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IN VITRO EVALUATION OF ANTIBACTERIAL EFFECTS OF ZINC OXIDE NANOPARTICLES AND ANTIBIOTICS AGAINST MRSA

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Abstract

The increases bacteria resistance to antibiotics multiple have led to novel methods for producing nanoparticles with biological method and effects for resistance bacteria , antimicrobial agents are well known (ZnO NPs) and are lower toxicity and biology safety Zinc oxide nanoparticles (ZnO NPs) are well known antimicrobial agents and are regarded as nontoxic and bio-safe. In this study, ZnO nanoparticle was synthesized using bioassayed on MRSA bacteria by (WAD) method. In addition, minimum inhibitory concentration (MIC) different concentrations (512, 256, 128, 64, 32 and 16) mg/ml of ZnO NPs, Antibiotic resistance different such (Gentamicin, Azithromycin, Cefoxitin, Trimethoprim and Levofloxacin) by disc diffusion assay. The goal of this research study zinc oxide (ZnO) nanoparticles synthesis and antibacterial properties against gram-positive bacteria compare Levofloxacin.
Keywords: *Staphylococcus epidermidis*, ZnO, MRSA

Introduction

Infection diseases, divided (intracellular or extracellular), always been a large problem to health human, causing of deaths each year, Nowadays resisted Bacterial to antibiotics has become big problem, (Moravej *et al.,* 2018), Metal oxide nanoparticles have appeared to be promising candidates during the last few years. has significantly advanced due to its wide application (Suresh *et al.,* 2016). Nanoparticles cover wide-ranging area from uses, most antibacterial inorganic materials are metal oxide nanoparticles such as (silver, copper, titanium oxide, and zinc oxide) (Bradley *et al.,* 2011; AlKhafaji and Hashim, 2019). Over the past few decades, development with novel action modes, and resistance of bacterial enzymes to modification along with improvements to efficiency of drug delivery (Van *et al.,* 2019). *S. aureus* have the ability to evolution of bacteria in the antibiotic, by a respond to each new antibiotic with a resistance mechanism (Annalisa *et al.,* 2009). These *staphylococci* are part of the animal normal flora, the pathogens flora, colonizing both the skin and mucous membranes, counting the nares (Levy *et al*., 2013).

Material and Methods

Isolates and Culture media

All Thirty swaps were collected from (AlYarmouk Hospital-Baghdad-Iraq), after that cultured on (MSA) and incubated at (37 ºC for 24 hrs) and biochemical characteristics (Coagulase, Catalase reagent and Oxidase reagent) (Namasivayam *et al.,* 2012). The *S. epidermidis* was obtained from Department of Biology it was confirmed by using Api-Staph identification System and vitek 2 system, further confirmation was made by PCR.

Antibiotic Sensitivity Test

For inoculums, standard homogenized *S. aureus* was prepared in normal saline and the suspending was diluted to 0.5×10^8 CFU ml compared with McFarland tubes 10 by disk diffusion method against (Gentamicin, Azithromycin, Cefoxitin, Trimethoprim and Levofloxacin) the zone of inhibition (in mm) Clinical and Laboratory Standards Institute (AlKhafaji and Hashim, 2019).

DNA extraction

Genomic DNA was extracted from the detected bacterial isolates according to the protocol of Wizard Genomic DNA Purification Kit, Promega. Quantus Florometer was used to detect the concentration of extracted DNA

Primers Selection

The set of primers 27F (AGAGTTTGATCTTGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT) was used for amplification of 16s rRNA for identification of bacteria at gene level ((Hashim and AlKhafaji, 2018).

Prepare a suspension of *S. epidermidis*

Suspension prepare on cultured M H B that was incubated for 18 h at 37° C. The bacteria were centrifuged at the 10000 g for (10 min at 4° C). than compare with turbidity of 0.5 McFarland (Carson *et al*., 2002).

Preparation stock solution of $Zn.2H_2O(CH_3CO_2)_2$

A stock solution of Zinc acetate $Zn.2H_2O(CH_3CO_2)_2$ was prepared by dissolving 0.01 g $Zn.2H_2O(CH_3CO_2)_2$ in 50 ml deionized water.

