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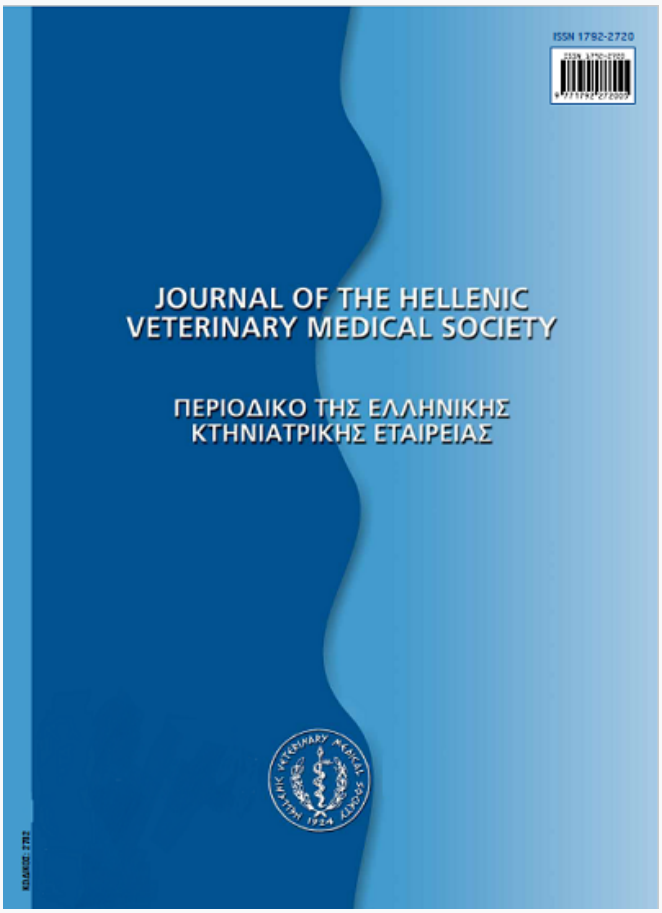

Effects of iron nano-particle's on expression of tetracycline resistance encoding genes in Staphylococcus aureus by Real Time-PCR

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Research article
Ερευνητικό άρθρο

Effects of iron nano-particle's on expression of tetracycline resistance encoding genes in *Staphylococcus aureus* by Real Time-PCR

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ABSTRACT. Increasing bacterial resistance towards traditional/conventional antibiotics is a major global health concern worldwide. Iron oxide nanoparticles (Fe nanoparticles, with average size of 20 nm) have considerable potential as antimicrobial agents in food safety applications due to their structure, surface properties, and stability. The aim of this work was to investigate the antibacterial effects and mechanism of action of iron nanoparticles against the expression of the tetA gene in Tetracycline Resistant *Staphylococcus aureus* strains by real time PCR. In the cross-sectional study, a total of 60 *S. aureus* were collected. Antibiotic susceptibility test was performed on the muller hinton agar according to the Clinical and Laboratory Standards Institute (CLSI). Then all strains were evaluated for tetA, tetB, tetC and tetD genes by multiplex-PCR method. In-vitro activity of iron oxide nanoparticles was evaluated against all resistant strains by microbroth dilution method. Therefore, the expression of tetA gene was measured in treated with iron oxide nanoparticles and untreated resistant *S. aureus* strain by Real time PCR. Our results indicated 25 (41.66%) strains resistant to Tetracycline. The prevalence of tetA, tetB, tetC and tetD genes were 5 (8.33%), 2 (2.33%), 20 (33.33%) and 10 (10.67%), respectively. The expression of tetA genes in resistant *S. aureus* strains treated with Iron oxide nanoparticles was lower than the untreated isolates. Iron oxide nanoparticles have strong antibacterial activity against resistant to Tetracycline *S. aureus* strains. In addition to, these nanoparticles reduce the expression of antibiotic resistance gene.

