

# The overview of Clarithromycin treatment for *Helicobacter pylori* infection

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**Abstract.** *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacillus that causes infections worldwide. *Helicobacter pylori*'s drug resistance and infectivity are vital factors causing *Helicobacter pylori* infection. The virulence factors of *Helicobacter pylori* include acid escape virulence factors and epithelial cell colonizing factors, which can directly or indirectly cause chronic gastritis or gastric ulcer. As the resistance of *Helicobacter pylori* increases, a combination of multiple antibiotics is now the recommended therapy, and clarithromycin is one of them. The production process of clarithromycin and its effectiveness are essential points of discussion.

**Keywords:** Bacterial infection, *Helicobacter pylori*, Virulence factors, Clarithromycin

## 1. Introduction

*Helicobacter pylori* is a bacterial infection with a high prevalence that is extensively dispersed worldwide, impacting around 50% of the global population. Even though around 90% of individuals infected with *H. pylori* do not exhibit symptoms, this infection poses significant health concerns and has become progressively more challenging to manage due to the rise in antibiotic resistance in recent times [1]. Research has indicated that the occurrence of *H. pylori*-positive status exhibits variability based on various parameters, including age, geographical location, living conditions, and socioeconomic position. The primary mode of *H. pylori* transmission appears to be through oral-oral transfer [2]. Although *H. pylori* infection is highly prevalent, only a certain group of patients infected with this bacterium suffer severe gastroduodenal pathology. The aetiology of *H. pylori* infection and its subsequent illness manifestation are thought to be regulated by a multifaceted interplay of several variables., including the host, environment, and virulence factors of the bacteria. *H. pylori* has successfully acclimated to the challenging conditions of the human stomach by acquiring a repertoire of virulence genes. These genes facilitate the bacterium's ability to withstand the environment characterized by acidity, navigate to the gastric epithelium, and establish adhesion to gastric epithelial cells. The presence of these virulence factors facilitates the effective establishment of colonization inside the gastric mucosa and maintains a continuous infection of *H. pylori*. This, in turn, induces chronic inflammation and results in tissue damage, potentially culminating in the formation of peptic ulcers or even gastric cancer [3]. The most effective treatment options now available for *H. pylori* infection involve the use of a proton pump inhibitor (PPI) and ranitidine bismuth citrate in combination with two other antibiotics, known as triple

treatments. Alternatively, a quadruple therapy approach can be employed, which includes the use of bismuth, tetracycline, metronidazole, and PPI. Clarithromycin is a highly effective antibacterial agent in combating *Helicobacter pylori* infections. The compound in question is a macrolide that exhibits acid stability and possesses a wide range of antibacterial properties. It is efficiently absorbed by the body and has a broad distribution throughout various tissue. Additionally, it is associated with few adverse effects. The minimum inhibitory concentration (MIC50) of Clarithromycin against *H. pylori* is rather low, the efficacy of the treatment is augmented through the suppression of acid. When the PPI or ranitidine bismuth citrate is coupled with amoxicillin or metronidazole, it is possible to achieve eradication rates above 95% for susceptible organisms [4, 5].

## 2. Pathogen virulence factors

*H. pylori* are successfully acclimated to the hostile conditions in the human stomach by acquiring a repertoire of virulent genes. These genes facilitate the bacterium's ability to endure the acidic environment, navigate towards the gastric epithelium, and establish adhesion to gastric epithelial cells. The presence of these virulence factors facilitates the effective establishment of colonization inside the stomach mucosa and supports the maintenance of a prolonged *H. pylori* infection. This, in turn, induces chronic inflammation and results in tissue damage, potentially culminating in the formation of peptic ulcers or even gastric cancer. A multitude of research has been conducted to examine the frequency and significance of purported *Helicobacter pylori* virulence genes in the development of disease. Although numerous virulence factors with diverse activities have been found, the connections with diseases seem to be less apparent, particularly when considering different study groups [3].

Acid escape virulence factors:

Urease:

1. The synthesis of ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) has the ability to neutralize stomach acidity.
2. The process of angiogenesis has been identified as a contributing factor in the progression of stomach cancer.
3. The progression of malignancies is enhanced through the PI3K-AKT-mTOR pathway activation.

Bacterial shape:

The helical structure of bacteria has the potential to facilitate their entry into the mucous layer, so providing a protective advantage to the bacteria.

