

# Antibacterial Susceptibility Pattern of the *Pseudomonas aeruginosa* and *Staphylococcus aureus* after Exposure to Electromagnetic Waves Emitted from Mobile Phone Simulator

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## ABSTRACT

**Background:** The increasing use of telecommunication devices such as Wi-Fi modems and mobile phones in the recent years can change the cellular structure of microorganisms so the generation of electromagnetic waves has led to concern in the community whenever be exposed to these fields and may have harmful effects on human health.

**Material and Methods:** Standard strains of bacteria were prepared on Mueller-Hinton agar for bacterial growth to obtain 0.5 McFarland turbidity ( $1.5 \times 10^8$  CFU) of bacteria. Antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method was done. For *Staphylococcus aureus* and *Pseudomonas aeruginosa*, antibiotics susceptibility test was conducted. The test group was exposed to electromagnetic waves emitted by mobile phone simulator with a frequency of 900 MHz and the control group didn't exposed.

**Results:** The results revealed that increasing duration of exposure to electromagnetic waves emitted by the mobile simulators with a frequency of 900 MHz especially after 24 h of exposure, can increase bacterial resistance in *S. aureus*, and *P. aeruginosa*.

**Conclusion:** Several factors can cause bacterial resistance against antibiotics. One of these factors is the electromagnetic waves emitted from mobile simulator with a frequency of 900 MHz, which can increase the permeability of the cell wall of bacteria.

## Keywords

Antibacterial susceptibility, Electromagnetic waves, Mobile simulator, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

## Introduction

Antibiotic resistance is considered to be a major problem in the treatment and control of the infection. In recent years, bacterial resistance has become widespread to common antibiotics worldwide, so that the use of antimicrobials with this resistance pattern has been considered as a major problem in the medical community. In addition, since the detection of this resistance pattern due to their rapid spreads, an abundance of the antibiotic resistance across the universe

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has been observed. This can often occur due to the unnecessary consumption of antibiotics. On the other hand, patients' specific conditions, long-term hospitalization, immunodeficiency and extensive use of immunosuppressive drugs and aggressive therapies (such as a catheter, intravascular shunt, and endotracheal tube), and other factors increase the drug resistance in different parts of the hospitals [1]. Therefore, the optimal applying and appropriate therapeutic strategies in order to control the infection and reducing the prevalence of antibiotic-resistant organisms is necessary [2]. *P. aeruginosa* is a hospital pathogen that causes infectious wounds and burns, and opportunistic diseases such as meningitis, urinary tract infection [3], respiratory system involvement [4], otitis media in swimmers [5], eye infections, and sepsis in infants or people with disabilities [6], especially in people with immune system deficiencies [7]. Also, *S. aureus* is a major pathogen, which is found everywhere, especially in infant ward, ICUs, surgery rooms and chemotherapy departments [8, 9], and will lead to various types of infections such as food poisoning or life-threatening infections [10] caused by direct infection of the wound, such as Staphylococcus wound infection [11] after surgery or infection following trauma [10, 12].

On the other hand, in recent years, the use of electromagnetic sources and telecommunication devices such as mobile phones have increased, and concerns have been raised for people in the community who are exposed to these fields, whose effects on the human health are under investigation [13-15]. Various classifications regarding the types of effects of waves on the human body have been made. In one classification, the effects of waves are divided into two categories. The first group has a reversible effect, such as the heat that increases its temperature, but after cooling, the nature of it remains preserved [16]. The second group, the irreversible effect, such as heat on the egg, increases the temperature and changes its nature, and does not return to the

first state after cooling down [17].

In another classification, these damages are divided as thermal and non-thermal [18]. thermal damage means raising the temperature of cells and tissues without triggering temperature-sensitive receptors on the skin surface. Due to increased temperature in the tissues, chemical changes occur in the cells, which may lead to various disorders and illnesses. For example, due to increased body temperature, changes in enzymatic processes and cytokine levels [19], disturbances in calcium metabolism, and changes in growth and cell proliferation will occur [18]. Until recently, only thermal damages were considered and unhealthy electromagnetic waves were unaware [20]. Unhealthy damage causes permanent changes in the structure of the cell. Because of unhealthy damage due to the heat and the intensity of the wave, these damages may also be caused by the weakest wave. Some of these damages including altering blood chemistry such as reducing red blood cells, affecting the reproductive system, causing cancer, nervous disorders, increased irritability, headache, dizziness, nausea, eye irritation, and feeling of a foreign body in the eye. Mobile waves, with similar effects on the body, can cause discomfort and diseases such as cataracts [21]. The third-generation (3G) a new version of mobile phone technology is an important wireless communication device that meets the needs of modern societies and is rapidly increasing worldwide. 3G mobile phones run on radiofrequency of 900-1800 MHz [22, 23].

