far the largest producer country.

## Therapeutic benefits of garlic

The importance of garlic has been highlighted in both ancient as well as in modern medicine. Its usages for medicinal purpose dated back to 1550 B.C. At ancient times it was also even considered as a valuable exchange resource. In Roman times, soldiers used to chew garlic before battles claiming that it protects from snake bites. African fisherman smears their bodies with garlic extract to protect against crocodiles [1]. Garlic is used as traditional medicine in different parts of the world. In Nepal, East Asia, and the Middle East, it has been used to treat all manner of illnesses including fevers, diabetes, rheumatism, intestinal worms, colic, flatulence, dysentery, liver disorders, tuberculosis, facial paralysis, high blood pressure, and bronchitis [2]. These days though synthetic medicines are used extensively but it has been associated with different side effects, which may have long or short term reactions. So, efforts are currently being made to look for the products of natural origin [3]. In modern medicine, it is used in the treatment of hypertension, hypercholesterolemia, diabetes, cold, atherosclerosis, and tumors. It has also significant role in immune system improvement. Garlic is also the good source of different vitamins like vitamin C, vitamin B1, magnesium, and calcium. Although, selenium and germanium are present in trace amount, it is very important element in reducing the division of cancer cells [4]. It has also got antimicrobial properties against gram negative and gram positive bacteria like *Staphylococcus* spp., *E. coli*, *Salmonella* spp., *Clostridium* spp., *Mycobacterium tuberculosis*. It is also effective against some antibiotic resistant bacteria. So, in the modern day, it is used in synergism with different antibiotics. It is believed that the antibacterial activity is caused by garlic components, such as allicin and other allyl groups. A feature unique to allicin is that most bacteria has the inability to produce resistance to these substances. So, it is effective in some infections which are antibiotic resistant [4]. Garlic consumption helps in fat metabolism, lowering blood cholesterol level (increases HDL and lowers LDL) by reducing the activity of the enzyme HMG CoA reductase. It is a powerful antioxidant and its Sulphur components have anti-tumor properties. A high intake of garlic reduces the risk of prostate cancer up to 50%. It protects brain from a loss of intellectual capacity, memory, and depression, and has preventive therapeutic effect in the treatment of Alzheimer's disease [5]. Garlic is sometimes deployed in gardens to deter pests. It is often grown among flowers or root vegetables as a companion plant, with the aim of protecting other plants from pests [6].

## Chemistry

Allium vegetables, particularly garlic (*Allium sativum* L.) exhibit a broad antibiotic spectrum against both gram positive and gram negative bacteria like *Bacillus* spp., *Citrobacter* spp., *Clostridium* spp., *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., etc. Most of its therapeutic effects are due to oil and water-soluble organo-sulfur compounds which are responsible for the typical odor and flavor of garlic. The compounds contained in garlic synergistically influence each other so that they can have different effects [7]. During cutting and crushing of the clove, the amino acid alliin, present in the garlic clove is metabolized by the enzyme allinase to yield "allicin". Chopping will activate allinase enzymes in some of the cells, and will allow those enzymes to convert some of the garlic's alliin into allicin. The concentration of allicin (main active ingredient) and the source of garlic's distinctive odor depend on processing method [8].