MIC determination

Used to determine (minimum inhibitory concentration). doubling dilutions serial of ZnONPS were prepared using MHB, added to each test culture bacteria was tube then incubated using shaking incubator for 24 hours at 37° C. Inhibition of cell growth was defined by counting the amount of CFUs on the plates or by the turbidities of the cell cultures. The first test tube no change in turbidity were further proved for bacterial by spreading 100-µl of the broth cultures onto agar plates to determine effect of ZnONPS (Xie *et al.,* 2011).

Zn NPs biosynthesis

For the synthesis of the Zn NPs, (7 ml) of the *S. epidermidis* filtrate was added to 3 ml of 1 mM $Zn.2H_2O(CH_3CO_2)_2$ (final concentration) at room temperature 37 ºC (AlKhafaji and Hashim, 2019).

Result and Discussion

Isolation and Identification *S. aureus*

Fifty specimens were collected over a period of a month between August and December 2019. While the isolates on Blood agar showed yellow-gray colonies are (4-3) mm in diameter on the zones of β-hemolysis according to (Mais and Sana. 2018) (Figure 1)

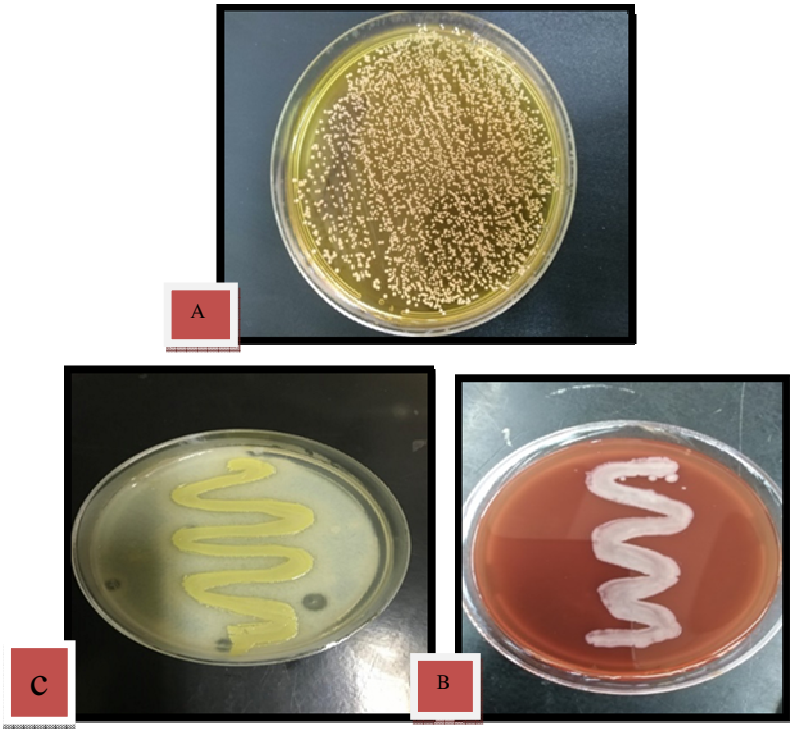

Fig. 1 : *S. aureus*: A) On mannitol salt agar. B) Blood agar. C) Milk agar at 37°C for 24 hrs (AST).

Figure (2) show various levels different antibiotics among isolates that were observed by Disk diffusion method. The isolates of *S. aureus* (n=22) showed different susceptibility towards many antimicrobial agents shown 14(%) resist to Azithromycin, 12(%) resist to cefoxitin. There was less resistance to Levofloxacin 4(%) than other antibiotics.

Isolates was multi-resistance for antibiotics with a high level against, Gentamicin, Azithromycin, Cefoxitin, Trimethoprim and Levofloxacin result was similar to that acquired by (Mais and Sana, 2018).

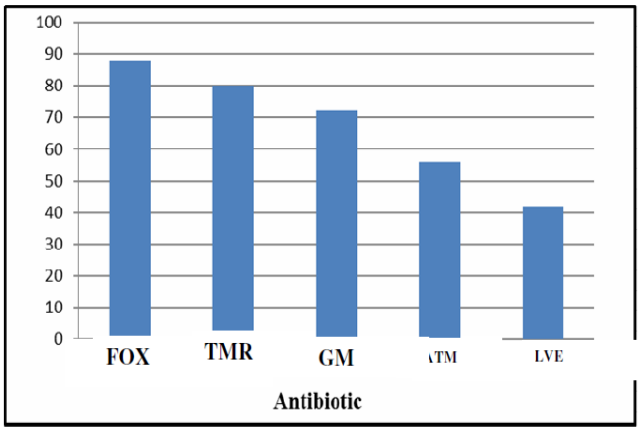

Fig. 2 : Antibiotic susceptibility test of *S.aureus*

Confirmation of *S. aureus* and *S. epidermidis* using Vitek 2 System Vitek 2 system gives confirmation of positive results for *S. aureus, S epidermidis* and *P. aeruginosa* as a selected organism with probability (98-99)%

