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# N-Acetylcysteine Potentiates the Antimicrobial Activity of Gold Nanoparticles: A Paradigm Shift in Treatment of Multidrug Resistant Pathogens

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**Received:** June 30, 2017; **Published:** August 03, 2017

## Abstract

Antimicrobial activity of gold nanoparticles (Au NPs) was tested against standard strains of *Staphylococcus aureus* besides 25 clinical isolates collected from cancer patients. Au NPs exhibited a robust antimicrobial activity against *S. aureus*. N-acetylcysteine (NAC) substantially potentiated the antimicrobial activity of tested Au NPs and synergism between Au NPs and NAC has been confirmed by checkerboard assay. Au NPs abrogated the activity of respiratory chain dehydrogenase of *S. aureus* and released muramic acid content from *S. aureus* in culture. The cytotoxic effect of Au NPs alone and in combination with NAC was examined using human HepG2 cells and this revealed no cytotoxicity at MIC values of Au NPs and interestingly, NAC reduced the cytotoxic effect of Au NPs at concentrations higher than their MIC values. Taken together, Au NPs have robust antimicrobial activity and NAC significantly enhances their antimicrobial activities against MDR which would provide a novel safe, effective and inexpensive therapeutic approach to control the prevalence of MDR pathogens.

***Keywords:*** *Nanoparticles; Bacteria; Staphylococcus aureus; N-Acetylcysteine; Synergism; MDR*

## Introduction

Antimicrobial resistance is an eminent problem that threatens the effective prevention and treatment of infectious diseases. This poses a major global public health hazard and a big challenge. As per World Health Organization (WHO), the cost of health care for patients having resistant infections is much higher than care for patients having non-resistant infections which could be attributed to the extended duration of sickness, additional tests and use of more expensive drugs. The continual intake of antibiotics is frequently coupled with the development of multiple drug resistance (MDR) [1]. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat infectious diseases, resulting in prolonged sickness, disability, and death [2]. Antimicrobial resistance occurs naturally over time, most likely via genetic changes, however, there are other factors that might accelerate this phenomenon such as misuse and abuse of antibiotics [3].

Antimicrobial resistant-microbes are ubiquitous where they have been shown to harbor a plethora of antibiotic resistance genes which would in turn render treating such bacteria so difficult [4,5]. Therefore, there have been several attempts to find novel strategies to interrupt microbial growth through targeting specific pathway or essential process e.g., quorum sensing inhibition which may offer a decisive solution for such predicament [6].

Nanotechnology-based products have offered quite diversified applications particularly in the medical field suggesting nanomaterials as prospective antimicrobial agents that could substitute conventional drugs [7,8]. Nanoscale technologies offer novel materials that

possess exceptional and distinct properties over their macroscopic counterparts due to increase in surface area to volume ratio thereby altering mechanical, thermal and catalytic properties of materials [7]. Moreover, the ease to control the physical and chemical properties of nanoscale materials is of great importance for the development of novel antimicrobial agents [3].

Antimicrobial effects of various metal-based nanoparticles (including Zinc, Silver and gold nanoparticles) have been well documented. Silver-based compounds have long been shown to be extremely toxic to various types of pathogens [9,10]. Gold nanoparticles (Au NPs) may have a potential in the development of antibacterial strategies because of their acceptable toxicity, versatility in surface modification, polyvalent and photothermal effects [11].

The interaction between nanoparticles and microorganisms or biomolecules is an expanding field of research, which as yet is largely unexplored. The aim of the current study was to examine the antimicrobial and cytotoxic activities of Au NPs against standard strains as well as clinical isolates of *S. aureus* from cancer patients. Furthermore, this work aimed at studying the impact of addition of NAC to Au NPs on their antimicrobial and cytotoxic activities.

## Material and Methods

### Microorganisms

*Staphylococcus aureus* (ATCC 9144) was a gift from department of Microbiology and Immunology, Faculty of Pharmacy, Tanta University. Additionally, 25 clinical isolates were collected from cancer patients at the National Cancer Institute (NCI, Cairo, Egypt). All chemicals and media used were of high analytical grade and were purchased from Sigma-Aldrich (St Louis, MO, USA).

