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Combined Effects of Extremely High Frequency Electromagnetic Field and Antibiotics on *Enterococcus Hirae* Growth and Survival

(Submitted by corresponding member of NAS RA A. H. Trchounian 6/IX 2011)

Key words: *extremely high frequency electromagnetic irradiation, ampicillin, dalacin, Enterococcus hirae, bacterial growth and survival*

Enterococcus genus is highly dispersed gram-positive bacteria. Different strains of *Enterococci* emerge almost everywhere. Some of the strains are essentially antibiotic-resistant human pathogens but on the other hand there are also commensal strains existing in intestines of humans; there are many strains which are used in different kinds of industry [1, 2]. Particularly, *Enterococci* are commonly used in cheese production in addition to main bacterial groups present during cheese development [1].

In previous study conducted in our laboratory the effect of extremely high frequency electromagnetic irradiation (EMI) was demonstrated particularly when the object of investigation was *Escherichia coli* [3-7] or *Enterococcus hirae* [8]. The decrease of bacterial growth specific rate (μ) and the increase in bacterial sensitivity towards antibiotics and consequently possible increase in antibiotic-resistance after irradiation were shown; the affecting antibiotics were tetracycline and chloramphenicol [6]. The effects of kanamycin and cefriaxone antibiotics were also determined [7].

To establish mechanisms of the effects of EMI, researchers suggest different targets of vulnerability (i.e. cell wall or membrane, genome or DNA) [9-11]. The mechanisms of affection are explained with resonant frequencies; particularly the 51.8 GHz and 53 GHz frequencies are the

resonant frequencies of water – the main component of cell and cell environment [11, 12]. There is also an accepted hypothesis that irradiation with 51.8 GHz and 53 GHz frequencies alter the permeability and accessibility of the cell wall, making the cell more vulnerable to various affecting agents [7].

Nowadays, fundamental knowledge about EMI is very important, because of ubiquitous existence of this radiation in almost any environment. There are either natural or artificial sources of EMI (i.e. sun, radio-electrical devices, mobile and telecommunication, etc.) therefore the effects on environment apparently increase intensively. Moreover, bacteria may communicate to each other by electromagnetic waves [13] so disturbance by EMI with even small intensity could be dramatic.

Thus, the point of the present study was investigation of growth, survival and development of antibiotic-resistance of *E. hirae* under extremely high frequency EMI in correlation with two other commonly used antibiotics (i.e. ampicillin and dalacin). The selection of antibiotics was focused on their affecting mechanisms and targets; particularly ampicillin as a beta-lactam antibiotic is effectively used against gram-positive bacteria, affecting cell walls, while dalacin (clindamycin) is commonly used antibiotic as bacterial protein synthesis inhibitor affecting ribosomal translocation of anaerobic bacteria [14, 15]. The focus of the research had two primarily goals. First, analyze *E. hirae* survival in minimal growth conditions, because previous bacterial study was conducted analyzing only bacterial growth [5, 8]. Here the *E. hirae* growth was not analyzed only in extreme conditions but also their survival was evaluated using minimal conditions and aggressive agents (i.e. antibiotics). Second, combined effects of physical and chemical agents on growth and survival of *E. hirae* were observed.

Materials and methods. *Bacteria and preparation. E. hirae ATCC9790* wild type stain was used throughout. They were grown until stationary growth phase (18-20 h) in tryptone medium (pH=8.0) under anaerobic conditions upon fermentation of glucose (0.2%) as described [5, 6]. Grown bacterial cells were concentrated by centrifugation (15 min), washed and diluted in bi-distilled water. Then, the bacterial suspension was transferred into the plate for electromagnetic irradiation. The thickness of bacterial suspension in the plate was ~1mm.

Electromagnetic irradiation of bacteria. Bacterial suspension was irradiated by EMI generator; model G4-141 (with conical antenna) radiating the coherent in time electromagnetic waves with frequencies of 51.8 GHz and 53 GHz [5, 6] in the option of amplitude modulation with frequency of 1 Hz (frequency stability was 0.05%); the flux capacity was 0.06 mW/cm^2. The generator was

assembled in the Institute of Radiophysics and Electronics of the National Academy of Science of Armenia (Ashtarak City) and generously supplied by Dr. V. Kalantaryan (Department of High Frequencies Radiophysics and Telecommunication, Yerevan State University, Yerevan, Armenia). The conditions of irradiation of bacteria were as alike as described previously [5, 6]. After direct irradiation of bacterial suspension for 1 h (the optimal duration for irradiation that was established earlier) [5-7], cells were immediately transferred into the fresh growth or minimal medium. It should be mentioned that the effects of this EMI are the same for different concentrations of exposed *E. hirae* cells [8] but they might differ due to the medium composition.

