Republic of Iraq Ministry of Higher Education and Scientific Research
University of Diyala
College of Education for Pure Science
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Effect of nanoparticles on the expression of some antibiotics resistance genes in *Staphylococcus aureus* isolated from clinical samples in Baquba city/Iraq

A Thesis

Submitted to the Council of the College of Education for Pure Science, University of Diyala, in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology

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2022 A.D **1443 A.H**

1.1 Introduction

Staphylococcus aureus in the 21st century endures worldwide as a common cause of nosocomial and community bloodstream infections, with high risks of metastatic complications and death and most of the human population is likely exposed to this bacterial species on a daily basis (Michael, 2019).

S. aureus is commensal bacterium that colonizes body parts of (30%) of healthy people. It plays essential roles in infecting both community and hospitals causing life threating diseases. This microorganism causes infections due to various virulent genes that encode different virulent factors including enzymes and toxins among others (Rasheed and Hussein, 2021).

The opportunistic pathogen *S. aureus* can actually colonize any niche throughout the human's, because of its capability to rapidly adapt to harsh conditions, and evades the immune system of hosts. *S. aureus* can cause various diseases, mostly skin and soft tissue diseases as well as toxin-mediated and systemic diseases (Hardey *et al*., 2020). The development of infections caused by *S. aureus* is often associated with hospitalized and immunocompromised conditions (Costa *et al*., 2019).

Genetic plasticity is a prominent feature of bacteria, which will enable bacteria to respond to various threats in the environment, including the existence of antibiotic molecules that may endanger their survival. The main bactericidal mechanisms of antibiotics are: hindering the synthesis of cell wall, inhibiting nucleic acid and protein synthesis, affecting bacterial metabolism and the function of cell membrane (Munita and Arias, 2016).

Over the past decade, an excessive and inappropriate use of antibiotics for human and animal treatment, as well as animal feed supplements for growth promotion, has led to an increase in a number of staphylococci acquiring cross-resistance to macrolides, lincosamides, and streptogramins (MLS) antibiotics.

(Mišić *et al*., 2017). Multi drug resistance (MDR) is defined as resistance to at least one agent in three or more antimicrobial classes, It is the most pronounced feature of Methicillin resistant Staphylococcus aureus (MRSA) isolates as they show sensitivity only to the glycopeptide antibiotics including Vancomycin (Diaz *et al*., 2018).

Tetracyclines is one of the most widely used classes of antibiotics in clinical and agriculture fields due to their excellent therapeutic index, low cost, oral administration, and few side effects. the prevalence of tetracycline resistance has alarmingly increased and become one of the most abundant antibiotic resistances among clinical and commensal microorganisms. conferring resistance primarily through three mechanisms: active efflux, ribosomal protection and enzymatic inactivation of the antibiotics. The first two mechanisms currently predominate in clinical settings (Wang *et al.*,2017).

Resistance can be achieved through antibiotic target modification, drug inactivation or drug export by efflux pumps. *S. aureus* encodes different multidrug resistance efflux pumps (Gupta *et al*., 2017).

Efflux is an important mechanism of antimicrobial agent and biocide resistance in *S aureus*. It is an important mechanism of antimicrobial agent and biocide resistance in *S. aureus*. The efflux process is mediated by membrane-based proteins capable of transporting a single class or several structurally distinct compounds, using either an ion gradient (commonly H) or ATP cleavage to provide the required energy (Patel *et al*., 2010). Recently, nanoparticles have been used as an alternative method for treatment of various antibiotic-resistant bacterial infections and may solve the problem of multidrug-resistant bacteria; in particular, silver nanoparticles (AgONPS) have received a great deal of attention. (Wang *et al*., 2017)

Silver has been previously utilized as an antimicrobial agent against multiple types of bacteria because of its low cytotoxicity. Silver has exhibited antimicrobial activity against *S. aureus* and is also highly effective against methicillin-resistant *S. aureus* (MRSA). The advantages of using AgONPs as antimicrobial agents include their extensive range of activity, cost effectiveness, and high efficacy (Patra *et al*, 2017), also Zinc oxide (ZnO) has been shown to be one of the most promising metallic nanomaterials. ZnO nanoparticles have received increasing attention as antibacterial agents in recent years because of their stability and safety for humans. It has been suggested that the mechanism of the antibacterial activity of ZnONPS is based on its ability to induce oxidative stress. (Fontecha *et al*.,2020). Titanium oxide nanoparticles these days have attracted a great deal of Interest because it has properties for achieving high effect in biological, pharmaceutical applications. The bactericidal effect of TiO2NPS on bacteria is extreme importance due to ability of pathogenic bacteria to enter in ecosystem food chain. (Mahdy *et al*., 2017).

