

## ASSESSMENT OF GOLD NANOPARTICLES AS POTENTIAL ANTIMICROBIAL AGENTS IN MULTIDRUG RESISTANT E. COLI FROM VETERINARY SOURCE

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### Abstract

Multidrug-resistant (MDR) *Escherichia coli* are a zoonotic pathogen with limited therapeutic options that are a serious threat in veterinary settings worldwide. The present study aimed to assess the antimicrobial activity of gold nanoparticles (AuNPs) against MDR *E. coli* of veterinary origin. 100 samples (faecal, milk, nasal and environmental swabs) were collected and processed for bacterial isolation and identification by the application of standard microbiological, biochemical and analytical techniques. Antibiotic susceptibility testing was conducted using the Kirby-Bauer method, and MDR isolates were chosen for further analysis. The gold nanoparticles were prepared by a chemical reduction process, and the prepared gold nanoparticles were characterized by UV-visible spectroscopy and transmission electron microscopy (TEM). The zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated by using antibacterial activity using the agar well diffusion method and broth micro dilution method. Of the 68 confirmed *E. coli* isolates, 52 (76.4%) were MDR. AuNPs showed good antibacterial activity with increasing concentration. The MIC and MBC values indicated strong bactericidal activity at lower concentrations. The morphological analysis showed the presence of structural damage to the bacterial cell wall, with the disruption of the membrane and the leakage of the cytoplasmic content, thus indicating a multi-targeted action against the bacteria. The results showed that AuNPs have a great potential to be used as alternative antimicrobial agents in veterinary medicine against MDR *E. coli*. The resistance mechanisms that they have to overcome, such as efflux pumps and biofilm formation, provide the promise that they could help solve the global problem of antimicrobial resistance.

## Introduction

Antimicrobial resistance (AMR) is a significant health risk in human and animal health and has become a serious global problem as it is emerging and spreading quickly amongst bacterial pathogens (Serwecińska, 2020). *E. coli* is one of the most problematic organisms because it is ubiquitous, is able to survive in different environments and can transfer and spread resistance determinants. Multidrug-resistant (MDR) *E. coli* strains, especially those of veterinary origin, pose a serious risk, as they can be spread to humans by direct contact, environmental contamination, or the food chain (Bondad-Reantaso et al., 2023). The zoonotic potential calls for alternate antimicrobial strategies that are effective, safe and resistant to the current resistance mechanisms.

Antibiotic use in food animals has been extensive and is not always controlled, which has increased selection pressure for the development of resistant populations of bacteria (Tomczyk et al., 2020). Antibiotics are being increasingly used routinely for growth promotion and prophylaxis in many areas, in addition to their therapeutic role. This has led to increased detection of MDR *E. coli* in animals, animal products and the farm environment (Prevention, 2019). Many of these strains are also multi-resistant, often to antibiotics of several different families, such as  $\beta$ -lactams, aminoglycosides, tetracyclines and fluoroquinolones, which diminishes treatment options and results in the potential for therapeutic failure. The horizontal transfer of resistance genes through the transfer of plasmids, transposons and integrons and the rapid transfer of resistance between and within species are made worse (Martínez-Álvarez et al., 2024).

Nanotechnology, a field of interest, now offers promising innovative solutions to combat antimicrobial resistance in this context (Huang et al., 2024). Gold nanoparticles (AuNPs) have emerged as one of the most promising nanomaterials because of their unique physicochemical characteristics, such as their high surface area-to-volume ratio, tunable size and shape and ease of functionalization. All these properties make it possible for AuNPs to interact properly with microbial cells and thus increase their antimicrobial activity (Querido, 2021).

Gold nanoparticles can have multiple mechanisms of action, unlike conventional antibiotics, which target the specific processes, making the development of resistance less likely.

