Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Urease Enzyme: Structure, Function, and Its Role in Helicobacter pylori Pathogenesis and Treatment Strategies

Muhammad Akhlaq*
Research Department Hamdard University Karachi. Corresponding Author Email: muhammadakhlaq377@gmail.com
Dr. Ghazala H. Rizwani
HMI Institute of Pharmacology and Herbal Sciences, Hamdard University Karachi, Pakistan
Sabira Sultana
Department of Eastern Medicine Government College University Faisalabad-Pakistan.
Rizwana Dilshad
Swedish College of Pharmacy and Allied Health Sciences, Rahim Yar Khan, Pakistan
Maqsood Ahmad
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, 63100, Bahawalpur, Pakistan
Rabia Nisar
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Punjab, Pakistan
Aqsa Afzal
Faculty of Pharmacy, Department of Pharmaceutics, Bahauddin Zakaria University Multan
Rimsha Zainab
Department of Botany, Shaheed Benazir Bhutto Women University, Peshawar, Pakistan
Fethi Ahmet Ozdemir
Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Bingol, 12000, Türkiye
Gawel Solowski
Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Bingol, 12000, Türkiye
Shafqat Rasool
School of Eastern Medicine, Minhaj University Lahore, Pakistan
Iftikhar Younis Mallhi
School of Human Nutrition and Dietetics, Minhaj University Lahore, Pakistan
Muhammad Mudassar
College of Allied Health Sciences, Faculty of medical sciences, Government College University Faisalabad, Pakistan
Mohsin Rafique
Department of Eastern Medicine, Superior University Lahore, Pakistan
Muhammad Akram*
Department of Eastern Medicine, Government College University Faisalabad Pakistan. Corresponding Author Email: muhammadakram@gcuf.edu.pk

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

ABSTRACT

The metalloenzyme urease requires nickel as a cofactor to break down urea molecules, producing ammonia and carbon dioxide through hydrolysis. This biochemical process is fundamental to nitrogen cycling within soil ecosystems and various biological environments. Among disease-causing microorganisms, particularly *Helicobacter pylori*, this enzyme serves as a crucial pathogenic mechanism that allows bacterial survival within the harsh acidic conditions of gastric tissue. By generating ammonia, the organism effectively buffers stomach acid, establishing a protective alkaline microenvironment that supports bacterial colonization and long-term persistence. Such acid neutralization leads to gastric tissue irritation, inflammatory responses, and subsequent development of conditions including gastritis, ulcerative disease, and malignant gastric tumors. The enzyme's importance in *H. pylori* infection has made it valuable for clinical detection methods, including breath-based urea testing and rapid enzymatic assays. While conventional therapeutic approaches combine acid-suppressing medications with antimicrobial agents, increasing bacterial resistance to antibiotics has driven research into alternative strategies. Scientists are investigating both laboratory-synthesized and plant-derived urease inhibitors as supplementary treatment options. Current research examines urease's molecular architecture and catalytic mechanisms, its contribution to bacterial pathogenicity and survival, its utility in medical diagnostics, existing therapeutic protocols, and emerging possibilities for urease-targeting compounds, emphasizing recent scientific progress and future therapeutic development opportunities

Keywords: Urease, *Helicobacter Pylori*, Gastric Colonization, Urease Inhibitors, Pathogenesis, Natural Therapies, Antibiotic Resistance

1. Introduction

Urease is one of the earliest enzymes discovered and characterized, first crystallized in 1926 by James B. Sumner from jack bean (*Canavalia ensiformis*) (Mobley et al., 1995). As a nickel-dependent enzyme, urease catalyzes the breakdown of urea into ammonia and carbon dioxide, a process critical to nitrogen recycling in many ecosystems. While urease is widely expressed in plants, fungi, and bacteria, its presence in certain pathogenic

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

microorganisms, such as *Helicobacter pylori*, has attracted considerable attention due to its pivotal role in disease progression.

H. pylorus is a spiral-shaped, Gram-negative bacterium that colonizes the human gastric mucosa. It infects more than 50% of the global population, often asymptomatically, but can lead to severe gastrointestinal disorders, including chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric carcinoma (Suerbaum & Michetti, 2002). One of the most important mechanisms that allow *H. pylori* to survive in the highly acidic stomach environment is its robust urease activity.

The enzyme produces ammonia from urea, creating a local buffering zone around the bacteria. This neutral microenvironment not only survival aids but also contributes to epithelial damage, inflammation, and immune system evasion (Ansorg et al., 1991). Therefore, urease serves a dual function: a survival tool and a virulence factor. Its diagnostic utility has also been harnessed in the form of rapid urease tests and urea breath tests, making it a biomarker of infection.

Traditional treatment for *H. pylori* has relied on antibiotics combined with proton pump inhibitors (PPIs), but rising antibiotic resistance has reduced the success of these regimens. As a result, researchers are increasingly investigating urease inhibitors—derived from both synthetic chemicals and natural plants—as possible alternative or complementary treatment options.

This article examines the structure and function of urease, its importance for *H. pylori* survival and disease development, and its potential use in both diagnosis and therapy. Particular attention is given to recent studies on plant-based urease inhibitors and the growing difficulties of managing *H. pylori* infections in the context of multidrug resistance.

