MODULATION OF THE ANTIBIOTIC SUSCEPTIBILITY PROFILES OF SOME MICROBIAL STRAINS ISOLATED FROM WASTEWATER UNDER THE INFLUENCE OF THE ELECTROMAGNETIC FIELD

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Abstract

The wastewater treatment plants are considered to be hotspots for antibiotic resistance selection, transfer and dissemination. Wastewater offers favorable conditions for the development of antibiotic resistant bacteria because it contains high amounts of organic matter, favoring the rapid multiplication of microbial cells and the development of biofilms in which microbial cells are in close proximity, the presence of pollutants (pesticides, heavy metals, antibiotics) acting as selective pressure agents for resistance.
The aim of this paper is to investigate how the antibiotics susceptibility profiles of some microbial strains isolated from wastewater are influenced by electromagnetic fields.
A number of 10 bacterial strains isolated from wastewater (*E. coli, Salmonella sp., Enterobacter sp., Enterococcus sp., Citrobacter sp., Klebsiella sp.*) were exposed to an electromagnetic field (50Hz electric field at different voltages) for 24 hours, at 37°C. Thereafter, the antibiotic susceptibility testing was performed for both treated and control strains, by disk diffusion method, according to CLSI 2016.
The obtained results proved that the electromagnetic field induced in some cases a decrease of the growth inhibition diameters such as penicillins, cephalosporins, aminoglycosides and vancomycin, while in other cases an increase of bacterial strains susceptibility to the tested antibiotics.
These preliminary results demonstrate that the electromagnetic filed in addition with other selective factors which are present in the wastewaters could modulate the environmental reservoir of antibiotic resistance and influence the frequency of the selection of resistant bacteria and the magnitude of the potential risk of dissemination into the environment and of contamination of animals and humans.

Keywords*: antibiotic susceptibility, bacteria, electromagnetic field, wastewater.*

1. Introduction

In recent years, due to the intensive use of antibiotics for humans health (domestic or clinical use), in veterinary purposes or in agriculture, the compounds are released constantly in the environment by anthropogenic sources such as wastewater treatment plants, considered to be hotspots for antibiotic resistance selection, transfer and dissemination in natural ecosystem (1-6). The wastewater treatment plant offers favorable conditions for the development of antibiotic resistant bacteria because it contains high amounts of organic matter, favoring the rapid multiplication of microbial cells and the development of biofilms in which microbial cells are in close proximity, the presence of pollutants (pesticides, heavy metals, antibiotics) acting as selective pressure agents for resistance (7-12). Presently, antibiotic resistance has become a

global problem, being reported more and more clinical infections, diseases and deaths caused by resistant microorganisms (8; 13-14). Beside the chemical pollution caused by the release of toxic substances in wastewater, the use of antibiotics induces the development of antibiotic resistant bacteria and antibiotic resistance genes which pose an increase risks to human and animals health (15).
The contamination of the natural environment with both antibiotic resistant bacteria and antibiotic resistance genes could have a negative impact on the structure and metabolism of bacterial populations (16-17). Therefore, it is necessary to identify their abundance and tolerance in polluted environments, such as the wastewater from wastewater treatment plants. The severity and persistence of antibiotic resistance genes in the natural environment represent an international priority problem (18-21). Investigation of the occurrence of antibiotics in wastewater has been conducted in several countries in Europe (22-25; 4). Boxali (2004) and Kummerer (2009) reported two important comprehensive studies regarding the ecotoxicity of antibiotics (26; 4). Different studies regarding the spread of antibiotic resistance bacteria and antibiotic resistance genes in wastewater were reported over time (27-32).

