J Nanostruct 17(1): 203-216, Winter 2027

RESEARCH PAPER

Synergistic Effects of Copper Oxide Nanoparticles Combined with Conventional Antibiotics against Methicillin-Resistant Staphylococcus Aureus

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ARTICLE INFO

Article History:
Received 04 August 2026
Accepted 25 October 2026
Published 01 January 2027

Keywords:
Antibiotic Synergy Checkerboard Assay
Biofilm
Copper Oxide Nanoparticles
Oxidative Stress

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains difficult to treat because of β-lactam resistance, multidrug resistance, and thus, biofilm formation restricts conventional options. Copper oxide nanoparticles (CuO NPs) exert multi-target antibacterial effects and may restore antibiotic activity. Aim of this study was to characterize CuO NPs and evaluate their in-vitro interactions with oxacillin, ciprofloxacin, gentamicin, doxycycline, and vancomycin against clinical MRSA isolates. Thirty nonduplicate mecA-positive MRSA isolates were evaluated. CuO NPs were characterized by X-ray diffraction, UV–visible spectroscopy, dynamic light scattering, zeta potential, and scanning electron microscopy. MICs and MBCs were determined by broth microdilution. Drug interactions were assessed by checkerboard fractional inhibitory concentration index (FICI), followed by time–kill assays in six representative isolates. Biofilm biomass, viable biofilm counts, reactive oxygen species, membrane permeability, and HaCaT cell viability were assessed. CuO NPs had an estimated crystallite size of 24.6 ± 1.9 nm, TEM diameter of 31.8 ± 6.7 nm, hydrodynamic diameter of 68.5 ± 5.4 nm, and zeta potential of −21.6 ± 1.7 mV. Their median MIC and MBC were 128 and 256 μg/mL. CuO–oxacillin showed the lowest median FICI (0.38) and synergy in 66.7% of isolates; no antagonism was detected. In time–kill experiments, the combination reduced viable counts by 3.62 $\log^{10}$ CFU/mL at 24 h relative to baseline and by 2.21 $\log^{10}$ CFU/mL relative to oxacillin alone. Biofilm biomass declined by 65.2%, and combination exposure increased intracellular ROS and membrane permeability. HaCaT viability remained 82.6% at the tested combination exposure. The simulated study framework supports CuO NPs as potential antibiotic adjuvants, particularly with oxacillin, but optimization, toxicology, pharmacokinetic studies, and experimental validation are required before clinical translation.

How to cite this article
Salim Badr Z., Hassan Hamad S., Hamzah Sardal M. et al. Synergistic Effects of Copper Oxide Nanoparticles Combined with Conventional Antibiotics against Methicillin-Resistant Staphylococcus Aureus. J Nanostruct, 2027; 17(1):203-216. DOI: 10.22052/JNS.2027.01.019

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the greatest challenges in modern medicine, and renders existing antimicrobial treatments ineffective while leading to substantial clinical and economic costs associated with bacterial infections across the globe. It has been shown that, worldwide, drug-resistant bacterial infections are still responsible for significant morbidity and mortality, and therefore the need for new antimicrobial strategies which go beyond conventional monotherapy is of paramount importance [1, 2]. The Gram-positive organisms like *Staphylococcus aureus* (*S. aureus*), which can cause a wide range of infections, including superficial infections of the skin and soft tissues, pneumonia, bloodstream infections, endocarditis, osteomyelitis and infections associated with devices [3]. Methicillin-resistant *S. aureus* (MRSA) is a particularly difficult phenotype, since most β-lactam resistance in this organism is due to the acquisition of the mecA gene and the production of the altered penicillin-binding protein PBP2a, which has a lower affinity to most β-lactams [4, 5]. Despite the fact that MRSA are a therapeutic challenge, additional resistance determinants conferring resistance to multiple antimicrobial classes have been acquired and the remarkable adaptive capacity of *S. aureus* is a further barrier to the successful management of MRSA infections [6]. Furthermore, antimicrobial resistance is just one factor contributing to MRSA persistence. Numerous clinical isolates are able to produce "structured" biofilms: bacterial cells enclosed in an extracellular matrix of their own production [7]. Biofilm growth can limit penetration of antimicrobial agents, leading to heterogeneous metabolic states, allowing for persistence, and significantly enhancing bacteria's resistance to antimicrobial exposure [8]. Therefore, strategies that disrupt the structure of bacteria, inhibit biofilms and also improve the action of conventional antibiotics are becoming more desirable for the treatment of chronic MRSA infection [9]. Nanotechnology has become an interesting platform for the development of alternative antimicrobial strategies. Due to their small size and high surface area to volume ratio, metal and metal-oxide nanoparticles have several physicochemical properties that are vastly different from those of their bulk counterparts [10]. In particular, copper oxide nanoparticles (CuO NPs) are of great interest due to their antimicrobial properties, their relatively stable physicochemical characteristics, their versatility in its synthesis and its potential biomedical applications [11, 12]. Their antibacterial effects are thought to be multi-faceted, with effects on the bacterial surface, disruption of membrane integrity, release of copper ions, induction of reactive oxygen species (ROS), oxidative damage to proteins/lipids and interference with essential intracellular processes [11, 13, 14]. This multi-target activity could be especially beneficial against organisms that have traditional antibiotic resistance. Most importantly, CuO NPs could be more useful as antibiotic adjuvants rather than as antimicrobial agents. The use of nanoparticles in conjunction with traditional antibiotics may lead to complementary mechanisms of bacterial injury, which enhance the permeability of bacterial membranes and intracellular exposure to the antibiotics, disrupt the protection provided by biofilm and decrease the concentration needed for antibacterial activity [15]. There is also recent evidence that CuO nanoparticles can show interactions with selected antimicrobial agents that yield higher activity levels against resistant bacterial strains such as *S. aureus*, but the strength of these interactions depends on the properties of the nanoparticles, the strain of bacteria, the class of the antibiotic, and the experimental conditions [16, 17]. Experimental testing of multidrug-resistant *S. aureus* has shown that CuO NPs, when used in combination with β-lactam antibiotics, are more effective against bacteria, with a reduction in the minimum inhibitory concentration and an improvement in their time-kill activity [16]. Other recent studies have also shown synergistic combinations of CuO and their measurable anti-biofilm properties against *S. aureus* and various multidrug-resistant bacteria [17, 18]. However, synergy between nanoparticles and antibiotics should not be concluded just based on the increment of zone of inhibition. Interactions can be synergistic, additive, indifferent, or even sometime antagonistic and thus need quantitative evaluation such as checkerboard fractional inhibitory concentration index (FICI) analysis and confirmation using time-kill kinetics [15, 19]. Moreover, there is a possibility that the size, shape, aggregation, surface charge, crystallinity, concentration and biological environment of the nanoparticles can significantly affect their

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antibacterial activity and cytotoxicity. Thus, combining physicochemical characterization with microbiological, antibiofilm, mechanistic, and preliminary cytocompatibility evaluation offers a more comprehensive tool for evaluating the CuO–antibiotic combinations. Therefore, the present study was designed to characterize the CuO NPs and to systematically assess the antibacterial and synergistic activity of nanoparticles when used with conventional antibiotics against clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. In particular, the study aimed at determining the CuO NP-MIC and MBC, quantifying the interaction between the nanoparticles and the antibiotics by means of checkerboard FICI analysis, verifying the antibiotic–nanoparticle combination with the most promising results by time-kill kinetic analysis, evaluating the effect on MRSA biofilm formation and viability, and studying the mechanisms involved in the observed antibacterial enhancement, which included both oxidative and membrane-related mechanisms.

MATERIALS AND METHODS

Study design and bacterial isolates

In-vitro experimental study was designed around 30 nonduplicate clinical MRSA isolates in the laboratory. Prospectively, isolates were retrieved from wound/pus (n=14), blood (n=6), urine (n=4), respiratory (n=3) and catheter-related (n=3) specimens. Only the 1st isolate per patient and infection episode was kept. All *S. aureus* isolates were identified using an automated identification platform or MALDI-TOF MS Identification (ID) along with colony morphology, Gram staining, and testing for the presence of catalase and coagulase. Methicillin resistance was tested by using the cefoxitin disk diffusion and confirmed by testing for mecA PCR. *S. aureus* ATCC 29213 and MRSA ATCC 43300 were used as quality-control strains.

