Effects of hydrogen peroxide and ferrous ions on the ability of *Helicobacter pylori* isolates to the production of urease and protease.

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Abstract

Introduction: The free radicals produced as a result of exposing the pathogenic bacteria *Helicobacter pylori* to hydrogen peroxide(H2O2), ferrous ions(Fe^{+2}), and hydrogen peroxide with ferrous ions affect the intensity of these free radicals and their ability to produce virulence factors in order to control and thus reduce the bacteria's pathogenicity.

Methods: Collecting 83 samples from clinical cases that included duodenitis, peptic ulcer disease, and gastric carcinoma, and of different ages from Samarra General Hospital in Samarra city in the Salah El-Din governorate for period December 2023 to May 2024. A total of 57 *H. pylori* isolates were successfully identified through biochemical and molecular methods, The minimum inhibitory concentration(MIC) and sub-inhibitory concentration (Sub-MIC) were determined for hydrogen peroxide(H_2O_2), ferrous ion(Fe^{+2}), and hydrogen peroxide with ferrous ion treatments. The effect of the (Sub-MIC) on *H. pylori* isolates ability to produce urease and protease enzymes was also investigated.

Results: The studied *H. pylori* isolates exhibited strong urease and protease before treatment. During exposure to hydrogen peroxide, the activity was low to moderate, particularly in the isolate from patients with Gastritis and Duodenitis, while the peptic ulcer isolates showed when exposed to H_2O_2 an increase in urease activity, whereas, the duodenal and peptic ulcer isolates showed a cessation in protease production. Also, the treatment with Fe^{+2} alone resulted in an observed decrease in both urease and protease activity.

Conclusion : The current research was concluded that the have varying effects on the clinical bacterial isolates, and the treatment by hydrogen peroxide and iron have the greatest inhibition to the activity of urease and protease enzyme in the bacteria.

Keywords: *Helicobacter pylori*; Oxidative stress; Urease; Protease; Hydrogen peroxide.

1. Introduction

Pathogenic bacteria *Helicobacter pylori* is a gram-negative, multiflagellate microaerophilic, spiral-shaped bacterium that colonizes the gastric mucosa of nearly half of the global population, considered to be among the most common and enduring bacterial infections in the world (Salvatori et al., 2026). *Helicobacter pylori* is strongly associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric carcinoma. The pathogenicity of *H. pylori* is primarily mediated through its virulence factors, including cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), urease, and proteases (Ali & AlHussaini, 2024).

The complex system of enzymes that *H. pylori* possesses is used for a variety of purposes, including colonization, host epithelial damage, and the supply of vital metabolic substrates. Urease and the effects of phospholipases and proteases on mucus and the mucosal barrier promote colonization (Nilius & Malfertheiner, 1996). *Helicobacter pylori* produces a lot of urease (6–10% of the total protein), which is essential for its survival, hydrolyzing urea to ammonia and CO_2 which increases the pH of the surrounding environment and protects against stomach acid damage environment by generation of a neutral microenvironment. A part from its function in colonization, urease triggers inflammatory reactions by stimulating neutrophils and monocytes, which cause harm to the stomach epithelial cells (Abd Alhasan et al., 2025; Shaalan et al., 2024) .

Protease is one of the most important virulence factors for bacteria, as it works to break down proteins and peptides into amino acids by affecting peptide bonds. Bacteria that release proteolytic enzymes do so either to protect themselves from toxic proteins or to weaken host cells (Seder et al., 2023).

A free radical is a molecule that has an unpaired electron in one of its atomic orbitals and is capable of independent existence. The characteristic that sets a free radical apart from other compounds is this unpaired electron. Reactive oxygen species (ROS) the most prevalent and important form of free radicals. This reaction between free radicals and organic compounds called oxidative stress (Pooja et al., 2025). Exposure of bacteria to free radicals (ROS) can damage a variety of macromolecules in the bacterial cell, and may be lead to mutations or cell death. However, free radicals may at the same time be considered useful compounds as signaling molecules that may be lead to a coordinated response in the bacterial cell under oxidative stress conditions (Kashmiri & Mankar, 2014).