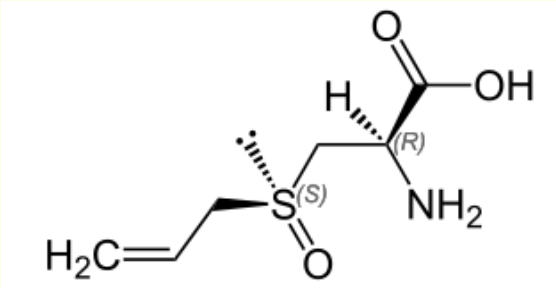


***Figure 1:*** *Alliin, a Sulphur containing compound found in garlic.*

A large number of sulfur compounds contribute to the smell and taste of garlic. Allicin has been found to be the compound most responsible for the "hot" sensation of raw garlic. The process of cooking garlic removes allicin, thus, reducing its spiciness. When eaten in quantity, garlic may be strongly evident in the diner's sweat and garlic breath the following day. This is because garlic's strong-smelling sulfur compounds are metabolized, forming Allyl Methyl Sulfide (AMS). AMS cannot be digested and is passed into the blood. It is carried to the lungs and the skin, where it is excreted. Since digestion takes several hours and release of AMS several hours more, the effect of eating garlic may be present for a long time [9]. This research paper highlights the therapeutic approach of Chinese and Nepalese varieties of garlic (*Allium sativum*), available in the Nepalese market in the treatment of gastritis involving *Helicobacter pylori* (Shown below figure 2).

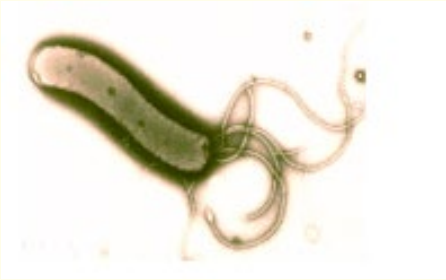

***Figure 2:*** *Helicobacter pylori (Diagrammatic representation).*

## Methodology

Laboratory analysis was performed to find out the antibiotic susceptibility of garlic against *Helicobacter pylori*. The study was conducted in two varieties of garlic, i.e. Nepalese and Chinese varieties. These two different varieties were collected from different vegetable markets of Nepal such as Bhaktapur, Morang, Dhading, Makawanpur, Kathmandu, Kavrepalanchowk and Lalitpur. Thirty samples of each Nepalese and Chinese varieties were taken for laboratory analysis.

### Isolation and identification of *Helicobacter pylori*

Isolates of *H. pylori* was obtained from gastric biopsy of *H. pylori* infected person. Morphological and cultural identification of bacteria was done using standard protocol [10]. The antibiotic susceptibility method of analysis were done as per qualitative field disc assay method as described by association of official analytical chemist (AOAC, 2000). The media used for antibiotic assay was Muller Hinton Agar (MHA) and Muller Hinton Agar enriched with sheep blood (7 - 10%). Isolated colonies of *Helicobacter pylori* was identified by gram staining and other biochemical tests like catalase test, oxidase test, urease test and $H_2S$ production test.

### Maintenance of microaerophilic condition

*Helicobacter pylori* is a microaerophilic organism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere [11]. So, microaerophilic condition was maintained burning a candle in an anaerobic jar. The candle burnt using oxygen gas and emitted carbon dioxide gas, and, thus, maintained a suitable condition.

### Gram staining

On a clean grease free slide, a thin smear of bacteria was prepared, and it was air dried and heat fixed. The smear was covered with crystal violet dye for 1 minute, and then it was washed properly with distilled water. After that iodine was put over the smear for 1

minute, and was again washed with distilled water. The decolorizer consisting of acetone alcohol was poured over the smear till the clear flow comes. It was again washed with distilled water, and safranin was poured over the smear and left for 1 minute. Finally the excess safranin dye was washed with distilled water. The prepared slide was observed under the microscope for classifying gram positive or gram negative bacteria [12].

### Catalase test

Certain bacteria possess catalase enzyme which break down hydrogen peroxide ($H_2O_2$) into oxygen ($O_2$) and water ($H_2O$). A loopful of bacterial isolate was put in a clean grease free slide. A drop of hydrogen peroxide was added to it and bubbles of oxygen were observed. So, the positive test shows the effervescence of oxygen gas [13].

### Oxidase test

A loopful of organism was scrapped over the oxidase strip which contains 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride. Oxidase positive organism contained cytochrome c as part of their respiratory chain and was oxidase-positive and turned the oxidase strip blue/purple. Organisms lacking cytochrome c as part of their respiratory chain did not oxidize the reagent, leaving it colorless, and were oxidase-negative [13,14].

