Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Studies on Methicillin resistant Staph aureus (MRSA) infection resistance for antibiotic using nanoparticles and essential oil

M. A. [1,2] , S. H. A.[1], N. E.[3], I. M. E[4] , and A. A. A.[1]

[1] Department of Botany and Microbiology, Faculty of Science, Benha University, Benha, Egypt.

[2] security forces laboratories in Giza - Ministry of Interior

[3] Department of Tropical, Faculty of Medicine, Tanta University, Tanta, Egypt.

[4] the R&D Department of Egyptian company for blood transfusion services (EGyBLOOD-VACSERA), Cairo, Egypt.

*Corresponding Authors: Marwa Mohamed Ahmed Amer, marwa_amer1012@yahoo.com

ABSTRACT

Background:Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that has developed resistance to nearly all β-lactam antibiotics, posing serious treatment challenges. Recent studies have shown that metal oxide nanoparticles, particularly copper oxide (CuO), possess strong antimicrobial properties against various pathogens, including *S. aureus*. Additionally, rosemary and its essential oil are commonly used in the food industry due to their pleasant flavor and potent antioxidant effects. Therefore, this study aims to improve the effectiveness of antibiotic treatments for MRSA infections through the use of copper oxide nanoparticles in combination with natural rosemary oil.

Patients: This study was conducted on fifty blood samples collected from Patients infected with methicillin resistant *Staphylococcus aureus* (MRSA) were obtained from clinical specimens. Patient samples will be recruited from ICUs, Tanta University hospital, from October 2020 to May 2023.

Methods: The samples were inoculated into blood agar and MacConkey agar, then incubated both aerobically at 37°c for 24hours.

Results: various combinations to nanoparticles and essential oil showed an enhanced activity to different antibiotic at incubation for 24 h compared to antibiotics individually. Where, the combination of copper oxide 50% concentration with Rosemary essential oil 60% concentration (inhibition zone mean 15.14 mm) showed improved to inhibition zone for different antibiotics to MRSA strains.

Conclusions: copper oxide nanoparticles have good effect for MRSA at high concentration. Also, zinc oxide nanoparticles showed good effect at low concentration but less than copper oxide nanoparticles .

Keywords: MRSA, Methicillin resistant *Staph aureus*; ICUs, Intensive care units.

Submit Date 2025-05-27
Accept Date 2025-06-16
Published Date 2025-07-01
Doi: 10.21608/jbes.2025.389805.1020

1. INTRODUTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a variant of *S. aureus* that has evolved, through natural selection, to resist the full spectrum of penicillins and related antimicrobial agents (1).

In recent years, the management of bacterial infections has become increasingly challenging due to the growing resistance to conventional antibiotics. One of the major obstacles lies in the treatment of intracellular infections, as many antibiotics are unable to effectively penetrate cellular membranes, resulting in limited therapeutic efficacy within host cells. This highlights the urgent need to explore alternative therapeutic strategies (2).

Mineral-based nanoparticles, particularly metal oxides, have demonstrated outstanding antimicrobial properties and selective toxicity toward microbial systems. These features underscore their promise in nanomedicine applications, including surgical tools, diagnostic technologies, targeted therapies, and antimicrobial treatments. Among the various metal nanoparticles, zinc oxide and copper oxide have received considerable attention for their potent antibacterial activities. Interestingly, methicillin-resistant *Streptococcus pyogenes* and *Staphylococcus epidermidis* have also exhibited notable sensitivity, ranking closely after MRSA in susceptibility (3).

Nanoparticles derived from metals and metal oxides have been widely researched for their broad-spectrum antibacterial effects against numerous pathogenic strains (4).

Furthermore, the use of dual antimicrobial strategies combining two antibiotics or antimicrobial agents has gained traction due to their enhanced efficacy. The concept of synergistic treatment has been thoroughly explored and offers several benefits compared to single agent therapy (5).

Combination approaches not only enhance microbial eradication but also reduce the risk of developing resistance. Additionally, they often decrease the required dosage and duration of treatment, potentially minimizing toxicity to host tissues (6).

