Archive of SID

Journal of Sciences, Islamic Republic of Iran 31(2): 105 - 111 (2020) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Evaluation of the Effects of Iron Oxide Nanoparticles on Expression of TEM Type Beta-Lactamase Genes in *Pseudomonas Aeruginosa*

M. Lotfpour, K. Amini*

Department of Microbiology, Faculty of Basic Sciences, Saveh Branch, Islamic Azad University, Saveh, Islamic Republic of Iran

Received: 27 September 2019 / Revised: 17 January 2020 / Accepted: 18 February 2020

Abstract

Pseudomonas aeruginosa is a common cause of surgical-site infections and healthcare-associated infections in the bloodstream, and urinary tract. Iron oxide nanoparticles (IONPs) have shown, to possess antibacterial features. The nanoparticles' status as emerging therapeutic elements has motivated investigators to assess the effects of iron nanoparticles on the expression of TEM type beta-lactamase genes in *P. aeruginosa*. In this descriptive-analytic study, 60 clinical isolates of *P. aeruginosa* were isolated from burn wounds and respiratory excretions of Pasargad Research Laboratory of Tehran, Iran. All isolates were characterized using differential biochemical tests and confirmed samples as *P. aeruginosa*. Their genomic DNA was extracted and PCR reaction was performed to screen TEM-gene carrying isolates. Then MIC of IONPs against these strains was determined and finally, Real-time PCR performed to the determination of the expression of the TEM gene. Results showed that 8 isolates (13/33%) had the TEM beta-lactamase gene. The MIC and MBC of IONPs against *P. aeruginosa* strains were observed at 256 µg/mL or 125 µg/mL, while the MBC was determined at 500 µg/mL. In addition, statistical analysis of Real-time PCR data showed that there is a statistically significant difference between gene expression levels of IONPs treated isolates and non-treated ones. The results showed that TEM gene expression levels in two isolates treated with IONPs were 78% and 75% lower than untreated bacteria ($P<0.001$; $r= 0.958$). Our findings confirmed that IONPs are potential antibacterial agents and can be considered as promising treatments for recalcitrant *P. aeruginosa* infections.

Keywords: *P. aeruginosa*; TEM beta-lactamase; Iron oxide nanoparticles.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a rod-shaped, encapsulated and gram-negative opportunistic pathogen that colonizes immunocompromised hosts using its virulence factors. [1]. *P. aeruginosa* is responsible for both nosocomial and community-acquired infections, so finding efficient medicine to

* Corresponding author: Tel: +989125454074, Fax:+982122119260 ; Email: Dr_kumarss_amini@yahoo.com

www.SID.ir

Archive of SID

treat its related infectious is necessary to improve the clinical outcome [2]. Typically, the organism is harmless, but it can become a persistent opportunist pathogen in hosts with a weakened immune system and hospitalized patients. The bacteria are a common cause of a wide range of infections, including ventilator-associated pneumonia, bacteremia, skin, and surgical-site infections. There is a great amount of data implicating that infections caused by drug-resistant *P. aeruginosa* are related to significant rises in mortality and morbidity rate, length of hospital stays, chronic care, and need for surgical intervention [1, 2].

Antibiotic-resistant (AR) infections and lack of new antibiotic development by the pharmaceutical industry, are already widespread across the world and has reached a crisis point in many societies [3]. AR *P. aeruginosa* is one of the significant agents of healthcare-associated infections, that are a global health issue because of the cumulative development of multidrug-resistant strains. This microorganism is fundamentally resistant to most antibiotics because of expression of impermeable proteins in the outer membrane. Additionally, *P. aeruginosa* utilizes different mechanisms including the secretion of inactivating enzymes, expression of efflux pumps and chromosomal mutations to resist against antibiotics [4, 5]. *P. aeruginosa* expresses many beta-lactamase enzymes, including Metallo-beta-lactamases (MBL), chromosomal cephalosporinase (AmpC) and extended-spectrum beta-lactamases (ESBL). Nowadays, the incidence of ESBL-producing bacteria has been progressively growing in acute care hospitals [4, 6].

