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# Antibacterial activity nanoscale (Zinc, Silver, Titanium) of *Staphylococcus aureus* isolated from dental decay

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**Keywords:**
Antibacterial activity, nanoscale, dental decay

**ABSTRACT**

The current study aimed to know the bacterial causes of dental caries, and for this purpose, for both sexes, at different ages ranging from (8-50) years, Then they were collected and transported directly to the laboratory and the samples were planted by plotting on plates of nutritious acar and then acaral blood and mkonkiAnd in solid saline mannitol medium under aerobic conditions, the type Staphylococcus aureus appeared at 248 isolates, or 31%. The sensitivity of the bacteria isolates under study was tested against 3 types of ntibioticsClindomycin, Metronidazole, Amoxicillin. Where wasolates of bacteria showed-*S. aureus* Resistance against all antibiotics. For isolates of *Streptococcus* It showed a difference in the response to antibiotics. The sensitivity of bacterial isolates to some types of manufactured nanomaterials was tested. Zinc, Sliver, Titanium with different concentrations, where the concentrations (100, 75, 50, 25) were used. The presence of the inhibition circuit indicated that zinc nanoparticles are a good antibacterial in varying proportions. Not all isolates appeared in response to silver and titanium nanomaterials, after which the treatment was treated with the synergistic effect. For nanomaterials on bacterial isolates, they gave better results than using them alone. The results showed the variation in the response of the isolates to the synergistic effect of the antibiotic with the nanomaterials and the difference in their sensitivity and resistance from one isolate to another. The results of the study showed using the technique of RT-PCR differentiated response to virulence factors protein A, haemolysin, TSST to bacterial isolates *S. Aureus* For the effect of zinc nanoparticles used in concentrations (2,1,0.5,0.25mg). And we got the highest inhibition at the concentration (0.5 mg). The results of the current study confirmed the response to the virulence factor (haemolysin) for the effect of zinc nanoparticles used in concentrations (2,1,0.5,0.25mg), and we obtained the highest inhibition of gene expression at the concentration (0.5 mg). The results of the current study confirmed the response to the virulence factor (Protein A) has the effect of zinc nanoparticles used at concentrations of (2,1,0.5,0.25mg), and a decrease in the gene expression of the factor was noted and the best inhibition was observed at the concentration (0.5 mg). In the current study, we obtained the effect of gene expression of virulence factor (TSST-1) in response to zinc nanoparticles used at different concentrations (2,1,0.5,0.25mg), and a decrease in the gene expression of the agent was noted and the best inhibition was observed at the concentration (0.5 mg).

## 1. INTRODUCTION

Tooth decay topic Dental caries, the interest of many researchers and specialists in the field of dentistry, so It is a very difficult problem, OK that van Tooth decay accompany him Severe pain is much stronger than other diseases, in addition to the fact that tooth decay remains the factor responsible for losing most teeth at all ages without other causes [15].

that the science of nanotechnology Nanotechnology is a newly emerging science that includes the process of manufacturing and developing materials into nanoparticles. The rate of nanoparticle sizes ranges between 1-100 nanometers, and they can be used for applications in energy, medicine, diagnosis, optics, electronics, as well as water treatment systems [14].

The use of modern technologies resulted in the great development in microbiology and genetic engineering techniques, which led to the possibility of detecting the genes of virulence factors or antibiotic resistance, as well as genetic elements related to pathogenesis without resorting to antibiotic sensitivity testing, isolation and diagnosis [2].

Nanosciences and technologies have opened the door to many and varied applications, including what is known as nanomedicine, and it includes a group of modern medical technologies that fall under the umbrella of nanotechnology, including the use of nanoparticles as anti- bacterials, as these nanoparticles showed high effectiveness in this field [1].

The importance of virulence factors lies in the fact that they give bacteria the ability to invade host tissues, grow and reproduce, and increase pathogenicity. The most important virulence factors for Staphylococcus aureus (Abdulwahab, 2015).

