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Research Article

Effect of gold nanoparticles on the expression of efflux pump *mexA* and *mexB* genes of *Pseudomonas aeruginosa* strains by Quantitative real-time PCR

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Abstract

Antibiotic-resistant *Pseudomonas aeruginosa* infections are usually difficult to treat, and there are limited antibiotics for treating them. Increased antibiotic resistance of this bacterium, especially in a multidrug form, has caused many problems for treatment. Nowadays, metal nanoparticles are considered as appropriate alternatives to antibiotics. The objective of the present study was to investigate the effect of gold nanoparticles on the expression of *MexB* and *MexA* genes in *Pseudomonas aeruginosa* isolates.*Pseudomonas aeruginosa* isolate was identified using biochemical tests and an API kit. The antibiotic sensitivitytest for different antibiotics was performed with-the Kirby-Bauer test according to the CLSI standard. The presence of *MexB* and *MexA* genes was assessed by PCR. The effect of gold nanoparticles was investigated by microdilution to evaluate the minimum inhibitory concentration, and the expression of *MexB* and *MexA* treated genes was done with silver nanoparticles by the Real-Time PCR method.40 *Pseudomonas aeruginosa* isolates were detected and identified. These isolates showed significant resistance to various antibiotics. All strains were carriers of *MexB* and *MexA* genes, and finally, in the expression of *MexB* and *MexA* genes,a significant decrease in the expression of these genes was observed in the samples treated with gold nanoparticles compared to non-treated samples.One of the mechanisms of antibacterial activity of gold nanoparticles is through reducing the expression of *mexA* and *mexB* genes and thus reducing the number of active efflux pumps at the cell surface.

Keywords

Pseudomonas aeruginosa,gold nanoparticles, *MexA*, *MexB*, Real Time PCR

Introduction

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that is a major cause of death in immunocompromised patients due tothe creation of life-threatening infections (Sadikot et al. 2005; Gellatly et al. 2013). *Pseudomonas aeruginosa* is one of the main causes of septicemia, urinary tract infection, and endocarditis, as well as skin, eye, and ear infections in hospitalized burn-injured patients (Ghazi et al. 2012; Jafari et al. 2013). *Pseudomonas aeruginosa* is resistant to antimicrobials through various mechanisms; for instance, chromosomal mechanisms of antibiotic resistance, induced

by efflux pump systems, have been reported in the membrane of this microorganism (Munita and Arias 2016). *Pseudomonas aeruginosa* has the potential to express 12 types of multidrug leakage pumps called Mex-Type Multidrug Resistance Efflux Pump; the five of them named *MexAB-oprM*, *MexXYoprM*, *MexEF-oprN*, *MexCD-oprT*, and *MexJK-oprM* are the most significant factors of resistance to antibiotics. *MexAB-oprM* is the most important system for removing antimicrobial compounds from the cell and resolving inherent drug resistance in *Pseudomonas aeruginosa* (Goli et al. 2018). Moreover, unlike other pumps that are of the acquired type and less important clinically, *MexAB-oprM* is the only leakage pump that is inherently expressed in wild strains (Nehme and Poole 2007). This situation prompts the development of alternative therapeutic strategies for bacteria, and if appropriate inhibitors are used, it can disrupt the function of these pumps to inhibit multidrug resistance. Due to their antimicrobial potential, some nanoparticles, such as gold nanoparticles, can be effective as an alternative method of antibiotics to fight bacterial infections. Gold has antibacterial, viral, and fungal properties (Kalishwaralal et al. 2010). Gold nanoparticles can also simultaneously affect vital parts of the microbial cell such as protein, energy, and DNA production(Rai et al. 2009). The purpose of the present study is to determine the antibacterial effect of gold nanoparticles on the expression of important genes of the *MexAB-oprM* efflux pump system called *mexA* and *mexB* in *Pseudomonas aeruginosa* isolates.

Materials and methods

The present study was conducted on *Pseudomonas aeruginosa* strains in patients suspected of having *Pseudomonas aeruginosa* infections and referred to hospitals and health centers in the south of Fars province, Iran, within 12 months from September 2018 to September 2019. The clinical samples were burn, throat, nose, and 6 from the referred patients.

Identification and detection

Identification and detection of *Pseudomonas* were made biochemically using an API kit. In the biochemical method, gram staining and biochemical detection tests including oxidase, evaluation of fermentation on TSI, oxidation of glucose in the OF medium, evaluation of motility on SIM medium, indole, growth at 42 °C, and arginine dihydrolase tests, were performed to identify and detect *Pseudomonas aeruginosa*. In the method of using API kit, *Pseudomonas aeruginosa* samples were confirmed, and *Pseudomonas aeruginosa* ATCC27853 was considered as a positive control.

Antibiotic sensitivity

In order to evaluate the antibiotic sensitivity of *Pseudomonas aeruginosa* strains, the disc diffusion method was used by employing Mueller-Hinton agar culture medium as a growth medium for bacteria and antibiotic discs Ciprofloxacin,Imipenem,Amikacin,Aztreonam, Cefepime, Ceftazidime,Ertapenem,Fosfomycin, Gentamicin, Piperacillin,Colistin,Polymyxin B,Rifampin, Tetracycline,Ticarcillin, Tobramycin,Trimethoprim,Tigecycline and Meropenem according to CLSI standard.

