**RESEARCH ARTICLE**

# Antibacterial Activity of Copper Oxide Nanoparticles Against Methicillin Resistant *Staphylococcus Aureus* (Mrsa)

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## ABSTRACT

One hundred wound swab samples were collected from wound patients who visited in the Teaching Hospital Wound Unit in Hillah, Babylon Province, Iraq. *S. aureus* was identified morphologically and biochemically. Total of 30 (30%) from total samples exhibited positive culture for *S. aureus*. Out of 30 *S. aureus*, 8 (26.6%) isolates were Methicillin-resistant *S. aureus* (MRSA); Antibiotic susceptibility was tested for eight antibiotics for MRSA that appeared sensitive toward tetracycline, rifampin and ciprofloxacin (62.5%) but (37.5%) were resistant. For penicillin G and cefoxitin, all isolates were resistant (100%). For Clindamycin, (25%) of isolates were resistant, but (75%) were sensitive. About (62.5%) were resistant to Erythromycin, but (37.5%) were sensitive. Isolates showed resistance to Gentamycin in 50% and 50% sensitive. Anti-bacterial activity of copper oxide nanoparticles (CuO NPs) against *S. aureus* displays excessive widespread spectrum antibacterial action against established bacteria with increase zone of inhibition diameter that is proportional to the increase in nanoparticle concentration. The MIC of CuO NPs ranged from 75–150µg/mL, and the MBC ranged from 150-300µg/mL. CuO NPs is recommended as an efficient anti-MRSA alternative.

**Keywords:** CuO, Nanoparticles, *S. aureus*, MRSA.

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## INTRODUCTION

There are numerous organic and chemical composites with antibacterial action for example, penicillins and natural produces which eradicate bacterial or depressed their growing. Among them, nanoparticles have ne wly gotten further attention.[1,2] Reactive oxygen species (ROS) for example hydrogen peroxide, organic hydroperoxides, hydroxyl radicals and superoxide nanoparticles (NPs) statement scheduled the external of microorganisms and NPs gathering in the cytoplasm-periplasmic area of bacteria can be clue to microorganisms kill.[3] In the situation of bacteria, ROS can cause destruction of cellular components counting peptidoglycan, lipids, DNA, and proteins creating ROS through NPs.[4] Copper NPs are substantial constituents for search in nanomedicine ground. This attention is linked to shape and size built on physical-chemical things. External area to size percentage of NPs is an essential element for these things.[5-7] In this situation, one of the essential pragmatic ingredients in manufacturing is a copper oxide (CuO) and its compound in nanometers rule.[8] These copper NPs can be used as an effective substitute for gold and silver NPs.[9] Amongst several metallic oxide NPs, CuO has got more attention for being the greenest fellow of the intimate of copper composites and displays a variety of beneficial physical stuffs, for example, extraordinary heat conductivity electron link special effects and gyration diminuendos.[8,9]

*S. aureus* is a Gram-positive pathogen that causes severe contagions and diseases related to important sickness and death. The incidence of novel drug-resistant of this bacteria for uncontrolled usage of antibiotics is presently the main issue to concern.[10] *S. aureus* is one of the furthermost hurtful species of Staphylococci met. It is the main cause of myocarditis, osteomyelitis, bacteremia, pneumonia, pericarditis, meningitis, encephalitis, mastitis, and scalded skin syndrome.[11] MRSA strains of multidrug-resistant *S. aureus*, primarily designated in 1960s, rose in the latter period as a source of hospital infections implicated for quickly advanced latent lethal diseases counting hostile life pneumonia, fasciitis, osteomyelitis, severe sepsis, endocarditis, necrotizing and toxinoses (toxic shock syndrome).[12] A safe and efficient alternative is highly needed for continuously emerging life-threatening resistant strains.

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## MATERIALS AND METHODS

### Culture media:

Antibiotics discs were purchased from (Bioanalyse/Turkey) and are listed below.

### Specimen Collections and Isolation:

Bacterial isolates were acquired from the patients who stay in the Teaching Hospital wound unit in Hillah, Babylon Province, Iraq. Totally 100 specimens were collected using Ames transport media then inoculated into brain heart infusion broth (BHI) and incubated at 37°C for 24 hours. Mannitol Salt Agar (MSA) plates were inoculated and incubated at 37°C for 24 hrs for *S. aureus* isolation.[13] Isolated colonies were purified on nutrient agar (NA).