## 2. Materials and working methods

### *2.1 Sample collection Collection of samples*

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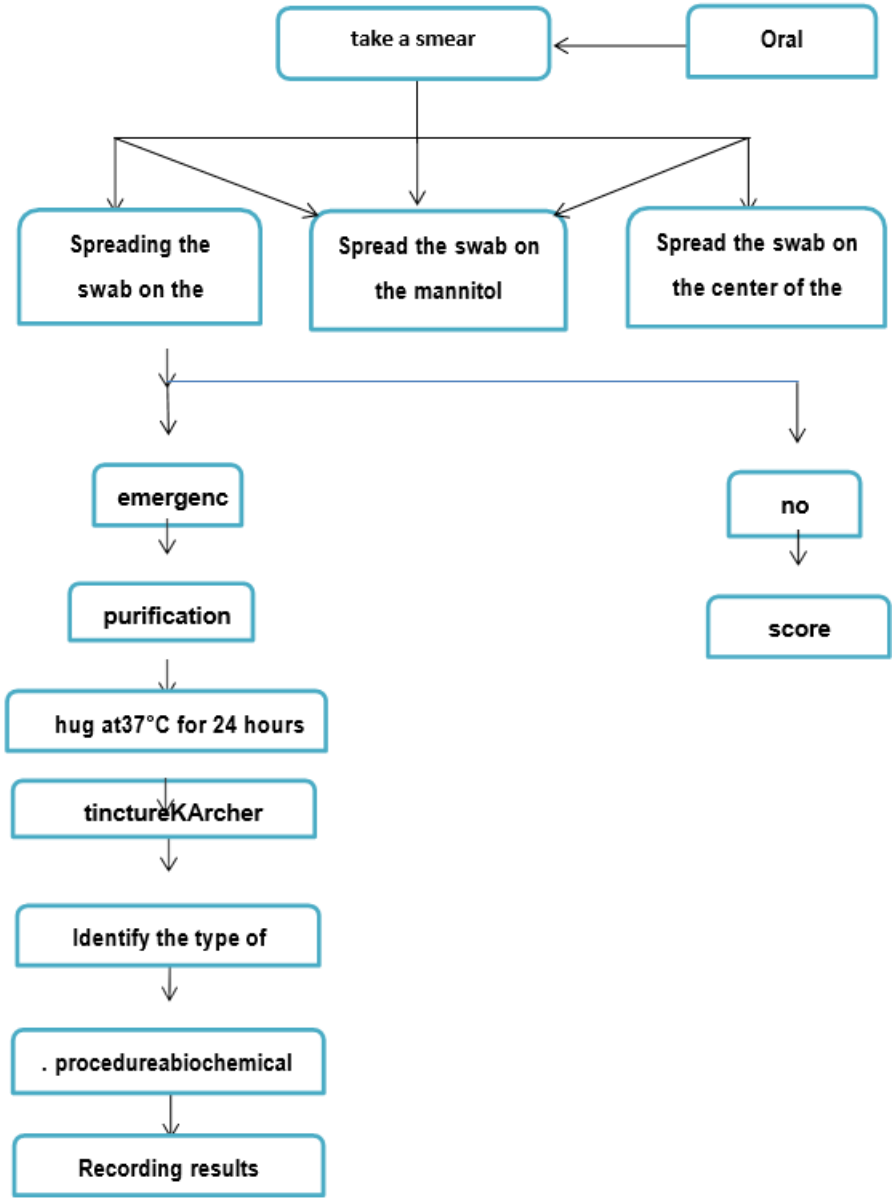



Phases of bacterial isolation and diagnosis

***2.2 Antibiotic sensitivity testAntibiotics Sensitivity test***

An antibiotic susceptibility test was carried out on the isolates under study by the disc diffusion method according to the method the Baure method - Kiby modified and described by the World Health Organization [22], and 10 types of antibiotic tablets were used using Muller Hinton's medium. Suspended bacterial isolates were prepared and a number of pure colonies were transferred to tubes containing Nutrient broth medium and the tubes were incubated. at a temperature of 35 °C for 18–24 hours, and then the growth in the tubes was compared with the tube containing MacFarland's solution, which is approximately 1.5 * 10 cells / ml, Using a sterile cotton swab, the bacteria were spread on the medium of Muller-Hinton acar and left for a period of time15 minutes for the dishes to dry, then the antibiotic tablets were distributed by sterile forceps on the surface of the medium, and the dishes were incubated for 24 hours at a temperature of 37 °C. Resistant, sensitive and moderately sensitive bacteria to antibiotics.