Molecular diagnosis of *S. aureus* and *S epidermidis* :

The results showed that multiplex PCR analysis for both strain were confirmed by AST and Vitek 2 system gave positive results for multiplex Polymerase chain reaction (PCR). Shown in figure (3).

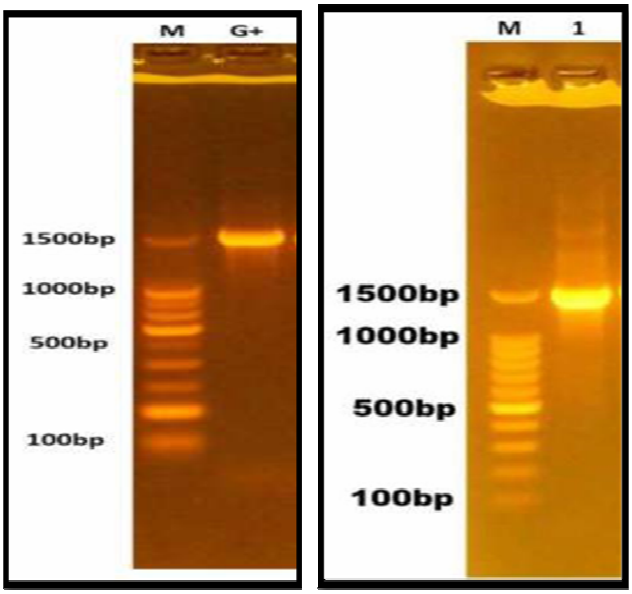

Fig. 3 : Agarose gel (1%) electrophoresis (100v/mAmp for 90min) of amplified *16s rRNA* (1500pb) from bacterial DNA stained with ethidium bromide. Lane M. 100 bp DNA ladder, Lane 1. Unknown bacterial isolates

Study of Zn NPs Nanoparticles compounds characterization:

- **Spectral Properties of the ZnONPs Nanoparticles**

Figure (4) revealed a strong surface plasmon placed around 400 nm. This indicates the creation of ZnONPs. It is not likely at 280 nm since it does not comprise any aromatic amino acids, the 215 nm was selection

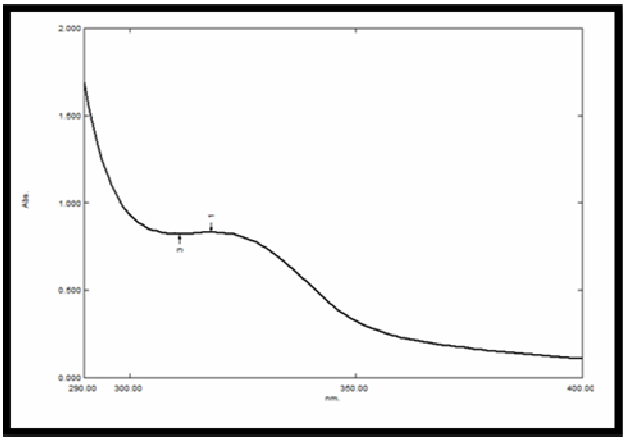

Fig. 4 : Absorption spectra of Zn NPs nanoparticles.

Atomic Force Electron Microscopy (AFM)

The AFM micrograph acquired for the Zn NPs Figure (5) shows the surface roughness alterations and the surface roughness change [root mean square (Rp)] values were recognized and the section analysis of the sample's grain size value was (19.76) nm.