Key words: Iron oxide nanoparticles**,** *Staphylococcus aureus***,** *tet* genes, Real time PCR.

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INTRODUCTION

Staphylococcus aureus is a gram-positive, round-shaped bacterium that considered as a natural bacterial flora in different tissues such as the nose, respiratory tract, and on the skin. The biochemical analysis showed that it is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen (Masalha et al, 2001). Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S.aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development there is no approved vaccine for *S. aureus* (Tsiodras et al, 2001). Tetracycline is a broad spectrum antibiotic that its general usefulness is reduced because of onset of antibiotic resistance, but still remains the treatment choice for specific indications in different bacterial infections (Tiwari et al, 2006). The Tetracycline family antibiotics are generally used in the treatment of infections of the urinary tract, respiratory tract, and the intestines and are also used in the treatment of chlamydia, especially in patients allergic to β-lactams and macrolides. However, their use for these indications is less popular than it was due to widespread development of resistance in the causative organisms (Chow et al, 1975).

Mechanisms in which the bacteria became resistant to Tetracycline are cytoplasmic exocytosis channels, ribosomal conservation and deactivation of enzymatic system. TetB gene encodes exocytosis pumps that makes the bacterium resistant to Tetracycline and Minocycline. Most of the exocytosis pumps in bacteria are encodes by TetA, TetB, TetC, TetD and TetG genes (Noble et al, 1992). Metallic element's nanoparticles can disrupt the transcription and translation in bacteria (Soenen et al, 2010), these elements can also effect gene expression by inducing breaks in DNA molecules (Panáček et al, 2006). Recent studies showed that some of chemical element oxides of Calcium, Magnesium and nanoparticles of Zinc and Copper have a noticeable antibacterial activity (Hadi et al 2011, Ohira et al, 2008). The ion of these elements by attaching to –SH groups of enzymes, will react with proteins and finally deactivate them (Tawale et al, 2010). Iron oxide nanoparticles (Fe nanoparticles, with average size of 20 nm) have considerable potential as antimicrobial agents in food safety applications due to their structure, surface properties, and stability. The aim of this work was to investigate the antibacterial effects and mechanism of action of iron nanoparticles against the expression of the tetA gene in Tetracycline resistant *Staphylococcus aureus* strains by real time PCR.

MATERIAL AND METHODS

Samples were taken from different tissue sources in patients referred to the department of infectious diseases in 3 major hospitals in Tehran-Iran. 60 *Staphylococcus aureus* positive samples were detected by biochemical and microbiological analysis on cultured colonies on Blood Agar medium. The disc diffusion test based on CLSI (Clinical and Laboratory Standards Institute, 2014) was performed to identify resistant strains of S. *aureus* against Tetracycline according to the Kirby and Bauer protocol. Minimum inhibitory concentration (MIC) on the iron nanoparticles was done according to broth dilution method. *S. aureus* reference strain (accession number: ATCC25923) was used as positive control. All the 60 isolated strains were used for culture on blood-agar and incubated for 24 hours at 37°C. 0.5 McFarland bacterial suspension made in PBS and the bacterial MIC in Fe nanoparticle suspension were recorded according to the CLSI standard method.

DNA Extraction and primer design

DNA was extracted from selected samples using DNA Extraction Kit (MBST-Iran) according to the manufacturer protocol. The quantitative evaluation of Extracted DNA samples was done by OD measuring with spectrophotometry. The quality of DNA samples was evaluated by electrophoresis on agarose gel at 100 V (data not shown). Specific primer pairs were designed for amplifying Tetracycline resistance inducing encoding genes showed in Table 1.

