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Artículos originales

Evaluation of the Antibacterial Potential of Reduced Graphene Oxide Against Methicillin-Resistant *Staphylococcus aureus*

Evaluación del potencial antibacteriano del óxido de grafeno reducido frente a *Staphylococcus aureus* resistente a la meticilina

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Conflict of interest

The authors declare no conflict of interest.

Resumen

Introducción: *Staphylococcus aureus* es un patógeno oportunista reconocido por causar diversas infecciones clínicas, especialmente en pacientes inmunocomprometidos, y está asociado con altas tasas de morbilidad y resistencia a múltiples antimicrobianos.

Métodos: Se recolectaron 24 muestras clínicas de hisopos provenientes de sitios sospechosos de infección de pacientes en Najaf, Irak. Las muestras se cultivaron en medios selectivos, y el crecimiento bacteriano positivo se observó en 15 muestras 62,5 %. Diez aislamientos fueron identificados como *S. aureus* mediante evaluación macroscópica y microscópica, pruebas bioquímicas y confirmación con el sistema VITEK 2.

Resultados: Las pruebas de susceptibilidad antimicrobiana revelaron que el 70 % de los aislamientos eran resistentes a ceftriaxona. La resistencia a gentamicina, cefalosporinas, eritromicina y tetraciclina se detectó en un 60 % de los aislamientos. Se observó resistencia intermedia al cloranfenicol en un 50 %, mientras que rifampicina y levofloxacino mostraron las tasas más bajas de resistencia 40 %. Un 70 % de los aislamientos presentaron resistencia a múltiples clases de antibióticos (MDR), y el 40 % fueron confirmados como *S. aureus* resistente a metilicina (MRSA). El óxido de grafeno reducido (rGO) no mostró efecto inhibitorio observable frente a *S. aureus* en el ensayo de difusión en agar bajo las condiciones evaluadas.

Conclusión: Estos resultados subrayan la necesidad de desarrollar estrategias terapéuticas innovadoras para combatir la creciente resistencia bacteriana, especialmente dado el fracaso del rGO no modificado en inhibir efectivamente a *S. aureus* en las condiciones experimentales empleadas.

Palabras clave: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, óxido de grafeno reducido, resistencia a múltiples fármacos.

Abstract

Introduction: *Staphylococcus aureus* is recognized for its role in diverse clinical conditions, especially causing severe manifestations in patients with suppressed immune function.

Methods: To evaluate bacterial presence, 24 clinical swab samples were systematically collected from suspected infection sites from patients in Najaf, Iraq. Bacterial culture yielded positive growth in 15 samples 62.5 %, and 10 isolates were confirmed as *S. aureus* through macroscopy, microscopy, biochemical tests, and VITEK 2 identification.

Results: Antibiotic susceptibility tests revealed high resistance rates, with 70 % of isolates resistant to ceftriaxone, followed by gentamicin, cephalosporin, erythromycin, and tetracycline, each at 60 %. Intermediate resistance was observed with chloramphenicol at 50 %, while the lowest rates were noted for rifampin and levofloxacin at 40 %. A significant proportion of the isolates 70 % exhibited resistance to multiple antimicrobial classes, while methicillin resistance was confirmed in 40 % of the *S. aureus* strains. When tested against these isolates, reduced graphene oxide (rGO) showed no inhibitory growth of *S. aureus* using the agar well diffusion assay.

Conclusion: The findings highlight a pressing demand for innovative treatment approaches to address this growing resistance, given the increasing resistance to antibiotics and the failure of unmodified rGO to provide effective bacterial inhibition under the conditions tested.

Keywords: *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, Reduced graphene oxide, Multidrug resistance.

Highlights

S. aureus, particularly Methicillin-resistant *Staphylococcus aureus*, is notorious for its high resistance to multiple antibiotics, creating significant treatment challenges and driving the search for novel antibacterial agents.

In this study, it was demonstrated that unmodified Reduced graphene oxide failed to inhibit the growth of clinical Methicillin-resistant *Staphylococcus aureus* isolates under the tested experimental conditions, providing new insights into its limited efficacy.

These results underscore the urgent need to optimize nanomaterials or explore alternative therapeutic strategies to effectively combat Methicillin-resistant *Staphylococcus aureus*, informing future antimicrobial research, clinical practice, and infection control efforts.

Introduction

The worldwide escalation of antibiotic resistance continues to endanger public health by increasing rates of illness, death, and treatment complications. With the diminishing effectiveness of standard antibiotics, attention has increasingly turned toward alternative antimicrobial solutions. Among these, nanomaterials, due to their distinctive physicochemical features, have gained recognition as potential tools in the fight against drug-resistant microorganisms⁽¹⁾. Among these, reduced graphene oxide (rGO) has gained attention due to its large surface area, electrical conductivity, and modifiable surface chemistry. Several structural characteristics, such as lateral sheet dimensions, surface oxygen groups, and lattice defects, are known to influence its antimicrobial performance⁽²⁾.