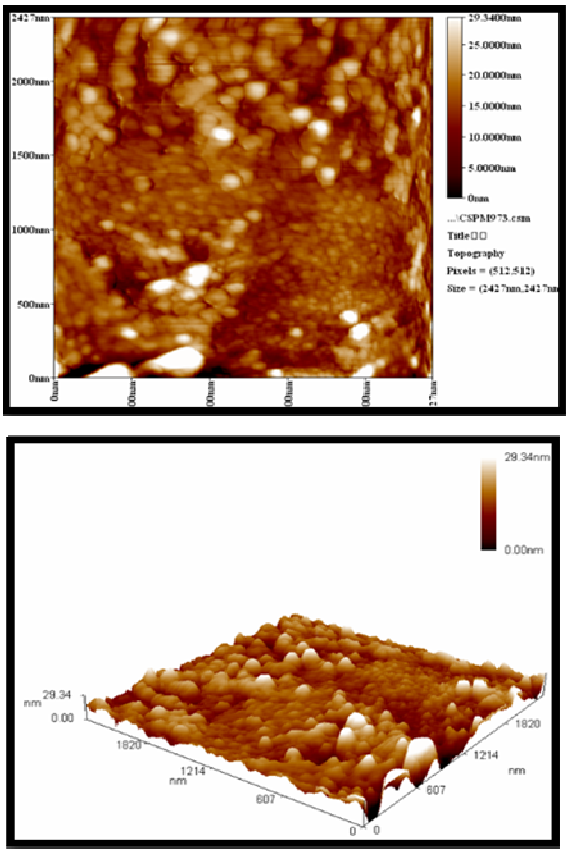

Fig. 5 : AFM for Zn NPs nanoparticles

TEM Analysis:

For transmission electron microscopy (TEM) imaging, the Zn NPs suspensions according (Sadowski. 2015).

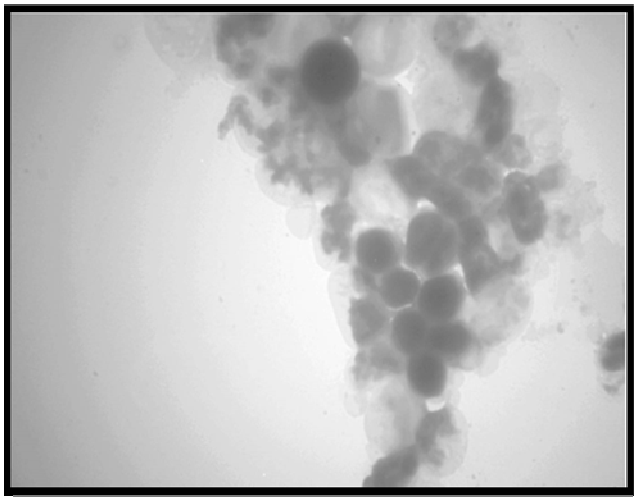

Fig. 6 : TEM for ZnO NPs

Zn NPs Assay :

The well diffusion agar method (WDA) was used to detection *S. aureus* sensitivity to word Zn NPs. The result was recorded below in Figure (6) The concentration (512,256 and 128) µg\ ml was appeared the inhibition zone size is (26, 19 and 17) mm respectively while (Mirhosseini and Arjmand, 2014) proved the activity of ZnO NPs with acetic acid on *Staph. aureus* in mutton meat.

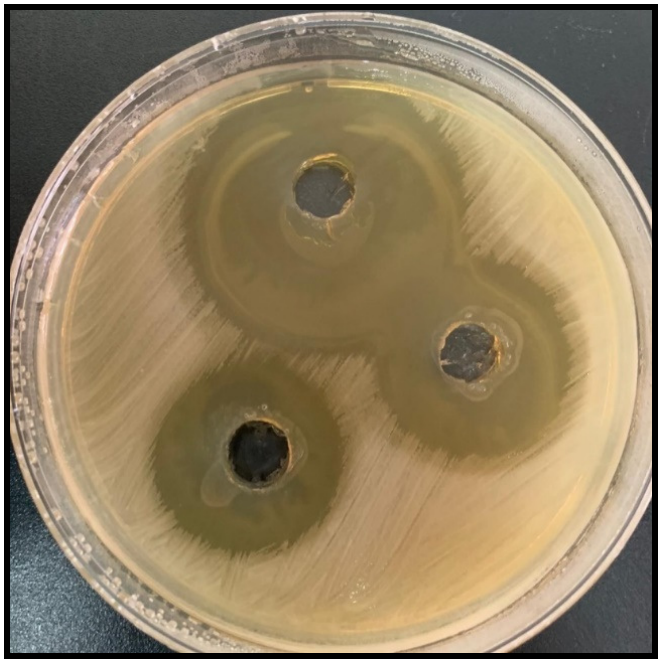

Fig. 6 : MIC for Zn NPs at different concentration on (MHA) at 37°C for 24 hr.

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