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# Efficacy of Ozone Therapy as an Antibacterial Agent: A Narrative Review

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**ABSTRACT**
Bacterial infections pose a significant threat to public health, especially with the emergence of antibiotic resistance. Understanding the intricate interactions between bacterial resistance and host virulence is crucial for devising effective strategies to combat infections. The host employs various defence mechanisms, including the expression of antimicrobial peptides and the activation of signalling pathways, to protect against bacterial invasion. However, bacterial pathogenicity remains a significant challenge, hindering the development of new drugs and vaccines. Bacterial pathogenesis involves exposure, adhesion, invasion, infection and transmission and is influenced by virulence factors encoded in bacterial DNA. The rise of multiple drug resistance (MDR) highlights the urgency of exploring alternative treatment options. Ozone therapy has demonstrated potent antibacterial properties and has been studied against various bacterial strains, offering a potential safer and more economical alternative to conventional antibiotics. Ozone's mechanism of action primarily involves oxidative stress on pathogens, leaving healthy cells relatively unharmed. This targeted approach may reduce adverse effects and help combat antimicrobial resistance. However, further research and clinical trials are necessary to validate its efficacy and safety.

## INTRODUCTION

Bacterial infections can arise when there is an imbalance between bacterial resistance and host virulence[1]. However, the host has several defence mechanisms that act as barriers to prevent such infections. These defence mechanisms include various chemical and physical factors contributing to the body's homeostasis and limiting bacterial invasion. One significant line of defence involves the presence of antimicrobial peptides (AMPs) in mucosal and epithelial surfaces. These AMPs play a crucial role in fending off potential bacterial invaders. For instance, neutrophil alpha-defensins and other AMPs are expressed in various body regions, such as the gingiva, cornea of the eye and Paneth cells in the intestine[1]. The host employs three main pathways of virulence against bacterial infections, each playing a unique role in its defence mechanism[2]. The first pathway involves the activation of Toll-like receptors (TLRs) that induce the production of AMPs upon recognising bacterial components.

The second pathway, the Immune deficiency signalling cascade (IMD), also produces AMP when bacteria are detected. Lastly, the Janus Kinase and signal transducer and activator of transcription (JAK/STAT) pathway contribute to expressing AMPs, further bolstering the host's defence. Despite these defence mechanisms, the pathogenicity of bacteria remains challenging to comprehend, which hinders the development of new drugs and vaccines[3]. Understanding the mechanisms by which bacteria cause diseases is essential for devising effective strategies to combat infections. The current discussion is focused on gaining a deeper understanding of bacterial pathogenicity in terms of ozone therapy. Scientists are exploring bacterial virulence factors, how bacteria interact with the host's immune system and the genetic basis of bacterial infections. Research may lead to developing novel therapeutic approaches and more targeted vaccines to combat bacterial infections effectively. Bacterial infections arise when there is a disruption in the balance between bacterial resistance and host virulence. However, the host employs various defence mechanisms, including the expression of antimicrobial peptides and the activation of different signalling pathways, to protect against bacterial invasion. Nevertheless, the complexity of bacterial pathogenicity remains a significant challenge for developing new treatment options, underscoring the importance of ongoing research in this field.

**Bacterial pathogenesis:** Bacterial infections encompass various diseases that vary in severity and type. The pathogenicity of bacteria and the presence of specific virulence factors are critical determinants in their ability to cause diseases. The virulence of a bacterium can differ based on various factors, including its route of entry into the host, host defence mechanisms and the expression of virulence-related factors like toxins, surface coats and receptors that facilitate binding to host cells. These virulence factors can be encoded in bacterial chromosomal DNA, plasmids, or bacteriophage DNA. Bacterial pathogenesis has five main stages: exposure, adhesion, invasion, infection and transmission. During exposure, the bacterium comes into contact with the host. Adhesion occurs when the bacterium attaches itself to specific receptors on host cells, facilitating its ability to colonise and cause infection.

