Pathogenesis and clinical management of *Helicobacter pylori* gastric infection

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Abstract

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that infects approximately 4.4 billion individuals worldwide. However, its prevalence varies among different geographic areas, and is influenced by several factors. The infection can be acquired by means of oral-oral or fecal-oral transmission, and the pathogen possesses various mechanisms that improve its capacity of mobility, adherence and manipulation of the gastric microenvironment, making possible the colonization of an organ with a highly acidic lumen. In addition, *H. pylori* presents a large variety of virulence factors that improve its pathogenicity, of which we highlight cytotoxin associated antigen A, vacuolating cytotoxin, duodenal ulcer promoting gene A protein, outer inflammatory protein and gamma-glutamyl transpeptidase. The host immune system, mainly by means of a Th1-polarized response, also plays a crucial role in the infection course. Although most *H. pylori*-positive individuals remain asymptomatic, the infection predisposes the development of various clinical conditions as peptic ulcers, gastric adenocarcinomas and mucosa-associated lymphoid tissue lymphomas. Invasive and non-invasive diagnostic methods, each of them with their related advantages and limitations, have been applied in *H. pylori* detection. Moreover, bacterial resistance to antimicrobial therapy is a major challenge in the treatment of this infection, and new therapy alternatives are being tested to improve *H. pylori* eradication. Last but not least, the development of effective vaccines against *H. pylori* infection have been the aim of several research studies.

Key words: *Helicobacter pylori*; Virulence factors; Immune response; Antibiotics; Vaccines

Core tip: *Helicobacter pylori* (*H. pylori*) is a bacterium that infects more than half of the world's population. The mechanisms of such infections are complex and deeply studied. In addition, the clinical outcomes are variable and depend on both pathogen and host characteristics. Moreover, the adequate clinical management by means of proper diagnosis and effective treatment is crucial for reaching success in bacterial eradication. This article aims to provide a broad overview of *H. pylori* infection, from pathogenesis to clinical management.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that inhabits the gastric environment of more than half of the world population[1]. Studies have demonstrated that the prevalence of *H. pylori*-positive status varies according to different factors such as age, geographical area, living condition and socioeconomic status[2]. Oral-oral transmission seems to be the main route of *H. pylori* transmission. This explains the common occurrence of the infection among members of the same family, such as parents and children. In this way, the sharing of utensils during feeding seems to be important for infection establishment[3]. Fecal-oral transmission is another form of infection that occurs through ingestion of contaminated water mainly due to unsatisfactory basic sanitation conditions[4]. Therefore, it is important to highlight that increasing socioeconomic status and the improvement of living conditions are factors that greatly influence the reduction in *H. pylori* infection prevalence[5].

Until Warren and Marshall's discovery of *H. pylori* infection in gastric mucosa, it was believed that the gastric environment was sterile because of its high acidity[6,7]. Aiming for successful colonization under such hostile conditions, the bacterium uses a wide range of mechanisms that provide improved mobility, robust adherence to epithelial cells and an enzymatic apparatus that allows the establishment of an appropriate microenvironment for infection perpetuation[8-10]. In addition, the potential of pathogenicity of this infection is provided by certain virulence factors such as cytotoxin associated antigen A (CagA), vacuolating cytotoxin (VacA), duodenal ulcer promoting gene A protein (DupA), outer inflammatory protein (OipA) and gamma-glutamyl transpeptidase (GGT)[11-15]. Moreover, the host immune system plays a crucial role in the course of the infection, likely by means of a Th1-polarized response against the pathogen (Figure 1)[16].

Although most *H. pylori*-positive individuals are asymptomatic, such infections predispose the development of diseases like peptic ulcers and gastric adenocarcinomas[17]. In this way, proper clinical management with a well-made diagnosis followed by effective treatment are important steps in the improvement of a patient's clinical outcome[18]. A variety of invasive and non-invasive diagnostic methods have been used for *H. pylori* detection and, regarding treatment, bacterial resistance represents a major challenge in infection eradication[19,20]. In this sense, new therapy regimens as well as probiotic implementation have been tried in order to improve treatment results[21,22]. Moreover, the efforts of several researchers have been directed towards the development of vaccines against *H. pylori* infection.