The antimicrobial activity of AuNPs was explained by different mechanisms, such as interaction with the bacterial cell membrane, generation of reactive oxygen species (ROS), inhibition of intracellular components, including proteins and DNA, and inhibition of enzymatic pathways (Sidhu et al., 2022). In addition, AuNPs can be functionalized with drugs or other bioactive molecules to facilitate targeted delivery that allows the delivery and effects of the bioactive molecules to be increased. The multipurpose nature of gold nanoparticles will pave the way towards the development of future antimicrobial drugs (Dutta et al., 2023).

A number of recent studies demonstrated the ability of gold nanoparticles as effective tools for the treatment of various bacterial pathogens, including MDR (Lombardo et al., 2019). However, antimicrobial activity can be different depending on the particle size, concentration, surface charge and synthesis method, and this can have a significant effect on the activity against different strains of bacteria, including veterinary strains. Furthermore, very limited knowledge exists on the interaction of AuNPs with bacterial cells from veterinary medicine, particularly with MDR *E. coli* (Rehan et al., 2024). It is essential to comprehend these interactions if the potential of using gold nanoparticles in veterinary medicine is to be assessed for practical use and to understand their potential in reducing zoonotic spread of resistant pathogens.

The biocompatibility and safety profile of gold nanoparticles is another crucial factor to consider. AuNPs are less toxic than the other metallic nanoparticles, yet there is a need to carefully evaluate their effect on animals and human beings before clinical/field use (Shanbhag et al., 2023). Dosage, route of exposure and accumulation must be carefully considered to provide safe use. The present study is designed to evaluate the antimicrobial activity of gold nanoparticles against the multidrug-resistant *E. coli* isolated from veterinary sources due to the urgency to find some alternative

antimicrobial methods (Wypij et al., 2021). The aim of this research is to examine their effectiveness, how they work, and any possible drawbacks, which will aid in expanding knowledge about nanotechnology-based interventions for the battle against antimicrobial resistance. In conclusion, the results might help develop new, effective and sustainable methods to manage MDR pathogens in veterinary settings and limit their contribution to public health.

### Literature Review

The rise of antimicrobial resistance (AMR) has complicated the search for alternative treatments for multidrug-resistant (MDR) *Escherichia coli* that originate from veterinary sources (Sharma et al., 2024). *E. coli* is a normal intestinal flora and an opportunistic pathogen of animals, associated with infections like mastitis and infections of the urinary tract and enteric diseases. But the global and indiscriminate use of antibiotics in animal farming, however, has helped the spread of MDR strains, representing a grave risk to animal and human health (Mohapatra et al., 2021). An indirect risk is that the bacteria could be transmitted from earlier in the food chain to humans via animal contact and the environment, making some new approaches to dealing with antimicrobial resistance necessary.

AMR has been a promising interdisciplinary area to leverage new tools through nanotechnology. Nanoparticles, particles between 1 and 100 nm, exhibit such properties that they are uniquely reactive, bioactive and changeable in surface morphology and surface area, which are considered key properties of biological activity (Mallakpour & Hussain, 2025). These properties allow nanoparticles to interact well with the microbial cells; thus, they are excellent candidates for antimicrobial applications. In veterinary medicine, nanoparticles have been shown to have bactericidal, antiviral, antifungal and anti-biofilm properties, indicating their potential for a wide spectrum against infectious agents (Roman et al., 2023).

Among other nanomaterials, the technology of gold nanoparticles (AuNPs) has attracted special attention for their potential in a number of applications due to their stability, drug responsiveness, and biocompatibility (Saleh & Hassan, 2023). Unlike the pathway-specific

antibiotics that are commonly used, AuNPs show multiple target mechanisms for antimicrobial activity. They involve membrane disruption of bacteria, generating reactive oxygen species (ROS), affecting the processes of DNA replication and protein synthesis, and even inhibiting vital enzyme activity (Guo et al., 2024). All of these complex effects prevent the likelihood of bacteria developing resistance, and thus AuNPs offer hope of replacing traditional antimicrobials.