Flagella:

1. Motility facilitates bacterial locomotion away from acidic environments.
2. Flagellin activation exhibits increased motility, hence providing protection for the bacteria.

Epithelial cells colonizing factors:

BabA:

It is capable of binding to the epithelial cell receptor, Leb, hence facilitating attachment of bacterial and colonization. Additionally, it has been observed to enhance the translocation of CagA and produce double strand breaks in host cells.

SabA:

It is a bacterial adhesin that specifically interacts with the sialyl-Lex antigen, facilitating the attachment and colonisation of bacteria.

The OipA:

It facilitates the attachment of bacteria to the gastric epithelium, resulting in potential harm to the mucosal layer. This process triggers the release of interleukin (IL)-8 and leads to the death of host cells.

The HopQ:

It facilitates the attachment of bacteria to the stomach epithelium, hence impeding the functioning of immune cells.

Epithelial cells pathogenicity factors:

CagPAI:

The genetic material under consideration encompasses a type IV secretion system (T4SS) which serves the purpose of aiding the transportation of CagA and peptidoglycan. CagT:

It functions as a critical component within the T4S system, facilitating the translocation process of CagA.

CagY:

It is capable of binding with integrin, thereby exerting influence over the immune response, facilitating bacterial persistence, and inducing modifications in the functions of the Type IV secretion system, also called T4SS.

Cag $\zeta$ :

It is involved in the functioning of the Type IV Secretion System (T4SS) and has a role in facilitating the transport of CagA.

CagL:

It functions as a central protein within the Type IV secretion system (T4SS) and interacts with integrin, facilitating the CagA translocation and triggering the production of interleukin-8 (IL-8).

CagA:

It induces cellular proliferation by the process of tyrosine phosphorylation. This phosphorylation event also leads to the upregulation of IL-8 production and cell elongation. Additionally, CagA is involved in the downregulation of heat shock protein VacA:

1. It induces vacuolization in epithelial cells, leading to cellular necrosis or apoptosis.
2. The autophagy activation and subsequent rise in cellular death is augmented by endoplasmic reticulum stress.

HtrA:

It functions as a protease, responsible for the degradation of misfolded proteins. Additionally, the facilitation of CagA dissemination is of utmost importance. Moreover, it cleaves tight junction proteins, namely occludin, claudin-8, and E-cadherin.

Outer membrane vesicles:

The internalization of outer membrane vesicles, whether clathrin-dependent or independent, serves as a protective mechanism for pathogens against the detrimental impact of reactive oxygen species. Additionally, this process hinders normal cellular activities while also stimulating dendritic cell functions.

$\gamma$ -glutamyl transpeptidase:

1. It is involved in transpeptidation and amino acid production, which have been found to promote cell death and suppress cellular growth and cell cycle arrest.
2. The vacuolation of epithelial cells, which leads to the death of these cells.

2. Clarithromycin

2.1. History

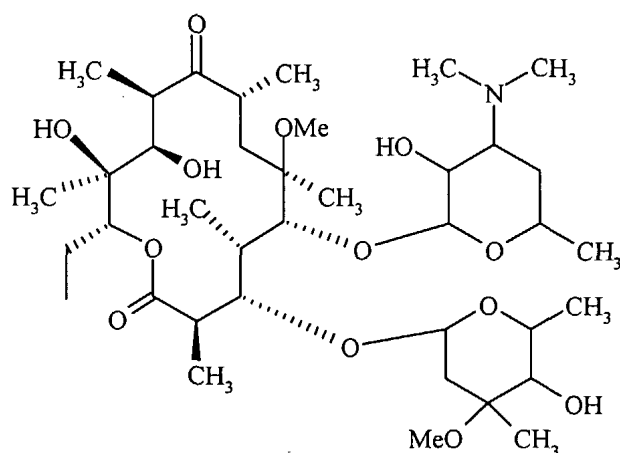
The discovery of Clarithromycin can be attributed to the efforts of researchers employed at Taisho Pharmaceutical, a prominent Japanese pharmaceutical business, in the year 1980. The development of the product was driven by the objective of creating a variant of the antibiotic erythromycin that would not undergo acid instability within the gastrointestinal tract, hence mitigating adverse effects such as nausea and stomachache. The pharmaceutical company Taisho initiated the process of seeking patent protection for their medicine in approximately 1980. Following this, they brought a proprietary variant of the drug, named Clarith, to the Japanese market in 1991. In the year 1985, Taisho entered a partnership with the American corporation Abbott Laboratories to acquire the international rights for their product. Additionally, Abbott Laboratories successfully obtained permission from the *Food and Drug Administration* for Biaxin in October 1991. The pharmaceutical product transitioned to a generic form in Europe in the year 2004, followed by its generic availability in the United States during the middle of 2005 [6].

