http://dx.doi.org/10.4314/ajtcam.v12i1.15

ANTIMICROBIAL EFFECT OF VANCOMYCIN ELECTRO-TRANSFERRED WATER AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* VARIANT

J. Antonio Heredia-Rojas[a], Licet Villarreal-Treviño[b], Abraham O. Rodríguez-De la Fuente*[a], Laura I. Herrera-Menchaca[b], Ricardo Gomez-Flores[b], Benito David Mata-Cárdenas[c], Laura E. Rodríguez-Flores[d].

[a] Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Ciencias Exactas y Desarrollo Humano, , Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, 66451. México. [b] Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Microbiología e Inmunología, , Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, 66451. México. [c] Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas, Laboratorio de Biotecnología, , Av. Universidad S/N Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, 66451. México. [d] Universidad Autónoma de Nuevo León, Facultad de Medicina, Departamento de Patología. Av. Francisco I. Madero pte. S/N, Col. Mitras Centro, Monterrey, Nuevo León,CP 66460 México.
*****Corresponding author's **E-mail**: abraham.rodriguezd@uanl.edu.mx.

Abstract

Background: There is a number of alternative and complementary therapeutics that are unproven or have not been properly tested. For past twenty years, the transfer of bio-energetic information has been recognized as a novel scientific approach capable of contributing to improved therapy in the management of several diseases through the so-called bio-resonance therapy (BRT). Although BRT was discovered in the late 1980s, it is still poorly studied. The aim of this study was to evaluate the antibacterial effect of water samples transferred with electronic information of vancomycin, a well known drug against methicillin-resistant *Staphylococcus aureus* (MRSA), by using a BRT device on bacterial cultures.
Material and Methods: MRSA cultures were treated with vancomycin electro-transferred water samples, vancomycin (4.0 and 8.0 µg/mL), sham electro-transferred (water to water) and non-transferred water samples (medium alone). Growth inhibition was evaluated in liquid and solid culture medium, spectrophotometrically and by CFU determination respectively.
Results: The obtained data showed that by transferring vancomycin (4.0 and 8.0 µg/mL) information to water samples, the growth of cultured MRSA was significantly ($p< 0.05$) inhibited (up to 35%), compared with those cultures treated with electro-transferred water to water or cultured in medium alone (0% growth inhibition).
Conclusion: This *in vitro* study suggests that water samples that are electronically transferred with vibration sustained information of vancomycin are capable of inhibiting growth of axenically cultured methicillin resistant *S. aureus*.

Key words: Antimicrobial effect, electro-transferred water, bio-resonance, vancomycin, *Sthaphylococcus aureus.*

Introduction

Since the 1980s, a promising procedure known as biophysical-information therapy or bio-resonance therapy (BRT) has emerged as an alternative therapeutic method (Fedorowski et al. 2004), but has not yet been properly evaluated. There is an on-going trend toward the use of alternative and complementary therapeutics, mainly in controlling microbial illnesses. Considering this trend and inability of reaching a consensus on the efficacy of these therapies, the need arises to examine if there is a significant and measurable biological effect of such unorthodox medical procedures.

There is a number of products known as capable of inhibiting microbial growth, including the conventional (orthodox medicine) antibiotics or antimicrobial agents (Mc Donnell and Rusell, 1999; Lavin, 2000; Takahashi et al., 2003) or by using unconventional approaches such as electric and magnetic fields; for instance, an early contribution from Rowley et al. (1974), showed an inhibition of infecting microorganisms in human wounds by exposure to alternated electric fields. More recently, Qin et al. (1996) reported that processing liquid foods with high-intensity pulsed electric fields, and inactivated micro-organisms. Conversely, it is known that magnetic fields affect the growth and reproduction of micro-organisms and may be used as an antimicrobial procedure (Pothakamury et al., 1993).