Determination of bacterial growth and survival. Bacterial specific growth rate was assessed by measuring optical density (OD) changes in bacterial suspension (growth medium with glucose) with a Spectro UV-VIS Auto spectrophotometer (Labomed, USA) at wavelength of 600 nm. μ was calculated by $\mu = 0.693/g$: g- OD doubling time. The lag-phase duration was determined as described before [8].

Bacterial survival was determined by displacement of irradiated bacteria into a minimal salt medium during four days [16, 17]. The number of bacteria was assessed by counting of colony-forming units grown on plates with solid nutrient medium, when appropriately diluted bacterial suspension was plated.

Antibiotics used; data processing. Ampicillin (Troge, Germany) and dalacin (Pfizer, USA) were used. They were added into the medium with their minimal effective concentrations 1.4mM and 0.4mM which were determined experimentally. All assays were carried out under anaerobic conditions at 37 ^{o}C. The average data processed to be statistical from two or three replicates with determination of the standard error which was not more than 3 % and of the Student's validity criteria (P) for the difference between experimental and appropriate control measurements [7, 8] when not mentioned, $P < 0.01$.

Results and discussion. It is noticeable that under extremely high frequency EMI specific growth rate dramatically decreased [5-7]. In order to observe all possible correlations and combined effects different combinations of irradiation and/or antibiotics were investigated. From this, several perspective groups of bacteria were analyzed. First group was the control group which was not either radiated or treated with antibiotics. Second group was the radiated group; bacterial mixture was exposed to 51.8 GHz irradiation. Bacterial suspension in third group was also exposed to irradiation of 53 GHz frequency. Bacterial suspension in forth group was treated with ampicillin

only and fifth group was treated with dalacin only. While the sixth group was either radiated with 51.8 GHz or treated with ampicillin and seventh group was radiated with 53 GHz and treated with ampicillin. Eighth group was radiated with 51.8 GHz and treated with dalacin and ninth group was radiated with 53 GHz and treated with dalacin.

Thus, particularly when the bacterial mass was radiated with 51.8 GHz electromagnetic waves, μ had decreased by approximately 30%, while with 53 GHz - the decrease was 40% comparable to control group of bacteria that were not exposed to irradiation and antibiotics (Fig. 1A). These data were similar to the findings reported before [8]. Consequently, the results showed that ampicillin decreases μ also by 30% when the agent is affecting separately, while dalacin decreases μ by about 50% (Fig. 1A). Additionally, the combined effect was shown. Antibiotics in combination with EMI affect even more severely. The combinations of 51.8 GHz and ampicillin; 53 GHz and ampicilin; 51.8 GHz and dalacin; 53 GHz and dalacin more effectively decrease μ of bacteria by 45%, 60%, 44% and 65% respectively (Fig. 1A). In parallel with μ determination, lag phase duration was also recorded as a secondary indicator of electromagnetic suppression effect. In comparison with control group, the lag phase was prolonged about 20% and 45% when the bacterial mixture was irradiated with 51.8 GHz and 53 GHz respectively (Fig. 1B). The combination of 51.8 GHz and 53 GHz frequencies with ampicillin prolonged the lag phase on 25% and 83% respectively, while ampicillin only prolonged the duration of lag phase on 20%. The combination of 51.8 GHz and 53 GHz frequencies with dalacin prolonged the lag phase duration on 48% and 2.4 fold respectively, while the lag phase duration in the group treated only with dalacin was prolonged on 23%.

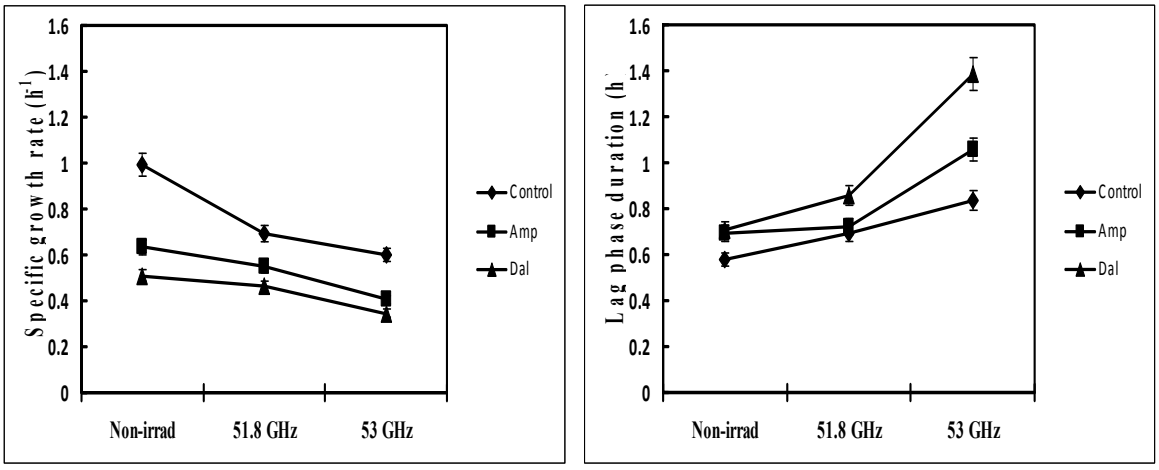

Fig. 1. Changes in μ (A) and growth lag phase duration (B) for *E. hirae* ATCC9790 by EMI of 51.3 and 53 GHz and ampicillin and dalacin.

