**Effect of Andrographolide and Its Analogs on Bacterial Infection: A Review**

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**Keywords**
Bacterial infections · Andrographolide · Antibacterial · Anti-inflammatory

**Abstract**
Bacterial infections remain the leading cause of death in children, the elderly, and immunocompromised patients. Andrographolide (AG), the main active component of the herb *Andrographis paniculata*, has been used for many years for anti-inflammatory and antibacterial infections. AG has an antibacterial effect on a wide variety of bacteria, which is reflected in the inhibition of bacterial pathogenic factors and the regulation of immunity to downregulate infectious inflammation caused by bacteria. In the current climate of frequently occurring antibiotic resistance, AG might be considered a promising lead for new antibacterial drug development. This review outlines the therapeutic potential of AG and its analogs in combating various bacterial infections, focusing on the mechanisms of action.

**Introduction**
Vaccines and antibiotics have dramatically reduced the mortality rate caused by bacterial infections worldwide [1]. However, bacterial infections remain the leading cause of death among children, the elderly, and immunocompromised patients, especially in low-income countries [1]. Antibiotics are usually the main treatment for bacterial infections, but a variety of antibiotic resistance reactions have occurred in long-term applications [2]. In addition, antibiotic treatment may lead to toxicity [3], allergic reactions [3], and even dual infections following long-term treatment [4]. Therefore, effective, non-resistant, or weakly resistant antibacterial drugs in natural products have attracted the attention of scientists.

*Andrographis paniculata*, commonly known as the “King of Bitters” in China, is a member of the Acanthaceae family of plants [5]. For centuries, *A. paniculata* has been used as a kind of medicinal food and has been widely used in China, India, Japan, Korea, and other Asian countries for >2,000 years for the treatment of human diseases, including myocardial ischemia, pharyngitis, and respiratory infections [6, 7]. This plant has exhibited antimicrobial [8], anti-inflammatory [9], anti-atherosclerosis [10], and anti-platelet aggregation actions [11], as well as other properties. Clinical studies of *A. paniculata* extract in adults with acute upper respiratory tract infections, cold symptoms, and sore throat and fever have demonstrated certain benefits [12, 13].
Andrographolide (AG), a labdane diterpenoid and the major constituent of *A. paniculata*, has been used clinically in China [14]. This compound is effective in the relief of symptoms of inflammation, fever, and pain caused by bacterial infections [15, 16]. The antibacterial pharmacological action of AG mainly lies in the inhibition of bacterial pathogenic factors and the regulation of the immune system to reduce the inflammatory response [17]. Several studies have shown that AG may be a potential quorum sensing (QS) inhibitor and a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) inhibitor, which confirms the remarkable antibacterial activity of this compound [17–20]. AG has synthesized a number of AG analogs in past studies, such as the less toxic 14-alpha-lipoxy AG (AL-1) [18] and the more water-soluble AG-β-cyclodextrin inclusion complex (AD-β-CD) [19]. These analogs enhance the antibacterial efficacy of AG and represent a new mechanism of action on pathogenic bacteria. In this study, we summarize the mechanism of AG and its analogs against bacterial infections and explore its potential as a new antibacterial drug (Table 1).

**Mechanisms of Influence on *Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* is an opportunistic human pathogen responsible for infections of various damaged human tissues. This pathogen is one of the bacteria that causes in-hospital (hospital-acquired) infection and exhibits certain resistance to the commonly used quinolones and β-lactams [21]. This resistance can be caused by various factors such as biofilm and efflux pumps [22]. *P. aeruginosa* biofilms grow vigorously [23]. Once this structure forms, the colony is protected from the patient’s immune system and is less susceptible to drug treatments. Biofilm formation is believed to be one of the main causes of persistent infection [24]. AG has a multifaceted role in the treatment of infections caused by *P. aeruginosa* (Fig. 1).