Invasion refers to the bacterium's ability to enter and multiply within the host's tissues. Infection represents the development and manifestation of the disease within the host. Lastly, transmission involves the spread of the bacterium to other hosts, ensuring its survival and continuation. Most bacterial infections share common virulence factors and antibiotic-resistance genes, which can be transferred between bacteria through mechanisms like bacterial transposons and plasmids[4]. This horizontal gene transfer contributes to the rapid spread of antibiotic resistance, posing a significant public health concern. Bacterial pathogenesis can occur through various mechanisms, including host-mediated pathogenesis and intracellular growth. Sometimes, the host's immune response can cause tissue damage while trying to eliminate the bacterial infection, leading to the proliferation of resistant bacteria. Some bacteria adhere to epithelial cells and secrete toxins, causing diseases without invading the host tissues[1]. The endotoxins secreted by bacteria have various biological effects on the host. For instance, they can stimulate B lymphocytes, increasing resistance to viral and bacterial infections. They also induce gamma interferon production by T lymphocytes, which enhances the antiviral state, facilitates the rejection of tumour cells and activates macrophages and natural killer cells.

Additionally, these toxins can activate the complement cascade, which leads to C3a and C5a. Furthermore, they induce the production of interleukin-1 by macrophages and interleukin-2 and other mediators by T lymphocytes, further modulating the host immune response[1,4]. Bacterial infections are complex and diverse, with the capacity to cause various diseases. The pathogenicity and virulence of bacteria play critical roles in determining the severity of infections. Understanding the mechanisms of bacterial pathogenesis is essential for developing effective treatments, combating antibiotic resistance and protecting public health. Ongoing research in this field continues to shed light on the intricate interactions between bacteria and their hosts, offering promising avenues for future medical advancements.

**Development of bacterial resistance against drugs:** Multiple drug resistance (MDR) poses a growing global health threat[5]. Antimicrobial resistance (AMR) is a significant concern and by 2050, it is estimated that it could lead to over 10 million deaths and cause a staggering global economic loss of US$100 trillion. India has earned the reputation of being the "AMR capital of the world," with approximately 70% resistance observed in gram-negative bacteria[6]. A significant proportion of bacterial strains in India exhibit resistance to necessary antibiotics. For example, under the auspices of the Government of India, a study conducted in 2017 revealed that E. coli, Klebsiella, Acinetobacter baumannii and Pseudomonas aeruginosa were resistant to fluoroquinolones and third-generation cephalosporins.

Moreover, a high percentage (82%) of Shigella strains demonstrated resistance to ciprofloxacin and cotrimoxazole[7]. The emergence of specific MDR bacteria is closely linked to the widespread use of broad-spectrum antibiotics for empiric and definitive therapy. This excessive and indiscriminate use of antibiotics contributes to the development of resistance in bacterial populations. Bacteria have evolved multiple molecular mechanisms to inactivate antibiotics and render them ineffective.

These mechanisms include[8]:

- **Production of inactivating enzymes:** Bacteria may produce enzymes like beta-lactamases that hydrolyse beta-lactam antibiotics, making them inactive
- **Changes and alterations in antibiotic targets:** Bacteria can modify the structures of antibiotic targets, reducing the affinity of the antibiotic for its target without disrupting essential protein synthesis. For example, in erythromycin resistance, methylation of an adenine residue in the 23S rRNA of the peptidyl transferase reduces its binding affinity for the antibiotic
- **Reduced cellular permeability:** Some bacteria can develop resistance by altering their cell surface structures, making it difficult for antibiotics to penetrate the bacterial cell. Changes or quantitative decreases in proteins responsible for antibiotic penetration in gram-negative bacteria can lead to intrinsic resistance
- **Increased drug efflux:** Bacteria employ energy-driven drug efflux systems to pump antibiotics out of their cells, preventing the drug from reaching effective concentrations
- **Acquisition of extrachromosomal elements:** Bacteria can acquire extrachromosomal elements, such as plasmids, transposons and integrons, from other bacteria in their environment. These elements may carry resistance genes, allowing the bacteria to develop resistance to multiple antibiotics

**Approaches to addressing bacterial resistance:** Addressing antimicrobial resistance requires a multifaceted approach. Apart from promoting responsible antibiotic use, it is vital to develop new antibiotics and alternative therapies and implement robust infection control measures to combat the rise of antimicrobial resistance and safeguard public health. Bacterial resistance can result from various mechanisms, including mutations in normal cells or the acquisition of foreign resistant genes through horizontal gene transfer. These resistant genes can accumulate on mobile genetic elements, such as plasmids, transposons and integrons, facilitating the transfer of multi-drug resistance phenotypes to susceptible bacteria in a single genetic event[1,4,9]. The horizontal transfer of resistance genes plays a significant role in rapidly disseminating antibiotic resistance among bacterial populations. Researchers and healthcare professionals are exploring various alternative treatment options as conventional therapy struggles to combat multidrug-resistant (MDR) infections.