Beta-lactamase enzymes are the most prominent reason for resistance to beta-lactam antibiotics, which are the most common treatment for Gram-negative bacterial infections. ESBL producing gram-negative bacteria, especially *P. aeruginosa*, are characterized by their ability to express a broad-spectrum beta-lactamase enzyme to hydrolyze a wide variety of beta-lactam antibiotics such as penicillins, cephamycins, cephalosporins, and carbapenems [7].

Some studies showed that the most serious infections due to gram-negative bacteria occur in healthcare places and are frequently caused by *Acinetobacter*, *Klebsiella pneumonia*, and *P. aeruginosa*. *P. aeruginosa* can develop resistance to antimicrobial agents either through mutations that alter the expression and/or function of genomic encoded mechanisms or through the acquisition of resistance genes on plasmids [8]. Ampicillin and penicillin resistance in *P. aeruginosa* isolates are attributed to the TEM enzyme. The microorganism relies on iron as a nutrient source to grow but excessively high levels of iron can be toxic to *P. aeruginosa* [9]. In addition, iron plays a key role in extracellular DNA (eDNA) release, biofilm formation and is a dominant controller of gene expression in *P. aeruginosa* which is intermediated through the quorum sensing pathways and/or the ferric uptake regulator (Fur) repressor protein [10, 11]. The invention of nanoparticles has started a new way in medical diagnoses, drug deliveries, and treatments. Iron oxide nanoparticles (IONPs) are one of the most commercially important types of these particles and exhibit some unique characteristics that enhance their ability to be used against pathogenic bacteria [11, 12]. The augmented use of nanoparticles and their status as emerging therapeutic elements has motivated the evaluation of the effects of iron nanoparticles on TEM type beta-lactamase genes expression in *P. aeruginosa*.

Materials and Methods

Bacterial strains

In our descriptive-analytic investigation, sixty strains of *P. aeruginosa* were gathered from burn wounds and respiratory excretions of Pasargad Research Laboratory of Tehran, Iran. All isolates were characterized using differential tests (gram staining, catalase, oxidase, indole, citrate, urease, H_2S and cetrimide tests) through the procedures explained in Bergey's manual of determinative microbiology (13). Confirmed samples as *P. aeruginosa* were cultured in Luria Bertani medium (30% glycerol) (Merck, USA), stored at -70 °C and subjected to further molecular identification. Then, isolates were transferred into tryptic soy agar (TSA) (Merck, USA) and incubated at 37 °C for overnight.

Molecular analysis

Isolates genomic DNA was extracted using the Gram-negative DNA extraction kit (Qiagen, Germany) according to product guidelines (14). The quantity and quality of extracted DNA were checked by determining the ratio of OD 260/280 (NanoDrop 2000c; Thermo Fisher Scientific) and 1.5% agarose gel, respectively. Then the PCR reaction was performed with a final volume of 20 µl, containing 4 µl template DNA (200 ng), 1 µl forward primer, 1 µl reverse primers (Table 1), 4 µl of distilled water and 10 µl of Amplicon 2x master mix. Briefly, one cycle of early denaturation at 94°C for 160 seconds, followed by forty cycles of 94°C for fifteen seconds, 55°C for twenty-five seconds, and 70°C for thirty seconds were the thermocycle conditions. The amplicons were separated by 1.2% agarose gel electrophoresis at for two hours. After electrophoresis, fragments were stained by ethidium bromide and visualized using ultraviolet light.

www.SID.ir

Archive of SID

Table 1. Oligonucleotide primers were used for the detection of *TEM* genes

Primer	Sequence	Base pair
TEM-F	(5’-ACGCTCACCGGCTCCAGATTTAT-3’)	93bp
TEM-R	(5’-TGGATGGAGGCGGATAAAGTTGC-3’)	93bp
16S-F	(5’- GGGACCCGCACAAGCGGTGG -3’)	191bp
16S-R	(5’- GGGTTGCGCTCGTTGCGGGA -3’)	191bp

