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Magnesium oxide nanoparticles' antibacterial and antibiofilm-forming properties against MDR *P. mirabilis* isolated from various clinical infections

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Abstract:

Background: *P. Mirabilis* has been connected to several respiratory disorders in humans, skin, ear, eye, and gastrointestinal disorders. Due to the possibility of catheter-associated UTIs, this is particularly worrisome for patients receiving prolonged urine catheterization in an indwelling device. The unique ability of *P. mirabilis* is to form a special class of biofilms, which are usually crystalline. complicates these infections. Additionally, *P. mirabilis* resistance is growing more complex and serious every year, particularly the manifestation of resistance to carbapenem drugs, making disease prevention and control more difficult. **Objective**: The purpose is to look at how magnesium The impact of oxide nanoparticles on MDR *P. mirabilis* that has been isolated from various clinical infections. **Approach**: Individuals suffering from a range of illnesses, including blood infections, burns, urinary tract infections, and otitis media, yielded 120 isolates of P. mirabilis. The University of Kufa's Faculty of Science's Advanced Microbiology Laboratory received specimens from patients who were admitted from the Hospital. **Results:** The results showed that antibacterial susceptibility test and biofilm formation showed that 60 isolates of *P. mirabilis* are *MDR.* The results proved that MgO NPs are effective in inhibiting MDR *P. mirabilis* demonstrated the inhibitory of *P. mirabilis* progressively grows when increasing MgO NP and decreases biofilm formation with increasing MgO NP concentrations using various concentrations (100, 200, 300, and 400) µg/ml. **Conclusion:** showed inhibitory MDR *P. mirabilis* progressively grows with more MgO NPs when employing varying doses of MgO NPs, and reduces the production of biofilms as MgO NP concentrations rise.

Keywords: Clinical infection, MDR *Proteus mirabilis,* zinc oxide nanoparticles,

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1. Introduction

P. mirabilis facultative anaerobic, Gram- that is a member of the Enterobacilli family. Lactose cannot be fermented by it, although maltose can (1,2). Additionally, *P. mirabilis* demonstrates self-elongation, motility, and polysaccharide production. This facilitates attachment and easy motility along surfaces (such as medical equipment) when in contact with solid surfaces (3-5). *P. mirabilis's* flagella are responsible for this motility, which not only supports colonization but has also been linked to its capacity to form biofilms and is thought to contribute to resistance to host defenses and specific antibiotics (6-8). According to reports, antibiotic resistance happens when a medication can no longer effectively stop germs from growing; as a result, the bacteria become "resistant" and keep growing. when therapeutic levels of antibiotics are present (9,10). These bacteria are usually called resistant and multiply in an environment where antibiotics are

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present, whenever the antibiotics are effective against these microbes (11,12). However, when they become less sensitive or resistant, a higher concentration of the identical medication is required to produce an effect (13,14). Some strains of antibiotic-resistant enteric bacteria have been isolated. Gram-negative bacteria are inherently resistant to a number of kinds of antibiotics due to the existence of another molecule called OM that these antibiotics cannot penetrate, unlike Gram-positive bacteria (15,16).

2. Aim of this research: investigating the effectiveness of magnesium oxide nanoparticles against isolates of MDR Proteus mirabilis from various illnesses.

3. Materials and Methods

3.1: Specimen collection and bacterial identification

120 patients with various clinical conditions (such as blood infections, otitis media, UTIs, burn infections, wounds, and blood) who were hospitalized at the AL-Najaf Governorate's center for research between October 2024 and January 2025 were gathered and worked on. In order to prevent any potential contamination, all specimens were gathered carefully. They were then taken and sealed until they were moved to the University of Kufa's Faculty of Science's Advanced Microbiology Laboratory, where they were cultured on various media for 24 hours at 37°C to diagnose bacteria.

3.2: Ethics-based approval

In accordance with the legislation and the guidelines of human rights organizations, which provide sufficient information in an ethical way, the study's specimens obtained the consent of the young patients and the agreement of the adult patients.

3.3: Type of this study: Academic-Medical Research

3.4: Recognizing *P. mirabilis* isolated from various infections

The first diagnosis was made using biochemical testing, selective and differential culture medium, microscopic analysis, and bacterial colony characteristics (17).