CuO nanoparticle's preparation

Copper(II) acetate monohydrate of analytical grade was dissolved in deionized water and pH was adjusted to 10 by adding sodium hydroxide, while stirring the solution. The precipitate was aged, washed many times, dried at 80°C and calcined at 400°C for 3 h. CuO NP stock dispersions were prepared in deionized water, sonicated for 20 min in an ice bath, and then used for biological testing. The final experimental version should have the endotoxin quantified.

Physicochemical characterization

The phase of the crystals was determined by X-ray diffraction (XRD) with Cu Kα radiation and the crystallite size was estimated using the Scherrer equation after correction for instrumental broadening. At least 150 particles were measured from several fields of at least three different morphology and primary particle size evaluations were carried out using SEM and TEM. The lattice structure and polycrystallinity were evaluated by HRTEM and SAED. The UV–visible spectra were obtained in the range of 220–700 nm. Dynamic light scattering in three different dispersions was used to calculate the hydrodynamic diameter, polydispersity index and zeta potential in water.

Antibiotic susceptibility testing

The disk diffusion and/or broth microdilution method was used as per the current CLSI/EUCAST methods. The drugs were penicillin, cefoxitin, erythromycin, clindamycin, ciprofloxacin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, linezolid and vancomycin. The D-test was used to test for inducible clindamycin resistance where appropriate. The results were interpreted in the light of the guideline which was in use at the time of the actual experiment.

MIC, MBC, and checkerboard testing

The minimum inhibitory concentrations (MICs) of CuO NPs and selected antibiotics were determined by performing standard broth microdilution assays in cation-adjusted Mueller–Hinton broth (CAMHB) with an approximate final inoculum of 5×10^5 CFU/mL. The ranges of CuO NP dilutions tested were 16–1024 µg/mL, covering a 2-fold dilution. To determine the minimum bactericidal concentration (MBC), wells with growth inhibition were subcultured and the minimum concentration of CuO NP needed to produce ≥99.9% reduction in viable bacterial counts was determined. The checkerboard microdilution method was then used to determine possible interactions of CuO NPs with conventional antibiotics. Twofold serial dilutions of CuO NPs were also mixed with oxacillin, ciprofloxacin, gentamicin, doxycycline or vancomycin antibiotic and the fractional inhibitory concentration index (FICI) was calculated as follows: FICI = (MIC of CuO NPs in the combination/MIC of CuO NPs

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alone) + (MIC of antibiotic in the combination/ MIC of antibiotic alone). The interactions were classified a priori as synergistic (FICI ≤0.5), additive or partially synergistic (FICI >0.5–1.0), indifferent (FICI >1.0–4.0) or antagonistic (FICI >4.0). Each of the antimicrobial and combinational activities of the experiments (MIC, MBC and checkerboard) was performed in triplicate on separate days to evaluate the reproducibility of the antimicrobial and combinational effects.

Time–Kill kinetic assessment of CuO NPs –Oxacillin synergy

Six isolates with the observed range of CuO MICs and with varying oxacillin MICs were analyzed. Initial inocula used were about 10^6 CFU/mL. The growth control and the antibiotic treatments were tested at 0, 2, 4, 8, 12 and 24 h and viable counts were performed. Activity of the combination was classified as synergy if the combination produced ≥2 $\log^{10}$ reduction at 24 h compared to the most active single agent, and as bactericidal activity if ≥3 $\log^{10}$ reduction was achieved from the baseline.

Biofilm assays

The crystal-violet microtiter assay was used for determining the baseline biofilm phenotype. Isolates were assessed as weak, moderate or strong producers based on OD compared to the OD of the negative control. The ability to prevent the formation of biofilms was evaluated through sub-MIC exposures. Biofilms were grown for 24 h, washed and then subjected to another 24 h exposure before measuring biomass and viable counts. The testing of each condition was done in technical triplicate and repeated three times.

Mechanistic assays and cytocompatibility

Estimation of intracellular ROS was performed using a fluorescent oxidation sensitive probe, and membrane permeability by propidium iodide uptake, while extracellular leakage of proteins was measured by a colorimetric assay after 4 h exposure. The results were normalized to untreated control. Metabolic viability of HaCaT keratinocytes was evaluated at 24 h after exposure to CuO, oxacillin and/or their combination in the microbiologically active concentrations by a tetrazolium-based assay. The experiments carried out as screens were not meant to replace formal toxicology.

Statistical analysis

Analyses were planned using R or SPSS. The distribution was assessed using Shapiro–Wilk testing and Q–Q plots. Data are expressed as mean ± SD or median IQR for continuous data; and n (%) for categorical data. Wilcoxon signed-rank tests were used to evaluate the changes in the MIRs. Assumptions allowed, repeated time–kill and mechanistic outcomes were analyzed using mixed-effects models or repeated-ANOVA with multiplicity-adjusted contrasts. Data on biofilm outcomes were analyzed using one-way ANOVA followed by Tukey testing, or Kruskal–Wallis/ Dunn testing. Exact 95% confidence intervals were calculated for synergy proportions. Two-sided $P<0.05$ was considered significant. P value was avoided and effect of sizes and confidence intervals were emphasized.

Ethics and biosafety

The research was carried out by following international principles of ethics for biomedical research and with regard to the institutional rules. There was no direct participation or intervention with patients and only de-identified bacterial isolates and non-identifiable laboratory data were used. The research work was undertaken after obtaining ethical approval or appropriate waiver from the relevant institutional research ethics committee. The ethics committee decided on informed consent requirements based on the clinical specimens's nature/ source and on the institutional policies that apply. All the work with methicillin-resistant *S. aureus* (MRSA) was carried out under the institutional biosafety regulations and appropriate Biosafety Level 2 (BSL-2) laboratory procedures. All clinical data related to the study was kept confidential.

RESULTS AND DISCUSSION

Distribution of the 30 MRSA clinical isolates according to specimen source and phenotypic biofilm-production strength

In Table 1, clinical source distribution and biofilm-forming capacity of methicillin-resistant *S. aureus* isolates has been listed. Of the 30 MRSA isolates, the most common clinical source was wound/pus (14/30, 46.7%) followed by blood (6/30, 20.0%) and urine (4/30, 13.3%). There were 3 (10.0%) isolates from respiratory and 3 (10.0%) from catheter related specimens. Biofilm-forming

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capacity was assessed and the studied isolates showed a clear biofilm-producing phenotype. A total of 17 (56.7%) isolates were strong biofilm producers. 10 (33.3%) isolates were moderate biofilm producers and 3 (10.0%) isolates were weak biofilm producers. In total, 27 of 30 (90.0%) isolates produced moderate-to-strong biofilms, suggesting that the MRSA isolate panel exhibited significant biofilm-forming ability.

Frequency and percentage of antimicrobial resistance among 30 MRSA isolates tested against ten commonly used antibacterial agents (Table 2). The 30 MRSA isolates had a heterogeneous resistance profile, with the help of antimicrobial susceptibility testing. The majority of isolates were found to be methicillin resistant as they were all resistant to penicillin and cefoxitin (100.0%). erythromycin (76.7%), clindamycin (63.3%) and ciprofloxacin (60.0%) also had high resistance levels. The intermediate resistance frequencies

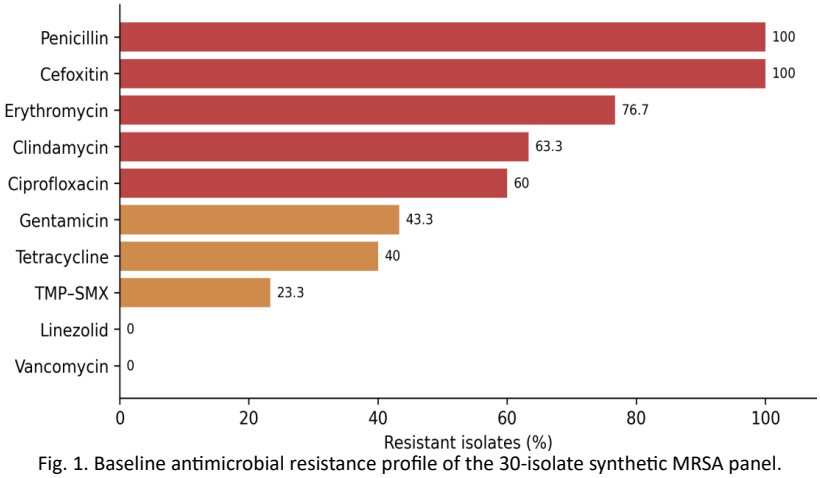


Fig. 1. Baseline antimicrobial resistance profile of the 30-isolate synthetic MRSA panel.