PCR

After culturing the identified isolates of *Pseudomonas aeruginosa* on MacConkey agar culture medium, the genomes of all isolates were extracted using a DNA extraction kit (Bioneer Co. Korea). The polymerase chain reaction was performed with a final volume of 25 µL. This reaction involves 12.5 µLof mastermix (Amplicon, Denmark), 1.5 µL of forward primer, and 1.5 µLof reverse primer (offered by Bioneer Co. Korea), 2.5 µLof DNA template, and 7 µl of distilled water. The information related to primer sequence and conditions used for replication of *gyrB*, *rhlR*, *exoT*, *lasR*, *PelA*, and *toxA* genes are presented in Table 1 (3–5). The electrophoresis of PCR products was performed by 1% agarose gel, and the bands were then photographed by the Gel Doc imager. Moreover, a 50 bp marker of GenedireX Co., a joint production of Taiwan and the United States, was employed to detect PCR products.

Polymerase chain reaction for identification of *mexA* and *mexB*

After culturing the identified isolates of *Pseudomonas aeruginosa* on MacConkey agar culture medium, the DNAs of all isolates were extracted using a DNA extraction kit (Bioneer Co. Korea). The polymerase chain reaction was performed with a final volume of 25 µL. This reaction included 12.5 µLof Master mix (Amplicon, Denmark), 1.5 µL of forward primer, 1.5 µL of reverse primer (offered

Table 1. Frequency of *gyrA*, *rhR*, *exoT*, *lasT*, *Pela* and *toxA* resistance genes in different antibiotics.

MEM	TGC	TS	TN	TC	T	RP	PRL	GM	FOT	ETP	CAZ	CPM	ATM	AK	IMI	CIP	Gene
0 (0%)	32 (80%)	32 (80%)	4 (10%)	10 (25%)	32 (80%)	32 (80%)	4 (10%)	5 (12.5%)	18 (45%)	21 (52.5%)	4 (10%)	28 (70%)	8 (20%)	2 (5%)	2 (5%)	3 (7.5%)	exo.T
1 (2.5%)	40 (100%)	40 (100%)	5 (12.5%)	10 (25%)	40 (100%)	40 (100%)	5 (12.5%)	6 (15%)	21 (52.5%)	25 (62.5%)	4 (10%)	34 (85%)	9 (22.5%)	3 (7.5%)	3 (7.5%)	3 (7.5%)	rhl.R
(2.5%)	40 (100%)	40 (100%)	5 (12.5%)	10 (25%)	40 (100%)	40 (100%)	5 (12.5%)	6 (15%)	21 (52.5%	25 (62.5%)	4 (10%)	6 (15%)	9 (22.5%)	3 (7.5%)	1 (2.5%)	3 (7.5%)	lasR
1 (2.5%)	40 (100%)	40 (100%)	5 (12.5%)	10 (25%)	40 (100%)	40 (100%)	5 (12.5%)	6 (15%)	21 (52.5%	25 (62.5%)	4 (10%)	6 (15%)	9 (22.5%)	3 (7.5%)	3 (7.5%)	3 (7.5%)	Tox.A
0 (0%)	37 (92.5%)	37 (92.5%)	4 (10%)	10 (25%)	37 (92.5%)	37 (92.5%)	4 (10%)	5 (12.5%)	20 (50%)	23 (57.5%)	4 (10%)	5 (12.5%)	8 (20%)	2 (5%)	2 (5%)	3 (7.5%)	pel.A
1	40 (100%)	40 (100%)	5 (12.5%)	10 (25%)	40 (100%)	40 (100%)	5 (12.5%)	6 (15%)	18 (45%)	25 (62.5%)	4 (10%)	6 (15%)	9 (22.5%)	3 (7.5%)	3 (7.5%)	3 (7.5%)	gyrB

by Bioneer Co. Korea), 2.5 µLof template DNA, and 7 µl of distilled water. The information related to primer sequence and conditions used to replicate the *mexA* and *mexB* genes are indicated in Table 1 (3–7). Electrophoresis of PCR products by 1% agarose gel. The bands were then photographed by the Gel Doc imager. Moreover, a 1 kb marker from GenedireX Co., a joint production of Taiwan and the United States, was employed to detect PCR products.

The characteristics of the gold nanoparticles

The gold nanoparticles with a size of 40 nanometers, purity of 99.95%, the concentration of 100 ppm, and with an international number (CAS: 744-57-5) ordered from the Nano Sadra Company (Mashhad – Iran) were used in this study (Fig. 1).

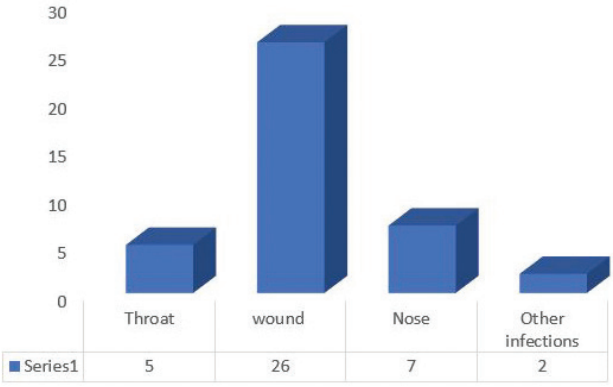

Figure 1. Frequency of clinical samples.