Biosynthesis iron oxide nanoparticles

Fe_3O_4 nanoparticles were synthesized according to Khatami et al. According to that study instruction [15], 30 grams of *Rosmarinus officinalis* leaves were collected, sterilized, dried and powdered. *R. officinalis* leaves powder was added to 1500 mL sterilized DW and heated at 85°C for 30 minutes. Twenty-five milliliters of the obtained extract was mixed with 1 mM of the iron (III) chloride hexahydrate 98% stock solution (0.1 M) under constant stirring. The production of IONPs realized when the liquid yellow color changed to black [15]. IONPs size and morphology were evaluated with scanning electron microscopy (SEM) (Hitachi, Japan).

Determination of the antimicrobial activity of IONPs

Well-diffusion assay method used for evaluation of IONPs antimicrobial activity against bacterial strains carrying the TEM beta-lactamase gene (n=8). The pure cultures of microorganisms were subjected to Mueller Hinton Broth (MHB) and incubated at 37 °C for 24 hours. Following 24 hours, the optical density of the MHB culture was checked to be around 0.1 at 600 nm to get 1×10^8 CFU/ml. Each strain was swabbed precisely onto the individual plates via sterile cotton swabs. Using a micropipette, different concentrations (2000, 1000, 500, 250 and 125 µg/mL) of IONPs were added to test wells (6-mm diameter). Following incubation at 35 °C for 24 h, growth inhibition zones were measured.

Minimum inhibitory concentration and Minimum Bactericidal Concentration of IONPs

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of IONPs against strains with positive TEM beta-lactamase gene (n=8) had been performed as previously defined by Seifi et al. [16]. To prevent the occurrence of errors, nanoparticle suspensions were prepared simultaneously with the implementation of microbial tests. *P. aeruginosa* ATCC 25923 was used as standard strain and tests were carried out in two replicates. To evaluate the MIC and MBC of IONPs, serial dilutions of nanoparticle powder in MHB (2000, 1000, 500, 250 and 125 µg/mL) were prepared according to the Clinical and Laboratory Standards Institute (CLSI) standard [17]. Suspension of cells in MHB containing 1.5×10^8 CFU/mL was added to each concentration. The tubes were assessed for turbidity at 600 nm after overnight incubation at 37 °C. Then, MIC was determined as the lowest concentration of IONP which prevents bacterial growth in the culture medium. To ascertain the MBC, a loopful of broth was collected from MIC determination tubes that did not demonstrated any growth and inoculated on sterile MHA. The dishes were incubated for overnight at 37 °C. After incubation, the lowest concentration at which no visible growth was recorded as the minimum bacterial concentration.

Real-Time PCR

To assessment of the effects of iron nanoparticles on TEM gene expression profile, after treatment of 8 isolates (with the TEM gene) with MIC concentration of IONPs using microdilution method, the expression of TEM gene in treated (n=8) and non-treated (n=8) isolates was studied by the Real-time method. Total RNA was purified using an RNA Extraction Mini Kit (CinnaGen, Iran), and quantified using a spectrophotometer, and the complementary DNA (cDNA) synthesis was carried out in reaction volumes using the CinnaGen cDNA Reverse Transcriptase Kit (Thermo Fisher Scientific) according to manufacturer procedure. The primers for the PCR amplification of cDNA were designed using the Oligo 7 available at the website (http://www.oligo.net/) (Table 1).

