Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 112 - 119
Received 25 January 2023; Accepted 05 February 2023.

Use of Nanoparticles as Alternative Way for Treatment of Methicillin Resistant Staphylococcus Aureus

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Introduction

Staphylococcus aureus are gram positive cocci , arrangement in cluster , non-motile, non- spore forming,non capsulated, aerobic or Facultative anaerobes,growth in mannitol salt agar, it able to fermentation of mannitol, glucose, lactose andsucrose. It give positive result in catalases test , MR-VR test and urease test while it gave negative result in oxidase and indol test (Quinn *et al*., 2006).
It caused many disease to human like Staphylococcal scalded skin syndrome, Myositis, Urogenital Infection, Arthritis, Osteomyelitis, and Food poisoning (Murray *et al*., 2002).
They are many bacterial causes of wound infection, like *Staphylococcus aureus*, streptococcus , *Pseudomonas aeruginosa*, and other enterobacteriaceae (Ahmad& Iranzo,2003).

In recent yearsmethicillin resistant *staphylococcus aureus* were more prevalence, and became a problem in treatment. Nanoparticles (NPs) have been established as a promising approach to solve this problem, it broad-spectrum antibacterial effect against both Gram- positive and Gram-negative bacteria(Edmundson *et al*.,2013).

Materials and methods

- **Sample collection**: 80 infected wound swabs were taken from infected traumatic or surgical wounds.

- **Bacteriological study**

a- Bacterial isolation: all swabs transport in trypton soya broth and cultivation for 24hours at 37° C. then sub culturing in mannitol salt agar. and cultivation for 24hours at 37° C.

b- Bacterial identification: gram stain and a group of biochemical tests (catalase, oxidase, urease, DNA ase, and coagulase) were applied.

- **Antibiotic and nanoparticles sensitivity test**

a- Antibiotic sensitivity test were applied according to (Ryan& Ray,2014).

b- Minimum inhibitory concentration (MIC) were applied by tube methods with bacteria concentration $1.5X10^8$ CFU/ML) and nanoparticles were added in concentration (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) (Roselli*et al*.,2013).

c- Determination of inhibitory zone: Holes in plate were done by cut aseptically with sterile cork borer, then 0.1 ml of $1.5X10^8$ CFU/ml of bacterial suspension were disseminate inagar media, after that 100µl of Nps were put in hole and incubation at 37^oC for 24h. the inhibition zone were measured using caliber.

Genetic study:

A- DNA extraction : one colony were dissolve in 200µl of DNase free water then heated in

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water bath at 100c for 10 minutes. Eppendorf tube transmitted to ice then centrifuged at 12000c/m for 20 seconds . supernatant were taken and kept in -20c. (oie,2009).

B- Detection of *staphylococcus aureus* specie by used of ***Nuc*** gene

1- Compounds used in preparation of Reaction Mixture for confirmation of *staphylococcus aureus* : as in table (1) and according to (Kuźma et al., 2003).

Table 1: PCR reaction mixture Compounds

Compound	Amount
Taq PCR Master Mix KIT (Qiagen, Germany) Which contain Taq DNA Polymerase (2.5 Unit), PCR Buffer with 3mM MgCL2, 200µMdNTP (Qiagen, Germany)	25µl
Nuc gene F: 5'-GCGATTGATGGTGATACGGTT -3' (out product size 270 bp).	1.4µl
Nuc gene R: 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	1.4µl
DNA template	3µl
DNA free water	21.4µl
Total	50

2- Thermocyclar programs : the program began by initial denaturation step of 95°C for 2 min, then 30 cycles, each cycle consist from 3steps : a denaturation step of 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2mints; and a final extension step of 72°C for 10 min

C- Detection of methicillin resistant staphylococcus aureus (***Mec A*** gene):

1- Compounds used in preparation of Reaction Mixture for confirmation of Methicillin resistant staphylococcus aureus (***Mec A*** gene) : as in table (2) and according to(Karmakar*et al*, 2016)

Table (2): PCR reaction mixture Compounds

Compound	Amount
Taq PCR Master Mix KIT (Qiagen, Germany) Which contain Taq DNA Polymerase (2.5 Unit), PCR Buffer with 3mM MgCL2, 200µMdNTP (Qiagen, Germany)	25µl
Mec A gene 5'-GTAGAAATGACTGAACGTCCGATGA-3' (out product size 310 bp).	1.4µl
Mec A gene R: 5'-CCAATTCCACATTGTTTCGGTCTAA-3'	1.4µl
DNA template	3µl
DNA free water	21.4µl
Total	50

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2- Thermocyclar programs : the program began by initial denaturation step of 95°C for 2 min, then 30 cycles, each cycle consist from 3steps : a denaturation step of 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2mints; and a final extension step of 72°C for 10 min.