### Preparation and characterization of gold nanoparticles (Au NPs)

Au NPs were prepared by citrate reduction of Gold (III) chloride trihydrate following the method of Turkevich., *et al.* (1951) which was later refined by [12] with some modifications [13]. A hundred milliliters of an aqueous solution of gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) at concentration of 1 mM was brought to boiling condition and stirred continuously. Afterwards, a solution of 10ml of 38.8 mM sodium citrate tribasic dihydrate ($C6H_5Na_3O_7.2H_2O$) was added quickly at one time, resulting in a change in solution color from pale yellow to black to deep red. The prepared nanoparticles were characterized using UV-Vis spectrophotometry ($T80^+$ PG instrument) and their size was determined using FEI Tecnai G2 F20 X-TWIN Transmission Electron Microscope (TEM).

### Bacterial identification and *in vitro* susceptibility testing

Bacterial clinical isolates were identified using Microscan technique at NCI, Cairo, Egypt. Clinical isolates as well as standard strains of *S. aureus* were used in the *in vitro* antimicrobial susceptibility testing to Au NPs.

### Evaluation of the antimicrobial activity of Au NPs by disc diffusion method

The antimicrobial susceptibilities of bacterial and fungal standard strains as well as clinical isolates to Au NPs were determined by standard disc diffusion test as described by the clinical laboratory standards institute (CLSI) [14]. Briefly, colonies from an overnight culture of *S. aureus* grown on nutrient agar were suspended in 3 ml saline and turbidity was adjusted to 0.5 McFarland (1 - 2 x $10^8$ CFU/ml). The samples were inoculated ($10^8$ CFU/ml) in petri dishes with nutrient agar or sabouraud agar. Subsequently, paper discs of 6 mm diameter were laid on the inoculated test pathogens, which was instilled with different concentrations (0.4, 0.2, 0.1, 0.05 and 0.025 mM) of Au NPs suspensions. Plates were incubated at 37°C for 24h and antimicrobial activity was examined by measuring the zones of inhibition around each disc.

### Determination of minimum inhibitory concentration (MIC) of Au NPs

The MIC values of Au NPs against *S. aureus* were estimated by standard broth microdilution method as described by CLSI [14]. Briefly, bacterial suspensions were prepared as previously described under disc diffusion test then diluted 1:1000 to give (1 - 2 x $10^5$ CFU/ml).

*S. aureus* was grown on nutrient broth in shaking incubator (150 rpm) and MIC values of tested nanoparticles were determined using the two-fold serial dilution technique at concentrations ranging from 0.025 to 0.2 mM of Au NPs suspensions. The cultures were incubated at 37°C for 18 - 20h. Results were estimated by measuring optical density (OD) at 600nm.

**Evaluation of the antimicrobial activity of N-acetyl cysteine and Au NPs alone and in combination**

MIC values of NAC alone were determined as previously described using two-fold serial dilution technique against *S. aureus* standard strains and clinical isolates at concentrations ranging from 0.4 to 50 mg/ml. The impact of addition of NAC to Au NPs was determined using two-fold serial dilution technique with the ranges tested being 0.4 - 25 mg/ml and 0.0025 - 0.2 mM for NAC and Au NPs suspensions, respectively. The cultures were incubated at 37°C for 18 - 20h and the results were evaluated by measuring OD at 600nm.

**Examining the synergism between nanoparticles and NAC (checkerboard dilution method)**

The combined effect of NAC with Au NPs was evaluated by checkerboard method to obtain the fractional inhibitory concentration (FIC) index [15]. The checkerboard consisted of column in which each well contains the same amount of antimicrobial agents diluted fourfold along x-axis. The rows in which each well contained the same amount of the plant extract and its component were diluted fourfold along the y-axis on a 96-well plate. The FIC index was calculated according to the equation: FIC index = FICA+FICB= (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). Synergism was defined as an FIC index = 0.5, the additive effect as an FIC index of 0.5 - 2.0, and antagonism as an FIC index = 2.0 [15].

