Alteration of Bacterial Antibiotic Sensitivity After Short-Term Exposure to Diagnostic Ultrasound

Seyed Mohammad Javad Mortazavi,1,2,* Leili Darvish,1 Mohammad Abounajmi,3 Samira Zarei,4 Tahereh Zare,1 Mohammad Taheri,1 and Samaneh Nematollahi6

1Department of Medical Physics and Medical Engineering, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
2Department of Radiology, Faculty of ParaMedicine, Hormozgan University of Medical Sciences, Bandar Abbas, IR Iran
3Department of Radiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
4Department of Microbiology, School of Medicine, Kerman University of Medical Sciences, Kerman, IR Iran
5Department of Biostatistics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
6Corresponding Author: Seyed Mohammad Javad Mortazavi, Department of Medical Physics and Medical Engineering, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran. E-mail: mmmortazavi@sums.ac.ir; Leili Darvish, Department of Medical Physics and Medical Engineering, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran. E-mail: leili.darvish@gmail.com

Received 2015 January 5; Revised 2015 March 27; Accepted 2015 April 14.

Abstract

Background: Many pathogenic bacteria show different levels of antibiotic resistance. Furthermore, a lot of hospital-acquired infections are caused by highly resistant or multidrug-resistant Gram-negative bacteria. According to WHO, patients with drug-resistant infections have higher morbidity and mortality. Moreover, patients infected with bacteria that are resistant to antibiotics considerably consume more healthcare resources.

Objectives: In this study, we explored a physical method of converting drug-resistant bacteria to drug-sensitive ones.

Materials and Methods: This is an in vitro case-control study, performed at the Ionizing and Non-ionizing Radiation Protection Research Center (INRPRC), Shiraz University of Medical Sciences (SUMS), Shiraz, Iran in 2014. All experiments were carried out using Gram-negative bacteria Klebsiella pneumoniae and E. coli and Gram-positive Staphylococcus aureus and Streptococcus group A, isolated from hospitalized patients. The bacterial strains were obtained from the Persian Type Culture Collection, BOST, Iran (Klebsiella pneumoniae PTCC 1290) and Bacteriology Department of Shahid Faghihi Teaching Hospital, Shiraz, Iran (E. coli, Staphylococcus aureus, and Streptococcus group A). The bacteria in culture plates were exposed to diagnostic ultrasound using a MyLab70XVG sonography system for 5 minutes. Then, the bacteria were cultured on Mueller-Hinton agar and incubated at 35°C for 18 hours. Finally, antibiotic susceptibility test was performed and the inhibition zone in both control and exposed groups were measured. Three replicate agar plates were used for each test and the inhibition zones of the plates were recorded.

Results: Compared with the results obtained from unexposed bacteria, statistically significant variations of sensitivity to antibiotics were found in some strains after short-term exposure. In particular, we found major differences (making antibiotic-resistant bacteria susceptible or vice versa) in the diameters of inhibition zones in exposed and non-exposed samples of Klebsiella pneumoniae and Streptococcus.

Conclusions: This study clearly shows that short-term exposure of microorganisms to diagnostic ultrasonic waves can significantly alter their sensitivity to antibiotics. We believe that this physical method of making the antibiotic-resistant population susceptible can open new horizons in antibiotic therapy of a broad range of diseases, including tuberculosis.

Keywords: Drug Resistance, Ultrasound, Infection, Antibiotics

1. Background

The World Health Organization (WHO) believes that antimicrobial resistance (AMR) is a progressive and serious threat to global public health that endangers the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses, and fungi. According to WHO, as AMR can be found in all parts of the world, international actions are needed to overcome this problem as new resistance mechanisms emerge and spread globally.

Diagnostic sonography as a very safe, reliable, and economic way to observe various organs of the body (1, 2) uses ultrasound waves in the frequency range of 1 - 20 MHz (however, frequencies up to 50 - 100 MHz have been used experimentally in ultrasound biomicroscopy, a technique used for obtaining high-resolution in vivo imaging of special regions of the body such as the anterior chamber of the eye). The annual number of ultrasound examinations has increased dramatically over the past decade.