2. Structure and Biochemistry of Urease (Expanded)

Urease metalloenzyme containing two nickel atoms having protein puzzles and splits amine bonding of carbamide (Shalan et al., 2024). The urease is one of oldest investigated compounds (firstly in 1926in jack beans by James B. Sumner) (Mobley et al., 1995). . It functions in the hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2). This biochemical reaction is important in the nitrogen cycle of living organisms enabling fertilization, Due to being indicator of presence of many illnesses; the enzyme is isolated

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

and crystallizes from bacteria like *Klebsiella aerogenes Mobley, H. L. (2001)*. In human health, urease gains prominence due to its key involvement in microbial pathogenesis—most notably in *Helicobacter pylori*, a gastric pathogen strongly associated with ulcers and gastric carcinoma.

The enzymatic breakdown of urea generates ammonia, which elevates the local pH by neutralizing stomach acid. This ammonia production creates an alkaline microenvironment within the gastric mucosa, disrupting the normally acidic conditions. The pH shift causes structural changes in acid-adapted proteins and enzymes, leading to their dysfunction and potential degradation. Additionally, the alkaline conditions and associated inflammatory processes can cause cellular damage, including harm to nuclear components such as DNA and RNA. This cellular injury, combined with chronic inflammation, may contribute to mutagenic changes that increase the risk of malignant transformation and tumor development.

The altered pH environment also disrupts the protective mechanisms of gastric epithelial cells, making them more susceptible to further bacterial colonization and tissue damage. These combined effects of direct cellular injury, protein denaturation, and chronic inflammatory responses create conditions that favor the progression from initial infection to more severe pathological outcomes, including gastric carcinogenesis (Zhang et al., 2025).

2.1 Molecular Architecture and Subunits

The complete *H. pylori* urease enzyme comprises twelve individual protein components organized in a twelve-membered assembly, incorporating UreA and UreB proteins in a specific $(UreA–UreB)_{34}$ arrangement.

The UreA component functions as the smaller structural element within this complex, while UreB contains the enzymatic ally active region and coordinates with helper proteins during enzyme maturation. This multi-subunit organization creates a protective spherical framework that shields the reactive center from environmental damage. Such structural design provides exceptional resilience under low-pH conditions, representing an evolutionary adaptation characteristic of ureases produced by acid-tolerant bacterial species (Benini et al., 1999; Ha et al., 2001).

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

2.3 Catalytic Mechanism of Urease

The reaction starts as urea molecules move into the enzyme's active region and position themselves between the pair of nickel ions. A hydroxide group, which sits bridging these two metal centers, then strikes the carbon atom in urea's carbonyl group, creating an unstable four-sided intermediate structure. This collapses into carbamate and ammonia, and the carbamate spontaneously hydrolyzes into a second molecule of ammonia and carbon dioxide (Carter et al., 2009).

This two-step process occurs with high efficiency, enabling *H. pylori* to survive even at pH levels as low as 1.5 by rapidly increasing the pH of its immediate environment.

2.4 pH Adaptation and Structural Stability

Most ureases have an optimal pH of 7.0–8.5. However, *H. pylori* urease is uniquely adapted to function under acidic stress. This adaptation is enhanced by:

- The protective mucosal layer in the stomach.
- Urease-rich microenvironments generated through ammonia production.
- Proton-gated channels in bacterial membranes that maintain cytoplasmic neutrality (Scott et al., 1998).

Such adaptations allow the bacteria to inhabit the acidic gastric niche where few other organisms survive.

2.5 Genetic Regulation of Urease in *H. pylori*

The urease gene cluster in *H. pylori* includes ureA**,** ureB**,** and ureI**,** along with accessory genes ureE**,** ureF**,** ureG, and ureH, which facilitate nickel transport and insertion into the enzyme.

- **UreI** encodes a urea channel, activated by low pH, allowing urea to enter the cytoplasm for hydrolysis.
- **NikR**, a nickel-responsive regulator, controls the expression of urease genes based on environmental nickel availability (Ernst et al., 2006).

This tight regulation ensures energy-efficient use of urea and nickel, critical for bacterial survival and virulence.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

2.6 Importance of Nickel Acquisition

Nickel is a scarce but essential micronutrient for *H. pylori*. The bacterium uses NixA, a high-affinity nickel transporter, to scavenge nickel from the gastric environment (Stähler et al., 2006). Without adequate nickel, urease cannot fold correctly or function.

As a result, chelating agents that bind nickel have been proposed as potential therapies to inactivate urease and suppress *H. pylori* colonization.

2.7 Implications for Pathogenicity

The biochemical structure of urease is not only optimized for acid resistance but also contributes directly to pathogenesis:

- The ammonia generated damages gastric epithelial cells and disrupts tight junctions.
- Alkaline stress and oxidative damage from urease activity stimulate inflammatory responses.
- Urease also acts as a neutrophil activator, enhancing host tissue injury (Weeks et al., 2000).

These multifactorial effects underscore why urease is regarded as a central virulence determinant of H. pylori.

3. Urease in Helicobacter pylori Survival and Gastric Colonization

The survival and successful colonization of *Helicobacter pylori* (*H. pylori*) in the human stomach—a highly acidic and inhospitable environment—are largely dependent on the activity of urease. While many microorganisms cannot withstand the stomach's pH of 1.5–3.5, H. pylori thrives by leveraging urease to neutralize gastric acid, allowing it to reach the gastric mucosa and establish chronic infection. This section explores the physiological role of urease in bacterial survival, the acid-neutralizing mechanism, colonization pathways, and interactions with gastric epithelial cells.

3.1 Challenges of the Gastric Environment

The human stomach presents multiple barriers to microbial survival:

- High acidity (pH 1.5–3.5)**:** Lethal to most bacteria.
- Pepsin and proteolytic enzymes**:** Degrade proteins and cellular structures.
- Mucus layer**:** Acts as a physical and chemical barrier.