Real-time PCR amplifications were performed in 20 µl reactions containing 10 µl qPCR Mix (QuantiTect SYBR Green), 1.5 µl cDNA, 2 µL of primers, 6.5 µL of DW and 1 µL of DNA-mixture with the following thermocycle conditions; initial denaturation at 94°C for 10 minutes, 40 cycles of 96°C for 5 sec, 55°C for 22 sec, then 72°C for 1 minute. The relative changes in target gene expression were calculated using the equation $2^{-\Delta\Delta CT}$, where all values were normalized with respect to the 16SrRNA levels [18].

Statistical analysis

Statistical analysis and descriptive statistics are conducted by Statistical Package for the Social Sciences (SPSS) software using paired samples T-test ($P < 0.05$, 0.01). The Kolmogorov-Smirnov test used for the evaluation of the normality of data. Student’s t-test was

www.SID.ir

Archive of SID

performed to determine statistical differences. The reproducibility of data was determined by the coefficient of variation (CV). Bio-Rad Real-Time PCR analysis software used to collection and analysis of RT-PCR data.

Results

Antimicrobial activity, MIC and MBC determination of IONPs

The evaluation of antimicrobial effects of IONPs using the microdilution technique and the assessment of MIC and MBC against eight *P. aeruginosa* strains carrying the TEM beta-lactamase gene were performed (Table 2). The antimicrobial activity of IONPs was examined at different concentrations. The lowest concentration of IONPs that inhibited the growth of *P. aeruginosa* (MIC) was observed at 256 µg/mL or 125 µg/mL, while the MBC of IONPs against *P. aeruginosa* was determined at 500 µg/mL.

Scanning electron microscopy (SEM) analysis of synthesized IONPs

SEM images of biosynthesized IONPs are detailed in Figure1. The nanoparticle size range was from 13 to 41 nm. The IONPs shape were mainly spherical and approximately monodisperse.

Molecular analysis

The results of the PCR test showed that 8 (13.33%) isolates had the TEM beta-lactamase gene (Figure 2). Bacterial growth was completely stopped after 2 hours of treatment with a concentration of 500 µg / ml of IONPs. In addition, statistical analysis of Real-time PCR data showed that there is a statistically significant difference between gene expression levels of two IONPs treated isolates and non-treated ones. The results showed that TEM gene expression levels in two isolates treated with iron oxide nanoparticles were 78% and 75% lower than untreated bacteria ($P<0.001$; $r = 0.958$) (Figure 3). The decreases in TEM beta-lactamase gene

Table 2. Antibacterial effects of iron oxide nanoparticles against strains with positive TEM beta-lactamase gene

Microorganism Code	Iron oxide nanoparticles		
	Zone inhibition (mm)	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)
PS1	26	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS12	32	256 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS3	24	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS18	20	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS51	22	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS47	21	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS40	26	128 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$
PS13	26	256 $\mu g\ ml^{-1}$	500 $\mu g\ ml^{-1}$

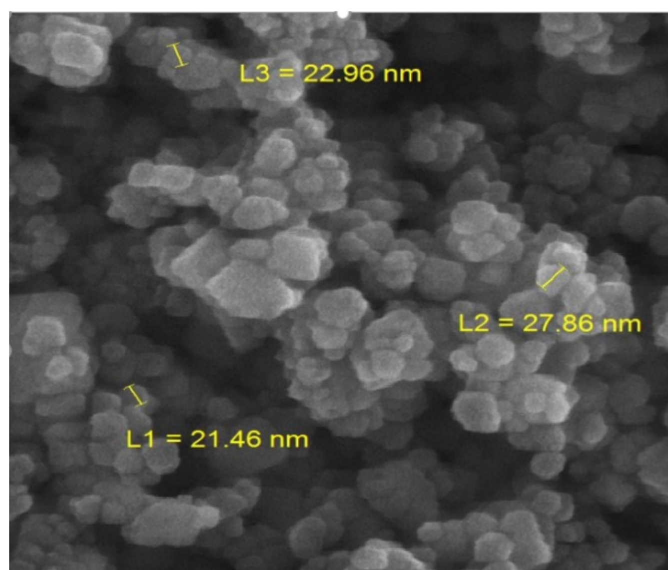

Figure 1. SEM image of iron oxide nanoparticles showing that the size of the nanoparticle is in range of 13 to 41 nm.