Regarding microbial growth inhibition using unconventional procedures, we have previously observed *Entamoeba invadens* trophozoite growth and encystation inhibition, using 60 Hz sinusoidal magnetic fields (Rodríguez-De la Fuente et al., 2008). On the other hand, we recently demonstrated that by transferring metronidazole (a well known cytotoxic drug against parasites) information to water samples via an electronic amplifier (BRT device), the growth of axenically-cultured trophozoites of *Entamoeba histolytica* and *Trichomonas vaginalis* was significantly inhibited, compared with those cultures treated with sham electro-transferred water samples (Heredia-Rojas et al., 2011). Furthermore, we observed that by transferring amphotericin B information to water samples by a BRT apparatus, the growth of cultured *Candida albicans* was significantly inhibited (Heredia-Rojas et al., 2012). These results suggest that it is possible to transfer and store biological information to pure water, and that water sample that has undergone such an information transfer, can effectively interact with other biological systems, such as amoeba and yeasts.

Recently, some researchers reported experimental evidence of the developing concept of Electro Magnetic Information Transfer (EMIT) of specific molecular signals directly and continuously on target cell picking up the molecular signals from the source chemical effector (Thomas et al., 2000; Jerman et al., 2005; Foletti et al., 2012).These observations support the hypotheses that water could record and replay the EMIT from biologic active chemical molecules to biological targets.

Contrary to mid-20th century expectations, bacterial infectious diseases have proven increasingly resistant to conventional therapy. Many hospitals encounter nosocomial infections of *Staphylococcus aureus*, especially in its more recent, methicillin-resistant *S. aureus* (Chambers and Deleo, 2009; Fry and Barie, 2011; Velázquez-Meza et al., 2013).

http://dx.doi.org/10.4314/ajtcam.v12i1.15

In view of this interesting issue involving unorthodox antimicrobial strategies and the possibility to transfer drug information to water molecules by means of resonant circuits, we have undertaken in the present study to further evaluate the antibiotic effect of water samples transferred with electronic information of vancomycin, a well known drug against methicillin resistant *S. aureus*, by using a BRT device.

Materials and methods

Reagents, culture media, and microbial strain

Vancomycin was obtained from Aventis Pharmaceuticals Inc. (Sommeret, NJ, USA). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a clinical isolate provided by Instituto Mexicano del Seguro Social (I.M.S.S.). The strain was identified by the use of biochemical profiles according to the recommendations of the Manual of Clinical Microbiology (Murray et al., 1999). Bacteria were maintained in nutrient agar (BD Bioxon, Becton Dickinson de México) tubes at 4°C. Before testing, nutrient agar tubes were placed at room temperature for 2 hr, and then a bacteria inoculum was transferred to a nutrient broth (BD Bioxon) tube and aerobically incubated at 35°C for 24 hr. From this, a nutrient agar plate was inoculated and incubated at 35°C for 24 hr, and colonies were isolated and suspended in 0.85% NaCl solution. The bacteria suspension was prepared by adjusting its turbidity to match the 0.5 McFarland$_{635nm}$ standard in 0.85% NaCl solution. To determine cefoxitin strain resistant, the disc diffusion method was used. In brief, 9-cm diameter Petri dishes were prepared with 10 ml of Müeller–Hinton agar medium (BD Bioxon) and inoculated with an embedded Q-tip of the 0.5 McFarland suspension, described above, plated on the agar surface. After drying in a sterile hood, 6-mm diameter disks soaked with 15 µl of cefoxitin were used as controls. The dishes were incubated at 35°C for 24 hr. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. The growth inhibition diameter was an average of four measurements, taken at four different directions. Tests were performed in triplicate.

Transmission apparatus

The equipment used for electronic transmission comprised a bio-resonance therapy oscillator device, Bicom version 4.4 by Regumed (Regulative Medizine Technik GmbH, Germany) serial number 202057299.

Vancomycin transmission to water

The source flask containing 4.0 and 8.0 µg/mL of vancomycin respectively, in a total volume of 25.0 ml of bi-distilled water were placed (separately) inside the input coil coupled to a bio-resonance amplifier, whereas in the output coil, a flask containing 25.0 ml of pure bi-distilled and sterile water was allocated at room temperature. Vancomycin solutions and bi-distilled water were sterilized by filtration. The oscillator was then turned on for the 15 min transmission period; during this procedure, parameters such as power, voltage, capacitance and impedance remained constant. Thus, the nature of the source flask (vancomycin *versus* vehicle) was the only variable. According to the bio-resonance device manufacturer, a specific program labelled as #196 was used for electronic transmission substance to substance. At the end of the transmission period, the flasks were kept away from light and stored at room temperature for 1 h before being used in bioassays. For bioassays, 500µL of transferred-water or sham-treated water were added to each culture tube.