Some of these innovative approaches include[8]:

- **Vaccines:** The development of vaccines against specific bacterial pathogens can help prevent infections and reduce the need for antibiotic use
- **Stem cells and antimicrobial peptides (AMPs):** Research is ongoing to explore the potential of stem cells and AMPs as therapeutic agents against bacterial infections
- **Nano-biotics and enzybiotics:** Nanotechnology-based antibiotics (nano-biotics) and Enzybiotics (enzymes targeting bacterial cell walls) are promising alternative treatments for MDR infections
- **Immunotherapeutic approaches:** Immunotherapies, which stimulate the body's immune response to fight infections, are being investigated as potential treatments for MDR bacterial infections
- **Phage therapy:** Bacteriophages, viruses that target and infect specific bacteria, are being researched as a treatment option for MDR infections
- **CRISPR-Cas/editors:** Gene-editing technologies like CRISPR-Cas are being explored as potential tools to modify bacterial genes responsible for antibiotic resistance

Additionally, developing alternative therapies, such as bacteriophage therapy and phage-derived products, shows great promise in combating antimicrobial resistance. Bacteriophages are viruses that specifically target and destroy harmful bacteria while leaving beneficial ones unharmed. This

personalised and targeted approach could revolutionise the treatment of bacterial infections, especially those that have become resistant to conventional antibiotics. Clinical trials and research in this area are ongoing and collaboration among scientists, clinicians and regulatory bodies is essential to ensure the safe and effective implementation of bacteriophage therapy. Furthermore, using probiotics as a preventative measure against infections and to support gut health is gaining traction. Probiotics are living microorganisms that can confer health benefits when administered adequately. Research has indicated that specific probiotics can contribute to maintaining a balanced microbial environment in the body and enhance the immune system, thereby reducing the risk of infections and potentially decreasing the reliance on antibiotics[10-13]. Exploring the potential of probiotics in combination with traditional antibiotics or other alternative therapies could provide a multifaceted approach to combatting antimicrobial resistance.

Advancements in nanotechnology offer exciting possibilities in the fight against resistant microbes. Nanoparticles can be engineered to have antimicrobial properties and may serve as an alternative treatment strategy[14]. These nanoparticles can target specific pathogens and disrupt their cell membranes, rendering them ineffective and reducing the risk of resistance development. Integrating nanotechnology into the existing arsenal of antimicrobial strategies could pave the way for more efficient and targeted treatment options.

Moreover, implementing robust antibiotic stewardship programs in healthcare settings is crucial to ensure the responsible use of antibiotics. These programs involve a coordinated effort among healthcare professionals to optimise antibiotic prescribing practices, reduce unnecessary use and monitor resistance patterns. By promoting judicious antibiotic use and raising awareness among healthcare providers and the general public, we can slow the development and spread of antimicrobial resistance[14,15]. Lastly, public education and awareness campaigns are pivotal in addressing antimicrobial resistance globally[15]. By educating individuals about the significance of practising proper hygiene, getting vaccinated and using antibiotics responsibly, communities can be empowered to engage in the battle against antimicrobial resistance actively. Governments and health organisations need to work together to disseminate accurate information and encourage the adoption of preventive measures, fostering a collective effort to preserve the effectiveness of antibiotics and safeguard public health for generations to come[14,15].