**Effect on QS System**

A QS system is a communication system that exists between bacteria. A QS system enables a given bacterial species to sense when a critical (i.e., quorate) population density has been reached in the host and, in response, activate expression of target genes required for succession. This system is an effective antibacterial drug target that affects the formation of *P. aeruginosa* biofilms and regulates the production of bacterial efflux pumps and virulence factors [25]. Bacteria that use QS produce and secrete certain signaling compounds (called auto-inducers [AI]) to signal one another and to coordinate their activities. For example, Gram-positive bacteria use small peptides to signal one another [26]. Among Gram-negative bacteria, the best-studied signaling system is the N-acylhomoserine lactone system [27, 28]. In *P. aeruginosa*, N- (3-oxopododecanoyl) homoserine lactone (3-oxo-C12-HSL) and N-butyryl homoserine lactone (C4-HSL) are used as signaling molecules upstream of LasR and RhlR. The QS regulators LasR and RhlR control the expression of 100 of genes in *P. aeruginosa*, many of which encode central metabolic functions [29]. LasR and RhlR interact with and are activated by 3-oxo-C12-HSL and C4-HSL, respectively. LasR-3-oxo-C 12-HSL is the key to the opening of the *P. aeruginosa* QS system, and the cascade triggers the RhlR-C4-HSL system [30].

The AG analog AL-1 has an effect on the *P. aeruginosa* QS system, especially the Las and Rhl systems. Existing reports have found that the formation of *P. aeruginosa* biofilms is effectively inhibited by AL-1, which acts by reducing the expression of Psl polysaccharides necessary for the initiation and maintenance of biofilms that are positively regulated by the Las system [17]. PslA to pslL are positively regulated by the Las system according to previous research [31]. The presence of AL-1 as a potential inhibitor of the QS system affects the expression of LasR, thereby inhibiting the production of LasR-3-oxo-C 12-HSL and reducing the level of psl production. RsmA is known to be a small RNA-binding protein that negatively regulates pathogenic determinants, such as motility, N-acylhomoserine lactones, and secondary metabolite production [32–34]. Previous reports concluded that RsmA acts as a translational repressor of psl [35]. Existing research also confirmed that the rsmA level of the experimental group using AL-1 was increased approximately 3-fold with respect to the level of the control [17] (Table 1). The decreased psl translational level may be due to the increased level of rsmA mediated by AL-1. *P. aeruginosa* accumulates with an extracellular matrix that often includes exopolysaccharides (EPS), proteins, and DNA, thereby providing an effective protection barrier against host immune responses and antibiotics. EPS are key matrix components for adhering substrates and biofilms required to maintain structure, as they contribute to overall biofilm structure and resistance [36–38]. The production of EPS is influenced by the pel biosynthetic operon regulated by the Las and Rhl systems. The inhibition of the Las and Rhl systems by AL-1 can indirectly regulate the EPS content [39]. Previous research demonstrated that AL-1 alone can inhibit the production of biofilm by reducing the production of EPS, and the effect of combining AL-1 with antibiotics is
remarkable [18] (Table 1). AL-1 has multiple effects on the QS system of *P. aeruginosa*, inhibits the formation of biofilms, and makes bacteria sensitive to various antibiotics, which has a significant synergistic effect.

**Inhibition of Virulence Factors**

The existence of virulence factors determines the type and severity of bacterial diseases and even the prognosis. AG inhibits the production of virulence factors of *P. aeruginosa* to achieve anti-infective effects. Current studies have shown that AL-1 significantly reduces the expression levels of lasR, lasl, rhlR, and rhlI in the QS system in a dose-dependent manner [17, 40]. AL-1 effectively inhibits the synthesis of elastase B regulated by the las system and the production of various virulence factors, such as pyocyanin, required for RhlR to regulate the pathogenesis [41, 42] (Table 1). Pyocyanin is the hallmark virulence factor product of *P. aeruginosa* [43, 44]. Elastase B degrades immune components and causes tissue damage [45]. AG and its derivatives have been used to treat bacterial infections for many years, and these drugs have been found to have minimal or no direct inhibition of bacterial growth, especially on *P. aeruginosa* [40, 46]. Thus, the antibacterial and anti-inflammatory effects of AG have been attributed to stimulation of the immune system. Similar to the action of QS inhibitors, AG can inhibit the formation of biofilm and the production of virulence factors by effectively inhibiting the QS system of *P. aeruginosa* to achieve anti-infective effects.

