Introduction

Enterococci are one of the most frequent causes of nosocomial infections in the intensive care unit; they appear in this sector along with clumsily use of cephalosporins and other antibiotics to which enterococci are resistant. These bacteria are contagious and usually cause infection in the urinary tract, wound, bile duct, and blood at the hospital; also, they can cause meningitis in children and endocarditis in adults. 

Enterococcus faecalis (E. faecalis) is one of the most common species of enterococcus which causes 85 to 90% of enterococcal infections. Gram-positive bacteria were previously classified as Group D Streptococcus, due to specific antigen which is teichoic acid. Most of these bacteria are non-hemolytic and sometimes are alpha-hemolytic which can be found in natural intestinal flora.

These bacteria are resistant to many antibiotics like Meropenem, Gentamicin, Ceftriaxone, Ceftazidime, Cefixime, Trimethoprim/Sulfamethoxazole, Erythromycin, and Clindamycin with above 60% resistance frequency rate and sensitivity to Vancomycin, Teicoplanin, and Nitrofurantoin based on report

Iron oxide due to its biocompatibility and magnetic feature has been widely used in biomedical research. Nanoparticles (NP) of Iron oxide with certain sizes (almost less than 100 nm), are applied for targeted drug delivery as carriers for many types of cancers. Moreover, nanoparticles are used for drug delivery system which were extended for directing nanoparticles by using an external magnetic field in particular places due to accurate treatment. Therefore, it is assumed that Reactive Oxygen Species (ROS) produced by nanoparticles have a bacteriostatic potency without damaging eukaryotic cells.

The aim of this study was to find a way to prevent drug resistance by using lower doses of antibiotics to prevent
treat the bacterial infections.

**Materials and Methods**

The reference strain, *E. faecalis* (ATCC 11700), was used as the control strain in all steps for comparison purposes.

**Preparation of culture medium**

In total, 20 culture-positive specimens of *E. faecalis* were collected from patients at Tehran University of Medical Sciences and cultured on Bile Esculin Agar (Merck, Germany) \(^{17,18}\).

**Kirby-Bauer Susceptibility Test**

In order to test the resistance of *E. faecalis*, clinical-positive specimens of these bacteria were cultured on Mueller Hinton agar medium (Merck, Germany). Disk diffusion was performed using Kirby-Bauer method (Clindamycin 2 μg, Oxacillin 1 μg, Erythromycin 15 μg, Cefotaxime 30 μg, Ceftazidime 30 μg, Tetracycline 30 μg, Chloramphenicol 30 μg, Vancomycin 30 μg disks, Mast Group Ltd Company, UK). Incubation was performed for 24 hr at 37°C with 5% CO\(_2\) (Semi-aerobic conditions). The isolate with higher resistance was selected for further study.

**Preparation of nanoparticle (NP) suspension**

Fe\(_3\)O\(_4\) nanoparticles (Purity over 99.7%) with 10-50 nm range size were purchased from US NANO. Nanoparticles stock solution was prepared by suspending one gram of nanoparticles into 100 ml sterile medium and dispersion was done by Electro sonic system (Bandelier Sonorex RK 31H) for 35 min. The microbial tests and preparation of nanoparticle suspensions were performed simultaneously in order to reduce probable errors.

**Microbial suspension preparation**

At first, bacterial cells were collected from BEA culture medium and were mixed in 10 ml Phosphate-Buffered Saline (PBS) in order to prepare samples with 0.5 McFarland Turbidity [1-1.5x10\(^6\) Colony-Forming Unit (CFU)] and the accuracy was measured by spectrophotometer (UNICO-2100; USA) at 620 nm wavelength range and absorbance was set at the range from 0.08 to 0.1 nm.

**MIC test and bacteriostatic potency**

The Clinical and Laboratory Standards Institute (CLSI) recommendations were used for Minimal Inhibitory Concentration (MIC) calculation of the sample in contact with the NP suspension. Gradient concentrations of Fe\(_3\)O\(_4\)NPs suspension, both NPs with bacteria, were prepared according to a conducted study by Khavarami et al. \(^{18,19}\).

**Nanoparticles impregnated discs preparation**

Sterile blank discs (Crude) were placed in a plate, then nanoparticle suspension with desired concentration was poured into the plate, where the discs were not immersed, then incubated about 24 hr at room temperature until the suspension was completely absorbed by blank discs. \(^{20}\).