D- RAPD technique (Random Amplification of Polymorphic DNA) used with 10 primers (as in table 3) and according to (Sambrok & Russei, 2001).

Table (3): The primers sequences used in RAPD technique.

Number	Primer code	Sequences
1	OP G-5	CTGAGACGGA
2	OP H-14	ACCAGGTTGC
3	OP M-01	GTTGGTGGCT
4	OP J-01	CCCGGCATAA
5	OP P-04	GTGTCTCAGG
6	OP Q-02	TCTGTCGCTC
7	OP R-10	CCATTCCCCA
8	OP V-20	CAGCATGGTC
9	OP U-12	TCACCAGCCA
10	OP W-17	GTCCTGGGTT

D- Agarose Gel Electrophoresis according to (Sambrok & Russei, 2001). The agarose gel were prepared (1.5%).

Results

- Result of bacterial isolation: out of 80 wound samples, 42 Staphylococcus spp. Were isolate, 28 of them belong to *Staphylococcus aureus*and only 24 isolates were Methicillin resistant. As in table (4).

Table (4): number and ratio of staphylococcus isolates

No of sample	Staphylococcus spp.		Staphylococcus aureus		Methicillin resistant Staphylococcus aureus	
80	No	Ratio	No	Ratio	No	Ratio
	42	52.5%	28	35%	24	30%

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Staphylococcus aureus given yellow colony on Mannitol salt agar (figure 1) and positive reaction in coagulase and DNase test.

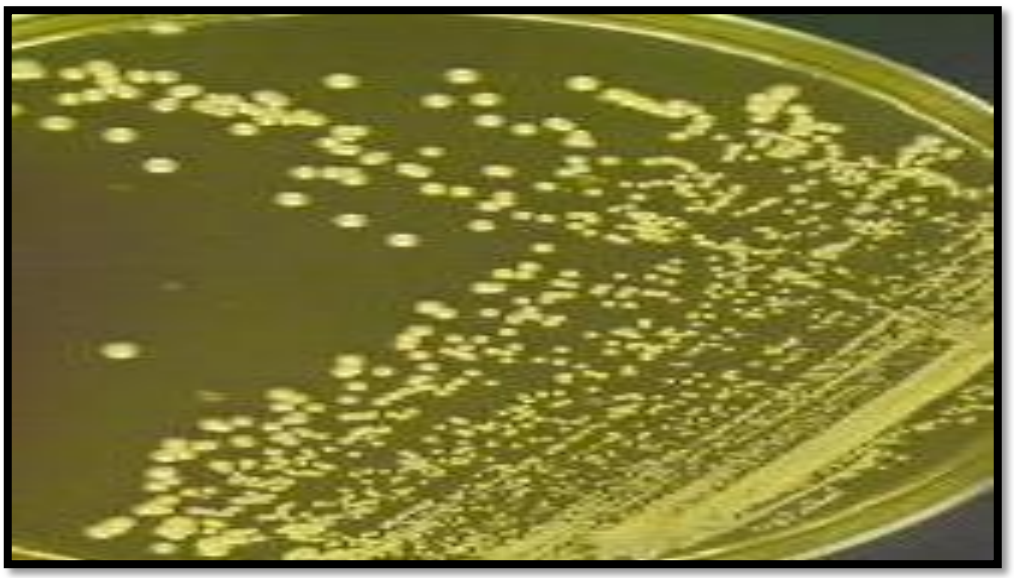

Figure (1): Mannitol salt agar, show yellow colony of *staphylococcus aureus*

In figure (2) show the positive result of *Staphylococcus aureus* by using of primer***Nuc*** gene, which given band in size 270bp.

