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EFFECT OF ZINC, SILVER AND TITANIUM NANOPARTICLES ON THE GENES OF VIRULENCE FACTORS OF BACTERIA ISOLATED FROM DENTAL CARIES.

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Abstract:

The sensitivity of Gram-positive and negative 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 ofRT-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).

Keywords: Gram-positive, .Zinc, Sliver, Titanium

INTRODUCTION:

that the science of nanotechnologyNanotechnology 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 (Al-Saadi, 2021).
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 (Al-Hormozi, 2016).
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 (Abdul-Wahab, 2015).
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).

MATERIALS AND WORKING METHODS:

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 obtainedMIC 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 (Sorkh et al., 2017)

RANDOM POLYMERASE CHAIN REACTION TEST:

extractionDNA:

DNA extraction has been doneDNA according to what was mentioned (Onasanya et al., 2003) 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 tubeEpendrof 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 fromDNA 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.

Diagnostic method using technologyReal-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 (Lau et al., 2013), which consists of several steps:

1: prepare a mixtureReal-Time PCR master mix:

A reaction mixture has been prepared**Real-Time PCR**using severalAccuPower 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 componentsReal-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 placedReal-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 Qpcr step	temperature Temperature	the time Time	number of coursesRepeat cycle
Initial Denaturation	95℃	3 min	**1**
Denaturation	95℃	10 sec	**45**
Annealing / Extention Detection (scan)	58℃	30 sec	

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 (Al-Torfi, 2014) 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 Qpcr step	temperature Temperature	the time 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**

RESULTS AND DISCUSSION :

The effectiveness of nanomaterials against bacterial isolates:

It was completedSensitivity 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 testedThe sensitivity of isolates to nanomaterials based on the Kirby-Bauer method by measuring the inhibition area of the bacterial isolates used.

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 diametermm10.

As for the concentrations of 50 and 75%, the inhibitory diameters were the bacteria of . *S.aureus* 14mm and 15mm, respectively, as for Strep. pyogenes concentrations 50, 75%, the inhibitory diameters were 10 mm for both concentrations, for Strep.muntans bacteria, concentrations 50, 75%, the inhibitory diameters were 14 mm, 10 mm, respectively, for E.coli bacteria, concentrations 50, 75%, the inhibitory diameters were 12 mm for both concentrations. K. pneumoniae bacteria did not give any inhibitory activity at all concentrations as shown in Table (5).

Table (5) Effect of zinc nanoparticles on a group of Gram-positive and Gram-negative bacterial isolates

25%	50%	75%	100%	bacteria
10mm	14mm	15mm	-	***S.aureus***
-	10mm	10mm	-	***Strep.pyogenes***
-	14mm	10mm	-	***Strep.muntans***
-	12mm	12mm	-	***E.coli***
-	14mm	10mm	-	***K. pneumonia***

The presence of the inhibition circuit is an indicator that the nanoparticles of (Zinc is a good anti-bacterial. The researcher Karvani and Chehrazi (2011) 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 (H2O2) 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 (Khan et al., 2019).

male researcher *et al*. Aleaghi (2016) 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.

For isolates ofStreptococcus showed a difference in the response to zinc nanoparticles in different species, as S. pyogenes produced in response to nanoparticles measuring (10 mm, 10 mm) to (75, 50)%, respectively, while the effect of nanoparticles on S. muntans was (14mm, 10mm) for concentrations of (75, 50%) respectively, in contrast, no effect of 25 and 100% concentrations was seen on all Streptococcus isolates.

The researcher made(2020) Liang et al., Test of zinc nanoparticles on S. pyogenes and noted that nanoparticles bind to polypeptides and glycogen present in the cell wall of bacteria, leading to wall destruction and then cell death.

Several studies on the bacterium *S. muntans*To measure the effect of zinc nanoparticles, a response was obtained at the lowest concentration user (Ahari et al., 2015,Ramazanzadeh et al., 2015,), in contrast, the researcher Eshed et al., (2012(No effect was recorded, this difference in results may be due to the different isolates and concentrations used, in addition to the fact that nanoparticles have a decreasing effect with time)Jatania and Shivalinga, 2014).

nanoparticles manufactured from(Zinc showed a slight effect on gram-negative bacteria and as shown in the table (), for isolates of E. coli bacteria produced an inhibition cycle with the same measurement despite the difference in concentration of the nanomaterial, where the size of the inhibition circle was (12 mm, 12 mm) for concentrations (50, 75).) %, respectively, and the concentration (25 and 100%) did not show any effect.