3.5: Antibiotic susceptibility test for *P. Mirabilis*

It was performed according to Clinical Laboratory Standard Institute by using the Kirby-Bauer process on the Muller-Hinton agar according to (18). The antibiotic disk, as reported by Bio Analyses, a Turkish business.

3.6: Biofilm formation detection for isolates of *P. mirabilis*

Using microtiter plates, they took samples from the brain and heart, where a biofilm of P. mirabilis bacteria (BHI) was found. They took some of them and placed them in a microtiter plate, then added After adding 20 µl of the bacterial solution. It was incubated for 24 hours at 37°C. Following a water wash, each well received 200 µl of phosphate-buffered saline, which was then left for ten minutes. After that, the microtiter plate is cleaned with distal water, 200 µl of crystal violet dye is added to each well, and it is incubated for 15 minutes at 37 Cº. After that, it is thoroughly cleaned with distal water and dried, and finally, 99% ethanol is added. A spectrophotometer with an optical density (OD) of 0.5 and a wavelength of 630 nm is used to measure the findings after alcohol has been added to each well (19,20).

3.7: MgO NPs' Antibacterial Activity

Using Agar well diffusion techniques, the antibacterial properties of MgO NPs were evaluated against MDR *P. mirabilis* isolated from various illnesses. After bacteria were added to the Mueller-Hinton agar plate using cotton dipped in the solution, magnesium oxide nanoparticles (80 µL) at the 100, 200, 300, and 400 µg/mL concentrations were introduced into 5 mm holes created with a sterile cork enrichment tool. After the Petri dishes were incubated for 24 h at 37°C, the diameter of the

growth inhibition zones was measured with a metric ruler (21).

3.8: Magnesium oxide nanoparticles' antibiofilm activity against MDR P. mirabilis isolates at varying concentrations

Results showed that magnesium oxide NPs expressed high anti-biofilm activity via the plate method against MDR *P. mirabilis* isolates using different concentrations of Magnesium NPs (100, 200, 300, and 400) µg/ml (21).

3.9: Statistical analysis

Graph Pad Prism version 6 was the computer program used to do statistical analysis, and for each data point, a mean value and standard error (SE) were determined. For the statistical analysis, P values less than 0.05 were deemed statistically significant.

4: RESULTS

4.1: Distribution of patient specimens for different infections

The patients specimens of different infections were obtained from hospitals and specialized centers in Najaf province, a total of specimens 120 (100%), which included urine specimens 60(50%), burn swabs 15(12.5%), wound infections 15(12.5%), ear swabs 15(12.5%), Blood infections 15(12.5%), as shown table (1).

On the other hand, 60 (63.15 %) of *P. mirabilis* isolates where found in different clinical cases included 30 (31.57 %) isolates in urine, 15(15.78 %) isolates in burn swabs, 5(5.26 %) isolates in wound infections, 8(8.42%) isolates in ear swabs, 2(2.10 %) isolates from the blood, as shown in table (2).

4.2: Identification of *P. mirabilis* isolated from different infections

Initial identification of *P.mirabilis* isolates based on morphological characteristics of the colonies on blood agar, CHROM agar, Xylose lysine deoxycholate agar (XLD), and MacConkey agar In the MacConkey agar medium, Proteus does not ferment lactose and produces smooth, pale, or colorless (NLF) colonies, however on the Blood agar plate, it grows in successive waves to create a thin filmy layer of concentric circles (swarming) and without hemolysis. The colonies of *P.mirabilis* appeared as light brown colonies on CHROM agar, and then differentiated these bacteria from *E.coli* colonies. All the isolates were cultured on Xylose lysine deoxycholate agar (XLD). *P.mirabili*s appeared red colony on XLD agar as shown in Figure 1.

4.3: Biochemical tests of bacterial species

Table 3 shows the results of the biochemical tests of *P. mirabilis*. The biochemical tests include the catalase test, methyl red test, motility test, production of Indole, Urease test, TSI (triple sugar iron agar), Voges-Proskauer test, Citrate, and Oxidase test.

