INTRODUCTION

Topic’s significance. Bacteria and other organisms are sensitive to electromagnetic irradiation (EMI) of extremely high frequency (range from 30 GHz to 300 GHz) or millimeter waves (length of a wave from 10 to 0.1 mm). Especially great interests have low-energetic intensities (power flux density till 10 mW cm\(^{-2}\)) of EMI, which have non-thermal action (Betskii et al., 2000; Guofen et al., 2002; Banik et al., 2003). Only the properties of these fields (frequency, amplitude and waveform) make EMI biologically active, when electromagnetic field is constant not only in time (intervals) but also in space (covering the exposed cells across the entire surface), in order to act like a signal (Hyland, 2008; Torgomyan, Trchounian, 2013). Investigation of these EMI effects started immediately, following the utilization of these frequencies in satellite communication, security screening and weapons systems, radio astronomy and for radar and remote sensing technologies (Pakhomov, Murphy, 2000; Da Silva, 2001; Cotgreave, 2005; Belyaev 2005; Hyland, 2008; Torgomyan, Trchounian, 2013). These devices produce EMI of much higher degree of coherence than do the sources of natural origin. So, this EMI intensity increased 1 mln times compared with the natural intensity. This feature facilitates EMI discernment by organisms (Ruediger, 2009). That is why the fundamental knowledge about EMI and its possible effects is very important. These effects are depending on EMI frequency and intensity, irradiation duration, mediated and repeated irradiation, composition of growth media, genetic features, and peculiarities of metabolism and membrane properties in bacteria (Banik, 2003; Belyaev, 2005; Torgomyan, Trchounian, 2013). Also, it is known that EMI is used for long-range interactions between bacteria and different cells using water as a medium (Nikolaev, 2000; Trushin, 2003; Reguera, 2011). It was defined that EMI of extremely high frequencies increases bacterial sensitivity toward antibiotics (Torgomyan, Trchounian, 2012; 2013).

Due to the feature to cause biological, especially bactericidal effects, EMI is used in therapeutic practice as a mono-therapy or combined with drugs. Moreover, this EMI has disinfecting applications at moderately low temperatures in treatment of agricultural wastewater and meat, rice, juices and other food products (Torgomyan, Trchounian, 2013).

There is evidence that, regardless of intensities of EMI, 41.5, 51.8, 70.6 and 99 GHz are resonant frequencies for *Escherichia coli* (Dardalhon et al., 1981; Belyaev et al., 1996; Torgomyan, Trchounian, 2011; 2013). Interestingly, the elevated decrease in *E. coli* growth rate under anaerobic conditions was shown with coherent (in time) EMI of 45–53 GHz; especially with 51.8 and 53 GHz and with “noise” EMI (broadband frequencies and randomly changing phases). Moreover, the other frequencies EMI as 70.6 and 99 GHz are
not resonant frequencies for water (Sinitsyn et al., 2000) and their effects on *E. coli* should be clarified.

The most significant targets responsible for the cellular effects of EMI at resonant frequencies are water, bacterial membrane and its surface characteristics, which are responsible for substance transport and energy-conversing processes, and genome and in other cellular structures (Pakhomov, Murphy, 2000; Banik et al., 2003; Beneduci et al., 2005; Torgomyan, Trchounian, 2013). With the most probability EMI affects directly on cell membrane or that mediated by water structure changes (Fesenko et al, 1995; Cojocaru et al., 2005). However, the specific mechanisms of induction of biological effects are not clear yet.

**Research goals and tasks.** The aim of this study was to investigate the effects of coherent EMI of 70.6 and 73 GHz on *E. coli*. Constituted tasks of the research were to:

1. study the effects of 70.6 and 73 GHz EMI on *E. coli* cell growth, viability, cell morphology and on membrane functional activity (ATPase activity, \(H_2\) production, \(H^+/K^+\) exchange, the number of accessible SH groups, membrane potential);
2. reveal the role of medium (distilled water, minimal salt solution, growth medium) and period of irradiation on bacterial effects;
3. establish the EMI effects on bidistilled water properties (pH, conductivity, optical density, redox potential and surface tansion) and determine the role of this effect on bacteria;
4. investigate the irradiated bacterial growth dependence on medium pH values and the presence of reducer;
5. determine the medium (distilled water, minimal salt solution, growth medium) mediated effects on bacterial growth;
6. reveal the combined effects of EMI and some antibiotics on bacterial growth, survival and on \(H^+/K^+\) exchange across membrane;
7. compare the effects of different frequencies – 70.6; 73 GHz and 51.8; 53 GHz on *E. coli*.

**Scientific novelty and applied value of the study.** Within the scopes of this work was established that coherent 70.6 and 73 GHz EMI depressed *E. coli* growth. Such effect can be connected with EMI primary effects on membranes or mediated by the changes in water clusters structures (Fesenko et al, 1995; Cojocaru et al., 2005). EMI bacterial effects with the main probability connected with its membrane and about that indicated the changes in \(H^+\) and \(K^+\) ions fluxes across the membrane, \(H_2\) production process, also structural changes in membrane. At the same time the water physicochemical properties also were changed. EMI effects on bacterial growth and survival were dependent on medium pH. Also, after EMI bacteria become more sensitive toward the chemicals (DTT, DCCD, antibiotics). During the
comparative research it became clear that EMI effects on bacteria were more visible with 53 GHz, and then with 73 GHz than with 51.8 GHz and 70.6 GHz.

The study of effects of different extremely high frequencies EMI on bacteria is essential for investigation of fundamental knowledge about EMI action mechanisms. Also it will give an opportunity to find out more effective frequencies for their use in microbiology and in biotechnology – in controlling microorganisms, in food prevention and in medicine as a mono-therapy or combined with drugs (such as antibiotics) - treating the range of bacterial diseases.

**Main points to present at defense.**

1. The effect of low intensity 70.6 and 73 GHz EMI on *E. coli*, particularly on their growth, viability, cell morphology and cell membrane function.
2. The effect of 70.6 and 73 GHz EMI on water physicochemical properties and the role of liquid medium on EMI bacterial effects.
3. The straightened combined effects of 70.6 and 73 GHz EMI and some antibiotics on bacteria.