Experimental Design

The experiments using axenically cultured MRSA included the following treatment regimens and controls: (*a*) cells treated with electronically-transferred 4.0 µg/mL, and (*b*) 8.0 µg/mL vancomycin water samples, as explained above, (*c*) cells treated with sham electro-transferred water samples, this is transferring the information from pure water to water, used as the control for a possible artefact effect induced by the bio-resonance device on water samples, (*d*) cells treated with non-transferred water, or medium alone, as a negative control, (*e*) cells treated with 4.0µg/mL of vancomycin as a positive control and, (*f*) cells treated with 8.0µg/mL of vancomycin as a positive control. Three cultures were included for each treatment regimens and controls. In all experiments in which transmitted vancomycin was compared with transmitted vehicle, the source flasks were randomized and blinded before each experiment. Therefore, the observed effect cannot be attributed to uncontrolled systematic factors (operator bias, temperature, time…) but to the independent variable, the content of the source flask.

Antimicrobial activity of electronically-transmitted vancomycin to water

We selected methicillin resistant *S. aureus* since it is a bacterium that is resistant to many antibiotics and is associated with nosocomial infection, particularly nosocomial pneumonia, surgical wound infection, and bloodstream infection. The percentage of microbial growth inhibition by electronically-transmitted vancomycin to water in liquid medium by spectrophotometry was determined as follows: One mililiter of MRSA suspensions (106 CFU/ml) was added to 4-ml (10 X 75 mm) round-bottom screw cap borosilicate culture tubes (VWR International S. de RL de C.V., Tultitán, Estado de México) containing 0.5 ml Müeller-Hinton nutrient broth (BD Bioxon) (5 X 105 CFU/ml, final bacteria concentration). After this, 0.5 ml vancomycin at 4 µg/ml and 8 µg/ml were independently added to 3 of these culture tubes; 0.5 ml electronically-transmitted vancomycin to water at 4 µg/ml and 8 µg/ml were independently added to other three of the culture tubes, and bacterial suspensions treated with sham electro-transferred water samples and non-transferred water, considered as medium alone (negative control), to a final volume of 2 ml for all culture tubes. Treatment and control culture tubes in triplicates were then incubated for 24 hr at 35°C, after which optical densities at 625nm were read in a Turner SP-830 spectrophotometer (Barnstead, Dubuque, IA, USA). For CFU determination, 100 µl of 1:10-8 dilutions of the treatment and control culture tube microbial suspensions used in the preceding experiment, were plated in nutrient agar (BD Bioxon) by surface spreading using a sterile glass rod. Plates were then incubated at 35oC for 24 hr and colonies were counted in a colony counter (ULB-100, Lightbox 37864-2000, Scienceware BEL-ART products, Pequannock, NJ, USA).

Statistical Analysis

The statistical differences were calculated among groups for bacterial growth by using analysis of variance for normal distributions and the correspondent Tukey test for establishing individual differences. The normality of the data was estimated by means of Kolmogorov-Smirnov test ($p<0.05$). All analyses were done using the SPSS package version 20.0. Differences were considered to be significant when the probability values were lower than 0.05.

http://dx.doi.org/10.4314/ajtcam.v12i1.15

Results

The present study evaluated the effect of electronically transmitted vancomycin to water samples on *in vitro* MRSA growth. It was observed a similar pattern of MRSA growth inhibition on liquid and solid culture media; electro-transferred vancomycin at either 4 µg/ml or 8 µg/ml, and vancomycin alone caused significant ($p<0.05$) up to 35% and/or up to 99% bacterial growth inhibition, respectively, compared with electrotransferred water to water and medium alone controls. The antimicrobial effect of electro-transferred vancomycin was not concentration-dependent (Figs. 1 and 2).