Microbes rely upon iron as a cofactor for many enzymes in their central metabolic processes. The superoxide and hydrogen peroxide (H_2O_2) are classify as a part of ROS and may react rapidly with iron, and inside cells they can generate both enzyme and DNA damage, in addition, ROS such

as hydrogen peroxide may also modulate bacterial virulence (Seixas et al., 2022). Also, free radicals also attack and destroy the components of cells to cause severe damage, and may cause many diseases such as cardiovascular diseases, cancer, metabolic, and neurodegenerative diseases (I.A. Abd-alwahab et al., 2024; Manful et al., 2025). Free radicals that resulting from the interaction between hydrogen peroxide and ferrous oxides can modify or change the virulence of bacteria (Jasim and Al-juboory,2023). This study aims to elucidate the impact of sub- Minimum inhibitory concentration (sub-MIC) levels of H_2O_2 , Fe^{+2} and sub-MIC of H_2O_2 with Fe^{+2} ions on *H. pylori* isolates, focusing on their ability to maintain urease and protease production under oxidative stress.

2. Materials and Methods

First, Sample collection: 83 gastric biopsy and stool samples between December 2023 and May 2024 were collected from patients with gastritis, duodenitis, peptic ulcer, or gastric carcinoma patients of different ages and both genders of Samarra General Hospital in Samarra city in Salah al-Din Governorate as in Table 2. Bacterial isolation on Columbia agar base under microaerophilic conditions and incubated at 24 hours at 37°C and identification Biochemical test which included (Gram stain, Cell morphology, Urease, Catalase, Oxidase, Motility, Growth under microaerophilic, Indole, Lactose fermentation , Gelatin hydrolysis, Citrate utilization) confirmed the identity of 57 *H. pylori* isolates Based on what was stated in (Holt et al., 1994),with confirmation using PCR for *ureA* and *cagA* genes. Hydrogen peroxide (30%) from GmbH & Co., Germany, and Ferrous chloride ($FeCl_2$), as a source of Ferrous ion (Fe^{+2}), supplied by Thomas Baker, India.

The culture media and materials which used in the diagnosis and the study, they were supplied by international companies and different origins such as Oxoid, Himedia, and Difco. MIC and sub-MIC values for hydrogen peroxide only concentration (210-300) mM , MIC and sub-MIC values for ferrous ions only concentrations (40-90)mM and both ferrous ions with Sub MIC hydrogen peroxide (4-8)mM were determined according to the dilution method in the medium by using Mueller-Hintton broth (MIC determination medium). All prepared concentrations were then inoculated with 0.1 ml of bacterial suspension, which had a turbidity similar to that of a 0.5 ml McFarland tube were thoroughly mixed and incubated under standard conditions at 37 °C for 24 hours. Visually, growth was then confirmed. 0.1 ml was taken from each vial and inoculated onto nutrient agar suitable for bacterial growth. (Collee et al., 1996; Vinckx et al., 2008), Urease activity was assessed on Christensen's urea agar and the change of the colour of the medium from yellow to pink indicated to positive result, due to the ammonia formed (Forbes *et al*., 2007), as in Table 4, and protease production was determined on skim milk agar by observation of transparent areas around the growing colonies indicates that the bacteria are protease-producing (Benosn, 2002), as in Table 5.

The statistical analysis was conducted by using IBM SPSS Statistics 26.0. The data were

summarized using frequencies and percentages. To examine differences in *Helicobacter pylori* prevalence among patient groups, Pearson's Chi-square test was employed rather than more basic approaches, And One-way ANOVA analysis using to demonstrated a significant variances in MIC and sub-MIC values of treatment, significant differences were estimated by using Duncan's test, significant level was a p-value below 0.01.

3. Results

Isolation rates, MIC values, and enzymatic activity were determined as follows:

3.1. Diagnosis of bacterial isolates

The diagnosis was initially made based on morphological and culture characteristics after bacterial growth on culture media. Cell characteristics were then studied after Gram staining and microscopic examination to characterize their morphology. The diagnosis was confirmed by physiological and biochemical tests, in which the studied bacteria were identified using tests such as urease, catalase, oxidase, motility and Growth under microaerophilic conditions tests, all of which showed positive results. In contrast, the bacteria were negative in tests, indole tests, lactose fermentation tests, gelatin liquefaction tests, and citrate tests (Pitt, 2018). The diagnosis of *Helicobacter pylori* was further confirmed using the PCR technique for *ureA* and *cagA* genes, as shown in Table 1.