www.SID.ir

Archive of SID

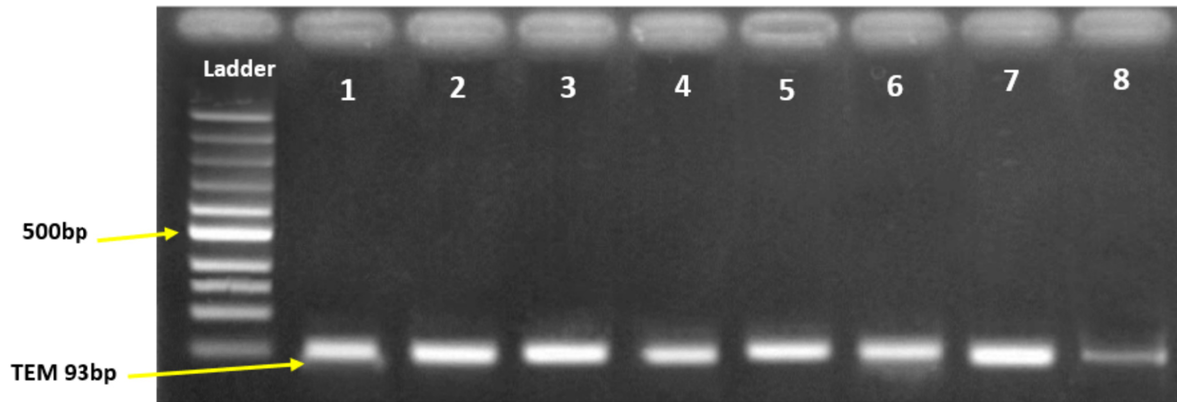

Figure 2. Agarose gel electrophoresis of PCR products amplified from TEM gene carrying *P. aeroginosa* strains. Arrow indicates the expected 93 bp PCR product. Ladder: 1 kb plus DNA ladder. Lanes from 1 to 8: Samples.

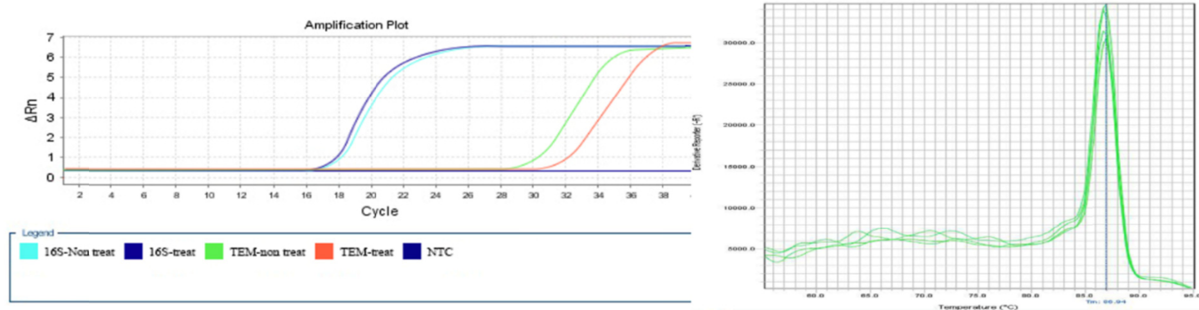

Figure 3. Comparison of gene expression levels in isolates of this study (Left) and melting curve (right).

expression levels observed in another six isolates but were not statistically significant.

Discussion

The expression of beta-lactamase by Gram-negative bacteria is one of the most effective and predominant mechanisms of resistance to beta-lactam antibiotics [5]. Due to the fact that the production of beta-lactamase enzymes leads to failure of treatment with extended-spectrum antibiotics, infection due to beta-lactamase-producing bacteria, especially TEM-derived ESBL producing gram-negative bacteria, are an increasing problem worldwide [19]. The current study aimed at the evaluation of the effects of IONPs on TEM type beta-lactamase gene expression in isolated strains of *P. aeruginosa*.