Conducted by researcher (2011)Karvani and Chehrazi tested the response of S. aureus and E. coli to zinc nanoparticles, and noted that these nanoparticles have more effect on S. aureus than E. coli, as the inhibition circuit for S. aureus is equal to (29 mm), while The inhibitory circle of E. coli is equal to (19 mm) in a concentration of (10 mg/ml.

For isolates of*K. pneumoniae*showed a difference in response to nanoparticles to (Zinc produced in response to nanoparticles measuring (14 mm, 10 mm) to concentrations (75, 50)%, respectively, in contrast, no effect was shown for concentrations of 25 and 100% on all K. pneumoniae isolates.

The researcher made(2016) Abdulrahman et al. Testing zinc nanoparticles on E. coli, K. pneumoniae bacteria, and the result was that E. coli bacteria are more inhibited and responsive to nanoparticles, and he mentioned that the response increases with increasing concentrations of nanoparticles, and this contradicts the results we obtained in this research. The concentration of 100% did not record inhibition for all isolates of Gram-negative bacteria, while the researcher Nazoori and Kariminik (2018) conducted a test for zinc nanoparticles against a group of isolates, including K. pneumoniae, and obtained inhibition for all isolates with the lowest concentration of nanoparticles, although these The isolates were resistant to antibiotics, thus we get an antibacterial with less toxicity and higher effect.

did notBacteria appear in response to nanomaterials (Sliver, Titanium), while researcher (2019) Yaseen et al. It obtained a weak response of gram-negative and gram-negative bacteria towards silver nanoparticles, for titanium nanoparticles, the researcher (Abdulazeem et al. 2019) obtained an inhibition of the growth of gram-positive and gram-negative bacteria with titanium nanoparticles stronger than the inhibition produced by using the antibiotic, and

this does not agree with the current search results. Made by Researcher(2020) Albukhaty et al. Titanium nanoparticles with different concentrations (25, 100, 50, %) and different temperatures obtained inhibition of E. coli and S. aureus bacteria with the manufactured nanoparticles, which were very efficient due to their chemical and physical properties.

Synergistic effect of nanomaterials:

It was completedSensitivity 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%).

hasThe 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 (6)

schedule (6) The synergistic effect of nanomaterials on a group of Gram-positive and negative bacterial isolates

(ZnO+Ag)NPs	ZnO+TiO2	(ZnO+Ag+TiO2)NPs	bacteria
8mm	10mm	11mm	***S.aureus***
-	-	-	***S. pyogenes***
15mm	13mm	15mm	***S. muntans***
-	8mm	10mm	***E. coli***
10mm	10mm	10mm	***K. pneumonia***

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, for the Streptococci isolates showed a difference in the response to the synergism of nanoparticles in different species as S. pyogenes did not show any synergistic response, while the result of the synergistic response of nanoparticles to S. muntans was (ZnO+Ag+TiO2, ZnO+TiO2, ZnO+Ag) in size (15mm, 13mm, 15mm) respectively.

The researcher made (Asamoah et al. 2020) 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 et al. Bednář (2019) obtained a five-fold inhibition of S. aureus by the synergistic antibacterial agent of silver nanoparticles.

The results of the isolates of the bacterium E. coli showed a weak response to the synergy of nanoparticles ((ZnO+Ag+TiO2, ZnO+TiO2 (10mm and 8mm), respectively, and the synergy between silver and zinc showed no effect, for K. pneumoniae isolates showed a response to the synergy of nanoparticles where it was The result is (ZnO+Ag+TiO2, ZnO+TiO2, ZnO+Ag) measuring (10mm, 10mm (10mm) respectively.

The result of the synergy of nanoparticles obtained by the researcher *et al*. Bednář (2019) showed that the effect against E. coli was twice that of silver nanoparticles, and Asamoah et al. (2020) A synergy between silver nanoparticles and zinc nanoparticles showed efficiency in affecting E. coli bacteria with an inhibition rate of 89.3%.