**Repression of MexAB-OprM Exhaust Pump**

The MexAB-OprM efflux pump is an important factor in the multidrug resistance mechanism of *P. aeruginosa* as a drug-resistant strain. Overexpression of the pump results in multidrug resistance of *P. aeruginosa* to quinolone, macrolide, β-lactam, and other similar antibiotics [47]. The MexAB-OprM efflux pump belongs to the ribonucleoprotein superfamily and consists of an inner membrane, MexB, a periplasmic membrane fusion protein,
Mechanisms of Influence on Staphylococcus aureus

Staphylococcus aureus is one of the most prominent pathogens for hospital-acquired infections and acquired immune-acquired bacterial infections [52]. It can cause pneumonia, mastitis, osteomyelitis, endocarditis, skin infections, abscesses, food poisoning, toxic shock syndrome, and sepsis. Among these infections, bacterial pneumonia is dominant [53]. The treatment of S. aureus infection by AG not only acts on bacteria themselves but also plays a certain role in the regulation of the body’s immune system (Fig. 2).

Regulating Body Immunity

Bacterial infection generally initiates immune responses, leading to an increase in inflammatory cytokines. The immune system kills or eliminates the bacteria through such processes as phagocytosis. However, overexpression of immune responses will damage normal functional cells. Therefore, the appropriate regulation of immune responses may eliminate bacteria, reduce damage, and facilitate the recovery of normal functions for the treatment of bacterial pneumonia [54–56]. AD-β-CD is formed by trapping hydrophobic AG into a macrocycle composed of 7 α-D-glucopyranoside units, which improves drug solubility, chemistry stability, and bioavailability and reduces drug toxicity [57, 58]. AD-β-CD is regarded as an NF-κB inhibitor that regulates immune responses [59, 60]. AG reduces the inflammatory reactions in macrophages by inhibiting cyclooxygenase-2, NF-κB activation, and apoptotic proteins expression and by regulating cytokines (interleukin 6 [IL-6] and tumor necrosis factor alpha [TNF-α]) [61] (Table 1). Bacterial pneumonia significantly promotes NF-κB p65 phosphorylation, and the levels of TNF-α and IL-6 were also high. Under the action of AG-β-CD, the expression level of all 3 was significantly reduced [19]. Existing research has reported that in the lungs after S. aureus infection, the amount of white blood cells and central granulocytes in the lungs increased rapidly [19]. The levels of these immune cells were downregulated by AG, AD-β-CD, and penicillin. However, the levels of immune cells were higher for AG and AD-β-CD than penicillin. Therefore, the local immune ability in the infected lungs after administration of AG and AD-β-CD remained at a level high enough to kill bacteria without strong immune damage. AG may be an efficient immunomodulatory agent that is effective in killing S. aureus by immunotherapy. At the same time, it is difficult for bacteria to develop resistance to drugs acting on the immune system, which also solves the problem of bacteria easily becoming resistant.

Suppression of the Transcriptional Regulator SarA

SarA is an important transcriptional regulator of S. aureus and is closely related to bacterial biofilm, adhesion, and virulence factors. The pathogenicity of S. aureus is mainly achieved through the coordinated expression of various virulence factors, such as extracellular proteins and cell wall-binding proteins. The expression of many virulence factors in S. aureus is regulated by global regulators, factors of virulence, and SarA is one of them. SarA increases the transcription level of RNAIII by binding to the P3 promoter region [62]. SarA can directly regulate the expression of various virulence factors, such as hemolysin, fibronectin-binding protein, toxic shock syndrome toxin 1, and enterotoxin B, thereby changing the pathogenicity of S. aureus [63]. Existing research has shown that the biofilm formation ability was weakened when S. aureus SarA was mutated, indicating that SarA may be one of the mechanisms promoting biofilm formation [64]. SarA can also upregulate the intercellular adhesion gene (Ica), which is also an essential gene for S. aureus biofilm formation [65]. When S. aureus SarA was mutated and Ica was downregulated, the biofilm formation ability was lowered. This finding indicates that the SarA mutation not only downregulates Ica but also reduces the ability of S. aureus to form a biofilm.
biofilm formation to a certain extent, which is one of the important mechanisms affecting the formation of biofilm. In the current research report, AG was able to effectively inhibit the biofilm formation of *S. aureus* MTCC 96 in a concentration-dependent manner [66] (Table 1). This inhibition may be related to the inhibitory effect of AG on the activity of SarA. This presumption can be demonstrated by the use of radiolabelled N-acetylglucosamine, leucine, thymidine, and uridine to determine the effects of AG on bacterial cell wall, protein, DNA, and RNA biosynthesis. Specific inhibition of intracellular DNA biosynthesis was observed in a dose-dependent manner by AG in *S. aureus* [66]. At the same time, the secondary effect on protein synthesis of *S. aureus* was also obvious at higher doses of AG. In addition to inhibiting the growth of *S. aureus* biofilm and the synthesis of virulence factors, AG itself also inhibits the growth of *S. aureus* in its antimicrobial activity. AG can be used as an inhibitor of SarA to reduce the pathogenicity of *S. aureus* to some extent.