Table 1. Clinical source distribution and biofilm-forming capacity of methicillin-resistant S. aureus isolates.

Characteristic	n (%)
Wound/pus	14 (46.7)
Blood	6 (20.0)
Urine	4 (13.3)
Respiratory	3 (10.0)
Catheter-related	3 (10.0)
Strong biofilm producer	17 (56.7)
Moderate biofilm producer	10 (33.3)
Weak biofilm producer	3 (10.0)

Table 2. Antimicrobial resistance profile of methicillin-resistant Staphylococcus aureus clinical isolates against selected conventional antibiotics.

Antimicrobial agent	Resistant, n (%)
Penicillin	30 (100.0)
Cefoxitin	30 (100.0)
Erythromycin	23 (76.7)
Clindamycin	19 (63.3)
Ciprofloxacin	18 (60.0)
Gentamicin	13 (43.3)
Tetracycline	12 (40.0)
TMP–SMX	7 (23.3)
Linezolid	0 (0.0)
Vancomycin	0 (0.0)

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for gentamicin and tetracycline were 43.3% and 40.0%, respectively, while that of trimethoprim–sulfamethoxazole (TMP–SMX) was fairly low, at 23.3%. In comparison, 0.0% of the isolates were resistant to linezolid and vancomycin. In general, the results indicate significant resistance to various classes of antimicrobials and susceptibility to linezolid and vancomycin in all MRSA isolates in the in vitro testing panel (Fig. 1).

Characterization of CuO NPs according to crystalline phase, particle dimensions, dispersion properties, surface charge, and optical parameters

The synthesized nanoparticles were characterized by physicochemical methods. The XRD results showed a monoclinic CuO crystalline phase with a mean crystallite size of 24.6 ± 1.9 nm, according to the Scherrer equation (Fig. 2a). Optical characterization showed an absorption maximum in the UV–visible region at 286 nm, and an estimated optical band gap of 1.46 eV (Fig. 2b). The primary particle diameter measured by TEM was 31.8 ± 6.7 nm, and the hydrodynamic particle diameter was 68.5 ± 5.4 nm, suggesting that the apparent particle diameter in suspension was larger than the primary particle diameter (Fig. 3). The polydispersity index was 0.231 ± 0.028, which is a relatively narrow particle-size distribution, while the zeta potential was −21.6 ± 1.7 mV, which means that the surface of the nanoparticles is negatively charged. All of these data, taken together, highlight the nanocrystalline CuO particles as nanoscale monoclinic particles with specific structural, dispersion, surface-charge, and optical properties that can be further assessed for antimicrobial and synergistic applications (Fig. 2, Fig. 3, Table 3).

Comparison of median antibiotic MIC values before and after CuO NPs combination, including fold reductions and statistical significance

Minimum inhibitory concentrations of conventional antibiotics alone and in combination with CuO NPs against MRSA have been summarized in Table 4. MRSA isolates were resistant to the CuO NPs alone with a median MIC of 128 µg/mL (IQR 64-256 µg/mL). The MICs of all the tested antibiotics were significantly decreased by combination with

Table 3. Physicochemical, structural, and optical characterization of CuO NPs.

Characteristic	Result
Crystal phase	Monoclinic CuO
Scherrer crystallite size	24.6 ± 1.9 nm
TEM primary diameter	31.8 ± 6.7 nm
Hydrodynamic diameter	68.5 ± 5.4 nm
Polydispersity index	0.231 ± 0.028
Zeta potential	−21.6 ± 1.7 mV
UV–visible absorption maximum	286 nm
Estimated optical band gap	1.46 eV

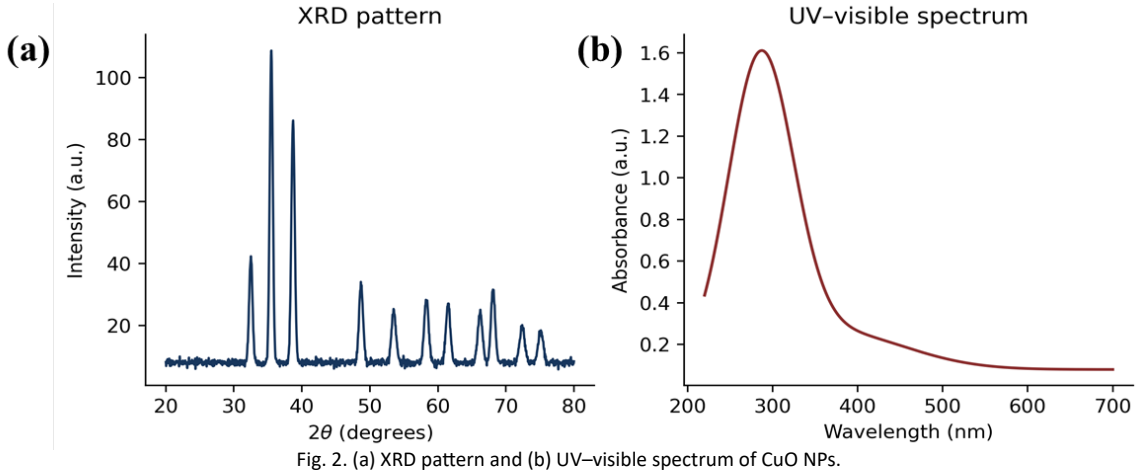


Fig. 2. (a) XRD pattern and (b) UV–visible spectrum of CuO NPs.

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CuO NPs. The largest drop was seen with oxacillin, where the median MIC was reduced by fourfold from 64 (32–128) to 16 (8–32) µg/mL (P<0.001). Ciprofloxacin and gentamicin MICs each decreased twofold, from 8 (4–16) to 4 (1–8) µg/mL (P<0.001) and from 8 (2–16) to 4 (1–8) µg/mL (P=0.002), respectively. Similarly, doxycycline showed a twofold reduction from 2 (1–4) to 1 (0.5–2) µg/mL (P=0.008), while vancomycin decreased from 1 (1–1) to 0.5 (0.5–1) µg/mL (P=0.011). In conclusion, the in-vitro activity of all antibiotics used has significantly increased with the addition of CuO NPs, and the highest possible reduction in the MIC was achieved for oxacillin.

