ORIGINAL ARTICLE

Antibacterial activity of Magnesium Oxide Nanoparticles biosynthesis from *bacillus subtilis* against Multi-Drug Resistant *Staphylococcus aureus* Isolated from Different Clinical Infections in Hospitals of Al-Najaf province /Iraq

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ABSTRACT

Background: *Staphylococcus aureus is an important pathogen with a significant impact on human health. Several infections caused by this bacterium occur in both community-acquired and hospital-acquired settings.* ***Objective:*** *This research aims to study the effect of magnesium oxide nanoparticles biosynthesized from Bacillus subtilis against multidrug-resistant (MDR) S. aureus isolated from various clinical infections.* ***Methodology:*** *A total of 230 specimens were collected from patients with different clinical infections referred to hospitals in Al-Najaf Province, Iraq, representing various age groups and both genders. Standard microbiological assays were used to identify S. aureus isolates, which were then subjected to antimicrobial susceptibility testing against 18 types of antibiotics using the disc diffusion method. Magnesium oxide nanoparticles (MgO NPs) were synthesized by mixing B. subtilis culture filtrate with magnesium nitrate solution. The color change from pale yellow to white indicated the formation of MgO NPs.* ***Results:*** *Out of the 230 specimens, 80 were identified as S. aureus isolates and tested for antimicrobial susceptibility. The results showed the highest resistance rate to Penicillin G (97.5%), while S. aureus showed no resistance to Vancomycin (0%). Among the 80 isolates, 45 (56.25%) were identified as multidrug-resistant (MDR S. aureus).The antibacterial activity of MgO nanoparticles at different concentrations (100, 200, 300, and 400 µg/ml) against S. aureus demonstrated that the inhibition zones increased proportionally with the concentration of MgO nanoparticles.* ***Conclusion:*** *This study concludes that higher concentrations of MgO nanoparticles biosynthesized by B. subtilis lead to an increased inhibition zone against MDR S. aureus, indicating a concentration-dependent antibacterial effect.*

INTRODUCTION

Staphylococcus aureus is a major human pathogen capable of adapting to diverse hosts and environmental conditions, causing a wide range of infections. Additionally, it is one of the leading causes of both hospital-acquired and community-acquired infections. It can cause infections of the bloodstream, skin and soft tissues, and lower respiratory tract; infections associated with medical instrumentation such as central-line-associated bloodstream infections; as well as serious deep-seated infections such as osteomyelitis and endocarditis[1].

S. aureus is among the most common bacterial pathogens in humans and is responsible for multiple infections, including infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, and scalded skin syndrome), septic arthritis, gastroenteritis, meningitis, urinary tract infections, pneumonia, and bloodstream infections[2].

Antimicrobial resistance is a critical global health concern driven by the misuse and overuse of antibiotics across various sectors. This has led to the emergence of resistant microorganisms, rendering many standard treatments ineffective and resulting in prolonged infections, increased healthcare costs, and higher mortality rates[3].Due to the increasing antibiotic resistance among bacterial species, there is an urgent need to develop new antibacterial agents to replace or supplement conventional antibiotics[4].

Nanobiotechnology, a modern branch of science, focuses on the production and application of nanoparticles. It has emerged from extensive research, giving rise to the interdisciplinary field known as *nanotechnology*[5]. Magnesium oxide (MgO) nanoparticles have demonstrated excellent antibacterial activity against various pathogenic microorganisms and therefore hold great promise as an effective therapeutic

alternative against infections caused by drug-resistant bacteria[6]. The aim of this study was the effect of magnesium oxide nanoparticles biosynthesized from *Bacillus subtilis* against multidrug-resistant (*MDR*) *S. aureus* isolated from various clinical infections.

METHODOLOGY

Specimens collection and bacterial identification

A total of 230 specimens were randomly collected from patients with different clinical infections who were referred to various hospitals in Al-Najaf Province, Iraq, including Al-Sadr Medical City, Al-Zahraa Hospital, Al-Hakim General Hospital, Najaf General Hospital, and the Burn Center in Najaf Province. Samples were obtained from patients of different age groups and both genders during the period from November 2024 to June 2025.

Ethical approval.

All participants supplied their informed agreement to be included in the study and were assured that their information would be kept private and used exclusively for this purpose.