In contrast to the usual pollutant factors, which are well known (for example chemical or acoustic pollution), the electromagnetic pollution of the natural environment and its effects on the living matter are relatively little known. The electromagnetic fields have been demonstrated to influence the biological systems both *in vivo* and *in vitro* models. The biological effects depend on the intensity, frequency, the modulation type and shape of the electromagnetic field, but also the time of exposure also influences the results (33). The effects of electromagnetic waves below 1kHz on the living cells were studied for the first time in 1977 by Andrew A. Marino and Robert O. Becker (34).
A large number of bioprocesses can be improved by electromagnetic and electrochemical stimulation, under proper conditions of microbial cultivation (electrolyte bioreactors) (33). The bio electromagnetic stimulation of the microbial strains represents an important area with various applications in biotechnologies and bioenergy (35). Several studies were reported regarding the influence of electromagnetic field on both prokaryotic (bacteria) and eukaryotic (molds, yeasts, algae) microorganisms (33).
The aim of this paper is to investigate how the antibiotics susceptibility profiles of some microbial strains isolated from wastewater are influenced by electromagnetic fields.

2. Materials and methods

A number of 10 bacterial strains isolated from wastewater were investigated. The laboratory code, isolation sites and taxonomic affiliation are summarized in Table 1.

Table 1. Investigated bacterial strains

No.	ID	Bacterial strains	Origin
1.	S18	*Salmonella arizonae*	Sewage water
2.	P34	*Klebsiella sp.*	Treated wastewater Bucharest
3.	S11	*Salmonella sp.*	Sewage water
4.	N85	*Enterobacter agglomerans*	Wastewater Fundeni Hospital
5.	N61	*Escherichia coli*	Wastewater Fundeni Hospital

6.	N60	*Citrobacter farmeri*	Wastewater Fundeni Hospital
7.	N67	*Klebsiella oxytoca*	Wastewater Fundeni Hospital
8.	O19	*Enterobacter sp.*	Domestic wastewater Bucharest
9.	P62	*Enterococcus sp.*	Treated wastewater Bucharest
10.	R73	*Enterococcus sp.*	Dambovita river water Bucharest

The bacterial strains were grown on Mueller-Hinton agar (MHA) and placed in an incubator for 24 hours at 35±2°C, in an electrical installation, for obtaining a homogeneous electric field of 50Hz electric field and different voltages (Table 2).

Table 2. Bacterial strains laboratory code and the type of the applied electric field

Bacterial strains	Voltage
N61 – N67	217 V
S11 – S18	420 V
P34 – R73	665 V
O19 – P62	875 V
N60 – N85	1120 V

The inoculum for testing the antibiotic susceptibility was represented by a direct colony suspension for each strain, equivalent to a 0.5 McFarland standard. Antibiotic susceptibility testing was performed for both treated and control strains, by disk diffusion method, according to Clinical and Laboratory Standards Institute guidelines (CLSI 2016). The treated and untreated bacterial strains were tested for susceptibility to 12 antimicrobial agents: amoxicillin-clavulanic acid (30 µg), penicillin (10 units), ampicillin (10 µg), cefuroxime (30 µg), ceftazidime (30 µg), imipenem (10 µg), gentamicin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), vancomycin (30 µg), erythromycin (15 µg). All antibiotic disks were provided by Oxoid. Antimicrobial susceptibility/resistance profiles of the tested strains were established after measuring the diameter of the growth inhibition zone.

Table 3. Antibiotic panels used for different bacterial strains, according to CLSI 2016

Antibiotics	Bacterial strains
Amoxicillin-clavulanic acid (AMC) Cefuroxime (CXM) Ceftazidime (CAZ) Imipenem (IMP) Gentamicin (CN) Tetracycline (TE)	Enterobacteriaceae, other than *Salmonella spp*.
Ampicillin (AMP) Nalidixic acid (NA)	*Salmonella spp.*
Penicillin (P) Ampicillin (AMP) Tetracycline (TE) Vancomycin (VA) Erythromycin (E)	*Enterococcus spp.*

3. Results and discussion

The present study shows some preliminary results regarding the study of the influence of electromagnetic field on the antibiotic susceptibility of some bacterial strains isolated from wastewater (Table 1). The results demonstrate that the electromagnetic field exhibited a variable influence on the antibiotic susceptibility patterns of the tested strains.
The electromagnetic field induced a decrease of the growth inhibition diameters for penicillin, cephalosporins (ceftazidime, cefuroxime) and tetracycline. The most significant changes were noticed for the second and third generation cephalosporins cefuroxime and ceftazidime against *Enterobacter sp., Citrobacter sp.* and *E. coli* tested strains.