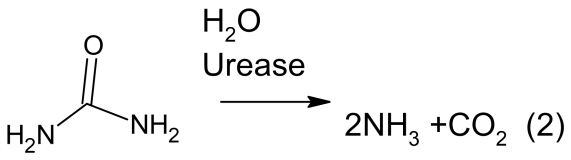
Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Yet H. pylori, a microaerophilic, spiral-shaped Gram-negative bacterium, have evolved several adaptive mechanisms. The most crucial among them is the expression of urease, which allows for immediate neutralization of stomach acid, facilitating both survival and motility in the gastric mucus layer (Montecucco & Rappuoli, 2001).

3.2 Acid Neutralization by Ammonia Production

Urease hydrolyzes urea, present in gastric juice, into ammonia (NH_3) and carbon dioxide (CO_2). Ammonia acts as a weak base, reacting with hydrogen ions (H^+) in gastric acid to form ammonium (NH_4^+) (reaction 2). This reaction raises the local pH around the bacterium, buffering the acidic environment:



$$NH_3 + H^+ \longrightarrow NH_4^+ \quad (3)$$

This process:

- Maintains *H. pylori*'s periplasmic and cytoplasmic pH near neutral (6.0–7.0)
- Prevents acid-mediated denaturation of bacterial proteins
- Enables survival during initial gastric entry (Weeks et al., 2000)

3.3 Role of the UreI Channel

A key adaptation in *H. pylori* is the UreI protein, a proton-gated urea channel embedded in the bacterial inner membrane. UreI opens only at low pH, allowing urea to enter the bacterial cytoplasm when it's most needed (Bauerfeind et al., 1997). This dynamic gating ensures:

- Efficient use of energy (urea is not constantly transported)
- Rapid acid neutralization upon gastric entry
- Protection of internal bacterial structures

When gastric pH is neutral, (in the duodenum), the UreI channel closes, conserving urea resources.

3.4 Motility and Penetration of Gastric Mucus

The ammonia generated by urease not only neutralizes acid but also:

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Increases the viscosity of the mucus layer, making it easier for *H. pylori* to penetrate.
- Destabilizes mucins by raising pH, as gastric mucus is gel-like at low pH and liquefies at neutral pH.

Coupled with flagellar motility, *H. pylori* swim through the loosened mucus and attaches to gastric epithelial cells, where pH is relatively neutral (Clyne et al., 2000).

3.5 Adhesion and Colonization

After penetrating the mucus, H. pylori uses several adhesins to bind to the epithelial surface, such as:

- BabA: Binds to Lewis b antigens.
- SabA: Binds to sialylated glycans.
- HopQ: Binds to CEACAMs, promoting type IV secretion system engagement.

Once adhered, *H. pylori* forms microcolonies protected by mucus and buffered by ammonia, allowing long-term colonization.

Urease plays a dual role**:**

1. Initial survival and acid buffering
2. Long-term immune evasion and chronic infection

3.6 Urease and Immune Modulation

Ammonia produced by urease is cytotoxic to gastric epithelial cells and causes:

- Apoptosis and tissue damage
- Release of inflammatory cytokines (e.g., IL-8, IL-1β)
- Recruitment of neutrophils and macrophages

This creates a chronic inflammatory state, contributing to:

- Mucosal ulceration
- DNA damage (through reactive oxygen species)
- Increased risk of gastric adenocarcinoma (Kusters et al., 2006)

In addition, urease is a potent immunogen. It stimulates both innate and adaptive immune responses, but *H. pylori* uses other virulence factors (e.g., VacA, CagA) to dampen immune clearance**,** leading to persistence.

3.7 Evidence from Mutant Studies

Animal and in vitro studies with urease-negative mutants of *H. pylori* have shown:

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Loss of colonization ability
- Failure to survive in acidic environments
- No induction of inflammation or gastritis

This confirms that urease is essential for both colonization and pathogenicity (Eaton et al., 1991).

4. Urease and Gastric Disease Pathogenesis

Helicobacter pylori colonization is linked to various disorders affecting the stomach and duodenum, spanning from mild, symptom-free stomach inflammation to serious conditions including ulcerative disease of the digestive tract, gastric tissue deterioration, cellular changes where intestinal-type cells replace gastric mucosa, and malignant tumors of the stomach lining.. Urease plays a central role in the transition from mere colonization to active pathogenesis. Its enzymatic action not only helps *H. pylori* survive acidic stress but also contributes directly to mucosal damage, immune activation, inflammation, and cellular transformation.

This portion examines the biochemical and cellular mechanisms by which urease promotes pathological development, focusing particularly on its function as a disease-causing factor, an inflammation-triggering compound, and a facilitator of tissue damage to the protective stomach lining.

4.1 Role of Ammonia in Gastric Mucosal Damage

The primary toxic product of urease activity is ammonia (NH_3). Once produced, ammonia reacts with hydrogen ions (H^+) in the gastric lumen to form ammonium (NH_4^+). While this neutralizes acid and supports bacterial survival, excess ammonia is cytotoxic.

Ammonia contributes to mucosal damage through several mechanisms:

- Direct cytotoxicity: Ammonia induces vacuolation and apoptosis in gastric epithelial cells.
- Disruption of tight junctions: Leads to increased permeability and exposure of deeper layers to acid and pathogens.
- Synergy with reactive oxygen species (ROS): Amplifies oxidative stress and causes DNA damage (Smoot et al., 2000).