Real-time PCR

Minimum inhibitory concentration (MIC)

In this method, 20 out of 40 *Pseudomonas aeruginosa* isolates were exposed to gold nanoparticles. The broth microdilution method was used for preparing Mc Farland Standard and minimum inhibitory concentration (MIC) of growth for gold nanoparticles, and a bacterial suspension with a suitable density of Mc Farland Standard barium sulfate was employed to study the effect of antimicrobial agents. In order to prepare this standard, 5% ml of 1% barium chloride was mixed with 9.95 ml of 1% sulfuric acid. Moreover, for preparing the suspension, the bacterium was taken from a newly cultured colony and dissolved in some sterile physiological saline, and the correct density of standard turbidity(determination of microbial concentration) was identified using absorption measurements in a spectrophotometer at a wavelength of 625 nm between 0.8% to 1%. The minimum inhibitory concentration of growth was identified using the standard method of Clinical and Laboratory Standards Institute (CLSI), and the broth dilution method was applied to determine the effect of gold nanoparticles in the case of exposure of bacteria to an antimicrobial suspension. MIC was considered as the lowest concentration of gold nanoparticles, which inhibits the growth of *Pseudomonas aeruginosa* isolates in culture medium was considered, and the broth microdilution method was used to assess the effect of different concentrations of gold nanoparticles on the expression of *mexA* and *mexB* genes of efflux pump on 20 isolates of *Pseudomonas aeruginosa*.

RNA extraction and cDNA synthesis

Extraction of RNA isolates treated with gold nanoparticles and non-treated in the logarithmic growth phase was performed using RNA extraction kit (Qiagen, USA) according to the instructions, and finally the DNase enzyme was employed to remove the remaining DNA, and then the concentration of RNA was determined by a nanodrop. The amount of 1 µg of RNA from the sample was used for synthesizing cDNA using a QuantiTect Reverse Transcription kit (Qiagen, USA). The quantitative reverse transcription PCR (qRT-PCR) using SYBR green-contained mastermix (Applied Biosystem, UK) was applied to evaluate the *mexA* and *mexB* genes of efflux pump.The materials used in 20 µL of mastermix were 2 µL of cDNA, 10 pM of forward and reverse primers, and 10 µL of SYBR green-contained mastermix performed on the Korean Bioneer device. The temperatures used in the qPCR were 90 °C for 10 minutes, 95 °C for 15 seconds, and 60 °C for 1 minute performed at 40 cycles. Moreover, the G74 gene was regarded as an internal control. Finally, the relative expression of *mexA* and *mexB* genes was calculated by the $\Delta\Delta C_T$ method.

Statistical analysis

Statistical calculation of the present research was performed using SPSS software, and the Real-Time PCR data were analyzed by the one-way ANOVA analysis. $P > 0.05$ was considered statistically meaningful.

Results

Clinical monitoring

Most strains of *Pseudomonas aeruginosa* were isolated from wound samples, and the least strains were isolated from nasal samples (Fig. 2). The studied hospitals were

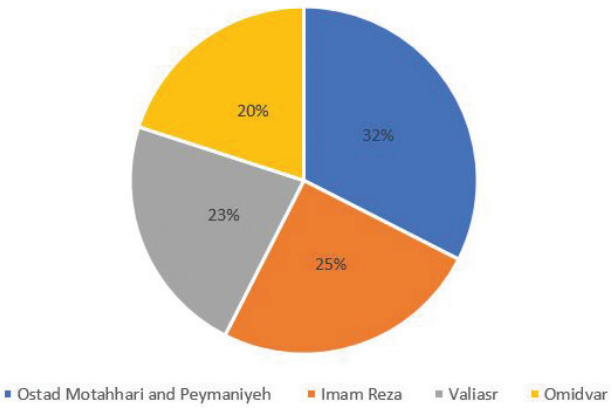

Figure 2. Distribution of clinical isolates of *Pseudomonas aeruginosa* in the studied hospitals.

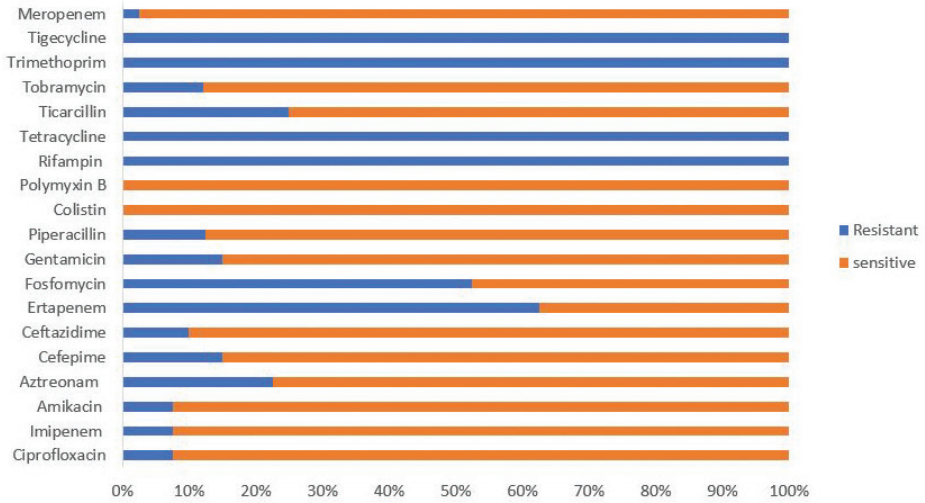

Figure 3. Susceptibility of *Pseudomonas aeruginosa* isolates to different antibiotics.

Ostad Motahhari and Peymaniyeh hospitals of Jahrom city in Iran,Imam Reza hospital of Lar city in Iran, Valiasr hospital of Lamerd city in Iran, and Omidvar hospital of Evaz city in Iran (Fig. 2). Forty non-duplicate strains of *Pseudomonas aeruginosa* were detected and identified from the studied hospitals using biochemical tests and API kits. Among 40 samples, 13 (32.5%), 10 (25%), 9 (22.5%), and 8 (20%) samples were collected from Ostad Motahhari and Peymaniyeh hospitals in Jahrom city, Imam Reza hospital in Lar city, Valiasr hospitals in Lamerd city, and Omidvar hospital in Evaz city, respectively (Fig. 3).