The induction of “adaptive response” in bacteria has been already reported (3). Adaptive response can be defined as the induction of repair by pre-exposure to a low
level chemical or physical stress. We have previously shown that pre-exposure of living organisms to low levels of ionizing (4-7) or a large dose of non-ionizing radiation (8-12), decrease the detrimental biological effects on these organisms compared to exposure to the large dose alone. Therefore, adaptive response in bacteria can also be observed as the decrease in lethal effects of antibiotics after exposure to a low level physical stress such as short exposure to electromagnetic radiation or ultrasound.

2. Objectives
On the other hand, we have previously shown that pre-exposure of laboratory animals to non-ionizing electromagnetic radiation in radiofrequency (RF) range can induce a survival adaptive response which can be observed as increased resistance to a subsequent Escherichia coli infection [13, 14]. Furthermore, over the past years, we have investigated the bio effects of physical stresses such as exposure to ultrasound for enhancing the sensitivity of bacteria to different antibiotics. This study aimed at developing an ultrasound-assisted method for increasing bacterial sensitivity to antibiotics.

3. Materials and Methods

3.1. Isolation and Identification of Isolates
This is an in vitro case-control study, performed at the Ionizing and Non-ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences (SUMS), Shiraz, Iran in 2014. The bacterial strains were obtained from the Persian Type Culture Collection, IROST, Iran (Klebsiella pneumonia PTCC 1290) and Bacteriology Department of Shahid Faghihi Teaching Hospital, Shiraz, Iran (E. coli, Staphylococcus aureus and Streptococcus group A).

The samples were cultured on blood agar and MacConkey agar for the isolation of microorganism. The culture plates were incubated at 37°C for 24 hours and observed for the presence or absence of visible bacterial growth.

3.2. Antibiotic Susceptibility Tests
We performed the antibiotic susceptibility tests by using the Kirby-Bauer disk diffusion method on Muller-Hinton agar (Figure 1). Drug susceptibility test was performed for nitrofurantoin, nalidixic acid (30 μg), gentamicin (10 μg), sulfamethoxazole, cephalaxin, ciprofloxacin (5 μg), and cephalothin for Gram-negative bacteria and vancomycin (30 μg), erythromycin (15 μg), amoxicillin (20 μg), penicillin (10 Units), clindamycin (2 μg), and cefixime (5 μg) for Gram-positive bacteria. All culture media and antibiotic disks were purchased from Merck (Germany) and HiMedia Laboratories (Mumbai, India), respectively. Results for antibiotic susceptibility pattern before and after exposure to ultrasound were recorded and analyzed. The inhibition zone of each plate was recorded as the average of 2 diameters (mm) measured at right angles to one another. Three replicate agar plates were used for each regime. According to the CLSI guidelines (2013), the result were categorized as sensitive, intermediate, and resistance.

3.3. Ultrasound Apparatus
The bacteria in culture plates were exposed to diagnostic ultrasound using a recently calibrated MyLab70XVG sonography system (EsaoteBiomedicalMyLab70XVG-Genova, Italia). All ultrasound exposures were performed by a 7.5 - 13 MHz linear array probe (type LA523) by an expert radiologist at Shahid Faghihi teaching Hospital, Shiraz, Iran.

3.4. Statistical Methods
The mean diameters of inhibition zones of the 3 replicates in exposed and non-exposed groups were compared using the nonparametric Mann-Whitney test. The significance level was considered at P < 0.05.

4. Results
Findings of this study are summarized in Tables 1 and 2. Compared to the results obtained from unexposed bacteria, statistically significant variations of sensitiv-
ity to antibiotics were found in some strains after short-term exposures. Tables 1 and 2 show the mean diameters of the inhibition zones of non-exposed Klebsiella and those exposed to diagnostic ultrasound in PenM, ResH and Doppler modes, respectively. This part of the study showed major differences in the diameters of zones of inhibition in exposed and non-exposed samples of *Klebsiella pneumonia* and *Staphylococcus aureus*. In two modes of ultrasound exposure (PenM and Doppler), ultrasonic waves made sensitive *Klebsiella pneumonia* resistant to cephalixin ($P = 0.001$). In ResH mode, ultrasound made sensitive *Klebsiella pneumonia* intermediate resistant to cephalixin ($P = 0.011$).

