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Original article

# Antimicrobial effect of meropenem-loaded mesoporous silica nanoparticles against carbapenem-resistant *Pseudomonas aeruginosa*

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**ABSTRACT**

**Background:** The emergence of antibiotic-resistant strains of *Pseudomonas aeruginosa (P. aeruginosa)* poses a significant threat to both hospital and community-acquired infections. The growing challenge of meropenem resistance in *P. aeruginosa* is a notable concern. Among the various mechanisms contributing to this resistance, efflux pumps MexAB-OprM systems are known to be the largest and the only intrinsic multidrug resistant efflux pumps within *P. aeruginosa*. The broad substrate specificity of the MexAB-OprM efflux pump system makes MexB gene overexpression crucial for the intrinsic resistance of *P. aeruginosa* to various antimicrobials. Meropenem loaded mesoporous silica nanoparticles (MSNs) have been postulated to act as an efflux pump inhibitor. This study aimed to determine the antimicrobial effect of meropenem-loaded MSNs on carbapenem-resistant *P. aeruginosa* clinical isolates and to identify its effect on expression of efflux pump (MexB) gene. **Methods:** Thirty *P. aeruginosa* isolates were confirmed to be meropenem-resistant using disc diffusion method. Minimum inhibitory concentration (MIC) of meropenem and meropenem-loaded MSNs were determined using microbroth dilution (MBD) test. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to measure MexB gene expression before and after treatment with meropenem loaded MSNs. **Results:** The present study found that meropenem resistance occurred in 30/30 (100%) isolates giving MIC > 32 µg/ml by MBD test. On repeating the test using meropenem-loaded MSNs (24/30, 80 %) of the tested isolates showed reduction in MIC with highly statistically significant difference from (512-32 µg/ml) by meropenem alone to (256-8 µg/ml) by using meropenem loaded MSNs. Real-Time RT PCR confirmed the anti-bacterial activity of meropenem-loaded MSNs by revealing a high statistically significant reduction in gene expression ($p$ value <0.001). **Conclusion**: Our results revealed that meropenem-loaded MSNs have an in vitro antibacterial action against meropenem- resistant *P. aeruginosa* isolates.

## Introduction

*Pseudomonas aeruginosa* is the most common Gram-negative opportunistic pathogen linked to hospital-acquired infection [1]. Its colonization and biofilm formation are responsible for numerous infections, including chronic

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infections in cystic fibrosis of lung, diabetic foot ulcers, burn wounds, urinary tract infections and eye infections, treatment of such infections may be difficult especially with inherited mechanisms of antibiotic resistance [2].

Beta lactams resistance in *P. aeruginosa* is mediated by efflux pumps, structural genes for at least 12 types of resistance-nodulation-cell division (RND) efflux systems have been identified, including MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM. Among these, MexAB-OprM is the most highly expressed multidrug efflux pump in *P. aeruginosa*, providing resistance to a broad spectrum of antimicrobials; it's composed of three components: MexB, a translocase solute/proton RND antiporter; OprM, an outer membrane porin; and MexA, a membrane fusion protein that connects MexB to OprM [3]. It was proposed that these operons overexpression led to increased *P. aeruginosa* resistance to carbapenems, chloramphenicol, tetracycline, nalidixic acid, ciprofloxacin, and streptonigrin. Conversely, disrupting these genes caused the mutants to become highly susceptible to these drugs [4].

Efflux pump inhibition can be achieved through several methods; reducing the expression of efflux pump genes by disrupting their genetic regulation, altering antibiotics so they are no longer recognized as substrates, blocking the active site to prevent drug binding and breaking down the energy system that powers these pumps [5].

Multidrug resistant (MDR) *P. aeruginosa* has evolved resistance to the majority of current antibiotics, including carbapenems [6]. Alternative therapies for carbapenem-resistant *P. aeruginosa* isolates include loading of antibiotic on nanoparticles as mesoporous silica nanoparticles (MSNs) [7]. Medical nanotechnology has emerged as an effective approach for curing some of the most difficult medical problems. This is achieved either through the direct antimicrobial activities of nanomaterials or by serving as drug delivery systems [8].

Mesoporous silica nanoparticles have been reported as an efficient delivery system for various antimicrobial agents, including but not limited to vancomycin, tetracycline, polymyxin B, gentamicin, rifampicin, and linezolid**.** Antibiotic-loaded MSNs has good antimicrobial activity against a wide range of microbes such as *Staphylococcus aureus*, as well as *P. aeruginosa*, *Enterobacteriaceae* and *Acinetobacter baumannii* [9].

This study aimed to determine the antimicrobial effect of meropenem loaded MSNs on carbapenem-resistant *P. aeruginosa* clinical isolates and to identify its effect on expression of efflux pump (MexB) gene.

## Methods

This experimental study was conducted on 30 meropenem resistant *P. aeruginosa* isolates obtained from laboratories of Ain Shams University hospitals. The study was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University (No. FMASU MS 494/2023).

### Data collection and species confirmation using conventional methods:

For each isolate, the type of specimen and its hospital distribution were recorded. Identification of the isolates was performed using traditional culture and identification techniques. The isolates were then preserved in glycerol at –80°C [10].

### Antibiotic susceptibility testing using disc diffusion method:

Antibiotic susceptibility of all isolates was done by disk diffusion method and results interpretation was done according to CLSI 2022 guidelines [11]. The following antibiotic discs were used: piperacillin- tazobactam (100 /10 μg), aztreonam (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5μg), fosfomycin (200 μg), colistin (10 μg), meropenem (10 μg), tobramycin (10 μg) and amikacin (30 μg) (Himedia, India) The diameter of the zones of inhibition was interpreted according to the standard cut off points to CLSI 2022 guidelines [11]. *Pseudomonas aeruginosa* ATCC (27853) was used as a control strain and isolates were classified as MDR if they showed resistance to at least one agent in at least three antibiotic classes.