Multiple hypotheses have been suggested to elucidate how rGO exerts its antibacterial effects, including mechanical disruption of bacterial membranes by nanosheet edges, induction of oxidative stress through reactive oxygen species generation, and the physical trapping of microbial cells that hinders their metabolic activity and overall viability⁽³⁾. To enhance its bactericidal effect, rGO has been incorporated into nanocomposites with metals like silver, zinc oxide, copper, and iron, resulting in synergistic antimicrobial activity⁽⁴⁾.

S. aureus continues to be a major causative agent of infections in both healthcare settings and the community, primarily due to its wide range of virulence factors. These include cell surface adhesins, the ability to form biofilms, and toxins like Panton-Valentine leukocidin (PVL), which aid in immune system evasion and promote tissue invasion⁽⁵⁾. Genetically, this bacterium employs regulatory systems such as the accessory gene regulator (*agr*) and the staphylococcal cassette chromosome *mec* (*SCCmec*) to coordinate the expression of resistance and virulence factors, enhancing its adaptability and survival in clinical environments⁽⁶⁾.

The primary objective of this research was to assess the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) among clinical isolates and to investigate the antibacterial efficacy of rGO as a potential alternative to traditional antibiotic treatments.

Methods

Specimens collection

Between September and December 2024, twenty-four sterile swab specimens were obtained from patients suspected of having bacterial infections in various healthcare facilities across Najaf Governorate, Iraq. Most patients had a documented history of irregular or non-prescribed antibiotic use. Samples were transported without delay to the Central Health Laboratory using appropriate transport media to maintain specimen viability. Ethical approval for the study was obtained from the Department of Pathological Analysis, Faculty of Science, University of Kufa. The Ethics Committee was fully briefed on the research objectives and methodology, with ongoing oversight provided throughout the study period.

Identification of bacterial isolates

Macroscopic examination:

Each clinical specimen was streaked onto three selective media: blood agar enriched with 5 % blood, Mannitol Salt Agar (MSA), and MacConkey agar (MAC), all obtained from HiMedia, India. After inoculation, the plates were subjected to aerobic incubation at 37°C for 24 hours to enable visible colony development. After that, colonies were examined for characteristic growth patterns, pigmentation, and hemolytic activity to facilitate preliminary bacterial identification.

Microscopic examination:

Smears were prepared from the isolated colonies and stained using the Gram staining technique (Bioanalyse, Turkey). The stained slides were examined under a light microscope to assess bacterial cell morphology, arrangement, and Gram reaction, providing initial clues for bacterial classification⁽⁷⁾.

Biochemical test

To identify *S. aureus* isolates and evaluate associated virulence factors, a series of biochemical tests was performed. Coagulase production was assessed using both slide and tube methods with human plasma, targeting bound and free coagulase, respectively. Catalase and oxidase tests were carried out using commercial diagnostic kits (Bioanalyse, Turkey), while citrate utilization was evaluated on selective media obtained from HiMedia, India. These assays are essential for differentiating *S. aureus* from other staphylococcal species⁽⁸⁾.

Vitek 2 compact system

Further confirmation of bacterial identity was achieved employing the VITEK 2 Compact automated system (bioMérieux, France). Isolated colonies were resuspended in sterile saline solution, and turbidity was adjusted to the required optical density using a DensiCHEK device. A 1.45 mL aliquot of the standardized suspension was transferred into identification cards, which were then inserted into the VITEK instrument. Identification was based on the analysis of biochemical profiles generated by the system⁽⁹⁾.

Antibiotic susceptibility test for *S. aureus*

Briefly, 2–3 colonies from fresh blood agar cultures were suspended in 10 mL of sterile saline. The turbidity of the resulting suspension was visually calibrated to the 0.5 McFarland standard to ensure consistency^(10,11). Following preparation, the inoculum was spread onto Mueller–Hinton agar (MHA) plates (HiMedia, India). Each test was performed in triplicate. Sterile water was used as a negative control, and a standard antibiotic disc served as a positive control to validate the assay. After incubation at 37 °C for 24 hours, inhibition zones were measured manually, and mean values \pm standard deviation (mean \pm SD) were calculated and interpreted according to CLSI 2024 guidelines.

Table 1. Names of antibiotics used in this study.

Class	Antibiotic	Abbreviation	Disc Concentration ($\mu\text{g}/\text{disc}$)	Manufacturer
Aminoglycoside	Gentamicin	CN	10	HiMedia, India
Amphenicol	Chloramphenicol	C	30	HiMedia, India
β -Lactam	Cephalosporin	KF	30	HiMedia, India
β -Lactam	Ceftriaxone	CT-R	30	HiMedia, India
Fluoroquinolone	Levofloxacin	LEV	5	HiMedia, India
Macrolide	Erythromycin	ETM	10	HiMedia, India
Rifamycins	Rifampin	RN	5	HiMedia, India
Tetracyclines	Tetracycline	TE	10	HiMedia, India

Assessment of MRSA through phenotypic methods

MRSA isolates were phenotypically assessed by the cefoxitin disk, which targets resistance mediated by the *mecA* gene. Cefoxitin discs (30 μg) were placed onto MHA plates inoculated with standardized bacterial suspensions. The assay was performed in triplicate for each isolate. Incubation and measurement of inhibition zones were performed following the antibiotic susceptibility testing procedures described above, adhering to CLSI 2024⁽¹²⁾.