***2.3 Preparation of dilute solutions of nanomaterials and their effect on resistant bacterial isolation***

A bacterial suspension equivalent to a MacFarland tube was prepared in different concentrations, which are (100, 75, 50, 25) as follows:

- The first concentration 100%: Prepare with melted 0.01 g/mL of nanomaterial and fill the volume with distilled water to 1 mL.
- Second concentration 75%: Mix 750 microliters of the first concentration and fill up the volume with 250 microliters of distilled water.
- Third concentration 50%: Prepare 500 microliters of the first concentration by mixing and fill the volume with 500 microliters of distilled water.
- Fourth concentration 25%: Mix 250 microliters of the first concentration and fill the volume with 750 microliters of distilled water.

After an incubation period of 24 hours at 37°C, the minimum inhibitory concentration was obtained MIC with the lowest dilution of nano-material and free of bacterial growth. As for the concentration of bacterial killer, it was determined by planting the minimum inhibitory concentration on solid culture media after an incubation period of 24 hours at a temperature of 37 °C and no growth was observed [12].

***2.4 Random polymerase chain reaction test: extractionDNA***

DNA extraction has been doneDNA according to what was mentioned as follows

1- Inoculate 5 ml of heart and brain broth mediumBrain heart broth was obtained from bacterial isolates, the tubes were incubated for 24 hours at a temperature of 37°C, then 2 lµ of it was transferred to Ependrof tubes and placed in a microcentrifuge at 14,000 rpm for 5 minutes, the sediment was removed and the precipitate was kept.

2- Cells were resuspended at 500. l µ of STE buffer to wash bacterial cells from plankton such as medium and others.

3- add 50µl of Lysozyme solution, then gently mixed and the tubes were incubated for one hour at 37°C to get rid of the bacterial cell wall.

4- After incubation add 500l µ of STE solution, 20 l µ of SDS solution and 10 µ of Proteinase K solution, the mixture was mixed and the tubes were incubated for an hour at 55 °C, as Proteinase K is used to inactivate proteins by breaking the peptide bonds of proteins. As for SDS, it is a powerful destroying agent. For proteins and a negatively charged ionic cleaner that denatures the cell membrane and works to separate the proteins associated with the DNA molecule.

5- Then add 750l µ of chloroform isomyl alcohol solution and mixed by turning the tubes and then separating the mixture by centrifugation 14000 rpm for 5 minutes.

6- The top layer was removed and transferred to a new tube ependrof tube 750l µ of chloroform-isoyl alcohol solution was added and the mixture was separated at 14,000 rpm for 5 minutes.

7- The top layer was also taken with a new tube, and a double volume of cooled ethanol was added, and 100l µ of C2H7NO2 ammonium acetate solution, then the tubes were placed at a temperature of 5 °C for 10 minutes to precipitate the DNA.

8- The mixture was precipitated by centrifugation at 14,000 rpm for 10 minutes. The precipitate was taken and the suspended matter was discarded.

9- 200 ml of 70% ethanol solution was added, then the tubes were precipitated in a centrifuge at 14,000 rpm for 5 minutes, after that the suspension was discarded, and the sediment was kept, and the tubes were dried in the incubator, as 70% ethanol works to short the DNA from DNA attached to it from the compounds.

10- Add 100l µ of distilled water after drying the ethanol and left the tubes for a little while to precipitate the DNA and then kept at -20°C until use.

