Antibacterial Activity of Magnesium Oxide Nanoparticles against MDR *Pseudomonas aeruginosa* Isolated from Different Clinical Infections

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**Abstract.** In this study, 180 isolates of *Pseudomonas aeruginosa* were isolated from patients suffering from various infections, including urinary tract infections, burns, ear infections, tonsillitis, and pneumonia. Specimens were taken from patients hospitalized in Al-Hakim General Hospital, Al-Sadr Medical City, and the Burn Center in Najaf Al-Ashraf, and they were transferred to the Microbiology Laboratory in the Biology Department in the College of Science. *P. aeruginosa* have been isolated from Specimens taken from patients with these various infections. Isolation methods were different, including culture on MacConkey medium, blood agar, as well as various biochemical and Vitek tests. Susceptibility testing was also performed on these bacteria for six families according to CLSI. These families are penicillins, aminoglycosides, carbopenems, cephalosporins, fluoroquinins, and lipopeptides. It was noted that forty isolates were multi-resistant to these antibiotics. It was also revealed that resistant isolates formed biofilms using a flat microliter. The effectiveness of domestic and imported magnesium oxide nanoparticles on resistant isolates was conducted. These two types were taken at concentrations of 100, 150, and 200 μg/ml. Imported MgO nanoparticles were more effective than domestic ones. It was also observed that the effect of magnesium oxide nanoparticles on resistant bacteria increased with increasing concentration. Antibiotics resistant to *Pseudomonas aeruginosa* bacteria were also taken and mixed with imported and local magnesium oxide nanoparticles with an optimal concentration of (200) μg/ml. The effect of magnesium oxide nanoparticles combined with antibiotics was greater than if the nanoparticles were alone.

1 Introduction

*Pseudomonas aeruginosa* is a Gram-negative opportunistic bacterium, able to adapt to difficult conditions and cause infectious diseases, such as cystic fibrosis, chronic wound and nosocomial infections, some infections can have high mortality due to its inherent and acquired resistance to broad-spectrum antibiotics [1]. Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively, Due to the

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antibiotic resistance developed by the bacterial species, therefore it became necessary to search for suitable alternatives to kill these species. Nano biotechnology is a modern branch of science that deals with the production and application of nanoparticles (NPs), after tremendous research effort, a new branch of science originated, known as “Nanotechnology” [2]. In the modern period, nanoparticles have a wide range of applications in the field of medicines, pharmacology, information technology, energy, environment, aerospace science [3,4]. Magnesium oxide used as semi conducting materials, catalyst in organic transformations, sorbent for organic and inorganic contaminants from wastewater and it also, possess good antibacterial, anticancer and antioxidant properties [5].

2 Materials and Methods

2.1 Specimens collection and bacterial identification

180 specimens were collected from patients with various injuries, including urinary tract infection, pneumonia, ear infection, tonsillitis, and burns. These samples were collected from patients in Al-Sadr Medical City, Al-Hakim General Hospital, and the burn center in Najaf Governorate during the period from (October 2022 to January 2023). All specimens were collected in a way to avoid any possible contamination, and the specimens were taken and closed until they were transferred to the Advanced Microbiology Laboratory / College of Science / University of Kufa and cultured on different media for 24 hours at a temperature of 37 degrees Celsius for bacterial diagnosis.

2.2 Antibiotics disk

The antibiotics disk according to the company Bio analyses / Turkey Which used in this study are listed in table (1).