Nowadays, exposure to electromagnetic waves is unavoidable; as a result, many home appliances such as a hairdryer and washing machine produce harmful electromagnetic waves whose intensity can be as strong as waves of the electromagnetic emissions released below the high-pressure electric shock welder are also higher [24]. According to the reports, every healthy person is exposed to 361 million roentgen radiation [25]. Therefore, we should be aware of the safety and protection

issues against the undesirable effects of electromagnetic waves [26]. In order to obtain a more accurate understanding of the effects of electromagnetic waves, the present study examined the effect of short-term exposure to electromagnetic waves emitted from mobile phone simulator with a frequency of 900 MHz as a mechanism of stress, on the sensitivity of pathogenic microorganisms, *P. aeruginosa* and *S. aureus* against multiple antibiotics.

## Material and Methods

### Materials

Materials used in this work include Muller Hinton Agar and nutrient broth culture media, *P. aeruginosa* ATCC No. 27853, *S. aureus* bacteria ATCC No. 25923, antimicrobial discs (ROSCO, Denmark) (Table 1). The equipment used in this research include Digital balance A & D Model 120, (Japan), 37 °C incubator (Behdad, Iran), UV/Vis Spectrophotometer 2100 (UNICO, America), RF Simulator (simulated mobile source with waves production of 900 MHz).

**Table 1:** Antimicrobial agents used in the present study.

Antimicrobials	Abbreviation	Potency
Piperacillin	PIPRA	100 µg
Cefotaxime	CTX	30 µg
Ceftriaxone	CTR	30 µg
Amikacin	AMI	40 µg
Imipenem	IMI	10 µg
Aztreonam	AZT	30 µg
Trimethoprim-Sulfamethoxazole	SXT	25 µg
Tetracycline	TET	10 µg
Vancomycin	VANCO	5 µg
Ciprofloxacin	CIPR	5 µg
Levofloxacin	LEVO	5 µg

### Preparation of culture media

At the first step, a specific amount of each medium powder was dissolved in distilled water according to manufacturing instructions. After that, sterile Muller Hinton Agar media was dispersed in the microbiological plates.

### Bacterial culture and antimicrobial susceptibility test

*P. aeruginosa* and *S. aureus* bacteria inoculated in the nutrient broth medium separately and incubated at 37 °C. Bacterial density was measured every 20 min using UV/Vis spectrophotometer at an optical density of 625 nm. When the bacterial concentration reached up to standard 0.5 Mc Farland, the fresh culture of each suspension was taken on, by the Muller Hinton Agar plates using sterile swabs. In order to examine the antibacterial susceptibility of the used microorganism, the antimicrobial discs were put on the Muller Hinton agar plates containing bacteria. Then these plates were classified as control (unexposed) and test or exposed group which were exposed to 900 MHz radiofrequency radiation emitted by mobile phone simulator for 2, 4, 6, 8, 10 and 24 h and incubated for 18 h at 37 °C. Finally, the average inhibition zone of each antimicrobial disc was recorded and compared with the control group.

### Statistical Analysis

Each experiment was carried out in triplicate to minimize the variations. Data reported as mean ± SD and data comparison was done using non-parametric Mann-Whitney test.

## Results

### The results of electromagnetic radiation from mobile simulator on *P. aeruginosa*

Inhibition zone diameter of *P. aeruginosa* bacteria after 2, 4, 6, 8, 10 and 24 h exposure to 900 MHz for each antibiotic was examined. As shown in Figure 1, for CTX after 2 and 4

h, for CTX and LEVO after 6 h, for CIPR, LEVO and IMI after 8 h, for CIPR and IMI after 10 h and finally for AMI, CIPR, CTX, PIPRA and LEVO after 24 h (Figure 2) bacterial exposure to 900 MHz radiofrequency radiation, antimicrobial susceptibility against *P. aeruginosa* decreased, hence the antibacterial resistance enhanced. In all measurements,

statistical analysis revealed that all aforementioned changes were significant (p-value  $\leq 0.05$ ).