PCR for detection Tetracycline resistance inducing encoding genes

For amplification of the target genes 10 ng of total DNA was subjected to Multiplex-PCR micro tubes in

Table 1: Primer sequences for amplification of *Target encoding genes.*

Target Gene	Primer sequences no	PCR fragment size (bp)
tet(A)	*5'-GCT ACA TCC TGC TTG CCT TC-3'* *5'-CAT AGA TCG CCG TGA AGA GG-3'*	210
tet(B)	*5'-TTG GTT AGG GGC AAG TTT TG-3'* *5'-GGTA ATG GGC CAA TAA CAC CG-3'*	659
tet(C)	*5'*-CTT GAG AGC CTT CAA CCC AG-*3'* *5'*-ATG GTC GTC ATC TAC CTG CC-*3'*	418
tet(D)	*5'*-AAA CCA TTA CGG CAT TCT GC-*3'* *5'*-GAC CGG ATA CAC CAT CCA TC-*3'*	787

100 microliter total volume including 10X PCR buffer, 2.5 U Taq polymerase enzyme (Cinnagen, Iran), 2 µl of each primers (20µM, Cinnagen, Iran), 2 µl of each dATP,dTTP,dGTP and dCTP (200µM Fermentase), 1.5 mM $MgCl_2$, in automated Thermo cycler (MWG, *Biotech* Primus, Germany) under the following program: Denaturation step for 10 min at 95° C, followed by 35 cycles of 30 S in 94°C, annealing step at 55°C for 35S and the elongation for 45S at 72°C.

10 µl of all PCR products were subjected to electrophoresis on 1.5% agarose gel in TBE buffer at 100 V and were visualized under UV light by Ethidium Bromide staining.

RNA extraction and cDNA synthesis

0.5 McFarland tetA positive *S. aureus* suspension were added to 0.1 mg/ml nanoparticles in 10 ml BHI broth and incubated for 15 h at 37°C. Fifteen hours after incubation RNA extraction was performed using RNA Extraction Kit (Cinnagen, Iran) and Trizol buffer (Life Technology, Belgium) according to the manufacturer protocol. One microgram of total RNA was subjected to cDNA synthesis using the AccuPower RT PreMix (Bioneer, South Korea) according to the manufacturer protocol. The synthesized Single strand DNA was then analyzed with agarose gel electrophoresis.

Real Time PCR

For quantification expression of the resistance against Tetracycline encoding genes, Real Time PCR was done using 1.5 microliter of single strand cDNA subjected in 12.5 µl SYBR Green I PCR Master Mix (Thermo, Denmark), 2 µg of each specific miRNA-212 designed primers (sequences not shown) and 4 µl double distilled water under the following conditions; Denaturation 30 seconds at 94 ° C followed by 45 amplification cycles of 5 S at 94 ° C (Denaturation step), 30 S at 59 °C (Annealing step) and 45 S at 72 ° C (Extension step). As housekeeping gene/s the Gyrase or/and 16S gene/s was used. The DDCT results were analyzed determining the ratio of each studied case. The graphs were drawn using Graphpad Design version 5 program.

RESULTS

60 isolated from all taken samples were detected as *Staphylococcus aureus* by biochemical and microbiological analysis on cultured colonies on Blood Agar medium. Twenty five out of 60 (41.66%) S. aureus samples detected as anti-Tetracycline resistant by disc diffusion test based on CLSI Standards.

Multiplex PCR results for detection *S. aureus* Tetracycline resistant strains

Multiplex PCR using four primer pairs (Table. 1) was done and the frequency of TetA, TetB, TetC and TetD were 8.33%, 2.33%, 33.33% and 16.67%, respectively. In one of the total 60 studied strains (1.6%) all the 4 target genes were detected (Figure 1).

Results of MIC broth dilution test of Fe nanoparticles on *S. aureus* Tetracycline resistant strains

MIC broth dilution test of Fe nanoparticles on incubated *S. aureus* Tetracycline resistant strains was recorded as 32µg/ml and the minimum bactericidal concentration was 64 µg/ml (data not shown).