PATHOGENESIS

Colonization

H. pylori successful colonization of the hostile gastric environment requires special mechanisms. Firstly, after reaching the gastric environment, *H. pylori* uses its crucial flagellar motility for swimming in gastric content, what allows the bacterium to get in the gastric mucus layer[8]. Four to eight sheathed flagella compose the flagellar group
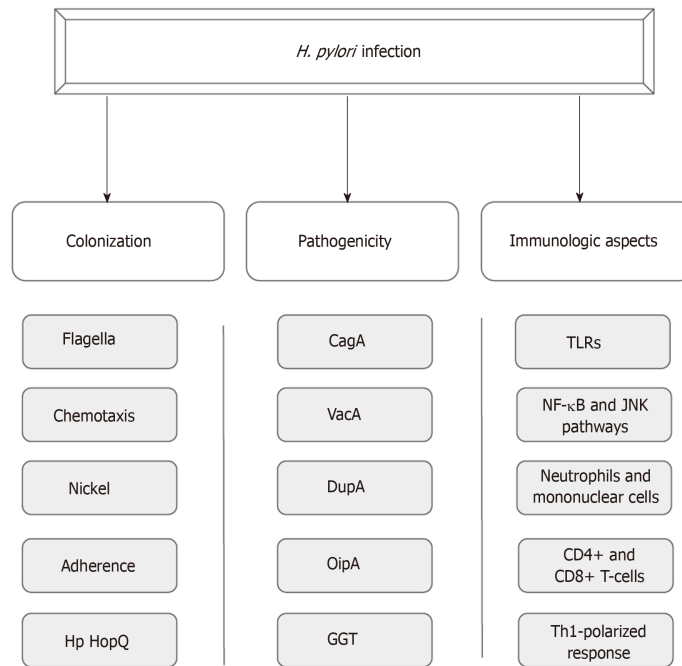
Figure 1 Aspects of *Helicobacter pylor* infection. CagA: Cytotoxin associated antigen A; VacA: Vacuolating cytotoxin; DupA: Duodenal ulcer promoting gene A protein; OipA: Outer inflammatory protein; GGT: Gamma-glutamyl transpeptidase; TLRs: Toll-like receptors.

situated on a single or on both poles of the bacterium[23-25]. *H. pylori* flagella can also provide different movements according to the media in which the bacterium is located. In liquid media, it presents a "swimming motility", whereas in soft agar and on the surface of solid media, "spreading" and "swarming" movements can be observed, respectively[25]. Various studies have shown that several mutations in genes that encode specific flagellar proteins such as fliD, FlaA and FlaB impair the proper motility of *H. pylori*, which can reduce or even cease its capacity to colonize the gastric mucosal layer[26-28].

Besides flagella, *H. pylori* mobility also depends on chemotaxic action in response to different molecules, such as mucin, sodium bicarbonate, urea, sodium chloride and some specific amino acids[29,30]. At least ten *H. pylori* genes are related to reception, signal transduction, and processing of chemotactic stimuli[31]. Different *H. pylori* chemoreceptors have been described: T1pA, B, C, and D, CheA kinase and various coupling proteins. These proteins are all crucial for bacterium colonization, as demonstrated by various studies over recent years[32].

In addition, some transition metals are essential for living organisms, as they serve as cofactors for enzymatic reactions and some physiological processes, especially for enzymes that carry out the genetic material replication and transcription, attenuation of oxidative stress, and cellular energy production. In bacteria, these metals are crucial for survival and successful infection[33]. Nickel is an indispensable metal for *H. pylori*, since it is the cofactor for two important enzymes: urease and hydrogenase. These enzymes have a strong role in the infection process[10]. The activity of *H. pylori* urease contributes to the colonization of the microorganism, once this enzyme catalyzes the hydrolysis of urea to carbon dioxide and ammonia, which are buffer substances that attenuate the acidity of the stomach environment[34]. In turn, hydrogenase is part of a signaling cascade that induces an alternative airway, allowing *H. pylori* to use molecular hydrogen as a source of energy for its metabolism[35].