Antibacterial activity of rGO against MRSA

Preparation of rGO suspension

A stock suspension of rGO was formulated using dispersing (10 mg) of powder in (10 mL) of sterile distilled water. The mixture was sonicated for 30 minutes to ensure homogeneous distribution. The rGO powder was obtained from Sigma-Aldrich (Merck, USA), with a stated purity of $\geq 98\%$ and particle sizes ranging from (0.5 to 5 μm). The degree of reduction and surface chemistry, including the presence of residual hydroxyl, carboxyl, and epoxy groups, was provided in the manufacturer's specifications. Under aseptic conditions, serial dilutions were performed to obtain working concentrations of 25, 50, 75, and 100 $\mu\text{g}/\text{mL}$ ^(13,14). This concentration range was selected based on previous studies reporting minimal cytotoxicity while allowing evaluation of potential antibacterial effects against MRSA.

Agar well diffusion method

A sterile cork punch was used to create 6 mm diameter wells in bacteria inoculated onto MHA plates. Each well was loaded with 100 μL of rGO suspension at concentrations of 25, 50, 75, and 100 $\mu\text{g}/\text{mL}$. Sterile distilled water was used as a negative control. The assay was performed in triplicate for each isolate. The plates were incubated at 37 °C for 24 h, and the diameter of the inhibition zones was measured in mm⁽¹⁵⁾.

Results

Identification of bacterial specimens

Out of the 24 clinical samples collected, fifteen 62.5 % samples showed positive growth of bacterial culture on different media, after 24 hours of incubation at a temperature of 37 °C, while nine 37.5% samples showed no visible growth, even after extended incubation for 48 hours under the same conditions (Figure 1).

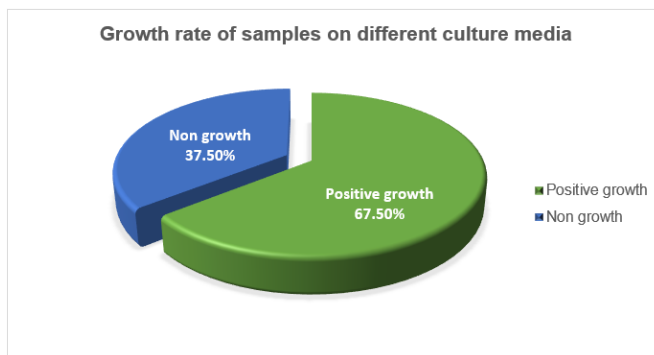


Figure 1. Distribution of positive and negative bacterial growth among clinical samples.

All specimens with bacterial growth produced visible colonies on blood agar. Of these, 10 isolates also exhibited growth on MSA, while those that grew on MAC were excluded due to their low number and the likelihood of being mixed Gram-negative populations. The 10 MSA-positive isolates were subsequently selected for further microbiological identification.

All ten isolates (Table 2) demonstrated mannitol fermentation, as indicated by the formation of yellow halos around colonies on MSA. This color shift results from acid production during fermentation, which lowers the pH and changes the phenol red indicator from red to yellow. Gram staining revealed Gram.-positive arranged a grape.-like clusters. On blood agar, all isolates exhibited β -hemolysis, evi-

denced by clear zones around the colonies, indicating complete erythrocyte lysis, a typical feature of virulent *S. aureus*.

All isolates exhibited catalase activity and tested positive for coagulase, confirming their identification as *S. aureus*. The coagulase test remains a critical diagnostic marker for distinguishing *S. aureus*. All isolates consumed citrate, while negative for the oxidase assay. These biochemical features enhance bacterial survival within host tissues and contribute to virulence.

Finally, the diagnosis was confirmed using the Vitek 2 compact system, which showed with a 99.9% probability that all bacterial isolates in this study were *S. aureus*.

Table 2. Biochemical and hemolytic characteristics of *S. aureus* isolates.

Test	Results
Gram stain	+
Hemolysis on blood agar	β
Mannitol fermentation	+
Catalase test	+
Slide (bound) coagulase	+
Tube (free) coagulase	+
Oxidase test	-
Citrate utilization test	+

Antibiotic susceptibility test of *S. aureus*

The antibiotic susceptibility profiles of the ten *S. aureus* isolates showed notable variation across the tested agents. Resistance was highest toward ceftriaxone (70 %), followed by gentamicin, cephalosporin, erythromycin, and tetracycline, each demonstrating resistance rates of 60 %. Chloramphenicol exhibited an intermediate resistance level of 50 %, whereas rifampin and levofloxacin showed the lowest resistance rates at 40 % (Table 3). Consistent with these patterns, one-way ANOVA revealed significant differences in the inhibitory effects of the antibiotics ($p < 0.05$), and Tukey’s HSD post-hoc analysis confirmed that ceftriaxone and cephalosporin produced significantly smaller inhibition zones compared with rifampin and levofloxacin. These findings underscore the pronounced variability in antimicrobial susceptibility among the isolates, reflecting the selective pressure imposed by commonly used antibiotics in clinical settings.

