IASJ المجلات الاكاديمية العراقية

Tikrit Journal of Pure Science

ISSN: 1813 – 1662 (Print) --- E-ISSN: 2415 – 1726 (Online)

Journal Homepage: http://tjps.tu.edu.iq/index.php/j

Silver and Cobalt nanoparticles as potential antibacterial agents *Staphylococcus aureus* isolated from wound infections

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https://doi.org/10.25130/tjps.v25i2.230

ARTICLE INFO.

Article history:
-Received: 4 / 12 / 2019
-Accepted: 12 / 1 / 2020
-Available online: / / 2020

Keywords: Nanoparticle, silver, cobalt, *Staphylococcus aureus*, MIC, MBC.

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ABSTRACT

The present study aimed to isolate and diagnose *S. aureus* from patients with wound infection after collecting (100) clinical samples of patients attending Salah Al-din General Hospital from the beginning of April to the end of September 2019 from different wound areas, and to study the effect of silver nanoparticles and cobalt on them. The results of the study showed that the two nanoparticles used have the ability to inhibit growth of *S. aureus* at different concentrations, The molecular study included the observation of the most important molecular changes at the level of DNA after treatment with nanomaterials, where many variations were observed on the bacteria studied, including these changes the emergence and disappearance of packages of DNA and different numbers when treated with nanomaterials, The results of the study showed that the laboratory rats when infected with *S.aureus*, appeared after about a week and the symptoms which include; clear skin scaling and hair loss at the site of infection. It is also clear that the recovery time was rapid in group of infected rats which treated with nanomaterials.

Introduction

Staphylococcus aureus causes many diseases like skin, wound and burn infection, folliculitis, pericarditis, tonsillitis, pneumonia, urinary tract infection and food poisoning [1,2].

Have many virulence factors like cell wall, capsule , Adhesions antigens, Exotoxin like *Staphylococcus* scalded skin toxin, Toxic shock syndrome toxin, Enterotoxins and enzymes (Deoxyribonucleas, Hyaluronidase, Fibrinolycin, Lipase and β–Lactamase) [3,4].

Wound infection are mainly treated with antibiotics and antimicrobials. Excessive or inappropriate use of these antibiotics can lead to the emergence of resistant bacteria which do not respond to antibiotic treatment, as seen in recent decades[5]. Nanoparticles (NPs) have been established as a promising approach to solve this problem. NPs are materials that have at least one dimension (1–100 nm) and have demonstrated broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria [6].

Materials and methods

Samples : 100 wound samples were collected from patients arrived to Salah Al-din hospital in period from March to November 2019. All sample cultured in Mannitol salt agar at 37˚ C for 24h. after colony appearing gram stain and group of biochemical tests were applied [7,8,9].

Effect of nanoparticles on *Staphylococcus aureus*.

Two type of nanoparticles were used which were:

1- Silver Ferrite($AgFe_2O_4$) Nanoparticles (SFNPs)
2- Cobalt Ferrite ($CoFe_2O_4$) Nanoparticles (CFNPs)

The study of Nanoparticles effects include:

a- Determination of Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC): *Staphylococcus aureus* isolate inoculated into brain heart infusion broth (final concentration 1.5X10 CFU/ml) and nanoparticles were added in concentration (200, 225, 250, 275 and 300 µg /ml), then incubated at 37˚ C for 24h. the lowest concentration tube in which growth not seen consider as MIC. And lowest concentration tube which is septic conceder as MBC [10].

b- RAPD PCR: which applied by:
a- DNA extraction according to [11].
b- Primers used in RAPD PCR : as in table (1)

Table 1: Primers used in RAPD PCR.

No.	Primer code	Nucleotide sequence 5 to 3
1	OP M-01	GTTGGTGGCT
2	OP H-14	ACCAGGTTGG
3	OP G-05	CTGAGACGGA
4	OP W-17	GTCCTGGGTT
5	OP Y-07	AGAGCCGTCA
6	CP R-10	CCATTCCCCS
7	CP U-12	TCACCAGCCA
8	OP V-20	CAGCATGGTC

- DNA bands detected by electrophoresis in 1% Agarose with red safe and according to [12,13].

Results and discussion

Results of *Staphylococcus aureus*: out of 100 wound samples, *Staphylococcus aureus* isolate in rate of 63% (63:100). Figure (1) shows *Staphylococcus aureus* colony on Mannitol salt agar.

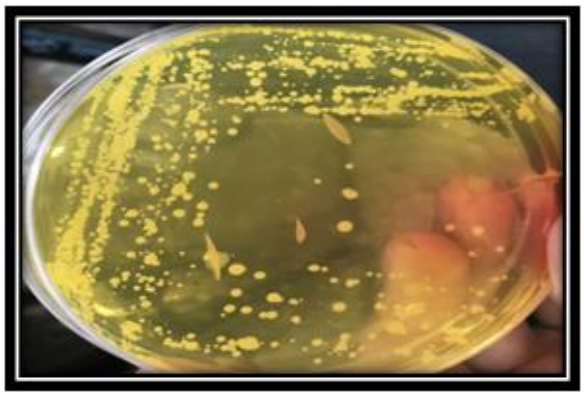

Fig. 1: *Staphylococcus aureus* colony on Mannitol salt agar, show yellow colonies and media

- Result of MIC and MBC: the MIC and MBC of both of AgFe2O4 and CoFe2O4 were 225 and 250 µg /ml respectively. As in table (2).