Tables 3 and 4 show the mean diameters of the inhibition zones of non-exposed *Staphylococcus epidermidis* and those exposed to diagnostic ultrasound in PenM, ResH and Doppler modes, respectively. Again, statistically significant variations of sensitivity to antibiotics were found in *Staphylococcus epidermidis* after short-term exposure to ultrasound. However, ultrasound was unable to make antibiotic-resistant bacteria susceptible or to make sensitive bacteria, resistant.

Tables 5 and 6 show the mean diameters of the inhibition zones of non-exposed *Staphylococcus aureus* and those exposed to diagnostic ultrasound in PenM, ResH and Doppler modes, respectively. As observed in previous tests, statistically significant variations of sensitivity to antibiotics were found in *Staphylococcus aureus* after short-term exposure to ultrasound. In this experiment, ultrasound was able to make antibiotic-resistant bacteria susceptible.

### Table 1. The Mean Diameters of the Inhibition Zones (mm) of *Klebsiella* (PTCC: 1290) in Bacteria Exposed to Diagnostic Ultrasound (PenM and ResH Modes) and Non-Exposed Bacteria$^a$

| Antibiotic     | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|----------------|------------------------------------------|----------------------|---------|
|                | Inhibition Zones$^b$ | Sensitivity | Inhibition Zones$^b$ | Sensitivity |
| **PenM Mode**  |                           |                      |         |        |
| Nitrofurantoin | 17.67 ± 0.58 | Sensitive | 16.34 ± 0.58 | Intermediate | 0.047 |
| Nalidixic acid | 20.34 ± 0.58 | Sensitive | 19.67 ± 0.58 | Sensitive | 0.230 |
| Gentamicin    | 14.34 ± 0.58 | Intermediate | 14.67 ± 0.58 | Intermediate | 0.519 |
| Sulfamethoxazol | 20.67 ± 0.58 | Sensitive | 21.34 ± 0.58 | Sensitive | 0.230 |
| Cephalexin    | 10.67 ± 1.15 | Resistant | 16.67 ± 0.58 | Sensitive | 0.001 |
| Ciprofloxacin | 19.67 ± 0.58 | Intermediate | 20.34 ± 0.58 | Intermediate | 0.230 |
| Cephalothin   | 16.67 ± 0.58 | Intermediate | 18.34 ± 0.58 | Sensitive | 0.024 |
| **Res H Mode** |                           |                      |         |        |
| Nitrofurantoin | 17.34 ± 0.58 | Sensitive | 16.34 ± 0.58 | Intermediate | 0.101 |
| Nalidixic acid | 19.34 ± 0.58 | Sensitive | 19.67 ± 0.58 | Sensitive | 0.519 |
| Gentamicin    | 13.67 ± 0.58 | Intermediate | 14.67 ± 0.58 | Intermediate | 0.101 |
| Sulfamethoxazol | 20.34 ± 0.58 | Sensitive | 21.34 ± 0.58 | Sensitive | 0.101 |
| Cephalexin    | 13.34 ± 1.15 | Intermediate | 16.67 ± 0.58 | Sensitive | 0.001 |
| Ciprofloxacin | 17.67 ± 0.58 | Intermediate | 20.34 ± 0.58 | Intermediate | 0.005 |
| Cephalothin   | 16.34 ± 0.58 | Intermediate | 18.34 ± 0.58 | Sensitive | 0.013 |

$^a$($N = 3$).

$^b$Data are presented as mean ± SD.

### Table 2. The Mean Diameters of the Inhibition Zones (mm) of *Klebsiella* (PTCC: 1290) in Bacteria Exposed to Diagnostic Ultrasound (Doppler) and Non-Exposed Bacteria$^a$