**Role of Naturopathy in drug resistant bacterial infection:** Naturopathy, as a branch of medical science, emerged as a response to the germ theory of disease, which revolutionized our understanding of health and disease transmission. This breakthrough led to a shift in perspective from viewing health as solely the absence of disease to a comprehensive and continuous state of well-being. Unlike conventional medicine, which often focuses on treating specific symptoms or diseases, Naturopathy takes a more holistic approach, considering the interconnectedness of various bodily systems and aiming to address the root causes of ailments. One of the fundamental principles of Naturopathy is its reliance on natural means to promote health and healing. Practitioners emphasize the use of non-invasive therapies, such as dietary changes, exercise, herbal remedies, hydrotherapy and lifestyle adjustments, to stimulate the body's innate healing mechanisms. By supporting the body's own ability to fight off diseases and maintain balance, Naturopathy reduces the dependency on pharmaceutical drugs, which often come with potential side effects. As Naturopathy primarily employs natural elements and lifestyle modifications, the risk of developing multi-drug resistant (MDR) bacteria is significantly lower compared to conventional medicine. The overuse and misuse of antibiotics in conventional medical practices have contributed to the rise of drug-resistant pathogens. In contrast, Naturopathy's emphasis on prevention and natural healing methods reduces the need for antibiotics and minimizes the likelihood of MDR bacteria emerging and spreading. Furthermore, Naturopathy considers the individual as a whole, taking into account physical, mental, emotional and environmental factors that influence health. This comprehensive approach helps patients achieve overall well-being, not just the temporary relief of symptoms. By addressing the root causes of health issues, Naturopathy aims to create lasting improvements and empower individuals to take an active role in maintaining their health. Ozone therapy is one such adjunct therapy used in Naturopathy. Ozone is a natural element found in the Earth's atmosphere and when applied in a controlled manner, it can have therapeutic benefits. Ozone therapy complements the principle of drugless therapy in Naturopathy by offering additional support to the body's healing processes. It is believed to enhance oxygen utilization at the cellular level, boost the immune system and help in detoxification. Another advantage of ozone therapy is that it reduces the risk of exposure to non-natural sources of treatment, which may cause harm to the body. Many conventional medical treatments involve the use of synthetic drugs or invasive procedures, which can lead to adverse reactions and complications. Ozone therapy, being a

natural treatment modality, is generally well-tolerated and has fewer side effects compared to some pharmaceutical interventions. Thus Naturopathy's approach of using natural means to stimulate the body's own healing mechanisms offers a compelling solution to the challenges posed by multi-drug resistant bacteria. By promoting overall well-being and reducing the reliance on pharmaceutical drugs, Naturopathy not only addresses health issues but also works towards preventing future illnesses. Ozone therapy, as a natural adjunct therapy, complements these principles and provides additional support in the pursuit of optimal health without compromising the body's natural defenses. As our understanding of health continues to evolve, Naturopathy and its holistic approach with ozone are likely to play an increasingly vital role in modern healthcare.

**Ozone as a potent antibacterial agent:** Over 150 years of clinical use, ozone therapy has been widely recognised for its disinfectant properties in various applications[16]. Ozone disinfects medical devices, hospital clothing and the food industry. It also finds applications in periodontics and water disinfection, further showcasing its versatility and effectiveness in killing bacteria and viruses[17]. However, the therapeutic use of ozone against bacteria and viruses is contingent upon several factors. The ozone concentration employed, the mode of administration and the volume and duration of exposure or treatment all play crucial roles in determining its effectiveness[16]. Ozone can be administered either locally or systemically (parenterally). The three primary application forms are ozonated water, ozonated oil and oxygen/ozone gas, each offering unique benefits depending on the specific medical condition[16]. Ozone therapy has been explored for various therapeutic effects to address various health conditions. It has shown promise in combating ischemia (restricted blood flow), joint diseases, immunosuppression, degenerative diseases and infections. As an immunomodulator, ozone can help regulate and enhance the immune system's response to infections and other challenges[17]. Additionally, the therapy's immunostimulant properties can aid in augmenting immunity, which can be beneficial in fighting microbial infections caused by pathogenic microorganisms[18].

It is worth noting that while ozone therapy holds potential as an alternative treatment for infections, its safety and efficacy need to be thoroughly studied and validated through scientific research and clinical trials. The appropriate dosage and mode of administration must be carefully determined to maximise therapeutic benefits while minimising potential side effects. Collaborative efforts among researchers, healthcare providers and regulatory bodies are essential in establishing evidence-based guidelines and protocols for the responsible use of ozone therapy in medical practice. Ozone therapy has a long history of use as a disinfectant in various settings and has shown promising therapeutic effects against different health conditions[19]. As research and understanding of its mechanisms improve, ozone therapy may become a valuable adjunct to conventional treatments in combating infections and supporting overall health. However, it is vital to approach its implementation cautiously, ensuring that evidence-based practices and safety measures are followed to optimise its potential benefits for global public health (Table 1).