4.2 Inflammatory Cascade and Immune Cell Recruitment

Urease stimulates both the innate and adaptive immune systems, primarily through:

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Ammonia and CO_2 production
- Interaction with immune receptors on epithelial cells

These effects lead to:

- Increased IL-8 production, which attracts neutrophils and macrophages
- Release of tumor necrosis factor-alpha (TNF-α), IL-1β, and other proinflammatory cytokines (Kusters et al., 2006)

Neutrophils, in response, release:

- Myeloperoxidase, proteases, and superoxide radicals, which cause collateral damage to host tissues.
- Chronic inflammation further damages the gastric epithelium and may contribute to gastric atrophy and metaplasia.

4.3 Synergistic Interaction with Other Virulence Factors

Urease does not act alone. It interacts with other *H. pylori* virulence proteins to aggravate disease:

VacA (Vacuolating cytotoxin A):

- VacA forms pores in epithelial cell membranes.
- Urease-generated ammonia enhances vacuole formation, amplifying VacA's toxic effect (Boncristiano et al., 2003).

CagA (Cytotoxin-associated gene A):

- Injected into host cells via type IV secretion system.
- Alters host cell signaling and proliferation.
- Urease helps maintain the microenvironment necessary for CagA activity by modulating pH and evading immune clearance (Hatakeyama, 2004).

Together, these factors create a toxic niche that supports long-term colonization and chronic inflammation.

4.4 Progression from Gastritis to Ulceration

Histological studies show that *H. pylori* infection typically begins with:

- Chronic active gastritis, characterized by lymphocytic and neutrophilic infiltration of the gastric mucosa.

If left untreated, this can progress to:

- Erosion of the mucosal layer

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Exposure of underlying tissues to acid and pepsin
- Ulcer formation, especially in the antrum or duodenum

Urease facilitates this process by:

- Weakening the mucosal barrier (via ammonia)
- Promoting epithelial apoptosis
- Enhancing local inflammation

4.5 Contribution to Gastric Carcinogenesis

Chronic H. pylori infection is classified as a Group I carcinogen by the WHO (IARC, 1994). Urease contributes to gastric cancer development via multiple pathways:

- Chronic inflammation causes oxidative stress and DNA damage.
- Ammonia alters cell cycle regulation and promotes mutagenesis.
- Immune evasion enables long-term colonization and sustained cellular stress.

Over time, this leads to:

1. Atrophic gastritis
2. Intestinal metaplasia
3. Dysplasia
4. Gastric adenocarcinoma

This process—often termed the Correa cascade—has been validated in multiple epidemiological and animal studies (Correa, 1992).

4.6 Urease-Induced Immune Tolerance and Chronicity

While urease activates immunity, H. pylori often **evades clearance**, leading to persistent infection. Urease may contribute to this by:

- Inducing tolerogenic dendritic cells
- Promoting regulatory T-cell (Treg) responses
- Inhibiting Th1/Th17 cell-mediated clearance

Such manipulation of the host's immune response enables the pathogen to establish long-term colonization spanning several decades, characterized by recurring inflammatory episodes and gradual deterioration of gastric tissue (Harris et al., 2015).

4.7 Clinical Correlation: Evidence from Human and Animal Models

- Patients with high urease activity often show higher levels of inflammation**,** more severe ulcers, and **a** higher risk of progression to cancer**.**

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- In urease-negative mutants of *H. pylori*, colonization and disease induction are significantly impaired (Eaton et al., 1991).
- Mouse and gerbil models confirm that inhibition or genetic deletion of urease leads to:
    - Reduced gastric damage
    - Decreased bacterial load
    - Absence of ulceration or neoplastic changes (Tan et al., 2009)

5. Diagnostic Applications of Urease Activity

5.1 Urea Breath Test (UBT)

Mechanism:

1. Patient ingests ^{13}C- or ^{14}C-labeled urea.
2. If H. pylori is present, urease breaks it down:
3. The $\text{Urea} \xrightarrow{\text{urease}} 2NH_3 + CO_2^*$ labeled CO_2 is exhaled and detected using isotope ratio mass spectrometry (for ^{13}C) or a scintillation counter (for ^{14}C).

Advantages:

- High sensitivity (90–95%) and specificity (95–100%)
- Safe, painless, and non-invasive
- Useful for initial diagnosis and post-treatment eradication confirmation

Limitations:

- Requires fasting before the test
- False negatives may occur after recent antibiotic or proton pump inhibitor (PPI) use
- Slightly less accurate in children and the elderly

Clinical Relevance

Urea breath test is recommended by guidelines (e.g., Maastricht VI/Florence Consensus) as the first-line test in symptomatic patients, especially in populations with high prevalence of H. pylori (Malfertheiner et al., 2022).

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

5.2 Rapid Urease Test (RUT or CLO Test)

The Rapid Urease Test (RUT), often called the CLO test (Campylobacter-like organism test), is an invasive, biopsy-based diagnostic method performed during endoscopy.

Mechanism:

1. A biopsy sample is obtained from the gastric mucosa.
2. The sample is placed into a medium containing urea and a pH indicator (usually phenol red).
3. If H. *pylori* is present, urease hydrolyzes urea → ammonia → local pH increases → color changes from yellow to pink.

Advantages:

- Fast results (minutes to hours)
- High specificity (close to 100%)
- Inexpensive and easy to use

Limitations:

- Sensitivity depends on bacterial density and site of biopsy
- False negatives if patient recently used PPIs, antibiotics, or bismuth
- Cannot quantify bacterial load or determine strain virulence

Clinical Relevance:

RUT is often used alongside histopathology or culture during endoscopy, particularly in patients with ulcers or suspected malignancy (Chey et al., 2017).