Antibiotic susceptibility testing

In the studied samples, the lowest levels of resistance were related to colestine (0%), polymyxin B (0%), meropenem (2.5%), imipenem (7.5%), amikacin (7.5%), and ciprofloxacin (7.5%), respectively. The highest resistance was observed against trimethoprim (100%), tigecycline (100%), tetracycline (100%), and rifampin (100%) (Fig. 4), respectively. According to the results, colestine and polymyxin B antibiotics are the best options for treating *Pseudomonas aeruginosa* infections.

Results obtained from gel electrophoresis of *gyrB*, *rhlR*, *exoT*, *lasR*, *Pela*, and *toxA* genes

The results indicated that the studied strains of *Pseudomonas aeruginosa* were carriers of most of these genes. The prevalence of *exoT*, *rhlR*, *lasR*, *gyrB*, *pelA*, and *toxA* genes in isolates were 32 (80%), 40 (100%), 40 (100%), 40 (100%), 37 (92.5%), and 40 (100%), respectively (Figs 5–11).

Since the antibiotic resistance in *Pseudomonas aeruginosa* has increased today than in the past, the presence of the above genes was investigated, which are effective factors in antibiotic resistance. According to the results, the prevalence of these genes was high in clinical isolates of *Pseudomonas aeruginosa*, indicating their role in antibiotic resistance in the evaluated isolates (Table 1).

PCR test results for *mexA* and *mexB* genes

The presence of *MexA* and *MexB* genes in 40 *Pseudomonas aeruginosa* isolates was evaluated before investigating the gene expression; the results demonstrated that the pump

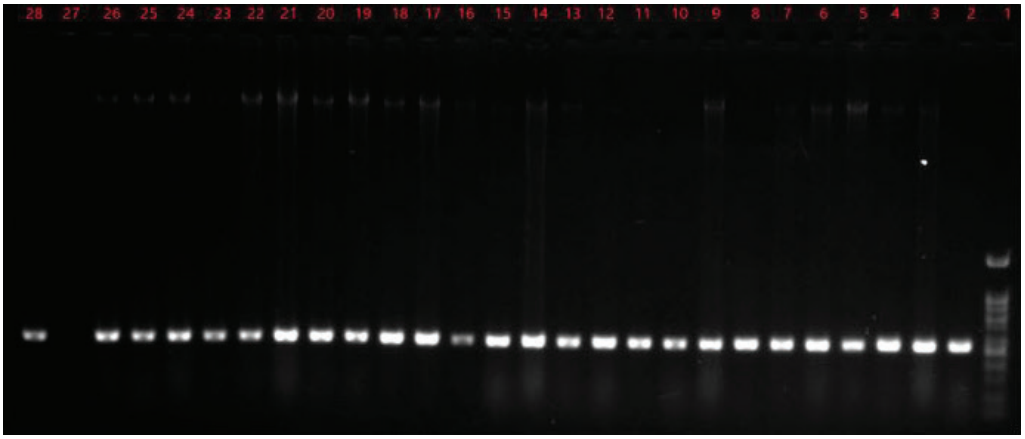

Figure 4. Results of *gyrB* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–26: positive samples of size 222 bp; Well 27: negative control; Well 28: positive control of *Pseudomonas aeruginosa* PTCC 17589.

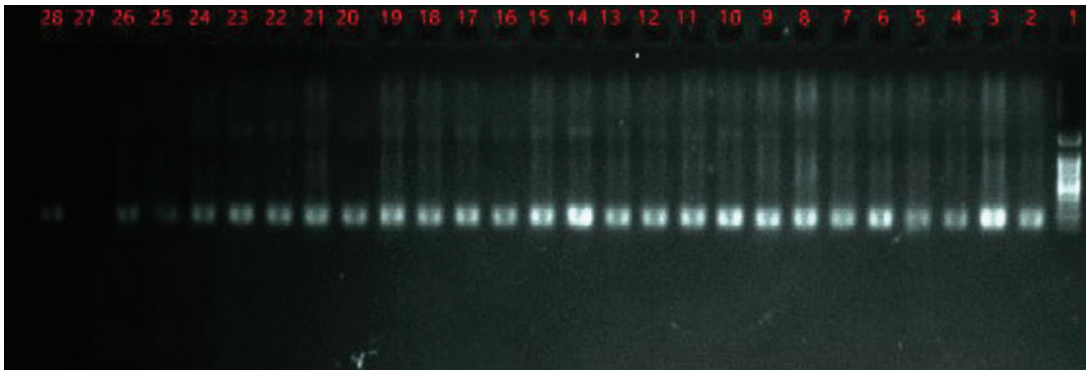

Figure 5. Results of *rhlR* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–26: positive samples of size 133 bp; Well 27: negative control; Well 28: positive control of *Pseudomonas aeruginosa* PTCC 17589.

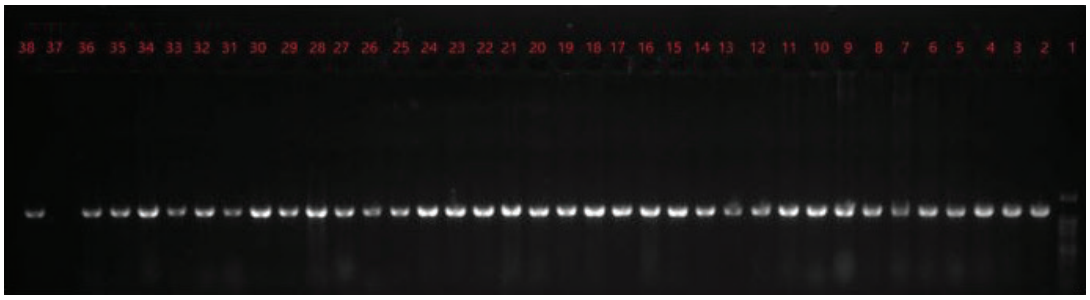

Figure 6. Results of *exoT* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–36: positive samples of size 471 bp; Well 37: negative control; Well 38: positive control of *Pseudomonas aeruginosa* PTCC 17589.