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

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

revealing ofHaemolysin genes :

The results of the study provedThe 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*et al.* Saghalli (2016) 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 (Kadhim et al 2019) conducted the test on bacteria (K. pneumonia, (P. aeruginosa, 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 (Divyakolu et al., 2019).

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

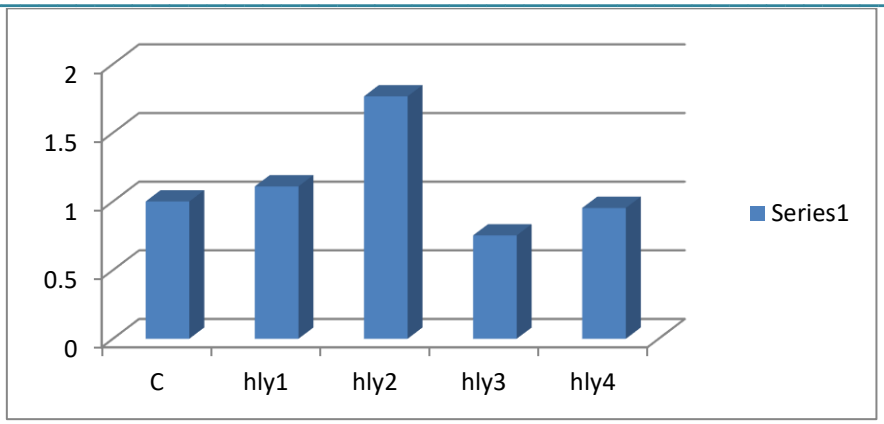


Figure 1: Effect of zinc nanoparticles on virulence factor gene expressionhaemolysin

Table (7) Effect of zinc nanoparticles on virulence factor gene expressionhaemolysin

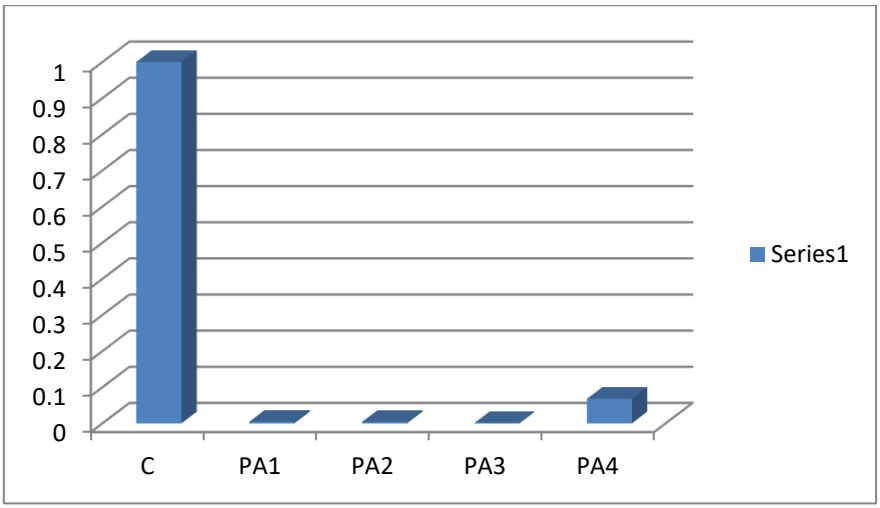
HLY	D CT	DD CT	FOLD
C			1
hly1	-0.26	-0.15	1.109569
hly2	-0.93	-0.82	1.765406
hly3	0.3	0.41	0.752623
hly4	-0.04	0.07	0.952638

revealing ofprotein A genes :

The results of the study provedThe 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 (8).

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 notedKadiyala et al. (2018) 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 Li et al. (2011) 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.



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

Table (9) 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 (10).).

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 (2016). (Aleaghil et al.,

Use Finder Kadiyala et al. ((2018) 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 (2021) Abdelraheem 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. aureus*It 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 ((Peng et al., 2021.

