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# Isolation and Diagnosis of *Escherichia coli bacteria* from Patients with Urinary Tract Infections and Bacteriosis and Studying their Sensitivity to Some Antibiotics and Nanobodies

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**ABSTRACT**

**The current study included the collection of 120 clinical samples (diuresis, blood) from patients visiting Baghdad city hospitals (Yarmouk Teaching Hospital, Central Children's Teaching Hospital, and Baghdad Teaching Hospital) for the period from November 2022 to February 2023. 30 discharge samples were collected from healthy people as a control group. The samples were within different ages and genders ranging from 14 years to 52 years. 10% of the E bacteria have been isolated coli of blood and 150% of blood based on phenotypic and culture characteristics and biochemical tests and the diagnosis was confirmed using the phytic device.**

**The results of the sensitivity test of bacterial isolates using the tablet method of diffusion showed that most of the isolates were resistant to the antibiotics used, where the percentage of resistance to Tetracycline (96%), (84%) Nitrofurantoin, (82%) Ceftriaxone, (66%) Meropenem, (52%) Ciprofloaxcine, (70%) Gentamicin, (80%) Amikacin, (46%) Imipenem, Nalidixic acid (40%).**

**The antimicrobial activity of gold and titanium nanoparticles was evaluated against 15 bacterial isolates (5 isolated from diuretic samples, 5 exit samples, 5 blood samples) using the diffusion method by gratigran. The results showed that the nanoparticles had a clear inhibitory effect against the studied isolates where the average inhibition diameters ranged between 17-22 mm and the MIC value (Minimal inhibitory concentration) was between 64-126.**

***Keywords***- Coliform Bacteria, Antibiotic Resistance, Nanoparticles, Bacteremia, Urinary Tract Infections.

## I. INTRODUCTION

Escherichia coli (E. coli) is a member of the enterobacteriaceae family that is a member of the enterobacteriaceae family that is negative for the dye Cram, bacillus form, motile, non-aerobic or anaerobic facultative anaerobic fermentation of lactose, most of which fermented ramenose and sorbitol sugar, producing the enzyme glucuronidase-β. Optimum temperature for growth of 37 °C (Wanger etal., 2017) positive for catalase and negative for oxidase indole and non-citrate consumption positive for red methylation (Hemraj et al., 2013). It lives naturally in the intestines of humans and animals and is at the same time opportunistic pathogens that cause many diseases such as diarrhea, meningitis, sepsis, bacteremia and is one of the most common bacterial types of urinary tract infections, as it causes about 90% of urinary tract infections in the world and is more common in childhood, (Hadi et al., 2014). The pathogenicity of these bacteria is due to the possession of

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many factors of virulence, and these factors are the possession of the factor cytotoxic necrotizing colicin, the toxic necrosis factor siderophores of iron chelates and their possession of surface structures such as flagella, capsule and polysaccharides (lipopolysaccharides (LPS), which give bacteria antigen characteristics by producing flagellar antigen (H), somatic antigen (O) and capsular antigen (K) also possess cilia (fimbriae). or pili) which helps it adhere to the host tissues and gives it the ability to form biofilm (Zowawi et al., 2015; Terlizz et).

Bacterial resistance to antibiotics has become one of the problems that threatens societies around the world, especially after the rise in infection and death rates, as most traditional antibiotics have become ineffective for treating bacterial infections, in addition to the decrease in effective treatments and the lack of successful prevention measures, which requires finding alternative treatments to eliminate bacterial infections. E. coli bacteria are characterized by their multi-resistance to antibiotics as a result of their possession of resistance enzymes, such as beta-lactamase enzymes that confer resistance to beta-lactam antibiotics and enzymes that confer resistance to aminoglycosides and ketolinolytics. The bacteria also possess other mechanisms by which they develop resistance to antibiotics, such as changing the permeability of the cell membrane, a change in Target location: inhibition of protein synthesis. (Kapoor et al., 2017) Nanotechnology may be an alternative treatment due to its high effectiveness against pathogenic germs and its safe use (Antao. et.al., 2009). Nanotechnology is one of the newly emerging sciences that includes the process of manufacturing and developing materials into nanoparticles, as the average size of nanoparticles ranges between 1-100 nanometers, so they can be used for applications in energy, medicine, diagnostics, optics, electronics, as well as water treatment systems (Al-Saadi, 2021).Therefore, our current study aimed to isolate and diagnose E. coli bacteria from different clinical sources and study their sensitivity to antibiotics and gold and titanium nanoparticles.