### 3. Process of traditional synthesis

Comprehensive Elucidation of the Innovation

A comprehensive investigation is currently being conducted to explore the large-scale manufacture and high purity of 6-O-Methyl-erythromycin, also called Clarithromycin, a highly effective inhibitor for gram-positive bacteria. Therefore, there was a regulated methylation of the 6-hydroxyl group of erythromycins, which had been protected at the 2' and 4'' positions. The current invention pertains to the manufacturing process of achieving an enhanced degree of purity for Clarithromycin (Formula I) [6].

Formula (I)

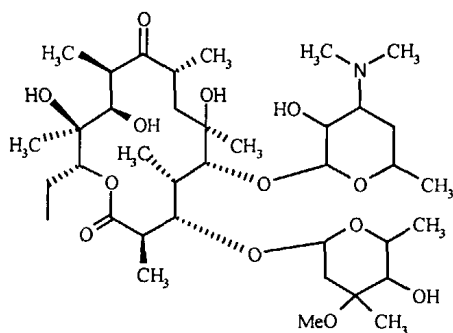


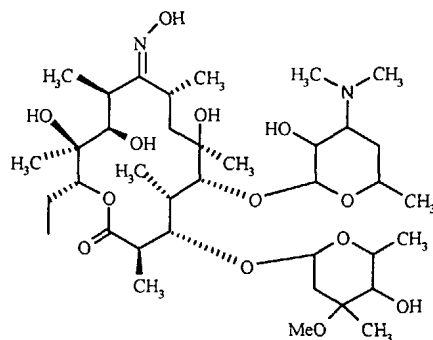
The initial iteration of Clarithromycin produced from this methodology exhibits a concentration of 6,11-O-dimethyl erythromycin-A, a possible byproduct, which is below 1.0%. The current innovation focuses on the industrial process of Clarithromycin, specifically aiming to achieve a controlled amount of side products. The finished product has been subjected to HPLC examination, which has led to the characterization of a total of eight contaminants. In accordance with a specific embodiment of the current invention, the synthesis of Clarithromycin Form II is achieved by the implementation of the subsequent reaction scheme:

(a) The reaction between Erythromycin-A and hydroxylamine hydrochloride, resulting in the formation of Erythromycin-A-9-Oxime.

The hydroxylamine hydrochloride compound is subjected to a reaction with caustic flakes in an aqueous solution of isopropyl alcohol within the range of 10°C to 20°C. This reaction results in the formation of the hydroxylamine base, which is dissolved in the solution. Subsequently, Erythromycin A is added to the solution [6].

The formula of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  isopropyl Alcohol