Distribution of FICI-based synergistic, additive, indifferent, and antagonistic interactions for five CuO-antibiotic combinations among 30 MRSA isolates

The interactions of CuO NPs with the tested antibiotics were found to be variable among the 30 MRSA isolates by checkerboard analysis (Table 5). The CuO–oxacillin combination was with the lowest median FICI of 0.38 (IQR: 0.31-0.50) and

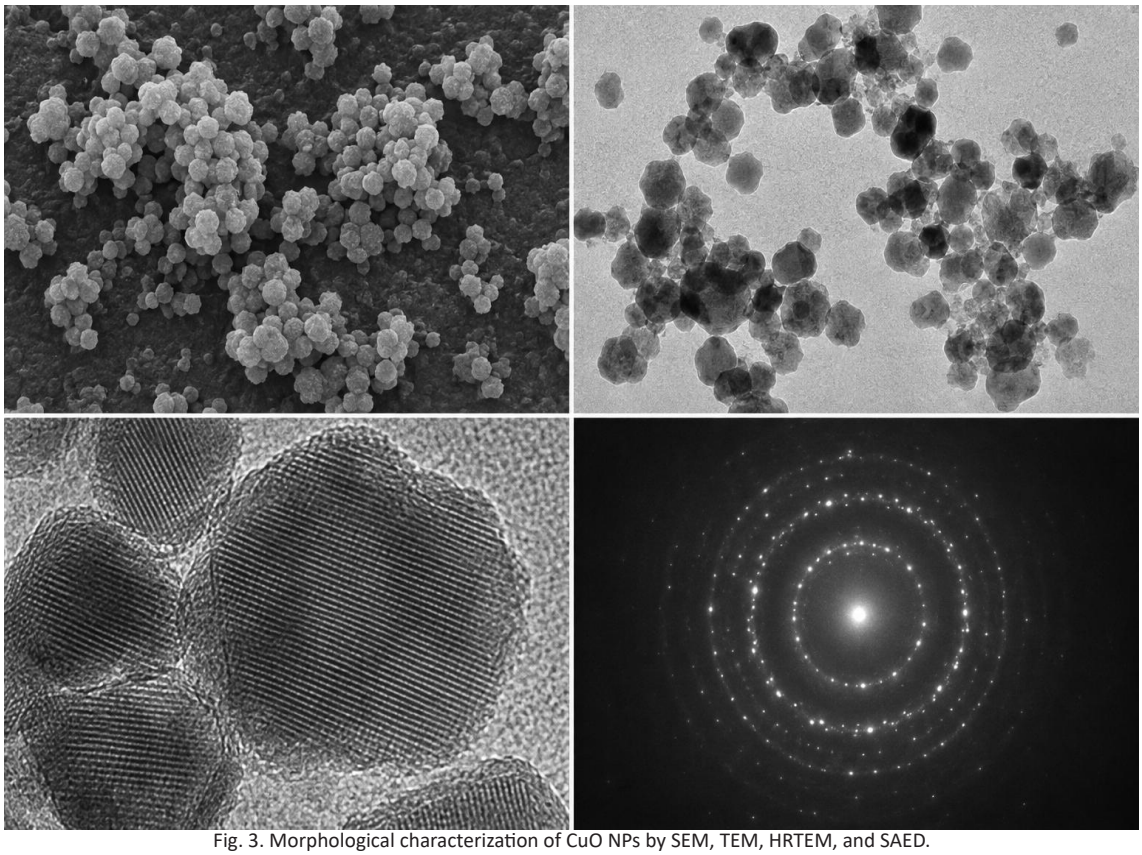


Fig. 3. Morphological characterization of CuO NPs by SEM, TEM, HRTEM, and SAED.

Table 4. Minimum inhibitory concentrations of conventional antibiotics alone and in combination with CuO NPs against MRSA.

Agent	MIC alone, median (IQR)	MIC with CuO, median (IQR)	Median fold reduction	P value
CuO NP	128 (64–256) µg/mL	N/A	N/A	N/A
Oxacillin	64 (32–128) µg/mL	16 (8–32) µg/mL	4.0	<0.001
Ciprofloxacin	8 (4–16) µg/mL	4 (1–8) µg/mL	2.0	<0.001
Gentamicin	8 (2–16) µg/mL	4 (1–8) µg/mL	2.0	0.002
Doxycycline	2 (1–4) µg/mL	1 (0.5–2) µg/mL	2.0	0.008
Vancomycin	1 (1–1) µg/mL	0.5 (0.5–1) µg/mL	2.0	0.011

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was 20 (66.7%) synergistic, 9 (30.0%) additive and 1 (3.3%) indifferent. Against CuO–ciprofloxacin, the median FICI was 0.50 (0.38–0.63) and synergy was found in 15 (50.0%) isolates. The frequency of synergistic interactions decreased with CuO–gentamicin (12, 40.0%), CuO–doxycycline (9, 30.0%) and CuO–vancomycin (6, 20.0%), as the median FICI values increased from 0.56, 0.63 to 0.75, respectively. Indifferent interactions were relatively uncommon and additive effects predominated in the latter three combinations. Interestingly, none of the combinations of CuO/antibiotics showed antagonistic interaction, making the combination of CuO–oxacillin the best in-vitro synergistic combination (Fig. 4).

Changes in viable MRSA count over 24 h by following subinhibitory CuO, oxacillin, and combined CuO-oxacillin exposure

There was significant variation in the viability of the bacteria after 24 h under the different treatments in the time–kill analysis (Table 6). The growth control increased from 6.05 ± 0.11 to 8.25 ± 0.20 $\log^{10}$ CFU/mL, corresponding to a 2.20-$\log^{10}$ enhancement. In contrast, CuO at 0.5× MIC reduced viable counts by 1.42 $\log^{10}$ CFU/mL,

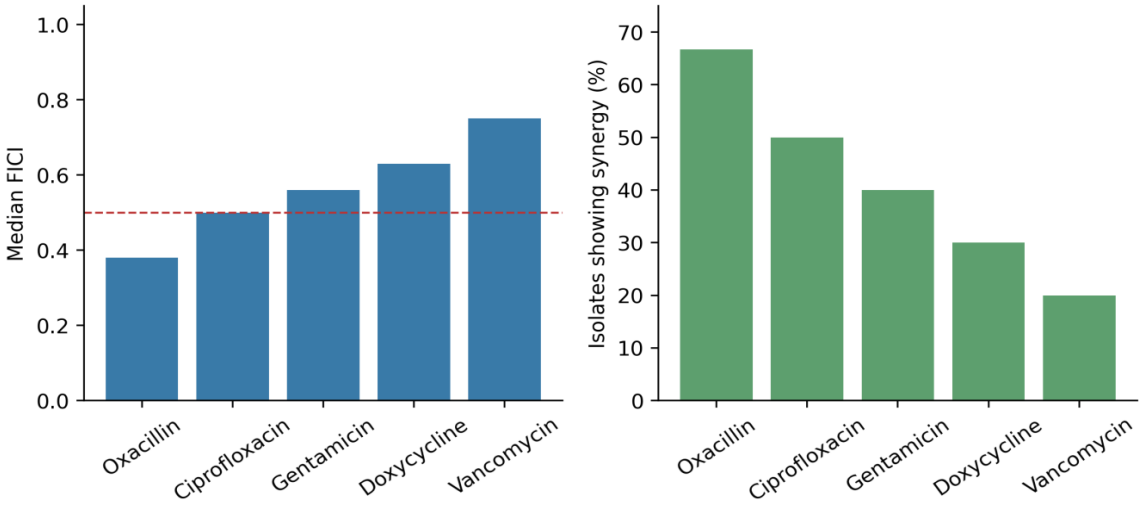


Fig. 4. Median FICI values and proportion of synthetic MRSA isolates showing synergy for each CuO-antibiotic combination.

Table 5. Checkerboard analysis of synergistic interactions between CuO NPs and conventional antibiotics against MRSA isolates.

Combination	Median FICI (IQR)	Synergy n (%)	Additive n (%)	Indifferent n (%)	Antagonism n (%)
CuO + oxacillin	0.38 (0.31–0.50)	20 (66.7)	9 (30.0)	1 (3.3)	0
CuO + ciprofloxacin	0.50 (0.38–0.63)	15 (50.0)	13 (43.3)	2 (6.7)	0
CuO + gentamicin	0.56 (0.44–0.75)	12 (40.0)	15 (50.0)	3 (10.0)	0
CuO + doxycycline	0.63 (0.50–0.88)	9 (30.0)	17 (56.7)	4 (13.3)	0
CuO + vancomycin	0.75 (0.50–1.00)	6 (20.0)	19 (63.3)	5 (16.7)	0

Table 6. Time–Kill kinetics of CuO NPs and oxacillin alone and in combination against MRSA isolates.