Table 3. Mean inhibition zones (mm) ± SD of antibiotics against *S. aureus* isolates and statistical comparison.

Antibiotic abbreviation	Mean inhibition zone (mm) ± SD	Resistance (%)
CN	16.8 ± 0.9	60
C	18.2 ± 1.0	50
KF	15.5 ± 0.7	60
CT-R	14.3 ± 0.5	70
LEV	20.1 ± 0.8	40
ETM	16.0 ± 0.7	60
RN	19.8 ± 0.6	40
TE	15.7 ± 0.7	60

Gentamicin (CN), Chloramphenicol (C), Cephalosporin (KF), Ceftriaxone (CT-R), Levofloxacin (LEV), Erythromycin (ETM), Rifampin (RN), Tetracycline (TE)

Out of the ten *S. aureus* isolates examined, seven 70% exhibited MDR, defined by non-susceptibility to at least three distinct antibiotic classes (Figure 2). This high frequency highlights the significant antimicrobial resistance burden within the clinical setting and underscores the therapeutic difficulties that arise when empirical treatments are administered without prior pathogen identification.

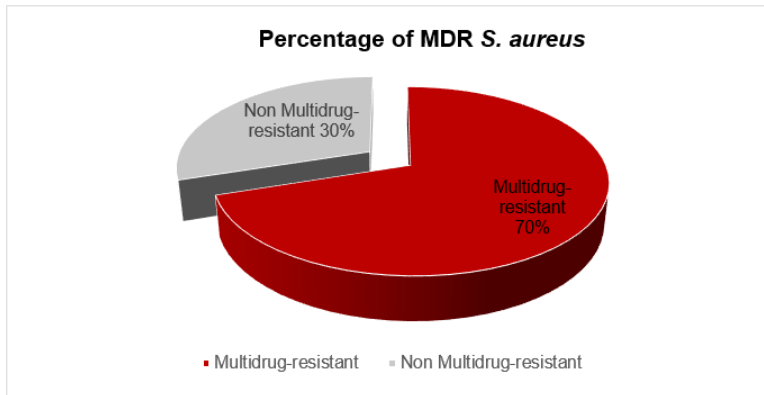


Figure 2. Proportion of *S.aureus* Isolates Exhibiting MDR

Phenotypic detection of MRSA

Phenotypic detection using the cefoxitin disk diffusion assay revealed methicillin resistance in 4 out of 10 *S. aureus* isolates, corresponding to a prevalence of 40%. This is because there is no inhibition zone around the antibiotic disc (Figure 3).

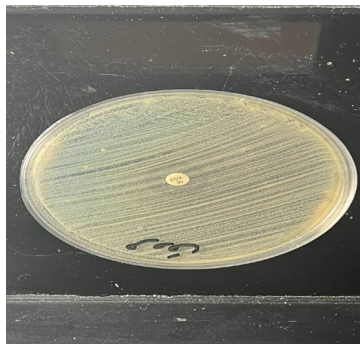


Figure 3. Illustrates a cefoxitin disk placed on an agar plate with no visible inhibition zone, confirming resistance in the tested isolate.

Antibacterial activity of rGO against MRSA

In this investigation, rGO was tested against MRSA isolates to assess its antibacterial potential. Contrary to previous findings that have shown notable antimicrobial properties for graphene-based materials, the rGO used in this study did not display any inhibitory effect on the MRSA strains under the current experimental conditions (Figure 4). This outcome highlights the complex and variable nature of interactions between nanomaterials and resistant bacterial strains, suggesting that the antibacterial effect

of rGO might be influenced by specific bacterial characteristics or by physicochemical variables such as concentration, particle morphology, and surface chemistry.

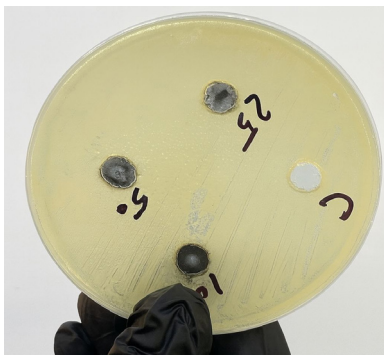


Figure 4. Demonstrates the absence of any inhibition zone around the rGO-treated area, indicating that bacterial growth was not affected.

Discussion

Identification of bacterial specimens

The consistent observation of β -hemolysis in all *S. aureus* isolates supports their virulent potential. This hemolytic activity is commonly attributed to cytotoxins such as α -hemolysin, which disrupt red blood cell membranes and contribute to host tissue damage. The uniform β -hemolytic pattern observed suggests that these isolates likely harbor the genetic determinants responsible for toxin production⁽¹⁶⁾.