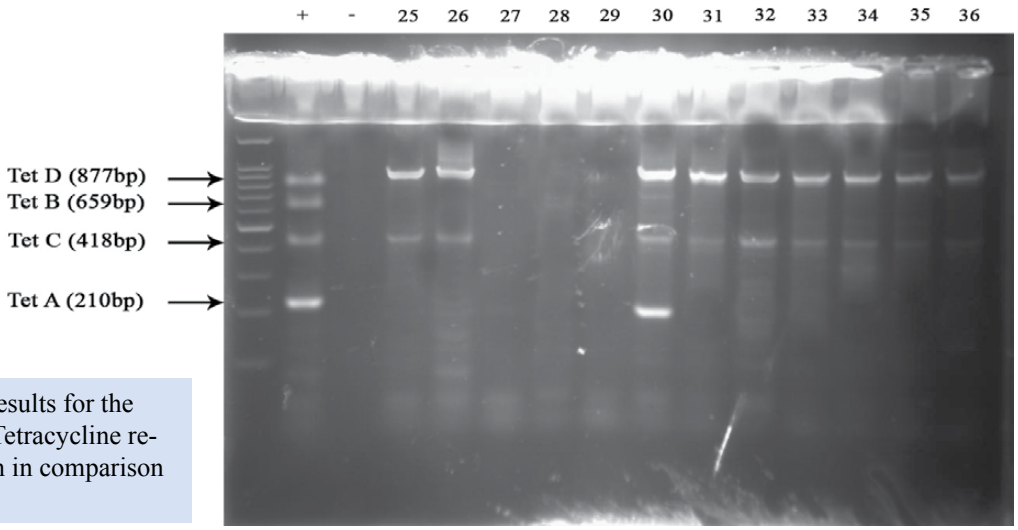

Fig 1: Multiplex PCR results for the detection of *S. aureus* Tetracycline resistant strains are shown in comparison to 100 bp DNA ladder.

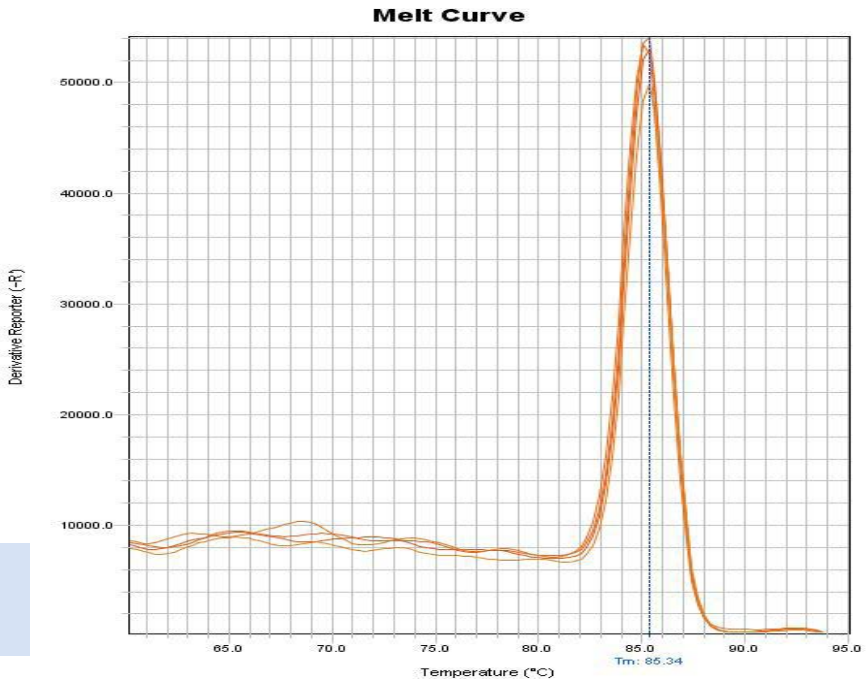

Fig 2: Melting curve recorded in Real Time-PCR on *S. aureus* strains treated with Fe Nano-particle

Real Time PCR results of quantification expression of the resistance against Tetracycline encoding genes in *S. aureus* strains treated by Fe Nano-particles

34.85 °C recorded as the best melting curve for TetA gene amplification by Real Time-PCR. It seems that the rate of TetA expression decreases in *S. aureus* treated with Fe nano-particles in comparison to untreated strains in our study (Figure 2).