Adhesion molecules (Table 1) and surface receptors of gastric cells are also important in the interaction between bacteria and host[9,36]. One of the most well-characterized molecules is the blood group antigen binding adhesin A (BabA), which carries out specific binding to Lewis H-1 antigens[37,38]. Bacteria with high BabA expression are more virulent, and cause duodenal ulcer and gastric adenocarcinoma pathogenesis[39]. Recently, another bacterial-host interaction was identified through the adhesion of the outer membrane Hp HopQ. These adhesins bind to the CEACAMs (cell adhesion molecules related to the carcinoembryonic antigen) 1, 3, 5 and 6. That binding gives rise to cell signaling mediated by the HopQ-CEACAM interaction, which allows the translocation of CagA, the main virulence factor of *H. pylori*, thus increasing proinflammatory mediators in the host cell[40-42].

Table 1 *H. pylori* adhesion molecules

Adhesin	Functions	Ref.
BabA	Specific binding to the b and H-1 Lewis antigens from the surface of the gastric epithelial cells	[105]
SabA	Binding to Le^x, which is upregulated in gastric epithelial cells by *H. pylori* after initial colonization mediated by BabA. Also allows the adherence of the bacterium to laminin, an extracellular matrix protein	[106,107]
AlpA and AlpB	Mediation of adherence to gastric mucosal cells and promotion of inflammatory intracellular signaling cascades (might induce IL-8 and IL-6)	[108]
OipA	Adhesion to the gastric mucosa cells and promotion of proinflammatory environment (associated with IL-8 increase, mucosal damage and duodenal ulcer)	[109]
HopQ	Interaction with CEACAM family proteins of gastric mucosal cells, allowing CagA translocation. Might inhibits the activity of natural killer cells and T cells	[110]
HopZ	Interaction with undetermined receptors, promoting adhesion to gastric cells	[111]

H. pylori: *Helicobacter pylori*; CagA: Cytotoxin associated antigen A; IL: Interleukin; BabA: Binding adhesin A; OipA: Outer inflammatory protein.

CagA

CagA is a bacterial protein that induces specific modifications in the morphology of epithelial cells while altering cell polarity, leading to a "hummingbird" phenotype. Changes in cytoskeleton associated with the development of gastric adenocarcinoma can also be triggered by this virulence factor[43]. The *Cag*A gene is contained in a *cag* pathogenicity island, a region that also possesses the coding sequence of a type IV secretion system (T4SS)[11]. This bacterial structure is responsible for performing the translocation of CagA, as well as peptidoglycans, into host cells[44]. Within the host cell, CagA undergoes tyrosine phosphorylation at a Glu-Pro-Ile-Tyr-Ala (EPIYA) motif, a variable C-terminal CagA region that can be composed by different EPIYA segments (EPIYA-A, EPIYA-B, EPIYA-C and EPIYA-D)[45]. EPIYA-A and EPIYA-B segments have been found in most *cag*A-positive *H. pylori* strains, while EPIYA-C and EPIYA-D segments are related to Western and Eastern strains, respectively[46]. *H. pylori* strains containing EPIYA-D or at least two EPIYA-C segments in its *cag*A gene are associated with a higher risk of cancer development[47]. In addition, Queiroz *et al*[48] demonstrated in a Brazilian population that first-degree relatives of patients with gastric cancer tend to be infected by *H. pylori* strains containing two or more EPIYA-C segments. After phosphorylation, CagA activates SHP-2 (SH2-containing protein-tyrosine phosphatase), which promotes the cell changes mentioned above[49].

Non-CagA virulence factors

Other various virulence factors have been related to an increased *H. pylori* capacity to impair gastric homeostasis. Among them, VacA is a determinant protein for *H. pylori* pathogenicity, and its gene is present in almost all bacterial strains. VacA promotes the formation of acidic vacuoles in the cytoplasm of gastric epithelial cells. Consequently, the integrity of mitochondria, cytoplasmic membrane, and endomembranous structures is destabilized, leading cells to collapse[50]. Moreover, this protein might also promote the activation and suppression of the immune response, inducing immune tolerance and persistent *H. pylori* infection through its activities on T-cells and antigen-presenting cells[51]. The set of changes performed by this virulence factor adds to enhanced gastritis, as well as to ulcer and cancer development[12].