<table>
<thead>
<tr>
<th>NO.</th>
<th>Antibiotics class</th>
<th>Antibiotics</th>
<th>Symbol</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Penicillins</td>
<td>Piperacillin</td>
<td>PIP</td>
<td>100 µg</td>
</tr>
<tr>
<td>2</td>
<td>Cephalosporins</td>
<td>Cefepime</td>
<td>FEP</td>
<td>30 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidem</td>
<td>CAZ</td>
<td>30 µg</td>
</tr>
<tr>
<td>3</td>
<td>Carbapenems</td>
<td>Doripenem</td>
<td>DOR</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem</td>
<td>IPM</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meropenem</td>
<td>MEM</td>
<td>10 µg</td>
</tr>
<tr>
<td>4</td>
<td>Aminoglycoside</td>
<td>Gentamicin</td>
<td>GEN</td>
<td>10 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Netilmicin</td>
<td>NET</td>
<td>30 µg</td>
</tr>
<tr>
<td>5</td>
<td>Fluroquinolones</td>
<td>Levofoxacin</td>
<td>LEV</td>
<td>5 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofoxacin</td>
<td>CIP</td>
<td>5 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gatifloxacin</td>
<td>GAT</td>
<td>5 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ofloxacin</td>
<td>OFX</td>
<td>5 µg</td>
</tr>
<tr>
<td>6</td>
<td>Lipopeptides</td>
<td>Colistin</td>
<td>CST</td>
<td>10 µg</td>
</tr>
</tbody>
</table>
3 Methods

3.1 Antibiotic Susceptibility Test for *P. aeruginosa* Isolates

Muller Hinton agar was prepared, it is sterilized in the autoclave and poured in petri dishes, then antibiotic resistance *p. aeruginosa* isolates were streaked by sterile swab on petri dish and placed antibiotics disc and incubated the dishes at 37 °C for 24 h, the diameter of inhibition zones was measured using a meter ruler [6].

3.2 Detection of biofilm formation for *p. aeruginosa* isolates

Biofilm was detected in the bacteria *P. aeruginosa* using a flat plate microtitre containing a 96 wells, where 200 microliters of Brain Heart Broth Infusion (BHBI) was taken and placed in each well of the microtitre plate, then 20 μl of the bacterial suspension was added to it, then incubated at a temperature of 37 °C for a period of 24 h, then it is washed with distal water, then phosphate buffer saline is added to each well in an amount of 200 μl and left for 10 minutes, then the microtiter plate is washed with distal water, then the crystal violet dye is added to each well with amount of 200 μl and incubated in the incubator at a temperature of 37 °C for 15 min, then it is washed well with distal water and dried well, then 99% ethanol alcohol is added to each well, then it is read using a spectrophotometer at an optical density (OD) of 0.5 with a wavelength of 630 nm [7].

3.3 Antibacterial Activity of MgO Nanoparticles (NPs)

3.3.1 Antibacterial Activity of Imported MgO NPs

The antibacterial activities of imported MgO NPs were tested using Agar well diffusion methods against MDR *P. aeruginosa* isolated from different infections. The dipping cotton swab was used to streaking the entire surface of a Mueller Hinton agar tray. Then, using a sterile cork Poorer, Pores (5 mm) were created and filled with imported MgO NPs (80ul) in three concentrations (100, 150 and 200 μg/ml). The Petri-dishes were then incubated at 37°C for 24 h. The diameter of the growth inhibition zones was measured using a meter ruler [8].

3.3.2 Antibacterial Activity of Domestic MgO NPs

This test is agreed in the same manner described in paragraph (3.3.1) excluding the use of Domestic MgO NPs.

3.3.3 Antibacterial Activity of Imported MgO with Domestic MgO NPs

The optimum concentration of imported MgO NPs (200 μg/ml) and optimum concentration of domestic MgO NPs (200 μg/ml) mix together, they were tested using Agar well diffusion methods against MDR *P. aeruginosa* isolated from different infections. The Petri-dishes of a Mueller Hinton agar were then incubated at 37°C for 24 h. The diameter of the growth inhibition zones was measured using a meter ruler [9].

3.3.4 The Relationship between Imported MgO NPs with some Resist Antibiotics
Antibacterial action of imported MgO NPs with some resist antibiotics against resistance *P. aeruginosa* isolates by disc diffusion method using MHA. the optimum concentration of imported MgO NPs (200 µg/mL) mix with some resist antibiotics (imipenem, gentamycin, ceftrazidime) discs were used. The size of the zone of inhibition for *P. aeruginosa* isolates were measured using a meter ruler [10].

3.3.5 The Relationship between Domestic MgO NPs with some Resist Antibiotics
This test is agreed in the same manner described in paragraph (3.3.4).