**Assay of respiratory chain dehydrogenase activity**

Dehydrogenase activity was determined using the iodonitrotetrazolium (INT) chloride assay [16]. Different concentrations (0.2, 0.1,0.05 and 0.025 mM) of Au NPs suspensions were mixed with 1 x $10^5$ CFU/ml of tested *S. aureus* and incubated for 100 min in nutrient broth. Negative and positive controls were included using cells that have been boiled for 20 min or cells without boiling in absence of nanoparticles, respectively. Afterwards, 1ml was sampled separately from the cultures, centrifuged at 12,000 rpm and the supernatants were discarded and the cell pellet was washed twice in PBS and resuspended in 0.9 ml PBS. INT solution (0.1 ml of 0.5%) was added, the culture was incubated at 37°C in dark for 2h, and 50 µl of formaldehyde was added to terminate the reaction. The culture was centrifuged to collect the bacteria, and 250 µl of 50% v/v acetone and ethanol were used to distill the iodonitrotetrazolium chloride-formazan (INF) twice. The supernatants were subsequently combined and the dehydrogenase activity was then calculated by measuring the absorbance of INF at 490 nm. The same test was performed using fixed concentration (0.4 mM) of Au NPs at various time intervals.

**Effect of Au NPs on bacterial cell wall stability**

In order to examine the effect of tested nanoparticles on the cell wall stability of *S. aureus* muramic acid release assay has been employed. Muramic acid release in the culture medium subsequent to treatment with Au NPs was determined using liquid chromatography–mass spectrometry (LC-MS) as recently described [10]. Bacterial suspension (prepared as previously described under MIC experiment) was probed with 0.4 mM of Au NPs for 18h. Afterwards, the biomass of the resulting suspension, cells without any treatment (negative control) and standard muramic acid in fresh culture medium (positive control) were centrifuged for 20 min at 6000 rpm, filtered through 0.2 mm filter, dried under reduced pressure and kept at 4°C until analysis. The following LC protocol was used: Mobile phase, A: 0.1% formic acid, B: Acetonitrile, run time: 3 min, flow rate: 250 µl /ml. Mass spectrometric analysis was carried out using a TSQ Quantum Access MAXtriple quadrupole system. Data acquisition and processing were performed using Thermo Scientific Xcalibur 2.1 software.

**Effect of Au NPs on bacterial cell wall stability (Transmission electron microscopy study)**

Bacterial suspensions were prepared as previously described under MIC experiment. *S. aureus* (1 - 2 x $10^5$ CFU/ml) were grown on nutrient broth and incubated with Au NPs (1mM) in shaking incubator (150rpm). The cultures were incubated at 37°C for 6 and 12h contact time. Control experiment was conducted in absence of Au NPs. The cultures were centrifuged at (6000 rpm) for 10 min and the super-

natants were discarded. Interaction between nanoparticles and microbial cells was examined by FEI Tecnai G2 F20 X-TWIN transmission electron microscopy.

### Assessment of cytotoxicity of Au NPs using human hepatocellular carcinoma cells

Human hepatocellular carcinoma cells (HepG2) were cultured at 0.5 x $10^5$ cells/well in Dulbecco's minimal eagle medium (DMEM) and plated in a flat bottom 96-well microplate. Cells were treated with 20 µl of different concentrations (0.4, 0.2, 0.1 and 0.05mM) of Au NPs for 48h at 37ºC, in a humidified 5% $CO_2$ atmosphere. Subsequently, media were removed and 40 µl MTT solution were added to each well and incubated for an additional 4h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol to each well and plate was shacked at room temperature. Absorbance was read at 570 nm using microplate ELISA reader. Data were expressed as the percentage of relative viability compared with the untreated cells.

## Results

### Preparation and characterization of Ag NPs and Au NPs

Au NPs, prepared by chemical reduction method using citrate salts, were characterized by measuring their absorption spectra, size and morphology. Figure 1 shows the absorption spectrum demonstrating a distinct peak at 520 nm for Au NPs. The particle size of Au NPs was determined using TEM (FEI Tecnai G2 F20 X-TWIN). TEM images revealed spherical shaped particles with an average size of 15 nm for Au NPs as shown in Figure 2.

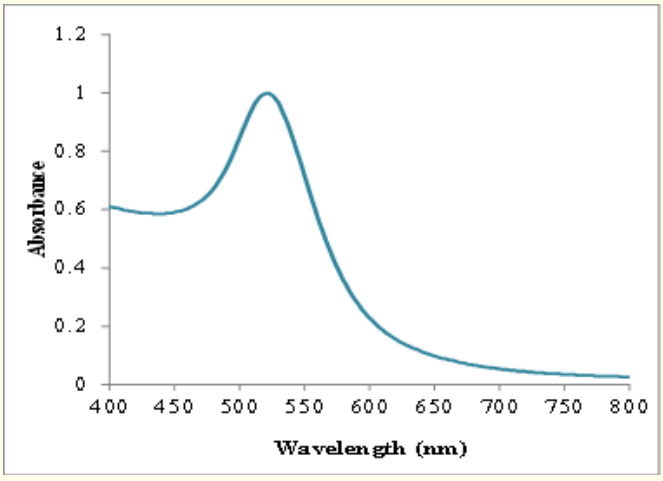


***Figure 1:*** *The absorption spectrum of Au NPs. The prepared Au NPs nanoparticles were characterized using UV-Vis spectrophotometry demonstrating a characteristic peak at 520 nm.*