A notably high rate of *S. aureus* isolation was observed in the current study. The present study demonstrated a high prevalence of *S. aureus* among clinical specimens, consistent with findings from both regional and international studies. For instance, *S. aureus* was identified as the most frequently isolated pathogen, particularly from wound and skin infections⁽¹⁷⁾. Similarly, in Saudi Arabia, an incidence rate of 35.3 % was reported⁽¹⁸⁾, while in Ethiopia, *S. aureus* was documented in 14.3% of 1360 clinical samples⁽¹⁹⁾. These variations likely reflect geographic, epidemiological, and healthcare practice differences, including infection control measures and antimicrobial usage patterns.

The elevated prevalence of *S. aureus* observed in this study may be attributed to its role as part of the normal skin microbiota, facilitating colonization of compromised tissues, particularly in hospitalized or immunocompromised patients. As an opportunistic pathogen, *S. aureus* expresses a broad range of virulence factors, including hemolysins, coagulase, protein A, lipoteichoic acid, and peptidoglycan that enable tissue invasion, immune evasion, and persistence in host environments^(5,16). In addition, the organism's ability to form biofilms and acquire resistance genes further enhances its survival and pathogenicity in nosocomial settings⁽⁶⁾.

Antibiotic susceptibility test of *S. aureus*

The high resistance rate to ceftriaxone observed in this study suggests a concerning trend, possibly linked to its widespread empirical use in both hospital and outpatient settings. A comparable resistance rate of 68 % to ceftriaxone in *S. aureus* isolates was documented⁽²⁰⁾, which aligns with the current study's observations of isolates from surgical site infections, attributing the resistance to the overuse of third-generation cephalosporins, which enhances selective pressure and facilitates the development of resistance.

Gentamicin, cephalosporins, erythromycin, and tetracyclines also showed a high resistance rate. Several studies have documented varying but concerning resistance rates of *S. aureus*, such as 62 % and 59 % for gentamicin and erythromycin, respectively⁽²¹⁾. A comprehensive study analyzing 13,024 MRSA clinical isolates between 2016 and 2021 reported high resistance to erythromycin 76.9% and moderate resistance to tetracycline 22.3%, while resistance to gentamicin remained relatively low at 3.6 %⁽²²⁾. In contrast, another community-based study reported an even higher tetracycline resistance rate of 50.7 %, while erythromycin resistance was 10.9%⁽²³⁾. These findings highlight the geographic and ecological variability in antibiotic resistance and underscore the urgent need for context-specific antimicrobial stewardship.

Resistance to gentamicin has become increasingly common, especially in nosocomial infections. This resistance often results from bacterial mechanisms, including enzymatic drug modification, decreased membrane permeability limiting antibiotic uptake, and the action of efflux systems that remove the drug from the cell⁽²⁴⁾. In a previous study on conjunctival isolates, detection of the *aac(3)-I* gene, which encodes an aminoglycoside acetyltransferase, was associated with reduced gentamicin efficacy⁽²⁵⁾. As for tetracycline, resistance in *S. aureus* is frequently driven by tet genes. The *tetK* and *tetL* variants are linked to efflux pump production, while *tetM* encodes a protein that protects the ribosome from tetracycline action⁽²⁶⁾.

Cephalosporin resistance frequently emerges due to the production of β -lactamase enzymes that can inactivate β -lactam antibiotics by breaking the β -lactam ring structure. In addition, alterations in penicillin-binding proteins (PBPs) result in decreased binding affinity, contributing further to antibiotic resistance⁽²⁷⁾.

In *S. aureus*, resistance to erythromycin is frequently associated with structural alterations at the antibiotic's binding site on the 50S ribosomal subunit, primarily caused by methylation via *erm* family genes. Additionally, efflux pumps encoded by genes such as *msrA* contribute to resistance by reducing intracellular drug accumulation through active expulsion⁽²⁸⁾.

Chloramphenicol showed a moderate resistance rate, with 53% resistance among *S. aureus* isolates in bloodstream infections⁽²⁹⁾. Resistance to chloramphenicol is conferred by the enzyme chloramphenicol acetyltransferase, which chemically modifies the drug and renders it inactive against bacterial cells.

The lowest resistance levels were recorded for levofloxacin and rifampin. Although this may indicate partial retention of efficacy, emerging resistance to these agents remains a concern. Similar findings were reported with 43 % resistance to levofloxacin and 38 % to rifampin⁽³⁰⁾. Levofloxacin resistance is associated with genetic changes in the *gyrA* and *parC* genes, which encode enzymes essential for DNA replication. In addition, active efflux systems may contribute by lowering intracellular drug concentrations. Rifampin resistance is commonly driven by mutations in the *rpoB* gene that alter the drug's binding site^(31, 32).

These results collectively underscore the evolving antimicrobial resistance landscape of *S. aureus* and reflect the organism's ability to adapt under selective pressure. The widespread resistance highlights the need for routine antimicrobial susceptibility testing and tighter control of antibiotic prescriptions to limit further resistance development.

