Axia SCIENTIFIC PUBLISHING

OPEN ACCESS JOURNALS

Axia Journal of Orthopedics

Comparative study of antimicrobial properties of gold nanoparticles

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Received: 03 June, 2025
Accepted: 18 June, 2025
Published: 23 June, 2025

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Citation: Santhosh Kumar T N, Comparative study of antimicrobial properties of gold nanoparticles.,Axia Journal of Orthopedics (AJO) 2025;1(1):1001.

Abstract

Introduction: Gold nanoparticles are known for its antimicrobial and immune response eliciting properties. It has been using for centuries in prevention of infectious diseases by increasing the immunity of the host.

Objective: The role of antibacterial therapy is the gold standard in all the established guidelines for prevention, mitigation, treatment and control of the known bacterial species which are harmful to human health. In this study we determined the antibacterial effectiveness of high dose of amoxicillin/potassium clavulanate and gold nanoparticles (AuNPs) sourced from different pharmaceutical companies.

Methodology: After obtaining the informed written consent from the subject, culture swabs were obtained from the Fournier's gangrene. The collected sample is made to grow on blood agar and MacConkey agar in ambient temperature of 370 Celsius over 24 hours. The growth is observed and confirmed as Klebsiella pneumonia a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Appearing as a mucoid lactose fermenter on MacConkey agar. Klebsiella pneumonia is isolated and inoculated on Mueller-Hinton Agar. The agar well diffusion method is carried out with 4 well containing AuNPs I, AuNPs II, amoxicillin/potassium clavulanate and blank respectively. The results are observed after 24 hours and 48 hours of inoculation.

Results: The Agar well diffusion method containing AuNPs I, AuNPs II, amoxicillin/potassium clavulnate and blank respectively after 24 hours showed higher Zone of Inhibition for amoxicillin/potassium clavulnate rather than AuNPs I & II and no Zone of inhibition around the blank well. The Zone of Inhibition observed after 48 hours of inoculation is 23 mm for amoxicillin/potassium clavulnate, 18 mm for AuNPs I and 15 mm for AuNPs II. No Zone of inhibition is observed around blank even after 48 hours.

Discussion and Conclusion: The rate of diffusion of gold nanoparticles in the particular agar is not known and due to heavy metal of gold and its insolubility property in water makes it difficult for liquid preparation. The Zone of Inhibition (ZOI), Minimum Inhibition Concentration (MIC) of the gold nanoparticles is not well established. The accuracy of the agar well diffusion method for evaluation of antimicrobial property of gold nanoparticles is not yet standardized. Keeping all these limitations of this study, we present our data about antimicrobial activity of gold nanoparticles against Klebsiella pneumoniais minimum as compared to amoxicillin/potassium clavulnate. The further studies can be carried with functionalized AuNPs in the prevention of antimicrobial resistance.

Keywords: Gold Nanoparticles (AuNPs), Agar well diffusion method, Zone of inhibition (ZoI), amoxicillin/potassium clavulnate and Klebsiella pneumonia.

INTRODUCTION

Antibiotic resistance is a major threat to global health. Resistant bacterial infections are currently resulting in over 1.2 million deaths every year1 and are projected to reach 10 million casualties annually by 2050. This crisis is due to the continuous emergence and spread of antibiotic-resistance genes across important human pathogens and the limited introduction of new, broad-acting, clinically useful antimicrobials since the 1970s2. Alongside genetic resistance, alternative bacterial lifestyles during infection, such as persistence3 and growth in biofilms4, also contribute to patient mortality due to ineffective antibiotic treatment. As such, it has become essential to not only boost our currently insufficient preclinical and clinical antimicrobial development pipelines5 but to also safeguard the longevity of our approved antibiotics. Both feats heavily rely on our ability to evaluate the efficacy of antimicrobial agents against bacteria, which underpins the identification of antibiotic-resistant strains in the clinic and enables testing of novel antibiotic or antibiotic-adjuvant candidates. A wide variety of medicinal products which originate from natural compounds and which are widely used to target and treat various appear diseases. The extraction of these complicated chemical molecules from plants, animals, microorganisms and minerals are common natural sources via several extraction processes, these compounds work as an initiator of future sinker molecules. Gold is inert and universally recognized as biocompatible. Until the recent past, it was only known as the metal. With the arrival of nanotechnology and the discovery of nanoparticles and the exploration of the physico-chemical properties of gold make it a supreme material for progress fields6,7. A nanoparticle is defined as a tiny particle with a size ranging between 1 and 100 nm. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts 8,9. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically. Zone of inhibition (ZOI), also known as a zone of clearing or a halo assay, refers to the clear zone surrounding an antimicrobial agent. These ZOIs result from a complete absence of bacteria on, or within a confluent bacterial lawn. The antimicrobial activity of the agent is screened against a test organism which is used to create a confluent lawn of bacterial growth on an agar plate. The ZOI is measured in mm after 24 to 48 hours of incubation. A primary function of the immune system is to protect the host from pathogenic microbial infections. Innate immune mechanisms dominate during the early phase of the antimicrobial immune response. As the innate response sometimes does not clear the infecting microbe, T lymphocytes and neutralizing antibodies may often be required for its complete elimination. The innate response is nonetheless indispensable for host survival since it prevents such microbes from spreading in an uncontrolled manner and thereby provides the host with the “grace period” of 3-4 d required for the activation of an efficient adaptive immune response.