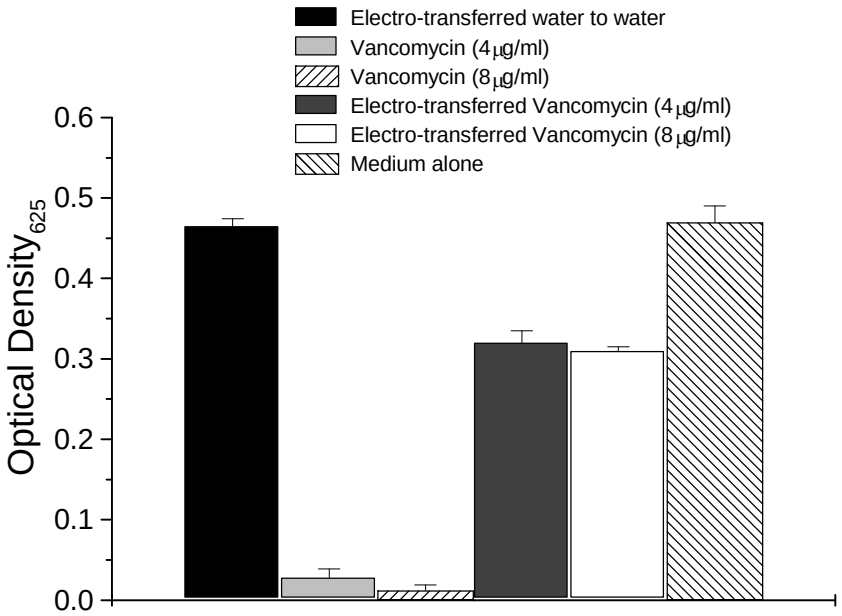

Figure 1: Effects of electro-transferred water on MRSA growth inhibition on liquid culture medium. MRSA growth after exposure to electronically-transmitted vancomycin to water in liquid culture medium was determined spectrophotometrically, as explained in the text. Controls used included vancomycin (4.0µg/mL and 8.0µg/mL) as a positive (antibacterial) control, and electro-transferred water to water (used as the control for a possible artefact effect induced by the bio-resonance device on water samples) and medium alone bacterial growth controls. Bars represent arithmetical grouped means ± SEM.

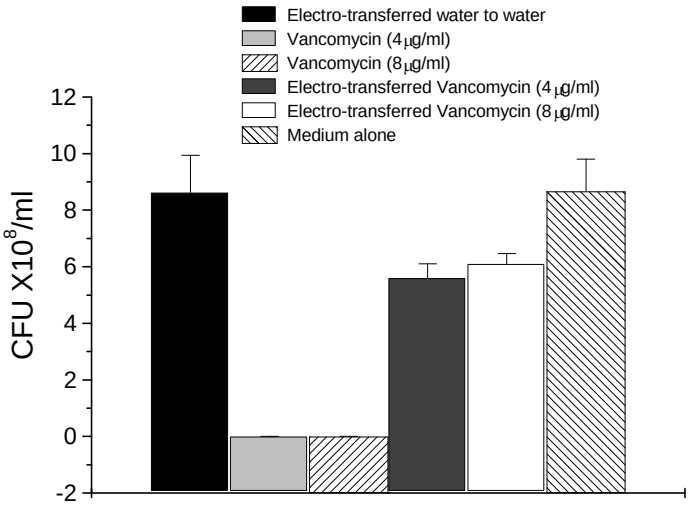

Figure 2: Effect of vancomycin electro-transferred water samples on MRSA growth inhibition on solid culture medium. MRSA growth after exposure to electronically-transmitted vancomycin to water in solid culture medium was determined by CFU determination, as explained in the text. Controls used included vancomycin (4.0µg/mL and 8.0µg/mL) as a positive (antibacterial) control, and electro-transferred water to water and medium alone bacterial growth controls. Bars represent arithmetical grouped means ± SEM.