4.4: Antibiotic susceptibility of *P. mirabilis* isolates

Antibiotic susceptibility testing was performed on 60 resistant isolates of P. mirabilis using the disk diffusion method using 14 antibiotics, and the results were calculated based on the diameter of the inhibition zone, as shown in the table. (4)

Table (1): Number and percentage of specimens collected from different infections

Type of Specimens	NO. of Specimens	Percentage (%)
Urine Specimens	60	50%
Burn Swabs	15	12.5%
Blood infection	15	12.5%
Otitis media Swabs	15	12.5%
wound infections	15	12.5%
Total	120	100%

Table (2): Number and percentage of *P. mirabilis* isolated from different infections

Type of Specimens	No. of Specimens	Male	Percentage%	Female	Percentage %	Total Percentage%
Urine Specimens	30	10	10.52 %	20	21.05%	31.57%
Burn Swabs	15	8	8.42%	7	7.36%	15.78 %
wound infections	5	3	3.15%	2	2.10%	5.26 %
Ear Swabs	8	5	5.26%	3	3.15%	8.42 %
Blood infections	2	1	1.05%	1	1.05%	2.10 %
Total	60	27	28.42%	33	34.73	63.15%

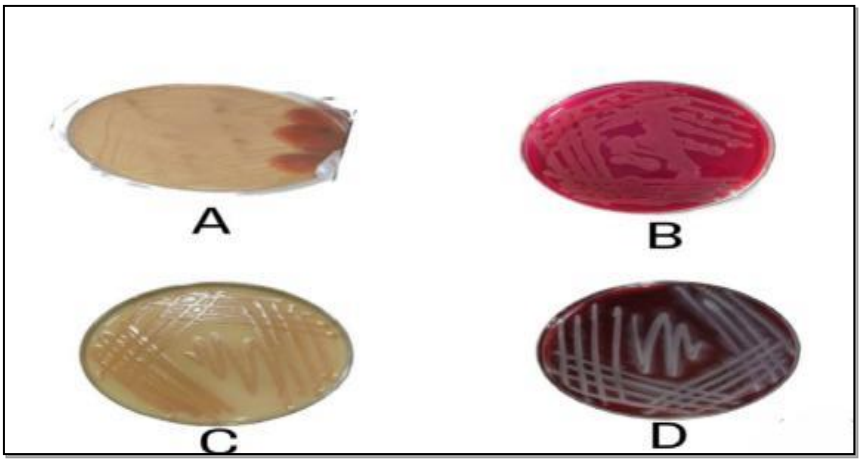

Figure 1: *P. mirabilis* on different culture media (A) MacConkey agar, (B) XLD agar, (C) CHROM agar, and (D) Blood agar.

Table 3: Biochemical test of bacterial species

Bacterial species	Catalase	**Voges-Proskauer**	**Indole** Production	**Methyl Red**	**Urease**	**Citrate**	**Motility**	**TSI**	**Oxidase**
P. mirabilis	+	-	-	+	+	+	+	**K/A + H_2S**	-

Table 4: Antibiotic susceptibility test for 60 *P. mirabilis* isolates

Antibiotic disk	**No.(%) of isolates exhibited**	
	Resistance	**sensitive**
Tetracycline	60/60(100%)	0/60(0%)
Doxycycline	59/60(98%)	1/60(1%)
Gentamycin	58/60(96%)	2/60(3%)
Nalidixic Acid	58/60(96%)	2/60(3%)
Tobramycin	54/60(90%)	6/60(10%)
Ceftazidime	54/60(90%)	6/60(10%)
Ceftriaxone	45/60(75%)	15/60(25%)
Ciprofloxacin	52/60(86%)	8/60(13%)
Trimethoprime-sulfamethaxazol	52/60(86%)	8/60(13%)
Amoxicillin-clavulanic	48/60(80%)	12/60(20%)
Cefotaxime	45/60(75%)	15/60(25%)
Impenem	6/60(10%)	54/60(90%)
Meropenem	2/60(3%)	58/60(96%)
Amikacin	0/60(0%)	60/60(100%)

4.5: Detection of Biofilm Formation by Congo Red Agar Method (CRA)

After detection of *P. mirabilis* that formed films by the phenotypic method, which usually includes the Congo red agar method, 100% of which were P. mirabilis. 45 (75%) were biofilm-producing when the CRA plates showed black, dried, crystalline colonies, and 15 (25%) were not biofilm-producing when the *P. mirabilis* colonies stayed pink or red in hue, figure (2)

4.6: *P. mirabilis* biofilm development using the Microtiter Plate Assay

The findings demonstrated that some P. mirabilis isolates have' ability to build biofilms. As indicated in the table, the microtiter plate assay (MPA) identified 60 (100%) isolates of P. mirabilis that were strong biofilm producers, 30 (50%) and 20 (37.5%) that were moderate, and 10 (12.5%) that were weak. (5)