Another bacterial protein, DupA, seems to provide a higher acid resistance to the bacterium, and also might promote an increase in the production of IL-8 in the antral gastric mucosa. Enhanced IL-8 levels lead to mucosal inflammation and polymorphonuclear leukocyte infiltration, which contributes to the emergence of gastritis and duodenal ulcers[13]. Interestingly, the relation between *dup*A-positive *H. pylori* strains and duodenal ulcers has been observed in Asian countries, but not in the Western population[52]. Furthermore, our group demonstrated that the presence of functional *dup*A in *H. pylori* strains has been considered as a protective factor for gastric carcinoma development[53]. The gene products of *dup*A are homologues of the

VirB4 ATPase, which is related to the mounting of the secretion apparatus, however, the probable association of *dup*A with *H. pylori* T4SS still needs to be better elucidated[13].

OipA, an outer membrane protein, contributes to both adhesion and increased inflammation by inducing enhanced IL-8 production[54,55]. The discovery of the relationship between OipA and the increased development of peptic ulcers and gastric cancer resulted in a larger number of studies on this *H. pylori* virulence factor[14]. The functional status of OipA has been described as an important factor in the outcome of the infection, since the expression of the *oip*A gene is regulated by a repair process called "slipped strand mispairing", which depends on the quantity of CT dinucleotide repeats in the *oip*A 5' region. Such a process determines whether *oip*A is nonfunctional or functional in a given bacterial strain, and the latter condition is related to increased gastric pathogenicity[56,57]. In addition, OipA might be related to changes in β-catenin signaling, cell proliferation and reduction of cell-cell junctions[58].

The enzyme GGT is a N-terminal nucleophile hydrolase also produced by *H. pylori* that catalyzes the conversion of glutamine into glutamate and ammonia, as well as the hydrolysis of glutathione into glutamate and cysteinylglycine[15]. Its activity leads to the production of reactive oxygen species (ROS), which, like ammonia, induce cell-cycle arrest, apoptosis and necrosis[59,60]. In addition, studies have demonstrated that this enzyme inhibits T cell proliferation and dendritic cell differentiation[61,62]. Higher GGT activity has been observed in peptic ulcer patients when compared to individuals with other gastroduodenal diseases[63].

Immunologic aspects

Complex host immune responses, embracing innate and adaptive mechanisms, are induced by *H. pylori* infection[64,65]. Given the initial contact with the pathogen, various *H. pylori* antigens such as lipoteichoic acid, lipoproteins, lipopolysaccharide, HSP-60, NapA, DNA, and RNA bind to gastric cell receptors, including toll-like receptor (TLR) 1, TLR2, TLR4, TLR5, TLR6, and TLR10 located on epithelial cell membranes, and TLR9, found in intracellular vesicles[66,67]. Such interaction promotes, among other signaling pathways, NF-κB and c-jun N-terminal kinase activation, followed by proinflammatory cytokine release[68]. Besides receptor activation by pathogen-associated molecular patterns, injection of CagA through T4SS also leads to the production of cytokines, in another NF-κB-dependent process[69].

Subsequently, gastric mucosa is infiltrated by neutrophils and mononuclear cells, resulting in the production of nitric oxide and ROS[70]. Moreover, CD4+ and CD8+ T cells, components of adaptive immunity, are also recruited. A preferential activation of CD4+ cells to the detriment of CD8+ cells might occur, and a specific response is directed to the bacterium[71]. Regarding general cytokine profiles in *H. pylori*-positive patients, studies have suggested a Th1-polarized response, characterized by scarce IL-4 (a Th2 cytokine) and enhanced levels of gamma interferon, tumor necrosis factor, IL-1β, IL-6, IL-7, IL-8, IL-10, and IL-18[72,73]. With the exception of IL-10, which seems to play a role in limiting the inflammatory response, other increased cytokines might promote proinflammatory effects during *H. pylori* infection. Furthermore, we demonstrated that an increase in IL-17 is also associated with *H. pylori* infection, especially in adults[74]. In regard to immunoglobulin production, *H. pylori*-specific serum IgM antibodies can be detected in patient serum 4 wk after infection[75]. In chronic infection, serum IgA and IgG immunoglobulins are directed toward several bacterial antigens[76,77]. Such inflammation is asymptomatic in most *H. pylori*-positive patients, however it increases the risk of duodenal and gastric ulcer disease, as well as gastric malignancy development[78].