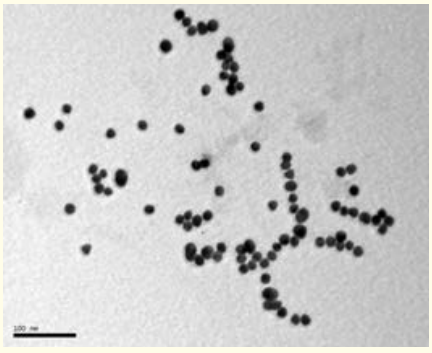

***Figure 2:*** *Transmission electron microscope (TEM) image of Au NPs. This shows nearly spherical particles shape with an average particle size of about 15 nm.*

### Identification of clinical isolates

A total of 25 *S. aureus* clinical isolates including 7 MRSA were identified using Microscan technique. All identified isolates were found to be MDR. The resistance profile of all tested *S. aureus* is shown in table 1.

***Table 1:*** *Antibiotic resistance patterns of S. aureus ATCC 9144 standard strains as well as 25 clinical isolates. Antibiogram showing the resistance profile of the 25 clinical isolates and one standard strains bacteria that have been recruited in the current study.*

| Microorganism | | Type | Antibiotic resistance pattern |
|---|---|---|---|
| *Staphylococcus aureus* | ATCC 9144 | Standard strain | SENSTIVIVE |
| | 1 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,IMP,MXF,OFL,OX,RIF,T/S |
| | 2 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S,TE,VA |
| | 3 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S |
| | 4 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S,TE,VA |
| | 5 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,,GAT,IMP,MXF,OFL,OX,RIF,T/S,TE,VA |
| | 6 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S |
| | 7 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,OFL,OX,RIF,TE,T/S |
| | 8 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 9 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 10 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 11 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 12 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OX,RIF,TE,T/S |
| | 13 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S |
| | 14 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 15 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S |
| | 16 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,T/S |
| | 17 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 18 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 19 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 20 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 21 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,MXF,OFL,OX,RIF,TE,T/S |
| | 22 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,OFL,OX,RIF,TE,T/S |
| | 23 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 24 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,MXF,OFL,OX,RIF,TE,T/S |
| | 25 | Clinical isolate | AUG,AZI,C,CAX,CFT,CFZ,CPE,E,GAT,IMP,OFL,OX,RIF,TE,T/S |

*AUG=Amoxacillin/clavulinic acid, AZI=Azithromycin, C= Chloramphenicol, CPE= Cefepeme, CFZ=Cefzolin, CFT=Ceftriaxone, CAX=Ceftriaxone, E=Erythromycin, GAT=Gatifloxacin, IMP=Imipenim MXF= Moxifloxacin, OX=Oxacillin, OFL=Ofloxacillin, RIF-Rifampicin, TE=Tetracycline, T/S=Trimethoprime/Sulphamethoxazole, V=Vancomycin*

### Antimicrobial activity of Au NPs

Au NPs were tested for their antimicrobial activity against *S. aureus* standard strains using Kirby Bauer disc diffusion method according to CLSI guidelines. Results as shown in table 2 revealed that Au NPs exhibited an antimicrobial activity at concentration of 0.4 mM against *S. aureus* (zone of inhibition = 15 mm). Lower concentrations of Au NPs showed no zones of inhibition against tested *S. aureus*.