4.7: MgO NPs' Antibacterial Activity

4.7.1: The effect of imported magnesium oxide nanoparticles against bacteria

The antibacterial qualities of imported magnesium oxide nanoparticles were assessed using the agar well technique against drug-resistant *P. mirabilis* that was obtained from a variety of diseases. A cotton swab soaked in a filter was used to dust the whole Mueller-Hinton surface. Next, using a bottle with a sterile cork, 80 µL of magnesium oxide nanoparticles were introduced at (one hundred, two hundred, three hundred, four hundred µg/ml), usually with a diameter of 5 µm. The Petri plates were cultured for twenty-four hours at 37°C, and the breadth of the growth inhibition zones was measured using a metric ruler. The results demonstrated that the inhibitory zone of P. mirabilis progressively expanded with the rise in dosages (100, 200, 300,

and 400) µg/ml. of concentrations of magnesium oxide nanoparticles, Figure (3), so that the 400 µg/ml concentration in the inhibitory zone was greater than the 300 µg/ml concentration and, ultimately, higher than the 100 µg/ml concentration. Consequently, the ideal concentration for inhibiting magnesium oxide nanoparticles (µg/ml) was 400 µg/ml, which was the concentration in the inhibition zone.

4.7.2: Study of the effect of synthetic magnesium oxide nanoparticles

The findings demonstrated that P. mirabilis was inhibited at several doses of 100, 200, 300, and 400 µg/ml, and that this inhibition progressively increased with the local magnesium oxide nanoparticles. The inhibition zone at 400 µg/ml was higher than that at 300, and finally, higher than the inhibition zone at 200 µg/ml. Consequently, the 400 µg/ml inhibitory zone was the ideal concentration for blocking the local magnesium oxide nanoparticles, which is µg/ml. As seen in the image, the inhibitory zone for the four local magnesium oxide nanoparticle concentrations was likewise smaller than that of the imported magnesium oxide nanoparticles. (4).

4.7.3: The combined impact of domestic and imported magnesium oxide nanoparticles on the majority of antibiotic resistance

The results showed that the presence of imported and domestic MgO NPs together with the some resistance antibiotic resulting in increase of the inhibition zone compared with the presence of imported MgO NPs alone as well as the case of the presence domestic MgO NPs alone with these antibiotic, as shown in Figures (5,6,and7), as well as, the statistical analysis, the results showed significant differences in the case of mixing both imported and domestic MgO NPs with antibiotics, compare with the case of imported and domestic MgO NPs alone with antibiotics.

4.8: Antibiofilm activity of different concentrations of magnesium oxide NPs against MDR *P. mirabilis* isolates

The results showed that MgO NPs expressed high anti-biofilm activity via the plate method against MDR *P. mirabilis* isolates using different concentrations of silver NPs (100, 200,300, and 400) µg/ml, as shown in Table 7.

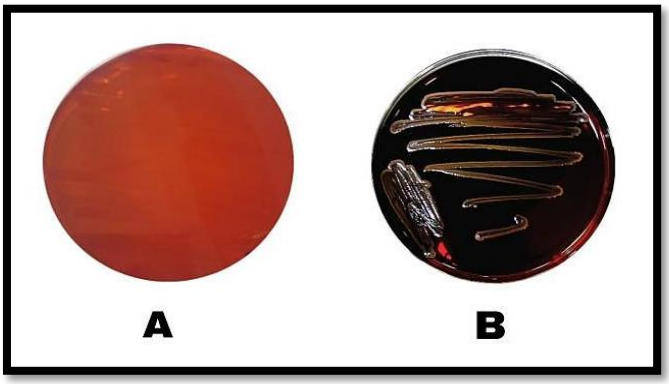

Figure 2: Biofilm formation by some *P. mirabilis* isolates (A) without Biofilm formation and (B) with Biofilm formation.