**Mechanisms of Influence on *Escherichia coli***

**Inhibition Signal Molecule Autoinducer-2**

Autoinducer-2 (AI-2) is a furanosyl boron diester that has been shown to be a signaling molecule for intra- and inter-species communication between Gram-negative and Gram-positive bacteria [67, 68]. *Escherichia coli* are a Gram-negative bacterium that can exist as a symbiont or pathogen in animals or humans [69]. The QS of *E. coli* is a key player in the expression of virulence genes in the stationary phase [70]. AG can significantly reduce the expression of LuxS, which is responsible for the production of AI-2 by *E. coli*, and thus inhibit AI-2 activity, thereby inhibiting the expression of virulence factors by regulating QS [71, 72] (Table 1). Hemolysin E and vacuolar autolysin motor toxin gene are virulence factors produced by *E. coli*. Hemolysin E promotes pore formation in the membrane of target cells and destroys the structural integrity of the cell, causing the release of cytokines and inflammatory respons-
Vacuolar autologous-motor toxin is similar to the cytotoxin of Helicobacter pylori, which causes vacuolization of fibroblasts [74]. AG reduces E. coli-induced cell damage by inhibiting the secretion of both factors.

**Reduction of E. coli Adhesion**

The adhesion of E. coli to airway epithelial cells is a critical initial step in respiratory colonization. F1 pili and P pili mediated this infection process, F1 pili can cause E. coli colonization in the trachea and alveoli, and P pili is present in the alveoli, lung, and viscera. Tsh is an important virulence factor that acts as an adhesin, especially in the initial stages of respiratory colonization. It has been reported in studies that AG can reduce the adhesion of E. coli to cells. According to previous research, AG significantly decreases the degree of adherence and the mRNA levels of fimA, papC, and tsh in E. coli [72]. These results indicate that AG inhibits bacterial adhesion to the cell sur-

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AL-1, 14-alpha-lipoyl AG; QS, quorum sensing; AG, andrographolide; Ica, intercellular adhesion; ACAND-HP-β-CD, hydroxypropyl-cyclodextrin inclusion AG; AD-β-CD, AGAndrographolide-β-cyclodextrin; NTHI, Nontypeable Haemophilus influenzae; COX-2, cyclooxygenase-2; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cell; CMI, cell-mediated immune; Nrf2, nuclear factor erythroid-2-related factor 2; Keap1, kelch-like ECH-associated protein 1; C4-HSL, N-butylryl homoserine lactone; AAC, aminoglycoside 2-N-acetyltransferase.
face by reducing the expression of F1 pili, P pili, and Tsh, thereby inhibiting the invasion of bacteria by cells and the proliferation of bacteria in the body [72] (Table 1).

**Destruction of the Integrity of E. coli**

Alkaline phosphatase (AKP) is an enzyme that exists between the cell wall and cell membrane [75]. β-galactosidase (β-gal) belongs to the intramembrane substance of the cell and is a detection index of cell membrane integrity [76]. When cells are damaged, the permeability of the cell wall increases, causing AKP and β-gal to leak out of the cell. Existing studies have reported that the levels of AKP and β-gal were elevated in E. coli supernatants under the action of hydroxypropyl-cyclodextrin inclusion Andrographolide (AcAND-HP-β-CD) [77]. AcAND-HP-β-CD is formed by hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion 14-acetyl AG (AcAND). The water solubility of AcAND-HP-β-CD is significantly improved, and the bacteriostatic activity is increased relative to AG. It can be speculated that AcAND-HP-β-CD can destroy the cell wall and cell membrane integrity of E. coli, resulting in increased cell permeability and exudation of intracellular biological enzymes and protein substances, leading to bacterial death (Table 1).