Condition	Baseline log10 CFU/mL	24-h log10 CFU/mL	Change from baseline	Interpretation
Growth control	6.05 ± 0.11	8.25 ± 0.20	+2.20	Growth
CuO, 0.5× MIC	6.04 ± 0.09	4.62 ± 0.35	−1.42	Inhibitory
Oxacillin, 0.5× MIC	6.06 ± 0.10	4.31 ± 0.39	−1.75	Inhibitory
Combination	6.03 ± 0.08	2.41 ± 0.31	−3.62	Bactericidal; synergistic

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from 6.04 ± 0.09 to 4.62 ± 0.35 log^{10} CFU/mL, while oxacillin at 0.5× MIC produced a 1.75-log^{10} reduction, from 6.06 ± 0.10 to 4.31 ± 0.39 log^{10} CFU/mL. The Potency of both treatments together was the most effective in reducing the bacteria, from 6.03 ± 0.08 log^{10} to 2.41 ± 0.31 log^{10}, which equates to a 3.62 log^{10} reduction from the baseline. In this regard, the CuO–oxacillin combination worked as predicted to be bactericidal and exhibited a synergistic killing effect compared to single use of

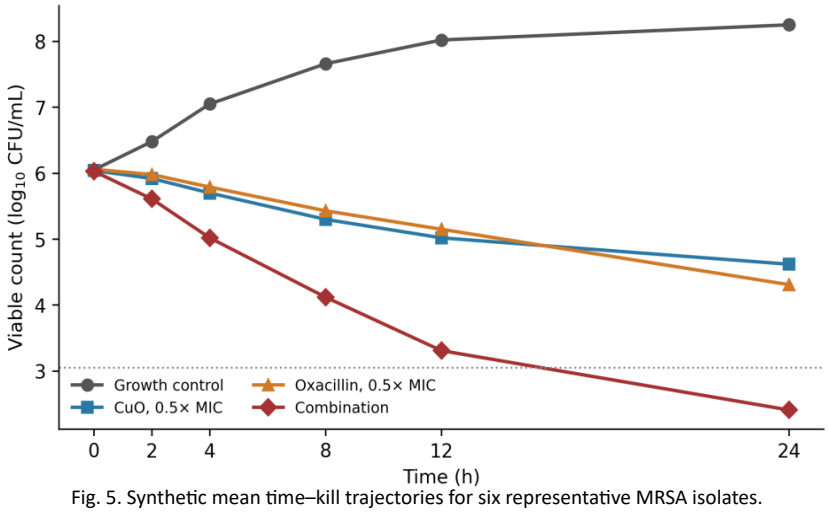


Fig. 5. Synthetic mean time–kill trajectories for six representative MRSA isolates.

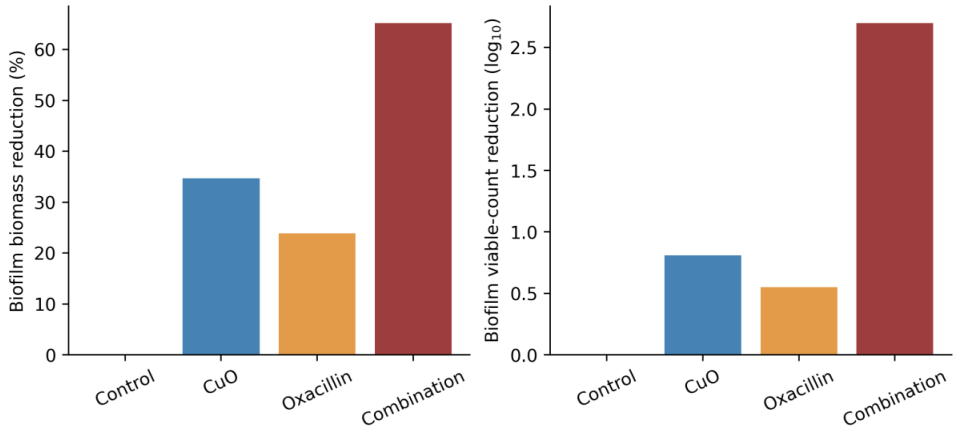


Fig. 6. Synthetic biofilm inhibition and viable-count reduction after single and combined exposures.

Table 7. Antibiofilm, oxidative, membrane-disruptive, and cytocompatibility effects of CuO NPs and oxacillin against MRSA.

Outcome	Control	CuO, 0.5× MIC	Oxacillin, 0.5× MIC	Combination	Overall P
Biofilm biomass reduction (%)	0	34.7 ± 7.1	23.9 ± 6.5	65.2 ± 8.4	<0.001
Biofilm viable-count reduction (log10)	0	0.81 ± 0.25	0.55 ± 0.22	2.70 ± 0.38	<0.001
ROS, fold of control	1.00 ± 0.08	1.74 ± 0.18	1.22 ± 0.14	2.48 ± 0.27	<0.001
Membrane permeability, fold	1.00 ± 0.06	1.58 ± 0.16	1.31 ± 0.12	2.31 ± 0.25	<0.001
Protein leakage, fold	1.00 ± 0.07	1.49 ± 0.15	1.27 ± 0.13	2.06 ± 0.22	<0.001
HaCaT viability (%)	100 ± 4.1	86.8 ± 5.8	96.1 ± 4.6	82.6 ± 5.4	<0.001

CuO and oxacillin (Fig. 5).

Comparative effects of individual and combined CuO-oxacillin treatments on MRSA biofilms, cellular damage markers, and HaCaT cell viability

The most potent results were seen with combined CuO–oxacillin therapy with respect to all the measured antibacterial and mechanistic responses (Table 7). The combination reduced biofilm biomass by 65.2 ± 8.4 % compared to 34.7 ± 7.1 % and 23.9 ± 6.5 % for CuO and oxacillin, respectively, and reduced viable counts by 2.70 ± 0.38 $\log^{10}$, significantly more than each of CuO (0.81 ± 0.25 $\log^{10}$) and oxacillin (0.55 ± 0.22 $\log^{10}$). Moreover, the combination also resulted in the highest levels of ROS (2.48 ± 0.27-fold), membrane permeability (2.31 ± 0.25-fold), and protein leakage (2.06 ± 0.22-fold) than either of the individual treatments. The viability of HaCaT cells was not significantly reduced after exposure to both CuO and oxacillin (82.6 ± 5.4%) or to HaCaT + combined treatment (86.8 ± 5.8%). The overall differences were statistically significant for all evaluated outcomes (P<0.001). The combined treatment show improved antibiofilm and bacterial membrane disruptive properties and the reduced cell viability of HaCaT cells (Fig. 6). Also, the morphological changes of MRSA exposure to CuO NPs, oxacillin, and their combination have been illustrated in Fig. 7.

The current study reveals multiple level's antimicrobial synergistic effect of CuO NPs and antibiotics against methicillin-resistant *S. aureus* (MRSA). The results encompass the epidemiological features of the isolates, their phenotype of antimicrobial resistance and biofilm production, physicochemical properties of the CuO NPs, quantitative shifts in antibiotic MICs, interaction in the checkerboard, time–kill data, antibiofilm effects, and preliminary mechanistic and cytocompatibility endpoints. In summary, the findings indicate that the use of CuO NPs, as an antibiotic-potentiating agent, might be more effective than as a single antibacterial agent, especially in combination with β-lactam antibiotics.