5.3 Urease-Based Stool Antigen Tests

Though not as directly urease-based as UBT or RUT, stool antigen tests detect H. pylori antigens, including urease components, using monoclonal antibodies.

Advantages:

- Non-invasive and cost-effective
- Suitable for screening and post-eradication confirmation
- Easy to administer, including in children

Limitations:

- Affected by sample quality and storage
- Less accurate than UBT in some populations

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

New-generation monoclonal-based ELISAs are approaching the sensitivity of UBT and may detect urease subunits specifically (Gisbert & Pajares, 2004).

5.4 Molecular and Biosensor-Based Tests Targeting Urease

Emerging technologies are now targeting urease gene sequences or enzymatic activity using:

- PCR-based assays for ureA, ureB, and ureC genes
- Urease-specific biosensors, which use electrical or optical signals to detect urea hydrolysis
- Microfluidic paper-based analytical devices (μPADs) that detect urease activity from gastric swabs

These novel methods promise:

- Point-of-care diagnostics
- Greater sensitivity, even at low bacterial load
- Rapid field-level screening in resource-limited settings (Zhang et al., 2020)

Comparison of Diagnostic Methods

Test	Type	Invasiveness	Sensitivity (%)	Specificity (%)	Time to Results
Urea Breath Test	Non-invasive	No	90–95	95–100	30–60 minutes
Rapid Urease Test	Invasive	Yes	85–95	95–100	15–60 minutes
Stool Antigen	Non-invasive	No	85–92	90–95	Few hours
PCR/Genetic	Invasive	Usually Yes	92–98	98–100	1–3 hours

5.6 Diagnostic Role in Monitoring Eradication Therapy

Since urease is rapidly lost following successful eradication, urease-based tests—especially UBT and stool antigen tests—are ideal for monitoring treatment success. Guidelines recommend:

- UBT or stool antigen test 4–6 weeks after therapy
- Avoidance of PPIs and antibiotics for at least 2 weeks before testing (Chey et al., 2017)

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Failure to eradicate *H. pylori* may lead to ulcer recurrence and higher cancer risk, so accurate post-treatment testing is critical.

6. Therapeutic Targeting of Urease – Pharmacological and Natural Approaches

Given the critical role of urease in *Helicobacter pylori* survival, colonization, and pathogenesis, the enzyme represents an attractive therapeutic target. Current *H. pylori* eradication regimens rely heavily on antibiotics and proton pump inhibitors (PPIs). However, rising antibiotic resistance and treatment failures have spurred interest in adjunctive or alternative strategies—particularly those that inhibit urease activity.

This section explores therapeutic approaches that directly or indirectly target urease, including:

- Synthetic urease inhibitors
- Comparison of PPIs vs anti-urease agents
- Natural plant-derived inhibitors and their pharmacological potential

6.1 Synthetic Urease Inhibitors

Synthetic compounds designed to inhibit urease activity fall into two main categories:

a. Sulfhydryl (thiol) inhibitors

These agents bind to the nickel ion at the urease active site, essential for enzymatic function. Examples include:

- Acetohydroxamic acid (AHA)
- Hydroxamic acid derivatives
- Phosphoramidates

Mechanism of Action

They form stable complexes with nickel, blocking the hydrolysis of urea and hence ammonia production (Mobley et al., 1995).

Limitations

- Poor gastrointestinal tolerance (especially AHA)
- Short half-life and systemic toxicity
- Not widely approved for clinical use

b. Competitive inhibitors

These mimic urea's structure and compete for the active site. Examples include:

- Imidazoles

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Benzohydrazides
- Thiourea analogs

Research status: Many are in preclinical or experimental phases, with promising in vitro activity against *H. pylori*, but limited in vivo testing.

6.2 Proton Pump Inhibitors (PPIs) vs Anti-Urease Agents

While PPIs like omeprazole or lansoprazole are not direct urease inhibitors, they remain essential in H. pylori therapy due to their ability to:

- Suppress gastric acid secretion
- Improve antibiotic efficacy
- Promote ulcer healing

However, PPIs do not affect urease activity. In contrast, anti-urease drugs target the root enzymatic mechanism that enables H. pylori survival.

Why not replace PPIs with anti-urease drugs?

- Urease inhibitors alone may not eliminate H. pylori.
- Combined regimens (e.g., urease inhibitors + antibiotics + PPIs) show promise in animal studies (Ha et al., 2014).
- PPIs improve gastric pH, which enhances antibiotic absorption and action.

The best approach may be adjunctive—using urease inhibitors to weaken H. pylori defenses and PPIs to reduce acid burden.

6.3 Natural Urease Inhibitors (Herbal and Plant-Derived Agents):

Phytotherapeutic Potential and Scientific Insights

The growing global resistance to conventional antibiotics in *Helicobacter pylori* treatment has catalyzed the search for alternative and adjunctive therapies. Among these, natural plant-derived compounds have gained increasing scientific interest due to their antimicrobial efficacy, low toxicity, and broad pharmacological spectrum. Traditional herbal remedies historically employed for treating digestive ailments have demonstrated significant enzyme-blocking properties against urease, presenting a viable therapeutic avenue for addressing *H. pylori* infections.

Urease, being a nickel-containing metalloenzyme, presents multiple binding sites for inhibition by phytochemicals. These natural compounds function through diverse mechanisms including direct binding to the urease active site, chelation of nickel ions

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

essential for catalytic function, or downregulation of urease gene expression. Inhibiting urease compromises *H. pylori*'s ability to neutralize gastric acid, thereby reducing its colonization efficiency and virulence.