**Work approbation.** Main results of the dissertation were discussed at seminars in Department of Biophysics, Biology Faculty of Yerevan State University, and at scientific conferences: 14th Int. School-Conference for Young Scientists (Pushchino, Russia, 2010), Int. symposium on salvation and ionic effects in biomolecules: theory to experiment (Tsakhkadzor, Armenia, 2010), IV Sissakian readings “Problems of biochemistry, radiation and space biology” (Alusta, Ukraine, 2010), Int. conference & DAAD Alumni seminar “Biotechnology and Health -4” (Yerevan, Armenia, 2010), 10th Int. Congress of the European Bioelectromagnetics Association (Rome, Italy, 2011), 17th Int. Biophysics Congress & 12th Chinese Biophysics congress (Beijing, China, 2011), 112th General Meeting of American Society for Microbiology (San Francisco, USA, 2012) and 6th Int. Congress on low and super low fields and radiations in biology and medicine (S.-Petersburg, Russia, 2012).

**Publications.** According to experimental data observed in dissertation 16 works, including 7 papers in peer-reviewed journals and 2 papers in proceedings, were published.

**Volume and structure of dissertation.** The dissertation contains following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), concluding remarks, conclusions and cited literature (total 164 papers and books). The document consists of 134 pages, 7 tables and 25 figures.

**MATERIALS AND METHODS**

**Bacteria, bacterial cultivation and preparation for experiments.** The *E. coli* wild type strain K-12(λ) was used in this study. Bacteria were grown under anaerobic conditions at 37°C in
the peptone medium that contains 0.2 % peptone, 0.5 % NaCl and 0.2 % K2HPO4, with addition of 0.2 % glucose at pH 7.3. Bacterial growth was monitored by changes in optical density (OD) of bacterial suspension using a spectrophotometer at a wave light of 600 nm. The bacterial suspension was washed and concentrated by centrifugation. Thereafter they were diluted into distilled water or appropriate medium and transferred into the plastic plate (Petri dish) for subsequent irradiation with suspension thickness of 1 mm.

The pH of mediums was measured by a pH-potentiometer with selective electrode.

**Bacterial irradiation.** The irradiation process was performed by using a generator of G4-142 type with coherent electromagnetic waves of 70.6 GHz and 73 GHz frequencies (4.23 and 4.11 mm wavelengths, flow flux intensity is 0.06 mW/cm²) which was supplied by Dr. V. Kalantaryan (Yerevan State University). The changes in temperature of exposed sample were below 0.1 °C during the exposure (non-thermal effects).

**Determination of bacterial growth characteristics and survival.** The lag growth phase duration as a period before bacterial culture absorbance doubling and the specific growth rate (μ) as a 0.693/time doubling of OD of bacterial suspension, were determined (Torgomyan, Trchounian, 2012; Torgomyan et al., 2013). The bacterial survival was determined by transferring them into saline medium (46 mM KHPO4, 23 mM KH2PO4, 8 mM (NH4)2SO4, 0.4 FeSO4, 6 µM MgSO4), (Markaryan et al., 2002; Torgomyan, Tadevosyan, et al., 2011). The quantity of bacteria in unit volume was determined in the same time during 4 days using spectrophotometer at 600 nm wave length and by counting colony forming units. In some experiments different antibiotics were added into the medium.

**Electron microscopy assays.** *E. coli* cells were visualized by transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Electron microscopy imaging analysis was performed using the program “Video-Test Structure-5, nanotechnology” (Torgomyan et al., 2012).

**Determination of surface tension.** The mediums suspension tension was measured by Du Noüy ring method; the surface tension is calculated from the diameter (0.6 cm) of the ring and the tear-off force (dyn/cm) (Torgomyan et al., 2012).

**Measuring of Eh and determination of H2 production.** Eh was measured by both platinum and titanium-silicate (Ti-Si) electrodes. H2 production rate was calculated through the difference between the initial rates of decrease in platinum (Pt) and titanium-silicate (Ti-Si) electrodes redox potentials (Eh). It was measured electrochemically per time and expressed in mV Eh min⁻¹ mg⁻¹ of dry weight of bacteria as reported elsewhere (Gabrielyan et al., 2010; Torgomyan, Trchounian, 2012).

**Proton and potassium ions transport study.** H⁺ and K⁺ fluxes through the bacterial membrane in the whole cells were studied by monitoring changes in their activity in the
medium with the use of appropriate selective electrodes (Tadevosyan et al., 2009; Torgomyan, 2012). Ion fluxes are expressed as the change in external activity of the ion in mM/min per number of cells in a unit of medium volume (ml).

**Determination of membrane potential.** The membrane potential ($\Delta \Psi$) was determined by the distribution of tetraphenylphosphonium cation (TPP$^+$) inside and outside of cell, which dependent on $\Delta \Psi$ value (Trchounian et al., 2012).

**Accessible SH-groups and ATPase assay.** Accessible SH groups of membrane vesicles were determined by Ellmann’s reagent (5,5’-dithiobis-2-nitrobenzoic acid); corrections were made for blanks without membrane vesicles. Right side out membrane vesicles were isolated by lysis of protoplasts with lyzosime using appropriate method (Konings and Kaback, 1973). Membrane vesicles were treated with the reagent and OD was measured after 2 hours. The level of SH- groups were expressed in nmol per mg protein (Torgomyan, Trchounian, 2011).

ATPase activity was measured by amount of liberated inorganic phosphate (Pi) after addition of 5 mM ATP that was determined by the method of Taussky and Shorr (Taussky and Shorr, 1953). Relative ATPase activity was expressed in nmol Pi per mg protein in 1 min.

**Data processing.** The average data are presented from three independent measurements; standard errors were not more 3 % if not indicated. The Student's validity criteria (p) were calculated to show the reliability of difference between changed values and control.