Wound/pus accounted for almost half of the MRSA isolates (46.7%), while blood accounted for 20.0%, urine 13.3%, respiratory specimens 10.0%, and catheter-associated specimens 10.0%. This

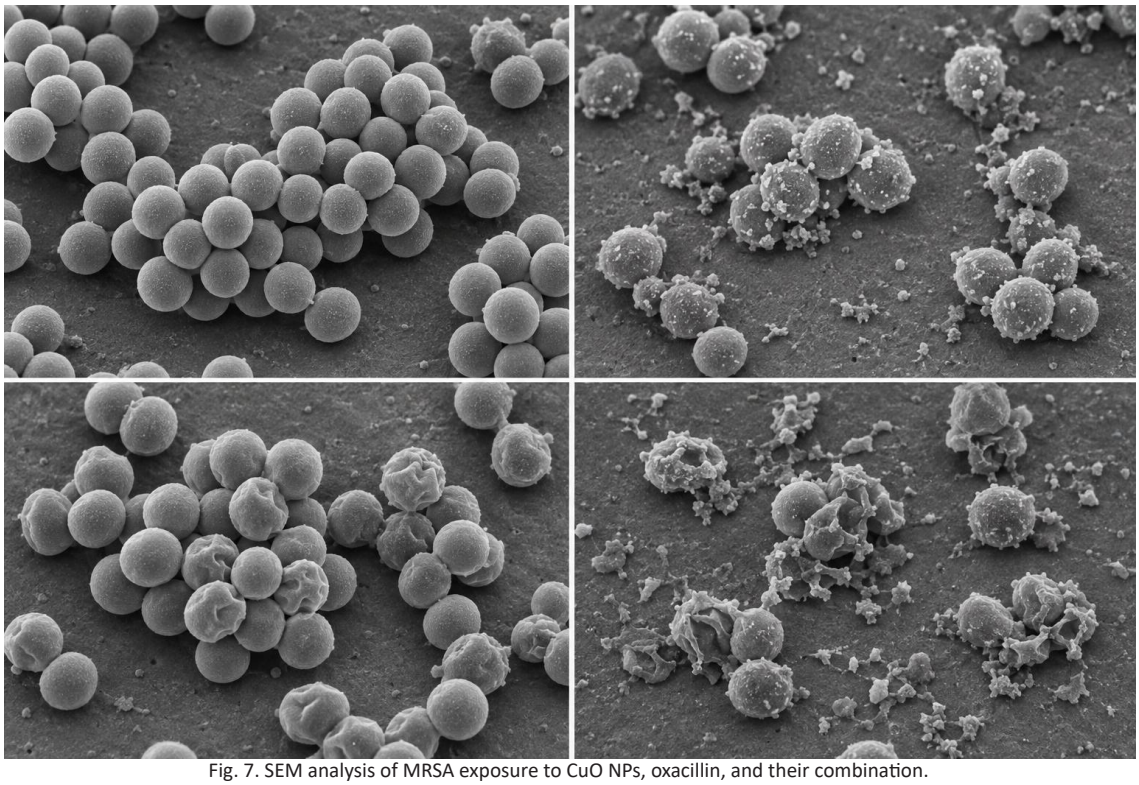


Fig. 7. SEM analysis of MRSA exposure to CuO NPs, oxacillin, and their combination.

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distribution has been confirmed by the known significance of MRSA in skin, soft-tissue, surgical-wound and device-related infections. A large percentage of MRSA samples from wound, pus, and burn samples from Iraq were recovered during an investigation [3]. This high presence of wound-associated isolates could be due to *S. aureus*'s capacity to colonize skin, adhere to damaged skin, evade host defenses, and survive in wound-associated biofilms. It is important to note that the differences in the distribution of isolates are likely to be caused by variations in sampling strategies for the clinical specimens, the patient population in the hospitals, the infection-control practices, the MRSA lineages, and inclusion criteria for the specimens in the different studies [3, 20].

Biofilm formation was seen as prominent with 56.7% isolates as strong biofilm producers and other 33.3% as moderate biofilm producers. Therefore, only 10.0% had a weak biofilm phenotype. The results from these observations confirm the biological relevance of the incorporation of antibiofilm endpoints in this current study. Hamad [20] reported that all *S. aureus* isolates obtained from Erbil were biofilm producers, but the percentages of strong and moderate MRSA biofilm producers were different, as compared to the present study. Likewise, Ali and Riaz [21] also reported significant biofilm-forming ability in clinical MRSA isolates and that the biofilm-associated phenotypes were associated with higher levels of antimicrobial resistance. Similarly, Rimi et al. [22] also reported that MRSA isolates could form extensive biofilms and exhibit multidrug resistance. The differences in the numbers of strong biofilm producers are biologically plausible due to the fact that biofilm formation varies from one producer strain to another, and is affected by ica genes, the regulation of agr, surface adhesins, production of extracellular DNA, nutrient composition, growth conditions, length of incubation, the type of phenotypic assay and the optical density cut-off used. However, recent clinical data highlight that biofilm MRSA are a unique therapeutic problem because of biofilm physiology is heterogeneous and the antimicrobial susceptibility is lower than of planktonic MRSA [23].

The baseline susceptibility profile showed highly resistant to penicillin and cefoxitin, which is consistent with methicillin-resistant phenotype. Some high resistances also were evident to gentamicin (43.3%), tetracycline (40.0%), and trimethoprim–sulfamethoxazole (23.3%), but low resistances to erythromycin (76.7%) and clindamycin (63.3%) and ciprofloxacin (60.0%). No isolates were found resistant to linezolid and vancomycin. The results are in agreement with the Iraqi results, indicating high resistance of MRSA isolates towards Penicillin, Cefoxitin, Erythromycin, Gentamicin, Tetracycline and other classes of antimicrobial agents [3]. High resistance to penicillin and macrolides and no vancomycin resistance were also reported by Hamad [20]. In addition, recent studies have shown that MRSA biofilms are often multidrug resistant phenotypes [21-23]. The lack of resistance to vancomycin and linezolid in this set of isolates should not be viewed as a general phenomenon. However, recent Iraqi studies have reported MRSA with very high MICs for these drugs [24]. Variations in susceptibility between studies may relate to antibiotic prescribing pressure, hospital clone structure, SCCmec type, carriage of resistance genes, antimicrobial stewardship (in hospital), geographic variation and the method used to determine susceptibility (*i.e.*, disk diffusion, automated testing, broth microdilution or other MIC-based method).

The results of physicochemical characterization showed that CuO was monoclinic and had a crystallite size of 24.6 ± 1.9 nm, according to XRD pattern and a diameter of 31.8 ± 6.7 nm, according to TEM. The hydrodynamic diameter was 68.5 ± 5.4 nm, with a PDI of 0.231 ± 0.028 and zeta potential of −21.6 ± 1.7 mV. The difference between the TEM and hydrodynamic dimensions can be expected due to the fact that the TEM is measuring dried primary particles, while the dynamic light scattering is measuring the solvated scattering entity, which is sensitive to aggregation of the nanoparticles. The XRD data obtained in the recent CuO studies also showed monoclinic crystallinity but showed different crystallite and particle sizes depending on the synthesis conditions [11, 25-27]. Some recent preparations of CuO were reported as having primary dimensions ranging from different nanoscales. For instance, Muslim and Naji reported a value near 15.6 nm for the primary dimension (crystallite size) [25] while other recent preparations indicated primary dimensions in other nanoscales [11, 26]. Nucleation, crystal growth, aggregation, zeta potential and optical behavior can vary with

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precursor concentration, pH, reducing/capping agents, calcination temperature, synthesis route, sonication, ionic strength, and surface functionalization. Intrinsic antibacterial activity of CuO NPs was indicated by a median MIC of 128 µg/mL, and the most remarkable effect of the CuO NPs was when it was used in combination with conventional antibiotics. The Oxacillin MIC was reduced from 64 (32–128) to 16 (8–32) µg/mL with a 4-fold median reduction ($P<0.001$). Each antibiotic (ciprofloxacin, gentamicin, doxycycline and vancomycin) exhibited a median MIC reduction of about twofold. These results indicate that CuO NPs do not have a consistent effect on reducing the MIC, but rather have an antibiotic-potentiating effect. Similarly, El-Sherbiny et al. [16] showed that CuO NPs were able to increase the activity of antibiotics against multidrug-resistant *S. aureus*, especially for a β-lactam, cefoxitin. Shoukani et al. [28] have reported improved anti-*S. aureus* activity when using PEG-coated CuO nanostructures. Several-fold increase in antibacterial activity has also been reported in other combination studies which utilize CuO [19]. These studies provide evidence that membrane injury and disruption of cellular homeostasis in bacteria can make them more susceptible to sub-optimal antibacterial agents when exposed to CuO. The checkerboard findings offer more quantitative evidence of this interaction. The antimicrobial combinations, CuO–oxacillin and CuO–ciprofloxacin, had the lowest median FICI values (0.38) and the most synergy (66.7%), followed by CuO–gentamicin (40.0%), CuO–doxycycline (30.0%) and CuO–vancomycin (20.0%). Importantly, there was no antagonistic interaction. It is biologically interesting that the oxacillin interaction is due mainly to altered affinity of β-lactam targets. Such membrane perturbations, oxidative stress, protein damage, and disruption of bacterial homeostasis may result in an additive physiological disadvantage of cell-wall inhibition to bacterial resistance, but without a genetic change to the mecA gene. The interaction between the strong β-lactam and other antibiotic is in accordance with El-Sherbiny et al. [16], who found strong synergy between CuO and cefoxitin. Other studies have reported significant interactions with the aminoglycosides and fluoroquinolones [19, 28, 29].