Table 4. Growth inhibition diameters and clinical category (susceptible, intermediate, resistant) before and after exposure to the electromagnetic field of Salmonella spp. and *Enterococcus spp. strains*

Microbial strain	**NA**		**AMP**		**P**		**VA**		**E**		**TE**	
	1*	2**	1*	2**	1*	2**	1*	2**	1*	2**	1*	2**
Salmonella arizonae	25 S	25 S	6 R	6 R								
Salmonella sp.	22 S	26 S	10 R	10 R								
Enterococcus sp.			15 R	11 R	18 S	15 S	23 S	23 S	21 I	21 I	11 R	8 R
Enterococcus sp.			6 R	6 R	18 S	15 S	20 S	6 R	16 I	14 I	17 I	15 I

Table 5. Growth inhibition diameters and clinical category (susceptible, intermediate, resistant) before and after exposure to the electromagnetic field of Enterobacteriaceae spp. strains

Microbial strain	**AMC**		**CXM**		**CAZ**		**IMP**		**CN**		**TE**	
	1*	2**	1*	2**	1*	2**	1*	2**	1*	2**	1*	2**
Enterobacter agglomerans	10 R	14 I	11 R	6 R	24 S	21 S	27 S	26 S	24 S	29 S	22 S	30 S
Escherichia coli	15 I	17 I	16 I	13 R	18 I	15 R	30 S	33 S	27 S	30 S	23 S	19 S
Citrobacter farmeri	16 I	16 I	18 S	13 R	30 S	21 S	23 S	24 S	16 S	15 S	15 S	18 S
Klebsiella oxytoca	6 R	6 R	6 R	6 R	26 S	31 S	32 S	32 S	31 S	31 S	19 S	20 S
Enterobacter sp.	19 S	17 I	14 R	13 R	29 S	15 R	21 I	17 R	21 S	12 R	21 S	18 S
Klebsiella sp.	34 S	26 S	18 S	18 S	6 R	6 R	40 S	36 S	34 S	31 S	31 S	31 S

*1= before (control sample - unexposed)
**2= after exposure to the electromagnetic field

In certain cases, the tested strains exhibited an increased resistance to the tested antibiotics, as revealed by the decrease of the growth inhibition diameters,

comparatively to the unexposed strains (control sample). This was the case for the Gram-positive *Enterococcus* sp. strain for vancomycin, of *Escherichia coli* N61 for cefuroxime and ceftazidime, of *Enterobacter* sp. N60 for ceftazidime, gentamicin, AMC, imipenem (Fig. 1) and of *Citrobacter farmeri* O19 for cefuroxime (Fig. 2).

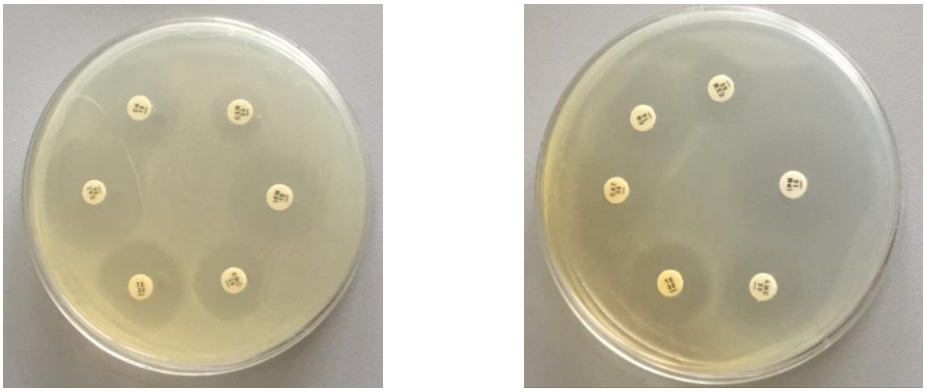

Figure 1. Sample O19 M (Control) Sample O19 (Exposed to the EM* field)