The present study revealed a notably elevated rate of MDR in *S. aureus* isolates, in line with findings from other studies. A 68 % rate was reported in isolates from a tertiary care center⁽³³⁾. In contrast, a lower rate of 45 % was observed in Ghanaian isolates⁽³⁴⁾.

The elevated MDR rate may be attributed to selective pressure from widespread antibiotic use, especially in settings with limited stewardship oversight. MDR in *S. aureus* is often driven by the acquisition of mobile DNA elements that carry multiple resistance genes and facilitate their dissemination. These elements enable the coexistence of several resistance mechanisms within a single strain⁽³⁵⁾.

Inappropriate antibiotic use, particularly empirical treatments without pathogen confirmation, further promotes the emergence of MDR strains. Healthcare institutions lacking regulated antimicrobial stewardship frameworks are especially vulnerable to elevated resistance rates⁽³⁶⁾.

However, it is important to acknowledge that the number of isolates obtained in this study was relatively limited. Only 24 specimens were processed, yielding 10 *S. aureus* isolates. Because of this small specimen size, the reported proportions of MRSA 40% and MDR 70% should be interpreted with caution, as they may not fully reflect the broader resistance patterns in the general population. Larger studies with more isolates are needed to provide more representative estimates.

Phenotypic detection of MRSA

The MRSA prevalence found in this study aligns with findings from similar investigations. For instance, research conducted in Egypt reported a 42% resistance rate using the same ceftioxin-based method⁽³⁷⁾, while a study in Sudan observed a 38 % rate⁽³⁸⁾. Such results highlight the widespread presence of MRSA in developing regions. In contrast, a lower prevalence of 18% was noted in Germany⁽³⁹⁾. This decline may reflect the impact of improved infection control practices and enhanced antibiotic stewardship strategies.

The development and persistence of MRSA involve both genetic acquisition and external environmental pressures. Located within the *SCCmec* element, the *mecA* gene can be horizontally transferred between strains, enhancing the rapid proliferation of methicillin resistance⁽⁴⁰⁾. The emergence of MRSA is closely associated with excessive use of β -lactam antibiotics and insufficient implementation of infection control protocols in healthcare environments⁽⁸⁾. In addition, individuals, whether patients or healthcare workers, who are colonized without symptoms may unknowingly contribute to the spread of the organism within clinical environments⁽¹⁶⁾.

The observed resistance rate in this research may thus reflect not only genetic determinants but also local practices in antibiotic use and infection control. These findings emphasize the importance of adopting integrated approaches that include accurate diagnostic tools, rational antibiotic use, and stringent hygiene protocols to curb the spread of MRSA.

It should be noted that MRSA identification in this study was based solely on phenotypic testing using ceftioxin disc diffusion, which is a widely accepted and recommended method in many studies for routine detection of methicillin resistance. Although molecular confirmation of the *mecA* gene via polymerase chain reaction (PCR) provides more definitive results, this was not performed due to resource limitations. Therefore, while the ceftioxin disc method is reliable, the reported MRSA prevalence may not fully reflect the actual presence of methicillin resistance in the isolates.

Antibacterial activity of rGO against MRSA

Although several reports have documented the effectiveness of rGO against clinical isolates, results remain inconsistent. For instance⁽⁴¹⁾ reported strong antibacterial activity of rGO against MRSA, attributing the mechanism to physical damage of the bacterial membrane and oxidative stress. In contrast⁽⁴²⁾ noted minimal or no effect in some MRSA isolates, emphasizing that synthesis techniques and experimental setups play a critical role in determining rGO efficacy. These conflicting outcomes underline the necessity of establishing standardized protocols and ensuring thorough material characterization before clinical application.

The lack of observed activity in the present study may be due to multiple factors. The functional properties of rGO, such as its surface charge, level of reduction, and lateral dimension, are key in determining its interaction with microbial cells. Insufficient optimization of these features could result in weak interactions and ineffective disruption of bacterial structures. Additionally, experimental parameters such as dosage, exposure duration, and bacterial load may have influenced the results. Moreover, MRSA's inherent resistance mechanisms, including its thickened cell wall and active efflux systems, may provide added protection against nanomaterials⁽⁴³⁾.

Future Directions

Advancing the antimicrobial potential of rGO requires detailed exploration of its structure–activity relationship. Future efforts should aim to optimize the material's morphology, chemical composition, and surface characteristics to improve its interaction with bacterial cells. Functionalizing rGO with

antimicrobial peptides, metal nanoparticles, or biocompatible polymers may enhance its bactericidal properties. Investigating its role as an adjunct to conventional antibiotics could reduce drug dosages and limit resistance development. To validate these strategies, robust *in vivo* testing in clinically relevant infection models is essential. Establishing standardized evaluation protocols will further facilitate comparison across studies and support potential clinical translation.

Conclusions

This study explored the antibacterial properties of rGO against MRSA obtained from clinical samples. The analysis identified a 40% prevalence of MRSA and a high incidence of 70 % of MDR among the isolates. However, rGO did not display any inhibitory effect under the conditions applied, differing from some earlier reports that suggested potential antimicrobial activity. This study's outcomes suggest that the antibacterial activity of rGO is highly dependent on its structural attributes, fabrication techniques, and testing conditions. Although rGO did not demonstrate inhibitory effects under the current parameters, the results provide valuable information regarding its interaction with bacterial cells. This highlights the necessity for further optimization and methodological standardization before any potential clinical application.