Table 6: Distribution of P. mirabilis isolates according to the development of biofilms

Type Biofilm	NO. of *P.mirabilis*
Strong(> 0.240 ± 0.022)	30(50%)
Moderate(0.120-0.240 ± 0.020)	20(37.5%)
Weak(< 0.12 0 ± 0.012)	10 (12.5%)
Total	60 (100%)

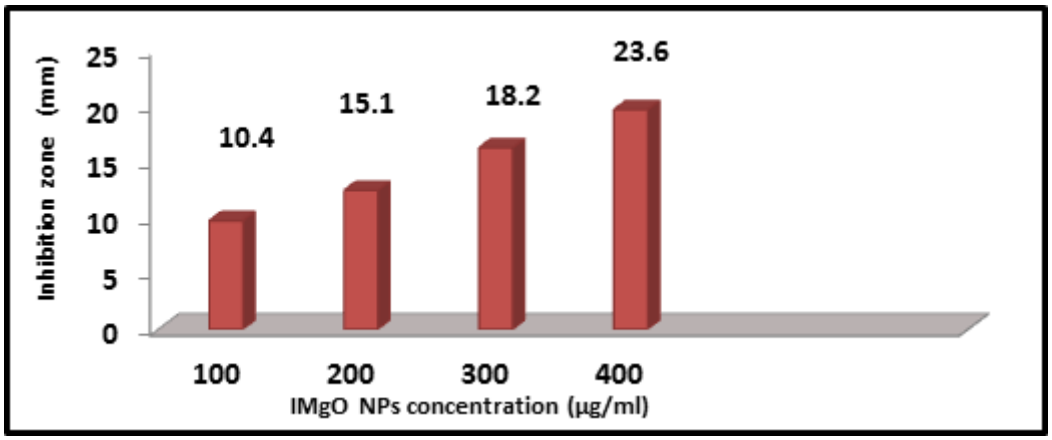

Figure 3: Impact of Varying Imported MgO NP Concentrations on MDR P. mirabilis Isolates

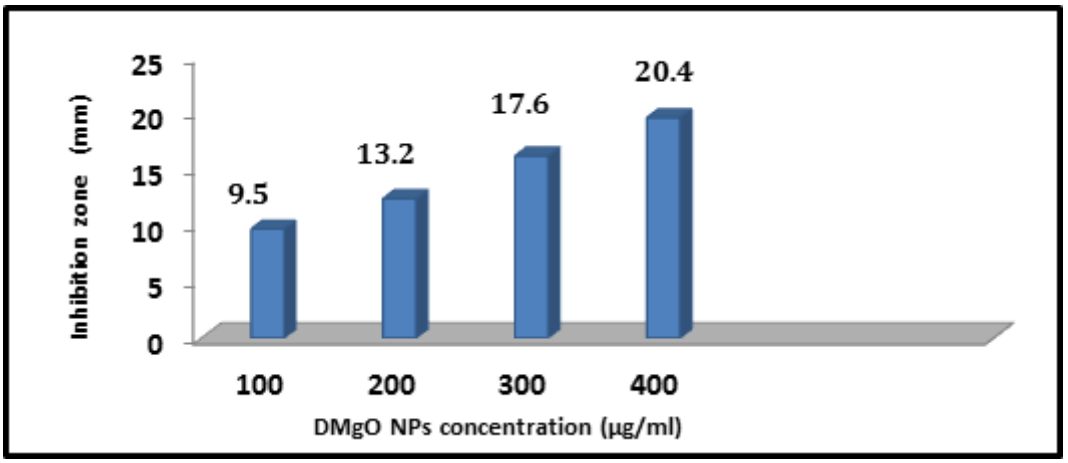

Figure (4): Impact of Varying Domestic Magnesium Oxide NP Concentrations on MDR P. mirabilis Isolates