AuNPs' strong antimicrobial activity against a range of pathogens, including MDR *E. coli*, *S. aureus*, *P. aeruginosa*, and *A. baumannii*, was observed in recent studies (Wang et al., 2023). Many physicochemical properties influence their efficiency: particle size, shape, surface charge density and functionalization. Squeeze these tiny particles because they've got a lot of surface area, which means antimicrobial activity has increased due to increased interactions with cells of the microbes (Koski-Karell, 2021). In addition, surface modifications can increase their antibacterial activity and allow targeted drug delivery.

One of the most important uses of AuNPs in veterinary practice is their applicability in the control of infection in farm animals with the aim of lowering the use of traditional antibiotics (Hamid et al., 2023). The delivery systems of drug formulations using nanoparticles have been investigated for many years, as it is possible to create nanosized particles that can deliver antimicrobial agents in a controlled and sustained manner, offering enhanced therapeutic potential. In addition, their antibiofilm activity is significant because biofilm is an important aspect that affects bacterial antibiotic resistance (Shehabeldine et al., 2022). The action of AuNPs breaks up biofilms, which increases the susceptibility of MDR pathogens to therapy.

Another intriguing finding about gold nanoparticles is that they can be used alongside current antibiotics in a combination therapy that is more effective than using the antibiotics alone (Gatou et al., 2024). Antibiotic-conjugated AuNPs have also been found to be quite effective against antibiotic-resistant bacteria such as an *E. coli* strain that produces the extended-spectrum  $\beta$ -lactamase (ESBL). This synergistic combination

not only enhances the antibacterial properties, but it also provides the decreased doses of the antibiotics and hence reduces their side effects as well as the emergence of bacterial resistance (Mendes et al., 2022).

Although these benefits were considerable, there are some difficulties associated with implementing these AuNPs. A variability of synthesis methods giving rise to different results regarding the antimicrobial activity indicates the necessity for standardizing the methods (Mittal et al., 2020). Also, prior to globalization, issues of toxicity, bioaccumulation and environmental effects will need to be addressed. While AuNPs are known to be biocompatible, their safety will be dependent on dosage, route of exposure, and particle properties, which can vary significantly based on the specific application and context in which they are used (Huang et al., 2024b).

## Materials and Methods

### Study Design and Sample Collection

The antimicrobial activity of the nanogold particles (AuNPs) against multidrug-resistant (MDR) *Escherichia coli* of veterinary origin was investigated by a cross-sectional in vitro study. One hundred clinical and environmental samples were collected under aseptic conditions from animal samples such as nasal swabs, milk, farm wastewater and excreta (faeces) of livestock, including those from the dairy farms and clinics. Samples were collected and sent to the microbiology lab in sterile sample containers with refrigeration (4 °C) and processed as soon as possible, but no later than 24 hours.

### Isolation and Identification of *E. coli*

The samples were cultured on MacConkey and Eosin Methylene Blue (EMB) agars at 37°C for 18-24 hours. Presumptive *E. coli* colonies - pink colonies on MacConkey agar and a metallic green sheen on EMB agar - were selected and purified. Conventional biochemical tests, such as indole, methyl red, and Voges-Proskauer tests, citrate utilization, and reaction with triple sugar iron (TSI), were used to confirm identification. Polymerase chain reaction (PCR) of the *uidA* gene was used to molecularly confirm the identity.

### Antibiotic Susceptibility Testing

The determination of the antimicrobial susceptibility of confirmed isolates was done by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per the Clinical and Laboratory Standards Institute (CLSI). A panel of popular antibiotics, namely ampicillin, tetracycline, ciprofloxacin, gentamicin and cefotaxime, was used. The isolates with resistance to three or more antibiotic classes were considered to be MDR and selected for further analysis.