CLINICAL MANAGEMENT

Clinical manifestations and diagnosis

As a consequence of the mechanisms explained above, *H. pylori*-positive individuals are under increased risk of presenting various clinical manifestations[17]. The course of infection is variable and strongly dependent on host factors. Besides this, the pattern of gastric mucosal involvement is correlated with the risk of initiation and progression of different gastric disorders. Development of antral-predominant gastritis is associated with duodenal ulcers, while a corpus-predominant gastritis and multifocal atrophy tend to turn into gastric ulcers, gastric atrophy, intestinal metaplasia and gastric carcinoma[79]. Among gastrointestinal conditions, dyspepsia and peptic ulcer disease are frequently observed in clinical practice, and bacterial detection, when it is present, followed by infection eradication are crucial steps in the management of such disorders[80]. In addition, recent studies have associated *H. pylori*

infection with a wide range of diseases. The infection was linked with the pathophysiology of neurological, dermatological, hematologic, cardiovascular, ocular, metabolic, hepatobiliary and allergic diseases[81].

Various diagnostic tests, with their specific advantages and disadvantages, are offered for *H. pylori* detection. Histology is the precursor method for *H. pylori* infection diagnosis, which, in such a technique, consists in the observation of typical bacteria associated with inflammatory reactions in the tissue slides. This method includes the use of several stains, such as Giemsa staining, and immunostaining to allow pathogen detection[19]. Another important *H. pylori* diagnostic method, the rapid urease test (RUT), detects an increase in reagent pH after the addition of a biopsy specimen containing *H. pylori* to the reagent. Such pH variation is caused by the conversion of the urea test reagent into ammonia. RUT is a relatively cheap, quick, easy, specific and widely available test[82]. Polymerase chain reaction (PCR) has also been applied for *H. pylori* detection. Al-Moayad *et al*[83] concluded that standardized PCR allows an accuracy superior to that observed in RUT, with improved detection in specimens with lower bacterial charge[84]. However, the necessity of endoscopy is an important limitation of the three methods mentioned above, and the advances in non-invasive diagnostic techniques have strengthened the idea of prioritizing the use of diagnostic alternatives for which endoscopy is dispensable.

The urea breath test (UBT) is now the main non-invasive method for such a diagnosis, gradually taking the place of RUT as the most suitable method for *H. pylori* detection. This test is based on the mechanism of bacterium degradation of 13C or 14C-labeled urea into CO_2, which can be measured in the exhaled air using a mass or infrared spectrometer[85]. A Brazilian study[86] evaluated the use of a locally manufactured isotope in UBT, trying to reduce the importation costs of this substance, which is considered a limitation for the performance of this method in many countries. The assay concluded that the substrate manufactured in Brazil with reduced costs had similar performance when compared to the one imported from foreign countries. Such a reduction in the costs of this substrate can contribute to the dissemination of UBT use around the world.

A less expensive option for UBT, stool antigen tests (SATs), are good alternatives for *H. pylori* diagnosis. SATs can be made by means of enzyme immunoassay or immuno-chromatography[87]. In addition, a new promising non-invasive method, the urine test for the diagnosis of *H. pylori* infection, has been largely studied as an alternative. A meta-analysis from 2017[88], which included 23 studies, showed that testing for antibodies in urine samples might be a good diagnostic option. However, further studies are necessary to confirm the accuracy of this method. Finally, new strategies for serologic diagnosis of *H. pylori* infection have been developed through the discovery of specific serological markers. A recent study evaluated the accuracy of the "hook-associated protein 2 homologue", FliD, as a marker of this infection. The use of the Flid ELISA method in the detection of *H. pylori* infection provided high specificity (99%) and sensibility (97%). Moreover, this method presents a simple technique at low cost[89].