*S. aureus* were identified depending on the morphological feature on culture media and biochemical tests according to Bergey's manual.[14]

### Antimicrobial Susceptibility Test by Disk diffusion Method:

In this test, the antibiotics names and its standard inhibition diameters were used as recommended by.[15] The Inhibition zone diameter was measured using a caliper and compared with those determined by the Clinical Laboratory Standards Institute (CLSI) to conclude the resistance (R) or sensitivity (S) to each antibiotic.

### Identification of Methicillin-Resistant *S. aureus* (MRSA):

Cefoxitin (30µg) disc was used as an alternative to Methicillin, A zone of inhibition diameter ≤19 mm was considered as resistant to Methicillin, and ≥20 mm was considered as sensitive.[15]

### Antibacterial activity of CuO NPs

CuO NPs (40nm) was acquired from (US research nanomaterials Inc USA). The antibacterial action was handed out as designated by the CLSI. Bacterial sensitivity to CuO NPs is tested by disk diffusion assay as mentioned earlier.[15]

**Table 1:** Culture media used in this study.

| *Media* | *Origin* |
|---|---|
| Ames transport, Blood agar base, Muller-Hinton agar, Nutrient agar, Nutrient broth, Mannitol salt agar, and Brain heart agar | Himedia -India |

**Table 2:** Antibiotic disks

| *Antibiotic Discs* | *Disc potency (µg)* | *Symbol* |
|---|---|---|
| Tetracycline | 30 | TE |
| Ciprofloxacin | 5 | CIP |
| Penicillin G | 10 | PCN |
| Cefoxitin | 30 | CF |
| Rifampin | 5 | RA |
| Clindamycin | 30 | DA |
| Erythromycin | 15 | E |
| Gentamycin | 10 | CN |

Triplicates of CuO NPs in dilutions of (600, 300, 150, 75, 37.5 and 18.7µg/mL) in sterile deionized water were prepared, and ready discs have emerged in each dilution before usage. The MRSA isolates were initially incubated for 15 minutes at 4ºC then incubated at 37ºC for 24 hour. The Positive results were documented after a zone of inhibition was noticed.[16]

### Minimum bactericidal concentration (MBC) determination) and Minimum inhibitory concentration (MIC):

Bacterial isolates were incubated overnight at 37ºC, which were used to mark 0.5 McFarland. About 10ml tubes of nutrient broth medium was prepared with five dilutions of CuO NPs (600, 300, 150, 75, 37.5 and 18.7µg/ml) in triplicates with negative control (without CuO NPs). Each tube was inoculated (aseptically) with 1ml of the bacterial suspension about108CFU/mL). The inoculated sets were incubated at 37ºC overnight. The lesser concentration with no turbidity is represented as the MIC. Tubes with no turbidity were cultured on nutrient agar plates and incubated at 37ºC overnight. Bacterial colonies growth was checked, and the concentration that displays no growth is represented as the MBC.[15,16]

## RESULTS AND DISCUSSION

### Isolation of specimens:

Among 100 wound swab samples administered through the study, 30 (30%) showed culture positivity for *S. aureus*. Only eight (26.6%) isolates were identified as MRSA.

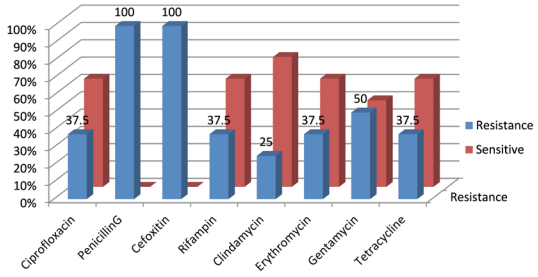
### Antibiotic Susceptibility Patterns and Antibacterial activity of CuO NPs for MRSA