**DDST susceptibility test**

The antibiotics to which the isolate showed resistance or semi-susceptibility were selected and assessed for DDST in Mueller Hinton agar medium with 20 mm center to be centered in the plate. Incubation was performed for 18 hr at 37°C and the inhibition zone from the edge of each disc was recorded \(^{19}\).

**Results**

**Kirby-Bauer Susceptibility Test**

The isolated bacteria were resistant to Oxacillin and Cefotaxime, and semi-sensitive to Tetracycline and Chloramphenicol acid antibiotics (Table 1).

**Preparation of the nanoparticle suspensions**

The X-ray powder diffraction (XRD) diagram of the commercial Fe\(_3\)O\(_4\) powder in figure 1A demonstrates the proper purity due to lack of any impurity diffraction pattern. Figure 1B presents the corresponding morphology and particle size distributions of prepared Fe\(_3\)O\(_4\) powders by TEM microscopy.

**MIC test and bacteriostatic potency**

According to the results, the antimicrobial activity of Iron oxide nanoparticles suspensions against *E. faecalis*, the MIC for the isolated bacteria and ATCC-11700 strain against Iron oxide NPs were Z=78.125 μl/μg\(^{-1}\) and 39.0625 ml/μg\(^{-1}\), respectively. The sensitivity coefficient for *E. faecalis* against the nanoparticle suspension was calculated for each sampling period. The mean of bacterial coefficient sensitivity to the nanoparticles is shown in figure 2.

### Table 1. Sensitivity of the *E. faecalis* to the applied antibiotics and in synergy with Fe\(_3\)O\(_4\) nanoparticles

| Antibiotic   | ATCC11700 | Clinical Sample |
|--------------|-----------|-----------------|
|              | μg        | Zone diameter (mm) | Zone diameter (mm) | Zone diameter (mm) |
| Clindamycin  | 2         | 10 Mast Disc       | 0 Mast Disc        | 12 DDST          |
| Oxacillin    | 1         | 0                | 0                 | 12               |
| Erythromycin | 15        | 25               | 23                | NA              |
| Cefotaxime   | 30        | 19               | 18                | NA              |
| Ceftazidime  | 30        | 17               | 0                 | 13               |
| Tetracycline | 30        | 13               | 13                | NA              |
| Chloramphenicol | 30     | 11               | 11                | 12               |
| Vancomycin   | 30        | 25               | 20                | NA              |

* (NA) did not test.
DDST susceptibility test

The results in table 1 show higher antibacterial activity for Clindamycin, Oxacillin, and Ceftazidime due to visibility of the zone; however, it seems Chloramphenicol in synergy with the applied Fe$_2$O$_3$ did not increase the inhibitory zone significantly.

Discussion

XRD and TEM images illustrate the appropriate crystal structure of synthesized Iron oxide NPs and have almost regular spherical shape in the size range of nano. Some bacteria have the potency in reducing metal oxide by mechanisms, therefore reducing the size of Iron NPs would not be a good idea for increasing toxicity of metal oxide; but nanoparticles with higher number of reactive groups on the surface like active sites for the formation of ROS which lead to oxidative stress could be good candidates.

Many studies reveal that in aquatic system, the antimicrobial activity of metal oxide compounds was mainly caused by soluble ions, and has effect on reduced cellular function due to aggregation of NPs in aqueous medium. But at efficient concentration, NPs at dilution condition can be more toxic for cells than the metal ionic form which is described as a nano-trojan horse type of mechanism. The pH values of Fe$_2$O$_3$ NPs have been calculated to be 5-7. Due to lack of significant difference between both pH values (pH of microorganism is about 2-4), this opinion could not be justifiable.

The results express that Chloramphenicol in synergy with NPs could not increase inhibitory effect significantly (Table 1). According to the given explanations, surface charge of bacteria is reduced due to repulsion. Therefore, greater concentration of NPs due to this electrostatic repulsion force cannot be a suitable option to overcome this inhibitory force.

Conclusion

Based on our findings, it can be inferred that Iron oxide nanoparticles suspension in different concentrations has growth inhibitory effects against bacteria that cause nosocomial infections, but at higher concentrations than the MIC, presumably it can make adaption to Fe$^{2+}$ ions which could be used as a source of energy in metabolic pathway. The use of bacteriostatic potency of these NPs against bacteria suspensions with certain concentration in combination with antibiotics can be a good option.
for inhibition of the bacterial infections in medical domains.

Conflict of Interest
Authors declare no conflict of interest.

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