### Synthesis and Characterization of Gold Nanoparticles

The chemical reduction technique was employed to prepare the nanoparticles of gold. In short, an aqueous solution of chloroauric acid ( $\text{HAuCl}_4$ ) was reduced using a solution of trisodium citrate while it was being stirred and heated (pumped) under control. The color change from pale yellow to ruby red was observed as the sign of the formation of AuNPs. Surface plasmon resonance was confirmed through the synthesized nanoparticles by UV-visible spectrophotometry; their size and morphology were analyzed by transmission electron microscopy (TEM), and particle size distribution by dynamic light scattering (DLS) was carried out for measuring the zeta potential of the synthesized nanoparticles.

### Preparation of AuNPs for antimicrobial Assay

A stock solution of AuNPs was prepared in sterile distilled water and filtered using a 0.22  $\mu\text{m}$  membrane filter. Sample dilutions were used to achieve various concentrations (10, 25, 50 and 100  $\mu\text{g}/\text{mL}$ ) for antimicrobial evaluations.

### Antimicrobial Activity Evaluation

AuNPs were evaluated for their antimicrobial activity by agar well diffusion and broth micro dilution methods against MDR *E. coli* isolates. To perform the agar well diffusion method, bacterial suspensions equivalent to the McFarland 0.5 were loaded onto Mueller-Hinton (MH) plates. Various sized AuNPs and concentrations were filled into the wells. These plates were then inoculated and incubated for 24 hours at 37°C, and the zone of inhibition was measured.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were obtained by a broth micro dilution assay on 96-well microtiter plates. Bacterial growth was examined visually and by an OD at 600 nm. The lowest concentration that did not show growth was recorded as MIC, and MBC was determined by sub-culturing on an agar medium.

#### Statistical Analysis

Each experiment was repeated three times, and the data presented as mean  $\pm$  SEM. The data obtained were analyzed using SPSS software (version 25.0). The one-way analysis of variance (ANOVA) was used to analyze the differences among groups with a p-value less than 0.05.

**Table 1:** Isolating and sampling distribution of *E. coli*.

Sample Type	No. of Samples	Positive Isolates	Prevalence (%)
Fecal samples	30	24	80
Milk samples	25	16	65
Nasal swabs	20	11	55
Environmental swabs	25	15	60
<b>Total</b>	<b>100</b>	<b>68</b>	<b>68</b>

#### Resistance to Multiple Antibiotics

Most bacteria showed various antibiotic resistance profiles. Out of the 68 bacteria sampled, 52, bacteria or 76.4%, showed

#### Ethical Considerations

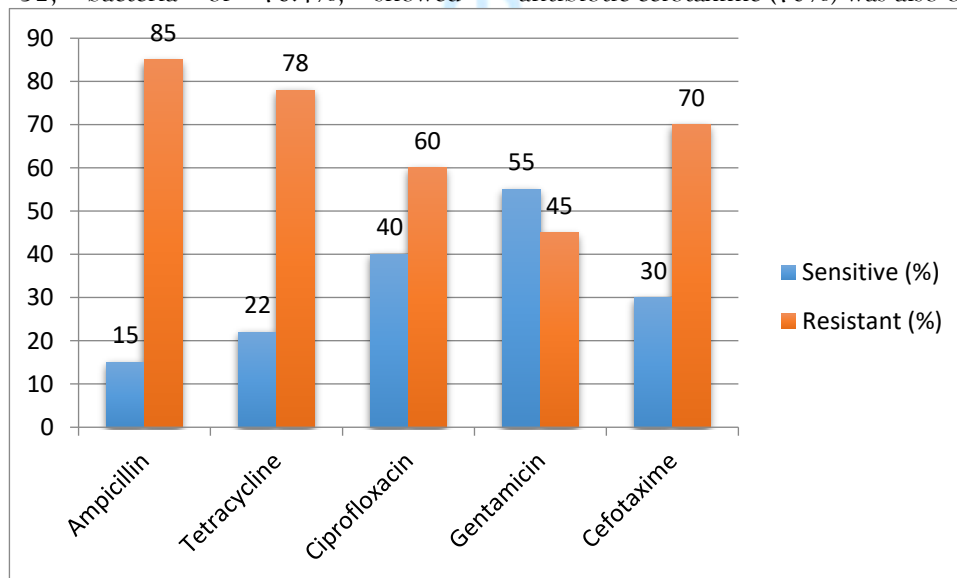
All the work with animals was carried out in an ethical manner within the confines of the institution. There was no animal-harm incidences noted as a result of the sample taking.