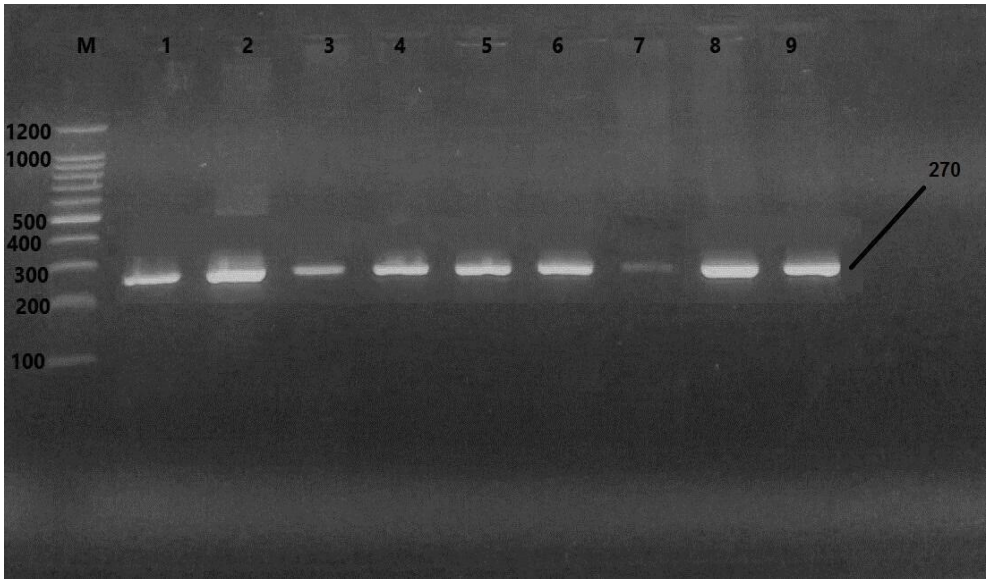

Figure (2):Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-9)positive result at 270 bp for *Staphylococcus aureus.*

In In figure (3) show the positive result of methicillin resistant *Staphylococcus aureus* byusing of primer ***mec A*** gene, which given band in size 310 bp.

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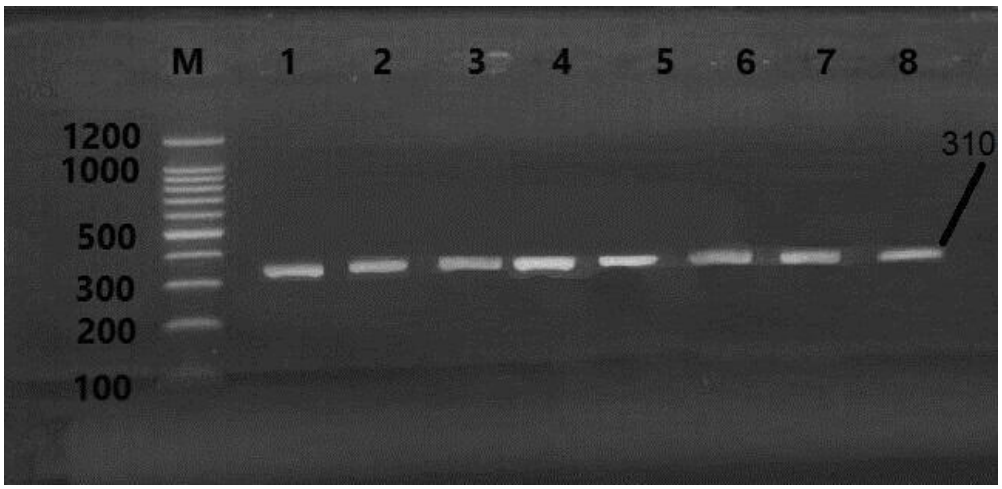
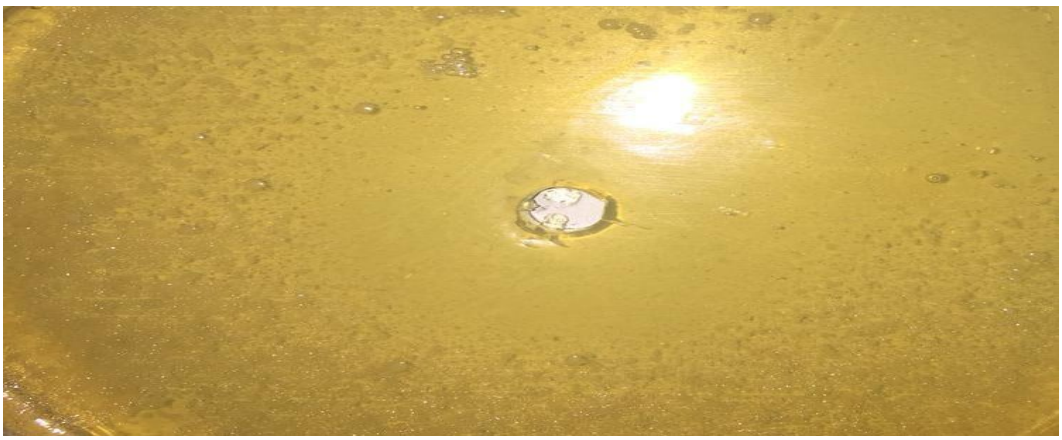