Discussion

The effect of vancomycin electro-transferred water samples on MRSA growth was evaluated. A statistically significant reduction of *S. aureus* growth was observed after pure cultures were treated with water samples electro-transferred in a BRT device by using a specific program called “substance to substance transference”.

http://dx.doi.org/10.4314/ajtcam.v12i1.15

The results of the present study agreed with others suggesting that signals could be transferred to target cells or aqueous systems by an oscillator (BRT instrument) when coupled to two electromagnetic coils, demonstrating the same biological activity, thus mimicking the biological function of the original chemical active molecule (Foletti et al., 2012). Earlier, Endler et al. (1995) demonstrated that the metamorphosis of tadpoles could be greatly slowed down by transferring information from a toxic solution of the hormone thyroxin to the aquarium water, in a number or parallel blind trials. It has been also reported that the transfer of the activity of 4-phorbol-12-B-myristate acetate, by electronic means, on the activation of human neutrophils (Thomas et al., 2000). In contrast, Jonas et al. (2006) found no effects from digital signals on the inhibition of thrombin/ fibrinogen coagulation by digital signal, used instead of the original molecule, suggesting that some biological signals can not be digitized nor electronically transmitted.

Furthermore, we have previously observed *E. histolytica* and *T. vaginalis* trophozoites growth inhibition after treatment with metronidazole electro-transferred water samples, using a BRT device with identical characteristics and conditions of the apparatus used in the present study (Heredia-Rojas et al., 2011). Moreover, we demonstrated that it is possible to transfer drug information to water molecules following the same protocol of transference "substance-to substance" and using the same BRT device in an antifungal model; a significant antimicrobial effect was observed in cultured *Candida albicans* (ATCC 32354 strain) previously treated with amphotericin B-electronically activated water samples (Heredia-Rojas et al., 2012).

A complete explanation on how it is possible to transfer biological information to pure water and to store it there is not yet clear; however, it is accepted that electromagnetic waves interact with water and induce water clusters formation (Baran et al., 2006). Some water structural changes were detected in experiments by the UV luminescence spectrophotometer and they have been related to different water structural "defects" that include specific centres of luminescence; the nuclear proton spins were considered to be a primary target of external magnetic fields, since proton lattice of water molecules is unstable and asymmetric (Binhi, 1998). Furthermore, Del Giudice et al. (2002) have shown the complex interactions between water and solute ions that occur after water solutions are exposed to extremely low frequency-magnetic fields. In the framework of coherent quantum electrodynamics, they suggested that water molecules in the liquid and solute ions are involved in their ground state in coherent ordered configurations and that ions are able to move without collisions among themselves in the interstices between water coherence domains. These interactions could explain some pathways for further interactions of electro-transferred water with biological systems**.** By the way, Teixeira (2007) proposed that the presence of other molecules (solute ions) and dissolved gases in the water samples, are responsible for anomalous states of the water and claims that there are no water clusters in pure liquid water. Even in small quantities, some solutes can modify substantially some properties of pure water. Additionally, it has been suggested that the "magnetic memory of water" is the combination of perturbations of the gas-liquid interface and the production of reactive oxygen species (Colic and Morse, 1999).

It is possible that despite the fact that water exhibits no magnetic properties, the water clusters could be altered in some way due to the action of electromagnetic waves, because electro-transferred water samples are under electromagnetic influence of the BRT apparatus. Since 1996, it is known that water clusters are extremely sensitive to the influence of physical factors such as electromagnetic fields, and even low and ultra-low fields (Liu et al., 1996). It has been proposed that this water capacity to acquire paradoxical configurations induced by low and extremely low intensity electromagnetic fields should be considered when trying to explain resonant intermolecular transfer of electromagnetic energy in liquid water samples (Woutersen and Bakker, 1999). Recently, it has been reported that electromagnetic transmission of chemical information can be stored in the electric dipole moments of water in close analogy to the manner in which magnetic moments store information on a computer disk (Widom et al., 2010). For the present study, one working hypothesis was that molecules can communicate with each other, exchanging information without being in physical contact and that at least some biological functions can be mimicked by certain energetic modes characteristics of a given molecule (Thomas, 2007). In addition, our results agreed with the hypothesis that aqueous system could be able to record, store, and transfer biophysical active information to biological targets (Smith, 2004; Foletti et al., 2013).