***2.5 Diagnostic method using technology Real-time PCR***

technique has been madeReal-Time PCR using the primers and probes for the genes of the bacteria S. aureus, which is responsible for tooth decay, according to the method [8], which consists of several steps:
1: prepare a mixtureReal-Time PCR master mix:
A reaction mixture has been prepared Real-Time PCR using several AccuPower Dualstar Qpcr Maater Mix supplied by the Korean company Bioneer and according to the company's instructions and as shown in Table (2):

**Table (1)** reaction components Real-Time PCR

| **mixPCR master mix** | **the sizeVolume** |
|---|---|
| DNA template | **5µl** |
| Heamolysin primer 10pmol | **µl1** |
| Protein A primer 10pmol | **1µl** |
| TSST primer 10pmol | **2µl** |
| DEPC water | **9µl** |
| Total | **18µl** |

Then the components of the reaction mixture were placed Real-Time PCR that was mentioned in the table above was transferred to sterile 0.2ml white tubes of Real-Time PCR machine, then all tubes were transferred to a vortex centrifuge at 3000 rpm for three minutes and then placed in the Real Time PCR machine.

-2: states of thermal cycles
Thermocycler conditions: Real-Time PCR
The thermal cycle program was applied to checkReal-Time PCR based on the instructions of the AccuPower Dualstar Qpcr Maater Mix kit by calculating the optimum temperature of the precursors using the MiniOpticcon Real-Time PCR system BioRad. USA. As in the table below (2):

**Table (2)** The program of thermal cycles for the reaction with technologyReal-Time PCR

| **Steps**<br>**Qpcr step** | **temperature**<br>**Temperature** | **the time**<br>**Time** | **number of coursesRepeat cycle** |
|---|---|---|---|
| Initial Denaturation | 95°C | 3 min | **1** |
| Denaturation | 95°C | 10 sec | **45** |

| | | | |
|---|---|---|---|
| Annealing / Extention Detection (scan) | 58°C | 30 sec | |

***2.6 Analysis of the results of the examination of Real-time PCR***

The results of the examination were analyzed Real-time PCR through the amplification plot based on the value Throushold cycler number (CT), where the sample is positive when it exceeds the threshold line.

: method of checking Real-time PCR for virulence genes:

technique has been madeReal-time PCR using primers for some bacterial virulence genes, according to the method used by as in several steps.

-1: reaction mixture Real-time PCR for virulence factor genes:

A reaction mixture has been prepared Real-time PCR using the AccuPower® 2X GreenStar™ Qpcr Master Mix kit supplied by the Korean company Bioneer and according to the company's instructions. As in Table (3):

**Table (3)** The reaction mixture for the study of virulence factors

| **mix PCRaster mix** | **the size Volnme** |
|---|---|
| 2x GreenStar master mix | **25µl** |
| DNA template | **5µl** |
| Heamolysin primer 10pmol | **2.5µl** |
| Protein A primer 10pmol | **2.5µl** |
| TSST primer 10pmol | **2µl** |
| DEPC water | **13µl** |
| Total | **50µl** |

Subsequently, the components of a reaction mixture were placedThe Real-Time PCR mentioned in the above table was transferred to sterile 0.2ml white tubes of the Real-Time PCR machine, then all tubes were transferred to a vortex centrifuge (Exispin) at a speed of 3000 rpm for three minutes and then placed in a vortex centrifuge. Real-time PCR device.

-2: states of thermal cycles

Thermocycler conditions: Real-Time PCR

The thermal cycle program was applied to checkReal - Time PCR based on the instructions of the AccuPower® 2X GreenStar™ Qpcr Master Mix kit by calculating the optimum temperature of the temperature primers using the MiniOpticcon Real - Time PCR system BioRad. USA, as in the table below (4):

**Table (4)** The program of thermal cycles for the reaction with technologyReal-Time PCR

| steps<br>Qpcr step | temperature<br>Temperature | the time<br>Time | number of courses Repeat cycle |
|---|---|---|---|
| Initial Denaturation | 95℃ | 3 min | **1** |
| Denaturation | 95℃ | 10 sec | **45** |
| Annealing / Extention Detection (scan) | 58℃ | 30 sec | |
| Melting | 65-95℃ | 0.5 Sec | **1** |

**3. Results and discussion**

***3.1 Bacterial isolation and diagnosis***

The results of the isolation showed850 samples Which were collected from people with tooth decay, that 50 samples did not show any growth That's 5.88%whileGrowth has been observed in a800Sample i.e. 94.11%. Includes isolates types of bacteriachromium cation. It the form of the species Staphylococcus aureus Which appeared in 248 isolates, or 31%. As shown in Table (5).