MATERIAL AND METHODS

The amoxicillin/potassium clavulanate is obtained from Karnataka antibiotic and pharmaceuticals limited plot no 14, II Phase, Peenya, Bengaluru 560058. AuNPs I is obtained from Shree Dhootapapeshwar Limited,135, Nanubhai Desai Rd., Khetwadi, Mumbai – 400 004. AuNPs II is obtained from Nanoshel United Kingdom through its subsidiary located in Punjab, India.

Composition of Blood Agar

Blood agar, like most other nutritional media, has one or more protein sources, salt, and beef extract for vitamins and minerals. Besides these components, 5% defibrinated mammalian blood is also added to the medium. The blood agar base is commercially sold by various vendors, or it can also be prepared in the laboratory if the necessary ingredients are available. The exact composition of the blood agar base is given below: [Table]

Table 1: Composition of Blood Agar.

S.N	Ingredients	Gram/liter
1.	Peptone	10.0
2.	Tryptose	10.0
3.	Sodium chloride	5.0
4.	Agar	15.0
Final pH at 25°C: 7.3 ±0.2		

Blood Agar and Hemolysis

Hemolysis is the lysis of red blood cells in the blood due to the extracellular enzymes produced by certain bacterial species. The extracellular enzymes produced by these bacteria are called hemolysins which radially diffuse outwards from the colonies, causing complete or partial lysis of the red blood cells. Four different types of hemolysis are observed on blood agar which can each be identified by a zone of hemolysis present around the growing colonies.

Alpha hemolysis:

- Alpha hemolysis is defined by a greenish-grey or brownish discoloration around the colony as a result of the partial lysis of the red blood cells.

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- During α-hemolysis, H2O2 produced by the bacteria causes haemoglobin present in the RBC of the medium is converted into methaemoglobin.
- Some of the α-haemolytic species are a part of the human normal flora, but some species like Streptococcus pneumonia cause pneumonia and other such severe infections.

Beta hemolysis:

- Beta hemolysis is defined by a clear zone of hemolysis under and around the colonies when grown on blood agar.
- The clear zone appears as a result of the complete lysis of the red blood cells present in the medium, causing denaturation of haemoglobin to form colourless products.
- β-haemolytic bacteria include group A streptococci like S. pyogenes and group B streptococcus like S. agalactiae, both of which are associated with severe infections in humans.

Gamma hemolysis:

- Gamma hemolysis is also called non-hemolysis as no lysis of red blood cells occurs.
- As a result, no change of coloration or no zone of hemolysis is observed under or around the colonies.
- Species like Neisseria meningitis are non-haemolytic or gamma-haemolytic.

Alpha prime or wide zone alpha hemolysis:

- Alpha prime hemolysis is defined by a small zone of intact erythrocytes adjacent to the bacterial colony, with a zone of complete lysis of RBCs surrounding the zone of intact erythrocytes.
- This might be confused with β-hemolysis due to the appearance of a clear zone around the colonies.

Principle of Kirby Bauer Disc Diffusion Method

The organism to be tested must be incubated overnight in broth and must be compared with the 0.5 McFarland Turbidity standard. Mueller- Hinton agar must be used as it does not inhibit sulphonamides and ensures reproducibility and with composition and PH of the medium. The agar when poured on Petri dishes should be 4mm.