| Antibiotic     | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|----------------|------------------------------------------|----------------------|---------|
|                | Inhibition Zones$^b$ | Sensitivity | Inhibition Zones$^b$ | Sensitivity |
| **Nitrofurantoin** | 16.34 ± 0.58 | Intermediate | 16.34 ± 0.58 | Intermediate | > 0.999 |
| **Nalidixic acid** | 18.34 ± 0.58 | Intermediate | 19.67 ± 0.58 | Sensitive | 0.047 |
| **Gentamicin** | 12.34 ± 0.58 | Resistant | 14.67 ± 0.58 | Intermediate | 0.008 |
| **Sulfamethoxazole** | 17.67 ± 0.58 | Sensitive | 21.34 ± 0.58 | Sensitive | 0.001 |
| **Cephalexin** | 12.34 ± 0.58 | Resistant | 16.67 ± 0.58 | Sensitive | 0.001 |
| **Ciprofloxacin** | 17.34 ± 0.58 | Intermediate | 20.34 ± 0.58 | Intermediate | 0.003 |
| **Cephalothin** | 16.34 ± 0.58 | Intermediate | 18.34 ± 0.58 | Sensitive | 0.013 |

$^a$($N = 1$).

$^b$Data are presented as mean ± SD.
In one mode of ultrasound exposure (PenM) ultrasound made resistant *Staphylococcus aureus* sensitive to amoxicillin (P = 0.003). However, ultrasound was unable to make antibiotic-resistant bacteria susceptible in other modes (ResH and Doppler). Tables 7 and 8 show the mean diameters of the inhibition zones of non-exposed *Salmonella* sp. and those exposed to diagnostic ultrasound in PenM, ResH and Doppler modes, respectively. Although statistically significant variations of sensitivity to antibiotics were found in *Salmonella* sp. after short-term exposure to ultrasound, conversion of antibiotic-resistant bacteria to susceptible or vice versa was not found.

**Table 3. The Mean Diameters of the Inhibition Zones (mm) of *Staphylococcus epidermidis* in bacteria Exposed to Diagnostic Ultrasound (PenM and Res H modes) and Non-Exposed Bacteria**

| Antibiotic | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|------------|------------------------------------------|----------------------|---------|
|            | Inhibition Zones<sup>a</sup> | Sensitivity | Inhibition Zones<sup>a</sup> | Sensitivity |
| **Pen M Mode** | | | | |
| Vancomycin | 16.34 ± 0.58 | Sensitive | 18.67 ± 0.58 | Sensitive | 0.008 |
| Erythromycin | 9.34 ± 0.58 | Resistant | 10.67 ± 0.58 | Resistant | 0.047 |
| Amoxicillin | 14.5 ± 0.5 | Resistant | 19.67 ± 0.58 | Resistant | 0.0001 |
| Penicillin | 17.34 ± 0.58 | Resistant | 19.67 ± 0.58 | Resistant | 0.008 |
| Cefixime | 9.84 ± 0.77 | Resistant | 9.34 ± 0.58 | Resistant | 0.417 |
| **ResH Mode** | | | | |
| Vancomycin | 16.67 ± 0.58 | Sensitive | 18.67 ± 0.58 | Sensitive | 0.013 |
| Erythromycin | 10 | Resistant | 10.67 ± 0.58 | Resistant | 0.184 |
| Amoxicillin | 15.5 ± 0.87 | Resistant | 19.67 ± 0.58 | Resistant | 0.002 |
| Penicillin | 19.67 ± 0.58 | Resistant | 19.67 ± 0.58 | Resistant | > 0.999 |
| Cefixime | 9.34 ± 0.58 | Resistant | 9.34 ± 0.58 | Resistant | > 0.999 |

<sup>a</sup>Data are presented as mean ± SD.

**Table 4. The Mean Diameters of the Inhibition Zones (mm) of *Staphylococcus epidermidis* in Bacteria Exposed to Diagnostic Ultrasound (Doppler) and Non-Exposed Bacteria<sup>b</sup>**