***Table 2:*** *Disc diffusion test demonstrating susceptibility of S. aureus standard strains to Au NPs. Discs instilled with 0.04mM Au NPs were placed in plates containing S aureus. Plates were incubated at 37°C for 24h and antimicrobial activity was investigated by measuring the zones of inhibition around each disc.*

| Disc content | Concentration (mM) | *S. aureus* |
|---|---|---|
| Au NPs | 0. 4 | 15mm |

**Determination of MIC values of Au NPs against *S. aureus***

MIC values of Au NPs against *S. aureus* were determined using standard microdilution technique according to CLSI guidelines. Figure 3 shows the optical densities (ODs) of *S. aureus* standard strains subsequent to treatment with different concentrations of Au NPs. MIC value of Au NPs against *S. aureus* was 0.4 mM including MRSA isolates.

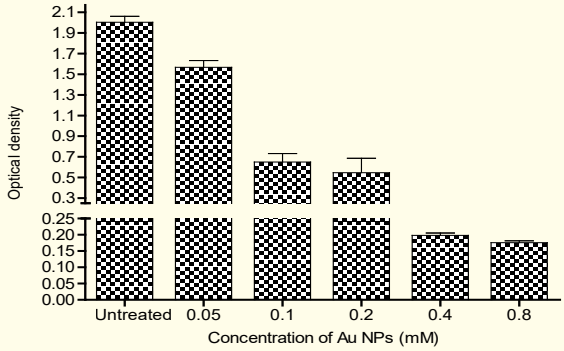


***Figure 3:*** *The optical densities of S. aureus standard strain subsequent to treatment with increasing concentrations of Au NPs. S. aureus was grown on corresponding media in shaking incubator (150 rpm) and MIC values of Au NPs were determined using the two-fold serial dilution technique at concentration ranging from 0.8 - 0.05 mM. The cultures were incubated at 37°C for 18 - 20h and MIC values were estimated by measuring (OD600 nm). Concentration-dependent reduction in OD values was obtained using increasing concentrations of Au NPs. n = 3 experiments; means ± standard deviations are shown.*

**NAC substantiates the antimicrobial activity of Au NPs**

The impact of addition of NAC to Au NPs has been examined. Results (Table 3) showed that combination of NAC and Au NPs has resulted in substantial reductions in MIC values of Au NPs by (33 fold) against tested *S. aureus.*

***Table 3:*** *MIC values for N-acetyl cysteine (NAC) (mg/ml), Au NPs (mM) and for Au NPs in combination with NAC against standard strains of S. aureus. MIC values of NAC alone were determined at concentrations ranging from 0.4 to 50 mg/ml. The impact of NAC addition to Au NPs was determined with the ranges tested being 0.4 - 25 mg/ml and 0.0025 - 0.2 mM for NAC and Au NPs, respectively. The cultures were incubated at 37°C for 18 - 20h and the results were assessed by measuring OD600 nm. Combination of Au NPs and NAC substantially reduced MIC values by 16-fold for S. aureus.*

| Microorganisms | MIC values of NAC (mg/ml) | MIC values of Au NPs (mM) | MIC values of Au NPs (mM) in presence of 0.4 mg/ml of NAC |
|---|---|---|---|
| *S. aureus* | 1.2 | 0.4 | 0.012 |

**NAC has a synergistic effect with Au NPs**

The FIC index of NAC in combination with Au NPs against tested *S. aureus* was 0.395. FIC index for Au NPs in combination with NAC was < 0.5 indicating synergistic interaction.

**Studying the potential mechanism of action of Au NPs as antimicrobial agents**

**Effect of Au NPs on respiratory chain dehydrogenase**

The effect of Au NPs on respiratory chain dehydrogenase of *S. aureus* has been examined. Results obtained (Figure 4) showed that Au NPs exhibited a concentration-dependent inhibitory effect on the activity of respiratory chain dehydrogenase of S. aureus. The effect of Au NPs on respiratory chain dehydrogenase of S. aureus has been examined. Results obtained (Figure 4) showed that Au NPs exhibited a concentration-dependent inhibitory effect on the activity of respiratory chain dehydrogenase of *S. aureus*.