The basis for an understanding of the antimicrobial effects of bio-resonance techniques is the assumption that the alternating electromagnetic fields, which are detectably emitted by living organisms and molecules and are characterized by intensity and frequency, contain biologically significant information that is used for transmitting a number of signals between cells, tissues, and even molecules (Likhoded et al., 2007). Recently, a novel property of DNA showing the capacity of some microbial DNA sequences to induce electromagnetic waves at high aqueous dilutions was reported (Montagnier et al., 2009*a*; Montagnier et al., 2009*b*). They found that electromagnetic signals of low frequency can be produced in aqueous dilutions of the human immunodeficiency virus DNA. This also opens the way to the development of highly sensitive detection system for some microbial illnesses in humans and animals.

To our knowledge, in the existing literature there are no original articles published on MRSA growth inhibition by transferring antibiotic drugs to water samples. On the other hand, as bio-resonance procedures are challenged by some, research trials conducted primarily in Russia, Germany, and Eastern Europe, indicated that electromagnetic waves can affect biology in single-cell models that can include micro-organisms (Islamov et al., 2002). Recently, a bacteriostatic effect was observed when cultures of *Staphylococcus aureus* Wood-46 were treated with the help of Molecular Resonance Effect Technology (MRET) a US-patented technology to activate water by means of electromagnetic waves (Vysotskii et al, 2009a). It was demonstrated that under definite conditions, activated MRET water possesses very strong bactericidal properties against *S. aureus* Wood-46 and can inhibit the development of pathogenic microbiological cultures by tens and hundreds of times (Smirnov, 2009; Vysotskii et al, 2009b).

There is a big challenge in this 21st century; serious infections caused by micro-organisms that have become resistant to commonly used antibiotics have become a major global healthcare problem. For instance, a question has been raised: are we in the post-antibiotic era? (Alanis, 2005). Independently of the answer, it is necessary to explore other antimicrobial strategies as the one shown in the present study. We consider that today´s biology, dominated by the molecular approaches developed since about 1940, is suffocated by an immense number of experimental data on molecular aspects of biological functions, which present an extremely fragmented view on the living state. Thus, the holistic approach to biological studies is a complement to the contemporary practice. Field theories, as a central element of holistic models, possibly will be dominant models in the future. In addition, we believe that it is important to draw the scientific community's attention to experimental results obtained by an unorthodox approach. Every really new approach is labelled as unconventional until we fully disclose its mechanism of action. As a matter of fact, in the biological domain we are more acquainted with chemical than with physical approaches, but the last ones, in a near future, may become the next step in the evolution of therapeutic methods.

In conclusion, our *in vitro* study suggests that water samples that are electronically transferred with vibration sustained information of vancomycin are capable of inhibiting growth of axenically cultured methicillin resistant *S. aureus*. However, with the results shown here, we are neither supporting any therapeutic technique nor recommending bio-resonance procedures, rather we showed evidence for a significant and measurable biological effect induced by electro-transferred water samples that acquire, or at least mimics, the chemical information transferring procedure, given the properties of vancomycin. Furthermore, an advantage of using a single-cell model for evaluating the effectiveness of bio-resonance technique is that the placebo effect, claimed by those who refuse any alternative or complementary therapeutics, is absent.

http://dx.doi.org/10.4314/ajtcam.v12i1.15

Acknowledgements

We are grateful to Dr. Antonio Cayetano Torres-Flores for his technical assistance in transferring water samples. We also thank Dra. Michaela Beltcheva of the Bulgarian Academy of Sciences, for critically revising the manuscript. The present study was partially funded by PAICYT grant CN981-11 to J. Antonio Heredia-Rojas.