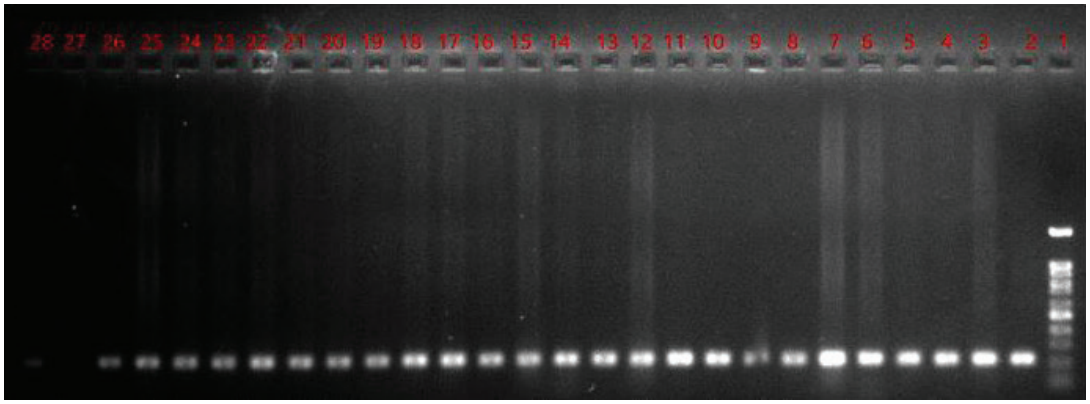

Figure 7. Results of *lasR* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–26: positive samples of size 130 bp; Well 27: negative control; Well 28: positive control of *Pseudomonas aeruginosa* PTCC 17589.

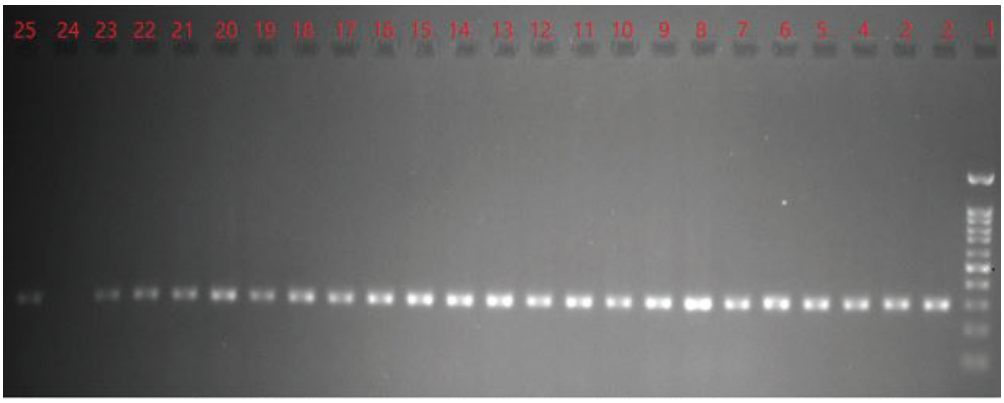

Figure 8. Results of *Pela* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–23: positive samples of size 148 bp; Well 24: negative control; Well 25: positive control of *Pseudomonas aeruginosa* PTCC 17589.

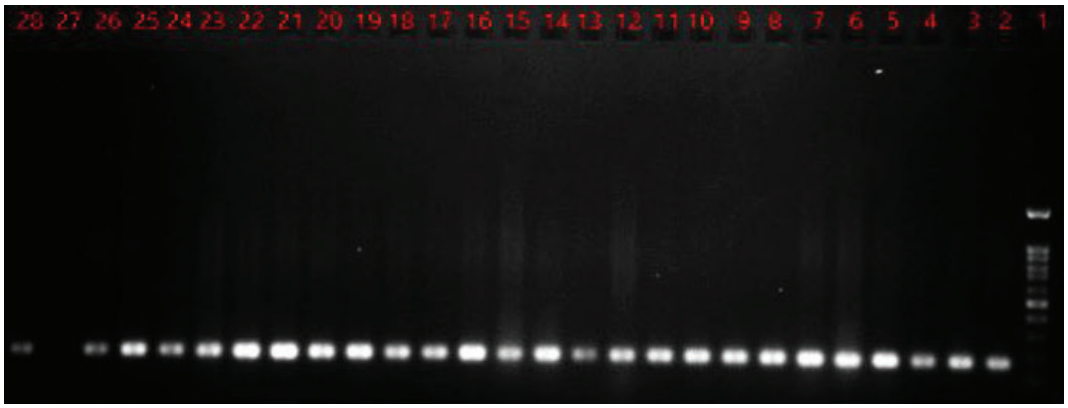

Figure 9. Results of *toxA* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 50 bp; Well 2–23: positive samples of size 85 bp; Well 24: negative control; Well 25: positive control of *Pseudomonas aeruginosa* PTCC 17589.

Figure 10. Percentage of *Pseudomonas aeruginosa* isolates carrying *gyrB*, *rhlR*, *exoT*, *lasR*, *Pela*, and *toxA*.