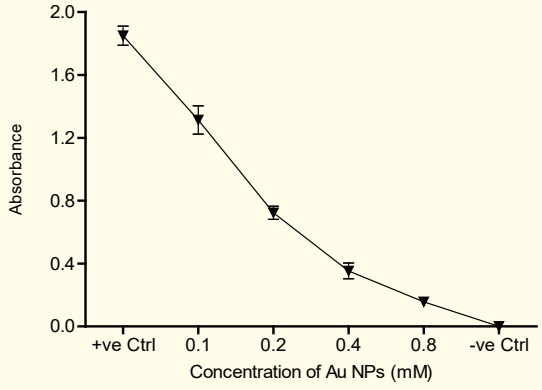


***Figure 4:*** *Respiratory chain dehydrogenase activity of S. aureus after treatment with increasing concentrations of Au NPs. S. aureus (1x105 CFU/ml) were incubated with increasing concentrations of Au NPs for 100 min. Cell pellets were mixed with iodonitrotetrazolium (INT) solution, incubated at 37°C in dark for 2h, and 50µl of formaldehyde was added to terminate the reaction. 250 µl of 50% v/v acetone and ethanol were used to distill the INF. Absorbance of was read at 490nm. The -ve Ctrl and +ve Ctrl represent the boiled and not boiled bacterial cells, respectively. n = 3 experiments; means ± standard deviations are shown.*

**Effect of Au NPs on bacterial cell wall stability**

**Assay of Muramic acid release by LC/MS**

The effect of Au NPs on bacterial cell wall stability of the Gram-positive pathogen *S. aureus* has been investigated. Au NPs (0.4mM) have been able to release of muramic acid content of *S. aureus* in the culture media. The released muramic acid was determined using LC/MS and the chromatogram peaks obtained subsequent to treatment with Au NPs are shown in Figures 5A and 5B.

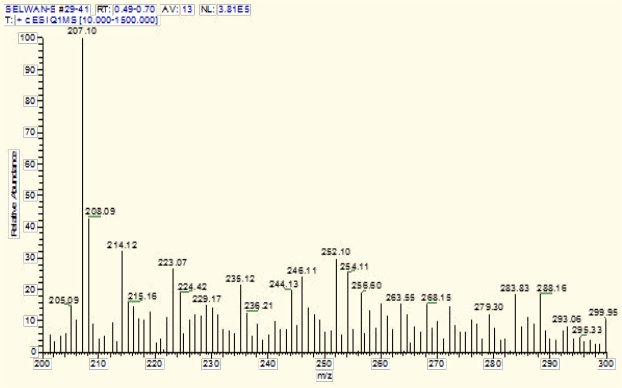


***Figure 5A:*** *LC/MS chromatogram demonstrating the release of muramic acid from S. aureus in culture medium subsequent to treatment with 0.4mM of Au NPs.*

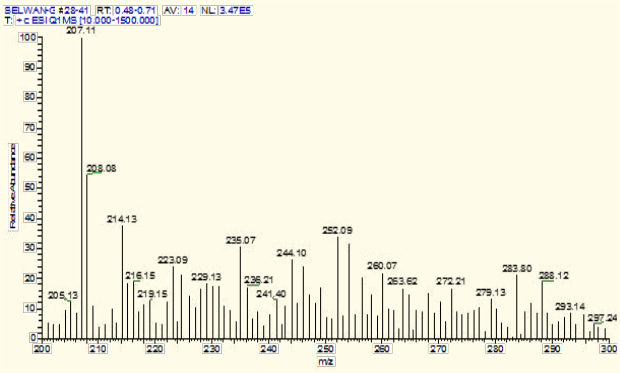


***Figure 5B:*** *LC/MS chromatogram demonstrating standard muramic acid in fresh culture medium (positive control).*

***Figure 5:*** *LC/MS chromatogram demonstrating the release of muramic acid from S. aureus in culture medium subsequent to treatment with 0.04 mM of Au NPs. Bacterial suspension was probed with 0.4 mM of Au NPs for 18h then the biomass of the resulting suspension (figure 5a), cells without any treatment (negative control) and standard muramic acid in fresh culture medium (figure 5b, positive control) were centrifuged, filtered, dried and analyzed by LC-MS.*

**Transmission Electron Microscope study**

TEM images of *S. aureus* cells that have been treated or not with Au NPs are depicted in Figure 6 (a, b and c). (a) the surface of untreated cells was smooth demonstrating typical characters, while cells treated with 0.4 mM Au NPs as shown in Figure 6 (b) showing pathogens after 6h contact time, Figure 6 (c) shows severe cell damage after 12h contact time. Some cells became indistinct showing massive leakage while others were distorted, deformed and twisted.

