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The inhibitory effect of some nanoparticles on biofilm formation of *Streptococcus agalactiae*

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ABSTRACT: Biofilm are a link between microorganisms in which germ cells stick together on living or non-living surfaces within a self-produced polymer.

OBJECTIVE: Evaluation of the inhibitory effectiveness of some nanoparticles : Zinc Oxide (ZnO), Titanium Oxide (TiO) and Copper Oxide (CuO) on the formation of Biofilm of *S. agalactiae* .

METHOD : In this study ,Sixteen isolates of *Streptococcus agalactiae* were isolated and diagnosed from a total of 850 clinical specimens (425 vaginal swabs and 425 rectal swabs) taken from expatriate women and women in the Women's and Children Teaching hospital, Afak General Hospital and Women's clinics in AL-Diwaniyah city during the period from July 2018 to April 2019. Clinical samples were collected from pregnant women with gestation of 35-37 week.

CONCLOSION: The results of the study showed the Efficiency of nano-oxide (TiO,ZnO,CuO) to inhibit the growth of *S.agalactiae* bacterium and on the formation of biofilms and quorum sensing , copper oxide was the most efficient among them**.**

Results: This study aim to detect the effect of some nanoparticles (CuO ,ZnO, TiO) to inhibit the biofilm formation of *Streptococcus agalactiae* its demonstrated the susceptibility of the *Streptococcus agalactiae* to form the Biofilms and the effectiveness of the three used nano metal oxide (CuO,ZnO,TiO) to inhibit it .Where, the high efficiency of preventing the formation of biofilm showed with used of copper oxide followed by the zinc oxide.While, the result from using titanium oxide has shown the low effectiveness in the inhibition of Biofilm formation.

KEYWORDS: *Streptococcus agalactiae* **,** Biofilms , Nano metal oxide.

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1. INTRODUCTION *Streptococcus agalactiae* (known as group B Streptococcus or GBS) Gram-positive cocci tend to form short or long chains, capsulated, some of which are facultative anaerobic and some are anaerobic ones, It grows at a temperature of 22-24 ° C and its growth rate is 37 ° C, negative for catalase , whole blood decomposition (β-hemolysis) The decomposition is clearly observed in the blood agar (Versalovic and James *et al.*, 2011).

They grow in normal nutrient agar and are better in blood agar or serum agar They appear in small, translucent, sparsely spaced colonies. The surface of the colony is not shiny on the fresh culture. In the secondary culture, the surface becomes bright and the surface is sticky when the capsule is formed. Growth in the liquid medium has a granular appearance with a deposition of powder in the bottom (Jawetiz *et al*,.2013) . *Streptococcus agalactiae* are pathogens that often form part of the naturally occurring microorganisms in the gastrointestinal tract and urinary tract in healthy women (Verani *et al*.,2010) ,can cause serious infection for newborns and adults.

Biofilm is a group of microbial cells trapped as a matrix in extracellular polymeric material. Using complex mechanisms produced by specialized genes, members of biofilms can speak, communicate and exchange virulence factors with each other according to a phenomenon called Quorum Sensing. The formation of biofilms by bacteria is a key factor in their pathogenicity and survival (D'Urzo *et al*., 2014).

Nano materials have become a promising and effective candidate that can replace traditional materials in all areas of science and technology, due to the small size of nanomaterials with a larger surface-to-surface ratio and an increasing number of active atoms on their outer surfaces (Azam *et al*.,2009). Some nanomaterials have been approved as microbial agents, including silver, gold and zinc, each having different properties and spectral events (Lansdown,2006 and Zhou *et al*.,2012).

2. MATERIALS AND METHODES

Preparation of TiO, ZnO, CuO Solution

The nanoparticles solution of (TiO, ZnO, CuO) prepared by dissolving 1 g of nanomaterials in 100 mL of non-ionic distilled water in a 250 mL sterile glass tube to prepare 1% of the nanoscale suspension as a storage solution and then ultrasonic suspension treatment (100w, 40 kHz) for 30 minutes and autoclave sterilization at 121 °C for 15 minutes, Various concentrations (100,250, 500) µg / mL are tested to detect the inhibitory effectiveness of nanoparticles against *S.agalactiae* bacteria(Hossinkhani *et al*.,2011; Zaki *et al*.,2016).