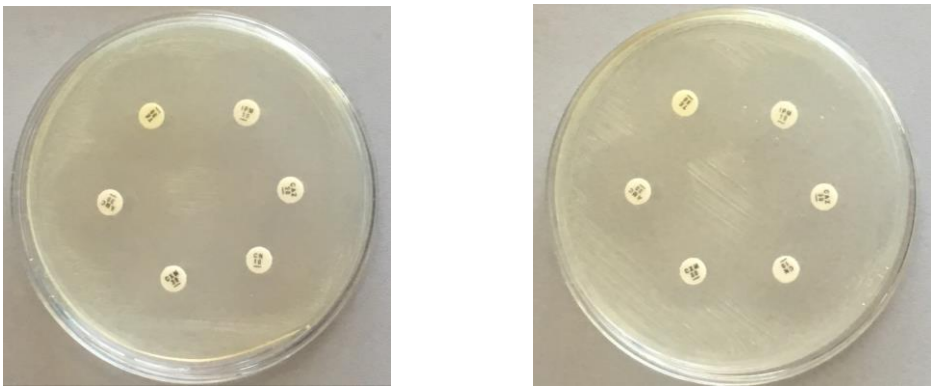

Figure 2. Sample N60 M (Control sample) Sample N60 (Exposed to the EM* field)

However, in case of the *Enterobacter agglomerans* N85 strain, all tested antibiotics exhibited slightly higher growth inhibition zone diameters (Fig. 3).

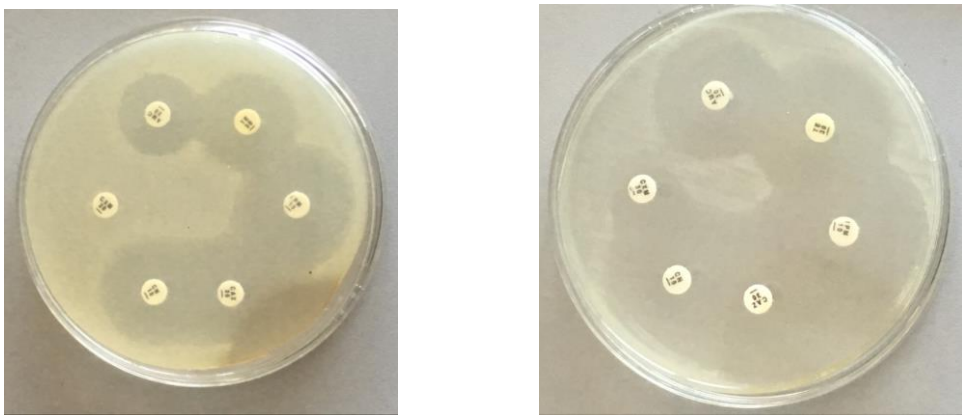

Figure 3. Sample N85 M (Control sample) Sample N85 (Exposed to the EM* field)
*EM= electromagnetic

4. Conclusions

The results regarding the antibiotic susceptibility of some bacterial strains isolated from wastewater and exposed to an electromagnetic field 50Hz at different voltages showed

that certain bacterial strains exhibited a decreased susceptibility and even turned into resistant to antibiotics comparatively to the control samples (unexposed to an electromagnetic field), while others exhibited an increased susceptibility to some of the tested antibiotics. These preliminary results demonstrate that the electromagnetic filed in addition with other selective factors which are present in the wastewaters could modulate the environmental reservoir of antibiotic resistance and influence the frequency of the selection of resistant bacteria with a potential risk of dissemination into the environment and of contamination of animals and humans.

Acknowledgements
This work was financiary suported by National Project no. 16 11 03 01/2016 and the research project PN-II-RU-TE- 2014-4-2037 from the Romanian National Authority of Scientific Research and Inovation.

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