Table 1. Diagnostic morphological and biochemical tests used in the diagnosis of *H. pylori* isolates.

Test	Result
Gram stain	- (Gram negative)
Cell morphology	Spiral, curved bacilli
Urease	+
Catalase	+
Oxidase	+
Motility	+
Growth under microaerophilic conditions	+
Indole	-
Lactose fermentation	-
Gelatin hydrolysis	-
Citrate utilization	-

(-) the negative result of the test. (+) the positive result of the test.

3.2. Isolates of *H. pylori* bacteria

The results in Table 2 showed a total of 83 patient samples were collected from individuals

experiencing different stomach and digestive tract diseases; from these samples, 57 tested positive for *Helicobacter pylori*, that form approximately 70%. The highest prevalence was observed in patients with ulcers, where infection was detected in just under 75% of cases. This was closely followed by those with gastritis, then by individuals diagnosed with stomach cancer or upper intestinal inflammation. Statistical analysis revealed minimal differences across the various conditions. Detection rates remained consistently high across all diagnoses, showing little variation despite the type of gastrointestinal disorder present.

Table 2. Number of isolates with percentage of *H. pylori* isolates studied.

Isolation source	**Total samples**	***H. pylori* isolates**	**Studied isolates (%)**
Gastritis	28	19	67.8
Duodenitis	20	13	65
Peptic ulcer disease	23	17	73.9
Gastric carcinoma	12	8	66.6
Total	83	57	

Chi-square (χ^2) = 0.450, P-value = 0.930 (N.S), N.S = Not Significant (P>0.01).

3.3. Minimum inhibitory concentration (MIC) and sub-MIC for H_2O_2, Fe^{+2} , and H_2O_2 with ferrous ion

The results in Table 3 showed the minimum inhibitory concentration (MIC) and sub-MIC for hydrogen peroxide, ferrous ion alone, and ferrous ion with hydrogen peroxide for the *H. pylori* isolates were determined according to the dilution method in the medium (Vinckx et al., 2008).

The results of statistical analysis revealed that hydrogen peroxide treatment, the lowest concentration of Sub MIC and MIC was (210-220) mM in the duodenum. The highest concentration was (290-300) mM with the gastric carcinoma isolate, while the gastritis isolate and Peptic ulcer isolate showed concentrations (240-250) mM and (250-260) mM consecutively. While The results showed when treated with ferrous ion alone that Sub MIC and MIC concentrations for gastritis and duodenitis isolates were (40,50) mM, While Sub MIC and MIC concentrations for Peptic ulcer isolate was (50,60) mM and (80,90) mM for Gastric carcinoma isolate.

The results showed that treatment with ferrous ions with Sub MIC of hydrogen peroxide determined through this research study of the studied isolates, resulted of Sub MIC and MIC as the following: The gastritis and duodenitis isolates (4,5) mM, while the Peptic ulcer was (6,7) mM and Gastric carcinoma isolates was (7,8) mM .

Table 3. Minimum inhibitory concentration and sub - Minimum inhibitory concentration of H_2O_2 , Fe^{+2} and H_2O_2 with Fe^{+2} for *H. pylori* isolates.

Isolation source	H_2O_2 Conc. (mM)		Fe^{2+} Conc. (mM)		Sub MIC H_2O_2 + Fe^{2+} (mM)	
	MIC	Sub-MIC	MIC	Sub- MIC	MIC	Sub-MIC
Gastritis	250	240b	50	40c	5	4c
Duodenitis	220	210c	50	40c	5	4c
Peptic ulcer	260	250b	60	50b	7	6b
Gastric carcinoma	300	290a	90	80a	8	7a

The different letters vertically mean there is a significant difference at $p \leq 0.01$.