The time–kill analysis confirmed the checkerboard results. No change was observed for untreated organisms, while CuO and oxacillin monotherapies caused reduction of 1.42 and 1.75 $\log^{10}$ CFU/mL at 24 h, respectively. Combined exposure gave a 3.62-$\log^{10}$ reduction from baseline with a 2.21-$\log^{10}$ advantage over oxacillin alone, meeting the criterion for bactericidal activity and synergy. This observation is especially relevant since there is no observation of whether enhanced activity continues over time when it is done with checkerboard FICI. El-Sherbiny et al. [16] also noted significant reduction in MRSA viable counts for CuO–antibiotic combinations, which was related to time. The combination studies using CuO have been shown the enhancement ≥3-log10 killing of bacteria when compared to the individual treatments [29]. Therefore, the time – kill information is orthogonal evidence that it is not just a result of MIC dilution boundaries. The results of the antibiofilm tests are a further extension of the planktonic susceptibility results. CuO alone had a 34.7% reduction in biofilm biomass and oxacillin alone had a 23.9% reduction, which combination resulted in a significantly greater 65.2% reduction ($P<0.001$). Likewise, the combined treatment reduced viable biofilm counts by 2.70 $\log^{10}$, whereas the CuO and oxacillin treatments reduced counts by 0.81 and 0.55 $\log^{10}$, respectively. The results are in good agreement with the recent reports showing antibiofilm activity of CuO-based materials against *S. aureus* and other resistant microorganisms [17-19]. They also align with other experimental findings, indicating synergistic effects between antibiotics and biofilm disrupting agents compared to their individual use to eradicate biofilm [30]. However, others have found a dose-dependent effect or less ability to inhibit the formation of biofilms. Differences may occur due to different maturity of the biofilm, biofilm matrix composition, production of polysaccharide intercellular adhesin by different strains, levels of protein and extracellular-DNA, penetration of nanoparticles, and assay of biomass, metabolic activity, or viable cells. In particular, the amount of biomass retained is measured by the level of crystal violet. This also cannot distinguish between viable and nonviable bacteria, and for this reason, the percentage biomass reduction does not necessarily correspond to the percentage CFU reduction.

The intracellular ROS level was elevated by 2.48-fold, membrane permeability by 2.31-fold, and leakage of extracellular proteins by 2.06-fold above the control levels in the combination treatment.

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These changes were larger than those resulting from the addition of either CuO or oxacillin. Modern evidence has confirmed that the effects of oxidative stress and membrane disruption are the most important mechanisms through which CuO displays its antibacterial activity [12]. The copper ions and surfaces of nanoparticles may disrupt the redox balance, impair membrane lipids and proteins, alter permeability and disrupt intracellular macromolecules. The higher protein leakage seen here corresponds to disruption of envelope integrity, which could be complemented by structural information based on SEM imaging. The shrinkages of cells and the inhibition of biofilm were also correlated to the synergistic antibacterial activity of CuO in the study [19]. Additionally, other recent investigations of anti-MRSA agents have correlated bactericidal activity with damage to the cell wall, cell form and leakage of intracellular proteins [31]. However, the fluorescence changes of ROS should be taken cautiously as the nanoparticles can influence the fluorescence by adsorption, scattering, quenching, and/or direct oxidation of the probe. Mechanistic attribution would be strengthened by orthogonal ROS measurements, copper-ion controls, antioxidant-rescue experiments, and membrane-potential assays.

The results from the HaCaT viability assays offer a welcome counterpoint to the results of antimicrobial activity. After oxacillin exposure, cell viability was 96.1%, 86.8% after CuO exposure and 82.6% after combined CuO–oxacillin exposure. Hence, the combination had relatively high viability for these conditions, and was found to be a significant reduction from untreated cells for these conditions. El-Sherbiny et al. [16] reported excellent cell compatibility at lower CuO concentrations, while Shoukani et al. [28] reported around 88% cell viability with a formulation of the antibiotic ciprofloxacin–PEG in another mammalian cell model. The cytotoxicity differences may be due to CuO concentration, exposure time, Cu ion dissolution, cell lineage, serum conditions, cell assay methodology, and coating, aggregation, and particle size. Therefore, the present viability result should be considered as preliminary cytocompatibility and not as proof of safety. Recent literature on nanoparticles has stressed the need to enhance the therapeutic window by regulating the dosage and surface modification [28, 32].

Collectively, the results suggest that CuO NPs possess intrinsic anti-bacterial and anti-biofilm properties and also can enhance the activity of conventional antibiotics against MRSA. This specific CuO–oxacillin interaction, which is corroborated by separate MIC reduction, FICI, time–kill, biofilm suppression, and membrane/oxidative endpoints, yields a logical mechanistic signal, and is not dependent on any single susceptibility test. However, the interactions between nanoparticles and antibiotics are still very formulation- and strain-specific [15, 33]. Validation through translation will need to be validated with a larger genetically diverse MRSA collection, replicate data at the level of the individual isolates, standardized batches of nanoparticles, dose–response cytotoxicity and hemolysis and primary-cell testing, pharmacokinetic evaluation, and relevant in-vivo infection models. In addition, new evidence has shown that chronic sublethal exposure to nanoparticles can change the physiology of bacteria and the sensitivity to antibiotics, which highlights the need to assess adaptive responses, rather than simply acute antibacterial activity [34]. Based on these results, CuO–antibiotic combinations represent a promising experimental approach, but need to be tested further before being used as an alternative for existing anti-MRSA drugs.