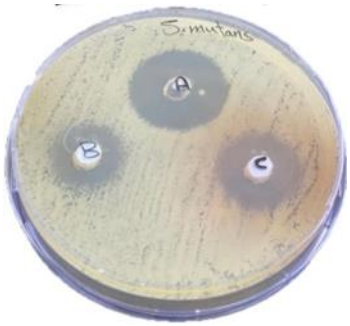
**Table (5)** CountingaDrand types of bacteriachromium cationIsolated from dental caries and their percentages

| The ratioCentennial % | Countingad isolates | theisolatesbacterial chromium cation |
|---|---|---|
| 31 | 248 | ***S. aureus*** |

Sensitivity of bacterial isolates to antibiotics

The results of the study showed variable response of Gram-positive bacteria isolates to a number of antibiotics used in this studywhich included (Clindomycin, Metronidazole, (Amoxicillin as described intable (6).

So bacterial isolates showed-S. aureus resistanttoall Species antibiotics And this The result agreed with [18]. The result of this study varies with what The researcher found it [11] where it was mentioned that bacterial resistancecation for antibiotics Clindamycin was only 20%. The researcher [13]. Bacterial resistance to-Clindamycin 80%, While the researcher [17]. A test of S. aureus bacteria on different types of antibiotics showed different results depending on the type of antibiotic Where it was stated that the resistance of bacteria to-Amoxicillin (77% while resistance to- Gentamycin is 26%, as the cause of resistance is due to a genetic change, i.e. a genetic mutation, such as deleting or replacing a specific gene or transferring a gene within the same sex or from another sex. The researcher said [10]. This antibiotic Metronidazole No It has the ability to inhibit aerobic bacteria.




S.aureus

**Picture (1):** Inhibitory activity of antibiotics on bacterial isolates. A- Amoxicillin B-Clindamycin C-Metronidazole

**Table (6)** Inhibitory activity of antibiotics on bacterial isolates

| C-Metronidazole | B-Clindamycin | A-Amoxicillin | Bacteria |
|---|---|---|---|
| - | - | - | ***S.aureus*** |

***3.2 The effectiveness of nanomaterials against bacterial isolates***

It was completed sensitivity testing of isolates of gram-positive bacteria and gram-negative bacteria under study towards types of nanomaterials (Zinc, Silver, Titanium) with different concentrations. has been tested the sensitivity of isolates to nanomaterials based on the Kirby-Bauer method by measuring the inhibition area of the bacterial isolates used.

***3.3 Efficacy of zinc nanomaterials for positive and negative isolates of chromium dye***

The results of the experiment showed that the zinc concentration of 100% did not give any effect to the positive and negative bacterial species, as well as the results of the concentration of 25% also did not show any effect only for the species. S. aureus inhibitory diametermm 10. As for the concentrations of 50 and 75%, the inhibitory diameters were the bacteria of. S.aureus 14mm and 15mm. shown in Table (7).

**Table (7)** Effect of zinc nanoparticles on a S.aureus bacterial isolates

| 25% | 50% | 75% | 100% | bacteria |
|---|---|---|---|---|
| 10mm | 14mm | 15mm | - | ***S.aureus*** |

The presence of the inhibition circuit is an indicator that the nanoparticles of (Zinc is a good anti-bacterial. The researcher [19] used zinc nanoparticles against S. aureus bacteria and got good results. He also noted that the inhibition increases as the size of the nanoparticles decreases as the surface area increases, and based on these results it was mentioned that zinc nanoparticles Zinc particles produce reactive oxygen compounds (ROS) and hydrogen peroxide ($H_2O_2$) on the cell surface that lead to the destruction of cell components such as protein, fat and DNA and thus cell death, and the most important elements that affect the work of nanoparticles are Size, shape, surface area, purity, and concentrations [20]. male researcher [4] showed that the response of S. aureus to zinc nanoparticles is higher and better than nanoparticles manufactured from other metals, and this matches what we have found.