References

1. Ventola CL. *The antibiotic resistance crisis: Part 1: Causes and threats*. P T. 2015;40(4):277–283.
2. Palmieri V, Perini G, De Spirito M, Papi M. *Graphene oxide and reduced graphene oxide as antibacterial agents: Mechanisms and potential applications*. Front Bioeng Biotechnol. 2023;11:1124456. doi:10.3389/fbioe.2023.1124456
3. Seabra AB, Paula AJ, De Lima R, Alves OL, Durán N. *Nanotoxicity of graphene and graphene oxide*. Chem Res Toxicol. 2014;27(2):159–168. doi:10.1021/tx400329h
4. Krishnamoorthy K, Veerapandian M, Zhang LH, Yun K, Kim SJ. Synergistic antibacterial effect of graphene oxide–zinc oxide nanocomposites. Mater Sci Eng C. 2022;142:110246. doi:10.1016/j.msec.2022.110246
5. Cheung GYC, Joo HS, Chatterjee SS, Otto M. *Pathogenicity and virulence of Staphylococcus aureus*. Annu Rev Microbiol. 2021;75:579–604. doi:10.1146/annurev-micro-020518-115759
6. Lakhundi S, Zhang K. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev. 2018;31(4):e00020-18. doi: 10.1128/CMR.00020-18.
7. Jabuk M, Al-Dujaili A, Al-Taei A. *Phenotypic methods for identification of Staphylococcus aureus*. Int J Med Microbiol. 2015;305(3):239–243. doi:10.1016/j.ijmm.2015.01.004
8. Becker K, Heilmann C, Peters G. *Clinical and molecular characteristics of Staphylococcus aureus infections*. Clin Microbiol Rev. 2021;34(2):e00120-20. doi:10.1128/CMR.00120-20
9. bioMérieux. *VITEK® 2 Compact System: Product information and instructions for use*. Marcy-l'Étoile (France): bioMérieux SA; 2023.
10. Kosikowska U, Andrzejczuk S, Pietrzak A. *Susceptibility testing methods in microbiological diagnostics*. J Med Microbiol. 2020;69(5):631–639. doi:10.1099/jmm.0.001195
11. Collee JG, Fraser AG, Marmion BP, Simmons A. *Mackie and McCartney practical medical microbiology*. 14th ed. Edinburgh: Churchill Livingstone; 1996.
12. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing*. 34th ed. CLSI supplement M100. Wayne (PA): CLSI; 2024.

13. Gurunathan S, Han JW, Dayem AA, Eppakayala V, Kim JH. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa*. *Int J Nanomedicine*. 2012;7:5901–5914. doi:10.2147/IJN.S35123
14. Li J, Wang G, Zhu H, Zhang M, Zheng X, Di Z. Antibacterial activity of large-area monolayer graphene film manipulated by charge transfer. *Sci Rep*. 2014;4:4359. doi:10.1038/srep04359
15. Balweri A, Singh M, Sharma R. Evaluation of antibacterial activity of reduced graphene oxide nanosheets against clinical isolates of *Staphylococcus aureus*. *J Nanoscience Nanotechnology*. 2016;16(7):6674–6680.
16. Otto M. *Staphylococcus aureus* toxins. *Curr Opin Microbiol*. 2014;17:32–37. doi:10.1016/j.mib.2013.11.004
17. Abdulbaqi A, Ibrahim AS. Molecular analysis of *Staphylococcus aureus* isolated from clinical samples and natural flora. *Cell Mol Biol*. 2023;69(1):145–149. doi:10.14715/cmb/2023.69.1.145
18. Almuhayawi MS. Epidemiology of *Staphylococcus aureus* in Saudi Arabia: A cross-sectional study. *Saudi J Biol Sci*. 2022;29(5):984–990. doi:10.1016/j.sjbs.2021.12.009
19. Dilnessa T, Bitew A. Prevalence and antimicrobial resistance pattern of *Staphylococcus aureus* in Ethiopia. *BMC Infect Dis*. 2016;16:687. doi:10.1186/s12879-016-2025-2
20. Khan SA, Ali A, Rehman T, Farooq U. Antibiotic resistance patterns of *Staphylococcus aureus* isolated from surgical site infections. *J Infect Dev Ctries*. 2022;16(1):45–52. doi:10.3855/jidc.15449
21. Shrestha B, Maharjan A, Adhikari N, Basnet R. Prevalence of antibiotic resistance among *Staphylococcus aureus* isolates in Nepalese hospitals. *BMC Microbiol*. 2020;20:146. doi:10.1186/s12866-020-01863-7
22. Li X, Chen Y, Xu X, Wang Y, Zhao J. Antimicrobial resistance profiles of 13,024 methicillin-resistant *Staphylococcus aureus* isolates from China, 2016–2021. *Front Cell Infect Microbiol*. 2023;13:1102779. doi:10.3389/fcimb.2023.1102779
23. Zhang Y, Wang X, Li H, Zhou X. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from community and hospital settings. *BMC Microbiol*. 2022;22:88. doi:10.1186/s12866-022-02526-6
24. Ramirez MS, Tolmasky ME. Aminoglycoside-modifying enzymes. *Drug Resist Updat*. 2017;33:10–21. doi:10.1016/j.drup.2017.03.002
25. Al-Buhamrah NA, Hussain JM, Saadon QK. Detection of the aac(3)-I gene in *Chlamydia trachomatis* isolated from conjunctivitis by using the PCR technique in Al-Najaf province, Iraq. *Microb Biosyst*. 2024;9(1):27–32. doi:10.3390/microbiosys901003
26. Chen Y, Wei X, Wu J. Molecular characterization of *agr* types and antibiotic resistance profiles of *Staphylococcus aureus* isolates from pediatric patients in Guangzhou, China. *BMC Microbiol*. 2023;23:123. doi:10.1186/s12866-023-02867-0
27. Edrees EE, Hussein AA, Al-Buhamrah NA. Phenotypic detection of some beta-lactamases and Enterobacterial Repetitive Intergenic Consensus Genotyping (ERIC) of natively isolated uropathogenic *Escherichia coli*. *Egypt J Med Microbiol*. 2024;33(4)
28. Roberts MC. Mechanisms of macrolide resistance in bacteria. *FEMS Microbiol Rev*. 2020;44(4):448–466. doi:10.1093/femsre/fuaa013
29. Nwankwo EO, Nas BA. Resistance profile of *Staphylococcus aureus* in bloodstream infections in northern Nigeria. *Afr Health Sci*. 2017;17(2):567–573. doi:10.4314/ahs.v17i2.25
30. Goudarzi M, Azad M, Seyedjavadi SS, Goudarzi H, Sabzehali F, Hourri H. Resistance patterns of *Staphylococcus aureus* in Iran: A nationwide surveillance study. *Microb Drug Resist*. 2021;27(2):144–155. doi:10.1089/mdr.2020.0266