#### Results

##### Isolation and Prevalence of *E. coli*

Of 100 veterinary samples, 68 (68%) were confirmed as *Escherichia coli* isolations. The highest percentage of prevalence across the sample types was demonstrated in faeces (80%), followed by milk (65%), nasal swabs (55%), and environmental samples (60%).

resistance to multiple antibiotics. Resistance levels were the highest when it came to ampicillin (85%) and tetracycline (78%). Resistance to the antibiotic cefotaxime (70%) was also observed.



**Figure 1:** Pattern of antibiotic resistance in MDR *E. coli*

#### Gold Nanoparticles (AuNPs) Characterization

The presence of an absorption peak at 525 nm in UV-visible spectroscopy confirmed that the

formation of AuNPs had occurred. The AuNPs' average size (18–30 nm) was also confirmed by TEM imaging.

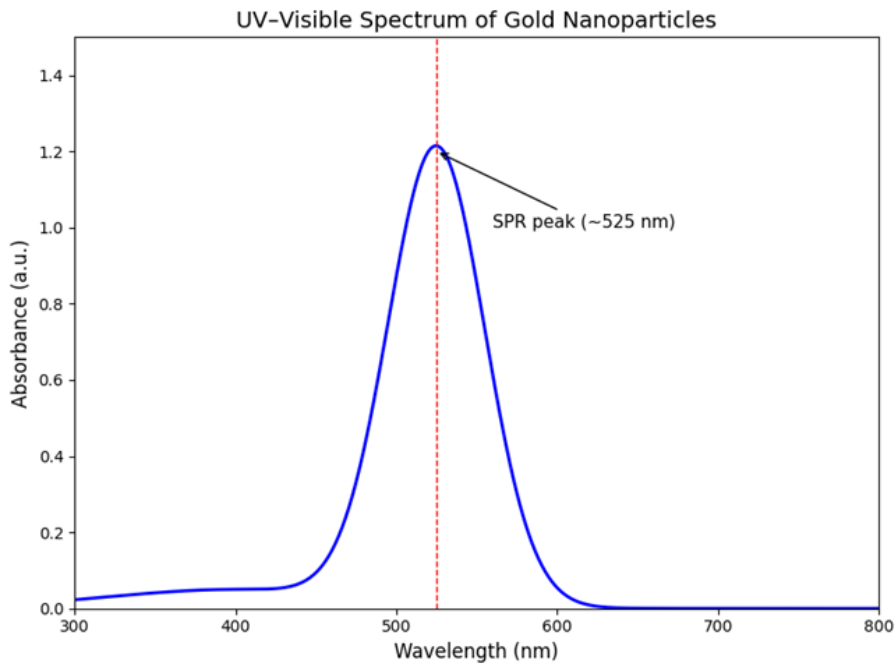


Figure 2: UV-visible range of the created gold nanoparticles (maximum at ~ 525 nm)