Aim of the study is phenotypic and molecular investigation of multidrug resistant (MDR) *S.aureus* and measure the expression of the erythromycin and tetracycline resistance genes and compare the gene expression in the present of the, ZnONPS, AgONPS , TiO2NPS and in the absence of them in order to improve the role of these genes in the resistance of *S. aureus* to nanoparticles.

For this aim, the following steps were performed:

1- Isolation and identification of *Staphylococcus aureus* isolates from clinical samples .

2- study the susceptibility of *S. aureus* isolates to some macrolide antibiotic and determine of MIC and MBC of antibiotic.

3- Molecular investigation of some genes encoded for resistance to erythromycin using PCR technique (*erm*A, *erm*B, *erm*C) .

4- Molecular investigation of some genes coded for resistance to tetracycline using PCR technique (*tet*K ,*tet*L, *tet*M).

5- Phenotypic and genotyping detection of efflux pumps genes *med*A , *tet*38 ,*tet*M, *lmr*S

6-determination of variation of *S. aureus* isolates by Box-PCR.

7- Study the gene expression of (*med*A, *tet*38,*erm*C,*tet*M) by RT-PCR By using nanoparticles ZnONPS, AgONPS, TiO2NPS .

أدت المعاملة باستخدام جزيئات ZnONPS النانوية إلى انخفاض في قيمة التعبير الجيني في أربعة عزلات لجين (*med*A) والتي كانت تقريبا (0.32) وبتركيز (325 ميكروكرام/مل) والتغيير من 0.61 to 0.08 ضعفًا في التعبير الجيني (*med*A) كان بسبب التعرض الفعال لمادة ZnONPS وبتركيز (325 ميكروكرام/مل) والتي اثرت وبشدة على التعبير الجيني لجين *med*A. ومن جانب اخر جميع العزلات كانت ذات تعبير جيني عالي عند معاملتها بمادة TiO2NPS واظهرت قيم Sub MIC ان التغير في قيمة fold كانت بحدود 12.20 حيث توجد فروق معنوية بين عزلة الحروق وعزلة الادرار قبل وبعد المعاملة بمادة TiO2NPS في حين لاتوجد فروق معنوية في عزلة الدم وعزلة الجروح

اظهر التعبيرالجيني *tet*38 أقل مستوى من folding بمعدل 0.54 وجد في حالة العزلات المعاملة بـمادة AgONPS بتركيز 40.62 ميكروغرام / مل ، كما كان هناك انخفاض في folding. لا توجد فروق ذات دلالة إحصائية في العزلات المعاملة بمادة AgONPS قبل وبعد المعاملة . أظهر التعبير الجيني عن tet38 أنه معدل مستوى folding يكون 0.97 في حالة العزلات التي التي تمت معاملتها بـ ZnONPS بتركيز 325 ميكروغرام / مل وأدى إلى انخفاض في التعبير الجيني لـ tet ، وكان sub-MIC لـ ZnONPS nanoparticles ذو فعالية واطئة على التعبير على كل من جينات *tet*38 و 16SrRNA في العزلات المستهدفة المختارة في حين لم تظهر فروق معنوي عند $p \geq 0.01$ بين العزلات المستهدفة ZnONPS. أظهر التعبير عن *tet*38 أنه تم الحصول على أعلى مستوى من التعبير بمتوسط 10.95 في حالة العزلات التي عولجت بـ TiO2NPS بتركيز 650 ميكروغرام / مل مع قيمة folding بين 2.12 – 20.18أ وظهرت عزلة واحدة (SA12) زيادة في التعبير عن tet38 مع تغييرfolding بنسبة 20.18. أدت المعالجة بـ TiO2NPS إلى زيادة قيمة التعبير الجيني في جميع العزلات الاربعة لـ tet38 بمتوسط 10.95 بتركيز 650 ميكروغرام / مل.

أظهر التعبير عن *erm*C أقل مستوى من folding بمتوسط 0.55 في حالة العزلات التي عولجت بـ AgNPS بتركيز 40.62 ميكروغرام / مل ، كما كان هناك انخفاض فيfolding ، ولا توجد فروق معنوية في ثلاث عزلات SA12، SA14، في حين أن SA13 لها فروق ذات دلالة إحصائية في AgONPS قبل العلاج وبعده. أظهر تعبير ermC أن مستوى folding بمتوسط 0.66 وجد في حالة العزلات التي عولجت بـ ZnONPS بتركيز 325 ميكروغرام / مل وأدى إلى انخفاض في التعبير الجيني لـ *ermC*. أظهر التعبير عن *erm*C أن أعلى مستوى من folding بمتوسط 41.47 وكانت هذه القيمة تعتبر الأعلى بين جميع المعاملات مع الجسيمات النانوية وجدت في حالة العزلات