The results of electromagnetic radiation from mobile simulator on *S. aureus*

Inhibition zone diameter in *S. aureus* bacte-

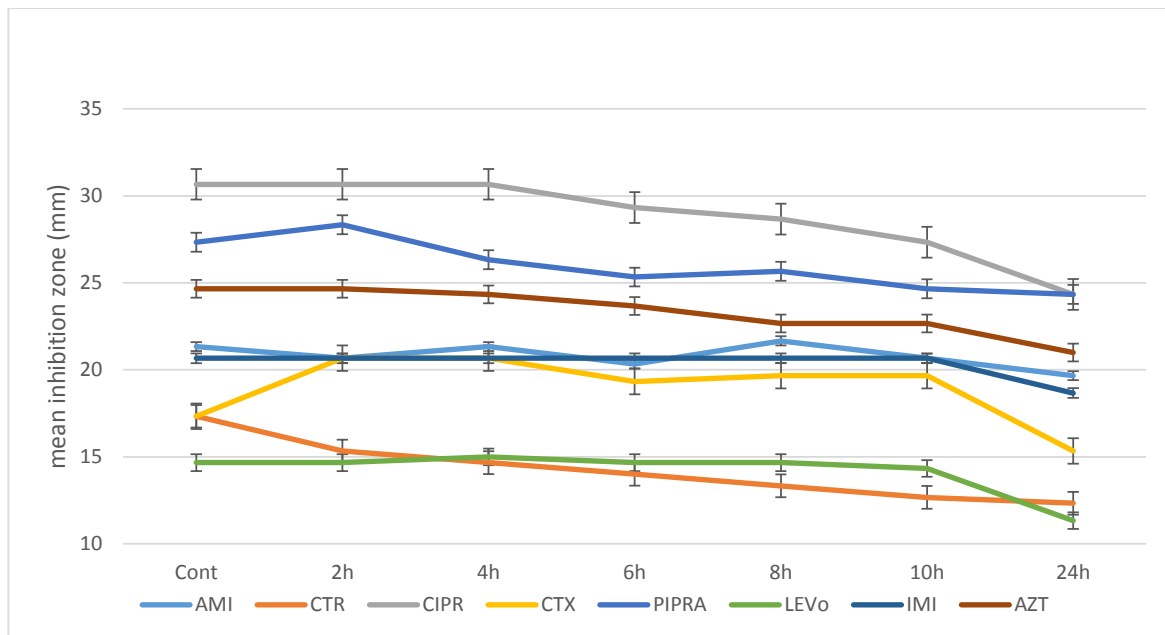


Figure 1: Mean inhibition zone diameter in *Pseudomonas aeruginosa* against various antibiotics during 24 hours.

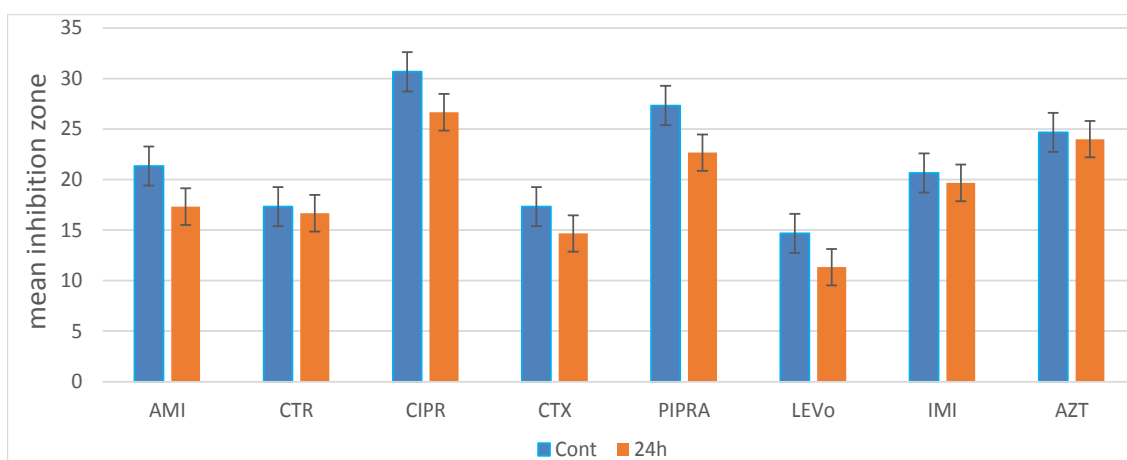


Figure 2: Mean inhibition zone diameter in *Pseudomonas aeruginosa* against various antibiotics in 24 hr exposure.