References

1. Alanis, A.J. (2005). Resistance to antibiotics: are we in the post-antibiotic era? Arch. Med. Res. **36(6)**: 697-705.
2. Baran, B., Berezyuk, O. and Golonzhka, V. (2006). Water systems after magnetic field action. Environ. Res. Eng., and Management. **4(38)**: 19-23.
3. Binhi, V.N. (1998). The structural defects of liquid water in magnetic and electric fields. Biomed. Radioelektron. **2**: 7-16.
4. Chambers, H.F. and Deleo, F.R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev. Microbiol. **7**: 629-641.
5. Colic, M. and Morse, D. (1999). The elusive mechanism of the magnetic "memory" of water. Colloids and Surfaces A: Physicochemical and Engineering Aspects **154**: 167-174.
6. Del Giudice, E., Fleischmann, M., Preparata, G. and Talpo, G. (2002). On the "unreasonable" effects of ELF magnetic fields upon a system of ions. Bioelectromagnetics. **23(7)**: 522-530.
7. Endler, P.C., Citro, M., Pongratz, W., Smith, C.W., Vinattieri, C. and Senekowitsch, F. (1995). Transfer of molecular information using a bioresonance instrument (BICOM) in amphibian trials. Acta Medica Empirica **44**: 1-16.
8. Fedorowski, A., Steciwko, A. and Rabczynski, J. (2004). Low-frequency electromagnetic stimulation may lead to regression of Morris hepatoma in buffalo rats. J. Altern Complement Med. **10(2)**: 251-260.
9. Foletti, A., Ledda, M., D'Emilia, E., Grimaldi, S. and Lisi, A. (2012). Experimental finding on the electromagenetic information transfer of specific molecular signals mediated through aqueous system on two human cellular models. J. Altern. Complement. Med. **18(3)**: 258-261.
10. Foletti, A., Grimaldi, S., Lisi, A., Ledda, M. and Liboff, R. (2013). Bioelectromagnetic medicine: The role of resonance signaling. Electromagn. Biol. Med. 32(4):484-99.
11. Fry, D.E. and Barie, P.S. (2011). The changing face of *Staphylococcus aureus*: a continuing surgical Challenge. Surg. Infect. **12**: 191-203.
12. Heredia-Rojas, J.A., Torres-Flores, A.C., Rodríguez-De la Fuente, A.O., Mata-Cárdenas, B.D., Rodríguez-Flores, L.E., Barrón-González, M.P., Torres-Pantoja, A.C. and Alcocer-González, J.M. (2011). *Entamoeba histolytica* and *Trichomonas vaginalis*: Trophozoite growth inhibition by metronidazole electro-transferred water. Exp. Parasitol. **127**: 80-83.
13. Heredia-Rojas, J.A., Gomez-Flores, R., Rodríguez-De la Fuente, A.O., Monreal-Cuevas E., Torres-Flores, A.C., Rodríguez-Flores, L.E., Beltcheva, M. and Torres-Pantoja, A.C. (2012). Antimicrobial effect of amphotericin B electronically-activated water against *Candida albicans*. Af. J. Microbiol. Res. **6(15)**:3684-3689.
14. Islamov, B.I., Balabanova, R.M., Funtikov, V.A., Gotovskii, Y.V. and Meizerov, E.E. (2002). Effect of bioresonance therapy on antioxidant system in lymphocytes of patients with rheumatoid arthritis. Bull. Exp. Biol. Med. **134**: 248-250.
15. Jerman, I., Ružic, R., Krašovec, R., Škarja, M. and Mogilnicky, L. (2005). Electrical transfer of molecule information into water, its storage, and bioeffects on plants and bacteria. Electromagn. Biol. Med. **24(3)**: 341-353.
16. Jonas, W.B., Ives, J.A., Rollwagen, F., Denman, D.W., Hintz, K., Hammer, M., Crawford, C. and Henry, K. (2006). Can specific biological signals be digitized? FASEB J. **20**: 23-28.
17. Lavin, B.S. (2000). Antibiotic cycling and marketing into the 21st century: a perspective from the Pharmaceutical industry. Infect. Control Hosp. Epidemiol. 21 (1 Suppl.): S32-S35.
18. Likhoded, V.G., Kuleshova, N.V., Sregieva, N.V., Konev Iu, V. and Trubnikova, I.A. (2007). Detection of endotoxins of gram-negative bacteria on the basis of electromagnetic radiation frequency spectrum. Zh Mikrobiol. Epidemiol. Immunobiol. **3**: 3-6.
19. Liu, K., Cruzan, J.D. and Saykally, R.J. (1996). Water clusters. Science **271**: 929-933.