**RESULTS AND DISCUSSION**

**EMI effects on E. coli K-12 growth and survival**

The growth rate of *E. coli* decreased after irradiation in bi-distilled water and in the assay buffer (Fig. 1) with 70.6 and 73 GHz EMI during 1 h: decrease of $\mu$ was 1.2 fold ($p = 0.011$) and 1.4 fold ($p = 0.003$), respectively. These data point out bactericidal effects of EMI with the frequencies of 70.6 and 73 GHz both. Note, 0.5 h was not sufficient exposure for such irradiation to revealing bactericidal effects (see Fig. 1) ($p$ values differs from 0.2 to 0.5; there were a statistically insignificant difference between the input groups). Moreover, the effect of 2 h EMI was essentially identical to that observed following 1 h one (interval of $p$ values for the comparisons differed from 0.001 to 0.005). This means that EMI exposure to observe valuable bactericidal effects was 1 h (Fig. 1). Besides, 2 h rest of the bacteria after EMI with 1 h exposure had renewal effect on *E. coli* growth: $\mu$ increased on 1.2-fold compared with the bacterial growth immediately after irradiation in the cases of two frequencies. Further repeated EMI of such bacteria had an insignificant action on $\mu$ compared with non-irradiated control (not shown). There were no noticeable differences in results obtained from irradiation performed in water or in buffer.
Also there was investigated the EMI effects on *E. coli* cells ability to form colony forming units. The EMI of bacteria was performed in water suspension or on solid growth medium (direct irradiation). So, the numbers of *E. coli* viable cells during 4 days decreased too after 70.6 and 73 GHz EMI exposures of bacterial suspension compared with control. These were 1.1 and 1.3 fold lower (Fig. 2). The results with direct irradiation of bacteria on solid growth medium with the frequencies of 70.6 and 73 GHz indicated the decreases of the settled colonies numbers compared with non-irradiated control was 1.3 fold ($p \leq 0.001$) with 70.6 GHz and 1.5 fold ($p \leq 0.001$) with 73 GHz (Fig. 3). Interestingly, EMI influenced not only on decreases of the colonies numbers, but also on their dimensions.

![Fig. 1](image1.png) The *E. coli* K12 specific growth rate after irradiation with the frequencies of 70.6 and 73 GHz in bi-distilled water and in the assay buffer. As a control non-irradiated bacteria were used.

![Fig. 2](image2.png) The effects of EMI of the 70.6 GHz and 73 GHz frequencies on *E. coli* K12(λ) survival. The control was without EMI. Bacteria were held in the minimal salt medium during 4 days.

![Fig. 3](image3.png) The changes in *E. coli* colony-forming units number of spread on solid growth medium irradiated with 70.6 and 73 GHz. In control, bacteria without irradiation (100%).

Bacterial growth and survival are important properties for characterization the influence of extremely high frequency EMI resulted to deep reorganizations in a membrane and inside a cell. The obtained results are indicated that 70.6 and 73 GHz EMI are “resonant” frequencies for *E. coli*. Compared with the earlier data of 51.8 and 53 GHz EMI,
it became clear that the effects on bacteria were more visible with 53 GHz, and then with 73 GHz than with 51.8 GHz and 70.6 GHz. Also, 51.8 GHz and 70.6 GHz EMI depressed bacterial growth similarly. But on *E. coli* viability 51.8 GHz EMI had more depressive effect (Torgomyan, Trchounian, 2012).

**EMI effects on *E. coli* cell sizes and structure**

To find out the changes in cell sizes and structure by EMI, electron microscopy analysis of *E. coli* cells was performed. It was shown that after EMI with 51.8, 53, 70.6 and 73 GHz compared with non-irradiated control bacterial cell sizes and structure changes were occurred (Fig. 4). Bacterial cells widths changes were negligible but the lengths increased significantly; more changes were observed with 70.6 GHz – 1.6 fold (p=0.03), then with 53 GHz – 1.4 fold (p=0.05) and with 73 GHz – 1.2 fold (p=0.01). Compared with non-irradiated control, after EMI with 53 and 73 GHz (see Fig. 4) and 51.8 and 70.6 GHz (not shown) the number of filamentous cell forms increased; outer membrane bulging, cytoplasm vacuolization and bacterial shape changes by EMI were also detected. The results obtained indicate that EMI affects on *E. coli* cell morphology and modifies the cytoplasmic membrane (Zhou et al., 2010; Shamis et al., 2011; Torgomyan et al., 2012).

![Fig. 4. Microphotographs of *E. coli* K12 cells in control (A) and after bacterial suspension irradiation at the frequencies of 53 (B) and 73 GHz (C).](image)

Besides, lipid membranes - water interfaces are known to be dielectric transition regions. Their local organizations are sensitive to weak electric field with dramatic consequences on the membrane structure and functioning. There is evidence, that low intensity EMI of extremely high frequency (at specific frequencies) can destabilize the structure of hydrogen bonds in water near to the membrane surface and within the cell (Kuznetsov et al., 2006). This affects on the cellular functions and creates conditions for biological response (Teissie, 2007; Torgomyan, Trchounian, 2013). It is not ruled out that the alterations in cell morphology might result by changes in water properties.
Irradiated *E. coli* growth at different medium pH values and in presence of reducer

*E. coli* growth depression by low intensity 70.6 and 73 GHz EMI has been determined at different pHs - pH 6.0; 7.3 (similar to pH 7.5) and 8.0. Inhibition of *E. coli* growth by EMI at the pH values was demonstrated. At pH 7.3 μ was depressed 1.3 and 1.4 folds \( p = 0.011 ; p = 0.003 \) by EMI of 70.6 and 73 GHz, correspondingly, but at pH 6.0 and 8.0 the inhibitions were lower – 1.1 and 1.2 folds only \( p = 0.002 \), compared with control (Fig. 5). Moreover, bacteria exposed to 70.6 GHz prolonged lag-phase 1.5 fold \( p = 0.003 \). But, bactericide effect of 73 GHz EMI was reflected clearly at pH 7.3, when lag-phase was prolonged 2 fold \( p = 0.05 \), (not shown).

![Fig. 5](image)

**Fig. 5.** The *E. coli* K12 specific growth rate after irradiation with the frequencies of 70.6 and 73 GHz in bi-distilled water and in the assay buffer. As a control non-irradiated bacteria were used.

DTT action on irradiated *E. coli* growth was established only at pH 7.3; the effect of DTT did not depend on pH (Kirakosyan et al., 2004). DTT (3 mM) (Fig. 6) had depressive effect on *E. coli* growth compared with control. DTT led to decrease of μ in 1.2 fold \( p = 0.003 \) and to prolong lag-phase (not shown) in 1.6 fold \( p = 0.003 \) in non-irradiated and even more in irradiated bacteria. The inhibitory effect of DTT on bacterial growth was not new, but its enhanced action by EMI was novel. Certainly, EMI changed bacterial sensitivity toward DTT, which was more effective in the case of 73 GHz, as the μ was decreased 1.6 fold \( p = 0.002 \) and the lag-phase was prolonged 2.2 fold compared with control. But with 70.6 GHz the changes in mentioned growth characteristics were lower 1.5 \( p = 0.002 \) and 1.9 folds, respectively.