## II. MATERIALS AND METHODS

***Samples of the study***

***Urine Samples***

150 samples were collected from patients at Yarmouk Teaching Hospital and outpatient clinics from people with urinary tract infections for the period from the beginning of November 2022 until the end of February 2023, as the information related to the patient from age, date of taking the sample, housing, etc. was recorded in a special questionnaire form, and the samples were planted on the middle of blood and maconki and methyl blue eocene medium by planning method, and the dishes were incubated at a temperature of 37 ° C for 24-18 hours for the purpose of diagnosing bacteria growing on the media. mentioned.

***Diarrhea Samples***

120 samples from different age groups of patients were collected at Yarmouk Teaching Hospital and outpatient clinics, where the samples were collected in sterile plastic bottles and immediately transferred to the microbiology laboratory for transplantation.

***Blood Samples***

10 blood samples from different age groups were collected from patients at Yarmouk Teaching Hospital and outpatient clinics, where the samples were collected in sterile tubes and immediately transferred to the microbiology laboratory for culture.

***Diagnosis of bacterial isolates***

The bacterial isolates under study were diagnosed based on Holt et al. (1994), as they were diagnosed phenotypic (based on phenotypic traits including colony shape, texture, smell on Mankoki medium and methyl blue eocene as well as their decomposition of red blood cells on the blood akar medium) and microscopic traits by dyeing them with Cram dye and examining them under a microscope to see the type of dye, the shape of the cells and the method of assembly. IMViC, catalysis, oxidase and uris tests were performed.

***Antibiotic susceptibility test***

Allergy testing was performed using the antibiotic tablet method, using 9 Types of antibiotics (Morello.et.al., 2006 5 ml of nutrient broth medium was inoculated with 2-3 colonies of pure bacterial cultures at 24 hours of age, the tubes were shaken well, and incubated with the incubator at 37 °C for 24 hours 108×cells/ml. Transfer 0.1 ml of bacterial stranded and spread over the center of Akar Müller Hunton, leave the dish for 5 minutes at room temperature until the culture is dry. Antibiotic tablets were transferred to the surface of the culture medium by means of sterile forceps at a rate of 9 Tablets for each dish. The dishes were incubated at 37°C for 24-18 hours after which the diameters of the inhibition zones around. Each tablet, the bacteria are considered sensitive (S), resistant (R) or medium intermediate (I) according to the standard specifications contained in (CLSI, 2016).

***Nanoparticles preparation***

One gram of titanium and 1 gram of gold were added to 50 ml of distilled water and the pH was adjusted to 4 by HCl (1 N) and placed in an ultrasonic path at 4500 kH for 25 seconds. The pH was adjusted to 10 by NaoH (1N). The mixture was mixed by a magnetic motor for two hours at 21 ° C. The mixture was incubated at 21°C for 18 hours and then the pH was reset to 7 by HCl (1 N) (Ghadi et al., 2014), and X-ray diffraction was measured.

***X- ray diffractions***

Analysis using an X-ray diffraction meter (XRD) is useful for knowing the structure phase (and the purity of manufactured gold and titanium and is generally used as a common method for studying the phase composition and crystal structure of gold and titanium. A thin film of regular suspended water from each type of

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nanoparticle was prepared on a glass slide and preserved for drying. X-ray diffraction pattern (XRD) was recorded using an X-ray diffraction meter In this technique, a glass slide containing PHB nanoparticles with a concentration of 20 was prepared mg / ml (several drops) to form a thin layer of suspension with a thickness of 0.5 mm. After that, it was checked with a device by exposing K and Cu rays on the model to be measured, at different angles (from 10 to 80 degrees), with a wavelength of 1.5406 Å, with a voltage of 40 kV, and an amount of 2 degrees. /One minute. Scherrer's equation has been applied to measure the size of nanoparticles (Rastogi et.al.,2016),