3.4. The ability of *H. pylori* isolates to the production of urease

The results shown in Table 4 indicate that all studied isolates of *H. pylori* were urease-producing before treatment, with the gastritis isolate showing a gradual decrease in enzyme production compared to its pre-treatment state as in Figure 1. The duodenitis isolate showed moderate enzyme productivity in its pre-treatment state and after treatment with H_2O_2, but its enzyme productivity decreased when treated with Fe^{+2} and Fe^{+2} with H_2O_2. While the isolate of Peptic ulcer showed moderate production of urease enzyme before treatment and when treated with Fe^{+2} alone, became ability highly enzyme-producing when treated with H_2O_2, and It loses its ability to produce the enzyme when treated with Fe^{+2} and H_2O_2 . While observe that gastric carcinoma isolates exhibit strong enzyme productivity when pre-treatment and treated with H_2O_2, and become productivity with weakens moderate state in the remaining studied treatments.

Table 4. Ability of *H. pylori* isolates to produce urease before and after exposure to sub - Minimum inhibitory concentration of H_2O_2 , Fe^{+2} and H_2O_2 with Fe^{+2} .

Isolates of *H. pylori*	Ability of isolates to production of urease			
	(A)	(B)	(C)	(D)
Gastritis	+++	++	+	+
Duodenitis	++	++	+	+
Peptic ulcer	++	+++	++	-
Gastric carcinoma	+++	+++	++	++

(A): Results of isolates before treatment. (B): Results of isolates after treatment with Sub MIC H_2O_2 only. (C): Results of isolates after treatment by Sub MIC ferrous ion . (D): Results of isolates after treatment by Sub MIC H_2O_2 with ferrous ion.

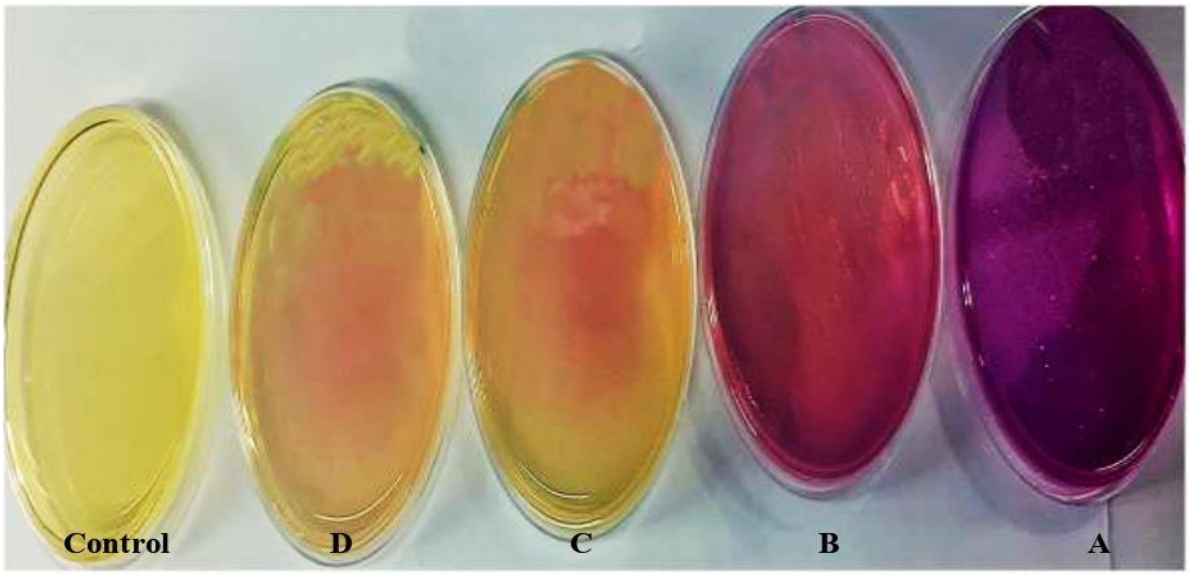

Figure 1. Urease production test to *H. pylori* isolates of Gastritis before and after exposure to sub-Minimum inhibitory concentration of H_2O_2 , Fe^{+2} and H_2O_2 with Fe^{+2}.

(A): Results of isolates before treatment. (B): Results of isolates after treatment with Sub MIC H_2O_2 only. (C): Results of isolates after treatment by Sub MIC ferrous ion . (D): Results of isolates after treatment by Sub MIC H_2O_2 with ferrous ion.