Polyphenols, flavonoids, alkaloids, tannins, and terpenoids have emerged as major classes of bioactive compounds with anti-urease activity. Green tea catechins, particularly epigallocatechin gallate (EGCG), have shown strong in vitro inhibitory effects on H. pylori urease, alongside antimicrobial and antioxidant properties. Similarly, curcumin—the principal curcuminoid in turmeric (*Curcuma longa*)—has demonstrated urease inhibition and significant attenuation of gastric inflammation in *H. pylori*-infected models.

Garlic (*Allium sativum*) exerts its anti-urease effect primarily through allicin, a sulfur-containing compound that interferes with the enzyme's cysteine residues and metal binding centers. In vitro and in vivo studies reveal that garlic not only inhibits urease but also suppresses bacterial growth and adhesion. The essential oil of clove (*Syzygium aromaticum*), rich in eugenol, displays both bactericidal and urease-inhibitory properties, alongside disruption of bacterial biofilms.

Neem (*Azadirachta indica*), widely utilized in South Asian traditional medicine, contains azadirachtin, nimbin, and quercetin—phytochemicals with demonstrated capacity to chelate nickel and inhibit urease activity. Additionally, neem extracts downregulate urease gene transcription and impair *H. pylori* motility and adhesion. Licorice (*Glycyrrhiza glabra*), traditionally used for gastritis and ulcers, contains flavonoids and saponins such as glycyrrhizin and liquiritigenin, which exhibit strong affinity for urease active sites and provide mucosal protection.

A comparative summary of notable plants and their anti-urease actions is presented in the following table:

Selected Medicinal Plants with Reported Urease Inhibitory Activity Against H. pylori

Plant/Compound	Active Components	Mechanism	References
Green tea (*Camellia sinensis*)	Catechins (EGCG, EGC)	Urease inhibition and antioxidant effects	Matsubara et al., 2003

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Turmeric (*Curcuma longa*)	Curcumin	Direct urease inhibition, anti-inflammatory	Mahady et al., 2002
Garlic (*Allium sativum*)	Allicin, ajoene	Urease suppression, bactericidal	O'Gara et al., 2000
Neem (*Azadirachta indica*)	Azadirachtin, nimbin	Urease and quorum sensing inhibition	Pandey et al., 2014
Clove (*Syzygium aromaticum*)	Eugenol	Anti-urease and anti-biofilm activity	Chami et al., 2005
Cranberry (*Vaccinium macrocarpon*)	Proanthocyanidins	Inhibit bacterial adhesion and urease	Burger et al., 2000
Licorice (*Glycyrrhiza glabra)*	Glycyrrhizin, flavonoids	Anti-H. pylori, gastroprotective	Fukai et al., 2002

Here's a rephrased version:

Beyond these findings, various botanical species, including *Terminalia chebula*, *Zingiber officinale* (commonly known as ginger), *Punica granatum* (pomegranate), and *Quercus infectoria* (oak galls) have shown substantial enzyme-blocking activity against urease during laboratory studies. These plant extracts frequently exhibit inhibitory concentrations required to reduce enzyme activity by half that match or surpass those of artificially produced compounds like acetohydroxamic acid.

Notably, these plants also offer gastroprotective effects by reducing oxidative stress, enhancing mucus secretion, or modulating host immune responses.

Despite encouraging laboratory results, the transition from bench to bedside for phytotherapeutic urease inhibitors is still limited. Variability in plant constituents due to ecological and seasonal factors, lack of standardization in extract preparation, and insufficient pharmacokinetic data hinder clinical application. Moreover, the bioavailability of many phytochemicals—such as curcumin and EGCG—remains a challenge, although recent innovations in nanoparticle encapsulation, liposomal delivery, and adjuvant co-administration (e.g., piperine with curcumin) are showing promise.

Recent advances in molecular docking and in silico modeling have further strengthened the potential of plant-derived inhibitors by demonstrating strong binding affinities of flavonoids, tannins, and phenolics with key amino acid residues of H. pylori

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

urease (ureA and ureB subunits), supporting experimental findings. These computational studies facilitate rational drug design based on phytochemicals, potentially accelerating their development into standardized botanical drugs.

In summary, natural urease inhibitors provide a scientifically substantiated, culturally acceptable, and potentially safer alternative or adjunct to conventional anti-H. pylori therapy. While more in vivo studies, clinical trials, and standardization protocols are needed, the accumulated evidence strongly supports their inclusion in future treatment paradigms, especially in populations with high H. pylori prevalence and antibiotic resistance.

6.4 Antibody-Based and Vaccine Strategies Against Urease

- Monoclonal antibodies against urease subunits (ureA, ureB) show potential in neutralizing enzyme activity.
- Recombinant urease-based vaccines have been tested in animal models with partial protection.
- Challenges remain with antigen stability, mucosal delivery, and variable immune responses.

6.5 Future Directions in Anti-Urease Therapy

- **Structure-guided drug design**: Using crystal structures of urease to develop potent, specific inhibitors
- **Nanoformulations**: Delivering plant-based urease inhibitors via nanoparticles for better absorption
- **Synergistic therapies**: Combining low-dose antibiotics, PPIs, probiotics, and urease inhibitors
- **Personalized treatment**: Based on urease activity levels and H. pylori genotype

7. Conclusion and Future Perspectives

Urease is essential for the survival of *Helicobacter pylori*, as it neutralizes gastric acid, enabling the bacterium to colonize the stomach lining and contribute to various gastric disorders. While standard therapies combining antibiotics with proton pump inhibitors have shown effectiveness, the increasing problem of antibiotic resistance and treatment-related side effects underscores the need for safer and more sustainable alternatives.. Natural urease inhibitors, such as curcumin, EGCG, and allicin, show promise due to their

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

enzyme-inhibiting, anti-inflammatory, and protective effects. However, clinical application is limited by challenges like variability in plant extracts and low bioavailability. Future research should prioritize standardization, advanced formulations, and clinical trials. Integrating natural urease inhibitors may lead to safer, more sustainable treatments for *H. pylori* infections.