### Urease test

The 24 hour broth culture was prepared from the isolated colonies of *Helicobacter pylori*. The urease slant media was prepared. From the broth culture, a loopful of organism was streaked over the slant of the media and incubated at 37°C for 18 to 24 hour. After incubation, the color of media was observed for the production of urease enzyme by the organism. The pink color of the medium showed the positive test result [14,15].

### H2S production test

A loopful of organism was streaked over the slant and stabbed over the Triple sugar Iron (TSI) agar. Tubes were incubated at 37°C for 18 to 24 hours. The results were observed for the presence of black color in the TSI slant.

### Preparation of working strain of pure ATCC culture of different pathogens

Different ATCC pure strains of bacteria were obtained from Microbiology lab of Department of Food Technology and Quality Control, Kathmandu. *E. coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028) and *Listeria monocytogenes* (ATCC 19115) were used for antibiotic susceptibility test of garlic.

### Preparation of different media

Different media like Muller Hinton Agar, Columbia Blood Agar, Nutrient Agar, Urease agar and TSI Agar slants were prepared following the manufacturer's (Hi - Media) instructions. Other biochemical tests were performed using Hi-Media Gram staining kit and different biochemical media.

### Extraction of garlic

Fresh garlic bulbs (both Nepalese and Chinese varieties) were purchased from the market of 7 districts of Nepal. The peeled garlic bulbs (100g) and 50 ml sterile distilled water were finely blended using a juicer. The resulting paste was centrifuged at 3000 rpm for 30 minutes and the supernatant was then sterilized by a filter (0.2 µm pore size, Millipore). The final concentration of fresh garlic extract (FGE) in aqueous solution was determined to be 40.7% (w/v) by subtracting the weight of the precipitate from the weight of the original peeled garlic bulbs. The FGE was stored in 1.5 ml micro test tubes at -20°C until used.

### Antibiotic susceptibility test

All the bacterial isolates used in the study were subjected to *in vitro* antibiotic susceptibility test by disc diffusion method of Kirby Bauer method as described in AOAC method. Three types of antibiotic disc, viz., penicillin, amoxycillin, ampicillin were used to study the zone of inhibition. Those standard discs of antibiotics were tested for sensitivity for different pathogens like *E. coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhimurium* (ATCC 14028) and *Listeria monocytogenes* (ATCC 19115). Isolated colonies of *Helicobacter pylori* were tested for antibiotic sensitivity test using garlic extract. All 60 garlic extract samples were tested for antibiotics susceptibility of *H. pylori*.

## Result and Discussion

### Proximate values of Nepalese garlic

Proximate analysis was done at the National Food and Feed Reference Laboratory of Department of Food technology and Quality Control, Kathmandu. Central Food Laboratory Manual was followed to calculate the proximate values. The obtained proximate values of garlic are tabulated as follows.

| Parameters | Unit | Nepalese Variety | Chinese Variety |
|---|---|---|---|
| | | Approximate Value | |
| Moisture | g | 61 | 65 |
| Protein | g | 5.8 | 5.6 |
| Fat | g | 0.1 | 0.1 |
| Carbohydrate | g | 30 | 28.5 |
| Energy | Kcal | 144.1 | 137.3.1 |

***Table 1:*** *Proximate values of Nepalese and Chinese variety of garlic.*

Both varieties of garlic showed little difference in their proximate values. Chinese variety of garlic had little more moisture content than Nepalese variety. Likewise, protein, carbohydrate and energy values were also lower compared to Nepalese variety. However, the fat values of both varieties of garlic were same.

### Antibiotic susceptibility test of different pathogens with commonly used antibiotics

Different standard antibiotic discs like amoxycillin (30 mcg), ampicillin (30 mcg) and penicillin (30 mcg) were tested against different pathogens, viz., *E. coli* (*E. coli* ATCC 8739), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115) and *Salmonella* typhi (ATCC 14028).