### Synthesis of meropenem-loaded MSNs [12]:

At room temperature, tetraethoxy saline (TEOS) was hydrolyzed using ammonia and cetyl trimethyl ammonium bromide as a surfactant template to create mesoporous silica nanospheres. In the beginning, the surfactant was dissolved in 100 mL of deionized water, and then an acetone, diethyl ether, and TEOS mixture were added to the mixture. After stirring the mixture overnight at room temperature, white MSN precipitate was formed. Methanol and deionized water were used to wash the

white powder that was collected and calcined at 550°C for 8 hours.

To load meropenem onto MSN, 50 mL of meropenem (200 mg) in a methanol/water solution was mixed with 200 mg of the calcined MSNs. After 24 hours stirring at room temperature, the solution was centrifuged and washed with deionized water. The loaded meropenem onto MSN nanoparticles was dried. After dissolving 100 mg of the meropenem-loaded MSNs in EDTA and stirring for 30 minutes, the drug loading efficiency was evaluated. Afterward, an ultraviolet- visible spectrophotometer was used to measure the meropenem loading effectiveness at 297 nm using 1 ml of the solution and using the following equations:

- Drug loading content = (drug content/total amount of drug-loaded MSNs) x100%.
- Drug loading efficiency = (drug content/total drug added) x100%.

**Determination of MIC for meropenem alone and meropenem-loaded MSNs:**

MIC of meropenem and meropenem-loaded MSNs was determined using the MBD test, according to CLSI 2022 guidelines for antimicrobial susceptibility testing. This involved preparing meropenem and meropenem-loaded MSNs serial dilutions ranging from 2 µg/ml to 512 µg/ml in a two 96-well separate microtiter plates, with the bacterial inoculum adjusted to $10^6$ CFU/ml. After 24 hours of incubation, 30 µL of resazurin was added to each well, and the plate was incubated for an additional 2 to 4 hours to observe color changes. Bacterial growth was indicated by the oxidation of resazurin from blue to pink. MIC results of meropenem were interpreted based on CLSI standard cut-off points: ≤2 µg /ml was considered susceptible, 4 µg /ml as intermediate, and ≥8 µg /ml as resistant [11]. The interpretation of MIC susceptibility break points to meropenem-loaded MSNs was determined as a two-fold decrease in MIC means sensitivity to meropenem-loaded MSNs [12].

**Genotypic detection of the inhibitory effect of meropenem loaded MSNs on the expression of MexB gene by real time RT-PCR:**

The levels of expression of MexB gene were quantified using real time RT-PCR as recommended by **Rodriguez et al., 2009** [13]. Expression levels results were standardized according to the transcription level of the house keeping gene 16S ribosomal RNA (16srRNA). Quantitative RT- PCR was performed using PureLink™ RNA Mini Kit (Thermo-fisher, USA), GoScript™ Reverse Transcriptase kit (Promega, USA), DreamTaq Green PCR Master Mix kit (Thermo-fisher, USA) and 2 pairs of primers specific for MexB genes[14] **(Table 1).**

RT-PCR was done with and without addition of sub-MIC values of meropenem loaded MSNs corresponding to each isolate.

The gene expression level of treated *P. aeruginosa* isolates was calculated relative to that in the untreated isolates using the livak method; Δ Ct = Ct gene test – Ct endogenous control [1**5**].

- Δ C is calculated for treated and untreated samples.
- Endogenous control =16srRNA
- ΔΔCt = ΔCt treated sample – ΔCt untreated sample
- RQ = Relative quantification = $2^{-\Delta\Delta Ct}$
- Fold of change in gene expression =$1/2^{-\Delta\Delta Ct}$

The cut-off value for sensitivity of isolates expressing Mex B gene to meropenem-loaded MSNs is two-fold or more change in gene expression [1**6**].

**Statistical analysis:** Data were analyzed using the Statistical Package for Social Sciences (SPSS version 25). The Kolmogorov-Smirnov test was used to assess normality for continuous variables. Descriptive analysis was performed to obtain the means and deviations for quantitative data when it is normally distributed, and median, interquartile range for skewed data. Numbers and frequencies for qualitative data.

Different types of graphs were used according to the type and distribution of data (bar, pie and error plot). Bivariate analyses were performed using the ANOVA, independent samples t test, Pearson correlation and paired t test.

## Results

### Origin of isolates

Over 12 months, 30 clinical isolates that were phenotypically confirmed to be *P. aeruginosa* were obtained from Ain Shams University hospitals.

Most isolates were collected from pus (16/30, **53.3**%) followed by blood (6/30, 20.0%) and others include (urine, drain, central line and pleural fluid (8/30, 26.7**%**) and were mostly isolated from patients admitted to intensive care units (ICUs) (21/30, 70%) **(Figure 1)**.

**Antimicrobial susceptibility pattern of isolates as determined by disc diffusion method**

16 isolates were resistant to 6 classes of antibiotics (16/30, 53 %), 8 isolates (27%) were resistant to 5 classes of antibiotics, 2 (7%) were resistant to 4 classes of antibiotics and 4 (13%) were resistant to 3 classes of antibiotics **(Figure 2)**.