4 Results and Discussion

4.1 Antibiotic Susceptibility for *P. aeruginosa* isolates
Because of the *P.aeuroginosa* is most frequent than the other bacterial species, antibacterial susceptibility test was conducted for 40 resist *P.aeuroginosa* isolates against 13 commonly used antibacterial agents by using the disk diffusion method, the results were interpreted according to the diameter of inhibition zone and compared with stander zones of inhibition determined by CLSI (2023), the results showed that *P. aeuroginosa* isolates has a great resistance to most commonly antibiotics used in treatment of different infections, the highest rate of resistance is seen with Gentamicin 36/40 (90%) followed by Piperacillin 30/40(75%), Ceftazidem 24/40(60%), Levofloxacine 24/40 (60%), Ciprofloxacin 20/40 (50%), Ofloxacin 20/40(50%), Gatifloxacine 16/40 (40%), Imipenem 12/40(30%), whereas the low rate of *P.aeuroginosa* within Colistin 0/40 (0%), Netilmicin 4/40(10%), Meropenem 2/40 (5 %), Doripenem 4/40 (10 %) and Cefepime 4/40(10 %) (Table 2).

**Table 2.** Antibiotic Susceptibility for *P. aeruginosa* isolates.

<table>
<thead>
<tr>
<th>NO.</th>
<th>Antibiotics class</th>
<th>Antibiotics</th>
<th>No. (%) of isolates exhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Resistance</td>
</tr>
<tr>
<td>1</td>
<td>Penicillins</td>
<td>Piperacillin</td>
<td>30/40(75%)</td>
</tr>
<tr>
<td>2</td>
<td>Cephalosporins</td>
<td>Cefepime</td>
<td>4/40(10 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidem</td>
<td>24/40(60%)</td>
</tr>
<tr>
<td>3</td>
<td>Carbapenems</td>
<td>Doripenem</td>
<td>4/40 (10 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem</td>
<td>12/40(30%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meropenem</td>
<td>2/40 (5 %)</td>
</tr>
<tr>
<td>4</td>
<td>Aminoglycoside</td>
<td>Gentamicin</td>
<td>36/40 (90%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Netilmicin</td>
<td>4/40(10%)</td>
</tr>
<tr>
<td>5</td>
<td>Fluroquinolones</td>
<td>Levofloxacine</td>
<td>24/40 (60%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>20/40 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gatifloxacine</td>
<td>16/40 (40%)</td>
</tr>
</tbody>
</table>
Beta-lactamase is an enzyme produced by several bacteria that provide resistance to β-lactam antibiotics such as penicillin, cephemycin, and carbapenem. β-lactamase provides antibiotic resistance by disrupting the structure of the antibiotic, and hydrolysis causes the β-lactamase enzyme to open the β-lactam ring, abolishing the antibacterial properties of the molecule [11].

Cephalosporins are broad-spectrum antimicrobial agents similar to penicillins, which have a β-lactam ring that inhibits synthesis of cell wall bacteria by binding to penicillin binding proteins (PBPs), eventually leading to cell lysis and death. The cephalosporins are chemically distributed into two types: oximinocephalosporins and methoxy cephalosporins. They are classified by classes of 5 generations focused on antibacterial activity [12].

Resistance to aminoglycosides is due to P. aeruginosa production of modified enzymes such as phosphotransferase and N-acetyl transferase, and the genes for these enzymes are carried on the plasmid or chromosome [13]. In addition, resistance also occurs due to a change in membrane permeability or the occurrence of chromosomal mutations [14].

As for colistin, the bacteria did not show any resistance to it (0%). This may be due to the lack or non-use of this drug in bacterial infections, and this is due to its danger and toxicity [15].

### 4.2 The ability of P. aeruginosa to biofilm formation by Microtiter Plate Flat

The results showed that the capacity of some P. aeruginosa isolates to biofilm formation. The microtiter plate assay (MPA) detected 67% (40/60) isolates as biofilm producers, from the 40 (100%) isolates of P. aeruginosa strong biofilm producers 20(50%), 15(38%) were moderate and 5 (13%) were weak. As shown in tables (3) and figure (1).