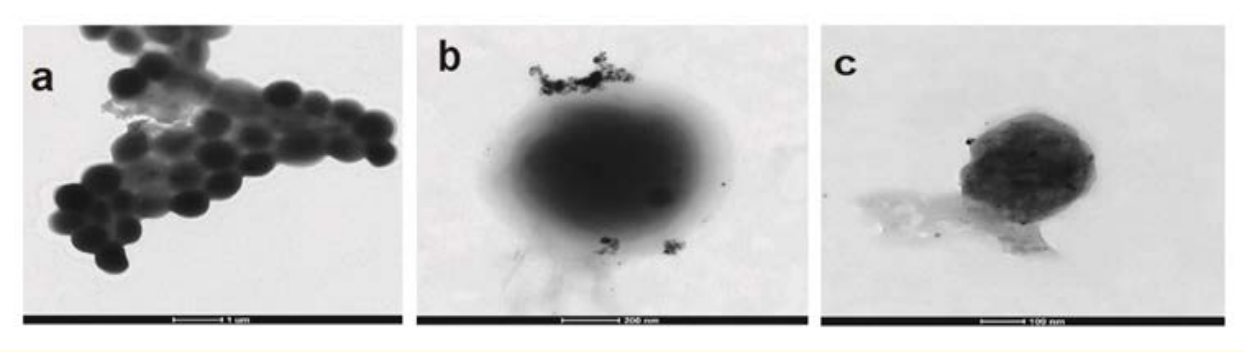


***Figure 6:*** *Transmission electron microscope images demonstrating the effect of Au NPs on S. aureus ATCC 9144. (a) S. aureus prior to exposure to Au NPs, (b) S. aureus subsequent to exposure to Ag NPs for 6h and (c) S. aureus subsequent to exposure to Au NPs for 12h. Membrane irregularities were observed in bacteria exposed to Au NPs.*

**Examining the cytotoxicity of Au NPs**

In order to investigate the cytotoxic effect of Au NPs, HepG2 cells have been employed. Results obtained have shown a concentration-dependent reduction in cell viability subsequent to using increasing concentrations (0.25, 0.5, 0.1 and 0.2 mM) of Au NPs. Au NPs did not show any cytotoxicity at their MIC values. Moreover, NAC, at concentration of 12.5 mg/ml, was added to examine its impact on cytotoxic effects exhibited by high concentrations of Au NPs on HepG2. Results obtained as shown in Figure 7 demonstrate that NAC reduced the cytotoxic effect of Au NPs at high concentrations.

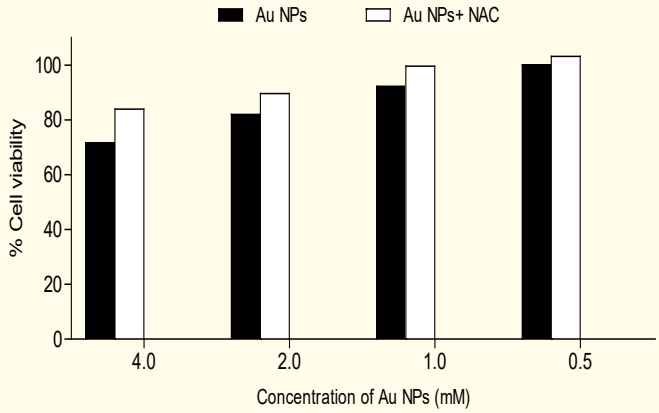


***Figure 7:*** *Effect of increasing the concentrations of Au NPs and N-acetyl cysteine (NAC) on cell viability of human hepatocellular carcinoma cells (HepG2). HepG2 cells were treated with 20 µl of increasing concentrations of Au NPs alone or in combination with increasing concentrations of NAC and were incubated for 48h at37ºC. Then, 40µl of MTT solution were added and incubated for an additional 4h and 180µl of acidified isopropanol were added. Absorbance was measured at 570nm and data are expressed as the percentage of relative viability compared with the untreated cells. A representative experiment out of three is shown.*

## Discussion

Au NPs, prepared by chemical reduction, were characterized using UV-Vis spectrophotometry and their absorption peaks were 520 nm. Their TEM images showed nearly spherical particles with 15 nm average size for Au NPs. The antimicrobial potential of Au NPs has been scrutinized. Susceptibility testing to Au NPs showed that Au NPs exhibited antibacterial activity against *S. aureus* at concentration of 0.4mM.