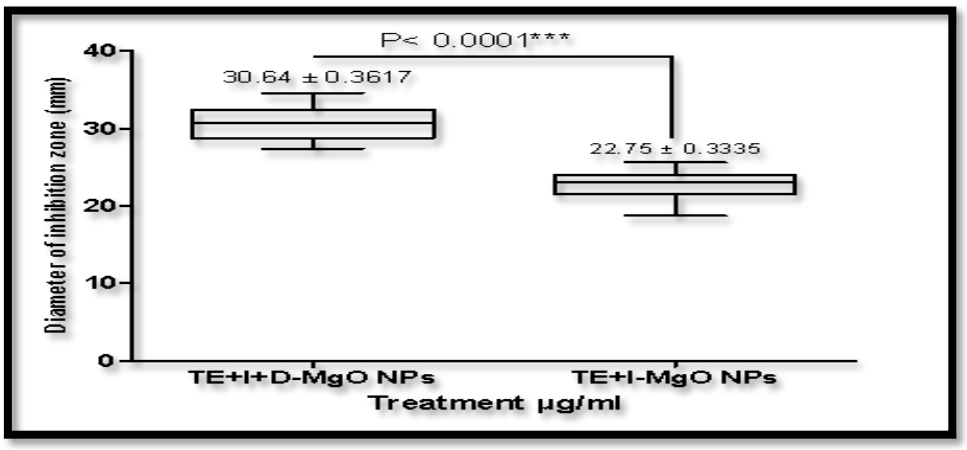

Figure (5): The combination effect of (TE+I+D MgO) NPs and (TE + I MgO) NPs on *P.mirabilis* inhibition zone

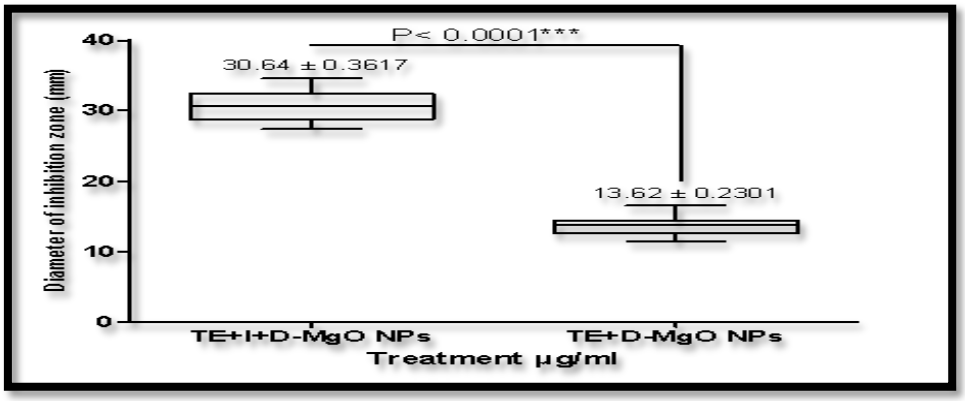

Figure (6): The combination effect of (TE+I+D MgO) NPs and (TE + D MgO) NPs on *P.mirabilis* inhibition zone

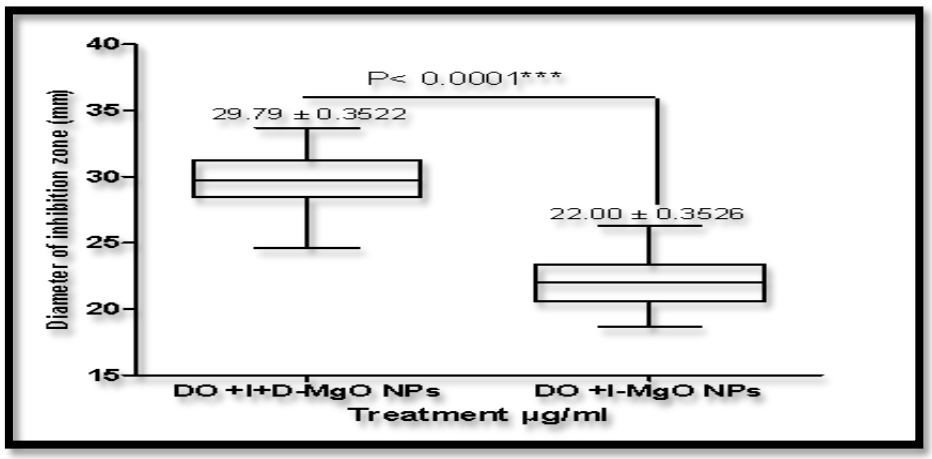

Figure (7): The combination effect of (DO+I+D MgO) NPs and (DO + I MgO) NPs on *P.mirabilis* inhibition zone