20. Mc Donnell, G. and Rusell, A.D. (1999). Antiseptics and disinfectants: activity, action and resistance. Clin. Microbiol. Rev. **12**: 147-179.
21. Montagnier, L., Aissa, J., Ferris, S., Montagnier, J.L. and Lavallée, C. (2009 a). Electromagnetic signals are produced by aqueous nanostructures derived from bacterial DNA sequences. Interdiscip. Sci. **1(2)**: 81-90.
22. Montagnier, L., Aissa, J., Lavallée, C., Mbamy, M., Varon, J. and Chenal, H. (2009 b). Electromagnetic detection of HIV DNA in the blood of AIDS patients treated by antiretroviral therapy. Interdiscip. Sci. **1**(4): 245-253.
23. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolken, R.H. (1999). Manual of Clinical Microbiology, 7th edn. Washington, DC: American Society for Microbiology.
24. Pothakamury, U.R., Barbosa-Cánovas, G.V. and Swanson, B.G. (1993). Magnetic-field inactivation of microorganisms and generation of biological changes. Food Technol. **47 (12)**: 85-93.
25. Qin, B.L., Pothakamury, U.R., Barbosa-Cánovas, G.V. and Swanson, B.G. (1996). Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. Crit. Rev. Food Sci. Nutr. **36**: 603-627.
26. Rodríguez-De la Fuente, A.O., Heredia-Rojas, J.A., Mata-Cárdenas, B.D., Vargas-Villarreal, J., Rodríguez-Flores, L.E., Balderas-Candanosa, I. and Alcocer-González, J.M. (2008). *Entamoeba invadens*: Influence of 60 Hz magnetic fields on growth and differentiation. Exp. Parasitol. **119**: 202-206.
27. Rowley, B., McKenna, J.M., Chase, G.R. and Wolcott, L.E. (1974). The influence of electrical current on an infecting microorganism in wounds, in Electrically Mediated Growth Mechanisms in Living Systems. , Liboff, A.R., Rinaldi, R.A., Eds. New York Academy of Sciences, New York U.S.A. p 238.
28. Smirnov, I.V. (2009). The effect of MRET activated water on staphylococcal infection *in vivo* in animal model and *in vitro* on the culture of *Staphylococcus aureus* wood-46. J. Ecol. Nat. Environ. **1(4)**:85-93.
29. Smith, C.W. (2004). Quanta and coherence effects in water and living systems. J. Altern. Complement. Med.**10(1)**: 69-78.
30. Takahashi, H., Hayakawa. I. and Akimoto, T. (2003). The history of the development and changes of quinolone antibacterial agents. Yakushigaku Zasshi **38 (2)**: 161-179.
31. Teixeira, J. (2007). Can water possibly have a memory? A sceptical view. Homeopathy **96**:158-162.
32. Thomas, Y., Schiff, M., Belkad, L., Jurgens, P., Kahhak, L. and Benveniste , J. (2000). Activation of human neutrophils by electronically transmitted phorbol-myristate acetate. Med. Hypotheses **54**: 33-39.
33. Thomas, Y. (2007). The history of the memory of water. Homeopathy **96**: 151-157.
34. Velázquez-Meza, M.E., Hernández-Salgado, M., Contreras-Cordero, J.F., Pérez-Cortes, P. and Villarreal-Treviño, L. (2013). Surveillance of methicillin-resistant *Staphylococcus aureus* causing nosocomial infections in five medical centers of Monterrey, Nuevo León, México from 2005-2009. Arch. Med. Res. **44**: 570-574.
35. Vysotskii, V.I., Kornilova, A.A. and Smirnov, I.V. (2009a). Applied Biophysics of Activated Water: the physical properties, biological effects and medical applications of MRET activated water. Chapter 2: Molecular Resonance Effect Technology as the basis method for activation of liquid substances. pp. 36-60. World Scientific Publishing.
36. Vysotskii, V.I., Kholodna, L.S. and Kornilova, A.A. (2009b). The effect of activated water on staphylococcal infection *in vivo* in animal model and *in vitro* on *Staphylococcus aureus* culture. IFMBE Proceedings **25**: 269-272.
37. Widom, A., Srivastava,Y. and Valenzi, V. (2010). The biophysical basis of Benveniste experiments: Entropy, structure, and information in water. Int. J. Quant. Chem. **110**: 252-256.
38. Woutersen, S. and Bakker, H.J. (1999). Resonant intrermolecular transfer of vibrational energy in liquid water. Nature **402**: 5407-5409.