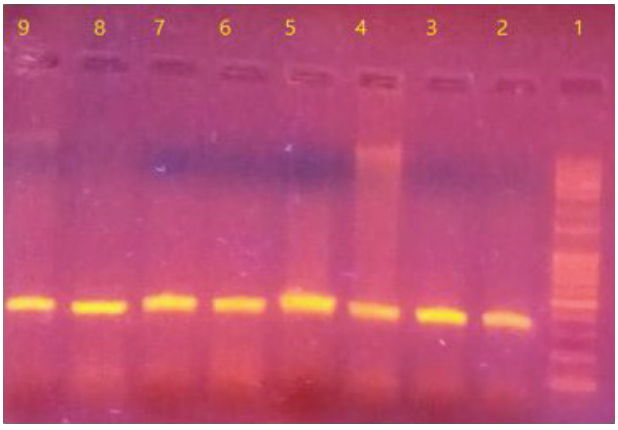

Figure 11. Results of *MexA* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 1: marker size 1 kb; Well 2–8: positive samples of size 316 bp; Well 9: positive control of *Pseudomonas aeruginosa* PTCC 17589.

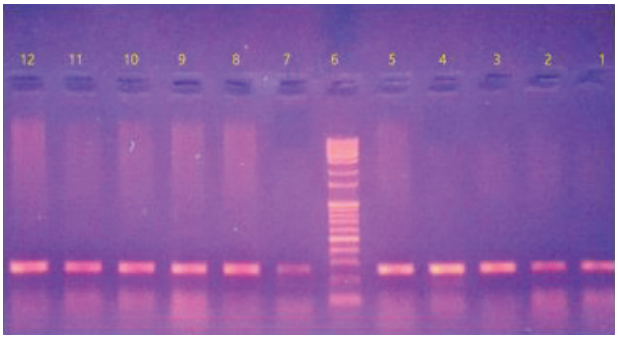

Figure 12. Results of *MexB* gene replication in clinical isolates of *Pseudomonas aeruginosa*. Well 6: marker size 1 kb; Well 1–5 and 8–12: positive samples of size 244 bp; Well 7: positive control of *Pseudomonas aeruginosa* PTCC 17589.

efflux genes (*MexA* and *MexB*) were present in all studied isolates (Figs 12 and 13).

Expression of *mexA* and *mexB* genes before and after contact with efflux pump inhibitors through real-time PCR

In order to compare the effect of gold nanoparticles on the expression of *MexA* and *MexB* genes after RNA extraction and cDNA synthesis, the changes of gene expression in isolates of two cell groups of "gold nanoparticles- treated" and "non-treated" was quantitatively investigated using melting curve analysis and amplification plot (Figs 14–17).

The expression rates of *MexA* and *MexB* genes in gold nanoparticle-treated isolates were meaning fully reduced compared to non-treated isolates, shown in Figs 18 and 19, respectively.The vertical and horizontal axes of the standard curve represent CT and quantity that indicates the number of copies of the gene per dilution. It is observed in the standard curves of *MexA* and *MexB* that the CT evaluation factors are indirectly related to concentration and increases as concentration is decreased.

According to the results, the inhibitory effect of gold nanoparticles on *MexA* gene is greater compared to *MexB* gene.

Discussion

Since no new antibiotics are available presently to replace the existing antibiotics for Gram-negative pathogens, and there is no extensively available vaccine against these infections, just one way to mitigate the effects of infections is to control their spread that can only be achieved in the case of fully understanding of causes, dynamics, and complexity of the prevalence of these organisms. The objective of the present studies was to contribute to this knowledge by studying *Pseudomonas aeruginosa*, which is the cause of nosocomial infections in burn-injured patients.

Much research has been carried out on the resistance of *pseudomonas*, the results of which are different in terms of time and place. In the present study, antibiotic resistance of 40 isolated strains from Omidvar hospital of Evaz city, Valiasr hospital of Lamerd city, Imam Reza hospital of Lar city,

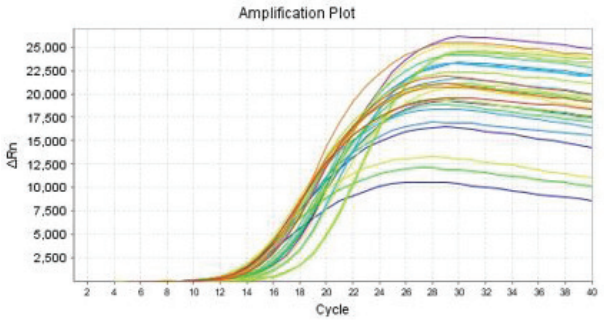

Figure 13. Results of *MexA* gene amplification curve in Real Time PCR by cycle.

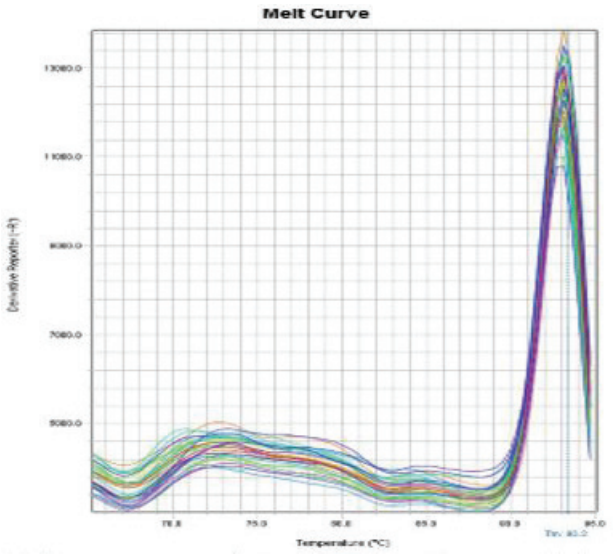

Figure 14. Melting curve analysis to ensure the specificity of the amplified fragments of the *MexA* gene; the measured curves of the gene in all samples are consistent and in the form of single-peak.