***3.4 Synergistic effect of nanomaterials***

It was completed sensitivity test of isolates of gram-positive bacteria and gram-negative bacteria under study towards the synergy of 3 types of nanomaterials (Zinc, Titanium, Silver at a concentration of 100, 75%).

Has the results showed the variation in the response of the isolates to the synergistic effect of nanomaterials and the difference in their sensitivity and resistance from one isolate to another. The sensitivity of the isolates to the synergy of nanomaterials was tested depending on the Kirby-Bauer method by measuring the inhibition area. The bacteria showed a different response toward the nanomaterials, as shown in Table (8)

**schedule (8)** The synergistic effect of nanomaterials on a group of S.aureus bacterial isolates

| (ZnO+Ag)NPs | ZnO+TiO2 | (ZnO+Ag+TiO2)NPs | bacteria |
|---|---|---|---|
| 8mm | 10mm | 11mm | ***S.aureus*** |

The results of the study showed Different response to bacterial isolates As for the isolates of S. aureus bacteria, they showed a weak response to the synergy of nanoparticles (ZnO+Ag+TiO2, ZnO+TiO2, ZnO+Ag) with a size of (11mm, 10mm, 8mm), respectively, as the use of zinc particles alone affected Better.

The researcher made [5] Testing the synergistic effect of zinc particles with silver nanoparticles on S. aureus bacteria. It was stated that silver was the main cause of inhibition, and this contradicts what we found, as silver did not show a significant effect on the isolates used in this research, and the researcher [16] obtained a five-fold inhibition of S. aureus by the synergistic antibacterial agent of silver nanoparticles.

***3.5 The virulence factors of the bacteria S. aureus***

The results of the study showed that using the RT-PCR differentiated response to virulence factors (protein A, haemolysin, TSST for Bacterial isolates). S. aureus For the effect of zinc nanoparticles used in concentrations (2,1,0.5,0.25mg), and we got the highest inhibition at the concentration (0.5 mg).

revealing of Haemolysin genes

The results of the study proved the current response of the virulence factor haemolysin to the effect of zinc nanoparticles used at concentrations (2,1,0.5,0.25mg), and we obtained the highest inhibition of gene expression at the concentration (0.5 mg) as shown in Table (7).

These results agree with the researcher [4] conducted a test for the effect of zinc nanoparticles on (haemolysin genes) and noticed a decrease in the gene expression of the enzyme after 12 hours, while the researcher conducted the test on bacteria S. aureus , He noticed that the ability to produce virulence factors, including hemolysin, decreased after treating bacteria with zinc nanoparticles with a concentration ranging between (25-50 mg/mL) the enzyme that dissolves blood cells controlled by (haemolysin genes), and this enzyme is one of the most important virulence factors found in bacteria.S. aureus Where the red blood cells of the host decompose and lead to the death of platelets and white blood cells [6].

The following figure (1) shows the effect of zinc nanoparticles on virulence factor gene expression haemolysin.