## III. RESULTS AND DISCUSSION

***Morphological Identification***

The isolates of E.coli bacteria were initially diagnosed based on phenotypic traits after growing them on MacConkey agar, Methylene Eosin EMB (agar Blue) and Blood agar, the results showed that the bacteria were fermented for lector sugar, giving pink, soft, shiny colonies with a sharp edge on the differential Akar medium containing bile salts and crystal violet dye that allows the growth of Cram-negative bacteria including Intestinal family inhibits the growth of bacteria that are positive for Cram tincture. As shown in Figure 1. The isolates gave shiny metallic green colonies on the medium of blue methyl eosine and this characteristic of the E. coli bacteria, which distinguishes it from other members of the intestinal family, as a result of containing the medium on the pigments of eocene and blue methylene that are deposited in the acidic medium after being associated with each other, giving a metallic green luster, which indicates that the bacteria produced organic acids as a result of fermentation of lactose and sucrose sugars as shown in Figure 1 (Barcella et al. . 2006).

**Figure 1: Lactose-fermented Escherichia coli colonies on central maconque and central akar eosine like blue amid blood acar.**

E. coli cells also appeared as Gram-negative short bacilli, and these results are consistent with Levinson (2016). The results of the biochemical tests also showed that all isolates were positive for the catalase test, negative for oxidase test and negative for urease test and these results are consistent with what was stated in. As for the results of the IMViC group tests, the bacteria were positive for both the indole test and the methyl red test, while negative for the Voges-Proskauer test and the Citrate test as shown in Figure 2, and these results are consistent with what was stated in both (Brown and Smith, 2017; Tille, 2017).


**Figure 2: A set of biochemical tests of E. coli.**

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A- The Simmon citrate test represents the appearance of the green color indicates the positive reaction as a result of consuming jackets.
B- IMViC group test.

***Antibiotic susceptibility test of isolated bacteria***

Multi-resistant bacteria are one of the serious and major problems on the medical side, and this causes the difficulty of choosing the appropriate treatment for the patient, and one of the main reasons for the emergence of resistance is the indiscriminate use of antibiotics without relying on sensitivity testing for these antibiotics. The diffusion method on the medium Muller-Hinton agar was used to determine the susceptibility of antibiotics against those isolated germs and was interpreted according to (CLSI, 2016). The results of this study showed that most of the isolates of E.coli bacteria were resistant to antibiotics (Table 1 and Fig. 1), the resistance rate was towards 96 Tetracycline % Ceftriaxone, Nitrofurantoin 84% 28%, Imipenem 80%, Amikacin 70%, Gentamicin 66%, Meropenem 52%, Ciprofloxacin 46%, while the resistance rate was the lowest towards Nalidixic acid 40%. The results of this study are almost consistent with the results of (Al-Saadi, 2019) where the resistance rate of Nitrofurantoin was 94% and not consistent with where the percentage was 25.5%. The results of our study regarding the antibiotic Gentamicin also agreed with where the resistance rate was 100%, but it was different from what it obtained where the resistance rate of Gentamicin was 17%. It agreed with and Khalid et al., 2013 regarding the antibiotic Norfloxacin where the resistance to this antibiotic was 75% and 56%, respectively. As for the antibiotics Erythromycin and Amoxicillin, the results obtained in our current study agreed with the results obtained with and, where the resistance rates to these two antibiotics were 88% and 83%, respectively. As for the results of antibiotic resistance, Meropenem The results of the current study showed that it showed the lowest percentage of resistance by coliform bacteria at 40%, and this percentage was close to the percentages obtained by both the researcher, as well as the researcher, whose results showed the emergence of low resistance to the antibiotic Meropenem, at rates of 16%, and 20%, respectively.

Treatment with chemical drugs, including antibiotics, plays an optional role in removing sensitive bacteria, especially in the urinary tract, as the high concentration of drugs in diuresis allows the survival of resistant bacteria and the removal of sensitive bacteria, which explains one of the reasons for the increase in the emergence of resistant bacterial strains in the urinary tract, and for this the treatment of infections caused by bacteria that carry resistance to antibiotics is one of the main problems in urinary tract infection , and these often appear Individuals are very highly resistant to beta-lactam Ii antagonists such as Ampicillin, Amoxicillin and first-generation cephalosporins, as these bacteria have usually acquired resistance enzymes to such antibodies that resistance to them has become a common and increasing phenomenon.