**Detection of the MIC of meropenem and meropenem-loaded MSNs on meropenem-resistant *P. aeruginosa* isolates *MBD* method**

All tested meropenem-resistant *P. aeruginosa* isolates were resistant to meropenem (30/30,100%) giving MIC > 32 μg/ml by MDB test. On repeating the test by using meropenem-loaded MSNs, 24/30 (80%) of the tested isolates showed a reduction of MIC from (512-32 μg/ml) by meropenem alone to (256-8 μg/ml) by using meropenem loaded MSNs. The mean (±SD) MIC of meropenem-loaded MSNs was decreased from 227 (±160) by meropenem alone to 65 (±74) (more than 2-fold decrease in MIC represents sensitivity to meropenem-loaded MSNs) (*p* value <0.001). A significant reduction in 95% confidence interval (CI) for MIC of meropenem alone from (165 -290) to (39.2-90) for meropenem- loaded MSNs suggesting enhanced antimicrobial activity (**Table 2, Figures 3, 4)**.

There was statistically significant difference between meropenem MIC& meropenem-loaded MSNs MIC for isolates from different sources, isolates from wounds were the most sensitive (significant *p* value) **(Table 3)**.

There was statistically difference between meropenem MIC& meropenem-loaded MSNs MIC for isolates from different healthcare settings; isolates from wards were more sensitive than other samples from ICUs **(Table 4)**.

**Effect of meropenem-loaded MSNs on the level of MexB gene expression in meropenem-resistant *P. aeruginosa* isolates**

Expression of the MexB efflux pump gene was compared in the 30 meropenem-resistant *P. aeruginosa* isolates before and after treatment with sub-MIC values of meropenem loaded MSNs. The expression of MexB gene showed statistically significant reduction after treatment with meropenem-loaded MSNs, a remarkable increase in MexB gene mean cycle threshold from 6.78 (±3.61 SD) to 11.50 (±4.96 SD), median increased from 5.93 to 10.15. The mean fold of decrease of MexB gene expression was 29.3 of MexB gene (±26.1 SD), median 19 (*p* value <0.001) after treatment by meropenem-loaded MSNs **(Table 5, Figures 6, 7).**

There was a statistically significant decrease in MexB gene expression after treatment with meropenem loaded MSNs in relation to sample type, isolates from wounds were more affected by treatment than others and in relation to healthcare setting, isolates from ICUs were the most affected by treatment **(Tables 6, 7**).

**Table 1.** Sequence of primers used in molecular studies [14].

| **Primer name** | **Sequence** |
|---|---|
| *MexB*-forward | *5′-CAAGGGCGTCGGTGACTTCCAG-3′* |
| *MexB*-reverse | *5′-ACCTGGGAACCGTCGGGATTGA-3′* |
| *16srRNA forward* | *5′-CAGCTCGTGTCGTGAGATGT- 3′* |
| *16srRNA reverse* | *5′-CGTAAGGGCCATGATGACTT-3′* |

**Table 2.** Comparison between MIC results of meropenem and MIC of meropenem-loaded MSNs on meropenem resistant *P. aeruginosa* isolates by paired t test.

| | MIC of meropenem | MIC of meropenem-loaded MSNs | Paired t test | |
|---|---|---|---|---|
| Mean | 227.20 | 64.80 | *p* value<0.001 | Highly significant |
| SD | 160.408 | 74.080 | | |
| Min -Max | 512-32 | 256-8 | | |

**Table 3.** MIC results in relation to sample types

| | Sample type | | | | | | *p* value |
|---|---|---|---|---|---|---|---|
| | Wound | | Blood | | Others | | |
| | Mean | SD | Mean | SD | Mean | SD | |
| Meropenem MIC | 194 | 170 | 363 | 170 | 192 | 68 | 0.064 |
| Meropenem- loaded MSNs MIC | 54 | 67 | 133 | 100 | 36 | 25 | 0.029* |

Test of sig "ANOVA" test,*Sig *p* value

**Table 4.** MIC results in relation to healthcare setting

| | location | | | | *p* value |
|---|---|---|---|---|---|
| | Ward | | ICU | | |
| | Mean | SD | Mean | SD | |
| Meropenem MIC | 206 | 135 | 236 | 172 | 0.647 |
| Meropenem- loaded MSNs MIC | 47 | 38 | 72 | 85 | 0.401 |

**Table 5.** The effect of meropenem-loaded MSNs on the expression of MexB gene among 30 meropenem-resistant *P. aeruginosa* isolates

| | Cycle threshold of MexB | | The fold of decrease of MexB expression |
|---|---|---|---|
| | Before treatment with meropenem-loaded MSNs | After treatment with meropenem-loaded MSNs | |
| Mean | 6.78 | 11.50 | 29.3 |
| SD | 3.61 | 4.96 | 26.1 |
| Median | 5.93 | 10.15 | 19 |
| *p* value | <0.001 | | |

**Table 6.** MexB gene cycle threshold before and after treatment with meropenem-loaded MSNs in relation to sample type:

| | Sample type | | | | | | *p* value |
|---|---|---|---|---|---|---|---|
| | Wound | | Blood | | others | | |
| | MexB | SD | Mean | SD | Mean | SD | |
| *58xB* gene cycle threshold before treatment with meropenem-loaded MSNs | 7.58 | 4.20 | 4.97 | 2.87 | 6.55 | 2.45 | 0.324 |
| *exB* gene cycle threshold after treatment with meropenem-loaded MSNs | 13.35 | 6.14 | 8.49 | 1.52 | 10.06 | 1.23 | 0.075 |

**Table 7.** MexB gene cycle threshold before and after treatment with meropenem-loaded MSNs in relation to healthcare setting:

| | Healthcare setting | | | | *p* value |
|---|---|---|---|---|---|
| | Ward | | ICU | | |
| | Mean | SD | Mean | SD | |
| MexB gene cycle threshold before treatment with meropenem-loaded MSNs | 6.24 | 2.23 | 7.02 | 4.09 | 0.597 |
| MexB gene cycle threshold after treatment with meropenem-loaded MSNs | 10.24 | 1.20 | 12.03 | 5.83 | 0.192 |