### Antibiotic sensitivity test with *E. coli* (ATCC 8739)

*E. coli* is the commonly found enteric pathogen associated with gastrointestinal disturbances, urine infections in most of the pregnant women.

| S. No. | Organism | Antibiotics | Zone of inhibition (mm) |
|---|---|---|---|
| 1. | *E. coli* | Amoxycillin | 26 |
| 2. | | Ampicillin | 30 |
| 3. | | Penicillin | 25 |

***Table 2:*** *Antibiotic susceptibility test with E. coli (ATCC 8739).*

Among three commonly used antibiotics, ampicillin seemed to be more sensitive to *E. coli*. The zone of inhibition given by ampicillin was 30 mm whereas amoxycillin gave slightly lower zone of 26 mm. The smallest zone of inhibition was given by penicillin of 25 mm only. Thus, *E. coli* was sensitive to all three antibiotics as the zone of inhibition was larger than 14 mm.

### Antibiotic sensitivity test with *Staphylococcus aureus* (ATCC 25923)

*Staphylococcus aureus* is the commensals organism of nostrils of human. However, it is the commonly found opportunistic pathogen associated with gastrointestinal disturbances and urine infections.

| S. No. | Organism | Antibiotics | Zone of inhibition (mm) |
|---|---|---|---|
| 1. | *S. aureus* | Amoxycillin | 32 |
| 2. | | Ampicillin | 28 |
| 3. | | Penicillin | 22 |

***Table 3:*** *Antibiotic susceptibility test with S. aureus (ATCC 25923).*

In the case of *S. aureus*, the largest zone of inhibition was given by amoxycillin of 32 mm, while ampicillin gave 28 mm of zone of inhibition. The lowest zone of inhibition was given by penicillin of 22 mm. So, the pure culture of *S. aureus* was found to be sensitive to all three antibiotics.

### Antibiotic sensitivity test with *L. monocytogenes* (ATCC 19115)

*L. monocytogenes* is gram positive organism capable of growing at low temperature as below as 0°C. It is a pathogenic microorganism causing Listeriosis. It can also cause meningitis in new born babies, and is also responsible for still birth.

| S. No. | Organism | Antibiotics | Zone of inhibition (mm) |
|---|---|---|---|
| 1. | *L. monocytogenes* | Amoxycillin | Not detected |
| 2. | | Ampicillin | 10 |
| 3. | | Penicillin | 30 |

***Table 4:*** *Showing antibiotic susceptibility test with Listeria monocytogenes (ATCC 19115).*

*L. monocytogenes* was tested for antibiotic sensitivity test against amoxycillin, ampicillin and penicillin. Penicillin showed 30 mm and ampicillin gave 10 mm of zone of inhibition. However, *Listeria monocytogenes* did not show any sensitivity towards amoxycillin. Thus, *L. monocytogenes* was found to be resistant to amoxycillin and ampicillin because the inhibition criteria for any antibiotics need to be 20 mm.

### Antibiotic sensitivity test with *Salmonella typhimurium* (ATCC 14028)

*S. typhimurium* is the gram negative bacteria. It is a pathogenic bacteria causing Salmonellosis, enteric fever, typhoid, and food poisoning. It is commonly found in the gut of infected animal and human being. Most human infections are caused by eating food or drinking water that has been contaminated by feces.

The antibiotic sensitivity of three different antibiotics against *S. typhimurium* was studied. Amoxycillin showed the highest zone of inhibition, i.e. 40 mm, ampicillin showed 20 mm while penicillin showed 16 mm zone of inhibition. Thus, *S. typhimurium* was seemed to be sensitive to all three antibiotics.

| S. No | Organism | Antibiotics | Zone of inhibition (mm) |
|---|---|---|---|
| 1. | *S. typhimurium* | Amoxycillin | 40 |
| 2. | | Ampicillin | 20 |
| 3. | | Penicillin | 16 |