Isolation and Identification of *Staphylococcus aureus*

All collected specimens were immediately transferred to the laboratory for bacterial isolation. Samples were first inoculated into sterilized brain heart infusion (BHI) broth and incubated at 37°C for 24 hours. Subsequently, cultures were streaked onto mannitol salt agar and blood agar and incubated overnight at 37°C under aerobic conditions for the presumptive diagnosis of *S. aureus*.

For bacterial identification, biochemical tests and Gram staining were performed [7]. A VITEK 2 Compact System was also used to identify bacterial isolates efficiently and accurately, minimizing the risk of contamination that could interfere with pathogen detection.

Antibiotics susceptibility testing

Muller-Hinton agar medium (Oxoid, UK) was used to evaluate the susceptibility of *Staphylococcus aureus* isolates using the disk diffusion method. A total of 18 antibiotic discs were applied to the agar surface. After overnight incubation at 37°C, the zones of inhibition were measured and interpreted according to the standard guidelines [8].

Synthesis of MgO NPs:

Isolation and Identification of *Bacillus subtilis*

Bacillus subtilis used for the biosynthesis of magnesium oxide nanoparticles (MgO NPs) was isolated from a soil sample. The bacterial isolate was identified based on morphological, microscopic, and biochemical characteristics, and its identification was further confirmed using the VITEK® 2 Compact System.

Synthesis of MgO nanoparticles using *B. subtilis*

Purified bacterial isolates were cultured in 1000 mL of brain heart infusion (BHI) broth and incubated in a shaking incubator at 37°C for 24 hours. After confirming bacterial growth, the culture was centrifuged at 10,000 rpm for 10 minutes at 4°C.Next, 0.5 M magnesium nitrate [$Mg(NO_3)_2$] was added to the bacterial supernatant. All the mixture was mixed in beaker (250ml) on magnetic stirrer at (400 rpm for 60 min in dark condition and heated to 80C), until the change in the color of the solution was observed and white precipitate formed. The purified white precipitate was collected in a sterile flask and dried in an oven at 50°C for 24 hours. The dried MgO nanoparticles were then stored for further use[9].

Characterization of Biosynthesized Magnesium Oxide Nanoparticles

The physical characteristics of the biosynthesized nanoparticles were analyzed using UV–Visible spectrophotometry. The optical properties of the nanoparticles, including their response to gamma irradiation, were confirmed by measuring absorbance within the wavelength range of 200–900 nm using a UV–Visible spectrophotometer.

Field Emission Scanning Electron Microscopy (FESEM) was employed to determine the surface morphology and particle size of the MgO nanoparticles in composite films. X-ray Diffraction (XRD) analysis was conducted to characterize the crystalline structure of the MgO NPs. These analyses were performed at the Electron Microscopy Unit, University of Tehran[10].

Antibacterial Activity of MgO NPs against S. *aureus* isolates

The antibacterial activity of the synthesized MgO nanoparticles was evaluated using the agar well diffusion method against multidrug-resistant (MDR) *S. aureus* isolates obtained from different clinical infections. A sterile cotton swab was used to evenly spread the bacterial suspension across the surface of Mueller-Hinton agar (MHA) plates.

Using a sterile cork borer, wells of 5 mm diameter were created and filled with 80 µL of MgO NPs at different concentrations (100, 200, 300, and 400 µg/mL). The plates were then incubated at 37°C for 24 hours, after which the diameters of the inhibition zones were measured using a metric ruler[11].

Antibacterial activity of MgO NPs from *B. subtilis* with some resistance antibiotics

The antibacterial activity of MgO nanoparticles synthesized by *Bacillus subtilis* was compared with that of selected antibiotics against resistant *S. aureus* isolates using the **disc diffusion method** on **Mueller-Hinton agar**.

For this assay, **100 µL of MgO NPs (400 µg/mL)** was added to antibiotic discs containing **cefoxitin, gentamicin, and ciprofloxacin**. After incubation, the **zones of inhibition** for *S. aureus* isolates were measured using a metric ruler[12].