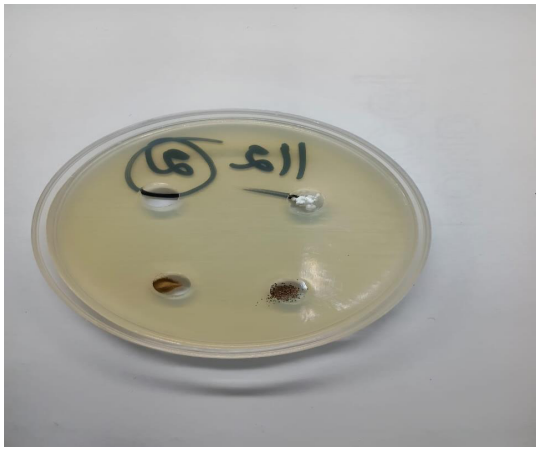
Materials Required

- Mueller- Hinton agar
- Antibiotic discs
- Cotton swabs
- Petri dishes
- 0.5 McFarland Turbidity standard
- Inoculum
- Forceps
- Metric ruler or caliper

After obtaining the informed written consent from the subject, culture swabs were obtained from the Fournier's gangrene. The collected sample is made to grow on blood agar and MacConkey agar in ambient temperature of 370 Celsius over 24 hours. The growth is observed and confirmed as Klebsiella pneumonia a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Appearing as a mucoid lactose fermenter on MacConkey agar. Klebsiella pneumonia is isolated and inoculated on Mueller-Hinton Agar. The agar well diffusion method is carried out with 4 well containing AuNPs I, AuNPs II, amoxicillin/potassium clavulnate and blank respectively. The results are observed after 24 hours and 48 hours of inoculation.

RESULTS

The Agar well diffusion method containing AuNPs I, AuNPs II, amoxicillin/potassium clavulnate and blank respectively after 24 hours showed higher Zone of Inhibition for amoxicillin/potassium clavulnate rather than AuNPs I & II and no Zone of inhibition around the blank well. The Zone of Inhibition observed after 48 hours of inoculation is 23 mm for amoxicillin/potassium clavulnate, 18 mm for AuNPs I and 15 mm for AuNPs II. No Zone of inhibition is observed around blank even after 48 hours. [Figures].



1. Initial adding of drugs to agar well with Klebsiella pneumonia on the Mueller-Hinton Agar.

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2. Obseravtion after 24 hours of inoculation.

3. Observation after 48 hours inoculation.

DISCUSSION

Antibiotic intervention is an effective treatment strategy for many bacterial infections and liberates bacterial antigens and stimulatory products that can induce an inflammatory response. Despite the opportunity for bacterial killing to enhance the development of adaptive immunity, patients treated successfully with antibiotics can suffer from reinfection. Since the discovery of penicillin in 1928, antibiotics have been widely used to treat bacterial infections, and as a result, bacteria have rapidly developed antibiotic resistance [1-2]. The development of multidrug-resistant (MDR) bacteria is now a critical issue in modern medicine, with the concern that serious bacterial infections will reemerge in the 21st century in the absence of effective treatment options [3-6]. Despite this important issue, antibiotics remain an effective treatment option for many common infectious diseases.

An adaptive immune response to infection is initiated by recognition of foreign protein antigens in the presence of local inflammation [7]. The contextual inflammatory cues come from innate immune cells that encounter bacterial products, and these signals profoundly affect the subsequent adaptive immune response [8]. This initial activation stage occurs within local lymph nodes and causes low-frequency naive T cells and B cells to produce an army of effector cells to eradicate a complex pathogen [9-10]. Effective antibiotic therapy will kill a large number of bacteria, thus liberating antigen for lymphocyte recognition and releasing bacterial products that can amplify local inflammatory responses. Thus, antibiotics have a direct effect on bacterial growth but also have the potential to enhance an ongoing pathogen-specific adaptive immune response. However, many studies have shown that antibiotic administration can paradoxically weaken immune memory, leaving a recovered host fully susceptible to reinfection with the same pathogen [11-13]. The mechanistic basis for this detrimental effect of antibiotics on immune memory and protection is incompletely understood. A more detailed understanding of this phenomenon might allow the development of targeted strategies to encourage immune memory development and support long-lasting protection from reinfection.

CONCLUSION

The gold nanoparticles act internally by increasing the host defence rather than the lysis of the cell as an antimicrobial.

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