Table 2: Results MIC and MBC on *Staphylococcus aureus*.

Type of nanoparticles	Concentration of nanoparticles (µg /ml)				
	200	225	250	275	300
CoFe2O4	Turbidity	Clear	Clear +septic	Clear +septic	Clear +septic
AgFe2O4	Turbidity	Clear	Clear +septic	Clear +septic	Clear +septic

Results of RAPD PCR test: from figure (2,3,4) it is obrions that both types of nanoparticles used in current study have effects on genetic material of *Staphylococcus aureus* which appeared as appearance or disappearance of DNA bands, increase or decrease in clarity of same bands [14,15,16].

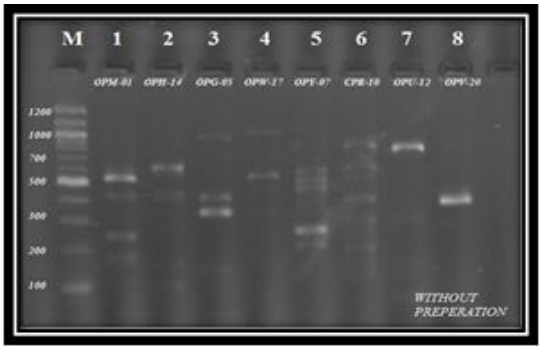

Fig. 2: Agarose gel electrophoresis of RAPD- PCR products. M: 100 bp DNA ladder, lines (1-8) positive result before treatment with Nanoparticles

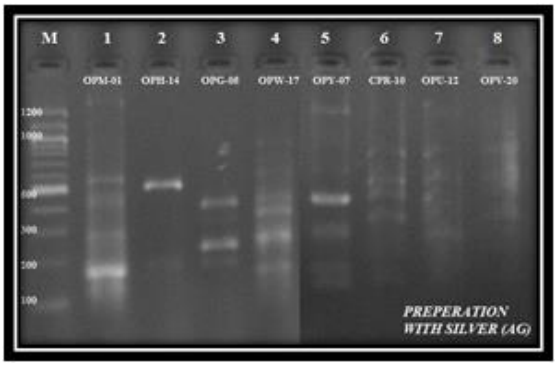

Fig. 3: Agarose gel electrophoresis of RAPD- PCR products. M: 100 bp DNA ladder, lines (1-8) positive result after treatment with AgFe2O4 Nanoparticles

Fig. 4: Agarose gel electrophoresis of RAPD- PCR products. M: 100 bp DNA ladder, lines (1-8) positive result after treatment with CoFe2O4 Nanoparticles.

Conclusions

1- The results showed that *Staphylococcus aureus* is the most common type of bacterium that causes skin infections in the studied cases.

2- It was noted that the nanoparticles (Ag) and (Co) used have good inhibitory activity against bacteria cause skin infection.

3- The results showed the emergence of variations (the disappearance or appearance of bundles and the difference in the number and difference in the intensity of shine) at the molecular level of bacteria studied treated with nanomaterials.

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الاجسام النانوية للفضة والكوبلت كمضاد بكتيري قوي للمكورات العنقودية الذهبية *Staphylococcus aureus* المعزولة من اصابات الجروح

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الملخص

هدفت الدراسة الحالية الى عزل البكتريا الجلدية من المرضى المصابين بالتهاب الجروح وتشخيصها بعد جمع (100) عينة سريرية من المرضى المراجعين لمستشفى صلاح الدين العام من بداية شهر نيسان من العام 2019 الى نهاية شهر ايلول من العام نفسه من مناطق الجروح المختلفة, ودراسة تأثير الاجسام النانوية للفضة والكوبلت عليها وأظهرت نتائج الدراسة التي اجريت على بكتريا المكورات العنقودية الذهبية *Staphylococcus aureus* إن الاجسام النانوية المستخدمة تمتلك القدرة على تثبيط نمو البكتريا باستخدام تراكيز مختلفة, وكان التركيز المثبط الأدنى MIC عند (225 مايكرو غرام) والتركيز القاتل MBC عند (250 مايكرو غرام) لكلا النوعين من الاجسام النانوية المستخدمة في الدراسة.

أما الدراسة الجزيئية فقد شملت ملاحظة اهم التغييرات الجزيئية على مستوى الدنا (DNA) قبل المعاملة بالمواد النانوية وبعدها, إذ لوحظت تغايرات كثيرة على البكتريا المدروسة وتشمل هذه التغايرات ظهور الحزم للدنا (DNA) واختفاءها واختلاف اعدادها عند معاملتها بالمواد النانوية.

اما نتائج الدراسة فتبين ان اصابة الجرذان المختبرية بالبكتريا *S.aureus* ظهرت بعد مرور أسبوع تقريباً وتمثلت الأعراض بتقشر واضح في الجلد وتساقط للشعر في مكان الإصابة, وعند معاملة الجرذان المصابة بالمواد النانوية وحسب المجاميع أوضحت النتائج أن وقت الشفاء كان سريعاً.