The variation of the results of bacterial sensitivity to antibiotics is due to several factors, including the irregular and random use of antibiotics, which makes some types of bacteria resist some antibiotics through the development of different resistance methods such as the presence of a protein in the outer wall that has the ability to excrete various antibiotics outward, as well as the bacteria's possession of R-Plasmid, which gives them the characteristic of resistance to many antibiotics. Plasmids have an important role in the transfer of resistance due to the presence of genes that code for this resistance, which are transferred to other sensitive bacteria to turn into antibiotic-resistant bacteria, and the transfer is carried out through several methods such as conjugation, transformation and transduction.

**Table 1: Multiple antibiotic resistance patterns for bacterial isolates**

| Percentage of Resistance | NA | CIP | NI | CN | AK | TE | TOB | MR | IMI | CRO | Antibiotic / Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Z | R | S | R | R | R | R | R | S | R | R | U1 |
| 90% | R | S | R | R | R | R | R | S | R | R | U2 |
| 60% | R | S | R | S | R | R | S | R | R | R | U3 |
| 70% | R | S | R | S | R | R | R | R | R | R | U4 |
| 50% | R | S | I | S | R | R | S | R | R | R | U5 |
| 60% | R | R | R | S | R | R | S | R | I | R | U6 |
| 60% | R | R | R | S | S | R | S | R | I | R | U7 |
| 30% | R | R | I | S | S | S | S | S | I | R | U8 |
| 60% | R | R | R | S | R | R | S | S | I | R | U9 |
| 70% | R | S | R | R | R | R | R | S | I | R | U10 |
| 60% | I | R | I | R | R | R | S | S | R | R | U11 |
| 50% | I | R | R | R | S | S | I | S | R | R | U12 |
| 60% | I | R | R | R | S | R | I | S | R | R | U13 |
| 60% | I | R | R | R | S | R | I | I | R | R | U14 |
| 50% | I | R | R | R | S | R | S | I | R | s | U15 |
| 20% | I | I | S | S | R | R | S | I | R | S | U16 |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20% | S | I | R | S | R | R | S | I | I | s | U17 |
| 30% | S | I | R | S | R | R | R | S | I | S | U18 |
| 50% | S | I | R | S | R | R | R | R | I | R | U19 |
| 80% | S | R | R | S | R | R | R | R | R | R | U20 |
| 80% | S | R | R | I | R | R | R | R | R | R | U21 |
| 70% | R | R | R | I | S | R | R | R | R | R | U22 |
| 80% | R | R | R | I | R | R | R | R | S | R | U23 |
| 80% | R | R | I | I | R | R | R | R | S | R | U24 |
| 70% | I | I | R | R | R | R | R | R | S | R | U25 |
| 70% | S | I | R | R | R | R | R | R | S | R | U26 |
| 60% | I | S | R | R | I | R | R | R | R | R | U27 |
| 70% | I | S | R | R | S | R | R | R | R | R | U28 |
| 60% | R | S | I | R | S | R | I | R | R | R | U29 |
| 60% | S | S | R | R | S | R | I | R | R | R | U30 |
| 70% | S | R | R | R | S | R | I | R | R | R | S1 |
| 60% | S | R | R | R | S | R | S | I | R | R | S2 |
| 60% | S | R | R | R | S | R | S | I | R | R | S3 |
| 70% | R | R | R | R | I | R | S | I | R | R | S4 |
| 40% | S | R | I | R | I | R | I | S | R | R | S5 |
| 40% | S | R | R | R | I | R | I | S | R | I | S6 |
| 50% | S | R | R | R | R | R | I | S | R | I | S7 |
| 60% | S | R | R | R | R | R | I | R | R | I | S8 |
| 70% | S | R | R | R | R | R | R | R | R | I | S9 |
| 80% | R | S | R | R | R | R | R | R | R | R | S10 |
| 70% | R | S | R | R | S | R | R | R | R | R | B1 |
| 60% | R | S | I | S | R | R | R | R | R | R | B2 |
| 70% | R | S | R | S | R | R | R | R | R | R | B3 |
| 70% | R | S | R | R | R | R | R | R | R | S | B4 |
| 80% | R | S | R | R | R | R | R | R | R | R | B5 |
| 60% | I | S | R | R | R | R | R | I | R | R | B6 |
| 50% | S | S | R | R | R | R | R | I | R | S | B7 |
| 60% | I | S | R | R | R | R | R | S | R | R | B8 |
| 50% | I | S | R | R | R | R | R | S | R | R | B9 |
| 60% | S | R | R | R | R | R | R | S | R | R | B10 |