DTT as a strong reducer resulted the loss of activity or specificity of membrane surface proteins to alter cell surface redox state or to induce oxidative stress with subsequent dramatic alterations within the cell. Perhaps, inhibition of μ and prolongation of lag-phase in control and in case of EMI exposed bacteria was a result of DTT stimuli, which elicited complex but functionally-coordinated alterations in functions of membrane proteins (Torgomyan, Trchounian, 2011).
EMI mediated effects on bacteria

The indirect effects of 70.6 and 73 GHz EMI on bacteria, by means of water, assay buffer and growth medium were examined (data not presented). After 15 min of incubation in preliminarily irradiated (1 h) mediums with the noted frequencies of the electromagnetic field, bacteria were transferred into growth medium. The mediated consequence of 70.6 and 73 GHz EMI on E. coli growth was absolutely insignificant (the specific growth rate and lag-phase duration were not changed) due to non-great difference in values of compared groups (p = 0.356). It is different for 51.8 and 53 GHz medium mediated effect, because clear inhibitory effects on bacterial growth were demonstrated, especially with 53 GHz (Torgomyan et al., 2012).

EMI effects on water

The analyses were made to determine the 70.6 and 73 GHz EMI effects on electrophysical parameters of bi-distilled water. Firstly, its OD was observed in wide spectra (200-1000 nm; not shown), but essential changes were presented only in near ultraviolet region (200-340 nm). Especially, 70.6 GHz exposure was the strongest one, because the absorption intensity elevated compared with non-irradiated water, and also with 73 GHz exposure (not shown). These effects could be connected with changes in the energetics of unshared pairs of electrons in water molecules and creation of ionic and orientation defects (Golovleva et al., 1997).

Interestingly, 1 h irradiation of these frequencies changed pH and conductivity of bi-distilled water; those were more revealed in the case of 73 GHz (Table 1). In the cases of water at pH 4.0 and 6.0 non-significant alkalizations occurred after 70.6 GHz. Moreover, the water with pH 8.0 after irradiation had changed to acidification. The evidence on that the energy of extremely high frequency EMI could be accumulated into the structures of water till critical values and increase of free H+ and OH− dispersions by modification of molecular cluster structuring (Sinitsyn et al., 2000) might explain these results.
The oscillating and fluctuating character of pH values in bi-distilled water depending on time, and other types of intra correlation between physical and chemical parameters of water can occur (Fesenko et al., 1995). And water conductivity changes might result by pH changes.

Thereby, after 70.6 and 73 GHz EMI of the water conductance more increased in the case of pH 6.0 – accordingly on 1.5 and 2.14 folds. In the remainder of the cases, the increases were less (Table 1). The increased conductivity might be explained due to the number of ions present in solution and their mobility connected with their charge and mass or size: ions with more charge conducted more current; larger ions conducted less. Further, the mentioned results could be connected also with more flexible cluster structuring of water at pH 6.0 (Torgomyan et al., 2011).

Table 1. The water pH and conductivity after 70.6 and 73 GHz EMI*

<table>
<thead>
<tr>
<th>pH</th>
<th>Control conductivity (µS)</th>
<th>70.6 GHz pH</th>
<th>70.6 GHz conductivity (µS)</th>
<th>73 GHz pH</th>
<th>73 GHz conductivity (µS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>47.20 ± 0.10</td>
<td>4.26 ± 0.05</td>
<td>48.60 ± 0.20</td>
<td>4.50 ± 0.10</td>
<td>49.10 ± 0.15</td>
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<tr>
<td></td>
<td>p ≤ 0.001</td>
<td>p = 0.003</td>
<td>p ≤ 0.003</td>
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<tr>
<td>6.0</td>
<td>1.54 ± 0.2</td>
<td>6.62 ± 0.15</td>
<td>2.32 ± 0.1</td>
<td>6.72 ± 0.05</td>
<td>3.30 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>p = 0.004</td>
<td>p ≤ 0.001</td>
<td>p ≤ 0.001</td>
<td>p ≤ 0.001</td>
<td>p ≤ 0.001</td>
</tr>
<tr>
<td>8.0</td>
<td>4.17 ± 0.20</td>
<td>7.73 ± 0.07</td>
<td>4.84 ± 0.10</td>
<td>7.53 ± 0.10</td>
<td>5.46 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>p ≤ 0.001</td>
<td>p ≤ 0.001</td>
<td>p = 0.002</td>
<td>p ≤ 0.001</td>
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*irradiation time was 1 h, in control the measurements were done after 1 h.

EMI effects on E. coli membrane vesicles ATPase activity and the number of accessible SH-groups

The influence of 51.8 and 53 GHz EMI on the H⁺-transporting FoF₁-ATPase activity as a result of conformational changes was suggested in earlier work (Tadevosyan et al., 2008). Interestingly, overall ATPase activity changed negligibly by 70.6 GHz and 73 GHz (not shown). 11% decrease in total ATPase activity occurred only with 70.6 GHz irradiation compared with control (p ≤ 0.001) (not shown). Similarly, compared with control, DCCD-sensitive ATPase activity decreased 12% (1.13 fold) only by 70.6 GHz (p ≤ 0.001). These results were in conformity with the data of DCCD-sensitive H⁺ efflux obtained. So, probably the conformational or other changes in FoF₁ suggested for the other frequencies did not occur with 70.6 and 73 GHz frequencies (Torgomyan, Trchounian, 2012).

For understanding the role of membranes in primary targeting of 70.6 and 73 GHz EMI signals, the number of accessible SH-groups of membrane vesicles was defined from non-irradiated and irradiated bacteria. The suggestion that low intensity EMI effects might be
related with modifications in structure, stability, interactions with each other, enzymatic activity and other function of membrane proteins could be supported with decreased number of SH-groups by 70.6 and 73 GHz EMI in 1.4 and 1.6 folds, correspondingly, compared with control (p = 0.003) (Fig. 7). Furthermore, it was shown that ATP increased the SH-groups number in control and in 70.6 GHz exposed cells 1.2 fold, and in 73 GHz exposed bacteria a little less - 1.1 fold. The treatment of membrane vesicles with DCCD also had enhancing effect on the number of SH-groups, but in control it was less (1.1 fold) than in 70.6 and 73 GHz irradiated samples - 1.2 fold. After DCCD treatment and subsequently ATP addition this number compared with their initial level in control (in membranes without ATP and DCCD) was decreased 1.3 fold, but in vesicles of 73 GHz irradiated bacteria it was increased 1.2 fold. This was not changed in the case of 70.6 GHz exposure. These data might be as a result of changed intra- and inter- disulfide-dithiol transitions and conformational changes in membrane proteins, especially by EMI.