3.5. The ability of *Helicobacter pylori* isolates to the production of protease.

The results in Table 5 showed that all of the colonies of *H. pylori* isolates have the ability to form a protease when grown on milk agar before treatment. The gastritis isolate showed strong protease enzyme production capacity before treatment and became weakly when exposed to H_2O_2, but lost the ability to produce the enzyme in the remaining studied treatments as in Figure 2. Also, the duodenitis isolate showed moderate protease enzyme production before treatment and became non-protease-producing when exposure to H_2O_2 , the treatment of the isolate with ferrous ions alone showed strong protease production capacity but the isolate became weakly protease-producing upon treatment with Fe^{+2} + H_2O_2 . The peptic ulcer isolate had a moderate ability to produce the protease enzyme before treatment, but treated with hydrogen peroxide alone and the treatment H_2O_2 with ferrous iron Fe^{+2} led to loss its ability to produce protease enzyme , but isolate showed a weak ability to produce enzyme when treated with Fe^{+2} alone. In contrast, gastric cancer isolates showed strong enzyme production before treatment, but treatment with H_2O_2 showed ability moderate, while treatment with Fe^{+2} alone and treatment with ferrous ions and H_2O_2 led to weak enzyme production in the isolate.

Table 5. Ability of *H. pylori* isolates to produce protease before and after exposure to Sub - Minimum inhibitory concentration of H_2O_2 , Fe^{+2} and H_2O_2 with Fe^{+2} .

Isolates of *H. pylori*	**Ability of isolates to production of protease**			
	(A)	(B)	(C)	(D)
Gastritis	+++	+	-	-
Duodenitis	++	-	+++	+
Peptic ulcer	++	-	+	-
Gastric carcinoma	+++	++	+	+

(A): Results of isolates before treatment. (B): Results of isolates after treatment with Sub MIC H_2O_2 only. (C): Results of isolates after treatment by Sub MIC ferrous ion **.** (D): Results of isolates after treatment by Sub MIC H_2O_2 with ferrous ion.

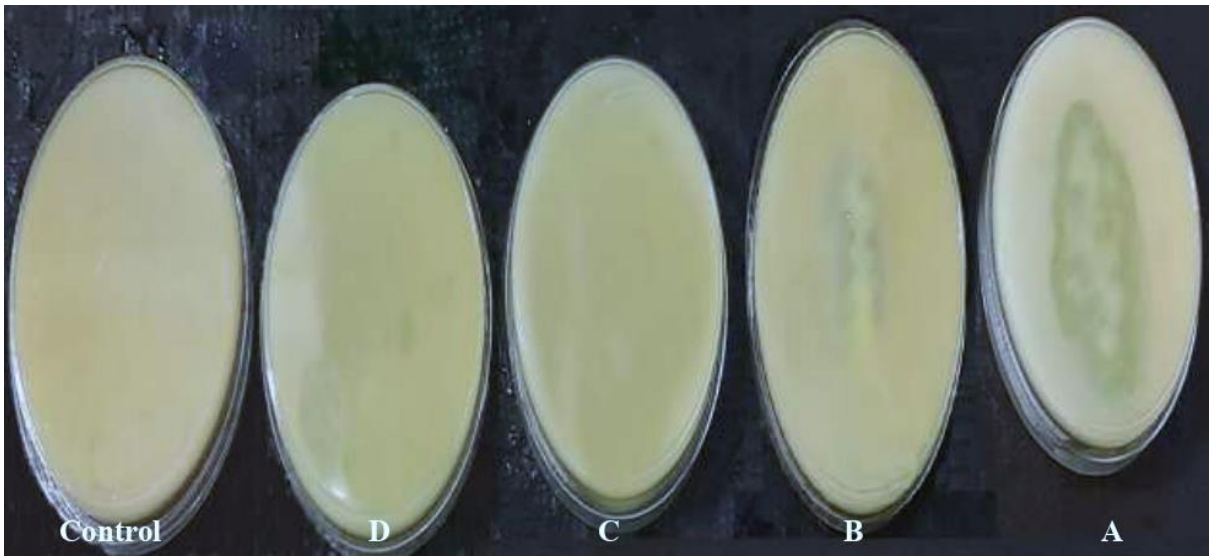

Figure 2. Protease production test to *H. pylori* isolates of Gastritis before and after exposure to sub-Minimum inhibitory concentration of H_2O_2 , Fe^{+2} and H_2O_2 with Fe^{+2}.