***Table 5:*** *Showing antibiotic susceptibility test with S. typhimurium (ATCC 14028).*

### Isolation of *Helicobacter pylori*

The best specimen to culture *H. pylori* are biopsy samples obtained during endoscopy. Thus, fresh gastric biopsy specimens from the patient were taken. Care must be taken to ensure that the patients did not receive antibiotics or anti secretory drugs, specially, proton pump inhibitors. The organism is extremely sensitive to desiccation and exposure to oxygen. The agar surface should be smooth and moist, but without excessive moisture. A non-selective medium such as Columbia Agar with 5% Sheep Blood was inoculated. The inoculated plates were incubated for 3 to 5 days at 35°C in a microaerophilic atmosphere (5 to 20% $CO_2$). After incubation, the plates showed isolated colonies. After isolation, the colony characteristics and biochemical tests were carried out.

### Cultural characteristics of *Helicobacter pylori*

*H. pylori* colonies were tiny to medium-sized and transparent colonies. A gram staining was done, and it was observed to be Gram negative, slightly curved rod shaped bacteria.

### Biochemical test and identification of *Helicobacter pylori*

Isolated colonies were streaked for two times into Nutrient Agar to purify the colonies. Different biochemical tests were performed from sub-cultured colonies of *H. pylori*. The colonies gave following biochemical reactions.

| S. No. | Biochemical tests performed | Positive reaction | Negative reaction | Result obtained |
|---|---|---|---|---|
| 1. | Urease test | Pink color | Off white color | Pink color |
| 2. | Oxidase test | Purple color on oxidase strip | No change in color of oxidase strip | Purple color was observed on oxidase strip. |
| 3. | Catalase test | Effervescence of gas seen with reaction of $H_2O_2$ | No effervescence seen | Gas effervescence seen on slide |

***Table 6:*** *Biochemical tests.*

Thus, the table showed that isolated colonies were of *H. pylori*.

### Antibiotic sensitivity test with *Helicobacter pylori*

Since, *Helicobacter pylori* is a gram negative microaerophilic bacteria, they can cause sores called ulcers in the lining of stomach or in the upper part of intestine. Both the acid and bacteria irritate the lining and cause an ulcer to form. If left untreated, a *H. pylori* infection can cause gastritis or it may also progress into peptic ulcer disease or stomach cancer later in life.

| S. No. | Organism | Antibiotics | Zone of inhibition (mm) |
|---|---|---|---|
| 1. | *H. pylori* | Amoxycillin | 22 |
| 2. | | Ampicillin | 10 |
| 3. | | Penicillin | 18 |

***Table 7:*** *Antibiotic susceptibility test with Helicobacter pylori.*

*Helicobacter pylori* showed low range of zone of inhibition with amoxycillin, ampicillin and penicillin. Ampicillin gave 10 mm of zone of inhibition, and penicillin gave 18 mm of zone of inhibition. So, both of them was resistant to *Helicobacter pylori*. However, amoxycillin gave 22 mm of zone of inhibition which showed that this antibiotic was intermediate to *Helicobacter pylori*.

### Antibiotic sensitivity test of garlic with *Helicobacter pylori*

Nepalese variety is the local breed of garlic, and many people like it for its strong and distinct flavor. From the proximate analysis also this variety was found to have less moisture content. Wells were made in the MHA plates which were enriched with 10% blood. After that *H. pylori* colony was swabbed in the agar plate and garlic extract was put in the wells of the MHA plates. Plates were left for half an hour for absorbing the extract, and were incubated at 37°C for 24 hour. The result of zone of inhibition after incubation is tabulated as below.