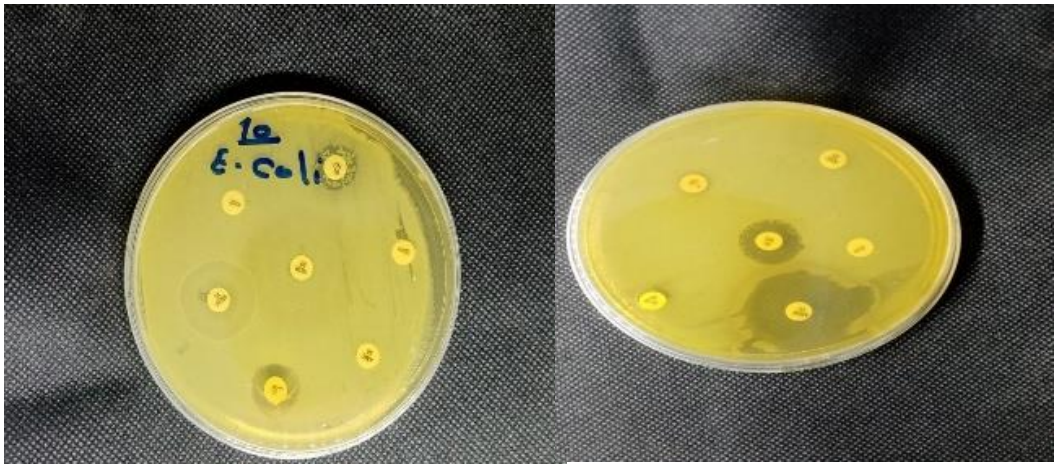


**Image No. 1: Antibiotic sensitivity test for E. coli bacteria**

***Diagnosis of gold nanotechnology with X-ray diffraction***

The measurement showed four peaks at 38.0203, 44.2211, 64.4701 and 77.3709 degrees, which are the characteristic peaks of nanogold attributed to Miller's coefficients 111, 200, 220 and 311, which indicate the presence of gold in the central cubic crystal system as shown in Figure 3. The presence of these peaks with the

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absence of other peaks of high intensity is evidence of the purity of the nanogold used.

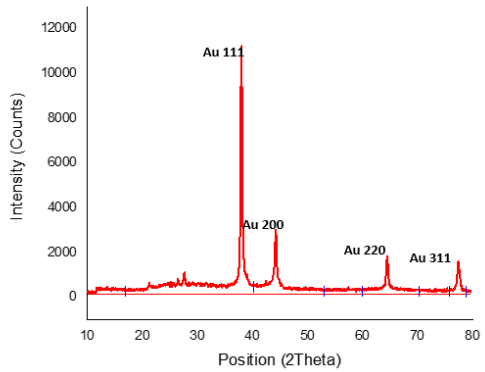


**Figure 3: X-ray diffraction pattern of gold nanoparticles**

***Diagnosis of titanium oxide nanoscale with X-ray diffraction***

The measurement showed four peaks at 25.42, 37.12, 37.97, 38.77, 48.12, 54.12, 55.27, 62.82, 69.27, 70.42 and 75.32°, which are the diagnosed peaks of anatase phase titanium oxide nanoparticles attributed to Miller's coefficients 101, 103, 004, 112, 200, 105, 211, 118, 116, 220 and 215, respectively and as shown in Figure 4. The presence of these peaks with the absence of other peaks of high intensity is evidence of the purity of the titanium oxide used.