Treatment

There is not a universally accepted regimen for the treatment of *H. pylori* infection. However, all of them target the regressing symptomatology and healing of the mucosa damaged by the infection process[20]. Since the 1997 Maarstricht consensus, the standard triple therapy with proton pump inhibitors (PPI) in standard dose, clarithromycin (500 mg), and amoxicillin (1 g) twice daily for 7 d have been employed in most countries as a first-line regimen to eradicate *H. pylori*. The quadruple therapy, with addition of bismuth (120 mg) to the regimen, has also been used as a first-line regimen[90]. However, the increase in microbial resistance to clarithromycin, whose prevalence varies with time and geographic region, is leading to changes in the therapeutic regimen. The indiscriminate use of azithromycin and erythromycin in the treatment of respiratory infections and cross-resistance among macrolide antibiotics may be responsible for the increased microbial resistance to clarithromycin[91]. As a consequence, longer therapeutic regimens have been used for *H. pylori* eradication[92]. In areas with high clarithromycin resistance, the addition of metronidazole (500 mg) concomitantly with PPI, clarithromycin and amoxicillin twice daily for 5 d, characterizing a quadruple therapy, improves the efficacy of the treatment, with an intention-to-treat higher than 90%[21]. Moreover, in regions with clarithromycin resistance above 15%-20%, and quinolone resistance below 10%, clarithromycin could be substituted by levofloxacin (250/500 mg) in triple therapy. Such exchange increases the per-protocol and intention-to-treat eradication rates of the treatment[21,93]. In addition, the use of hybrid therapy has been suggested as an alternative to the standard approaches in some countries. This therapeutic scheme consists of administering PPI and amoxicillin for 14 d, and then adding both clarithromycin and

nitroimidazole as a quadruple therapy for the final 7 d[94]. Finally, faced with such a situation, studies have proposed the use of tailored therapy as a possible new first-line treatment. Conducting tests for identifying the susceptibility of the bacterial strains to the different regimens appears to be a great alternative for bacterial eradication[95].

Probiotics are being used in the prevention and treatment of many gastrointestinal infections, so it is strongly believed that they might be useful for the treatment of *H. pylori* infection[20]. Research about the use of probiotics for this purpose are typically divided into treatments with and without antibiotics, and data available in the literature are still controversial[96]. Zagari *et al*[97] showed that probiotic supplementation did not improve either the efficacy or tolerability of the treatment, regardless of the species of microorganism used. On the other hand, some studies suggest that probiotics help in the restoration of the intestinal microbiota disturbed by antibiotics, leading to a decrease in side effects and, consequently, increased adherence to treatment, corroborating successful therapy[98]. However, no effect has been observed against *H. pylori* infection using treatment with probiotics alone. Other studies claim that the use of probiotics in combination with antimicrobial therapy has a potentiating effect by increasing eradication rates; however, the relationship with adverse effects is still uncertain[99]. The beneficial effects of probiotics on this infection may be associated with immunological and non-immunological mechanisms, such as substance production, gastric mucosal strengthening, and regulation of immune function[100]. As seen, the role of probiotics in this infection eradication is not well-established and consolidated, and the use of different species of microorganisms, doses and research methods contribute to such uncertainties.

Vaccines

The development of vaccines is a promising alternative that targets the prophylaxis and/or the treatment of the infection (Table 2)[101]. Recently, studies have focused on the development of reverse vaccines with the help of bioinformatics, and five antigenic epitopes have been prioritized as potential vaccine candidates: babA, sabA, fecA, vacA and omp16[20]. However, their development has been a major challenge in the *H. pylori* field, since many studies have not been successful in experimental models. In contrast, a randomized phase 3 study with children has been conducted in China, which was efficacious and safe in providing oral vaccines with recombinant B urease against *H. pylori*[102]. However, a more accurate evaluation of its long-term effect is required. In another study by Wang *et al*[103], intramuscular administration was compared with oral administration of the multi-epitope vaccine, evidencing a better protection rate by oral administration. The development of nanovaccines is also being explored, and presents a nice potential to become an excellent alternative in triggering an effective immunological response against *H. pylori* infection[90,104].

CONCLUSION

Although the knowledge about the different *H. pylori* infection characteristics have been expanded since its discovery, much still needs to be done for a broader understanding of its underlying mechanisms. Furthermore, the new diagnostic methods should be better explored in order to reduce health expenditure and to provide less invasive diagnostic alternatives to patients. Finally, the growing resistance of *H. pylori* to antimicrobial therapy alerts to the necessity of developing satisfactory strategies for bacterial eradication, as well as vaccine implementation aiming at reducing infection prevalence.

Table 2 Preliminary effects of developing vaccines against *Helicobacter pylori* infection

Vaccine	Prophylactic	Therapeutic
EpiVax/*Helicobacter pylori* vaccine	Yes	Yes
Helicovaxor®	Yes	No
Imevax/IMX101	Yes	No
Wuhu Kangwei Biological Technology	Yes	No

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