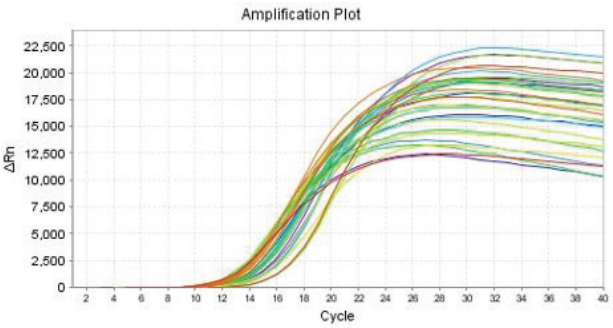

Figure 15. Melting curve analysis to ensure the specificity of the amplified fragments of the *MexB* gene that the measured curves of the gene in all samples are consistent and single-peaked.

and Ostad Motahhari and Peymaniyeh hospitals of Jahrom city were evaluated; the resistance rates was as follows:Colestine 0%, ciprofloxacin 3 (7.5%), imipenem 3 (7.5%), amikacin 3 (7.5%), aztreonam 9 (22.5%), cefepime 6 (15%), ceftazidime 40 (100%), ertapenem 25 (62.5%), fosfomycin21 (52.5%), gentamicin 6 (15%), piperacillin 5 (12.5%), polymyxin B 0%, Rifampin 40 (100%), tetracycline 40 (100%), ticarcillin 10 (25%), tobramycin 5 (12.5%), trimethoprim 40 (100%), tigecycline 40 (100%), and meropenem 1 (5/2%).

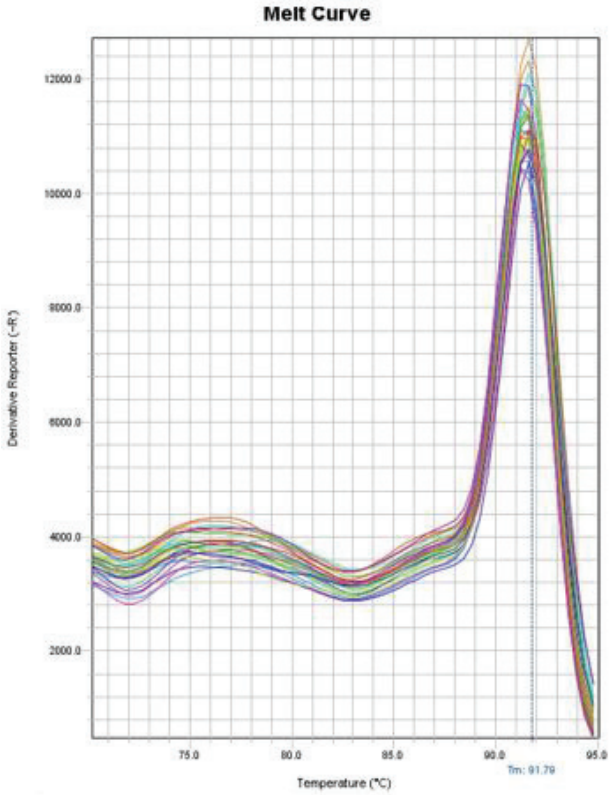

Figure 16. Melting curve analysis to ensure the specificity of the amplified fragments of the *MexB* gene; the measured curves of the gene in all samples are consistent and in the form of single-peak.

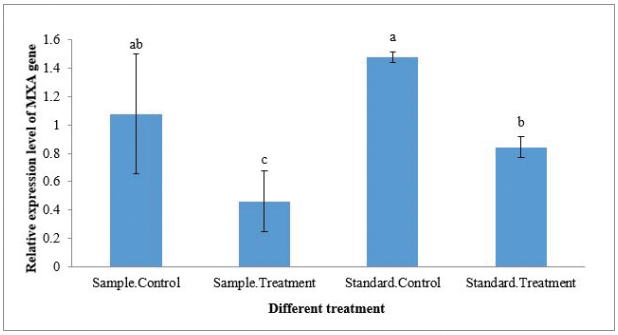

Figure 17. The expression rates of *MexA* gene in the gold nanoparticles-treated samples and non-treated samples; a significant decrease in gene expression was observed between treated and non-treated samples in comparison with the control group. The *G74* gene was used as an internal control.

In a study conducted by Shahid et al on *Pseudomonas aeruginosa* strains at the burn care units, the lowest and highest resistances were against the tetracycline (0%) and gentamicin (90%), respectively(Shahid et al. 2003). According to the present study, the results indicated an increase in drug resistance for the reason of overuse over a period of time. According to the results of the present research as well as the literature, the colestine and polymyxin B are the best therapeutic options against *Pseudomonas aeruginosa* infections.