**Figure 1.** Origin of isolates.

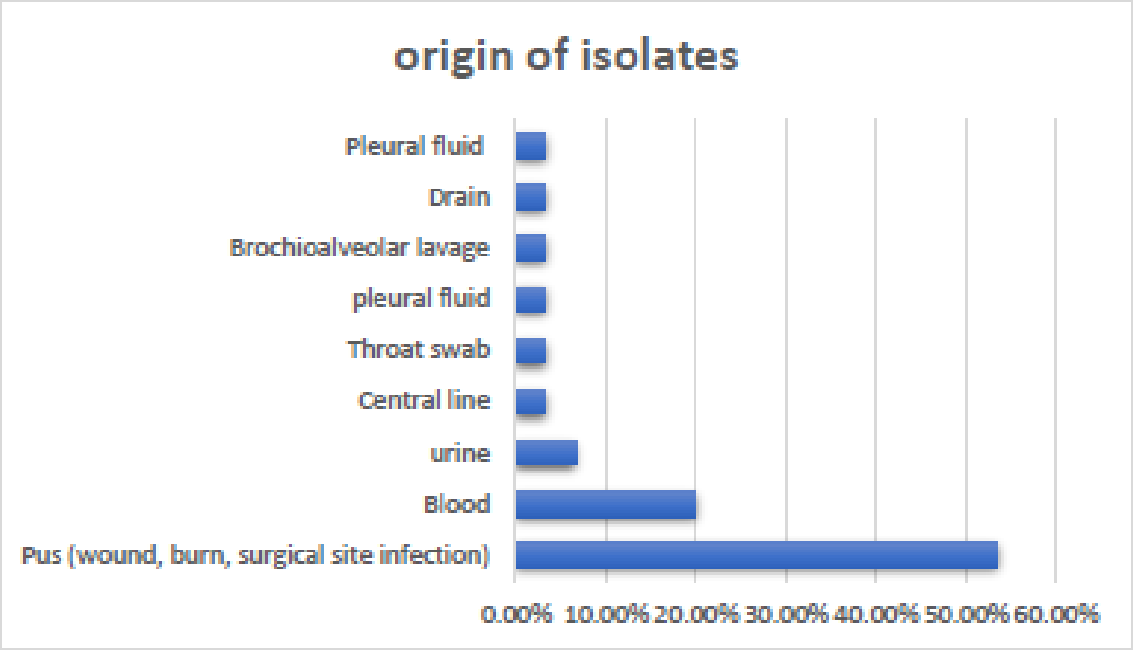



**Figure 2.** Frequency and percentage of resistant antibiotic classes

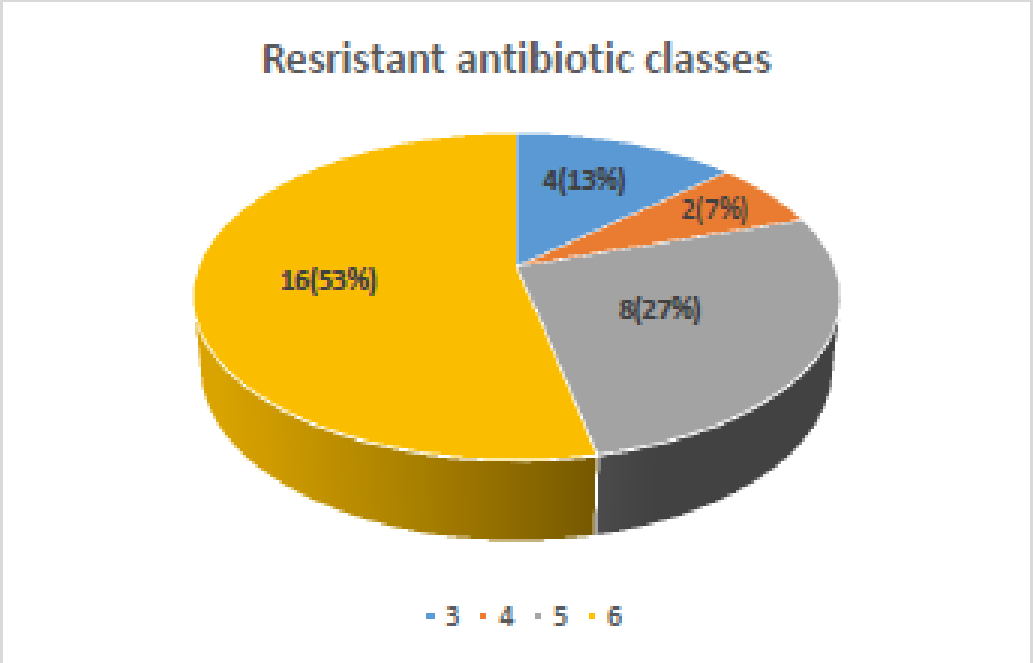


**Figure 3.** Different MIC results for meropenem alone [X] and meropenem-loaded MSNs [Y] against eight meropenem resistant P. aeruginosa isolates on microtiter plates by MDB test.
 Rows (A, B, C, D, E, F, G and H) represented testing (one isolate + meropenem) in the upper microtiter plate (X) and (one isolate+ meropenem loaded MSNs) in the lower plate (Y).
 +ve and -ve controls were added as shown.