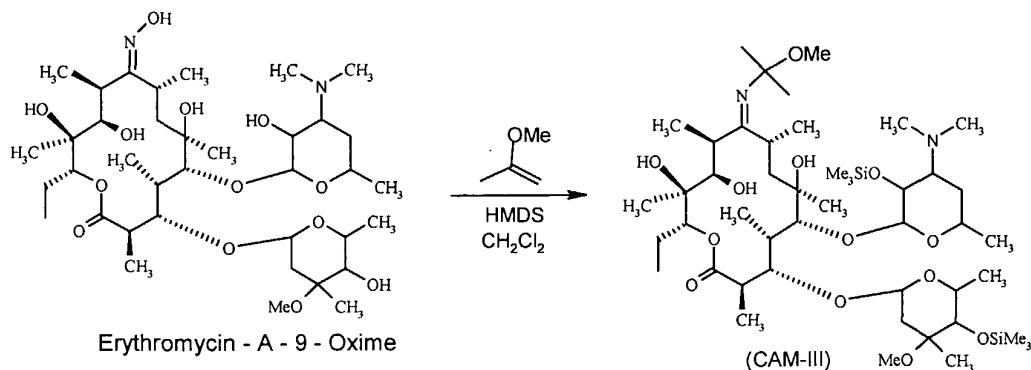




#### The formula of Erythromycin - A Erythromycin - A - 9 - Oxime

The pH of the reaction mixture is carefully regulated to a range from 6.5 to 7.0 with a gradual and precise supplement of glacial acetic acid. Erythromycin-A is introduced into the agitated reaction mixture, which is thereafter stirred for an additional duration of 28 hours at a temperature of 55°C. Following the conclusion of the reaction, the mixture is subjected to neutralization by the addition of aqueous ammonia and water. The resulting mixture is then subjected to continuous stirring for a duration of one hour. To achieve a precipitate of Erythromycin-A-9-Oxime with a yield ranging from 85% to 90%, an increased quantity of water is introduced into the reaction mixture [6].

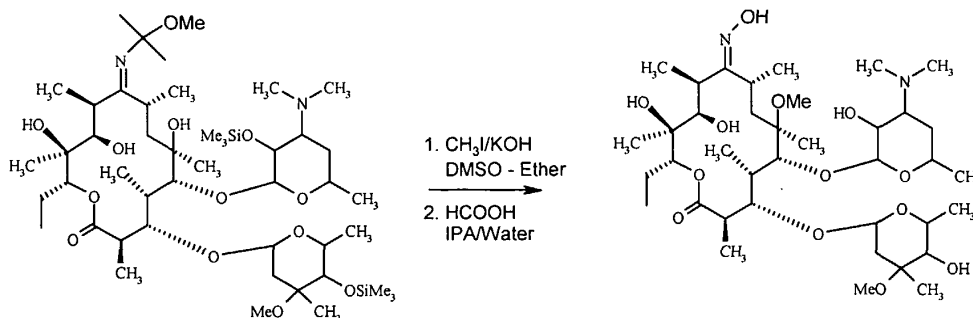
(b) The objective of this study is to investigate the oxime protection, T, with the 4''-OH functional groups of Erythromycin-A-9-Oxime. The compound known as Erythromycin-A-9-Oxime, which was acquired through the previously mentioned process, is subjected to a reaction involving 2-methoxy propene and pyridine hydrochloride from dichloromethane. The mixture is subjected to stirring for a duration of 6 hours within a temperature range of 8°C to 120°C, after which hexamethyldisilazane (HMDS) is introduced [6].



The stirring process is maintained for approximately 15 hours at a temperature consistent with the surrounding environment. Following the comprehensive protection of the oxime and 2' & 4'' -OH functionalities of Erythromycin - A-9-oxime, the fully protected compound is entirely protected through conventional work-up and filtration methods. CAM-III is obtained through the process of vacuum drying, resulting in a yield of above 90%. The melting point of the substance under consideration ranges from 125 degrees Celsius to 1270 degrees Celsius [6].

(c) The 6-OH group of CAM-III is subjected to methylation, followed by deprotection of the oxime and the 2' and 4'-OH functions.

To achieve this, 2',4''-O-Bis(trimethylsilyl)-erythromycin-A-9- [O- (1-methoxymethyl ethyl) oxime] is reacted with methyl iodide in the presence of potassium hydroxide powder in a suitable solvent system. This reaction results in the formation of 2',4''-disilylated-Clarithromycin-9-methoxypropyl oxime (CAM-IV). Upon further hydrolysis, CAM-IV yields Clarithromycin-9-Oxime, as illustrated below:



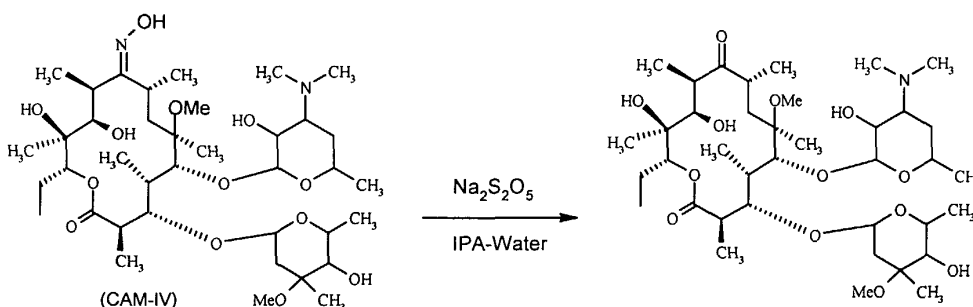
The term "computer-aided manufacturing" (CAM) pertains to the utilization of computer technology for manufacturing purposes. The chemical being examined is clarithromycin-9-oxime. One of the suitable solvent systems for this reaction includes a combination of DMSO and diethyl ether, among other potential options. The ideal proportion of diethyl ether in DMSO falls within the range of around 40% to 60%, with a preferred value of around 50%. The reaction is typically performed within the temperature range spanning between 10°C and 60°C, with a preferred range of approximately 15°C to 25°C. The duration of reactions often falls within a range of 40 minutes to 2 hours, with a favored interval of 80 to 100 minutes [6].