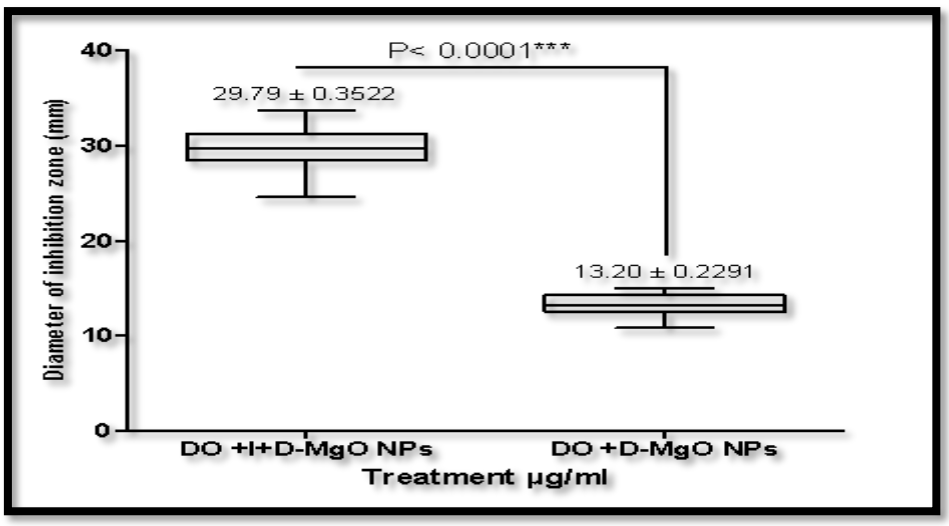

Figure (8): The combination effect of (DO+I+D MgO) NPs and (DO + D MgO) NPs on *P.mirabilis* inhibition zone

Table 7: Antibiofilm activity of MgO NPs by absorbance at 630 nm

No. of *P. mirabilis* isolates	Absorbance of MgO NPs (µg/ml)			
	100	200	300	400
1	0.182	0.128	0.121	0.094
2	0.200	0.178	0.124	0.089
3	0.159	0.158	0.145	0.080
4	0.193	0.137	0.100	0.077
5	0.199	0.178	0.151	0.121
6	0.211	0.188	0.143	0.136
7	0.186	0.166	0.108	0.100
8	0.157	0.146	0.096	0.093
9	0.187	0.166	0.140	0.122
10	0.149	0.139	0.130	0.111
11	0.201	0.177	0.159	0.148
12	0.150	0.141	0.110	0.103
13	0.193	0.167	0.145	0.134
14	0.129	0.120	0.105	0.077
15	0.217	0.189	0.180	0.157
16	0.196	0.178	0.149	0.138
17	0.170	0.169	0.143	0.129
18	0.204	0.194	0.176	0.150
19	0.158	0.140	0.107	0.099
20	0.187	0.159	0.137	0.121
21	0.192	0.187	0.155	0.137
22	0.180	0.162	0.135	0.120
23	0.207	0.194	0.170	0.133
24	0.160	0.144	0.123	0.115
25	0.179	0.163	0.131	0.109
26	0.195	0.181	0.152	0.141

DISCUSSION

P. mirabilis, an opportunistic pathogen linked to a number of human disorders affecting the respiratory, gastrointestinal, ocular, ear, and skin systems, The results of P. mirabilis's biochemical tests, which included the catalase, methyl red, motility, production of indole, urease, H2S, Voges Proskauer, citrate, and oxidase tests, revealed that all of the bacteria tested positive for the detection of the catalase, methyl red, citrate utilization, urease, and motility tests, but negative for oxidase., Proskauer and Indole Voges. Additionally, the triple sugar iron (TSI) test revealed that K/A = acid/alkaline with gas generation (22,23) and positive hydrogen sulfide (H2S).

60 resistant *P. mirabilis* isolates were subjected to an antibacterial susceptibility test; the findings were interpreted based on the width of the inhibition zone and contrasted with the standard inhibition established by (24). The treatment of infections is made more difficult by antibiotic resistance in P. mirabilis strains. As a result of the resistant strains' rapid increase and the inadequacy of present treatments, new antibiotic targets must be developed immediately (25). Numerous Gram-positive and Gram-negative bacterial infections can be treated with tetracycline antibiotics; however, high rates of tetracycline resistance in Enterobacteriaceae have been reported. *P. mirabilis* has a natural resistance to tetracycline, which may be the main factor contributing to its growing tolerance. New antibiotics must be developed in order to treat bacterial infections due to the increase in acquired resistance of Enterobacteriaceae (26).