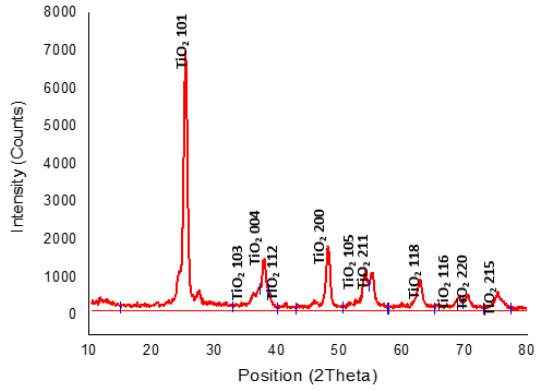


**Figure 4: X-ray diffraction pattern of titanium oxide nanoparticles**

***Effect of nanoparticles on the growth of E. coli bacteria***

In an attempt to find substances affecting the pathological isolates of antibiotic-resistant E. coli bacteria, the effect of gold and titanium nanomaterials on the growth of 15 pathological isolates taken from (diure=5, exit = 5, blood = 5) and by the method of propagation by drilling, the current results showed that there is a clear effect of the nanomaterial on the bacterial isolates tested, and the rate of inhibition diameters ranged between 17-22 mm, and the value of the minimum inhibitory concentration MIC of the nanomaterial ranged between 64-126 µg/. (Table 2) we notice that the middle color turns blue as an indication that the concentration is lethal and no longer has bacteria, red indicates the presence of live bacteria (Figure 5).

**Table 2: Minimum inhibitory concentration values (MIC) for nanomaterial**

| Samples | Mic | Sub Mic |
|---|---|---|
| Stool 1 | 126 | 64 |
| Stool 2 | 64 | 16 |
| Stool 3 | 126 | 64 |
| Stool 4 | 126 | 64 |
| Stool 5 | 126 | 126 |
| Blood 1 | 126 | 126 |
| Blood 2 | 126 | 64 |
| Blood 3 | 64 | 32 |
| Blood 4 | 64 | 32 |
| Blood 5 | 126 | 64 |
| Urine 1 | 126 | 64 |
| Urine 2 | 126 | 64 |
| Urine 3 | 126 | 64 |
| Urine 4 | 126 | 64 |
| Urine 5 | 126 | 64 |

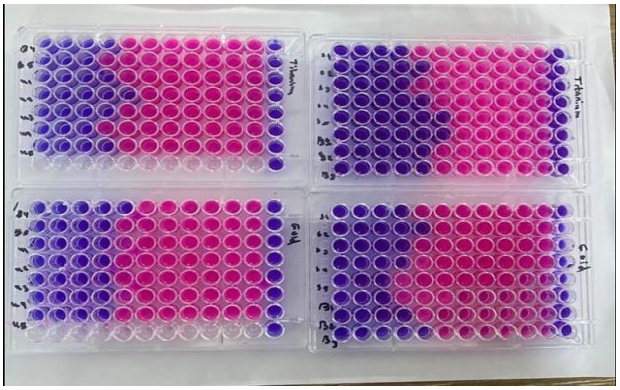

**Figure 5: Minimum inhibitory concentration MIC of nanomaterial**

Nanomaterials (1-1000 nm) are widely used nowadays due to their high tissue targeting ability. Reducing the size to the nanoscale can modify the properties of materials in terms of their chemical, mechanical, electrical, structural, morphological and optical properties. Cationic peptides form a new class of molecules with antimicrobial potential. The interaction of the positive charge on the surface of these peptides with negatively charged molecules on the surface of bacterial cells such as phosphate groups of gram-negative liposugars is proposed.

In addition, their super-branched nature provides chemically reactive groups to which other molecules can be attached to impart healing properties. As anti-biofilm agents, they possess many promising properties. Due to the small particle size, they can easily penetrate the

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expanded polystyrene matrix (EPS) to exert some bactericidal activity. Dendrimers have also been found to prevent EPS formation by inducing DNA mutations. They are also able to inhibit protein synthesis.

Another possible mechanism by which cationic dendritic peptides may work is their detergent-like effect. They interact with the anionic components of bacterial membranes and the biofilm matrix resulting in loss of membrane potential has shown the ability of membranes to reduce the biofilms of preformed bacteria by up to 83.5%. Thus, they provide a clinically useful alternative to antibiotics to eliminate pre-formed biofilms that can only be achieved through unattainable concentrations in vivo.

***Examination of E. coli sensitivity to gold and titanium nanoparticles***

The inhibitory ability of gold and titanium nanoparticles (Au-NPs + Ti-NPs) was tested at multiple concentrations against E.coli bacteria by tablet method by measuring the inhibition diameter area and comparing the results with the standard tables given in (CLSI, 2016). The solution of the nanoparticles was shown to have an inhibitory effect, as in Figure 6 and Table 3.