The second aspect of this study was to observe the *E. hirae* survival during 4 days with exposure to the same treatments (i.e. irradiation and/or antibiotics) compared to control group. Based on the results it is apparent that bacterial suspension radiated either with 51.8 GHz and 53 GHz waves eventually contains ~10% less viable bacteria than control (Fig. 2A).

The number of viable bacteria decreases gradually depending on antibiotic. While ampicillin had not significant effect on survival, dalacin had noticeable bactericide effect (Fig. 2B). So, the second antibiotic – dalacin decreases the number of viable bacteria comparable to control group in 4 days of survival observation by 7 %. The combination of 51.8 GHz and dalacin decreases the number of viable bacteria ~2.5 fold, while the combination of 53 GHz and dalacin has less effect (Fig. 2C).

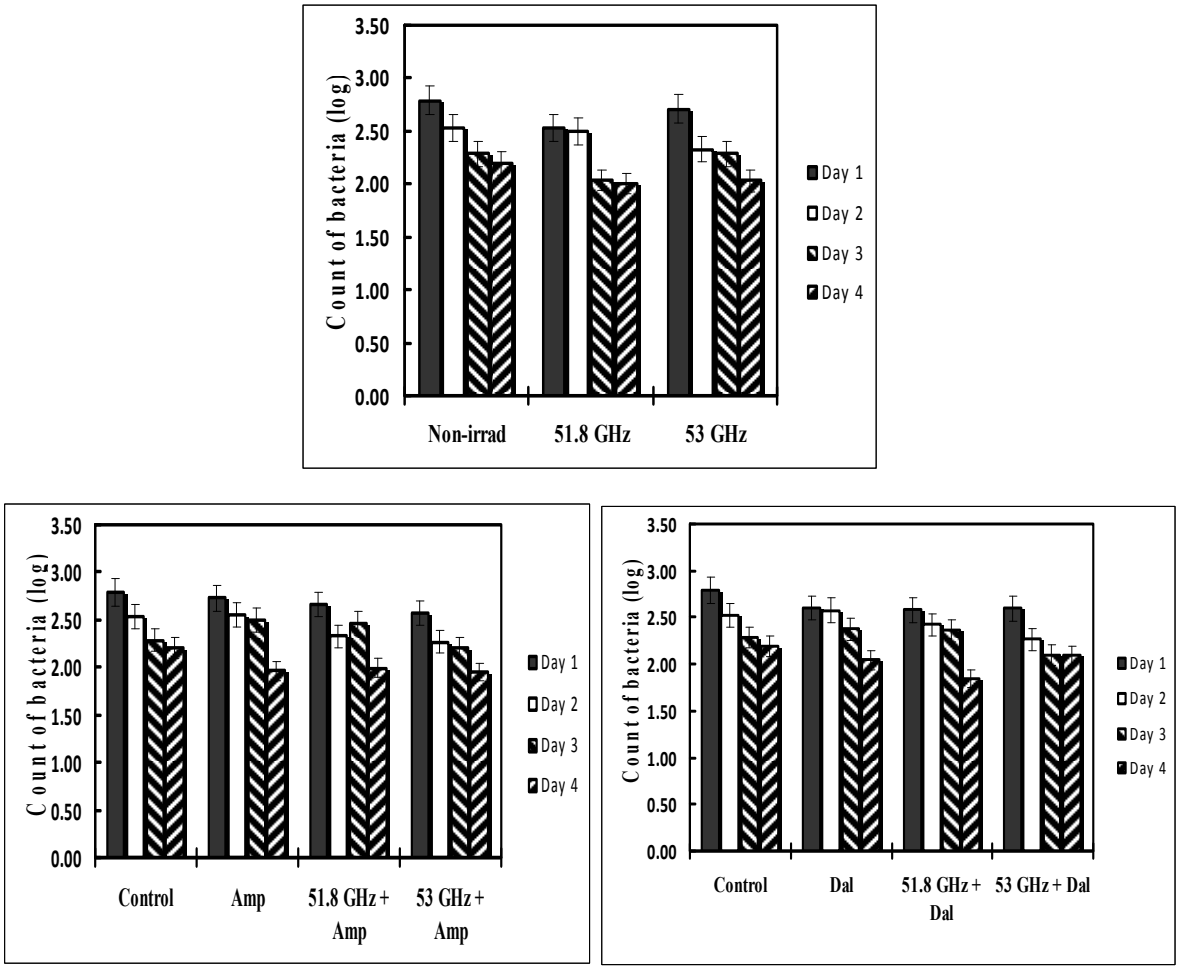

Fig. 2. *E. hirae* ATCC9790 survival histograms: non-irradiated versus irradiated with EMI of 51.8 and 53 GHz (A) and with ampicillin (B) or dalacin (C).