One of the most widely used medication classes, beta-lactam antibiotics, has a variety of medicinal applications. Their advent in the 1930s fundamentally altered the strategy for combating infectious bacterial illnesses. These antibiotics work through the following mechanisms: By inhibiting transpeptidase, an enzyme that helps peptides cross-link to form peptidoglycan, beta-lactam antibiotics prevent the last stage of peptidoglycan production. Mucilage, sometimes referred to as peptidoglycan, is a crucial part of the bacterial cell wall that gives both Gram-positive and Gram-negative membranes their

mechanical stability. Penicillin-binding proteins are the target of beta-lactam antibiotics. The terminal transpeptidase pathway is then disrupted by binding, which results in lysis and viability failure. The most significant resistance mechanism in Gram-negative bacilli is beta-lactamases, which use the bacterial cell's autolytic pathways. These enzymes have been categorized in a variety of ways according to their β-lactam antibiotics' amino acid composition and hydrolytic action as genetic technologies have grown in popularity (27,28). While plasmid-mediated enzymes are often constitutively produced, chromosomal beta-lactamases are typically the source of inducible beta-lactamase production in Gram-negative bacteria. Promoters that are expressed in genes that come before it frequently control this hydrolytic impact (29). Furthermore, in patients with functional or anatomical issues, it frequently results in complex UTIs; this is especially problematic for those who have more time with urinary catheters that are indwelling, as they might result in catheter-associated UTIs. P. mirabilis's special capacity to produce crystalline biofilms, which ultimately cause catheters to become coated and obstructed, complicates these infections (30). Concern over the rise and dissemination of bacteria resistant to antibiotics, which can cause major clinical and public health issues, has grown within the last ten years. Public health agencies must keep an eye on and report any changes in isolates that are resistant to antibiotics. The overuse, underuse, and abuse of antibiotics have become the primary sources of antibiotic resistance in bacteria, which is a significant clinical issue in the treatment of diseases (31). One of the main clinical issues in treating infections is antibiotic resistance. The high prevalence of antibiotic resistance in hospitals and the general population has been caused by a number of reasons, such as inadequate monitoring, overprescribing, and over-the-counter access. A number of illnesses with high morbidity and death rates from hospital-acquired and nonhospital-acquired infections are caused by *P. mirabilis*, one of the most significant opportunistic multidrug-resistant (MDR) Gram-negative bacteria (32). Urinary tract infections linked to catheter usage are frequently caused by P. mirabilis. Its capacity to induce these illnesses is mostly due to biofilm formation on catheter surfaces. To create biofilms, *P. mirabilis* produces a variety of virulence factors. Adhesion proteins, quorum-sensing molecules, lipopolysaccharides, efflux pumps, and urease are a few examples of these variations. *P. mirabilis* biofilms that form on catheter surfaces have a unique crystalline structure because of their urea-degrading biomineralization. Catheter crusting and blockage are prevalent, as are ascending urinary tract infections and urine retention. Bacteria become very resistant to the immune system and traditional antimicrobials when they grow into crystalline biofilms (33). The way that magnesium oxide nanoparticles work has been used to assess their antimicrobial action against bacteria that are both Gram-positive and Gram-negative. Magnesium oxide nanoparticles' antimicrobial properties entail a number of procedures, Reactive oxygen species production, for instance, the alkalizing effect, and the interaction of nanoparticles with bacteria that damages the bacterial cell (34). The effectiveness of magnesium oxide nanoparticles as antibacterials against Pseudomonas aeruginosa was shown in other research by (35). As the quantity of imported magnesium oxide nanoparticles increased, the inhibition zone progressively grew. By specifically targeting a single bacterial cell, nanoparticles can increase the effectiveness of antibacterial medications and stop resistance from developing (36). Many nanoparticles, such as magnesium oxide nanoparticles, are potential biopolymeric nanoparticles with superior physical, chemical, and biological properties that can be used to treat bacterial infections. Magnesium oxide has multiple uses in tissue engineering, drug delivery, water treatment, antibacterial agents, and packaging. One of its distinguishing properties is quantum size effects (37,38).

CONCLUSIONS

P. mirabilis. It is most frequently isolated from urinary tract infections, but it has also been found in individuals with burns, blood infections, wound infections, ear infections, and other illnesses. The MDR *P. mirabilis* isolates which the highest resistance rate was seen with Tetracycline (Te) (followed by Doxycycline (Do), while low resistance Amikacin (AK). The results, using various magnesium oxide nanoparticle concentrations, showed that the inhibitory zone for *P. mirabilis* bacteria progressively grows as the concentration of magnesium oxide nanoparticles rises.

Conflict of interest: NIL

Funding: NIL

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