ria after 2, 4, 6, 8, 10 and 24 h exposure to 900 MHz emitted from a mobile simulator for each antibiotic was examined. After 2 h for CTR and VANCO, after 4 and 6 h for CTR, AMI, and TET, after 8 h for CTR, LEVO, AMI, TET, SXT and AMP, and after 10 h for CTR, LEVO, AMI, TET, SXT and after 24 h exposure for LEVO, AMI, TET, SXT, AMP, and PIPRA were decreased in comparison with the non-exposed group. All of these differences were statistically significant ( $p < 0.05$ ). The results of the mean inhibition zone measurements in *S. aureus* were plotted in Figure 3. According to this chart, *S. aureus* bacteria had different changes in the response to antimicrobial discs at different times.

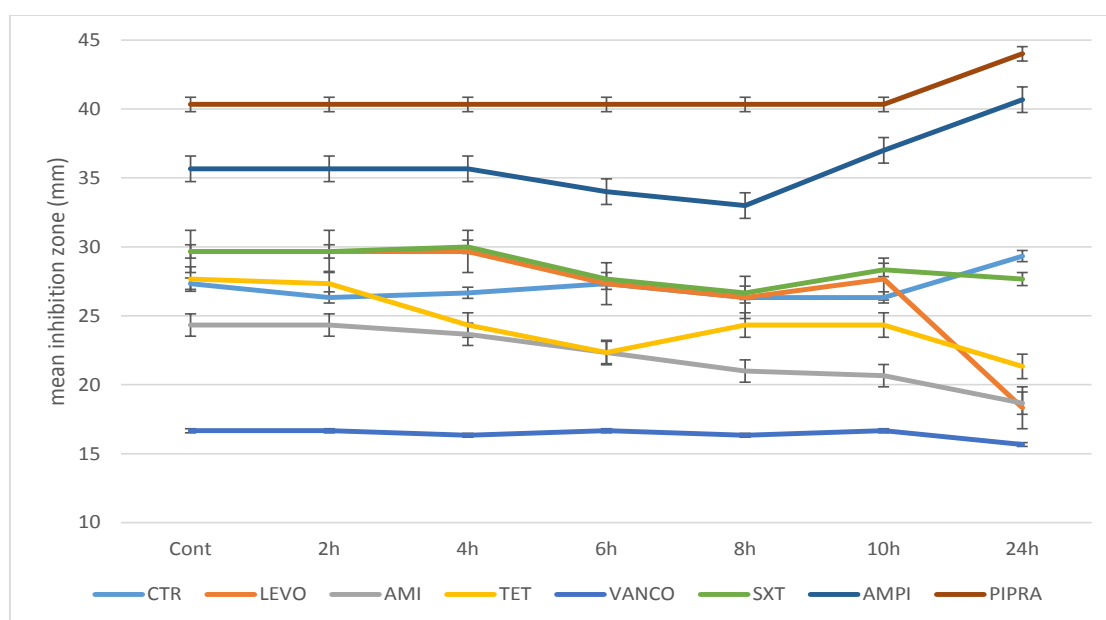
The effect of mobile simulator radiation with a frequency of 900 MHz on *S. aureus* bacteria, revealed that after 8 h exposure bacteria tend to be resistant against used antimicrobial agents. This change is more remarkable in amikacin and tetracycline, although all agents in the bacteria encountered a mobile simulator after 8 h exhibit resistance (Figure 4).