Pseudomonas aeruginosa is a stubborn microorganism in terms of resistance to various antibiotics and possesses three main mechanisms of limited adsorption resistance

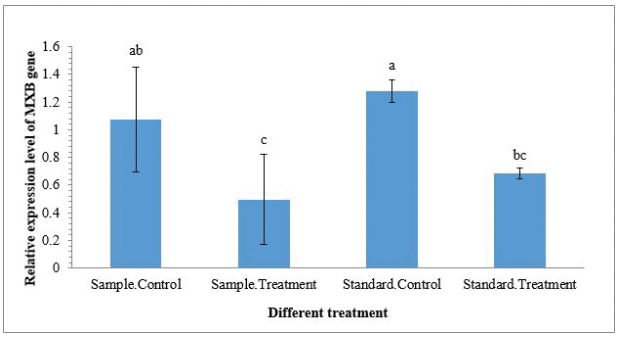

Figure 18. The expression rates of *MexB* gene in the gold nanoparticles-treated samples and non-treated samples; a significant decrease in gene expression was observed between treated and non-treated samples in comparison with the control group. The *G74* gene was used as an internal control.

Figure 19. The characteristics of the gold nanoparticles used.

and efflux, drug inactivation, and change in targets (Brinkman et al. 2000). Efflux pumps play an important role in the resistance of *Pseudomonas aeruginosa*. All antibiotic classes except polymyxins are sensitive to excretion by one or more of these efflux systems.

Among these systems, the *MexAB-oprM* is of great importance nowadays, which is responsible for the excretion of beta-lactams, quinolones, and an extensive range of anti-microorganisms. In the present article, the presence of *mexA* and *mexB* genes was investigated. The *MexAB-oprM* genes are chromosomal genes present in all wild strains, and their absence can be attributed to mutations. Arabestani et al. concluded that *mexAB-oprM* genes were present in 100% isolates of *Pseudomonas aeruginosa*(Arabestani et al. 2015). Moreover, Elgravi reported the presence of *mexAB-oprM* of septicemic *Pseudomonas aeruginosa* with the frequency of 100% of samples for both *mexA*, *mexB* operon genes indicating the chromosomal nature of the *mexAB-oprM* efflux pump genes (Al-Grawi 2012). In the present study, the presence of *MexA* and *MexB* genes in 40 isolates of *Pseudomonas aeruginosa* was evaluated before investigating the gene expression; the results demonstrated that the *MexA* and *MexB* efflux pump genes were present in all studied isolates, which was in line with the results of previous investigations.

Many investigation sindicate that the antimicrobial properties of antibiotics can be increased by destroying efflux pumps. The inhibitors of these pumps can be employed to disrupt the function of drug efflux pumps. Nowadays, various inhibitors, such as alanine-arginine beta-naphthylamide,can change the minimum concentration of inhibitors by excreting toxic substances. Gold nanoparticles currently have extensive applications in biomedicine, including bioimaging, gene transfer, drug delivery, plasmonic biosensing, colorimetric assays, tissue engineering, imaging therapy, and cancer therapy. The antibacterial mechanism for gold nanoparticles depends on their size; smaller gold nanoparticles create irreversible pores as they move through the bacterial cell membrane (Zheng et al. 2017). The antibacterial activity of gold nanoparticles involves several mechanisms, such asthe presence of a strong positive charge on gold nanoparticles that increases the tendency of these nanoparticles toward the negative charge of bacteria, which in turn disrupts the integrity of the cell membrane and leads to cell rupture. In the present study, the expression of *MexA* and *MexB* genes before and after contact with efflux pump inhibitors (gold nanoparticles) was investigated by the Real-Time PCR technique. Unfortunately, not much research has been conducted on the effect of gold nanoparticles on the function of efflux pump genes, but many studies have been carried out on the use of other nanoparticles on these genes.

Aria et alstudied the effect of vanilla and the applicaiton of gold nanoparticles on multidrug-resistant isolates of *Pseudomonas aeruginosa* and concluded that treatment with gold nanoparticles reduced the expression of *mexA* and *mexB* genes, which is in line with the results of the present paper(Arya et al. 2019), in which a reduction in the expression of *mexA* and *mexB* genes was observed in the gold nanoparticle-treated strains.

Furthermore, Mohammadipour et al. conducted a piece of researcher garding the effect of encapsulated silybin in nanoparticles on the expression of *oprM* gene in resistant strains of *Pseudomonas aeruginosa* and concluded thata decrease in *oprM* expression in resistant isolates caused a reduction in *mexAB-oprM*, *mexXY-oprM* on the cell surface and an increase in the sensitivity to antibiotics that were in line with the results of the present research (Mohammadipour et al. 2017). The gold nanoparticles reduce the expression of *mexA* and *mexB* genes and increase antibiotic sensitivity, indicating the positive effect of gold nanoparticles on the antibiotic-resistant isolates of *Pseudomonas aeruginosa*.

Rudbaraki conducted an study on the change of *mexB* gene expression in ciprofloxacin-resistant *Pseudomonas aeruginosa* isolates under treatment with encapsulated silibinin in nanoparticles and concluded that silibinin increases the capability of ciprofloxacin in inhibiting the growth of *Pseudomonas aeruginosa* by reducing the expression of genes involved in efflux pump systems, such as *mexB* gene (Ahmadi et al. 2017).

In the present study, the expression rate of *MexA* gene in gold nanoparticle-treated isolates was significantly reduced thanin non-treated isolates. An investigation on *MexB* expression indicated that gold nanoparticle-treated samples showed a significant decrease in expression compared to non-treated samples. According to the findings, the inhibitory effect of gold nanoparticles on *MexA* gene is higher than this effect on *MexB* gene.

Conclusion

The *MexAB-oprM* system is one of the main factors in the resistance of *Pseudomonas aeruginosa*. The results of the present investigation indicated that gold nanoparticles reduce the number of active efflux pumps on the cell surface by reducing the expression of *mexA* and *mexB* genes so that a decrease in the level of these efflux pumps reduces the excretion of antibiotics from the cell and subsequently causes lower concentrations of antibiotics to be able to kill cells.

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