Analyses of TEM gene expression in treated *P. aeruginosa* with iron oxide nanoparticles in this study exhibited that the expression levels of TEM was meaningfully decreased in two isolates. This may suggest that IONPs cause stress and inhibit *P. aeruginosa* by their impact on the expression of the multi-resistance response genes [20, 21]. In the present study out of the 60 *P. aeruginosa* isolates, eight (13.33%) were positive for the TEM-1 B-lactamases gene. Frequency of TEM-1 B-lactamases gene in previous clinical studies by Cavallo et al (1310 *P. aeruginosa* isolates), Bert et al (249 *P. aeruginosa* isolates) in France and a veterinary study by Elhariri et al (45 *P. aeruginosa* isolates) in Egypt was reported as 0%,1.9% and 33%, respectively [22-24]. In addition, a clinical study in Iran by Shojapour et al (175 *P. aeruginosa* isolates) demonstrated that 19 % of isolates were positive for the TEM-1 B-lactamases resistance gene which was in line with our findings [25].

In our study, the minimum concentration of Fe_3O_4 NPs required for the growth inhibition and bactericidal activity of TEM beta-lactamase positive *P. aeruginosa* was 256 or125 µg/ml and 500 µg/ml, respectively. These findings are in line with those studies

www.SID.ir

Archive of SID

demonstrating that the toxicity and bactericidal effects of copper, zinc or IONPs depends on concentration, species, and particle size [26, 27]. These can be attributed to the nanoparticles' diameter because they can penetrate deeply into the biofilm matrix. In addition, these nano agents have a high surface area to volume ratio, that provides an effective interaction with bacteria [28,26,10]. In the Khatami et al study, MIC and MBC of iron oxide nanoparticles against another gram-negative bacteria, *E. coli*, were obtained at 250 µg/mL and 500 µg/mL, respectively [29]. Masadeh et al. calculated MIC value of IONPs against *Enterobacter aerogenes*, *Proteus mirabilis*, and *Klebsiella pneumoniae* in the range of 10-320 µg/mL of IONPs [30].

Antimicrobial nanoparticles deal with many characteristic advantages, low cost, and selective toxicity when compared to conventional antibiotics. Previous studies have demonstrated that the application of low concentrations of iron nanomaterials may negatively affect the functionality of porin pumps [31, 32]. Ramezani et al. revealed the significance of the physicochemical features of iron nanoparticles and their effective concentration on different bacteria [10].

Conclusion

Our finding showed the effects of iron nanoparticles on TEM gene expression levels, as another antimicrobial feature. In conclusion, this study demonstrates that the iron nanoparticles downregulated the expression of the TEM gene and inhibits the growth of *P. aeruginosa*. Current survey findings support that iron oxide nanoparticles and its composites can be considered as potential antibacterial and therapeutic agents for recalcitrant *P. aeruginosa* infections.

Acknowledgement

We would like to extend our sincere gratitude and appreciation for all of the hard work and dedication provided by the Pasargad laboratory staff.