In addition, the effect of the mobile simulator

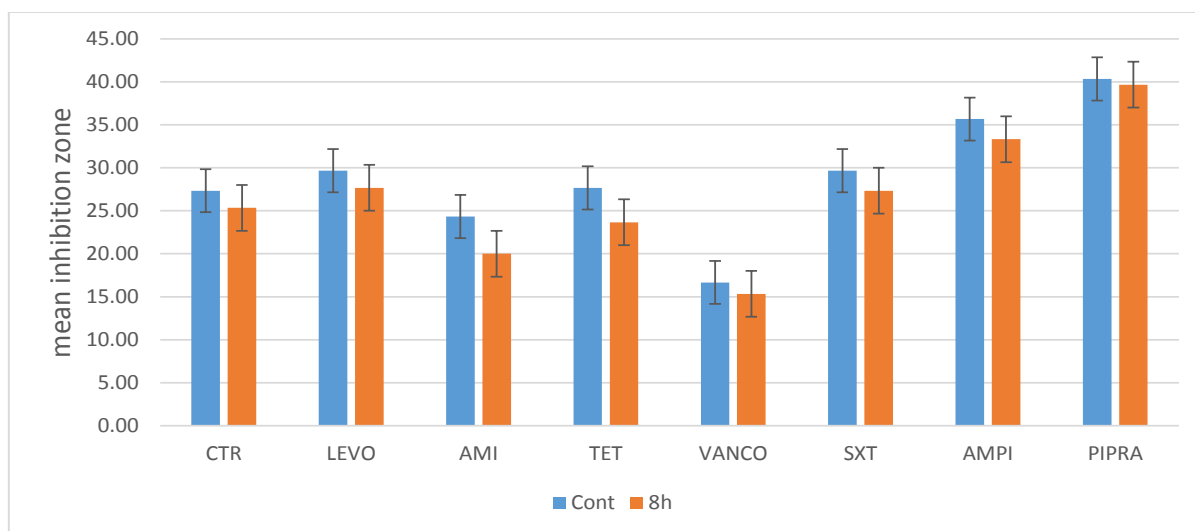
on the *S. aureus* bacteria was the most resistant to the group of sham after 24 h of radiation in the antibiotic of levofloxacin and increased sensitivity in the antibiotics of piperacillin and ampicillin. However, significant changes were observed in all antibiotics (Figure 5).

## Discussion

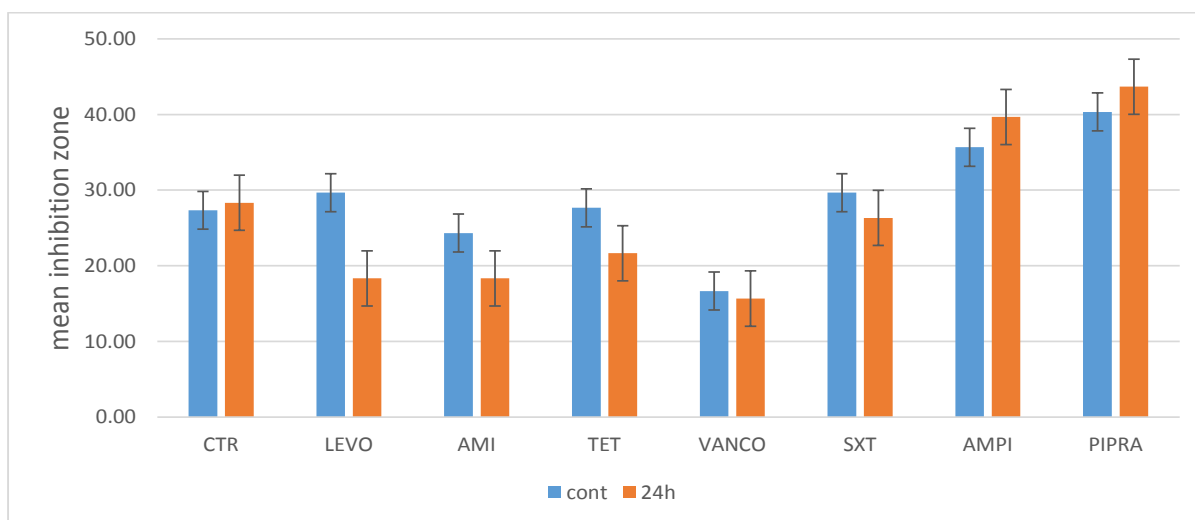
The purpose of this study was to investigate the effect of short-term exposure to radiofrequency radiation from mobile phone simulator on the susceptibility of pathogenic microorganisms' *P. aeruginosa* and *S. aureus* against multiple antibiotics. The results of this study showed that exposure to 900 MHz emitted from the mobile simulator for bacterial strains of *S. aureus* and *P. aeruginosa* during 24 h caused significant changes in structural properties and resistance to the antibiotics studied. So that *P. aeruginosa* bacteria were resistant to all antibiotics after 24 h of irradiation compared to the non-exposed (control) group, and *S. aureus* bacteria after 24 h of radiation compared with the control group to some antibiotics such as LEVO, TET, and SXT, were resis-



**Figure 3:** Mean inhibition zone diameter in *Staphylococcus aureus* against various antibiotics during 24 hours.



**Figure 4:** Mean inhibition zone diameter in *Staphylococcus aureus* against various antibiotics in 8 hr exposure.



**Figure 5:** Mean inhibition zone diameter in *Staphylococcus aureus* against various antibiotics in 24 hr exposure.

tant and to the antibiotics of PIPRA, AMPI, and CTR were susceptible.