The primary objective of this study was to assess the antimicrobial potential of individual metal oxide nanoparticles and to evaluate whether their combinations could produce a synergistic enhancement in antimicrobial action against clinically significant pathogens.

2. MATERIALS AND METHODS

This study was conducted on fifty blood samples collected from Patients infected with MRSA were obtained from clinical specimens. Patient samples will be recruited from ICUs, Tanta University hospital, from October 2020 to May 2023.

All isolates for all assays were grown overnight in MacConkey and blood agar at 37 ◦C in an aerobic environment. Then classic identifications to all plats by morphological diagnosis, gram stain and chemical diagnosis by catalase and coagulase reaction.

Followed by examination the samples by Vitec compact 2 according to manufacturer's directions to gram positive card.

By disc diffusion method; We determined of antimicrobial activity and inhibition zone for Rosmary essential oil and Cupper oxid nanoparticles suspension with different concentration (10, 20, 30, 40, 50 and 60%). The diameter of the inhibition zones were measured in millimeters. Nanoparticles, its used in the investigation, copper oxide (Cuo) were purchased from NanoTech Egypt for Photo-Electronics.

Statistical analysis : Data were presented as mean ± standard deviation, frequencies, and percentages where applicable. The Mann–Whitney U and Kruskal–Wallis tests were applied for non-parametric comparisons of continuous variables. Categorical variables were analyzed using the Paired Samples Test, and 95% confidence intervals were calculated. A p-value less than 0.05 was considered statistically significant. All analyses were conducted using SPSS version 29.0.2.0 for Windows. (Knapp, 2017).

3. RESULTS

Figure (1) Shows an average of inhibition zone for copper oxide nanoparticles six different concentration (10, 20, 30, 40, 50, and 60%)with 50 MRSA samples :copper oxide nanoparticles 10% concentration with 12.86 mm, copper oxide nanoparticles 20% concentration with 12.86 mm, copper oxide nanoparticles 30% concentration with 12.86 mm, copper oxide nanoparticles 40% concentration with 12.86 mm, copper oxide nanoparticles 50% concentration with 15.46 mm, and copper oxide nanoparticles 60% concentration with 12.86 mm.

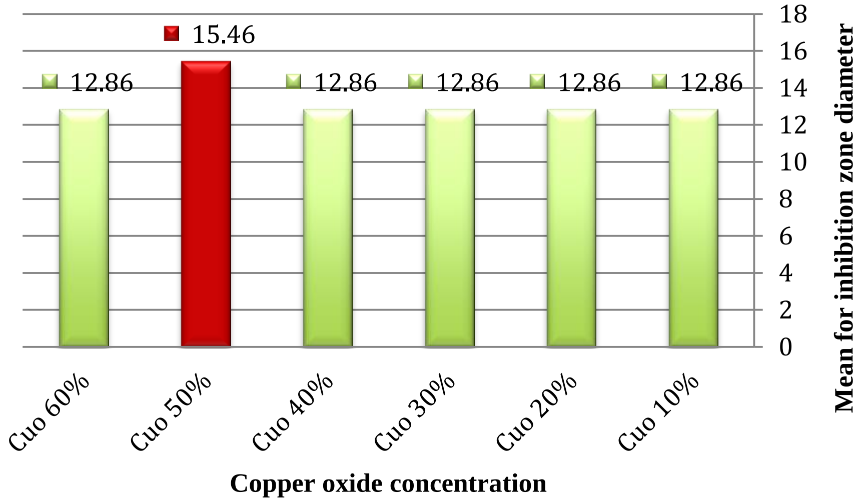

Figure (1): Means of inhibition zone diameter mean of Copper oxide nanoparticles concentration for Methicillin-resistant *Staphylococcus aureus* (MRSA) activity.