The modified AG derivative AcAND-HP-β-CD has better effect than AG itself and can kill E. coli directly, which is more conducive to the advancement of infection treatment.

**Maintenance of the Cytoskeleton**

F-actin plays an important role in the mobility and contraction of cells during cell division and is a constituent of the cell cytoskeleton [78]. Lactate dehydrogenase, a biological enzyme widely distributed in animals and plants, is a marker for common injuries. It has been observed that AG can significantly reduce the release of lactate dehydrogenase and F-actin cytoskeleton polymerization in chicken type II lung cells induced by E. coli [72]. AG can maintain normal cell morphology by decreasing F-actin cytoskeleton polymerization induced by E. coli (Table 1). In addition to the direct or indirect inhibition of E. coli, AG’s support for the cell itself is also indispensable for AG bacteriostatic treatment.

**Inhibition of Staphylococcus epidermidis Adhesion**

Staphylococcus epidermidis is the main pathogen of infection in intravenous catheters or other medical devices [79]. This pathogen does not cause disease as a normal flora on the surface of healthy human skin, but it can enter the human body with “foreign substances" and invade the bloodstream to cause sepsis [80]. Biofilm is its main cause of disease [81]. The formation of S. epidermidis biofilms is a dynamic process that is mainly divided into 2 stages. The first stage is the initial attachment of biological proteins by hydrophobic proteins or polysaccharide adhesins on the surface of bacteria to form a bacterial community. Subsequently, the bacteria are mainly mediated by polysaccharide intercellular adhesin and aggregate with each other to form a bacterial biofilm [82, 83]. Existing research on the effect of AG on S. epidermidis found that AG inhibits the adhesion of S. epidermidis and further inhibits biofilm formation [84] (Table 1). This inhibition of adhesion is independent of polysaccharide intercellular adhesin. AG has biofilm permeability, which is not available in antibiotics and acts on the bacteria itself in a dose-dependent manner through the protective barrier of the biofilm [85].

**Induction of Salmonella-Specific Cell-Mediated Immune Responses**

AG is reported to have an anti-inflammatory effect, which stimulates or enhances T cell activity in vivo or in vitro or inhibits adaptive immunity [15, 86, 87]. Salmonella typhimurium is a Gram-negative pathogen that infects a variety of hosts and is able to evade the host’s natural immune system, replicating in the host and causing disease [88]. Existing research found that AG enhanced not only Salmonella-specific serum IgG antibody levels in mice immunized with inactivated S. typhimurium vaccine, but also significantly increased IFN-γ levels in spleen cell supernatants stimulated by Salmonella lysates [89] (Table 1). This finding indicates that AG can induce a Salmonella-specific cell-mediated immune response, which has also been shown to be an important factor in preventing salmonellosis [90].

**Reduction of Inflammatory Cell Infiltration Caused by Nontypeable Haemophilus influenzae**

It has been reported that AG can effectively reduce macrophage and neutrophil pulmonary infiltration induced by cigarette smoke (CS)-induced nontypeable Haemophilus influenzae (NTHi) inflammation, reduces TNF-α, IL-1β, chemokine (C-X-C motif) ligand 1 CXCL1/KC, 8-hydroxy-2’-deoxy-guanosine levels in the lung, and inhibited NTHi-mediated increases in matrix metalloproteinase-8 and matrix metalloproteinase-9 gene expression [91]. NTHi is a type of Gram-negative bacilli without cap-
sules [92]. NTHi is often found in the human upper respiratory tract and is an important opportunistic pathogen that can cause otitis media, pneumonia and repeated respiratory infections in humans [93]. CS exposure can worsen lung inflammation, which is linked to a heightened risk of respiratory tract infection due to suppressed innate and adaptive immunity and delayed clearance of microorganisms [94–96]. The protective effect of AG on the respiratory tract and lungs may be due to increased activation of nuclear factor erythroid-2-related factor 2 and decreased function of Kelch-like ECH-associated protein 1 inhibition, resulting in enhanced expression of antioxidant enzyme genes, including haem oxygenase-1, glutathione reductase, glutathione peroxidase-2, glutamate-cysteine ligase modifier, and NAD(P)H quinone oxidoreductase 1 [91] (Table 1). AG effectively reduced CS-induced NTHi inflammation by downregulating inflammatory cytokines and oxidative damage. These findings support the therapeutic potential of AG in preventing lung inflammation caused by NTHi in cigarette smokers.