The following figure shows the effect of zinc on virulence factor gene expression ((TSST-1

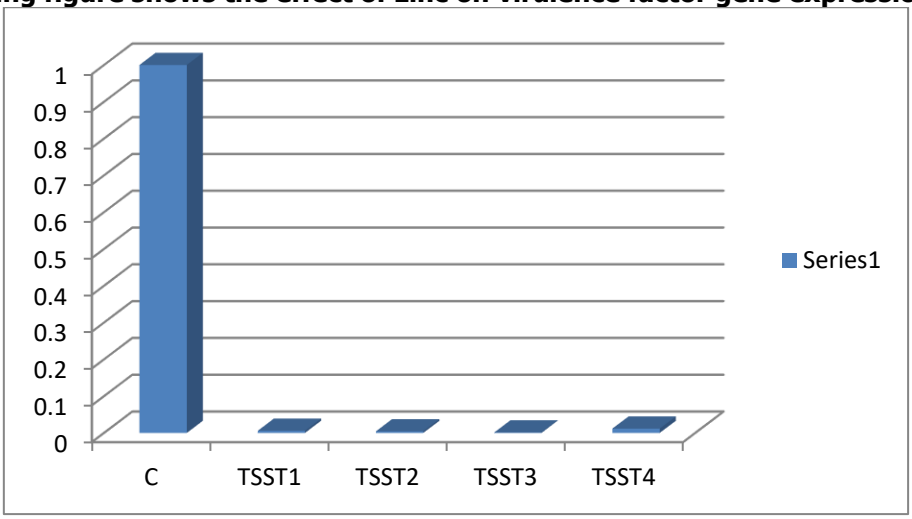


Figure 3: Effect of zinc nanoparticlesZnONPs on virulence factor-1 (TSST-1) gene expression.

Table (10) Effect of zinc nanoparticlesZnONPs 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

REFERENCE

Al-Jamili, Marwa Jassim, Bahjat, Najdat Mahdi, Khurshid, Sahma Abbas. (2019). Diagnosing some bacteria that cause gingivitis in people with type 2 diabetes and investigating some of their chemical and immune standards. Journal of Mesopotamia, Frozen 82, (3): 48-54.

Saleh, Mushtaq Talib, Turki, Ahmed Mohammed, Shehan Mayada Abdullah (2015). Diagnosis of Staphylococcus aureus genetically based on gene.nuc Anbar University Journal of Pure Sciences. 9:(3)

Fazza, Souad Abd. (2018). The effect of cloves water extract on some bacterial isolates that cause gingivitis. Journal of Life Sciences of Kufa. 9-1 : (1)5

Sorkh, M. A.; Shokoohizadeh, L.; Rashidi, N. and Tajbakhsh, E. (2017). Molecular Analysis of *Pseudomonas aeruginosa* Strains Isolated from Burn Patients by Repetitive Estrogenic Palindromic – PCR (rep-PCR). Iranian Red Crescent Medical Journal, 19(4).

Lau, K. Y., Vaughan, A. S., Chen, G., Hosier, I. L., & Holt, A. F. (2013). On the dielectric response of silica-based polyethylene nanocomposites. Journal of Physics D: Applied .Physics, 46(9), 095303

Al-Torfi, Z.A.H. (2014). A study virulence factors (Eh CP1 and Eh CP5) and some Immunobiological changes in human infeacted with *Entamoeba histolytica* in Al-Najaf Al-Ashraf Governorate . Athesis . Coll. Sci . Kufa university. 140 .

Karvani, Z. E. and Chehrazi, P. (2011).Antibacterial activity of ZnO nanoparticle on grampositive and gram-negative bacteria African Journal of .Microbiology Research. 5(12):1368-1373

Khan, A., Adam, A., Aziz, M.A., Ahmed, M.I., Yamani, Z.H. and Qamar, M. (2019). Shape-dependent performance of gold nanocrystals supported on TiO2 for photoelectrochemical water oxidation under different radiations. Int J Hydrogen Energy. 44(41):23054–23065.

Aleaghil, A S. A., Fattahy, E., Baei, B., Saghali, M., Bagheri, H., Javid, N. and Ghaemi, E. A. (2016). antibacterial activity of Zinc oxide nanoparticles on Staphylococcus aureus. International Journal of Advanced Biotechnology and Research (IJBR). 7:1569-.1575

Liang, S. X. T., Wong, L. S., Lim, Y. M., Lee, P.F., Djearamane, S. (2020). Effects of Zinc Oxide nanoparticles on *Streptococcus pyogenes.* South African Journal of Chemical Engineering. 34 : 63-71.