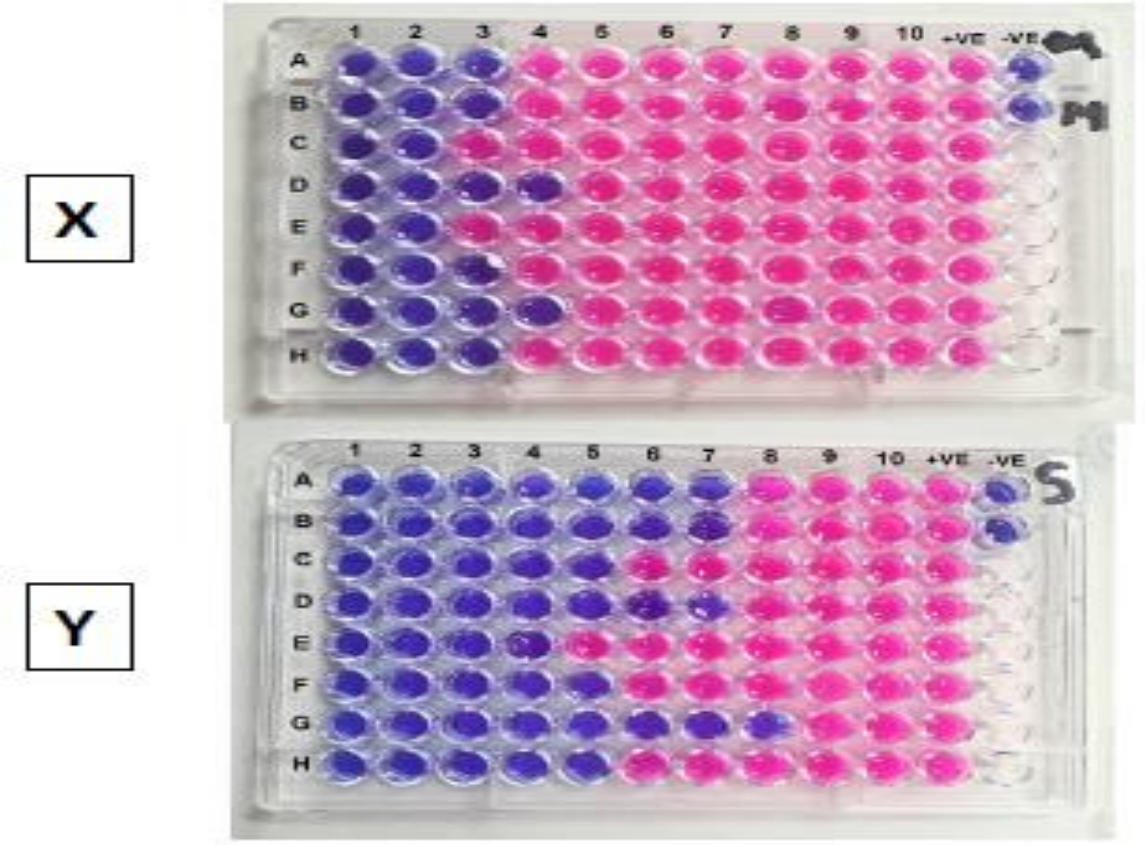



**Figure 4.** 95 % CI for MIC of meropenem and MIC of meropenem loaded MSNs.

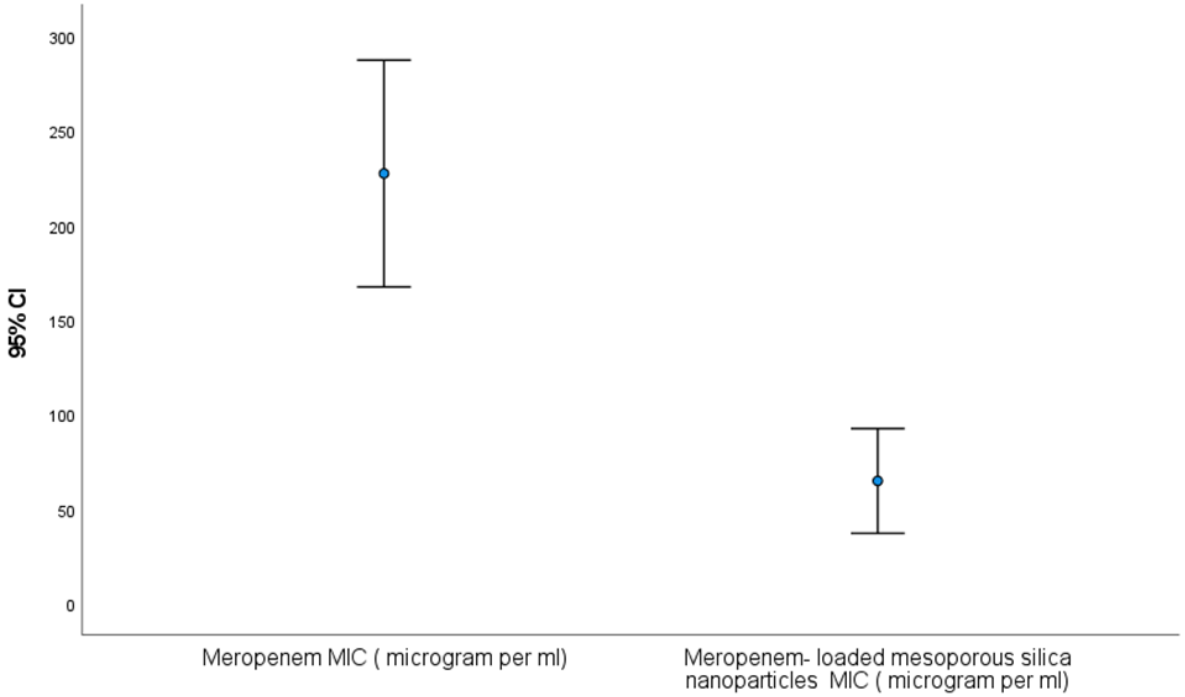