| Antibiotic | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|------------|------------------------------------------|----------------------|---------|
|            | Inhibition Zones<sup>b</sup> | Sensitivity | Inhibition Zones<sup>b</sup> | Sensitivity |
| **Pen M Mode** | | | | |
| Vancomycin | 16.34 ± 0.58 | Sensitive | 18.67 ± 0.58 | Sensitive | 0.008 |
| Erythromycin | 8.84 ± 0.29 | Resistant | 10.67 ± 0.58 | Resistant | 0.008 |
| Amoxicillin | 16.67 ± 0.58 | Resistant | 19.67 ± 0.58 | Resistant | 0.003 |
| Penicillin | 19.34 ± 0.58 | Resistant | 19.67 ± 0.58 | Resistant | 0.519 |
| Cefixime | 9.34 ± 0.58 | Resistant | 9.34 ± 0.58 | Resistant | > 0.999 |
| **ResH Mode** | | | | |
| Vancomycin | 16.67 ± 0.58 | Sensitive | 18.67 ± 0.58 | Sensitive | 0.013 |
| Erythromycin | 0 | Resistant | 8.34 ± 0.58 | Resistant | 0.002 |
| Amoxicillin | 20.34 ± 0.58 | Sensitive | 17.34 ± 0.58 | Resistant | 0.003 |
| Penicillin | 17.67 ± 0.58 | Resistant | 13.17 ± 0.77 | Resistant | 0.003 |
| Cefixime | 29.34 ± 0.58 | Sensitive | 26.34 ± 0.58 | Sensitive | 0.003 |
| Clindamycin | 15.17 ± 0.29(I) | Intermediate | 16.84 ± 0.29 | Intermediate | 0.002 |

<sup>b</sup>(N = 3).
<sup>bb</sup>Data are presented as mean ± SD.

**Table 5. The Mean Diameters of the Inhibition Zones (mm) of *Staphylococcus aureus* in Bacteria Exposed to Diagnostic Ultrasound (PenM and ResH Modes) and Non-Exposed Bacteria<sup>c</sup>**

| Antibiotic | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|------------|------------------------------------------|----------------------|---------|
|            | Inhibition Zones<sup>c</sup> | Sensitivity | Inhibition Zones<sup>c</sup> | Sensitivity |
| **Pen M Mode** | | | | |
| Vancomycin | 17.67 ± 0.58 | Sensitive | 14.67 ± 0.58 | Sensitive | 0.003 |
| Erythromycin | 0 | Resistant | 8.34 ± 0.58 | Resistant | 0.002 |
| Amoxicillin | 20.34 ± 0.58 | Sensitive | 17.34 ± 0.58 | Resistant | 0.003 |
| Penicillin | 17.67 ± 0.58 | Resistant | 13.17 ± 0.77 | Resistant | 0.001 |
| Clindamycin | 29.34 ± 0.58 | Sensitive | 26.34 ± 0.58 | Sensitive | 0.003 |
| Cefixime | 15.17 ± 0.29(I) | Intermediate | 16.84 ± 0.29 | Intermediate | 0.002 |
| **ResH Mode** | | | | |
| Vancomycin | 16.67 ± 0.58 | Sensitive | 14.67 ± 0.58 | Sensitive | 0.003 |
| Erythromycin | 0 | Resistant | 8.34 ± 0.58 | Resistant | 0.002 |
| Amoxicillin | 17.5 ± 0.87 | Resistant | 17.34 ± 0.58 | Resistant | 0.795 |
| Penicillin | 16.67 ± 0.58 | Resistant | 13.17 ± 0.77 | Resistant | 0.003 |
| Clindamycin | 29.67 ± 0.58 | Sensitive | 26.34 ± 0.58 | Sensitive | 0.002 |
| Cefixime | 15.34 ± 0.58 | Intermediate | 16.84 ± 0.29 | Intermediate | 0.016 |

<sup>c</sup>(N = 3).
<sup>cc</sup>Data are presented as mean ± SD.
Table 6. The Mean Diameters of the Inhibition Zones (mm) of *Staphylococcus aureus* in Bacteria Exposed to Diagnostic Ultrasound (Doppler) and Non-Exposed Bacteria<br>

| Antibiotic       | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|------------------|-------------------------------------------|----------------------|---------|
|                  | Inhibition Zones<sup>b</sup> | Sensitivity | Inhibition Zones<sup>b</sup> | Sensitivity |       |
| Vancomycin       | 18.67 ± 0.58 | Sensitive | 14.67 ± 0.58 | Sensitive | 0.001 |
| Erythromycin     | 0 | Resistant | 8.34 ± 0.58 | Resistant | 0.002 |
| Amoxicillin      | 18.84 ± 0.29 | Resistant | 17.34 ± 0.58 | Resistant | 0.016 |
| Penicillin       | 16.34 ± 0.58 | Resistant | 13.17 ± 0.77 | Resistant | 0.005 |
| Clindamycin      | 29.5 ± 0.87 | Sensitive | 26.34 ± 0.58 | Sensitive | 0.006 |
| Cefixime         | 15.5 ± 0.5 | Intermediate | 16.84 ± 0.29 | Intermediate | 0.016 |

<sup>a</sup>(N = 3).<br><sup>b</sup>Data are presented as mean ± SD.