31. Lee JS, Kim HY, Park KS. Molecular characteristics and prevalence of rifampin resistance in *Staphylococcus aureus* blood isolates in South Korea. *Infect Drug Resist.* 2023;16:245–256. doi:10.2147/IDR.S386214
32. Huynh TQ, Tran VN, Thai VC, Nguyen HA, Nguyen NTG, Tran MK, et al. Genomic alterations involved in fluoroquinolone resistance development in *Staphylococcus aureus*. *PLoS One.* 2023;18(7):e0287973. doi:10.1371/journal.pone.0287973
33. Sharma R, Gupta A, Mehta M, Singh N. Multidrug resistance in *Staphylococcus aureus*: A rising concern in India. *Indian J Med Microbiol.* 2020;38(2):183–188. doi:10.4103/ijmm.IJMM_19_334
34. Mensah SE, Osei-Tutu L, Kenu E, Afutu E, Badu-Peprah A. Antimicrobial susceptibility profile of *Staphylococcus aureus* isolated from clinical samples in Ghana. *Afr Health Sci.* 2019;19(2):1990–1998. doi:10.4314/ahs.v19i2.49
35. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev.* 2018;31(4):e00088–17. doi:10.1128/CMR.00088-17
36. Otter JA, French GL, Cooper BS. Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *J Hosp Infect.* 2013;85(3):213–218. doi:10.1016/j.jhin.2013.07.016
37. Hassan AA, Youssef RM, Khalil MS. Prevalence of methicillin-resistant *Staphylococcus aureus* in clinical isolates from Egypt. *Egypt J Med Microbiol.* 2022;31(2):145–152.
38. Ahmed MA, Ibrahim AM, Elhassan AM. Detection of MRSA among clinical isolates in Khartoum, Sudan. *Sudan J Med Sci.* 2021;16(1):22–29
39. Schmidt T, Kock R, Werner G. Epidemiology of MRSA in Germany: Decline and control measures. *Int J Med Microbiol.* 2020;310(4):151–160.
40. Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers.* 2020;6(1):34.
41. Gurunathan S, Han JW, Dayem AA, Eppakayala V, Kim JH, Song H. Antibacterial efficacy of reduced graphene oxide against methicillin-resistant *Staphylococcus aureus*: Mechanistic insights. *ACS Appl Mater Interfaces.* 2019;11(18):16351–16363. doi:10.1021/acsami.9b03101
42. Chen Y, Wei X, Wu J. Variable antibacterial activity of graphene oxide and reduced graphene oxide against MRSA: Role of physicochemical properties. *Nanomaterials.* 2021;11(7):1821. doi:10.3390/nano11071821
43. Li X, Zhang W, Zhang J, Sun J. Influence of physicochemical characteristics of graphene oxide on its antibacterial activity: A review. *Front Microbiol.* 2020;11:346. doi:10.3389/fmicb.2020.00346