(A): Results of isolates before treatment. (B): Results of isolates after treatment with Sub MIC H_2O_2 only. (C): Results of isolates after treatment by Sub MIC ferrous ion . (D): Results of isolates after treatment by Sub MIC H_2O_2 with ferrous ion.

4. Discussion

The results of this study highlight the significant impact of oxidative stress on the virulence associated enzymes of *Helicobacter pylori* particularly urease and protease. Urease, which is indispensable for colonization and acid resistance, remained robust under oxidative stress conditions. This resilience is likely due to the strong genetic regulation of *ureA* and *ureB* genes, as well as the stabilization of the urease enzyme complex by accessory proteins and nickel incorporation. The urease gene cluster includes seven accessory genes that are crucial for nickel ion insertion and enzyme activation in addition to these two structural genes (Collins & D'Orazio, 1993; Shaalan et al., 2024).

Two structural subunits (UreA and UreB) and six accessory subunits (UreE, UreF, UreG, UreI,

and UreD) make up *H. pylori* urease. The UreB subunit contains two nickel ions (Mobley *et al*., 1995), therefore, the urease enzyme exhibit greater structural flexibility compared to protease enzyme (Li et al., 2025).

Hydrogen peroxide (H_2O_2) exhibited moderate inhibitory effects on the studied isolates, particularly on urease activity. The Peptic ulcer isolate showed increased urease expression after treatment, which may be attributed to increased expression of genes responsible for producing this enzyme (Reis *et al*.,2017). H_2O_2 reacting with *H. pylori* and effected on their regulatory the pathways of redox, by effecting on some of genes such as *OxyR* and *PerR* genes. The present results reffer to the evidence that H_2O_2 modify the virulence of bacterial mechanisms (Al-Assie & Nijris, 2025; Suerbaum & Michetti, 2002) .

Hydrogen peroxide affects the production of protease enzyme through its oxidative effect on the bacterial cell wall and internal enzyme systems, which leads to their oxidation (Kirthika et al., 2022). Also, exposure the isolates to ferrous ions (Fe^{+2}) only lead to high decrease in activity of protease and urease, this result may due to attributed of Fe^{+2} ability to effected in oxidation and reduction cycles, and resulting free radicals, and ROS can caused damage in proteins such as enzymes or influenced on transcription of virulence-related genes, and subsequently, modulated of gene expression (Imlay, 2013; Mahmood et al., 2026).

Additionally, these bacteria contain a protein called Fur, which is essential for controlling a complex system that regulates iron uptake and storage according to the conditions the bacteria are exposed to, and protects the bacteria from oxidative stress. When the number of free radicals increases, this protein can also stimulate the synthesis of antioxidant enzymes such as catalase. The expression of genes that control Fur is inhibited due to its binding to regulatory regions in the DNA promoter known as Fur boxes. Typically, binding is controlled by iron, so it can either activate or inhibit gene expression, which also regulates fur growth at low pH (Fekrirad et al., 2026; Gancz et al., 2006).

The react between of H_2O_2 and Fe^{+2} to generate the higher inhibitory products by Fenton reaction, such as hydroxyl radical ($OH^{\bullet}$), which caused damage foe enzymes, and may affected on their active site (Zhang et al., 2026).

In addition, the current results of urease activity in carcinoma isolates in spite of exposed to oxidative stress that may refers to adaptive responses in long-term to chronic inflammation. There are articles suggest that some of bacterial strains have elevated tolerance to high levels of ROS, that may be enhanced survival status and thereby to increasing of tissue damage and carcinogenic development (Hooi et al., 2017; Sharafutdinov et al., 2023). On the other side, the effect of exposure to ferrous ion

with hydrogen peroxide on the studied bacterial isolates was observed, some of the isolates lost their ability to produce the enzyme, or their productivity was weakened, or they became able to produce the enzyme as a result of the oxidative stress generated by the treatment. This may affect the genes responsible for its formation, or it may cause an increase in the copies of the necessary genes and their translation, and thus it may increase the ability to produce, or it may weaken its productivity, or it may lead to mutation in the genes, or the stress may affect the enzymes, and thus the isolate becomes non-producing of the enzyme (Fasnacht & Polacek, 2021; Fekrirad et al., 2026).