Table 7. The Mean Diameters of the Inhibition Zones (mm) of *Salmonella* in Bacteria Exposed to Diagnostic Ultrasound (PEN M and RES H Modes) and Non-Exposed Bacteria<br>

| Antibiotic            | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|-----------------------|-------------------------------------------|----------------------|---------|
|                       | Inhibition Zones<sup>b</sup> | Sensitivity | Inhibition Zones<sup>b</sup> | Sensitivity |       |
| Ciprofloxacin         | 28.34 ± 0.58 | Sensitive | 34.34 ± 0.58 | Sensitive | 0.0001 |
| Cefixime              | 23.67 ± 0.58 | Sensitive | 25.67 ± 0.58 | Sensitive | 0.013 |
| Amikacin              | 21.67 ± 0.58 | Sensitive | 21.67 ± 0.53 | Sensitive | > 0.999 |
| Sulfamethoxazole/trimethoprim | 26.67 ± 0.58 | Sensitive | 29.67 ± 0.58 | Sensitive | 0.003 |
| Cephalexin            | 26.34 ± 0.58 | Sensitive | 24.34 ± 0.58 | Sensitive | 0.013 |
| Gentamycin            | 20.67 ± 0.58 | Sensitive | 20.67 ± 0.58 | Sensitive | > 0.999 |

<sup>a</sup>(N = 3).<br><sup>b</sup>Data are presented as mean ± SD.

Table 8. The Mean Diameters of the Inhibition Zones (mm) of *Salmonella* in Bacteria Exposed to Diagnostic Ultrasound (Doppler) and Non-Exposed Bacteria

| Antibiotic            | Bacteria Exposed to Diagnostic Ultrasound | Non-Exposed Bacteria | P Value |
|-----------------------|-------------------------------------------|----------------------|---------|
|                       | Inhibition Zones<sup>b</sup> | Sensitivity | Inhibition Zones<sup>b</sup> | Sensitivity |       |
| Ciprofloxacin         | 27.67 ± 0.58 | Sensitive | 34.34 ± 0.58 | Sensitive | 0.0001 |
| Cefixime              | 23.34 ± 0.58 | Sensitive | 25.67 ± 0.58 | Sensitive | 0.008 |
| Amikacin              | 21.34 ± 0.58 | Sensitive | 21.67 ± 0.53 | Sensitive | 0.519 |
| Sulfamethoxazole/trimethoprim | 28.84 ± 0.77 | Sensitive | 29.67 ± 0.58 | Sensitive | 0.206 |
| Cephalexin            | 23.67 ± 0.58 | Sensitive | 24.34 ± 0.58 | Sensitive | 0.203 |
| Gentamycin            | 18.5 ± 0.5 | Sensitive | 20.67 ± 0.58 | Sensitive | 0.008 |

<sup>a</sup>(N = 1).<br><sup>b</sup>Data are presented as mean ± SD.
5. Discussion

To the best of our knowledge this is the first study that explores the effect of ultrasound exposure as a mechanical stress on the antibiotic susceptibility of some microorganisms. In this study, we found some major alterations in the diameters of the inhibition zones in Klebsiella pneumonia and Staphylococcus aureus after exposure to ultrasound waves. Interestingly, ultrasound was capable of making some antibiotic-resistant bacteria susceptible as well as making some sensitive bacteria, resistant. Antibiotic resistance can be defined as the ability of microorganisms to resist the lethal effects of specific antibiotics. This phenomenon occurs when the effectiveness of drugs to cure or prevent infections reduces or vanishes (15). Lattimer et al. (16) in a paper published in JAMA in 1961 reported that in spite of great advances in medicine, scientists are losing the battle against drug resistance. At that time, they believed that the speed of discovery and development of new drugs was not fast enough to take over the significant ability of some microorganisms to develop resistant mutants. Therefore, they predicted that humans might encounter lethal epidemics in the future if they could not control drug-resistant microorganisms (16). Now, we should confess that the situation has not changed significantly since the publication of this paper more than 50 years ago.