References

1. Chalhoub H, Pletzer D, Weingart H, Braun Y, Tunney MM, Elborn JS, Rodriguez-Villalobos H, Plésiat P, Kahl BC, Denis O, Winterhalter M. Mechanisms of intrinsic resistance and acquired susceptibility of Pseudomonas aeruginosa isolated from cystic fibrosis patients to temocillin, a revived antibiotic. *Sci. Rep*. **7**:40208; (2017).
2. Chevalier S, Bouffartigues E, Bodilis J, Maillot O, Lesouhaitier O, Feuilloley MG, Orange N, Dufour A, Cornelis P. Structure, function and regulation of Pseudomonas aeruginosa porins. *FEMS Microbiol. Rev*. **41**(5):698-722; (2017).
3. Li X-Z, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin. Microbiol. Rev*. **28**(2):337-418; (2015).
4. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *Pharm & Therap*. **40**(4):277; (2015).
5. Pasa O, Ozer B, Duran N, Inci M, Yula E. Beta-lactamase Enzymes of Clinical Pseudomonas aeruginosa Strains. *West Indian Med. J*. **65**(1):40-5; (2016).
6. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infect. Dis*. **14**(1):13; (2014).
7. Shanmugam K, Thyagarajan R, Katragadda R, Vajravelu L, Lakshmy A. Biofilm formation and Extended Spectrum Beta Lactamases (ESBL) producers among the gram negative bacteria causing Urinary tract infections. *Int. J. of Med. Microb. and Trop. Dis*. **3**(3):86-90; (2017).
8. Gellatly SL, Bains M, Breidenstein EB, Strehmel J, Reffuveille F, Taylor PK, Yeung AT, Overhage J, Hancock RE. Novel roles for two-component regulatory systems in cytotoxicity and virulence-related properties in Pseudomonas aeruginosa. *AIMS Microbiol*. **4**(1):173-91; (2018).
9. Oglesby AG, Farrow JM, Lee JH, Tomaras AP, Greenberg EP, Pesci EC, Vasil ML. The influence of iron on Pseudomonas aeruginosa physiology a regulatory link between iron and quorum sensing. *J. Biol. Chem*. **283**(23):15558-67; (2008).
10. Ramezani Ali Akbari K, Abdi Ali A. Study of antimicrobial effects of several antibiotics and iron oxide nanoparticles on biofilm producing pseudomonas aeruginosa. *NMJ*. **4**(1):37-43; (2017).
11. Lerner RN, Lu Q, Zeng H, Liu Y. The effects of biofilm on the transport of stabilized zerovalent iron nanoparticles in saturated porous media. *Water Res*. **46**(4):975-85; (2012).
12. Fahmy HM, Mohamed FM, Marzouq MH, Mustafa AB, Alsoudi AM, Ali OA, Mohamed MA, Mahmoud FA. Review of Green Methods of Iron Nanoparticles Synthesis and Applications. *J. Bionanosci*. **14** (5):1-13; (2018).
13. De Vos P, Garrity GM. Bergey's manual of systematic bacteriology: *Springer*;p123-191; (2009).
14. Phillips K, McCallum N, Welch L. A comparison of methods for forensic DNA extraction: Chelex-100® and the QIAGEN DNA Investigator Kit (manual and automated). *Forensic Sci. Int*. **6**(2):282-5; (2012).
15. Khatami M, Alijani H, Sharifi I, Sharifi F, Pourseyedi S, Kharazi S, Lima Nobre MA, Khatami M. Leishmanicidal activity of biogenic Fe3O4 nanoparticles. *Sci. Pharm*. **85**(4):36; (2017).
16. Seifi Mansour S, Ezzatzadeh E, Safarkar R. In vitro evaluation of its antimicrobial effect of the synthesized Fe3O4 nanoparticles using Persea Americana extract as a green approach on two standard strains. *Asi. J. Gre. Chem*. **3**:353-65; (2019).