Table(1): Comparison between Copper oxide nanoparticles (Cuo) 50% concentrations and Rosmary essential oil (Ros.) 60% concentrations with MRSA through clear zone, and antibiotic susceptibility

Variable	Antibiotics susceptibility (N = 50) Inhibition zone diameters (mm) Mean ± SD	Antibiotics susceptibility combine with Cuo 50% and Ros 60% (N = 50) Inhibition zone diameters (mm) Mean ± SD	P=value
VA-30	9.5 ± 5.175	15.42 ± 4.768	0.0001
Fox-30	5.22 ± 5.14	12.22 ± 4.510	0.0001
LZD-30	12.78 ± 5.44	19.76 ± 6.489	0.0001
Lev-5	9.26 ± 5.68	15.70 ± 5.45	0.0001
CEF-30	13.04 ± 5.99	19.54 ± 6.879	0.0001
AK-30	11.38 ± 3.136	16.52 ± 4.161	0.0001

**p-value > 0.05 not significant*

Cuo, Copper oxide nanoparticles; Ros, Rosmary essential oil ; VA-30, Vancomycine 30 mg ; Fox-30, Cefoxitin 30 mg ; LZD-30, Linezolid 30 mg ; Lev-5, Levofloxacin 5 mg; CEF-30, Cefepime 30 mg ; AK-30, Amikacin 30 mg.

Table (1) show the mean of antibiotic susceptibility for 50 MRSA samples of VA-30 (9.50 vs 15.42 mm; P= 0.0001), Fox-30 (5.22 vs 12.22; P= 0.0001), LZD-30 (12.78 vs 19.76; P= 0.0001), Lev-5 (9.26 vs 15.70; P= 0.0001), CEF-30 (13.04 vs 19.54; P= 0.0001) , and Ak-30 (11.38 vs 16.52; P= 0.0001) respctivly, were very high significant in antibiotics susceptibility combine with Cuo 50% and Ros 60% than antibiotics susceptibility only.

Table (2): Comparison between (Rosmary essential oil 60% concentrations and Copper oxide nanoparticles 50% concentrations) and (Rosmary essential oil 60% concentrations and Copper oxide nanoparticles 10% concentrations) for MRSA through clear zone means.

Combine between Cuo 50% and Ros 60% (N ═ 50) Inhibition zone diameters (mm) Mean ± SD	Combine between Cuo 10% and Ros 60% (N ═ 50) Inhibition zone diameters (mm) Mean ± SD	P=value
15.14 ± 3.458	13.50 ± 3.157	0.0001

p-value > 0.05 not significant

Data present as mean ± SD, p- value comparison was done between inhibition zone at (Copper oxide nanoparticles 50% concentration and Rosmary essential oil 60% concentration) and (Copper oxide nanoparticles 10% concentration, and Rosmary essential oil 60% concentration) for 50 MRSA samples, Cuo; copper oxide nanoparticles, Ros; rosmary essential oil.

Table (2): show the comparsion between the mean of inhibition zone for 50 MRSA samples combine with (Copper oxide nanoparticles 50% concentration and Rosmary essential oil 60% concentrations) and the mean of inhibition zone for 50 MRSA samples combine with (Copper oxide nanoparticles 10% concentration and Rosmary essential oil 60% concentrations) were lower significant (15.14 vs 13.50; P= 0.0001).

4. Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) displays resistance to all β-lactam antibiotics due to the presence of the mecA gene, which facilitates the production of penicillin-binding protein 2a (PBP2a) (7). Growing concerns over multidrug resistance in*S. aureus* strains have shifted research towards alternative therapeutic strategies.

According to Allaker (8), the likelihood of resistance development against metal nanoparticles is significantly lower than that for conventional antibiotics, as metals act on multiple microbial targets simultaneously. In this study, copper oxide nanoparticles and rosemary essential oil demonstrated notable antibacterial effects against MRSA. The average inhibition zone was 15.46 mm for copper oxide and 7.78 mm for rosemary oil. Copper oxide nanoparticles produced a zone of 12.86 mm even at the lowest tested concentration, outperforming some conventional antibiotics (9).