TetA expression level in *S. aureus* treated with Fe Nano-particles was two times lesser than the TetA expression level in untreated strains. The decrease of the expression level of TetA genes in treated strains is shown in Fig. 3.

Gene expression decreased in Fe nano-particle treated strains (green and blue curves) in comparison to non -treated ones (purple and red curves).

ΔΔCt Analysis Method showed that the gene expression in Fe nano-particles treated strains was decreased 2 times than the non-treated strains.

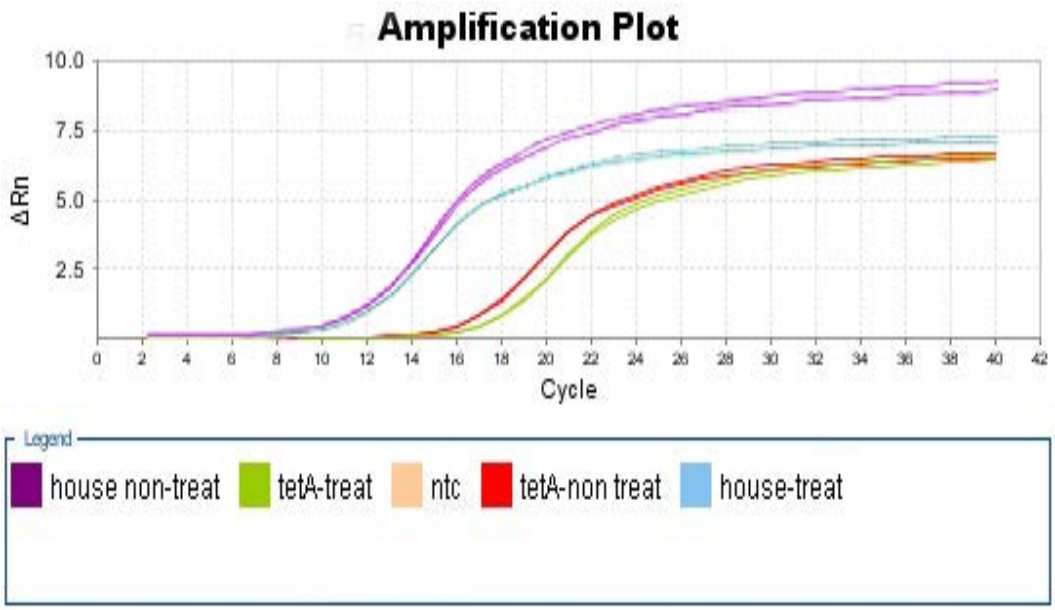

Fig 3: Comparison of the TetA expression level in *S. aureus* treated with Fe Nano-particles and non-treated strains.