5. Conclusion

The conclusion of the current research that clinical isolates of *H. pylori* possess a distinctive ability to oxidative stress resistance, as well as a difference in the strength of their expression of urease and protease enzymes when treated with the studied treatments. It was observed that the greatest inhibition or cessation of enzyme production occurred with treatment with hydrogen peroxide and iron, and may be a result of the effect of free radicals that generated by the treatment on DNA or protein damage, in addition, isolates associated with gastric cancer maintained higher urease activity under oxidative stress, and may be influence on bacterial virulence factors, and subsequently, effect on disease severity.

6. References

Abd Alhasan, T. M. A., Abbas, M. K., & Mohammed, M. K. (2025). Assessment of Some Aminoglycoside Drugs Repurposing as Helicobacter pylori Urease Enzyme Inhibitors by In silico and In vitro. *Medical Journal of Babylon*, *22*(3), 776–786. https://doi.org/10.4103/MJBL.MJBL_1067_23

Al-Assie, R. J. J., & Nijris, O. N. (2025). Effects of Hydrogen Peroxide on Biofilm Formation and Antibiotic Susceptibility of Clinical Bacterial Isolates. *Journal of Physiology and Biology Sciences*, *1*(2), 72–77. https://doi.org/10.69792/jpbs.2025.vol.2.3

Ali, A., & AlHussaini, K. I. (2024). Helicobacter pylori: A Contemporary Perspective on Pathogenesis, Diagnosis and Treatment Strategies. *Microorganisms*, *12*(1), 222. https://doi.org/10.3390/microorganisms12010222

Collee, J. G., Fraser, G. A., Marmion, P. B., & Simmons, A. (1996). Practical Medical Microbiology. 4 th. *Churchill Livingstone, New York*, 413–418.

Collins, C. M., & D'Orazio, S. E. F. (1993). Bacterial ureases: structure, regulation of expression and role in pathogenesis. *Molecular Microbiology*, *9*(5), 907–913. https://doi.org/10.1111/j.1365-2958.1993.tb01220.x

Fasnacht, M., & Polacek, N. (2021). Oxidative Stress in Bacteria and the Central Dogma of Molecular Biology. *Frontiers in Molecular Biosciences*, *8*. https://doi.org/10.3389/fmolb.2021.671037

Fekrirad, Z., Darbouy, M., & Molabashi, Z. A. (2026). Oxidative stress and the response of bacteria to it. In *Microbial Stress Survival* (pp. 217–238). Elsevier. https://doi.org/10.1016/B978-0-443-27622-4.00024-9

Gancz, H., Censini, S., & Merrell, D. S. (2006). Iron and pH Homeostasis Intersect at the Level of Fur Regulation in the Gastric Pathogen Helicobacter pylori. *Infection and Immunity*, *74*(1), 602–614. https://doi.org/10.1128/IAI.74.1.602-614.2006

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's Manual of determinate bacteriology*.

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., Malfertheiner, P., Graham, D. Y., Wong, V. W. S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., Kaplan, G. G., & Ng, S. C. (2017). Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology*, *153*(2), 420–429. https://doi.org/10.1053/j.gastro.2017.04.022

I.A. Abd-alwahab, W., J. J. Al-Assie, R., Kamil Azeez, A., & Kamil Ghadir, G. (2024). The effect of carotenoids of Rhodotorula glutinis and probiotic of Lactobacillus acidophilus on some physiological and histological variables of the pancreas and liver in male rats exposed to ultraviolet radiation. *Tikrit Journal for Agricultural Sciences*, *24*(2), 235–245. https://doi.org/10.25130/tjas.24.2.17

Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nature Reviews Microbiology*, *11*(7), 443–454. https://doi.org/10.1038/nrmicro3032

Kashmiri, Z. N., & Mankar, S. A. (2014). Free radicals and oxidative stress in bacteria. *Int. J. Curr. Microbiol. Appl. Sci*, *3*(9), 34–40.