A growing body of evidence affirms the antibacterial and antiviral activities of NAC, although weak, as well as its potential adjuvant role with some classes of antibiotics [17,18]. A recent work from our group showed that silver nanoparticles have a robust antimicrobial activity against MDR pathogens and NAC significantly enhances the antimicrobial activity of silver nanoparticles [10]. As such, it was interesting to test whether Au NPs behave similarly as silver nanoparticles and whether NAC could also potentiate the antimicrobial activity of gold nanoparticles.

This has shown that NAC had a weak antimicrobial activity against on *S. aureus* which is in line with reported data [19]. Interestingly, addition of NAC to Au NPs has led to substantial enhancement of the antimicrobial activities of Au NPs as revealed by the massive reduction in their MIC values. Interaction between Au NPs and NAC has been further confirmed by checkerboard assay. Checkerboard assay revealed that FIC index for Au NPs in combination with NAC against tested *S. aureus* was < 0.5 indicating synergistic interaction. To the best of our knowledge, this is the first study to report such a robust synergism between NAC and Au NPs.

The antimicrobial activity of Au NPs is apparently mediated through strong electrostatic attractions to the negatively charged lipids of the plasma membrane [20]. This has also been propped by the observation that cationic particles exhibited more toxic activities compare to anionic ones [21]. It has previously been reported that Au NPs conjugated with antimicrobial agents and antibodies exhibit selective antimicrobial activity [22]. Muhling and colleagues have reported that strong laser-induced hyperthermic effects coincide with bubble-formation around Au NPs effectively damaged bacteria [23]. Several studies have reported a plethora of medical applications including antimicrobial activity of Au/drug nanocomposites against an array of sensitive and resistant microorganism [24-26].

The exact detailed antimicrobial mechanism of action of Au NPs remains elusive, and in an attempt to unravel that, the mechanisms of action of Au NPs that might be, at least in part, involved in their antimicrobial activities have been examined. First, the effect of Au NPs on respiratory chain dehydrogenases has been examined using INT chloride assay. This showed that Au NPs inhibited the respiratory chain dehydrogenase activity on *S. aureus* in a concentration-dependent manner. Then, we set out to investigate other potential mechanisms of action that might be associated with Au NPs by scrutinizing their effect on the cell wall stability of *S. aureus*. Muramic acid is the main cell wall component of *S. aureus* and is commonly used as an indicator for cell wall stability. Muramic acid release assay was employed using LC/MS which showed that cell wall damage took place subsequent to treatment with Au NPs.

TEM has been employed to visualize the surface interaction between Au NPs and tested *S. aureus* to examine the surface morphology of both native bacteria and bacteria that have been probed with Au NPs. Images of *S. aureus* cells that have been treated or not with tested Au NPs were displayed in Figure 6. This showed that the surfaces of native cells (Figure 6a) were smooth and intact demonstrating typical characters; while cells treated with 0.4 mM Au NPs for 6h (Figures 6b) showed significant changes and signs of damage and lysis. On the other hand, membrane deformation and severe cell damage was obtained after exposure for 12h as shown in (Figures 6c) where some cells showed large leakage, others were twisted, deformed and distorted.

In spite of the enormous potential advantages of Au NPs in the field of biomedicine, studies are ongoing to explore their cytotoxic activities. Different cell models have been recruited to study the cellular effects of Au NPs. Since oral route may represent an important route of administration for Au NPs and the liver being the primary metabolic organ for orally-administered substances, therefore, HepG2 cells were recruited to investigate the cytotoxic effect of Au NPs on liver cells. The potential cytotoxicity of Au NPs on HepG2 cells was studied using MTT assay. Au NPs showed minimal or no cytotoxicity at low concentration and elevated toxicity at higher concentrations. Interestingly, NAC at a concentration of 12.5 mg/ml enhances the viability of HepG2 cells which would in turn render those nanoparticles safe for use (Figure 7).

## Conclusion

Taken together, Au NPs have been shown to exhibit a robust antimicrobial activity against *S. aureus*. Interestingly, we reported for the first time a robust synergistic effect between NAC and Au NPs thereby potentiating their antimicrobial activity. We have also shown that Au NPs inhibit the respiratory chain dehydrogenases and cause cell wall damage through release of muramic acid content of *S. aureus.* To conclude, this study emphasizes the potential of Au NPs as new promising non-cytotoxic antimicrobial agents against MDR *S. aureus*.

## Conflicts of Interest

The authors declare no conflict of interest.

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