Figure (3):Agarose gel electrophoresis of PCR products. M: 100 bp DNA ladder, lines (1-8)positive result at 310 bp for methicillin resistant *Staphylococcus aureus*

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC): from table (5) showed that MIC of Ago and Zno against Methicillin resistant *Staphylococcusaureus* were 20 µg/ml and 40 µg/ml respectively while the MBC of Ago and Zno against Methicillin resistant *Staphylococcus aureus* were 40 µg/ml and 80 µg/ml respectively, the inhibitory zone were 32mm and 28 mm.

Table (5): MIC of AgNPs and Zno against methicillin resistant *Staphylococcus aureus*

Type of nanoparticles	Nanoparticles concentration(µg/ml)					
	10	20	40	60	80	100
AgNPs	growth	No growth	No growth+ septic	No growth	No growth	No growth
Zno	growth	growth	No growth	No growth + septic	No growth	No growth



Figure(4)methicillin resistant *Staphylococcus aureus*growth on mannitol salt agar, showinhibitory zone of Ag NPs

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Result of RAPD test: from figure 5,6,7 showed that both Ago and Zno nanoparticles were effected in genetic materials which showed as appear or disappear and increase or decreasein thickness of bands

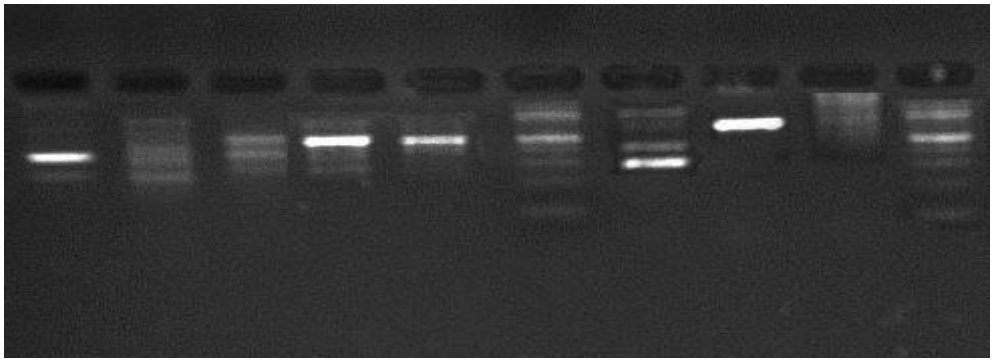

Figure (5): Agarose gel electrophoresis of RAPD- PCR products. lines (1-10) positive resultof methicillin resistant *Staphylococcus aureus* with 10 different primers, before treatment with Nanoparticles