![Graph showing the changes in number of SH-groups in membrane vesicles of E. coli after irradiation with 70.6 and 73 GHz](image)

**Fig. 7.** The changes in the number of accessible SH-groups in membrane vesicles of *E. coli* after irradiation with 70.6 and 73 GHz. Membrane vesicles were treated with DCCD (0.2 mM) for 10 min prior assays and ATP (3 mM) was added when mentioned. The value was a difference between the values in presence and absence of membranes in assay medium.

Similarly, the number of SH-groups in membrane vesicles was determined also after DTT treatment at the same conditions as those were above. In this case, the number of SH-groups was 5.8 fold lower than in control (p=0.003), and in irradiated with 70.6 and 73 GHz were - 10 and 4.7 folds less, respectively (not shown). However, DTT cannot reduce buried (solvent-inaccessible) disulfide bonds, so this could mean that 70.6 GHz, but not 73 GHz EMI decreased the accessibility of intra- and inter-molecular SH-groups. Therefore, EMI of the used frequencies most likely primarily affected not only the FoF1-ATPase, but preferably changed the interaction between membrane proteins, especially through disulfide cross links, by modifying their structure and stability (Torgomyan, Trchounian, 2011; 2012).

**EMI effects on *E. coli* membrane-associated energy-dependent processes**

The bactericidal effects of 70.6 GHz and 73 GHz EMI seems to be complex. They are not explained only by the changes of water physicochemical properties. The alterations in
conformations and functions of membrane proteins might be involved in these effects. So, energy (glucose) driven processes associated with the cell membrane – H⁺, K⁺ fluxes, H₂ production and membrane potential were assayed to reveal the role of bacterial membranes in EMI effects.

It was defined that EMI lowered the glucose-dependent H⁺ and K⁺ transport processes across bacterial membrane (Fig. 8A, B). Non-damaged _E. coli_ K-12 cells carried out H⁺ and K⁺ exchange during glucose fermentation by secreting 2 H⁺ with F₀F₁-ATPase and uptaking a K⁺ by TrkA system (Torgomyan, Trchounian, 2013). The EMI, however, showed no effect on the H⁺/K⁺ exchange direction and ratio (2/1). The overall H⁺ secretion from 70.6 and 73 GHz irradiated cells decreased 1.2 fold (p<0.035) and 1.3 fold (p<0.05), respectively (Fig. 8A). Addition of DCCD (0.2 and 0.4 mM) inhibited H⁺ efflux 1.6 fold (p<0.02) and 1.9 fold (p<0.035), respectively. DCCD also had depressive effect on irradiated bacteria. That effect was clearer with 0.4 mM DCCD. The H⁺ efflux in the presence of 0.4 mM DCCD from 70.6 GHz and 73 GHz irradiated bacteria was depressed 2 fold and 2.7 fold (p<0.05), respectively (Fig. 8A).

![Fig. 8. Glucose-dependent total H⁺ (A) and K⁺ (B) fluxes (in %) through the whole _E. coli_ K12 cell membrane after 70.6 and 73 GHz EMI. DCCD was added into assay medium with final concentrations of 0.2 mM and 0.4 mM.](image)

The K⁺ influx was only slightly depressed (1.1 fold; p<0.025) after the exposure to 73 GHz compared with the control flux (Fig. 8B). Furthermore, K⁺ flux in control (non-irradiated) after DCCD addition (0.2 and 0.4 mM) was inhibited 2.63 fold (p<0.05) and 11.5-fold (p<0.025), respectively. Such depressive effects for two DCCD concentrations on irradiated bacteria were 2.72 fold (p<0.01) and 22 fold (p<0.03) with irradiation of 70.6 GHz and 2.52 fold (p<0.01) and 19-fold (p<0.035) with irradiation of 73 GHz, respectively (Fig. 8B).
The change in ions fluxes, especially by 73 GHz EMI could be due to the alterations in the activities of $F_0F_1$ and TrkA and interaction between these proteins. This correlated well with the changed number of accessible SH-groups in membrane vesicles of irradiated bacteria (higher alterations were found with 73 GHz), as a consequence of rearrangement in intra- and inter-molecular disulfide bonds in membrane proteins (Torgomyan, Trchounian, 2011). Also, decrease of organic acids secretions, fermentation end products, might have effect on $H^+$ flux rate (Torgomyan, 2012). Addition of 0.4 mM DCCD in the case of irradiated bacteria exhibited higher inhibiting effect on both ion fluxes than in control. Thus, DCCD addition showed that 73 GHz had stronger effect on $H^+$ efflux and 70.6 GHz - on $K^+$ influx.

Glucose fermentation by *E. coli* at pH 7.5 yielded different organic acids and $H_2$ (Torgomyan, Trchounian, 2012; 2013). *E. coli* produces $H_2$ by formate hydrogen lyase (FHL), a membrane-bound multi-enzyme complex (Bagramyan et al., 2002). Interestingly, $H_2$ production was suggested to relate to the interactions between FHL and $F_0F_1$. The latter is supplying reducing equivalents from FHL to TrkA ($K^+$ transport) system. This might specify the relation between $H_2$ production by FHL, the $F_0F_1$-ATPase activity, and $H^+/K^+$ transporting processes that is occurred by dithiol-disulfide transitions. The modification of this interaction might initiate the disturbance of $H_2$ production.

**Fig. 9.** The changes in $H_2$ production rate by *E. coli* after EMI of 70.6 GHz and 73 GHz frequencies. The non-irradiated (control) and irradiated cells were without (overall) and with 0.5 mM DCCD (pre-incubation for 10 min).

$E_h$ measured by Pt electrode decreased down to low negative values $-640 \pm 24$ mV as a result of $H_2$ evolution by *E. coli* during glucose fermentation. The ability of bacteria to utilize glucose and to produce $H_2$ could be partly depressed by EMI. Indeed, irradiated bacteria had depressed $H_2$ production: at 70.6 GHz particularly $H_2$ production was only 81.3 % of the control (considered as 100 %); the inhibition was increased with 73 GHz $-64.2$ % (Fig. 9). Moreover, $H_2$ production was determined after DCCD treatment as well. DCCD inhibited $H_2$ production was in the control (45%) ($p = 0.002$) and even more in irradiated bacteria - 35% by 70.6 GHz and 37.1% by 73 GHz ($p \leq 0.001$). DCCD-sensitive $H_2$
production rate compared with control was more depressed by 73 GHz EMI – 2.04 fold (p = 0.002). Although by 70.6 GHz EMI the depression was only 1.2 fold (p = 0.004).