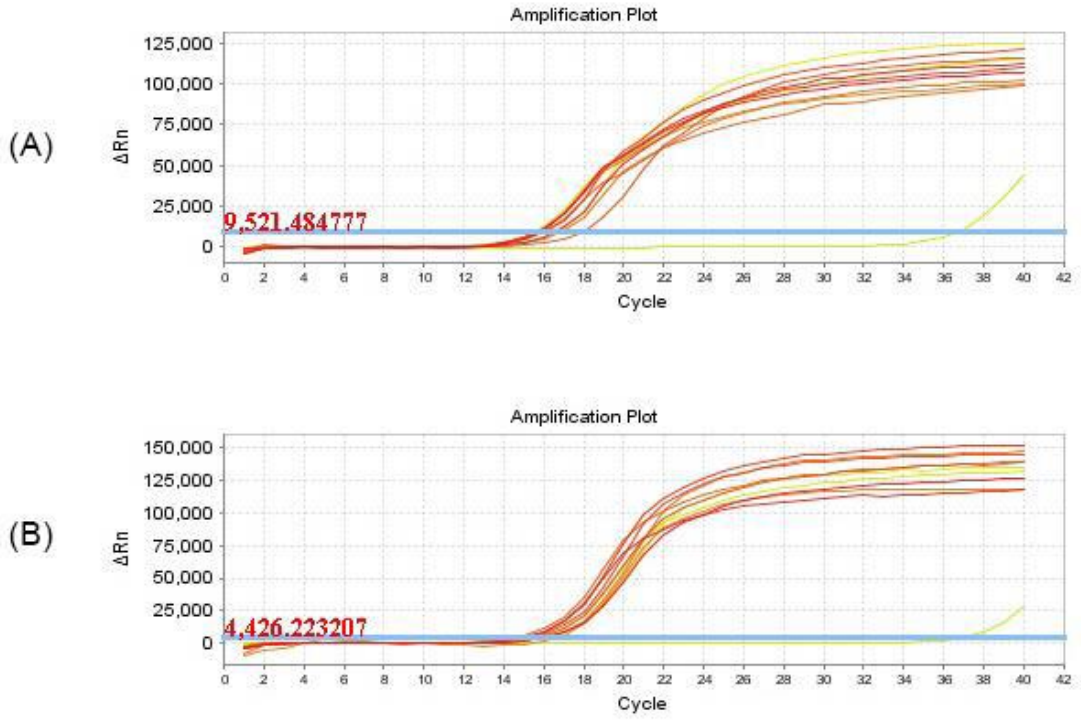


**Figure 5.** The cycle threshold of Mex B gene in *P. aeruginosa* isolates before (a) and after (b) treatment with the sub-MICs of meropenem-loaded MSNs.