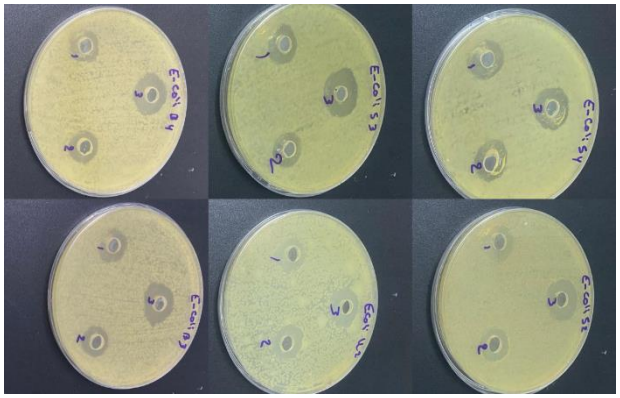

**Figure 6: Showing the effect of gold and titanium nanoparticles on bacteria**

**Table 3: Bacterial sensitivity test for nanoparticles**

| Samples | 1 (gold) mm | 2 (titanium) mm | 3 (gold+titanium) |
|---|---|---|---|
| Stool 1 | 12 | 14 | 19 |
| Stool 2 | 11 | 12 | 18 |
| Stool 3 | 13 | 13 | 20 |
| Stool 4 | 12 | 11 | 17 |
| Stool 5 | 13 | 12 | 16 |
| Blood 1 | 11 | 15 | 20 |
| Blood 2 | 12 | 14 | 21 |
| Blood 3 | 13 | 11 | 19 |
| Blood 4 | 13 | 12 | 18 |
| Blood 5 | 12 | 13 | 19 |
| Urine 1 | 13 | 12 | 20 |
| Urine 2 | 15 | 14 | 18 |
| Urine 3 | 14 | 13 | 19 |
| Urine 4 | 13 | 15 | 20 |
| Urine 5 | 14 | 13 | 20 |

***Determination of the minimum inhibitory concentration of gold nanoparticles***

The minimum inhibitory concentration of E.coli bacteria was determined using the microtyter method in solution. The sensitivity of these bacteria to gold nanoparticles was tested and the following was found:

Bacteria isolated from serum and blood showed lower inhibition concentration values in the range 64-256 µg/ml. Bacteria isolated from diuresis showed similar minimum inhibition concentration values, which were 128 µg/ml .From this it is clear that samples S5 and B1 (256 µg/ml) are isolates that required the highest lowest inhibitory concentration which means that they possess a higher biofilm. The isolates that required the lowest MIC were S2, B3 and B4, and the rest of the isolates had MIC values of 128 µg/ml as shown in Table 4 and Figure 7.

These values are much higher than those previously recorded for nanogold against this type of bacteria and this can be attributed to the size of the gold nanoparticles, as well as the fact that the bacteria did not work for Biofilm, so nanogold can be considered a good alternative in case of resistance of bacteria to antibiotics, so these values are largely consistent with the values recorded in another study.

**Table 4: Minimum inhibitory concentration values of E.coli bacteria from serum, blood and different diuretic isolates using gold nanoparticles**

| Sample | MIC | Sub-MIC |
|---|---|---|
| S1 | 128 | 64 |
| S2 | 64 | 32 |
| S3 | 128 | 64 |
| S4 | 128 | 64 |
| S5 | 256 | 128 |
| B1 | 256 | 128 |
| B2 | 128 | 64 |
| B3 | 64 | 32 |
| B4 | 64 | 32 |
| B5 | 128 | 64 |
| U1 | 128 | 64 |
| U2 | 128 | 64 |
| U3 | 128 | 64 |
| U4 | 128 | 64 |
| U5 | 128 | 64 |