#### Table 3. Distribution of P. aeruginosa isolates on the basis of biofilm production.

<table>
<thead>
<tr>
<th>Type</th>
<th>NO. of P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>20 (50%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>15 (38%)</td>
</tr>
<tr>
<td>Weak</td>
<td>5 (13%)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (100%)</td>
</tr>
</tbody>
</table>
Biofilms are adherent populations of microorganisms that secrete a biochemical and physical matrix for defense, reinforcement, and survival; this matrix acts as a semipermeable membrane, preventing the dissemination of molecules such as quorum-sensing molecules and antibiotics from reaching planktonic microbes, by slowing the inflammatory and maturation processes of chronic wound healing, biofilms have an effect on chronic wound healing [16]. The ability of a microorganism to form biofilm is an important virulence factor as it establishes a protective environment for the organisms to survive and evade antibiotics, these biofilms are the main cause of many chronic infections such as diabetic foot ulcers, and they pave the way for the re-emergence of multidrug-resistant strains and result in treatment failure [17]. Other studied reported by [18] they indicated that from 126 (100%) isolates of *P. aeruginosa* 95 (80%) were produced biofilms.

### 4.3 The effect of different concentrations of magnesium oxide nanoparticles in antibiotics resistance *p. aeruginosa* isolates growth

#### 4.3.1 Imported Magnesium Oxide Nanoparticles

The results, using different concentrations (100, 150, 200) μg/ml, indicated that the inhibition zone for *P. aeruginosa* bacteria gradually increases with increasing concentrations of imported magnesium oxide nanoparticles, figure (4-3), so that the inhibition zone with a concentration of 200 μg/ml was higher than the inhibition zone with a concentration of 150 and the last, it was higher than the inhibition zone with a concentration of 100 μg/ml, and therefore the inhibition zone with a concentration of 200 μg/ml represented the optimal concentration of inhibition for magnesium oxide nanoparticles μg/ml, as is clear in figure (2).

The antimicrobial action of magnesium oxide nanoparticles might be different according to several intrinsic factors including magnesium oxide source, the molecular weight that influences the penetration inside microorganisms, and the synthesis of new magnesium oxide derivatives with novel characteristics that usually improve the antimicrobial action of magnesium oxide, the principal mechanisms have been posited to elucidate magnesium oxide interaction with various kinds of microorganisms, which vary based on the cell wall structure and metabolic process, also the purify of magnesium oxide action may effects on microorganism [19].
This result agrees with [20] when they exposed that magnesium oxide NPs were effective antibacterial against *P. aeruginosa* so that inhibition zone gradually increases with increasing concentrations of imported magnesium oxide nanoparticles.

![Image](image1.png)

**Fig. 2.** Effect of Different Concentrations of Imported Magnesium Oxide NPs against MDR *P. aeruginosa* isolates.

![Image](image2.png)

**Fig. 3.** Effect of Different Concentrations of Imported Magnesium Oxide NPs on MDR *P. aeruginosa* isolates.

### 4.3.2 Domestic Magnesium Oxide Nanoparticles

The results, using different concentrations (100, 150, 200) μg/ml, indicated that the inhibition zone for *P. aeruginosa* bacteria gradually increases with increasing concentrations of domestic magnesium oxide nanoparticles figure (4-5), so that the inhibition zone with a concentration of 200 μg/ml was higher than the inhibition zone with a concentration of 150 and the last, it was higher than the inhibition zone with a concentration of 100 μg/ml, and therefore the inhibition zone with a concentration of 200 μg/ml represented the optimal concentration of inhibition for domestic magnesium oxide nanoparticles μg/ml, the results showed that the inhibition zone for the three concentrations of domestic magnesium oxide nanoparticles was lower than that of the imported magnesium oxide nanoparticles, as shown in the figure (4).
The increase in the level of inhibition in the concentrations of imported magnesium oxide nanoparticles relative to the concentrations of domestic magnesium oxide nanoparticles may be due to the difference in manufacturing sources for magnesium oxide nanoparticles, as well as the difference in techniques used in manufacturing [21].