CONCLUSION

The findings of this study illustrated that CuO NPs possess a great potential of increasing the antibiotic activity of the conventional antibiotics against MRSA. The combination of CuO NPs and oxacillin showed the highest synergistic effect; the antibiotic MIC was significantly lowered; the FICI was below 0.5; and bactericidal effect was observed in the time-kill study among all the combinations tested. Combination treatment also effectively decreased biofilm biomass and viable bacterial counts, and enhanced generation of ROS, membrane permeability and protein leakage, which are related to oxidative and membrane-disruptive mechanisms. The preliminary findings related to cytocompatibility were promising, but more dose–response, toxicity and in vivo studies are needed. In conclusion, CuO NPs have been shown to be a viable antibiotic-adjuvant approach for enhancing the antimicrobial activity of MRSA, a bacterium that is resistant to multiple antibiotics and biofilms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Meštrović T, Ikuta KS, Swetschinski L, Gray A, Aguilar GR, Han C, et al. The burden of bacterial antimicrobial resistance in Croatia in 2019: a country-level systematic analysis. Croat Med J. 2023;64(4):272-283.
2. Muhammed H, Nemmar A. A Comprehensive Review on Copper Oxide Nanoparticles: Physicochemical Parameters, Pharmacological Potential and Pathophysiological Effects. International Journal of Nanomedicine. 2026;Volume 21:1-35.
3. Study on the prevalence of methicillin-resistant Staphylococcus aureus infection, antibiotic resistance pattern, biofilms genes, and antibiotic resistance genes from clinical samples. Archives of Razi Institute. 2023:923-928.
4. Awad A, Hassoon S, Ali H, Mohammad S. CULTURAL AND BIOCHEMICAL IDENTIFICATION OF ANTIBIOTIC-RESISTANT BACTERIA IN THE EARS OF DOGS AND PATIENTS WITH OTITIS EXTERNA. Archives of Veterinary Medicine. 2025;18(1).
5. Goudarzi M, Nasiri MJ, Dadashi M, Goudarzi H. Hidden reservoirs in clinical settings: Clonal dissemination and virulence profile of multidrug-resistant Staphylococcus aureus from healthcare workers' mobile phones. Acta Microbiol Immunol Hung. 2026;73(3):317-329.
6. Ali Alghamdi B, Al-Johani I, Al-Shamrani JM, Musamed Alshamrani H, Al-Otaibi BG, Almazmomi K, et al. Antimicrobial resistance in methicillin-resistant staphylococcus aureus. Saudi J Biol Sci. 2023;30(4):103604.
7. Peng Q, Tang X, Dong W, Sun N, Yuan W. A Review of Biofilm Formation of Staphylococcus aureus and Its Regulation Mechanism. Antibiotics. 2022;12(1):12.
8. Guo H, Tong Y, Cheng J, Abbas Z, Li Z, Wang J, et al. Biofilm and Small Colony Variants—An Update on Staphylococcus aureus Strategies toward Drug Resistance. Int J Mol Sci. 2022;23(3):1241.
9. Jalalifar S, Mirzaei R, Motallebirad T, Razavi S, Talebi M. The Emerging Role of Probiotics and their Derivatives against Biofilm-Producing MRSA: A Scoping Review. BioMed Research International. 2022;2022(1).
10. Essa SH, Mohammad SQ, Kadhum DA, Jalil IS. Effectiveness of silver nanoparticles as antibacterial agents with natural wound healing cream with extracted Aloe vera gel. Regulatory Mechanisms in Biosystems. 2024;15(4):821-825.
11. Gudkov SV, Burmistrov DE, Fomina PA, Validov SZ, Kozlov VA. Antibacterial Properties of Copper Oxide Nanoparticles (Review). Int J Mol Sci. 2024;25(21):11563.
12. Devaraji M, Thanikachalam PV, Elumalai K. The potential of copper oxide nanoparticles in nanomedicine: A comprehensive review. Biotechnology Notes. 2024;5:80-99.
13. Gebreslassie YT, Gebremeskel FG. Green and cost-effective biofabrication of copper oxide nanoparticles: Exploring antimicrobial and anticancer applications. Biotechnology Reports. 2024;41:e00828.
14. Meghana S, Kabra P, Chakraborty S, Padmavathy N. Understanding the pathway of antibacterial activity of copper oxide nanoparticles. RSC Advances. 2015;5(16):12293-12299.
15. Abdullah, Jamil T, Atif M, Khalid S, Metwally K, Yahya G, et al. Recent Advances in the Development of Metal/Metal Oxide Nanoparticle and Antibiotic Conjugates (MNP–Antibiotics) to Address Antibiotic Resistance: Review and Perspective. Int J Mol Sci. 2024;25(16):8915.
16. El-Sherbiny GM, Kalaba MH, Sharaf MH, Moghannem SA, Radwan AA, Askar AA, et al. Biogenic synthesis of CuO-NPs as nanotherapeutics approaches to overcome multidrug-resistant Staphylococcus aureus (MDRSA). Artificial Cells, Nanomedicine, and Biotechnology. 2022;50(1):260-274.
17. Siddique MH, Sadia M, Muzammil S, Saqalein M, Ashraf A, Hayat S, et al. Biofabrication of copper oxide nanoparticles using Dalbergia sisso leaf extract for antibacterial, antibiofilm and antioxidant activities. Sci Rep. 2024;14(1).
18. Shehabeldine AM, Amin BH, Hagras FA, Ramadan AA, Kamel MR, Ahmed MA, et al. Potential Antimicrobial and Antibiofilm Properties of Copper Oxide Nanoparticles: Time-Kill Kinetic Essay and Ultrastructure of Pathogenic Bacterial Cells. Applied Biochemistry and Biotechnology. 2022;195(1):467-485.
19. Srivastava P, Kim Y, Cho H, Kim K-s. Synergistic Action between Copper Oxide (CuO) Nanoparticles and Anthraquinone-2-Carboxylic Acid (AQ) against Staphylococcus aureus. Journal of Composites Science. 2023;7(4):135.
20. Ahmad P. PHENOTYPIC AND MOLECULAR DETECTION OF BIOFILM FORMATION IN METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM DIFFERENT CLINICAL SOURCE IN ERBIL CITY. Mediterr J Hematol Infect Dis. 2023;15(1):e2023016.
21. Ali A, Riaz S. Emerging threats of high biofilm formation and antibiotic resistance in clinical methicillin-resistant Staphylococcus aureus (MRSA) isolates from Pakistan. Infection, Genetics and Evolution. 2024;121:105592.
22. Rimi SS, Ashraf MN, Sigma SH, Ahammed MT, Siddique MP, Zinnah MA, et al. Biofilm formation, agr typing and antibiotic resistance pattern in methicillin-resistant Staphylococcus aureus isolated from hospital environments. PLoS One. 2024;19(8):e0308282.
23. Kaushik A, Kest H, Sood M, Steussy B, Thieman C, Gupta S. Biofilm Producing Methicillin-Resistant Staphylococcus aureus (MRSA) Infections in Humans: Clinical Implications and Management. Pathogens. 2024;13(1):76.
24. Al-Zehhawi PAM, Mohammed Jaddoa NT. Linezolid versus vancomycin in vitro activity against methicillin-resistant Staphylococcus aureus biofilms. Microb Pathog. 2025;201:107371.
25. Muslim AM, Naji IS. Green Synthesis of CuO Nanoparticles Mediated Rhazya Stricta Plant Leaves Extract Characterization and Evaluation of their Antibacterial and Anticancer Activity (in vitro Study). Iraqi Journal of Physics. 2024;22(3):93-105.
26. Ul-Hamid A, Dafalla H, Hakeem AS, Haider A, Ikram M. In-Vitro Catalytic and Antibacterial Potential of Green Synthesized CuO Nanoparticles against Prevalent Multiple Drug Resistant Bovine Mastitogen Staphylococcus aureus. Int J Mol Sci. 2022;23(4):2335.
27. Pawar SM, Patil SS, Sonawane KD, More VB, Patil PS. Hydrothermally synthesized copper oxide nanoparticles: Rietveld analysis and antimicrobial studies. Surfaces and Interfaces. 2024;51:104598.
28. Ibne Shoukani H, Nisa S, Bibi Y, Ishfaq A, Ali A, Alharthi S, et al. Green synthesis of polyethylene glycol coated, ciprofloxacin loaded CuO nanoparticles and its antibacterial activity against Staphylococcus aureus. Sci Rep. 2024;14(1).
29. Abu-Hussien SH, King AL, Khan MA. Box-Behnken optimized copper oxide nanoparticles from Thymus vulgaris potentiate efficacy against multidrug-resistant bacterial pathogens and exhibit anticancer activity. Bioresources and Bioprocessing. 2026;13(1).
30. Deusenbery C, Carneiro O, Oberkfell C, Shukla A. Synergy of Antibiotics and Antibiofilm Agents against Methicillin-Resistant Staphylococcus aureus Biofilms. ACS Infectious Diseases. 2023;9(10):1949-1963.
31. Chen Q, Dong Z, Yao X, Sun H, Pan X, Liu J, et al. Bactericidal and biofilm eradication efficacy of a fluorinated benzimidazole derivative, TFBZ, against methicillin-resistant Staphylococcus aureus. Front Pharmacol. 2024;15.
32. Attarilar S, Yang J, Ebrahimi M, Wang Q, Liu J, Tang Y, et al. The Toxicity Phenomenon and the Related Occurrence in Metal and Metal Oxide Nanoparticles: A Brief Review From the Biomedical Perspective. Frontiers in Bioengineering and Biotechnology. 2020;8.
33. Jiang Y, Tang X, Wang A, Yang H, Jiang X, Zhang Y, et al. Shikonin-copper coordination nanoparticles for enhanced antibacterial and antibiofilm activity against Staphylococcus aureus. Sci Rep. 2025;15(1).
34. Liu X, Xu Y, Zhang L, Yu Y, Cui J, Yu Z, et al. Chronic CuO Nanoparticles Exposure Enhances Bacterial Antibiotic Sensitivity and Attenuates Bacterial Pathogenicity. ACS Sustainable Chemistry and Engineering. 2025;13(41):17575-17585.

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