**Inhibition of Propionibacterium acnes: Induced Cellular Inflammatory Factors**

As keratinocytes are the first barrier against exogenous pathogens in human skin, they form the primary barrier and alert the host in danger via releasing a large amount of cytokines and chemokines, such as IL-1, IL-8, TNF-α, and prostaglandins [97]. Keratinocytes play an important role in the process of inflammation that causes acne. The human skin symbiotic bacterium *Propionibacterium acnes* has long been associated with inflammatory acne vulgaris [98]. It has been demonstrated that live *P. acnes* can induce the secretion of the proinflammatory cytokines IL-8 and TNF-α by monocytes [99]. Existing research has reported that AG can produce IL-8 and TNF-α in a moderately inhibited effect on human keratinocyte HaCaT cells induced by heat-inactivated *P. acnes* at doses that are not cytotoxic [100] (Table 1). By this inhibition, skin acne caused by *P. acnes* is alleviated.

**Restoration of Antibiotic Susceptibility in Mycobacterium tuberculosis**

Aminoglycoside 2-N-acetyltransferase (AAC) is a 181-amino-acid-long protein in MTB that confers resistance to aminoglycosides [101]. The crystal structure of AAC is available as a complex with kanamycin [102]. Kanamycin is a commonly used antibiotic for the treatment of tuberculosis (TB) caused by MTB, and its resistance makes TB treatment more severe [103]. Current studies have found that AG shows effective anti-mycobacterial activity. AAC was identified and predicted as a potential target protein for AG by docking analysis [101]. Based on Genetic Optimization for Ligand Docking score, AG had the highest binding affinity to AAC protein compared with other target proteins [101] (Table 1). Furthermore, comparing the binding affinity of AG and kanamycin, AAC is predicted to have a better binding affinity to AG than kanamycin [101]. This finding suggests that AG can competitively inhibit the combination of AAC and kanamycin, reverse MTB resistance to kanamycin, restore antibiotic sensitivity, and thus play an antimicrobial role.

**Conclusion**

AG is an extract of a traditional Chinese medicine with a clear antibacterial effect. This review is intended to help compile and consolidate the current understanding of AG antibacterial activity discovery and inhibition mechanisms. As treatment of bacterial infections is a heavily researched subject in global public health, it is notable that AG and some of its synthetic AG analogs have good antibacterial effects in both in vitro and in vivo experimental studies. AG can inhibit the formation of bacterial biofilms, the production of virulence factors, the adhesion between bacteria, and the destruction of bacterial integrity in direct bacteriostatic action. Indirect bacteriostasis has a potential immunopotentiator effect on the treatment of bacterial infections by regulating the immune response and maintaining the cytoskeleton of the body. This antimicrobial property of AG and its analogs also exhibits notable properties in combination with antibiotics. AG can partially restore antibiotic susceptibility by inhibiting the expression of bacterial efflux pumps and competitively inhibiting antibiotic resistance sites in bacteria. However, there are still some difficulties in the clinical application of AG. For example, the poor solubility of AG has become one of the important reasons for limiting its efficacy [104]. Therefore, at present, many studies in drug development often modify the structure of AG to increase water solubility and optimize delivery systems, thereby enhancing pharmacological activity and increasing therapeutic index [105]. The effect of AG in regulating immunity and maintaining body cells has potential for discovery in future clinical antibacterial therapy. In addition, the potential characteristics of AG in pharmacological research are the improvement of anti-
biotic sensitivity in combination with antibiotics, which is an advantage in clinical treatment and represents a promising lead in the development of new antibacterial drugs.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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Author Contributions


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