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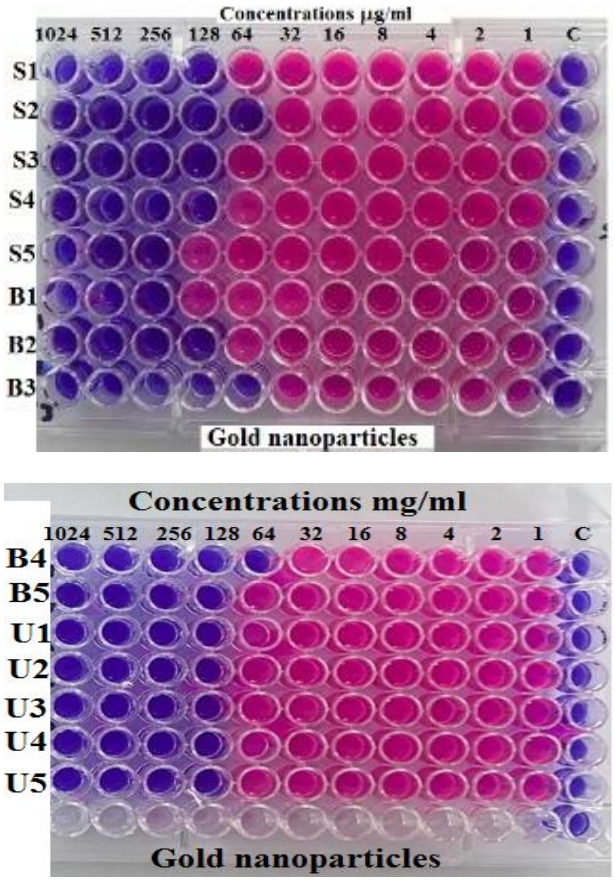


**Figure 7: Minimum inhibitory concentration of E.Coli bacteria from serum, blood and different diuretic isolates using gold nanoparticles**

***Determination of the minimum inhibitory concentration of titanium oxide nanoscale***

The minimum inhibitory concentration of E.coli bacteria was determined using the microtreader method in solution. The sensitivity of these bacteria to titanium oxide nanoparticles was tested and the following was found:

Bacteria isolated from serum and blood showed lower inhibition concentration values in the range 32-256 µg/ml .Bacteria isolated from diuresis showed lower inhibition concentration values in the range 64-256 µg/ml.

The results in Table 4 show that samples S5 and B1 (32 µg/ml) are isolates that required the lowest inhibitory concentration, which means that they have less biofilm, which means that titanium oxide nanoparticles have the opposite effect to nanogold. The isolates that required the highest MIC were B5 and U4, while the isolates S1, S3, B4, U1, U3 and U5 had MIC values of 128 µg/ml, and the rest of the isolates showed an inhibition value of 64 µg/ml as shown in Table 5 and Figure 8. These values are consistent with previous studies, where the study showed that the values of the minimum inhibitory concentration were within the range 31.25-125 µg/ml as well as identical to another study by researchers Hamid and Laith.

**Table 5: Minimum inhibitory concentration values for E.coli bacteria from serum, blood and different productions isolates using titanium oxide nanoparticles.**

| Sample | MIC | Sub-MIC |
|---|---|---|
| **S1** | 128 | 64 |
| **S2** | 64 | 32 |
| **S3** | 128 | 64 |
| **S4** | 64 | 32 |
| **S5** | 32 | 16 |
| **B1** | 32 | 16 |
| **B2** | 64 | 32 |
| **B3** | 64 | 32 |
| **B4** | 128 | 64 |
| **B5** | 256 | 128 |
| **U1** | 128 | 64 |
| **U2** | 64 | 32 |
| **U3** | 128 | 64 |
| **U4** | 256 | 128 |
| **U5** | 128 | 64 |

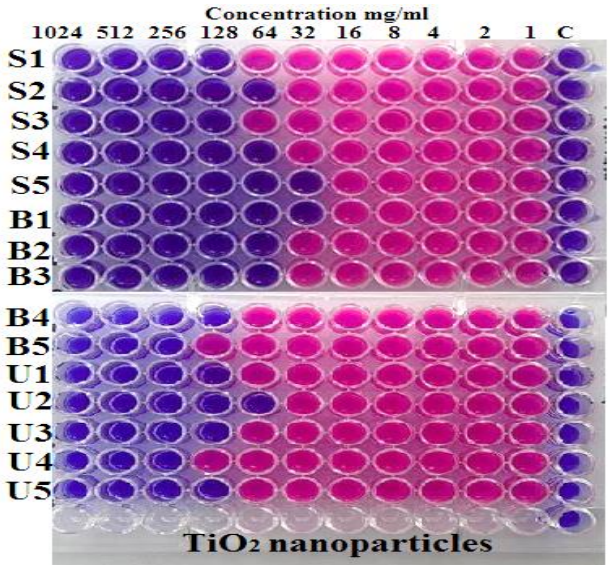


**Figure 8: Minimum inhibitory concentration of E.coli bacteria from serum, blood and different diuretic isolates using titanium oxide nanoparticles.**

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