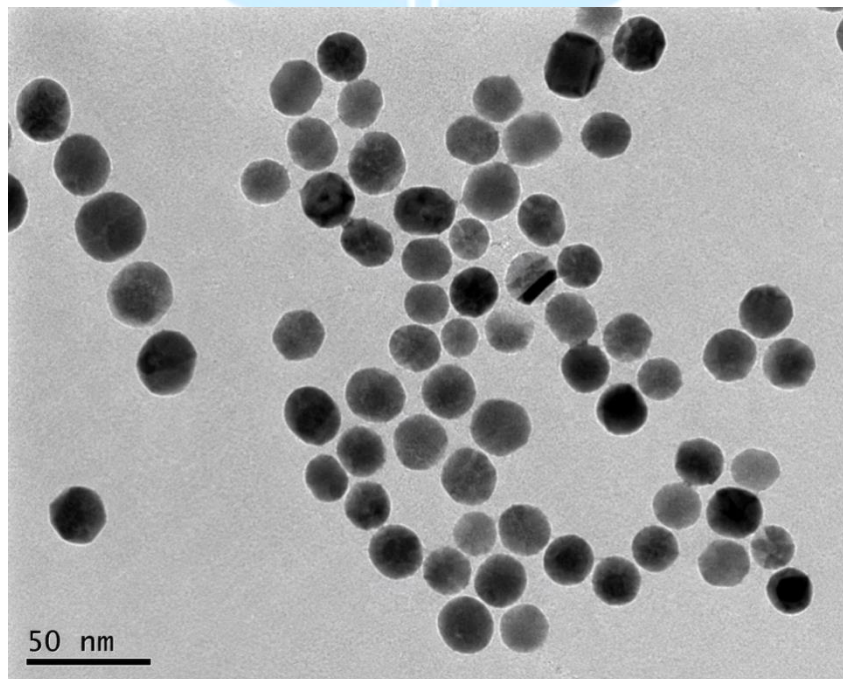


Figure 3: Spherical Gold Nanoparticle Detection with TEM Microscopy with Sizes from 18 nm to 30 nm

**AuNPs' Antimicrobial Effects**

AuNPs showed a dose-dependent inhibitory effect against drug-resistant strains of *E. coli*. With a dose as high as 100 µg/mL, it showed the

presence of a zone of inhibition which was largest among the samples ( $18.6 \pm 0.4$  mm). With the lowest dose of 10µg/mL, the largest zone of inhibition was  $9.2 \pm 0.3$  mm.

Table 2: Zone of inhibition (mm) for AuNPs against MDR *E. coli*

AuNP Concentration	Zone of Inhibition (mm)
10 µg/mL	$9.2 \pm 0.3$
25 µg/mL	$12.5 \pm 0.5$

50 µg/mL	15.8 ± 0.4
100 µg/mL	18.6 ± 0.4

### Defining MIC and MBC

The MIC of AuNPs was determined to be between 12.5 and 25 µg/mL, while the MBC was found to be between 25 and 50 µg/mL.