**Mechanism of ozone action:** O3 reacts with various bioactive molecules stimulating an endogenous cascade of responses by activating various substrates[23]. A study conducted by Costanzo *et al*.[24] explained the effects of O3 on Cytoskeletal Organization, Mitochondrial Activity and Nuclear Transcription *in vitro*[25]. In another *in vitro* study conducted by Sharma *et al*.[25], O3 effectively reduced the CFU concentrations of Acinetobacter baumannii, Clostridium difficile and methicillin-resistant Staphylococcus aureus in dry as well as wet samples[26]. Oxidative stress is the disturbance between prooxidants and antioxidants in the body. Excessive prooxidants potentially damage cellular components such as lipids, proteins and DNA by oxidation[27]. O3 is a potent bioactive oxidant that decomposes into oxygen and nascent oxygen. This molecule is highly reactive and quickly reacts with the functioning of carbohydrates, proteins and lipids, causing a breakdown of the bacterial cell wall[17,23].

O3 is readily soluble in plasma, extracellular fluid, respiratory tract and gut mucosa through passive diffusion. There it reacts with the bacterial cell envelope containing sulphydryl groups, which are abundant in microbial enzymes, causing its oxidation, leading to enzymatic degradation of the bacteria[23,24]. It also reacts with polysaccharides and liposome shells of the microorganism, disrupting the nucleic acid and damaging the bacterial membrane necessary for maintaining bacterial cell integrity. It causes increased permeability of ozone molecules into the cell[28]. There it further causes breakage of glycosidic bonds leading to the formation of aliphatic acid and aldehyde. The reaction of ozone with this aliphatic acid and aldehyde compounds causes the formation of hydroxyl radicals, which initiate irreversible damage to the bacterial cell wall and cytoplasmic membrane[23]. The peptidoglycans in gram-positive and gram-negative bacteria comprise N-acetylglucosamine and N-acetylmuramic acid, which are connected with beta-1,4 glycosidic bonds[28]. Ozone cleaves the glycosidic bonds and attacks the peptidoglycan molecule's ordinary nitrogen atoms or

Table 1: Recent studies In the field of ozone prove it to be a potent antibacterial agent:

| Author and year | Bacteria involved | Type of study | Test | Time of ozone exposure | Result |
|---|---|---|---|---|---|
| Rangel *et al.*[9]. | *Staphylococcus aureus*, *Salmonella enterica* subsp. enterica serovar *choleraesuis*, *Escherichia coli* *Pseudomonas aeruginosa* | *In vitro* | Effect of Ozone on bacterial Cell membrane permeability Intracellular ROS levels | 24 hours | *E. coli*- inhibition of about 30% and increase in intracellular ROS by 215%, *P. aeruginosa*-inhibition of about 25% increase in intracellular ROS by 173%, *A. baumannii*-inhibition by 15% and an increase in intracellular ROS by 175% |
| Bitter *et al.*[20] | Enterococcus faecalis | *Ex Vivo* | Effects of: Diode laser Gaseous Ozone Medical dressings in the root canal | Application of 60 s gaseous ozone via endodontic cannulas with a 100 mL $min^{-1}$ flow rate. (O3 concentration: 2100 ppm, equivalent to 4.49 g $m^{-3}$) | Combining gaseous ozone and laser irradiation with NaOCl irrigation showed significant bacterial reductions of *E. faecalis* to the application of medical dressings |
| Breidablik *et al.*[21] | *Escherichia coli* | *Ex Vivo* | Effects of hand disinfection with: Alcohol hand rub Ozonised water Soap water | The ozone concentration in the surrounding air was equal to or less than 0.01 parts per million (ppm) | Alcohol removed: bacteria in 10 out of 35 participants; ozonised water eradicated: Bacteria in 10 out of 55 participants Soap washing: Removal of bacteria in 6 out of 20 participants Thus ozonised water is a potential alternative and effective disinfectant against *E. coli* |
| Tonon *et al.*[22] | Porphyromonas gingivalis Fusobacterium nucleatum Streptococcus oralis | *Ex Vivo* | Antimicrobial effect of ozonised physiological saline solution in different concentrations against oral biofilms | Application of O3 saline solution at concentrations 25, 50 and 80 µg $NmL^{-1}$: 30 sec and 1 min | Ozonised treated biofilms showed antibacterial activity at a concentration of 80 µg $NmL^{-1}$ for 30 sec and 1 min, mainly against Porphyromonas gingivalis |

R-group or both. It releases hydroxy-hydroperoxides causing irreversible damage to the cell wall composition of the bacteria and better bacteriostatic and bactericidal effects[18,20]. A similar interaction occurs in virus-to-cell contact by damaging the viral capsid via lipoprotein and glycoprotein oxidation and damaging the reproductive viral cycle[24].