It is apparent that correlated exposure to affecting agents is more effective than singular treatment and particularly 53 GHz was more effective rather than 51.8 GHz irradiation regarding to specific growth suppression (see Fig. 1). However, for survival with antibiotics 51.8 GHz was more effective than 53 GHz (see Fig. 2). This might suggest different structures and pathways essential for bacterial growth and survival, a further study is required.

Obviously 51.8 GHz and 53 GHz EMI affect membranes of the cells [3, 5, 6]. It was already demonstrated that *E. coli* growth was also depressed because of these waves [8]. The results observed are comparable with the results when the object of interest was *E. coli*. Consequently, the analogous effect of EMI using different bacteria is evident, despite of the differences of *E. coli* and *E. hirae*. It is interesting, because these bacteria have different membrane structures and composition due to their types.

In order to make more in depth conclusions the affecting mechanisms of EMI are considerable. The targets are affected, because they resonantly interact with EMI. The cluster structure of water alters; DNA gains conformational changes and the permeability of membrane changes after the EMI exposure [6, 7, 9-11]. So, these alternations can lead to increase of accessibility/transparency of the membrane. This can be the reason why antibiotic and EMI combination dramatically enhances the bactericide effect. It is also apparent that the effect is significant not only during the bacterial growth but also during the survival. The survival time decreases when the bacterial suspension was treated with combined physical and chemical agents.

The experiments with antibiotics are relevant and interesting as a matter of fact. Probably, the same antibiotics used will be similarly effective against pathogen stains. Therefore, based on effects and affecting mechanisms of selected antibiotics, it is possible to make conclusion about the more efficient ways of struggle against the pathogen strains. The bactericide effect (using antibiotics) is increasing in combination with EMI. It is apparent, because minimal affecting concentrations of antibiotics were used.

The combined EMI/antibiotic effects revealed can have more useful practical application in either scientific or medical and industrial fields.

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Combined Effects of Extremely High Frequency Electromagnetic Field and Antibiotics on *Enterococcus Hirae* Growth and Survival

Combined effects of extremely high frequency electromagnetic field and antibiotics on *Enterococcus hirae ATCC 9790* bacterial growth and survival were investigated using 51.8 GHz and 53 GHz frequencies in combination with two commonly used antibiotics: ampicillin and dalacin. Results revealed that, despite bacterial type and membrane structure and properties, the combined effect, especially with 53 GHz and dalacin, suppresses bacterial growth and decreases their survival.

В. А. Оганян

Совокупное воздействие сверхвысоких частот электромагнитного поля и антибиотиков на рост и выж*и*ваемость *Enterococcus hirae*

Совокупное воздействие электромагнитного поля сверхвысоких частот и антибиотиков на рост и выживаемость *Enterococcus hirae ATCC 9790* исследовано с использованием частот 51.8 и 53 ГГц и двух часто применяемых антибиотиков: ампициллина и далацина. Показано, что, независимо от типа бактерий и структуры и свойств бактериальной мембраны, комбинированный эффект, в особенности 53 ГГц и далацина, сильнее подавляет рост бактерий и снижает их выживаемость.

Վ.Ա. Օհանյան

Գերբարձր հաճախության էլեկտրամագնիսական դաշտի և հակաբիոտիկների համատեղ ազդեցությունը *Enterococcus hirae* բակտերիաների աճի և կենսունակության վրա

Ուսումնասիրվել է *Enterococcus hirae ATCC9790* բակտերիայի աճի և գոյատևման վրա գերբարձր հաճախության էլեկտրամագնիսական դաշտի և հակաբիոտիկների ներգործության համատեղ ազդեցությունը՝ օգտագործելով 51.8 և 53 ԳՀց հաճախություններ ու երկու հաճախ օգտագործվող հակաբիոտիկներ՝ ամպիցիլին և դալացին: Արդյունքները ցույց տվեցին, որ անկախ բակտերիաների տիպից ու թաղանթի կառուցվածքից ու

հատկություններից, համակցված ներգործությունը, հատկապես 53 ԳՀց և դալացինը, ճնշում է բակտերիաների աճը և նվազեցնում նրանց կենսունակությունը:

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