MRSA maximum degree of susceptibility (62.5%) was perceived sensitive to Tetracycline, Rifampin, and Ciprofloxacin while (37.5%) were resistant. All isolates were resistant (100%) to Penicillin G and Cefoxitin. (25%) were resistant to Clindamycin but (75%) were sensitive. About (62.5%) were resistant to Erythromycin, while (37.5%) were sensitive. About (50%) of the isolates showed resistance to Gentamycin (3), (Figure 1). This result don't agree with[17,18] who found that Erythromycin, Tetracycline, Gentamycin, Ciprofloxacin and Rifampin with resistance percentages 37.7%, 34.9%, 28.5%, 29.2%, and 3.8%, separately but agree with the rates of resistant to Penicillin G and Cefoxitin (100%) and Clindamycin (32.8%). Also,[19] they observed that 60% of the isolates were resistant to Clindamycin. These variances in results might due to the difference in topographical zones, differences in isolates amount, and place of gathering. CuO NPs displays great extensive range antibacterial activity with MRSA. The result in Table 3, displayed the action of CuO NPs that showed a rise in the zone of inhibition diameter proportional with the rise in the concentration of nanoparticle. The 600µg/ml concentration exhibited a maximum zone of inhibition against the test organisms, as presented in Figures (2 and 3). This result approved with the study of Wen-Ru Li, *et al.*[20] who found the copper nanoparticles act as an antibacterial against *S. aureus*.

**Table 3:** Antibiotic susceptibility patterns and antibacterial activity of CuO NPs on *S. aureus*

| | *Inhibition zone diameter(mm)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Antimicrobial agent* | *No(1)* | *No(2)* | *No(3)* | *No(4)* | *No(5)* | *No (6)* | *No(8)* | *No(8)* |
| Tetracycline | 13(R) | 12 (R) | 21 (S) | 20(S) | 22(S) | 21(S) | 12 (R) | 21(S) |
| Ciprofloxacin | 22 (S) | 14(R) | 23(S) | 22(S) | 13(R) | 23(S) | 13(R) | 22(S) |
| Penicillin G | 21(R) | 19(R) | 20(R) | 18(R) | 19(R) | 22(R) | 22(R) | 21(R) |
| Cefoxitin | 18(R) | 20(R) | 19(R) | 18(R) | 19(R) | 17(R) | 18(R) | 20R) |
| Rifampin | 21 (S) | 14(R) | 22(S) | 15(R) | 20(S) | 23(S) | 15(R) | 23(S) |
| Clindamycin | 19(S) | 20(S) | 22(S) | 20(S) | 13(R) | 14(R) | 20(S) | 18(S) |
| Erythromycin | 13(R) | 24(S) | 12(R) | 11(R) | 12 (R) | 23(S) | 12(R) | 23(S |
| Gentamycin | 17(S) | 11(R) | 13(S) | 10(R) | 19(S) | 9(R) | 11(R) | 14(S) |
| CuO 600µg/mL | 22 | 23 | 20 | 24 | 22 | 23 | 22 | 24 |
| CuO 300µg/mL | 20 | 18 | 18 | 20 | 21 | 20 | 19 | 21 |
| CuO 150µg/mL | 18 | 17 | 16 | 18 | 16 | 18 | 19 | 17 |
| CuO 75µg/mL | 14 | 15 | 15 | 16 | 14 | 16 | !6 | 13 |
| CuO 37,5µg/mL | 12 | 11 | 10 | 9 | 11 | 10 | 12 | 11 |
| CuO 18,7µg/mL | 8 | 6 | 7 | 5 | 6 | 8 | 6 | 7 |

-R: Resistant. –S: Sensitive




**Figuer 1:** Antibiotic sensitivity of *S. aureus* (MRSA).

**Figuer 2:** Antibacterial activity of CuO NPs on *S. aureus* (MRSA).

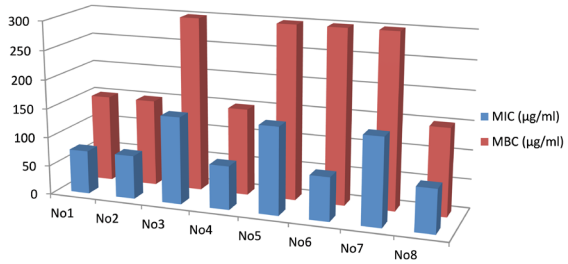



**Figure 3:** MIC and MBC of CuO NPs on *S. aureus* (MRSA).

## CONCLUSION

This results showed the methicillin-resistant strains, high rate resistance to penicillin G and cefoxitin (100%), erythromycin (62.5%), gentamycin resistance (50%), therefor this experiment found that CuO NPs is highly recommended as an alternative anti-MRSA agent with significant inhibitory and antibacterial effect.

## ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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