So, the changes in overall H+ efflux and H2 production indicated changed activity of FHL. This was probably a result of interaction disruption between FoF1 and FHL.

Also there were revealed that EMI had no effects on *E. coli* membrane potential (not shown).

**EMI and antibiotics effects on *E. coli* growth, viability and glucose-dependent H+ and K+ transport across membrane**

The effects of different antibiotics – chloramphenicol (Chl), ceftriaxone (Cef), kanamycin (Kan) and tetracycline (Tet), of minimal inhibitory concentrations (4 µM, 0.4 µM, 15 µM and 4 µM), on irradiated bacteria were examined by determination of bacterial growth and survival. Bactericidal effects were inferred from decreased µ (Fig. 10) and increased lag phase duration (not shown) of *E. coli* growth. 70.6 GHz and 73 GHz EMI exposed *E. coli* growth inhibitions compared with non-irradiated cells were 1.2 and 1.4, respectively (p = 0.002). Antibiotics enforced the bactericidal effect of EMI. Chl did not enforce the effect of 70.6 GHz irradiation. But the growth of 73 GHz irradiated bacteria with this antibiotic compared with control was 1.65 fold depressed (p = 0.002) (Fig. 10). The Cef effects on irradiated bacteria by 70.6 GHz and 73 GHz were enhanced 1.62 and 1.82 fold, respectively (p = 0.003) (if Cef only – 1.5 fold). But Kan and Tet had similar and more harmful effects on irradiated bacterial growth. Such as, 70.6 GHz and 73 GHz EMI depressed bacterial growth 1.95 fold and 2.2 fold (p = 0.004), correspondingly (on the control bacteria - 1.56 fold, p = 0.003).

The numbers of *E. coli* viable cells EMI decreased too. EMI also enforced the effects of antibiotics on *E. coli* survival in minimal salt medium by lowering the number of colonies within 4 days. The effects of antibiotics on viability decrease were 1.1 fold more compared with control. These were with more efficiency on EMI exposed *E. coli* dependent on antibiotics, in case of 73 GHz exposure it was from 1.6 to 2 (p = 0.007) and with 70.6 GHz – 1.4 to 1.7 fold (p = 0.003), (Table 2).

Again, on *E. coli* exposed by 70.6 and 73 GHz EMI similar to the growth results, Cef and Chl had less enhanced effects - 1.4 fold and 1.6 fold, respectively. More strong effects were with Kan (1.6 and 1.7 fold; p = 0.003) and tetracycline (1.7 and 2 fold). The depressive effects of these antibiotics on viability were higher at two frequencies. It was especially of Tet, when EMI damaged membrane and facilitated antibiotics permeability through it (Torgomyan, Trchounian, 2012). These data corroborate that combined stressful effects of EMI (especially of 73 GHz) and antibiotics on *E. coli* growth and survival were with more efficacy.
Table 2. Decrease in the ability of *E. coli* to form colony-forming units during 4 days after irradiations and in presence of different antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Decrease in the number of colonies (fold)</th>
<th>non-irradiated</th>
<th>70.6 GHz</th>
<th>73 GHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.15 ± 0.04</td>
<td>1.30 ± 0.04</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td></td>
<td>1.20 ± 0.04</td>
<td>1.40 ± 0.04</td>
<td>1.60 ± 0.05</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td></td>
<td>1.25 ± 0.04</td>
<td>1.40 ± 0.04</td>
<td>1.65 ± 0.05</td>
</tr>
<tr>
<td>Kanamycin</td>
<td></td>
<td>1.30 ± 0.04</td>
<td>1.62 ± 0.05</td>
<td>1.70 ± 0.05</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td>1.45 ± 0.04</td>
<td>1.70 ± 0.05</td>
<td>2.00 ± 0.06</td>
</tr>
</tbody>
</table>

*there were presented data of divisions of the number of colonies in the 1st day to the 4th day; control – without antibiotics, p ≤ 0.001, the number of replicates was five.

Fig. 10. The effects of EMI with frequencies of 70.6 GHz and 73 GHz in combination with antibiotics - chloramphenicol (Chl), ceftriaxone (Cef), kanamycin (Kan) and tetracycline (Tet) on *E. coli* specific growth rate. Control was without EMI and antibiotics.

The effects of EMI and antibiotics on energy-dependent H⁺ and K⁺ transport across the membrane of whole cells was also studied. Cef and Kan depressed H⁺ flux 1.25-fold (p<0.025) and 1.62-fold (p<0.01) (not shown) and K⁺ flux 1.13-fold and 1.5-fold (p<0.02), respectively. However, they did not change the H⁺/K⁺ exchange direction and the ratio. Addition of DCCD showed higher depressive effects with 0.4 mM on overall H⁺ and K⁺ fluxes. The effects of antibiotics on irradiated bacteria, especially on H⁺ flux were stronger compared with antibiotics alone. Cef and Kan depressed H⁺ flux of 70.6 GHz irradiated bacteria 1.4-fold (p<0.025), but of 73 GHz irradiated bacteria 1.9-fold (p<0.035) and 2.12-fold (p<0.02), respectively (not shown). Cef also depressed 70.6 and 73 GHz irradiated bacterial K⁺ flux 1.2-fold and 1.33-fold (p<0.015), respectively. DCCD (0.4 mM) inhibited glucose-dependent ion fluxes, especially H⁺ efflux. The H⁺ flux of 70.6 and 73 GHz irradiated bacteria with Cef was depressed 1.74-fold and 2-fold, respectively and with Kan 3.6-fold (p<0.025) and 4.7-fold (p<0.035), respectively. Interestingly, 70.6 GHz EMI and Kan also depressed K⁺ flux (11.5-fold; p<0.015), which was a little higher compared with Kan used alone.
The DCCD-sensitive ions fluxes, which primarily indicate the FoF1 ATPase operation was calculated as well. The DCCD-sensitive fluxes for both ions were more visible with 0.4 mM DCCD concentration (not shown). The decrease of DCCD-sensitive H+ flux was insignificant after EMI, but K+ flux was decreased 1.2-fold (p<0.025) after exposure to 73 GHz irradiation Ceftriaxone decreased these ions fluxes 1.2-fold (p<0.015) and kanamycin - 1.9-fold (p<0.035) compared with the control (non-irradiated; without antibiotics). On 70.6 GHz irradiated bacterial H+ and K+ fluxes, only ceftriaxone had higher decreasing effect 1.7-fold and 1.3-fold (p<0.02), respectively. In case of 73 GHz irradiated bacteria, two antibiotics depressed DCCD-sensitive H+ flux and decrease with ceftriaxone and kanamycin was 2-fold and 2.6-fold (p<0.05), respectively. Ceftriaxone had higher decreasing effect on 73 GHz irradiated bacterial DCCD-sensitive K+ flux; the flux was decreased 1.5-fold (p<0.025). However, kanamycin showed increasing effect on DCCD-sensitive K+ flux of 70.6 GHz irradiated bacteria, but had no effect on 73 GHz irradiated bacteria.