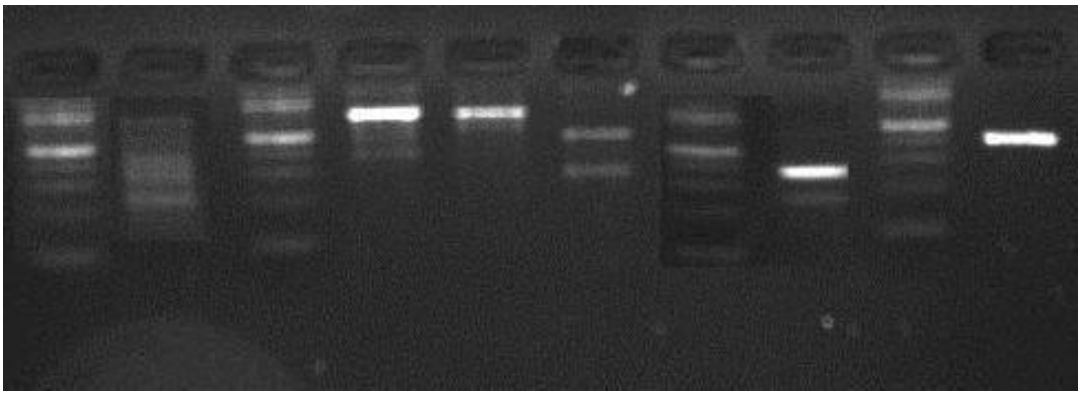

Figure (6): Agarose gel electrophoresis of RAPD- PCR products. lines (1-10) positive result of methicillin resistant *Staphylococcus aureus* with 10 different primers, after treatment withAgo Nanoparticles

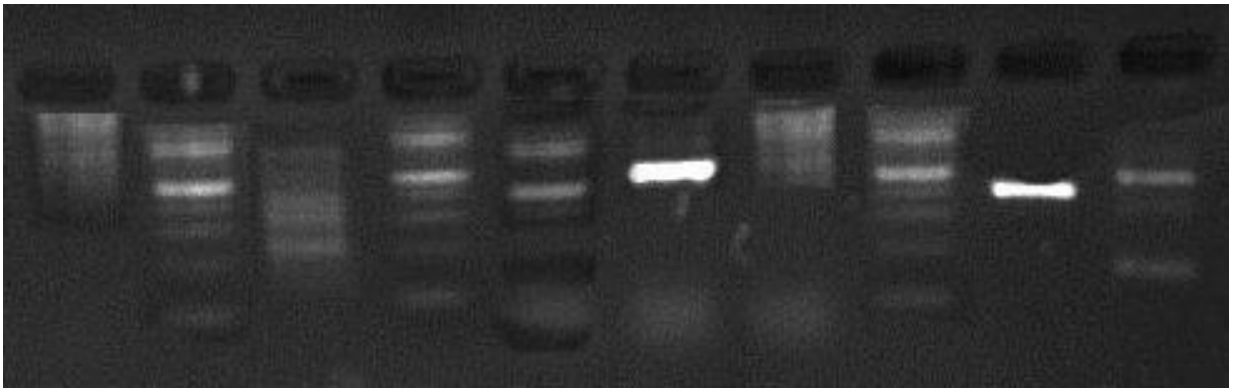

Figure (6): Agarose gel electrophoresis of RAPD- PCR products. lines (1-10) positive result of methicillin resistant *Staphylococcus aureus* with 10 different primers, after treatment withZno Nanoparticles

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Discussion

In the current study showed that Staphylococcus genius able to cause wound infection and main species of them were *Staphylococcus aureus.* This result agreement with (Almeida et al.,2014 , Lesseva& Hadjiiski, 1996). *Staphylococcus aureus* have many virulence factors which help him in pathogenesis like adherence factors , S. aureus Exoproteins, coagulase , DNase, Lipase and protase (Bien*et al*.,2011).

Most *Staphylococcus aureus* that isolated in current study were antibiotic resistant. This result agreement with (Almeida*et al*.,2014). That's maybe due to miss used of antibiotic, development of new bacterial strain have ability to resistant to antibiotic.

In current study show clear effect of nanoparticles against bacteria. That's agreement with (Liu*et al*.,2009). ZnO-NPs act as antibacterial by interact with membrane lipids and disorganize the membrane structure, which leads to loss of membrane integrity, malfunction, also it have ability to penetrate into bacterial cells at a nanoscale level and result in the production of toxic oxygen radicals, which damage cell membranes or cell proteins, all this mechanism lead to bacterial death (Krishnamurthy*et al*.,2012; Arzh et al.,2010). Also in current study show damage in genetic material, that agreement with (Vidic et al.,2013; Krishnamurthy*et al*.,2012).

The lethality of Ago nanoparticles by thiol-group reactions that inactivate enzymes Steuber and inhibits DNA replication, expression of ribosomal and other cellular proteins, and interferes with the bacterial electron transport chain (Yamanaka*et al*., 2005; Groom *et al*., 2000).

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