References

- Akhtar, M. F., Ashraf, M., Javeed, A., & Mujeeb, F. (2012). In vitro urease inhibition by extracts of some indigenous medicinal plants. *Journal of Medicinal Plants Research*, 6(3), 403–407.
- Bauerfeind, P., Garner, R., Dunn, B. E., & Mobley, H. L. (1997). Sensing environmental pH: a novel role for urease accessory proteins. *Proceedings of the National Academy of Sciences*, 94(19), 10447–10452. https://doi.org/10.1073/pnas.94.19.10447
- Benini, S., Rypniewski, W. R., Wilson, K. S., Ciurli, S., & Mangani, S. (1999). The urease mechanism of action: new insights from X-ray crystallography. *Journal of Biological Inorganic Chemistry*, 4(5), 556–563. https://doi.org/10.1007/s007750050358
- Boncristiano, M., Rossi, P., Cricco, G., Nuti, M., Covacci, A., & Montecucco, C. (2003). The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms. *The Journal of Experimental Medicine*, 198(12), 1887–1897. https://doi.org/10.1084/jem.20030963
- Burger, O., Weiss, E. I., Sharon, N., Tabak, M., Neeman, I., & Ofek, I. (2000). Inhibition of *Helicobacter pylori* adhesion to human gastric mucosa by cranberry juice. *New England Journal of Medicine*, 343(15), 1103. https://doi.org/10.1056/NEJM200010123431515
- Carter, E. L., Flugga, N., Boer, J. L., Mulrooney, S. B., & Hausinger, R. P. (2009). Interplay of metal ions and urease. *Metallomics*, 1(3), 207–221. https://doi.org/10.1039/B901325B
- Chami, N., Bennis, S., Chami, F., Aboussekhra, A., Remmal, A. (2005). Study of anticandidal activity of clove essential oil (Eugenia caryophyllata) and its two major constituents. *Phytotherapy Research*, 18(12), 990–995.

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Chey, W. D., Leontiadis, G. I., Howden, C. W., & Moss, S. F. (2017). ACG Clinical Guideline: Treatment of *Helicobacter pylori* infection. *American Journal of Gastroenterology*, 112(2), 212–239. https://doi.org/10.1038/ajg.2016.563
- Clyne, M., Labigne, A., & Drumm, B. (2000). Helicobacter pylori requires an acidic environment to colonize the mouse stomach in vivo. *Infection and Immunity*, 68(8), 4435–4439. https://doi.org/10.1128/IAI.68.8.4435-4439.2000
- Correa, P. (1992). Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Research*, 52(24), 6735–6740.
- Eaton, K. A., Morgan, D. R., & Krakowka, S. (1991). Motility as a factor in the colonization of gnotobiotic piglets by *Helicobacter pylori*. *Journal of Medical Microbiology*, 35(4), 263–266. https://doi.org/10.1099/00222615-35-4-263
- Ernst, F. D., Homuth, G., Stoof, J., Mäder, U., Waidner, B., Kuipers, E. J., ... & van Vliet, A. H. (2006). Iron- and nickel-responsive regulation of urease expression by the *Helicobacter pylori* NikR repressor. *Journal of Bacteriology*, 188(2), 478–487. https://doi.org/10.1128/JB.188.2.478-487.2006
- Fukai, T., Marumo, A., Kaitou, K., et al. (2002). Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sciences*, 71(12), 1449–1463. https://doi.org/10.1016/S0024-3205(02)01886-4
- Gisbert, J. P., & Pajares, J. M. (2004). Stool antigen test for the diagnosis of *Helicobacter pylori* infection: a systematic review. *Helicobacter*, 9(4), 347–368. https://doi.org/10.1111/j.1083-4389.2004.00241.x
- Ha, N. C., Oh, S. T., Sung, J. Y., Cha, K. A., Lee, M. H., & Oh, B. H. (2001). Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nature Structural Biology*, 8(6), 505–509. https://doi.org/10.1038/88585
- Ha, N. C., Oh, S. T., Sung, J. Y., et al. (2014). Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nature Structural & Molecular Biology*, 8(6), 505–509.
- Harris, P. R., Ernst, P. B., & Smith, P. D. (2015). *Helicobacter pylori* and the gastric epithelial cell: immunological recognition and response. *Cellular Microbiology*, 17(6), 725–731. https://doi.org/10.1111/cmi.12430