The antimicrobial action of nanoparticles is strongly influenced by their small size, enabling interaction with bacterial membranes and internal components (10). Their multi-targeted mechanism involves disrupting membrane integrity, interfering with respiratory processes, and interacting with DNA and proteins (11). Additionally, essential oils, especially those from rosemary, are more effective against Gram-positive bacteria (12). Rosemary oil exhibited inhibition zones of 7.78 mm at high concentration and 5.88 mm at low concentration.

Issabeagloo et al. (12) also noted that essential oil activity increases with concentration and can be comparable to vancomycin. Among several oils, rosemary ranked third after galbanum and fennel in efficacy. Similarly, Nakagawa et al. (13) found MIC values for rosemary extracts between 15.6–62.5 µg/mL for planktonic MRSA and 45–250 µg/mL for biofilms. Santoyo et al. (14) identified camphor, borneol, and verbenone as the most potent active components in rosemary extract.

Nanoparticles’ effectiveness is influenced by properties like surface charge and hydrophobicity (15). Smaller particles (1–10 nm) exhibit stronger antimicrobial activity, often by binding to cell membranes and generating reactive oxygen species (ROS), which disrupt cell function and DNA replication (16).

The combination of copper oxide nanoparticles with rosemary oil enhanced the antibacterial activity of antibiotics after 24 hours of incubation. Specifically, copper oxide at 50% with rosemary oil at 60% produced greater inhibition zones against MRSA than individual treatments. This combination was also more effective than copper oxide with zinc oxide nanoparticles. Although the exact mechanisms behind these combinations require further investigation, differences in chemical reactivity and target specificity likely contribute to their efficacy (17).

Nanoparticle behavior in suspension includes both direct interaction with bacterial membranes and indirect action via ion release. These metal ions, such as Cu^{2+}, can disrupt membranes, denature proteins, and induce cell death (18). Furthermore, copper ions generate

ROS that impair DNA synthesis and protein formation (19).

5. Conclusion

Copper nanoparticles not only damage membranes and genetic material through ROS production but also interfere with metabolic pathways. Hence, minimizing nanoparticle size is essential for maximizing antimicrobial performance (6).

Authors' Contributions

M.A. (Amer, M.M.A.): Conceptualization, methodology, data collection, and writing—original draft.
S.H.A. (Abdel-Aziz, S.H.): Supervision, validation, and editing of the final manuscript.
N.E. (Nadia Elwan): Literature review and preparation of figures/tables.
I.M.E. (I. M. Elkalamawy): Experimental design, statistical analysis, and technical support.
A.A.A. (Attia A. Attia): Project supervision, funding acquisition, and final manuscript approval.

6. References

(1) Leas, B.,F.; Pegues, D., A. and Mull, N., K. (2024): Active Surveillance Culturing of Clostridioides difficile and Multidrug-Resistant Organisms: Methicillin-Resistant Staphylococcus aureus, Carbapenem-Resistant Enterobacterales, and Candida auris: Rapid Response. Agency for Healthcare Research and Quality (US). Bookshelf ID: NBK604049.

(2) Uddin, T., M.; Chakraborty, A., J.; Khusro, A.; Zidan, B., R., M.; Mitra, S.; Emran, T., B.; Dhama, K.; Ripon, M., K., H.; Gajdács, M.; Sahibzada, M., U., K.; Hossain, M., J. and Koirala, N. (2021):Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *J. Infect Public Health*. 14(12): 1750-1766.

(3) Wang, L.;Hu, C. and Shao, L. (2017): The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine.*12(21): 1227–1249.

(4) Joudeh, N. and Linke, D. (2022): Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists.*J Nanobiotechnology*. 20(1):262.

(5) Kong, L.; Zhang, R.; Gong, J.; Wang, H.; Zhai, L.; Dang, D.; Liu, Q.; Zhao, Z. and Tang, B., Z. (2024): Aggregation-induced emission photosensitizer for antibacterial therapy of methicillin-resistant Staphylococcus aureus.*ChemCommun (Camb)*. 60(46):5960-5963.