In a study, Mortazavi et al. showed antimicrobial susceptibility of *Klebsiella* spp., *S. aureus* and streptococci (group A) bacteria after exposure to 7 - 13.5 MHz diagnostic ultrasound probes for 5 minutes were investigated. For *S. aureus* bacteria, vancomycin, er-

ythomycin and amoxicillin antibiotics and for streptococci and *Klebsiella* spp. nitrofurantoin, nalidixic acid, and gentamicin antibiotics were used. The results showed that *S. aureus* was resistant to erythromycin and sensitive to vancomycin [27]. In this study, ultrasound waves altered the antimicrobial susceptibility in staphylococcus bacteria that is inconsistent

with the present study findings.

In 2017, Taheri et al. reported the *Escherichia coli* and *Listeria monocytogenes* after 3, 6, 9, 18 and 24 h of radiation emitted by Wi-Fi modems with the frequency of 2.4 GHz and mobile simulator with a frequency of 900 MHz showed different responses. In this study, the antibiotics of piperacillin, levofloxacin, aztreonam, ciprofloxacin, and cefotaxime used. After 9 h exposure to Wi-Fi frequencies, the bacteria were resistant to ciprofloxacin antibiotic and were susceptible to ceftriaxone, cefotaxime and levofloxacin antibiotics [28]. However, in our study *S. aureus* bacteria were resistant to the antibiotics of levofloxacin and TET after 24 h of radiation with 900 MHz radiation made by the mobile simulator. This difference in response to antibiotic sensitivity can result in the difference in the radiation frequency, the duration of radiation, the type of antibiotic and the structure of the bacteria. Adebayo et al. (2014) investigated the effect of radiofrequency radiations emitted from telecommunication base stations on the resistance of Bacillus bacteria, which increased resistance to this bacterium [29]. This study, with our results, increased the resistance of both *S. aureus* and *P. aeruginosa* bacteria after 24 h exposure to 900 MHz emitted waves from the mobile simulator.

Several studies have highlighted the causes of antimicrobial susceptibility changes of bacteria under the influence of electromagnetic fields [29-31]. A study showed that antimicrobial susceptibility depends on the physical properties of the magnetic field, such as frequency, duration of radiation, and the type of bacteria [32]. Another factor that can affect the antimicrobial susceptibility is the structure of the bacterial cell wall and the nature of the peptidoglycan properties in the cell wall of the gram-positive and negative bacteria [33].

Torgomyan et al. showed that changes in proteins in the bacterial cell wall could be the most important membrane mechanism for low-intensity electromagnetic field radiation [34].

Overall, studies in this area have described the following effective mechanisms: first, due to the importance of the membrane potential of the bacterial cell wall, these waves can have an effect on the antibiotic susceptibility [32]. Second, increased antibiotic susceptibility may be due to the interaction of electromagnetic fields on the water molecules in the cell. Other suggested mechanisms including increase in the permeability of the bacteria and make the ion channels to be kept open in the bacterial wall, efflux pumps and ion channels in the cell wall, play an important role in the absorption of antibiotics, and finally, another factor that can affect bacterial sensitivity in magnetic conditions is the antibiotic structure [28, 35]. Hydrophilicity or hydrophobicity of the antibiotic, antibiotic activity and antibiotic molecule size are other factors [36].

## Conclusion

In the present study, it has been shown that the waves emitted from the mobile simulator with a frequency of 900 MHz can change the antimicrobial susceptibility of *S. aureus* and *P. aeruginosa* as a physical method. According to the obtained results, it can be concluded that the bacterial species used in this study were influenced by the electromagnetic field and responded differently.

## Acknowledgment

We would like to acknowledge Shiraz University of Medical Sciences, for the generous funding of this work through grant No 94-01-75-10643.

## Conflict of Interest

None

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