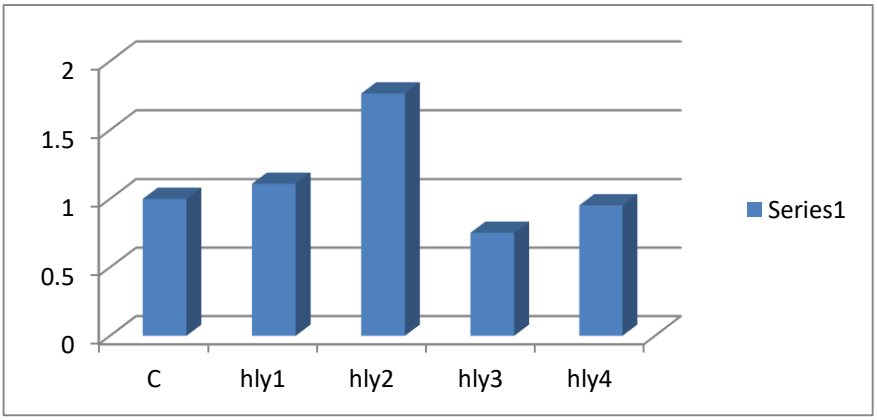



**Figure 1:** Effect of zinc nanoparticles on virulence factor gene expression haemolysin

**Table (9)** Effect of zinc nanoparticles on virulence factor gene expression haemolysin

| HLY | D CT | DD CT | FOLD |
|---|---|---|---|
| **C** | | | 1 |
| **hly1** | -0.26 | -0.15 | 1.109569 |
| **hly2** | -0.93 | -0.82 | 1.765406 |
| **hly3** | 0.3 | 0.41 | 0.752623 |
| **hly4** | -0.04 | 0.07 | 0.952638 |

revealing ofprotein A genes

The results of the study proved The current response of the virulence factor (protein A) to the effect of zinc nanoparticles used at concentrations (2,1,0.5,0.25mg), and a decrease in the gene expression of the factor was noted and the best inhibition was observed at the concentration (0.5 mg) as shown in Table (10).

Several studies have proven the efficiency of nanoparticles in inhibiting bacteria S. aureus This is by releasing the contents of the cell such as protein and sugar, which is an indicator of cell death, as the researcher noted [7] that the bacterial concentration decreases with the increase in the incubation time of bacteria with nanoparticles due to the loss of cell contents and the increase in the production of ROS and lipid peroxidation, and that the exposure of bacteria to ZnONP affects the construction of amino acids and causes inhibition of enzymes by entering the cell by breaking the wall, including the important enzyme β-galactosidase Bacterial metabolism or enzymes necessary for bacterial growth, and researcher [9] used silver nanoparticles as an antibiotic, which resulted in a change in the gene expression of many proteins, including recombinase A protein, where the expression of genes and the number of folds decreases. The following figure shows the effect of zinc nanoparticles on the gene expression of virulence factor (protein A).

In the following figure (2) shows the effect of zinc on the gene expression of virulence factor protein A.

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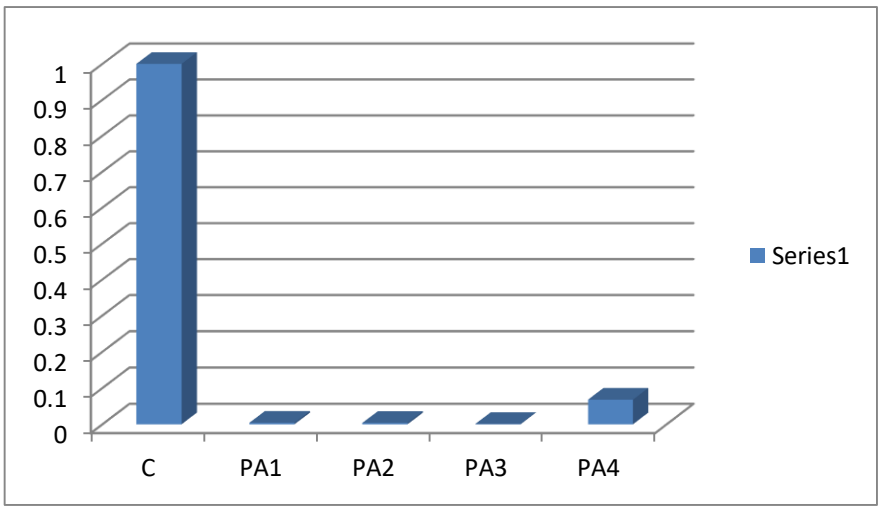