The decrease observed in the diameters of the inhibition zones in Klebsiella pneumonia and Staphylococcus aureus after exposure to ultrasound waves, can be interpreted as an adaptive response. Adaptive response can be defined as the acquisition of radiation resistance against exposure to high dose in cultured cells or organisms which had been previously pretreated with an adapting low dose radiation (this low dose radiation is also called “priming dose” or “conditioning dose”) (17). This observation is generally in line with our previous reports on the induction of adaptive response after exposure to low levels of ionizing (4-7) and non-ionizing radiation (8-12). More specifically, our findings are in line with the reports indicating that when bacteria are exposed to mild forms of different stresses (chemical and physical stresses), this stress improves their abilities to adapt and become resistant to any subsequent more extreme exposures (18-20). Also, that pre-exposure can increase the resistance to other exposures (e.g. exposure to antibiotics) and induce “cross-protection” phenomenon (3).

The main limitation of our experiment was the low number of bacterial strains studied. However, the unique interdepartmental cooperation in our study was a significant strength point. Based on these results, we believe that short-term exposure of microorganisms to diagnostic ultrasonic waves can significantly alter their sensitivity to antibiotics. It can be concluded that the physical methods of making the antibiotic-resistant population susceptible can open new horizons in antibiotic therapy fora broad range of diseases, including tuberculosis. On the other hand, when exposure to ultrasound makes the antibiotic-susceptible population resistant, this may endanger patients’ lives.

Footnotes

Authors’ Contribution: Study concept and design: Seyed Mohammad Javad Mortazavi. Acquisition of data: Seyed Mohammad Javad Mortazavi, Leili Darvish, Mohammad Abouajmii, Sina Zarei, Tahereh Zare, Mohammad Taheri, and Samaneh Nematollahi. Analysis and interpretation of data: Seyed Mohammad Javad Mortazavi and Samaneh Nematollahi. Drafting of the manuscript: Seyed Mohammad Javad Mortazavi. Critical revision of the manuscript for important intellectual content: Seyed Mohammad Javad Mortazavi. Statistical analysis: Samaneh Nematollahi. Study supervision: Seyed Mohammad Javad Mortazavi.

Funding/Support: This study was financially supported by the Ionizing and Non-Ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences (SUMS), Shiraz, Iran.