DISCUSSION

The Tetracycline family antibiotics are generally used in the treatment of infections of the urinary tract, respiratory tract, and the intestines and are also used in the treatment of chlamydia, especially in patients allergic to β-lactams and macrolides however, their use for these indications is less popular than it once was due to widespread development of resistance in the causative organisms (Chow et al, 1975). Bondarenko in 2012 showed that Cu Oxide nano particles react with Amin and Carboxyl groups on the surface of microbial cells and release Cu in Oxidation reactions which leads to antibacterial effects by releasing Hydroxyl radicals (Bodarenko et al, 2012). Sondi in 2004 showed that nano-particles have a reductive effect during DNA replication process (Sondi et al, 2004). Warsa in 1996 showed that all of the 215 studied strains of *S. aureus* isolated from Asian countries carried both of the TetK and TetM (Tetracycline and Minocycline resistant inducing genes, respectively), on the contrary, the tetK gene was not detected in isolated from Japan and Korea (Warsa et al, 1996) This study was carried out in order to evaluate the expression level of TetA encoding gene in *S. aureus* strains treated with Fe nanoparticles. Antibacterial agents are widely used in different levels of social hygiene, medicine and industrials (Aruoja et al, 2009). Growth of studied strains were suppressed after 2 hours treatment with 32µg/ml Fe nanoparticles which led to the conclusion that Fe nanoparticles at the least concentration play an effective role in decreasing the bacterial growth without time consuming side effects. Our results indicated 25 (41.66%) strains resistant to Tetracycline. The prevalence of tetA, tetB, tetC and tetD genes were 5 (8.33%), 2 (2.33%), 20 (33.33%) and 10 (10.67%), respectively. The expression of tetA genes in *S. aureus* resistant strain treated with Iron oxide nanoparticles was lower than the untreated isolates. Iron oxide nanoparticles have strong antibacterial activity against resistant to Tetracycline *S. aureus* strains. In addition to, these nanoparticles reduce the expression of antibiotic resistance gene. Azam in 2012 showed that the antibacterial activity of CuO nanoparticles was found to be size-dependent and the highly stable minimum-sized monodispersed copper oxide nanoparticles demonstrated a significant increase in antibacterial activities against both Gram-positive and -negative bacterial strains (Azam et al, 2012). So, further studies needed in order to evaluate the anti-bacterial effect of Fe nano-particles in different size and treatment conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Aruoja V, Dubourguier H-C, Kasemets K, Kahru A (2009) Toxicity of nanoparticles of CuO, ZnO and TiO 2 to microalgae Pseudokirchneriella subcapitata. Science of the total environment 407(4):1461-8.

Azam A, Ahmed A, Oves M, Khan MS, Memic A (1989) Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and -negative bacterial strains. Int J Nanomedicine 7: 3527-3535.

Bondarenko O, Ivask A, Käkinen A, Kahru A (2012) Sub-toxic effects of CuO nanoparticles on bacteria: kinetics, role of Cu ions and possible mechanisms of action. Environmental pollution.169:81-90.

Chow, Anthony W.; Patten, Valerie; Guze, Lucien B. (1975) Comparative Susceptibility of Anaerobic Bacteria to Minocycline, Doxycycline, and Tetracycline". Antimicrobial Agents and Chemotherapy 7 (1): 46–49.

Hadi M, Shokoohi R, Ebrahimzadeh Namvar A, Karimi M, Solaimany Aminabad M (2011) Antibiotic resistance of isolated bacteria from urban and hospital wastewaters in Hamadan City. Iranian Journal of Health and Environment 4(1):105-14.

Masalha M; et al. (2001) Analysis of Transcription of the Staphylococcus Aureus Aerobic Class Ib and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen. Journal of Bacteriology. 183 (24): 7260–7272.

Noble W, Virani Z, Cree RG (1992) Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS microbiology letters 93(2):195-8.

Ohira T, Yamamoto O, Iida Y, Nakagawa Z-e (2008) Antibacterial activity of ZnO powder with crystallographic orientation. Journal of Materials Science: Materials in Medicine 19(3):1407-12.

Panáček A, Kvítek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, et al (2006)Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. The Journal of Physical Chemistry B 110(33):248-253.

Soenen SJ, Himmelreich U, Nuytten N, Pisanic TR, Ferrari A, De Cuyper M (2010) Intracellular nanoparticle coating stability determines nanoparticle diagnostics efficacy and cell functionality. Small 6(19):2136-45.

Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science 275(1):177-82.

Tiwari HK, Sen MR (2006) Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious diseases 6(1):156.

Tawale J, Dey K, Pasricha R, Sood K, Srivastava A (2010) Synthesis and characterization of ZnO tetrapods for optical and antibacterial applications. Thin Solid Films 519(3):1244-7.

Tsiodras S, Gold H, Sakoulas G, Eliopoulos GM, Wennersten MD, Venkataraman L, Moellering Jr (2001) Linezolid resistance in a clinical isolate of Staphylococcus aureus. The Lancet 358: 207–208.