ΔRn: normalized reporter

**Figure 6.** Boxplot

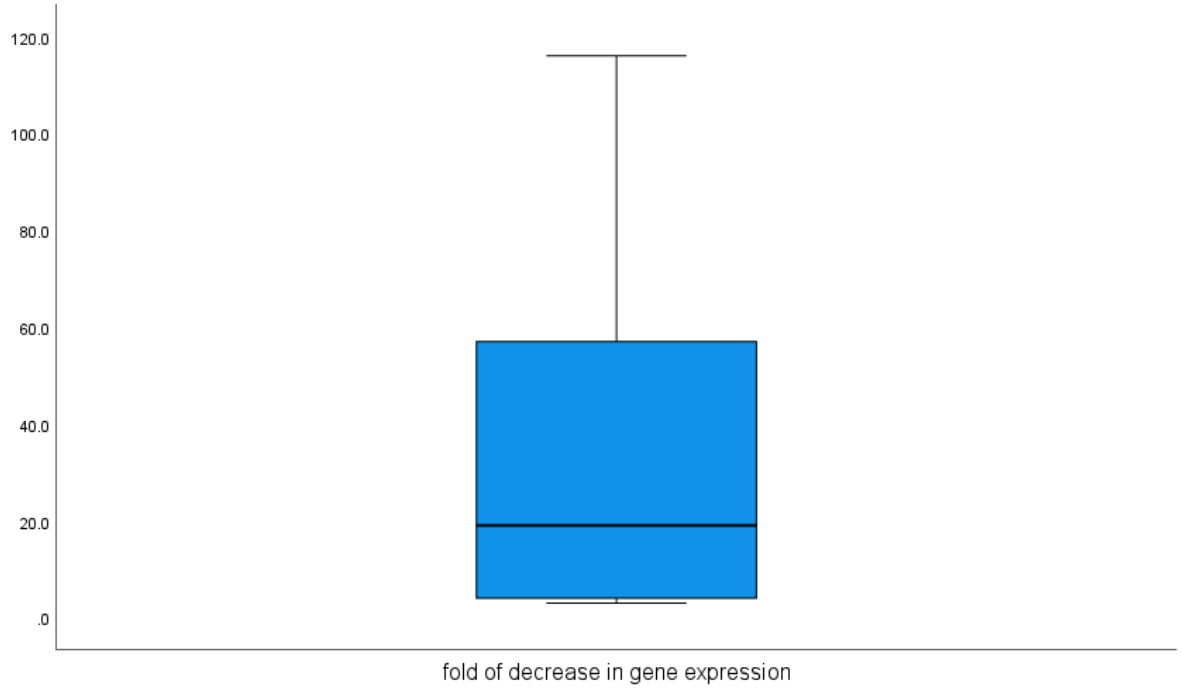
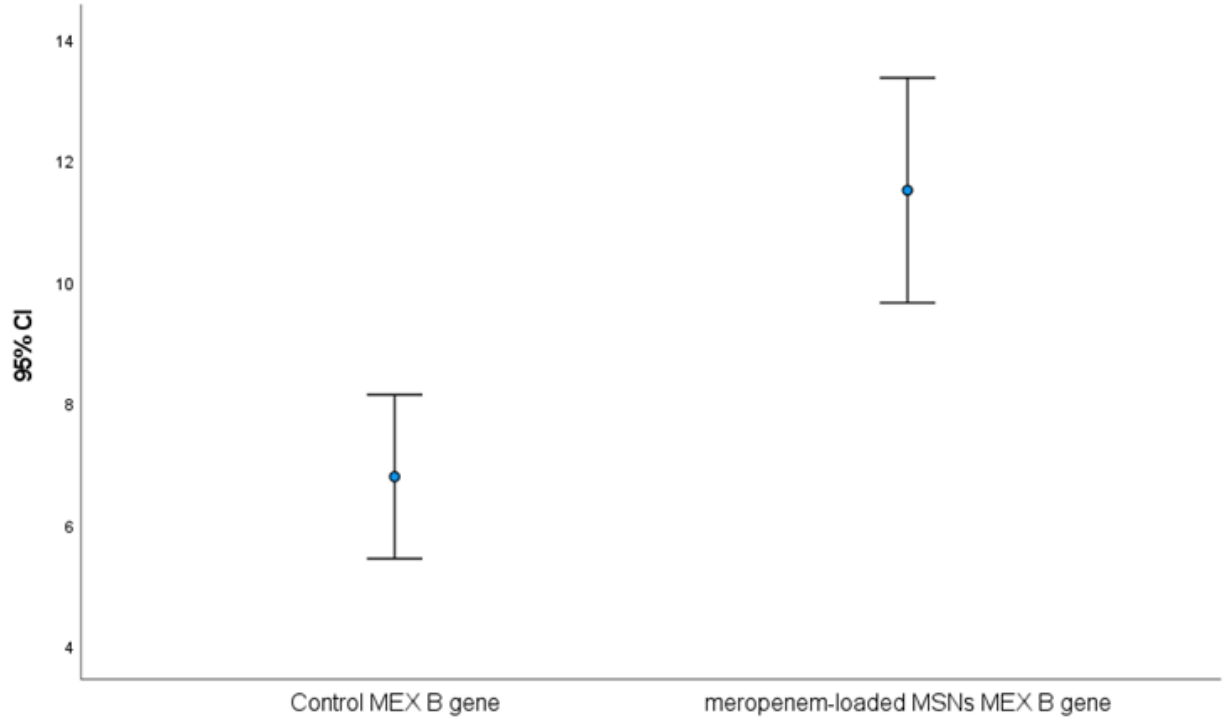


**Figure 7 .** Error bar




**Discussion**

*Pseudomonas aeruginosa* is an opportunistic pathogen that can cause outbreaks of hospital-acquired and life-threatening infections, especially among immunocompromised and critically ill patients. The mortality rate among burn patients infected with *P. aeruginosa* is higher compared to those who are not infected, especially when the infection involves MDR strains [17].

The present study was conducted on 30 isolates of meropenem-resistant *P. aeruginosa*. Most isolates were from pus samples (16/30, 53.3%) followed by blood (6/30, 20%) and others include (urine, drain, central line, pleural fluid (8/30, 26.7%). These results agree with **Hassuna et al.** [18] as they stated that most of meropenem-resistant *P. aeruginosa* isolates were from pus samples (227/634 isolates, 36%) but followed by tracheal aspirates 18% (116/634 isolates), **Al-Haik et al.** [19] also found that the highest isolation rate was (60%) from wounds and burns patients followed by sputum (26.7%), and the lowest rate from blood (16.6%). Wound infections and burns provide a suitable site for bacterial multiplication because of the larger area involved and longer duration of patient stay in the hospital [20].

The present study demonstrated that meropenem-resistant *P. aeruginosa* were more frequently isolated from patients attending ICUs (21/30, 70%). This agrees with **Bazghandi et al.** [21] who found that the highest rate of meropenem-resistant *P. aeruginosa* strains isolated belonged to the ICU (32.1%, 27/84), followed by internal departments (31%, 26/84), Intensive care patients are more susceptible to infection because of prolonged hospital stay and instrumentation [22]. In contrast to our results **Mirzaei et al.,** [23] found that the frequency of MDR isolates was higher in the department of surgery (71.8%).

Rising rates of MDR *P. aeruginosa* in healthcare associated infections and between hospitalized patients is a main public health problem. Resistance of *P. aeruginosa* is usually accompanied by the production of biofilms, active expulsion of antibiotics by efflux pump, and alteration of outer membrane protein expression [22].

In the current study, the antibiogram of *P. aeruginosa* isolates revealed that they were highly resistant to meropenem, amikacin (100%) followed by tobramycin, (90%), ceftazidime, cefepime and fosfomycin (83.3%), ciprofloxacin and piperacillin –tazobactam (80%), aztreonam (76.7%) and colistin (73.3%). **Ameen et al.** [24] reported highest resistance of MDR *P. aeruginosa* against imipenem (100%) followed by gentamycin (98%), amikacin (77.8%), piperacillin/tazobactam (68.1%). **Tam et al.** [25] conducted a study on MDR *P. aeruginosa*

isolated from blood, demonstrated 100% resistance to Carbapenems and Quinolones. In contrast, studies done in India reported that imipenem was 100% sensitive followed by piperacillin/tazobactam 72% [26].