Thus, Cef and Kan had effects on both ions fluxes with irradiation; especially 73 GHz strengthened the effect of Cef. Kan showed higher effect on H+ flux as compared with Cef, but the latter exhibited higher effect on DCCD depressed H+ and K+ fluxes than Kan. Interestingly, EMI and antibiotics alone had no effect on these ions ratios, which was 2 and 1 for H+ and K+ fluxes, respectively. However, the combined action of EMI of 73 GHz and antibiotics changed the ratio to 1.5-fold (p<0.01), but in case of 70.6 GHz, such change was only with Cef.

**CONCLUSIONS**

The following conclusions were made based on experimentally obtained data:

1. The irradiation of anaerobically grown (during glucose fermentation) *E. coli* K12(λ) bacteria with low intensity, coherent 70.6 and 73 GHz EMI led to inhibition of bacterial growth, viability and the ability to form colonies. Moreover, the 73 GHz EMI had stronger effects.
2. The irradiation effects on bacteria with these frequencies are dependent on irradiation period and irradiation medium.
3. The mentioned frequencies have no medium mediated effects on bacteria, although they changed water physicochemical properties.
4. The effects of these frequencies depend on medium redox state (different pH values, the presence of reducer).
5. These frequencies led to cell morphology and cytoplasmic membrane modifications, the activity of membrane transport and enzimatic complexes are changed as a result of altered specific interactions between protein components.
6. Theses frequencies, especially 73 GHz increased the bactericidal effect of some antibiotics in their minimal inhibitory concentrations, which evidenced their common and supplementary action mechanism on cells in which the main role belong to bacterial membrane.

7. The comparative analysis showed that 73 and 70.6 GHz EMI had on bacteria less depressive effects than 53 and 51.8 GHz EMI.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC


5. Torgomyan H., Trchounian A. (2012) Different antibiotics effects on bacteria after high frequency electromagnetic irradiation: *Escherichia coli* become more sensitive to tetracycline than to chloramphenicol, ceftriaxone or kanamycin. 112th General Meeting of American Society for Microbiology, June 16-19, San Francisco, California, USA, K-1841.


8. Torgomyan H., Trchounian A. (2011) Low-intensity electromagnetic irradiation of 70.6 and 73 GHz frequencies enhances the effects of disulfide bonds reducer on *Escherichia*
coli growth and affects the bacterial surface oxidation–reduction state. Biochemical and Biophysical Research Communications 414, 265-269.


Սահմանում ենթադրվող պատճառ

Բևեռական նկարագրություններ

Էսերիքիա կոլի՝ Escherichia coli բակտերիան

Այս հոդվածությունը բնոմենտի դատական պատմությունների համար

Անունների մեխանիզմները և այլ պատմություններ

Ժողովուրդի պատճառը՝ Escherichia coli, ինֆեկցիայունացված ծանրամոլոգիա, բակտերիայի սատ, թուրք կարմորող, բնոմենտի աշխատանքները, համակարգ H-Կ-այի սաղմանային, հայտնիականը:

Վիրուս սանրմուկների բոլոր օբյեկտների, սուրադիողների, պատանիների ու այլ նյութեր, որոնք դիմական միջավարության ծրագրերում կարող են հանդիպել ըստ Escherichia coli բակտերիայի ներգործություններ:

Pakhomov et al., 1998; Pakhomov, Murphy, 2000: Օրգանիզմներ են պարունակում միջազգային ծրագրեր, որոնք միջազգային ծրագրեր են պարունակում և հանդիպում են տարածաշրջանային ծրագրերի հետ.

Nikolaev, Trushin, 2003; Reguera, 2011: Հայտնի են բազմաթիվ ծրագրեր, որոնք մեծ շատի նյութեր ու գործոններ են առանձնացնում և հավանական են պարունակում տարածաշրջանային ծրագրեր.

Rojavin, Ziskin, 1998; Honga et al., 2004; Lagunas-Solar et al., 2006; Shamis et al., 2008; Ukuku et al., 2008; Geveke et al., 2009; Zand et al., 2010: Տարածաշրջանային ծրագրեր և բազմաթիվ նյութեր են պարունակում և հավանական են պարունակում տարածաշրջանային ծրագրեր.

Banik, 2003; Belyaev, 2005; Torgomyan, Trchounian, 2013: Շատ ավելի բազմաթիվ նյութեր են պարունակում և հավանական են պարունակում տարածաշրջանային ծրագրեր.

Fesenko et al., 1995; Cojocaru et al., 2005: Պատմական միջազգային ծրագրեր են պարունակում և հավանական են պարունակում տարածաշրջանային ծրագրեր.