**Ozone and cost-effectiveness in the Indian scenario:** Ozone therapy is a more economical and potentially safer alternative than the cost, side effects and conventional medical therapy against bacterial infections[16]. The economic advantage of ozone therapy lies in its relatively lower cost than conventional antibiotic treatments. With the rising concern over the financial burden of healthcare, ozone therapy offers an attractive option for patients and healthcare systems to explore. Conventional medical therapies, especially those involving antibiotics, can be costly due to pharmaceutical drug development, production and distribution. Additionally, the overuse or misuse of antibiotics can contribute to the emergence of antimicrobial resistance, leading to the need for even more expensive and potent antibiotics in the future.

In contrast, ozone therapy involves using ozone gas, which can be generated on-site, reducing the dependence on expensive pharmaceuticals. The cost-effectiveness of ozone therapy may significantly reduce medical expenses for patients and healthcare facilities. Furthermore, one of the essential advantages of ozone therapy is its potential to minimise adverse effects or side effects compared to conventional antibiotics. Antibiotics can sometimes lead to side effects, such as gastrointestinal disturbances, allergic reactions and disruptions in the body's natural microbiota. In contrast, ozone therapy's mechanism of action primarily involves oxidative stress on pathogens, leaving healthy cells relatively unharmed[16]. This targeted approach may translate into a reduced incidence of adverse effects and better patient tolerance, enhancing the overall safety profile of ozone therapy. Besides its economic advantages and potential safety benefits, ozone therapy also holds promise in addressing the growing concern of antimicrobial resistance. As ozone's mode of action differs from conventional antibiotics, it may present a new avenue for treating infections caused by drug-resistant bacteria. It could help preserve the effectiveness of existing antibiotics for other critical cases while offering an effective treatment option for infections that have developed resistance. In India, as in many other countries, the initial setup cost for establishing a clinical ozone therapy practice may be approximately 1 lakh 40 thousand rupees. While the initial investment may be a consideration for

healthcare providers and institutions, the long-term cost-effectiveness and potential positive impact on patient outcomes make it a worthwhile investment in the fight against bacterial infections and antimicrobial resistance.

However, it is essential to acknowledge that ozone therapy, like any medical intervention, should be administered responsibly and by evidence-based guidelines and protocols. More research and clinical trials are needed to further validate the efficacy and safety of ozone therapy for various bacterial infections. Collaborative efforts between healthcare professionals, researchers, policymakers and regulatory bodies are essential to promote responsible and effective integration of ozone therapy into medical practice to benefit patients and global public health.

## CONCLUSION

The development of bacterial resistance against drugs, particularly multiple drug resistance (MDR), has become a pressing concern. Antibiotic resistance not only complicates treatment but also threatens public health on a global scale. A multifaceted approach is necessary, which includes promoting responsible antibiotic use, developing new antibiotics and alternative therapies and implementing robust infection control measures. One alternative therapy is ozone, which has demonstrated potent antibacterial properties in recent studies. Ozone therapy is safer and more economical than conventional medical treatments. Its action primarily involves oxidative stress on pathogens, leaving healthy cells relatively unharmed, which could minimise adverse effects. Moreover, ozone therapy may offer a new avenue for treating infections caused by drug-resistant bacteria, contributing to the fight against antimicrobial resistance. While ozone therapy holds promise, further research and clinical trials are necessary to validate its efficacy and safety for various bacterial infections. Collaborative efforts among healthcare professionals, researchers, policymakers and regulatory bodies are crucial in establishing evidence-based guidelines and protocols for the responsible use of ozone therapy in medical practice. By doing so, we can optimise its potential benefits and foster a collective effort to preserve the effectiveness of antibiotics and safeguard public health for generations to come.

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