Antibiofilm activity of TiO, CuO, ZnO nanoparticles

The microtiter plate was used to measure the effectiveness of nanoparticles against the formation of biofilms in *S. agalactiae* bacteria in vitro .Three concentrations were Prepare (100,250,500) microgram/ml of each nanomaterial and then inoculate (1.9) ml of brain heart infusion broth by (0.1) of bacterial suspension of (0.5) concentration that read at 630 nm . The (150) microliters from the bacterial growth transferred to each well of 96 well in the plate, and 50 µl of nanoparticles at a concentration of 4x were added, 50 µl from the brain heart broth added into a single well for each bacterial test as a control group to confirm the production of the biofilms by the bacteria and inhibition by the used nanoparticles. An amount of 200 µL of sterilized distilled water was added to all peripheral wells (to reduce water loss). The plate was incubated for 16 hours at 37 °C. After incubation the plankton cells aspirated. Then fixed by methanol 99%. The plate is then washed twice with sterile phosphate buffer saline and dried. Then 200 µL of (0.2%) crystal violet solution were added to all the wells of the plate and left for 5 minutes, the excess solution was removed and the

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plate washed twice, and then dried with air, and the cells that associated with crystal violet dye dissolved in 33% acetic acid. The formation of biofilms was read at 630 nm using the ELISA reader (Shukla and Rao,2017).

3. Results and discussion

Results of the test of the biofilms formation and its inhibition by nano-oxides

The biofilme formation test for all *S.agalactiae* isolates was done by using the 96-well ELISA plate after an incubation period of 18 hours and at a density of 0.5 light and absorbance reading along a wavelength of 630 nanometers the result of this test more clarified in figure (1).

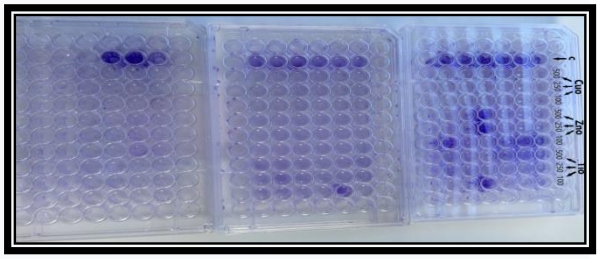

Fig (1): Inhibition of biofilms by nanoparticles using a crystal violet test

C: Control

The results showed that all GBS isolates have the ability to form biofilms that contribute to antibiotic resistance. This result is consistent with (Abdul-Lateef *et al*.,2018) Study that conducted in the province of Babylon, where all tested *Streptococcus agalactiae* were made up of biofilm

The study found that the copper oxide has a high efficiency in preventing the formation of biofilms, noting that the greater concentration of copper oxide, the nanoscale increased the rate of inhibition of biofilms and this result came close to what reached (Agarwala *et al*.,2014). Where the results indicated the efficiency of copper oxide in the inhibiting of the biofilms in both *Staphylococcus aureus and Escherichia coli.*

The toxicity of copper oxide to bacteria is done by several mechanisms that eventually lead to its death. The first objective of copper is to destroy the living cell envelope. Copper ions cause damage to DNA. The copper binding of DNA leads to the formation of many OH-free radicals near the link site, Nucleic acid in the region (Borkow and Gabby, 2009).

Zinc nano-oxide had a good potential for inhibition of biofilms but less than the effect of copper oxide. Many studies have shown their effectiveness against many bacterial species such as *Staphylococcus spp* (Huang *et al*.,2008) as well as against *K. pneumonia* (Lee,2009). Zinc oxide showed efficacy towards *Pseudomonas spp* (Jaing *et al*.,2009) ,and *Escherichia coli* (Zhang *et al*., 2009) . It was observed that when the concentration of nanoparticles was increased, the biofilm was increasingly inhibited The result came close to what he had reached (Rizwan *et al*., 2010) against *E. coli, S. aureus*

Titanium oxide has shown low effectiveness in inhibiting biochemical membranes, which is contrary to the findings (Roy *et al*.,2010) , which showed the the failure of titanium oxide in inhibiting microbial growth and inhibiting the formation of biofilms in *Staphylococcus aureus*.

Table (1) refers to the statistical analysis of the results of the composition of the biofilms and their inhibition using nano-oxides

Table (1): The results of the statistical analysis of inhibition of the biophysics include the rate of inhibition and standard error.

Nano materials	Concentrate µg / ml	Standard error ± inhibition rate	$LSD_{0.05}$
TiO	100	0.152±0.009	0.051

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	250	0.150±0.006	
	500	0.153±0.009	
	Control	0.289±0.003	
ZnO	100	0.154±0.009	0.060
	250	0.154±0.01	
	500	0.170±0.01	
	Control	0.289±0.003	
CuO	100	0.137±0.002	0.051
	250	0.141±0.002	
	500	0.143±0.005	
	Control	0.289±0.003	

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