(6) Raja, F. N.; Worthington, T; de Souza, L. P. L; Hanaei, S. B. ;and Martin, R. A. (2022): Synergistic antimicrobial metal oxide-doped phosphate glasses; a potential strategy to reduce antimicrobial resistance and host cell toxicity *ACS Biomater.Sci. Eng.* 8 1193–9

(7) Hepziba, E., R.; Soesanto, S. and Widyarman, A. S. (2023): Antibiofilm of Arumanis Mango Leaves (Mangifera indica L.) Ethanol Extract Against Staphylococcus aureus in vitro. *J. Indones. Dent. Assoc.* 5(2):99−105.

(8) Allaker, R. (2010): The Use of Nanoparticles to Control Oral Biofilm Formation. *J. Dent. Re*., 89(11):1175-1186.

(9) Niaz, N.; Bano, A.; Fasim, F.; Kausar, R.; Khan, B., A.; Zafar, N.; Khan, S. and Uzair, B. (2018): Synthesis, characterization and evaluation of antibacterial activity of copper oxide nanoparticles against clinical strains of *Staphylococcus aureus* . *Pak. J. Pharm. Sci*. 4(31):1549-1553.

(10) Raja, F., N., S.; Worthington, T.; and Martin, R., A. (2023): The antimicrobial efficacy of copper, cobalt, zinc and silver nanoparticles: alone and in combination .*Biomed. Mater.* 18 : 045003

(11) Sánchez-López, E.; Gomes, D.; Esteruelas, G.; Bonilla, L.;Lopez-Machado , A., L.; Galindo, R.;Cano, A.; Espina, M.;Ettcheto, M.;Camins, A.; Silva, A., M.; Durazzo, A.;Santini, A.;Garcia, M., L. and Souto, E., B (2020): Metal-Based Nanoparticles as Antimicrobial Agents: An Overview . *Nanomaterials (Basel).* 10(2):292.

(12) Issabeagloo, E.; Kermanizadeh, P.; Taghizadieh M., and Forughi, R. (2012): Antimicrobial effects of rosemary (*Rosmarinus officinalis L.*) essential oils against *Staphylococcus* spp. *Afr. J. Microbiol*. 6(23): 5039-5042.

(13) Nakagawa S., Hillebrand G.G., and Nunez G.(2020): Rosmarinus o_cinalis L. (Rosemary) Extracts Containing Carnosic Acid and Carnosol are Potent Quorum Sensing Inhibitors of Staphylococcus aureus Virulence. *Antibiotics.* 9(4):14

(14) Santoyo, S.; Cavero, S.; Jaime, L.; Ibañez, E.; Señoráns, F., J. and Reglero, G. (2005): Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction. *J. Food Prot.*, 68(4): 790-795.

(15) Nel, A. E.; Madler, L. and Velegol, D. (2009): Understanding biophysicochemical interactions at the nano-bio interface. *Nat. Mater*., 8(7):543-57.

(16) Claudel , M.; Schwarte, J., V. and Fromm, K., M. (2020): New Antimicrobial Strategies Based on Metal Complexes . *Chemistry* . 2(4): 849-899.

(17) Slavin, Y., N.; Asnis, J.; Häfeli, U., O. and Bach, H. (2017): Metal nanoparticles: understanding the mechanisms behind

antibacterial activity *J. Nanobiotechnol.* 15(65): 1–20.

(18) Ling, L., L.; Schneider, T.; Peoples, A., J.; Spoering A., L.; Engels, I.; Colon, B., P.; Mueller, A.; Schaberle, T., F; Hughes, D., E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V., A.; Cohen, D., R.; Felix, C., R.; Fetterman, K., A.; Millett, W., P.; Nitti, A.,G.; Zullo, A., M.; Chen, C. and Lewis, K.(2015): A new antibiotic kills pathogens without detectable resistance *Nature.* 517(7535) 455–459.

(19) Hulme, J. (2022): Application of Nanomaterials in the Prevention, Detection, and Treatment of Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Pharmaceutics*. 14(4): 805.