Kirthika, P., Jawalagatti, V., Senevirathne, A., & Lee, J. H. (2022). Coordinated interaction between Lon protease and catalase-peroxidase regulates virulence and oxidative stress management during Salmonellosis. *Gut Microbes*, *14*(1). https://doi.org/10.1080/19490976.2022.2064705

Li, Y., Guo, S., Zou, H., & Chen, Y. (2025). Structure difference of Jack bean urease and Helicobacter pylori urease on binding interactions with quercetin. *International Journal of Biological Macromolecules*, *307*, 141705. https://doi.org/10.1016/j.ijbiomac.2025.141705

Mahmood, I., Farhan, F., & Ajil, A. (2026). Comparative analysis of antioxidant enzyme activities under hydrogen peroxide induced oxidative stress in clinical isolates of pathogenic bacteria. *American Journal of Biology and Natural Sciences*, *3*(2), 98–108.

Manful, C. F., Fordjour, E., Subramaniam, D., Sey, A. A., Abbey, Lord, & Thomas, R. (2025). Antioxidants and Reactive Oxygen Species: Shaping Human Health and Disease Outcomes. *International Journal of Molecular Sciences*, *26*(15), 7520. https://doi.org/10.3390/ijms26157520

Nilius, M., & Malfertheiner, P. (1996). Helicobacter pylori enzymes. *Alimentary Pharmacology & Therapeutics*, *10*(Sup1), 65–71. https://doi.org/10.1046/j.1365-2036.1996.22164007.x

Pitt, S. J. (2018). *Clinical microbiology for diagnostic laboratory scientists*. John Wiley & Sons.

Pooja, G., Shweta, S., & Patel, P. (2025). Oxidative stress and free radicals in disease pathogenesis: a review. *Discover Medicine*, *2*(1), 104. https://doi.org/10.1007/s44337-025-00303-y

Salvatori, S., Marafini, I., De Vico, P., Fonsi, A., & Monteleone, G. (2026). Molecular Insights into Helicobacter pylori-Induced Gastritis and Gastric Cancer. *Cancers*, *18*(2), 331. https://doi.org/10.3390/cancers18020331

Seder, N., Rayyan, W. A., Al-Fawares, M. H. O., & Bakar, A. (2023). Pseudomonas aeruginosa virulence factors and antivirulence mechanisms to combat drug resistance; A systematic review. *Mortality*, *10*(11).

Seixas, A. F., Quendera, A. P., Sousa, J. P., Silva, A. F. Q., Arraiano, C. M., & Andrade, J. M. (2022). Bacterial Response to Oxidative Stress and RNA Oxidation. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.821535

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*. https://doi.org/10.3389/fmicb.2024.1464484

Sharafutdinov, I., Tegtmeyer, N., Linz, B., Rohde, M., Vieth, M., Tay, A. C.-Y., Lamichhane, B., Tuan, V. P., Fauzia, K. A., Sticht, H., Yamaoka, Y., Marshall, B. J., & Backert, S. (2023). A single-nucleotide polymorphism in Helicobacter pylori promotes gastric cancer development. *Cell Host & Microbe*, *31*(8), 1345-1358.e6. https://doi.org/10.1016/j.chom.2023.06.016

Suerbaum, S., & Michetti, P. (2002). Helicobacter pylori Infection. *New England Journal of Medicine*, *347*(15), 1175–1186. https://doi.org/10.1056/NEJMra020542

Vinckx, T., Matthijs, S., & Cornelis, P. (2008). Loss of the oxidative stress regulator OxyR in *Pseudomonas aeruginosa* PAO1 impairs growth under iron-limited conditions. *FEMS Microbiology Letters*, *288*(2), 258–265. https://doi.org/10.1111/j.1574-6968.2008.01360.x

Zhang, X., He, L., Liu, Z., Miao, R., Wang, Y., Zhou, H., & Wang, T. (2026). Selective H2O2 generation via 2e− oxygen reduction reaction over Cu Fe bimetallic oxides for electrocatalysis degradation sulfamethoxazole. *Separation and Purification Technology*, *395*, 137641. https://doi.org/10.1016/j.seppur.2026.137641