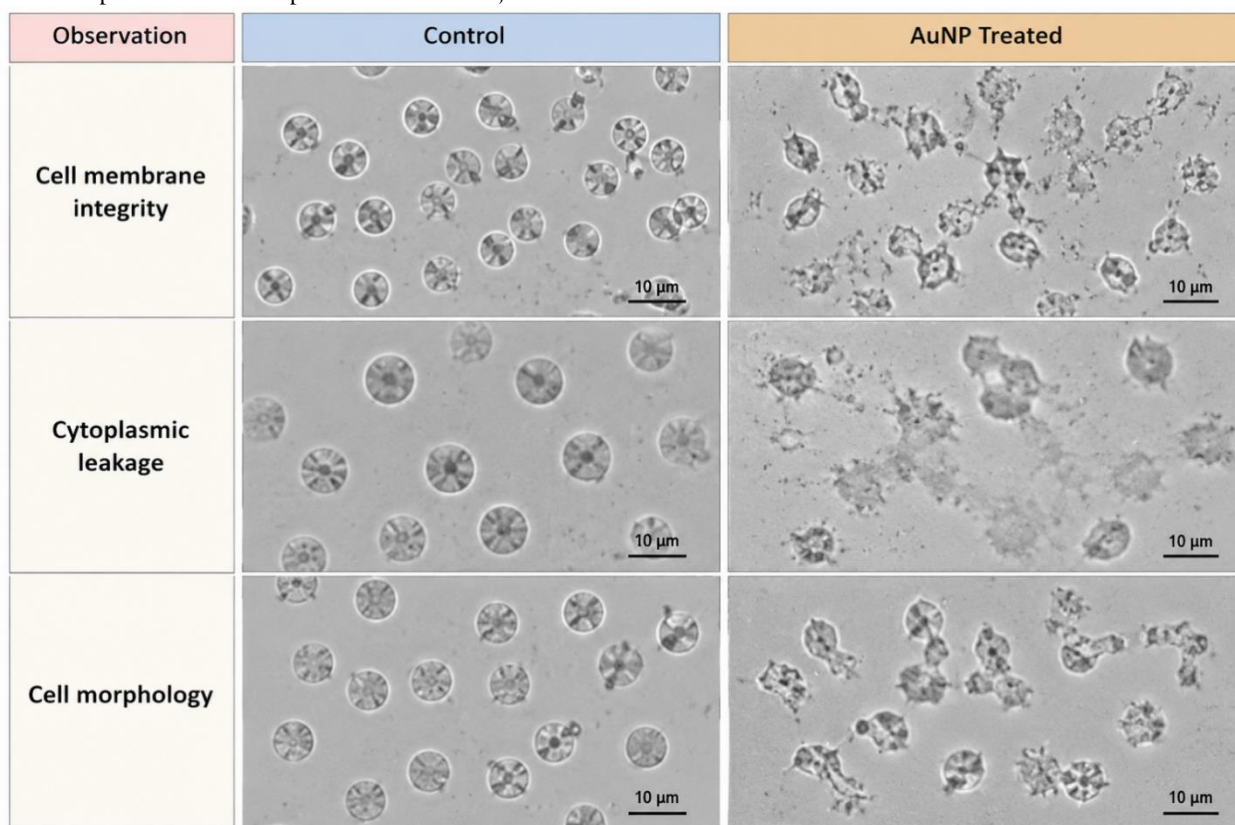
**Table 3:** MIC and MBC of Gold Nanoparticles against MDR *E. coli*

Isolate Type	MIC (µg/mL)	MBC (µg/mL)
MDR <i>E. coli</i>	12.5-25	25-50

### Antibacterial Mechanism Observation

Microscopy revealed that bacterial cell walls had been ruptured after exposure to AuNP, with

photographic samples of destructive membrane alteration and cytoplasm spill out.



**Figure 4:** Morphological changes in *E. coli* post-AuNP treatment

### Discussion

The current research declares the large antimicrobial abilities of gold nanoparticles and shows efficacy against multi-drug resistant *Escherichia coli* isolated from veterinary compartments. The *E. coli* prevalence (68%) shows a major representation within veterinary environments and signifies the need for research on safer handling of both commensal and opportunistic pathogens among livestock. Ribeiro et al. (2023) stated that the most coping locations for the microbial leakage, Fresco and

dairy, have been reported heavily, where mastitis and colonizing of the *E. coli* sub-strains are largely observed in the food-producing livestock professions. The zoonotic nature and food-chain transit of the multi-drug-resistant (MDR) strains are extremely dangerous (Jawad et al., 2025). The observed nature and coping locations are especially concerning.

A large majority of the MDR strains (76.4%), which are infamously resistant to common novel antibiotics such as ampicillin, tetracycline, and cefotaxime, were observed in this study and have

been attributed to the overuse of antibiotics in the veterinary field and the lack of regulation regarding such use, particularly in communities and countries (excluding India) that excessively use antibiotics. Countrywide, South Asia has been nearly unanimously cited as a region where resistance in livestock-associated *E. coli* is observed, and there is a significant lack of effective therapies in veterinary medicine, indicating no evidence of an out flux of multi-drug resistant strains (Mamba, 2019). Seddiqi et al. (2021) reported the need to prevent multi-drug resistant strains has been recognized almost as long as the need for alternative antimicrobial methods in veterinary medicine.