![Effect of Different Concentrations of (A) Imported Magnesium Oxide NPs and (B) Domestic Magnesium Oxide NPs against MDR P. aeruginosa isolates.](image1)

![Effect of Different Concentrations of Domestic Magnesium Oxide NPs on MDR P. aeruginosa isolates.](image2)

**Fig. 4.** Effect of Different Concentrations of (A) Imported Magnesium Oxide NPs and (B) Domestic Magnesium Oxide NPs against MDR *P. aeruginosa* isolates.

**Fig. 5.** Effect of Different Concentrations of Domestic Magnesium Oxide NPs on MDR *P. aeruginosa* isolates.

### 4.3.3 The Combination Imported and Domestic Magnesium Oxide Nanoparticles

The results showed when mixing concentrations (100, 150, 200) μg/ml of imported magnesium oxide nanoparticles with domestic magnesium oxide nanoparticles that there was an increase in the inhibition zone for each of the three concentrations compared to if the imported or domestic MgO Nps were alone, as shown in the figures (6) and (7).

The reason for this synergy and the increase in the inhibition levels for the three concentrations may be due to the fact that the imported magnesium oxide nanoparticles are of the appropriate molecular weight to reach the bacterial nucleic acid and inactivate it effectively. The reason for this inactivation of the nucleic acid reduced the resistance of the
bacteria to the domestic magnesium oxide nanoparticles, as was the case with the bacteria when adding domestic magnesium oxide nanoparticles concentrates to it, this interpretation agrees with that [22].

**Fig. 6.** Effect of Different Concentrations of Combination of Imported and Domestic Magnesium Oxide NPs against *P. aeruginosa* isolates.

**Fig. 7.** Effect of Different Concentrations of Combination of Imported and Domestic Magnesium Oxide NPs on MDR *P. aeruginosa* isolates.

### 4.3.4 The Synergism Effect of Imported MgO Nanoparticles with the most Resistance Antibiotics for *P. aeruginosa*

Imported magnesium oxide nanoparticles were added at their optimum concentration, which represents (200) µg/ml, with antibiotics that were resistant to the *P. aeruginosa*, the result of the mixing was positive, meaning that there was an increase in the inhibition zone compared to the inhibition zone in the magnesium oxide nanoparticles alone, as shown in figures (8) and (9).
Due to emergence of resistant infections, existing antibacterial drugs have become less effective or even ineffective. It is essential to reduce the emergence of resistant bacteria to maintain the efficacy of existing drugs for treating the common and life-threatening infections [23].

Various approaches have been developed and used to eradicate antibiotic resistance. Combination therapy reduces the adverse effects of antibiotics and increases the potency of antimicrobial agents against resistant pathogens. These agents can be used as stand-alone or adjunctive therapies. Improving the antibiotic efficacy against bacteria can also be enhanced by applying novel drug delivery systems. Nanoparticles such as MgO nanoparticles, are considered therapeutic agent delivery systems [24].

4.3.5 The Synergism Effect of Domestic MgO Nanoparticles with the most Resistance Antibiotics for P. aeruginosa

Domestic magnesium oxide nanoparticles were added at their optimum concentration, which represents (200) micrograms/ml, with the antibiotics that Pseudomonas aeruginosa is resistant to. The results showed that there was a positive effect on the inhibition zone, as there was an increase in the area of the inhibition zone compared to the magnesium oxide particles. Magnesium nanoparticles alone, as in figures (10) and (11).
The most paramount challenge faced globally by the physicians is to treat the infections caused by nosocomial multi-drug resistant microorganisms. These bacteria are endowed with unique efflux pumps, which are substrate specific and a major defense mechanism for the bacteria. In fact, among various etiologies, the expulsion of antibiotics through efflux pump/s stands alone as an important factor for such resistance which these bacteria develop with time [25]. Magnesium oxide nanoparticles have been used with antibiotics against multidrug-resistant efflux pumps, and the combination of nanoparticles with antibiotics together leads to the inhibition of the action of antibiotic-resistant efflux pumps [26].

**Reference**