The precise ratio of methyl iodide to potassium hydroxide powder is of utmost importance, as any variation from the appropriate proportion can lead to the generation of unwanted byproducts. Based on thorough examination, it has been shown that the combination of methyl iodide (1.75 moles) and potassium hydroxide (1.20 moles) results in the formation of a resultant substance containing regulated impurities. This substance can be subjected to subsequent purification processes, such as one or two recrystallizations. An advantage of employing the methylation approach in the current study using DMSO-Diethyl ether is the capacity to isolate the ether layer subsequent to halting the reaction mixture with a 40% dimethylamine solution. The ether layer is comprised solely of the methylated product, while the DMSO layer contains impurities. The DMSO layer is subsequently employed for the retrieval of DMSO, which functions as the solvent. In the second stage of the synthesis, the methylated product, referred to as CAM-IV, undergoes a reaction with 98% formic acid in a volume ratio of 1:1 between isopropyl alcohol and water. The aforementioned reaction is conducted over a period of 30 minutes, within the temperature range observed is between 25°C and 35°C. The intended result of this chemical reaction is the synthesis of Clarithromycin-9-Oxime [6].

(d) The process of converting to Clarithromycin from Clarithromycin-9-Oxime is examined in this study.

The production process of Clarithromycin involves the conversion of Clarithromycin-9-Oxime through a reaction with sodium metabisulphite in a solution consisting of Isopropyl alcohol and water in equal proportions (1:1, v/v). This reaction is carried out at the temperature of 80°C for a period of 6 to 8 hours [6].

Formula (I)



Clarithromycin-9-Oxime Clarithromycin  
 2.3 Mechanism of actions

Macrolides exert their mechanism of action by the binding process to ribosomes, specifically targeting the peptidyl transferase loop located in domain V of the 23S ribosomal RNA. This interaction effectively hinders the translocation of aminoacyl transfer-RNA, consequently impeding the subsequent process of protein synthesis. Clarithromycin exhibits a wide spectrum of antimicrobial action, comparable to that of erythromycin. It effectively inhibits a diverse array of microorganisms encompassing both Gram-positive and Gram-negative bacteria, atypical pathogens, anaerobic organisms. Nevertheless, the development of resistance to erythromycin typically indicates a concurrent resistance to clarithromycin. It is worth mentioning that clarithromycin exhibits activity, ranging from active to moderately active, against *Campylobacter* species. However, it demonstrates greater activity against *H. pylori* compared to erythromycin, azithromycin, and roxithromycin [6].

#### 2.4 Side effect and precautions

The most documented side effects of clarithromycin included nausea with 3.8%, diarrhea with 3.0%, stomach discomfort with 1.9%, and headache with 1.7% [6]. Taste disturbance is a frequently observed phenomenon that has a correlation with dosage. For instance, when administered concurrently with omeprazole, about two-thirds of patients reported the occurrence of a metallic taste sensation, which subsequently vanished entirely within a few days following the completion of the treatment [7]. Occasional adverse effects such as cholestasis, jaundice, hepatitis, and Steven Johnson syndrome have also been documented.

#### 4. Overview and conclusion

Clarithromycin, a macrolide antibiotic that is developed from erythromycin, serves as the foundation on the treatment of *H. pylori* infection due to its favorable attributes, including a low minimum inhibitory concentration (MIC), effective absorption into the mucosal lining, and minimal susceptibility to the acidic environment. The variability about the prevalence of clarithromycin resistance in *H. pylori*, which is the major determinant of the effectiveness of empirically prescribed conventional *H. pylori* eradication therapy, exhibits significant regional and international differences. The prevalence is contingent upon the selection pressure exerted by the specific group of antibiotics within a particular geographical region [8].

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