In the similar manner as above, MHA plates were enriched with blood to study antibiotic sensitivity of garlic. Wells were made on the agar plates with sterilized borer. Colonies of *H. pylori* were swabbed in the agar surface, and let it for some to absorb. Carefully garlic extracts of Chinese variety were put into the well, and were left for half an hour to absorb the extract, then incubated at 37°C for 24 hour. Results obtained after analysis are tabulated as below.

| S. No. | Varieties | Zone of inhibition (mm) | |
|---|---|---|---|
| | | Maximum | Minimum |
| 1. | Nepalese | 16 | 6 |
| 2. | Chinese | 11 | 4 |

***Table 8:*** *Comparative study of Nepalese variety and Chinese variety of garlic.*

While doing comparative study of both types of garlic, Nepalese variety was seemed to show larger zone of inhibition as compared to Chinese variety. The maximum zone of inhibition shown by Nepalese variety of garlic was 16 mm, while that of Chinese variety was 11 mm. Similarly, the minimum zone of inhibition given by Nepalese variety was 6 mm, while that of Chinese variety was 4 mm. So, the study showed that Nepalese variety of garlic exhibited high range of zone of inhibition as compared to Chinese variety of garlic.

## Conclusion

The incidence of microbial infection is alarmingly increasing with the increase of susceptible population of immuno-compromised. That is why there is growing worldwide concern regarding the problem of infectious diseases. In order to overcome this problem, many scientific researches have been done to discover antimicrobial drugs. Moreover, residues of these drugs produce different adverse effects such as allergic reaction in the intestinal flora, and emergence of resistant flora of microorganisms. Nepal is a developing country where conflict, economic shock, climate change and soaring prices of food and fertilizer are combining in effect. The staple food items are generally salty, sour and spicy. The incidence of *H. pylori* is significantly higher in Nepal whose dietary habits are spicy than those whose dietary habits are bland. As a result inflammation of the lining of stomach called gastritis has seen very common in Nepal. Research also showed that the most common endoscopic findings are gastritis in Nepal, which is caused by infection in association with *H. pylori* infection. Due to lack of proper access to modern medication, people are forced to rely on herbal medicines like garlic. So, these herbal

medicines are often used to provide first line and basic health services of people, especially those living in remote areas. It is a popular ingredient in cooking due to its strong smell and delicious taste. Beside its condiment properties, it is well documented for its medicinal benefits. *H. pylori* are the main etiological agent of gastritis and peptic ulcer. This study was conducted to study the antimicrobial effect of two varieties of garlic, i.e. Nepalese variety and Chinese variety. Different food borne pathogenic bacteria like *E. coli*, *S. typhimurium*, *S. aureus* and *L. monocytogenes* were tested with standard antibiotics discs like amoxycillin, ampicillin and penicillin. Except *Listeria* spp., the entire organisms gave clear zone of inhibition with standard antibiotic discs. The maximum zone of inhibition of 40 mm for amoxycillin was given by *S. typhimurium*, and the minimum zone of inhibition of 26 mm given by *E. coli*. Similarly in case of ampicillin, the maximum zone of 30 mm was shown by *E. coli*, and minimum of 10 mm was shown by *Listeria* spp. In case of penicillin, zone of inhibition of 30 mm was shown by Listeria spp., and minimum zone of inhibition of 16 mm was given by *Salmonella* spp. The study was also carried out to find out the antibiotic sensitivity of *H. pylori* and other pathogens (*E. coli*, *S. typhimurium*, *S. aureus*, and *L. monocytogenes*) with garlic extract.

*H. pylori* was isolated from gastric biopsy and its morphological, cultural and biochemical tests were carried out. After isolation, MHA enriched with blood was used to test for antibiotic sensitivity with garlic extract. Altogether 60 samples, 30 samples of each variety of garlic were tested from 7 districts of Nepal. In case of Nepalese variety, minimum zone of inhibition of 7 mm and maximum zone of 16 mm was obtained. Similarly, in case of Chinese variety, minimum zone of inhibition of 5 mm and maximum zone of 11 mm was obtained. The range of zone of inhibition obtained from both varieties of garlic during the study ranges from 5 mm to 16 mm. The susceptible breakpoint or interpretive criteria of any antibiotics is 20 mm and resistant breakpoint is 14 mm. However, results obtained from the study does not fulfill the criteria but generalized some inhibitory effect of garlic with *H. pylori*.

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