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Hatakeyama, M. (2004). Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. *Nature Reviews Cancer*, 4(9), 688–694. https://doi.org/10.1038/nrc1433
- International Agency for Research on Cancer, WHO/IARC Expert Committee on Cancer Statistics, World Health Organization, & IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (1994). *IARC monographs on the evaluation of the carcinogenic risks to humans: hepatitis viruses* (Vol. 59). International Agency for Research on Cancer World Health Organization.
- Kusters, J. G., van Vliet, A. H., & Kuipers, E. J. (2006). Pathogenesis of *Helicobacter pylori* infection. *Clinical Microbiology Reviews*, 19(3), 449–490. https://doi.org/10.1128/CMR.00054-05
- Mahady, G. B., Pendland, S. L., Yun, G. S., & Lu, Z. Z. (2002). Turmeric (*Curcuma longa*) and curcumin inhibit the growth of *Helicobacter pylori*, a group 1 carcinogen. *Anticancer Research*, 22(6C), 4179–4181.
- Malfertheiner, P., Megraud, F., Rokkas, T., Gisbert, J. P., Liou, J. M., Schulz, C., ... & El-Omar, E. M. (2022). Management of *Helicobacter pylori* infection: the Maastricht VI/Florence Consensus Report. *Gut*, 71(10), 1724–1762. https://doi.org/10.1136/gutjnl-2022-327745
- Matsubara, K., Kawamoto, T., & Takeuchi, H. (2003). Anti-*Helicobacter pylori* effects of catechins and other plant polyphenols. *Japan Journal of Infectious Diseases*, 56(6), 273–276.
- Mazzei, L., Ciurli, S., & Mangani, S. (2017). The crystal structure of urease: a review. *Biological Chemistry*, 398(8), 695–705. https://doi.org/10.1515/hsz-2017-0121
- Mobley, H. L. T., Island, M. D., & Hausinger, R. P. (1995). Molecular biology of microbial ureases. *Microbiological Reviews*, 59(3), 451–480. https://doi.org/10.1128/mr.59.3.451-480.1995
- Mobley, H. L. (2001). Urease. Helicobacter pylori: physiology and genetics, 177-191.
- Montecucco, C., & Rappuoli, R. (2001). Living dangerously: how *Helicobacter pylori* survives in the human stomach. *Nature Reviews Molecular Cell Biology*, 2(6), 457–466. https://doi.org/10.1038/35073084

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- O'Gara, E. A., Hill, D. J., & Maslin, D. J. (2000). Activities of garlic oil, allicin, and ajoene against *Helicobacter pylori*. *Applied and Environmental Microbiology*, 66(5), 2269–2273.
- Pandey, R., Mishra, A., & Singh, S. K. (2014). Inhibitory activity of *Azadirachta indica* (neem) leaves extract on bacterial urease: In vitro and in silico studies. *Journal of Pharmacy and Bioallied Sciences*, 6(2), 118. https://doi.org/10.4103/0975-7406.129177
- Scott, D. R., Weeks, D. L., Hong, C., Postius, S., & Sachs, G. (1998). The role of urease in acid resistance of *Helicobacter pylori*. *Gastroenterology*, 114(1), 58–70. https://doi.org/10.1016/S0016-5085(98)70637-0
- Shaalan, H., Azrad, M., & Peretz, A. (2024). The effect of three urease inhibitors on H. pylori viability, urease activity and urease gene expression. *Frontiers in Microbiology*, *15*, 1464484.
- Smoot, D. T., Mobley, H. L., Chippendale, G. R., Lewison, J. F., Resau, J. H., & Peek, R. M. (2000). *Helicobacter pylori* urease is a potent stimulus of mononuclear phagocyte chemotaxis and adhesion. *Gastroenterology*, 118(4), 885–896. https://doi.org/10.1016/S0016-5085(00)70266-5
- Stähler, F. N., Odenbreit, S., Haas, R., Wilrich, J., & van Vliet, A. H. (2006). The nickel transporter NixA of *Helicobacter pylori*: regulation and metal specificity. *Infection and Immunity*, 74(8), 5042–5049. https://doi.org/10.1128/IAI.00283-06
- Sugiyama, T., Uotani, T., Ichikawa, H., et al. (2004). Green tea polyphenol inhibits *Helicobacter pylori*-induced inflammatory responses. *Digestive Diseases and Sciences*, 49(10), 1478–1482. https://doi.org/10.1023/B:DDAS.0000042232.77965.aa
- Tan, S., Noto, J. M., & Romero-Gallo, J. (2009). Urease-dependent regulation of innate immunity by *Helicobacter pylori*. *Nature Medicine*, 15(7), 831–837. https://doi.org/10.1038/nm.1964
- Weeks, D. L., Eskandari, S., Scott, D. R., & Sachs, G. (2000). A H^+ -gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. *Science*, 287(5452), 482–485. https://doi.org/10.1126/science.287.5452.482
- Zhang, Y., Lu, S., & Xu, Q. (2020). Paper-based microfluidic devices for clinical diagnostic applications. *Biosensors and Bioelectronics*, 170, 112649. https://doi.org/10.1016/j.bios.2020.112649

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

- Zhang, T., Zheng, Y., Chen, T. *et al.* Biomaterials mediated 3R (remove-remodel-repair) strategy: holistic management of *Helicobacter pylori* infection. *J Nanobiotechnol* **23**, 475 (2025). https://doi.org/10.1186/s12951-025-03455-2
- Tan, S., Noto, J. M., & Romero-Gallo, J. (2009). Urease-dependent regulation of innate immunity by *Helicobacter pylori*. *Nature Medicine*, 15(7), 831–837. https://doi.org/10.1038/nm.1964
- Chami, N., Bennis, S., Chami, F., Aboussekhra, A., Remmal, A. (2005). Study of anticandidal activity of clove essential oil (Eugenia caryophyllata) and its two major constituents. *Phytotherapy Research*, 18(12), 990–995.
- Pandey, R., Mishra, A., & Singh, S. K. (2014). Inhibitory activity of *Azadirachta indica* (neem) leaves extract on bacterial urease: In vitro and in silico studies. *Journal of Pharmacy and Bioallied Sciences*, 6(2), 118. https://doi.org/10.4103/0975-7406.129177