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 6). Data were expressed as mean± standard error (SE). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Distribution of patient samples for different infections

The patients' samples of different infections were obtained from hospitals and specialized centers in Najaf province, The specimens included: wound swabs 46(20%), burn swabs 41(17.8 %), urine specimens 33(14.35%), nasal swabs 28(12.2%), tonsils swabs 23(10 %), ear swabs 21(9.13%), sputum specimens 20(8.7%), Vaginal swabs 10(4.35%) and blood specimens 8 (3.47%), also, the specimens were distributed according to gender as follows: 120(52.2%) of males and 110(47.8%) of females (Table 1).

Isolation and Identification of *S. aureus*

A total number of (230) patients submitted in this study whom suffered from different clinical infections. Among 195 isolates gave bacterial growth, only 80/195(84.21%) isolates were recognized as *S. aureus* by using the VITEK-2 automated compact system.

Antibiotic susceptibility test of *S. aureus* isolates

In this study, only (80) of *S. aureus* isolates were subjected to antimicrobial susceptibility test towards ١٨ antibiotic types, by using agar disc diffusion test (Kirby-Bauer method). The results antibiotics resistance test of *S. aureus* isolated from different clinical infections. The results showed the highest rates of resistance towards the antibiotic Penicillin G with a percentage of 78(97.5%) followed by Cefoxitin (57.5%) and Oxacillin (56.25%) while low resistance to Amikacin 30 (43.75%) and Levofloxacin 20 (25%) and no resistant to Vancomycin (0%) (Table 2).

Table 1: Number and percentage of specimens collected from patients with different infections

Type of specimens	No. of specimens	Percentage (%)	Gander			
			Male		Female	
			No.	%	No.	%
Wound swabs	46	20.0	28	12.17	18	7.8
Burn swabs	41	17.8	18	7.8	23	10
UTIs specimens	33	14.35	12	5.25	21	9.1
Nasal swabs	28	12.2	16	7	12	5.2
Tonsils swabs	23	10	16	6.96	7	3.04
Ear swabs	21	9.13	14	6.09	7	3.04
Sputum specimens	20	8.7	11	4.8	9	3.9
Vaginal swabs	10	4.35	0	0	10	4.35
Blood specimens	8	3.47	5	2.17	3	1.3
Total	**230**	**100**	**120**	**52.2**	**110**	**47.8**

Table 2: Antibiotics resistance percentage of (80) *S. aureus* isolated from different clinical infections

Name of Antibiotic		Diameter of Inhibition Zone (mm)		
		Sensitive (S) %	Intermediate (I) %	Resistance (R) %
β-lactams / Penicillins (penams)	Penicillin G (PEN)	2(2.5%)	0	78(97.5%)
	Oxacillin (OXA)	32(40%)	0	48(60%)
β-lactams / Cephems	Cefoxitin (FOX)	30(37.5%)	0	50 (62.5%)
Glycopeptides	Vancomycin (VAN)	78(97.5%)	2(2.5%)	0
Aminoglycoside	Gentamicin (GEN)	32(40%)	5 (6.25%)	43(53.75%)
	Amikacin (AK)	41(51.25%)	4(5%)	30(43.75%)
	Tobramycin (TOB)	37(46.25%)	3(3.75%)	40 (50%)
Macrolides	Azithromycin (AZM)	29 (36.25%)	2(2.5%)	49(61.25%)
	Erythromycin (Ery)	17(21.25%)	4(5%)	59(73.75%)
Tetracyclines	Tetracycline (TET)	24(30%)	4(5%)	52(65%)
	Doxycycline (DXT)	42(52.5%)	6(7.5%)	32(40%)
Ansamycins	Rifampicin	42 (52.5%)	2(2.5%)	36 (45%)
Lincosamides	Clindamycin (CLI)	41(51.25%)	3(3.75%)	36 (45%)
Quinolones / Fluoroquinolones	Ciprofloxacin (CIP)	34(42.5%)	2(2.5%)	44(55%)
	Levofloxacin (LEV)	59(73.75%)	1(1.25%)	20(25%)
Sulfonamides	Trimethoprim-sulfamethoxazole (SXT)	42(52.5%)	3(3.75%)	35(43 .75%)
Fusidance	Fusidic acid (FUS)	19(23.75%)	4(5%)	57(71.25%)
Phenicols class	Chloramphenicol (C)	24 (30%)	5(6.25%)	51(63.75%)

Synthesis of MgO nanoparticles using *B. subtilis*

B. subtilis was utilized for the biosynthesis of MgO NPs and it caught an efficient isolate for synthesis extracellular after using a supernatant that contained free cells-only as well as added magnesium nitrate under appropriate conditions previously controlled , it showed change in the features of the medium used in biosynthesis, especially from collected white precipitate in a sterile flask after it was dried in the oven at 50°C for 24 h and used in the manufacture of MgO NPs , as shown in (Figure 1).