Ramazanzadeh, B., Jahanbin, A., Yaghoubi, M., Shahtahmassbi, N., Ghazvini, K., Shakeri, M. and Shafaee, H. (2015). Comparison of antibacterial effects of ZnO and CuO nanoparticles coated brackets against .Streptococcus mutans. J. Dent. 16:200–205

Eshed, M., Lellouche, J., Matalon, S., Gedanken, A. and Banin, E. (2012). Sonochemical coatings of ZnO and CuO nanoparticles inhibit *Streptococcus mutans.* biofilm formation on teeth model. Langmuir. 28: 12288–12295.

Jatania, A. and Shivalinga, B.M. (2014). An in vitro study to evaluate the effects of addition of zinc oxide to an .orthodontic bonding agent. Eur. J. Dent. 8:112–117

Karvani, Z. E. and Chehrazi, P. (2011). Antibacterial activity of ZnO nanoparticle on grampositive and gram-negative bacteria African Journal of Microbiology Research. 5(12):1368-1373.

Abdulrahman, S. H. H., Karthikeyan, C., Ahamed, A. P., Thajuddin, N., Alharbi, N. S., Alharbi, S. A. and Ravi, G. (2016). In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae. .Scientific Reports. 6:24312

Nazoori, E. S. and Kariminik, A. (2018). In Vitro Evaluation of Antibacterial Properties of Zinc Oxide Nanoparticles on Pathogenic Prokaryotes. Journal of Applied Biotechnology Reports. J Appl Biotechnol Rep. 5(4):162-165.

Abdulazeem, L., AL-Amiedi, B. H., Alrubaei, H. A. and AL-Mawlah, Y. H. (2019). Titanium dioxide nanoparticles as antibacterial agents against some pathogenic bacteria. Drug . Invention Today .12 : 5

Albukhaty ,S. Al-Bayati, L., Al-Karagoly, H. and Al-Musawi, S. (2020). Preparation and characterization of titanium dioxide nanoparticles and *in vitro* investigation of their cytotoxicity and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* Animal Biotechnology, DOI: 10.1080/10495398. 1842751.

Asamoah, R. B., Annan, E., Mensah, B., Nbelayim, P. Apalangya, V., Agyeman, B. O. and Hindawi, A. Y. (2020). A Comparative

Study of Antibacterial Activity of CuO/Ag and ZnO/Ag Nanocomposites. Advances in Materials Science and Engineering. ID .7814324:18

Bednář, J. , Svoboda, L. , Rybková, Z. , Dvorský, R. , Malachová, K. , Stachurová T. , Matýsek D. and Foldyna, V. (2019). Antimicrobial Synergistic Effect Between Ag and Zn in Ag-ZnO·mSiO$_2$ Silicate Composite with High Specific Surface Area. Nanomaterials (Basel). 9(9): 1265 .

Divyakolu, S., Chikkala, R., Ratnakar, K. Suguna. and Sritharan V. (2019). Hemolysins of Staphylococcus aureus—An Update on Their Biology, Role in Pathogenesis and as Targets for Anti Virulence Therapy. Advances in .Infectious Diseases. 9:80-104

Kadiyala, U., Emre, E. S. T., Bahng, J. H. , Kotov, N. A. and Vanepps, J. S. (2018). Unexpected insights into antibacterial activity of zinc oxide nanoparticles against methicillin resistant *Staphylococcus aureus* (MRSA). Nanoscale. 8:10(10): 4927–4939.

Li, W.R., Xie, X.B., Shi, Q.S., Duan, S.S., Ouyang, Y. S. and Chen, Y. B. (2011). Antibacterial effect of silver nanoparticles on Staphylococcus aureus . Biometals. 24:135– .141

Peng, L., Jiang, J., Chen, T., Xu, D., Hou, F., Huang, Q., Peng, Y., Chao, Y. , Hu, D. and Fan, R. (2021). Toxic Shock Syndrome Toxin 1 Induces Immune Response via the Activation of NLRP3 Inflammasome. Toxins. 13: 68.