In this matter, gold Nano particulate compounds display an extreme scope of antimicrobial ability to positively be a back-up agent to the research shown within the study, where maximum resistance was recorded for ampicillin and tetracycline and cefotaxime compounds, and an extreme showing of no evidence. AuNPs achieved low MIC and MBC values within a short time. This seems to show their capability for effective microbial inhibition (Naseer et al., 2020). Shalaby et al. (2022) examined that these results reinforce the challenge of resistance development in AuNPs owing to their broad-spectrum antimicrobial activity against all kinds of bacteria.

AuNPs proved their capability to create visible morphologic damage to the cells. The damage included lesions of the membranes of the cells and was accompanied by cytoplasmic disorder. Direct interactions with the membranes of bacterial cells and their walls caused them to lyse. AuNPs proved their resistance to the challenge of oxidative damage through the generation of reactive oxygen species. The AuNPs inhibited the bacterial activities by preventing DNA replication and causing oxidative damage (D. Wang et al., 2021). These multimodal mechanisms of resistance development complemented the resistance prohibition. All these oxidative activities enhanced the resistance of the multi-drug-resistant *E. coli* organisms (Nomani et al., 2023).

AuNPs possess certain multi-drug resistant characteristics that other antimicrobial agents also possess. This is a great attribute in a

veterinary setting where repeated misuse and overuse of antibiotics encourages the development of resistance. The low concentrations of AuNPs support the conclusion of a substantial reduction in the risk of toxicity and antimicrobial use. This promotes a reduction in multiple doses of a veterinary product with continuous administration of it (LeBoff et al., 2022). Despite the benefits provided by AuNPs, there are significant barriers to their use in the veterinary field.

The variance in synthesis, stability, and surface structure of the particles affects their antimicrobial activity. To promote reproducibility, synthesis protocols must be standardized. While the biocompatibility of AuNPs is well received, it is not fully determined by the internal accumulation in tissues or the potential long-term effects and toxicity on the environment. Many in vivo studies are necessary to confirm the safety of AuNPs, as well as their distribution in the biological system after synthesis and any subsequent biological functionality (Sulaiman et al., 2020). Traditional antibiotics and AuNPs have the possibility of working synergistically. An enhancement in the delivery of the drug and the disruption of the bacterial protective mechanism has resulted in the nanoparticle-antibiotic conjugates reversing the efficacy of antibiotics in the resistant strains (Liu et al., 2020). Combination strategies of this sort can be a functional way to address the antimicrobial resistance problem in veterinary medicine.

### Conclusion

The research showed the potential of gold nanoparticles (AuNPs) as an effective antimicrobial agent against multi-drug resistant (MDR) *Escherichia coli* obtained from a veterinary context. The rapid spread of the MDR *E. coli* from this study underlines the developing worldwide issue of antimicrobial resistance, as well as the wide-ranging and sometimes unrestrained use of MDR *E. coli* in the veterinary context. This situation promotes the declining health and productivity of animals and increases the threat of chronic zoonosis to the populace through the food chain and other ecosystems. The research showed that gold nanoparticles

demonstrate the ability to kill and inhibit MDR *E. coli* and that the extent of inhibition and killing depends on the size of the particles. This research also showed large inhibition and killing. The research showed the gold nanoparticle induced disruption of the cell membrane of *E. coli* and the gold nanoparticle induced leakage of the *E. coli* cytoplasm. Despite gold nanoparticles being able to overcome classic antimicrobial resistance, this multi-target antimicrobial mechanism supports the gold nanoparticle-induced hyperactivity. Gold nanoparticles can be blended easily with other mechanisms to kill microorganisms violently and in a total way, which can be done at virtually no cost. This study shows gold nanoparticles have the potential to serve as an adjunct therapy to MDR pathogens of *Escherichia coli* in veterinary practice. This work presents a potential new technological innovation in veterinary practice, along with the prolonged time these formulations take to help kill and the extended half-life of these Nano sized particles.

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