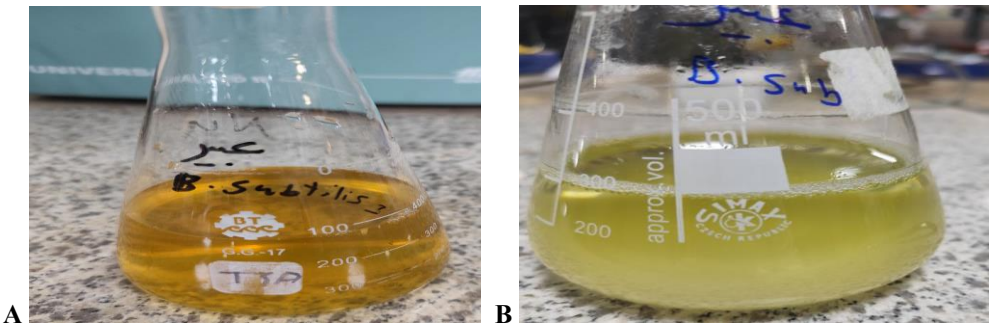

Fig. 1: Biosynthesis of MgO NPs in BHI broth using *B. subtilis* Before addition of Mg $(No_3)_2$ and After addition of Mg $(No_3)_2$ (A,B).

Characterization of biosynthesized MgO NPs according to physical and biological characters

UV-visible Spectroscopy

UV- Visible spectrophotometric is a proven technique for detecting the nanoparticles. The biosynthesis of nanoparticles can be confirmed by visual observation and measuring the absorbance band using UV-visible spectroscopy. The absorption spectrum of nanoparticles produced in the reaction mixture has a peak at 28°nm, as shown in figure (2).

Field Emission Scanning Electron Microscopy (FESEM) analysis

FESEM was used to determine the morphology and size of MgO particles. Well distributed and spherical shaped MgO nanoparticles were generated by *B. subtilis* with a size of between (59.55 – 82.27 nm). Their size average was 68.01 nm, figure (3).