All (30/30) meropenem resistant *P. aeruginosa* isolates were MDR. Most of MDR *P. aeruginosa isolates* were resistant to 6 groups of antibiotics (16/30, 53 %), 8 MDR isolates (27%) were resistant to 5 groups of antibiotics, 4 (13%) were resistant to 3groups of antibiotics and 2 (7%) were resistant to 4 groups of antibiotics.

This agrees with **Memar et al.** [12] who found that (10/10,100%) of collected *P. aeruginosa* isolates were MDR. Also, **Abd El-Baky et al.** [27] found that 96% of the isolated *P. aeruginosa* were MDR. **Parmar et al.** [28] reported a lower rate**,** 53 (23.24%) isolates were resistant to the 12 routine anti-pseudomonal drugs and out of 53 isolates, and 16 (30.18%) isolates were resistant to all drugs tested. This may reflect regional differences in antibiotic prescribing habits or variations in antimicrobial stewardship programs [29].

In the present study, the 30 tested isolates showed resistance to meropenem by detecting MIC using MBD test. The MIC ranges of meropenem were (512-32 µg /ml). These results came in agreement with **Memar et al.** [12] who found that all isolates (10/10,100%) were resistant to meropenem and the MICs values were higher than 8 µg/mL in all isolates. Similar results were recorded by **Srivastava et al.** [30], meropenem was found to be resistant in (2/2, 100%) with MIC range 128 µg and 256 µg, **Albiero et al.** [31] recorded MIC values ranging from 1024 to 0.25 µg/ml, 13/19 (68%) of the tested isolates were more than 4 µg/ml**.**

Meropenem resistance in *P. aeruginosa* could be attributed to a reduction in the outer membrane permeability, up-regulated expression of the efflux pumps genes and production of metallo-β-lactamases, which inactivate these drugs efficiently. There is urgent need for alternative treatment strategies and intensified infection control measures [18].

Mesoporous silica nanoparticles have been reported as an efficient delivery system for various antimicrobial agents, including but not limited to vancomycin, tetracycline, polymyxin B, gentamicin, rifampicin, and linezolid **.**Antibiotic loaded MSNs have good antimicrobial activity against a wide range of microbes such as *Staphylococcus aureus*, as well as *P. aeruginosa*, *Enterobacteriaceae* and *Acinetobacter baumannii* [32,33].

The bactericidal impact of meropenem-loaded MSNs demonstrated the successful integration of meropenem onto MSNs without compromising its antimicrobial efficacy. The interaction between positively charged MSNs and bacterial lipid bilayers has been documented in the literature [12].

According to the present study, after repeating the MBD test with meropenem-loaded MSNs, a significant reduction in MIC was detected, decreased from (512-32 µg/ml) to (256-8 µg/ml). MIC of 24/30 (80%) isolates decreased (2-4) folds and MIC of 6/30 (20%) isolates slightly decreased. The findings of the present study go in accordance with those of **Memar et al**. [32] who found MIC ranges were decreased from 64-4 µg/ml with meropenem to 16-1µg/ml with meropenem-loaded MSNs. **Lamb et al.,** [34] reported MIC of meropenem was 64-8 µg/ml decreased to 16-2 µg/ml with meropenem-loaded MSNs. This effect could be attributed to the interaction between MSNs and microorganisms, potentially enhancing the uptake of meropenem and consequently augmenting its antibacterial effect [12, 32, 33].

One of the most important mechanisms of meropenem resistance in *P. aeruginosa* is over-expression of efflux pumps. MexB is the primary transporter responsible for expelling antibiotics such as meropenem from the bacterial cell. Studies have shown that MexB often exhibits higher levels of expression in *P. aeruginosa* strains resistant to meropenem. [35].

In the present study, the expression of the MexB efflux pump gene was compared in the 30 meropenem-resistant *P. aeruginosa* isolates before and after treatment with sub-MIC values of meropenem loaded MSNs, revealed a high statistically significant reduction in gene expression ($p$ value <0.001) and the mean of fold of decrease in the MexB gene expression was (29.3)**.**

**Rudbaraki et al.** [36] conducted a study investigating the alteration of MexB gene expression in ciprofloxacin-resistant *P. aeruginosa* isolates during treatment with encapsulated silibinin in nanoparticles. There was a decrease in the expression of genes associated with efflux pump systems, including the MexB gene. Additionally,

**Arya et al.** [37] conducted a study examining the impact of vanilla and the application of gold nanoparticles on multidrug-resistant isolates of *P. aeruginosa*, they observed that treatment with gold nanoparticles led to a reduction in the expression of MexA and MexB genes.

**Conclusion**

According to the present study, meropenem-loaded MSNs exhibit an in vitro antibacterial effect against meropenem-resistant *P. aeruginosa*. This approach shows the potential for meropenem and MSNs to work together effectively to address the serious issue of MDR *P. aeruginosa*. The effect of meropenem and MSNs is highly attributed to the role of MSNs as inhibitors of efflux pumps, as evidenced by the reduced expression of the MexB efflux pump gene following treatment with meropenem-loaded MSNs in this study. To determine the optimal concentration of MSNs for effective use with meropenem, larger scale studies are recommended and more in vivo assessments on the effectiveness and compatibility of meropenem loaded MSNs for infections. This could potentially enable the use of lower concentrations of meropenem, thereby reducing its side effects and resistance rates.

**Conflict of interest**

There is no conflict of interest stated by the author.

**Author's contribution**

All of the listed authors participated in the work and approved it for publication.

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None.

**Data availability**

All data generated or analyzed during this study are included in this puplished article.

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