**the shape (2):** Effect of ZnONPs on the gene expression of virulence factor Protein A

**Table (10)** Effect of zinc nanoparticles ZnONPs on virulence factor protein A. gene expression

| PA | D CT | DD CT | FOLD |
|---|---|---|---|
| C | | | 1 |
| PA1 | 9.03 | 7.94 | 0.004072 |
| PA2 | 9.17 | 8.08 | 0.003696 |
| PA3 | 11.18 | 10.09 | 0.000918 |
| PA4 | 4.97 | 3.88 | 0.067921 |

revealing ofTSST-1 genes:

In the study We obtained the effect of the gene expression of the virulence factor (TSST-1) in response to zinc nanoparticles used at different concentrations (2,1,0.5,0.25mg), and a decrease in the gene expression of the factor was noted and the best inhibition was observed at the concentration (0.5 mg) as shown in Table (11).

Several studies have shown that zinc nanoparticles have the best effect on H. pylori S. aureus from particles made from other minerals such as Cuo, Ceo2, Al2o3, Tio2 [4].

Use Finder [7] RT-PCR technique to test the effect of ZnONP on many different genes of S. aureus, a difference in gene expression was noticed. Also the researcher Abdelraheem (2021) and Mohamed conducted a test of the effect of ZnONp nanoparticles on P. aeruginosa bacteria and noticed an inhibition in the production of Biofilm and many virulence factors. It was mentioned that the effect increases with increasing concentration and decreasing the size of nanoparticles.

(TSST-1), an antigen considered one of the most important virulence factors, is produced by 5 to 25% of the population S. aureusIt is expressed by moving genetic elements such as the jumping gene and is affected by

environmental conditions such as pH, iron and deficiency of some elements (such as (Mg, Ca, O2) and is associated with many chronic diseases, including toxic shock syndrome (TSS), so its inhibition is one of the most important factors to reduce toxicity Bacteria [21].

The following figure shows the effect of zinc on virulence factor gene expressionTSST-1

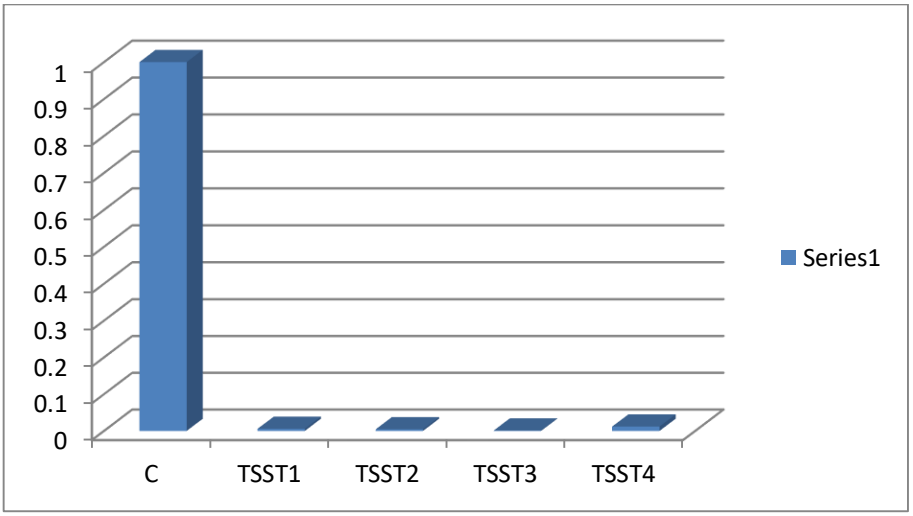


**Figure 3:** Effect of zinc nanoparticlesZnONPs on virulence factor-1 (TSST-1) gene expression.

**Table (11)** Effect of zinc nanoparticles ZnONPs on virulence factor-1 (TSST-1) gene expression.

| TSST | D CT | DD CT | FOLD |
|---|---|---|---|
| C | | | 1 |
| TSST1 | 7.93 | 7.43 | 0.005799 |
| TSST2 | 8.35 | 7.85 | 0.004334 |
| TSST3 | 10.2 | 9.7 | 0.001202 |
| TSST4 | 6.9 | 6.4 | 0.011842 |

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