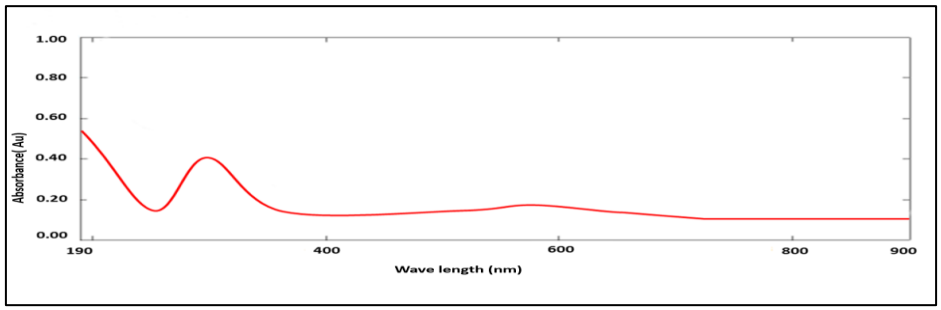

Fig 2: UV-visible spectroscopy analysis of MgO NPs synthesis by *B.subtilis*

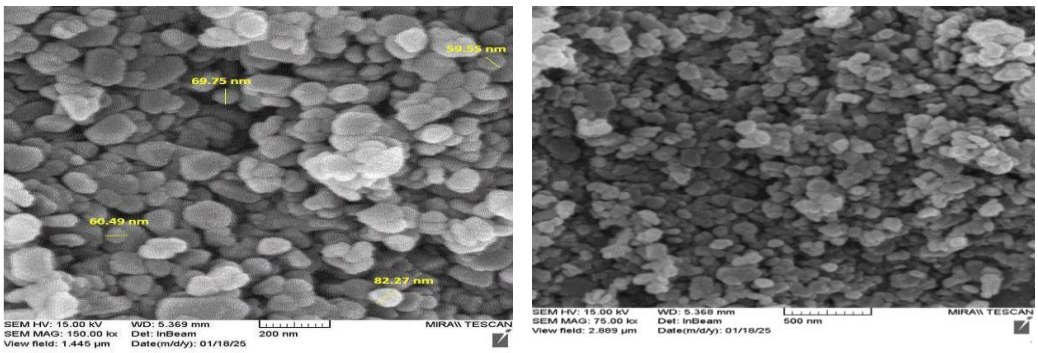

Fig 3 : FESEM Micrograph of biogenic MgO NPs synthesized by *B. subtilis* showed MgO NPs

X-Ray diffraction analysis (XRD)

X-ray diffraction (XRD) is a technique used to detect the means for the shape of crystalline phase in nanoparticles depended on peaks of diffraction, the cubic crystal system of the biosynthesized MgO NPs was confirmed by the XRD analysis, in the current study it showed the higher or sharp peak at 2θ=36.9° for *B. subtilis* corresponding to the (111) respectively crystallographic plane, as shown in figure (4).

Antibacterial effects of MgONPs synthesized by *B. Subtilis* against MDR *S. aureus*

The results of antibacterial activity test from MgO NPs synthesized by *B. Subtilis* against MDR *S. aureus* isolates with different concentrations (100, 200, 300, 400µg/ml). Specifically, the inhibition zone at 400 µg/ml surpassed that at 300 µg/ml, which in turn exceeded the inhibition zone at 100 µg/ml. Consequently, the inhibition zone at 400 µg/ml was identified as the optimal concentration for the inhibitory effect of imported zinc oxide nanoparticles, as depicted in figure (6). The results showed significant differences in different concentrations and the highest inhibition zone of MgO NPs which significantly increased (P< 0.0001) in concentration (400 µg/ml) were19.2±0.381. Also, the results showed that inhibition zone of MDR *S. aureus* isolates increase progressively with increase the MgO NPs concentrations in reaching a maximum inhibition in 400µg/ml, as shown in figure (7).

Correlations between MgONPs synthesized by *B. Subtilis* and the most resistant antibiotics

The inhibition zones produced by MgO NPs in combination with ciprofloxacin (13.64 ± 0.211 mm) and gentamicin (13.50 ± 0.205 mm) were significantly greater than those with cefoxitin (12.92 ± 0.188 mm) , the statistical analysis confirmed highly significant differences between ciprofloxacin and cefoxitin (P = 0.0005) as well as gentamicin and cefoxitin (P = 0.0193), whereas no significant difference was observed between ciprofloxacin and gentamicin (P = 0.3262) ,as shown in Figure (8).