www.SID.ir

Archive of SID

17. Turbett SE, Pierce VM. Overview of antibacterial susceptibility testing. *UpToDate Retrieved January*.;**12**:2019; (2017).
18. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill F3, Smith TF. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol. Rev*. **19**(1):165-256; (2006).
19. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing Enterobacteriaceae. *Clin. Microbiol. Rev*. **31**(2):e00079-17; (2018).
20. Sharma VK, Johnson N, Cizmas L, McDonald TJ, Kim H. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere*. **150**:702-14; (2016).
21. Colque C, Orio AA, Feliziani S, Marvig R, Tobares A, Johansen H. Hypermutator Pseudomonas aeruginosa exploits multiple genetic pathways to develop multidrug resistance during long-term infections in the airways of cystic fibrosis patients. *Bio. Rxiv*. **8**(3):80-89; (2019).
22. Cavallo J, Fabre R, Leblanc F, Nicolas-Chanoine M, Thabaut A. Antibiotic susceptibility and mechanisms of β-lactam resistance in 1310 strains of Pseudomonas aeruginosa: a French multicentre study (1996). *J Antimicrob. Chemother*. **46**(1):133-6; (2000).
23. Bert F, Branger C, Lambert-Zechovsky N. Identification of PSE and OXA β-lactamase genes in Pseudomonas aeruginosa using PCR–restriction fragment length polymorphism. *J. Antimicrob. Chemother*. **50**(1):11-8; (2002).
24. Elhariri M, Hamza D, Elhelw R, Dorgham SM. Extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa in camel in Egypt: potential human hazard. *Ann. Clin. Microbiol. Antimicrob*. **16**(1):21; (2017)..
25. Shojapour M, Shariati L, Karimi A, Zamanzad B. Prevalence of TEM-1 type beta-lactmase genes in Pseudomonas aeruginosa strains isolated from burn infections using Duplex PCR in Shahrekord, 2008. *J. Arak. Univ. Med. Sci*. **14**(1); (2011).
26. Neupane, Bishnu Prasad, Dinesh Chaudhary, Sanjita Paudel, Sangita Timsina, Bipin Chapagain, Nirmala Jamarkattel, and Bishnu Raj Tiwari. Himalayan honey loaded iron oxide nanoparticles: synthesis, characterization and study of antioxidant and antimicrobial activities. *Int. j. nanomed*. **14**:3533; (2019).
27. Khatami, Mehrdad, Hajar Q. Alijani, Mojtabi Haghighat, Mehdi Bamrovat, Sara Azhdari, Mohammad Ahmadian, Marcos Nobre, Mohammadreza Heidari, Mina Sarani, and Sanaz Khatami. Green Synthesis of Amorphous Iron Oxide Nanoparticles and their Antimicrobial Activity against Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli. *Iran. J. Biotechnol*. **10**:33-39; (2019).
28. Vasantharaj S, Sathiyavimal S, Senthilkumar P, LewisOscar F, Pugazhendhi A. Biosynthesis of iron oxide nanoparticles using leaf extract of Ruellia tuberosa: antimicrobial properties and their applications in photocatalytic degradation. *J. Photochem. Photobiol. Biol*. **192**:74-82; (2019).
29. Khatami, M., Aflatoonian, M.R., Azizi, H., Mosazade, F., Hoshmand, A., Nobre, M.A.L., Poodineh, F.M., Khatami, M., Khraazi, S. and Mirzaeei, H. Evaluation of Antibacterial Activity of Iron Oxide Nanoparticles Against Escherichia coli. *Practice*. **18**:19; (2017).
30. Masadeh, Majed M., Ghadah A. Karasneh, Mohammad A. Al-Akhras, Borhan A. Albiss, Khaled M. Aljarah, Sayer I. Al-Azzam, and Karem H. Alzoubi. Cerium oxide and iron oxide nanoparticles abolish the antibacterial activity of ciprofloxacin against gram positive and gram negative biofilm bacteria. *Cytotechnology*. **67**(3):427-35; (2015).
31. Borcherding J, Baltrusaitis J, Chen H, Stebounova L, Wu CM, Rubasinghege G, Mudunkotuwa IA, Caraballo JC, Zabner J, Grassian VH, Comellas AP. Iron oxide nanoparticles induce Pseudomonas aeruginosa growth, induce biofilm formation, and inhibit antimicrobial peptide function. *Envi. Sci: Nano*. **1**(2):123-32; (2014)..
32. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *Int. j. nano*. **7**:6003; (2012).

www.SID.ir