References

1. Liu J, Cao HY, Wang HW, Kong XY. The Role of Lung Ultrasound in Diagnosis of Respiratory Distress Syndrome in Newborn Infants. Iran J Pediatr. 2014;24(2):147-54. [PubMed: 25535352]
2. Ottonello G, Dessi A, Trudella M, Porcu C, Fanos V. A case of neonatal urosepsis with multifocal osteoarthritits: could ultrasonography change the clinical course? Iran J Radiol. 2013;10(3):269-71. doi: 10.5822/iranjradiol.4079. [PubMed: 23436605]
3. McMahon MA, Xu J, Moore J, Blair IS, McDowell DA. Environmental stress and antibiotic resistance in food-related pathogens. Appl Environ Microbiol. 2007;73(1):211-7. doi: 10.1128/AEM.00578-06. [PubMed: 17142599]
4. Mortazavi SMJ, Cameron IR, Niroomand-Rad A. The life saving role of radioadaptive responses in long-term interplanetary space journeys. Int Congr Ser. 2005;1276:265-7. doi: 10.1016/j.ics.2004.12.019.
5. Mortazavi SMJ, Shabestani-Monfared A, Ghiasi-Najad M, Mozdarani H. Radioadaptive responses induced in lymphocytes of the inhabitants in Ramsar, Iran. Int Congr Ser. 2005;1276:201-3. doi: 10.1016/j.ics.2004.12.002.
6. Mortazavi SJ, Mozdarani H. The search for a possible optimum adapting dose under the optimum irradiation time scheme in cultured human lymphocytes. Int J Low Radiat. 2006;3(1):174. doi: 10.1504/ijlr.2006.003000.
7. Mortazavi SMJ, Shirazi MAM, Meh dizadeh S, Rouintan MS, Ebrahimi J, Tamaddon M, et al. Short-term radon inhalation induces significant survival adaptive response in Balb/c mice. Int J Low Radiat. 2010;7(2):98. doi: 10.1504/ijlr.2010.032803.
8. Mortazavi SMJ, Mosleh-Shirazi MA, Tavassoli AR, Taheri M, Bagheri Z, Ghalandari R, et al. A comparative study on the increased radioresistance to lethal doses of gamma rays after exposure to microwave radiation and oral intake of flaxseed oil. Iran J Radiat Res. 2019;19(1):3-14.
9. Mortazavi SMJ. Space research and EMF-induced adaptive responses. J Med Hypotheses Ideas. 2013;7(1):1-2. doi: 10.1016/j. jmhri.2012.10.001.
10. Haghani M, Mortazavi SMJ, Sardari D, Mosleh-Shirazi M, Mansouri A. Assessment of the role of specific absorption rate of mobile phones on the induction of microwave-induced survival adaptive responses after exposure to lethal doses of gamma radiation. Int J Radiat Res. 2013;5(3):167-73.
11. Mortazavi SMJ, Mozdarani H. Deep space missions and the issue of overcoming the problem of space radiation. Int J Radiat Res. 2013;11(3):199-202.
12. Mortazavi SMJ. Is mobile phone radiofrequency radiation all bad? J Med Hypotheses Ideas. 2014;8(1):42–3. doi: 10.1016/j.jmhi.2013.08.003.

13. Mortazavi SMJ, Motamedifar M, Namdari G, Taheri M, Mortazavi AR, Shokrpour N. Non-Linear Adaptive Phenomena which Decrease the Risk of Infection After Pre-Exposure to Radiofrequency Radiation. Dose-Response. 2014;12(2):233–45. doi: 10.2203/dose-response.12-055.Mortazavi. [PubMed: 24910582]

14. Mortazavi SMJ, Motamedifar M, Namdari G, Taheri M, Mortazavi AR. Counterbalancing immunosuppression-induced infections during long-term stay of humans in space. J Med Hypotheses Ideas. 2013;7(1):8–10. doi: 10.1016/j.jmhi.2012.12.001.

15. Martinez JL, Baquero F. Emergence and spread of antibiotic resistance: setting a parameter space. Ups J Med Sci. 2014;119(2):68–77. doi: 10.3109/03009734.2014.901444. [PubMed: 24678768]

16. Lattimer JK, Seneca H, Zinser HH, Donovan JT. Drug-resistant bacteria made drug-susceptible by enzyme inhibitors. JAMA. 1961;178:764–6. [PubMed: 1446278]

17. Mortazavi SMJ, Mosleh-Shirazi MA, Tavassoli AR, Taheri M, Mehdi-zadeh AR, Namazi SAS, et al. Increased Radioreistance to Lethal Doses of Gamma Rays in Mice and Rats After Exposure to Microwave Radiation Emitted by a GSM Mobile Phone Simulator. Dose-Response. 2013;11(2):281–92. doi: 10.2203/dose-response.12-010.Mortazavi. [PubMed: 23930307]

18. Hill C, Cotter PD, Sleator RD, Gahan CGM. Bacterial stress response in Listeria monocytogenes: jumping the hurdles imposed by minimal processing. Int Dairy J. 2002;12(2-3):273–83. doi: 10.1016/s0958-6946(01)00125-x.

19. Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and Modulations of Antibiotic Resistance Gene Expression. Clin Microbiol Rev. 2007;20(1):79–114. doi: 10.1128/cmrr.00015-06. [PubMed: 17223624]

20. Al-Nabulsi AA, Oualal TM, Shaker RR, Olaimat AN, Jaradat ZW, Zain Elabedeen NA, et al. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of Listeria monocytogenes. Food Microbiol. 2015;46:554–60. doi: 10.1016/j.fm.2014.07.015. [PubMed: 25475279]