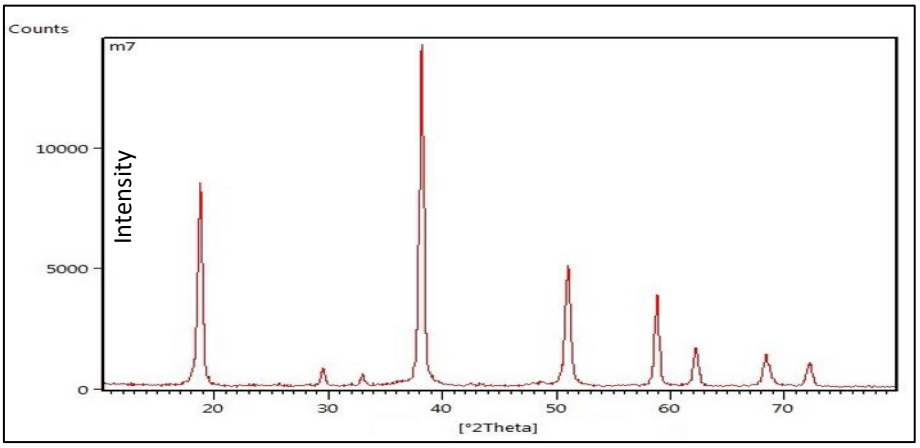

Fig 5: XRD analysis of MgO NPs synthesized by *B. subtilis*

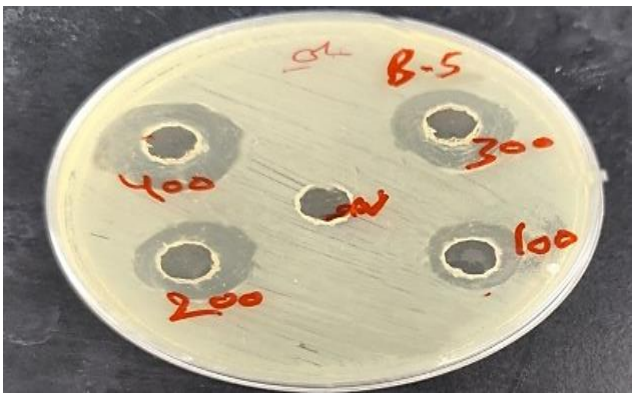

Fig. 6: Effect of Different Concentrations of MgO NPs against S. aureus isolates

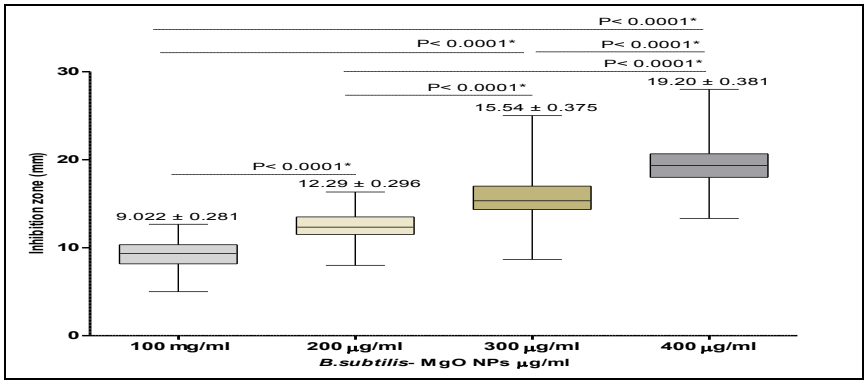

Fig. 7: The Effect of MgO NPs on MDR *S. aureus* isolates.

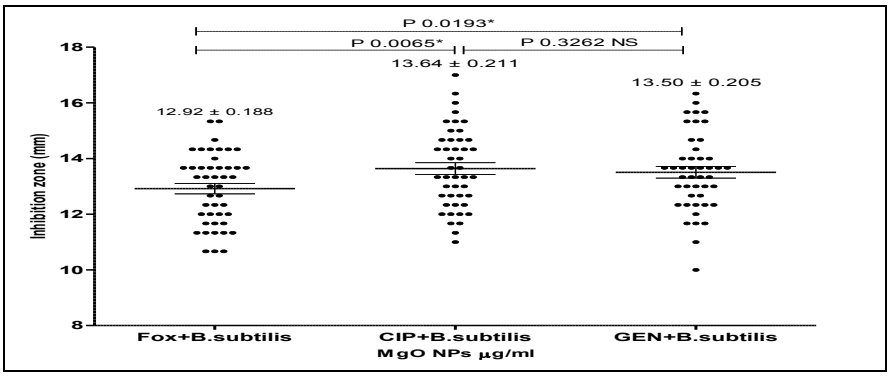

Fig. 8: Correlation between MgO NPs biosynthesized by *B. subtilis* with some resistance antibiotics

DISCUSSION

Staphylococcus aureus is one of the most common bacterial species found on the skin and mucous membranes of humans and animals. Moreover, it is an important opportunistic pathogen capable of causing multiple diseases, including skin and soft tissue infections, toxic shock syndrome, septic arthritis, endocarditis, and bacteremia[13].

The emergence and spread of antibiotic-resistant bacteria over the past decade represent a major concern for clinical and public health. Monitoring antimicrobial resistance patterns is particularly important at the national level for public health agencies[14].

β-lactamase is an enzyme produced by various bacterial species that confers resistance to β-lactam antibiotics by hydrolyzing the β-lactam ring, thereby eliminating the antibacterial activity of the molecule[15]. In *S. aureus*, the primary mechanism of β-lactam resistance is the expression of β-lactamase encoded by the blaZ gene, which hydrolyzes the β-lactam ring and renders the antibiotic inactive[16].