Այսպիսով, համարվում է, որ Escherichia coli բակտերիան կարող է բազմաթիվ նյութեր ու գործոններ պարունակել և հավանական է, որ այսպիսով պարունակված տարածաշրջանային ծրագրեր:
Ե. coli-ի հիպոթեզական ռազմական ժամանակաշրջանում շատ լայն տեղեկատվություն է տալիս, բայց մեծ տեսանկյուններով շատ մեծ սուրբծառուղություն է կունենում, որը շատ բազմաթիվ իրավիճակներում է պայմանավորվում ։ Մանրամասնություն, որը ներկա է բազմաթիվ իրավների շրջանում և համապատասխան հիպոթեզի տեղեկացություններ։ Այսպիսով, բազմաթիվ բնակչություններ մարդու միջազգային ու գրական տեղեկացությունները կազմում են ուսումնասիրական լայն ճանաչում։ Պատմական, մաշկի բնակեցման մեծ զանգվածը, թափանակ իրավիճակի տեղեկացություններ, Torgomyan, Kalantaryan et al., 2011; Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։ Սակայն, համապատասխան հիպոթեզի տեղեկացությունների մեծամասնությունը համապատասխան բնակչության մեջ չի գտնվում։ Պատմություն տատարերի համակարգում խաղաղ են կազմվում ։
МЕХАНИЗМЫ И СРАВНИТЕЛЬНЫЙ АНАЛИЗ ДЕЙСТВИЯ КОГЕРЕНТНОГО ЭЛЕКТРОМАГНИТИНОГО ИЗЛУЧЕНИЯ КРАЙНЕ ВЫСОКИХ ЧАСТОТ НА БАКТЕРИИ ESCHERICHIA COLI

РЕЗЮМЕ

Ключевые слова: Escherichia coli, электромагнитное излучение, рост бактерии, структура клетки, АТФазная активность, доступные SH-группы, H⁺-K⁺-ый обмен, антибиотики.

В последние годы все организмы, от одноклеточных до человека, стали больше подвергаться действию физического фактора окружающей среды – электромагнитного излучения крайне высоких частот (ЭМИ КВЧ), (Pakhomov et al., 1998; Pakhomov, Murphy, 2000). Это связано с тем, что ЭМИ КВЧ используется живыми организмами для управления основными физиологическими функциями. Было установлено, что бактерии и другие клетки могут взаимодействовать друг с другом через ЭМИ (Nikolaev, 2000; Trushin, 2003; Reguera, 2011). Показано, что ЭМИ КВЧ увеличивает чувствительность бактерий и их биофильтам к разным антибиотикам (Torgomyan, Trchounian, 2013). К тому же ЭМИ с эффективностью применяется в биотехнологии, в медицине, в животноводстве, в растениеводстве, в пищевой промышленности и т.д. (Rojavin, Ziskin, 1998; Honga et al., 2004; Lagunas-Solar et al., 2006; Usichenko et al., 2006; Shamis et al., 2008; Ukuku et al., 2008; Geveke et al., 2009; Zand et al., 2010).

Поглощение ЭМИ КВЧ биологическими системами зависит от частоты ЭМИ, интенсивности, продолжительности воздействия и др. (Banik, 2003; Belyaev, 2005; Torgomyan, Trchounian, 2013).

Есть несколько гипотез о возможном взаимодействии ЭМИ КВЧ с биологическими системами. Основными мишениями ЭМИ КВЧ на клеточном уровне являются молекулы воды, клеточные мембраны и клеточный геном (Pakhomov, Murphy, 2000; Banik et al., 2003; Beneduci et al., 2005; Torgomyan, Trchounian, 2013). Вероятно, что изменения, происходящие в воде и в мембране клетки после воздействия ЭМИ, создают условия для клеточного ответа. (Fesenko et al, 1995; Cojocaru et al., 2005). Физико-химические механизмы влияния ЭМИ КВЧ на клетку остаются до конца неизвестными.

В работе были поставлены следующие задачи – изучение влияния 70.6 и 73 ГГц ЭМИ на рост, выживаемость, число колоний, на структуру клетки и клеточную стенку, АТФазную активность, производство молекулярного водорода, H⁺/K⁺ обмен, количество доступных сульфгидрильных групп, мембранный потенциал. Также было изучено влияние ЭМИ КВЧ и разных антибиотиков на рост, выживаемость и H⁺/K⁺ обмен бактерий.

Исследования показали, что 70.6 и 73 ГГц ЭМИ являются эффективными частотами для E. coli, так как подавляют рост, выживаемость и образование колоний бактериями. Показано, что воздействие ЭМИ на бактерии зависит от состава среды.
(дистиллированная вода или солевой раствор) и продолжительности ЭМИ (Torgomyan, Kalantaryan et al., 2011). Было показано, что оптимальный период для воздействия 1 час. 70.6 и 73 ГГц ЭМИ оказывают разное влияние на бактерии при облучении в воде и в солевом растворе (Torgomyan et al., 2011). Повторное воздействие не влияет на рост бактерий (Torgomyan et al., 2011). Стало возможным предположение о том, что бактерии имеют восстановительные механизмы (Tadevosyan et al., 2007).

Установлено, что рост облученных бактерий зависит от рН среды и присутствия восстановителя. Примечательно, что наблюдается изменение чувствительности облученных бактерий к химическим соединениям (ДТТ, антибиотики), (Torgomyan, Trchounian, 2011; 2012; 2013).

Было показано, что 70.6 и 73 ГГц ЭМИ не имеет опосредованное средой (дистиллированная вода, солевой раствор, ростовая среда) влияние на бактерии. Но ЭМИ влияет на физико-химические характеристики воды, в результате изменяется рН, проводимость, оптическая плотность, окислительно-восстановительный потенциал воды, а поверхностное натяжение не меняется (Torgomyan et al., 2012). Похоже, что влияние ЭМИ на бактерий зависит как от окружающей среды, в частности от молекул воды, или же определяется прямым взаимодействием с бактериями.

Новизна работы также в том, что удалось показать, что ЭМИ изменяет структуру, размер E. coli и приводит к изменениям внешней мембраны (Torgomyan et al., 2012). Также о прямом влиянии на мембрану показывают изменения в АТФазной активности, производстве молекулярного водорода, Н⁺/К⁺ обмене и структурные изменения. Также было установлено, что 70.6 и 73 ГГц ЭМИ (особенно 73 ГГц) увеличивает депрессивное влияние антибиотиков – хлорамфеникола, цефтриаксона, канамицина и тетрациклина (Torgomyan, 2013; Torgomyan, Trchounian, 2012; 2013) на рост, выживаемость и на Н⁺/К⁺ обмен бактерий.

Такое воздействие может привести к изменениям в мембране и внутри клетки. ЭМИ прямо или опосредованно (с помощью изменения в структуре воды) вызывает изменения в мембранах клетки или мембранносвязанных процессах (Torgomyan, Trchounian, 2013). Антибиотики в свою очередь действуют на бактериальную мембрану, что отражается в свойствах мембраны, структуре и биохимических процессах, связанных с изменениями мембран.