Aprevious study reported high levels of resistance in *S. aureus* isolates. [17] it found that all isolates were resistant to penicillin and cefoxitin (100%), with a 77.8% resistance rate for oxacillin. Similarly, a study reported 100% resistance to penicillin and cefoxitin and 81.92% resistance to oxacillin[18]. Resistance to fluoroquinolones in *S. aureus* arises from mutations in genes encoding DNA gyrase and topoisomerase IV, overexpression of efflux pumps, plasmid-mediated resistance, and decreased intracellular drug accumulation[19].

Not all bacteria are capable of synthesizing nanoparticles due to differences in metabolic processes and enzyme activity. Extracellular biosynthesis is often preferred over intracellular methods because of its efficiency and ease of nanoparticle recovery. Bacteria have the potential to precipitate nanoparticles either extracellularly or intracellularly through metabolic activities involving specific reducing enzymes, such as nitrate-dependent reductase or NADH-dependent reductase, which are likely involved in nanoparticle formation[20].

UV–Visible spectroscopy analysis of the synthesized MgO nanoparticles showed maximum absorbance at 285 nm, which corresponds to surface plasmon resonance and confirms the formation of MgO nanoparticles[21]. Field Emission Scanning Electron Microscopy (FESEM) analysis revealed the surface morphology and variations in particle size of the synthesized MgO nanoparticles. Larger nanoparticles were observed due to aggregation, which may occur because of cellular components on the nanoparticle surface acting as capping agents[22].

X-ray Diffraction (XRD) analysis showed distinct peaks at 2θ values of 21.345°, 36.9°, 42.85°, 62.22°, 74.55°, and 78.51°, corresponding to the (101), (111),

(200), (220), (311), and (222) planes, respectively. These peaks confirm the formation of a hexagonal MgO phase, in agreement with the standard data from JCPDS card no. 01-075-0447[23].

MgO nanoparticles were selected as potential antibacterial agents due to their nanoscale size and strong antimicrobial activity. The main antibacterial mechanisms of MgO nanoparticles include: physical damage to bacterial cell walls, entrapment of bacteria in aggregated nanomaterials, oxidative stress, disruption of bacterial glycolysis, DNA damage, and degradation of proteins and cell structures, which lead to release of metal cations and alkaline effects[24].

Previous studies demonstrated the antibacterial activity of MgO nanoflowers (MgO NFs) against *S. aureus* at concentrations of 25, 50, 100, 150, and 200 µg/mL, with the highest activity observed at 200 µg/mL, producing inhibition zones of 16 ± 0.5 mm[25]. Combining MgO nanoparticles with conventional antibiotics has emerged as a promising strategy to combat multidrug-resistant (MDR) *S. aureus*. This synergistic approach enhances antibiotic potency, overcomes resistance mechanisms, and reduces required dosages. MgO nanoparticles contribute via reactive oxygen species (ROS) generation, membrane disruption, and improved antibiotic delivery, enhancing the efficacy of existing antibiotics[26].

Other studies reported that the bactericidal efficacy of MgO nanoparticles increased when combined with antibiotics such as amikacin, cefazidime, and meropenem, resulting in inhibition zones of 15, 8, and 22 mm, respectively[27]. Additionally, antibiotics containing MgO nanoparticles exhibited significant antibacterial activity against microbial pathogens[28]. Furthermore, MgO nanoparticles, whether imported or locally synthesized, enhanced the antibacterial activity of gentamicin, levofloxacin, and ceftazidime at 200 µg/mL, leading to effective growth inhibition of resistant bacterial isolates[29].

CONCLUSION

This study successfully demonstrated the biosynthesis of magnesium oxide (MgO) nanoparticles using *B. subtilis* as an efficient and eco-friendly method. The synthesized MgO nanoparticles exhibited significant antibacterial activity, particularly against multidrug-resistant *Staphylococcus aureus* isolates from various clinical infections.

Authors' contributions: All authors have contributed in the design, analysis, and interpretation of data, drafting and revising of the manuscript, and they have approved the manuscript as submitted.

Availability of data: All data are included in the manuscript and any other data are available upon reasonable request.

Conflict of interest: This study has not been published before and is not under consideration in any other reviewed media. All authors report no conflict of interest relevant to this work.

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