

SOIL MICROBIAL DIVERSITY AND ANTIBIOTIC RESISTANCE IN NATURAL AND TRANSFORMED ECOSYSTEMS

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ABSTRACT

Terrestrial ecosystems may provide an ideal setting for the acquisition and dissemination of antibiotic resistance, because they are frequently impacted by anthropogenic activities. The soil microbiome plays an important role in development and spread of antibiotic resistance in humans. The aim of our study was to detect the antibiotic resistance soil bacteria in different ecosystems: natural ecosystems, agroecosystems and urboecosystems. Were isolated 468 dominating bacteria, among them 79 antibiotic resistant bacteria. All isolates were multi-drug resistant, of which greater than 74,5% were resistant to 9 antibiotics. A study of soil samples from the primeval forests showed that the microbial community characterized by a low content of antibiotic-resistant microorganisms. Among 78 isolated bacteria only two of them *Bacillus cereus*, and *Pantoea agglomerans* demonstrated high level of resistance to antibiotics. A total 106 strains were isolated from the soil of medicinal plants, 13 of them were antibiotic-resistant. The greatest numbers of antibiotic-resistant bacteria have been isolated from soil of urboecosystems and agroecosystems contaminated by enrofloxacin. Among the 284 tested bacteria 64 were antibiotic resistant. Multi-resistance were such pathogenic and conditionally pathogenic bacteria as: *Enterococcus faecium*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus licheniformis*, *Serratia fonticola*, *Hafnia alvei*, *Bacillus cereus*, *Bacillus megaterium* and *Clostridium difficile*.

Keywords: ecosystem, soil, diversity, microbiome, antibiotic resistance.

INTRODUCTION

Soil plays a vital role in ecosystems, serving as the primary nutrient base and habitat for plants and organisms, and functioning as a giant bioreactor for degrading pollutants and facilitating nutrient transformation. Inevitably, soil is a hot spot for antibiotics to affect indigenous microbes since it receives a large portion of excreted antibiotics through application of manure and sewage sludge as fertilizers (Thiele-Bruhn, 2003). The higher density of microbes in the

soil environment encourages genetic exchanges, which could enhance the development of microbial resistance in the presence of antibiotics (Murray, 1996). A comparatively small proportion of bacterial species are pathogens but some of these are responsible for infectious diseases that can have devastating impacts on human and animal health. Humans have exploited the activities of antibiotic compounds, many of which are derived from nature, to fight bacterial infections for centuries. Development of antibiotic resistance in pathogens is a shining example of health issues in which understanding the central role of the environment is crucial to managing this potential risk (Keen and Patrick, 2013).

Environmental contamination by antibiotic compounds is inextricably linked to development of antimicrobial resistance in non-target species of bacteria. Whether the bacteria perform critical ecosystem services, pose a health threat as pathogens or have incompletely understood functions in nature, development of antimicrobial resistance as a result of human activities is problematic. Susceptibility characteristics of microbes can be altered by incorporation of genetic information encoding for resistance or by mutation in their DNA. Antibiotic resistance genes are recognized as important environmental contaminants (Kümmerer, 2004; Pruden et al., 2006; Wright, 2007). It is now accepted that resistance is a natural property of all bacteria (Martínez, 2008; Allen et al., 2010; Wright, 2010) and the term resistome is used to describe the framework that encompasses all forms of resistance and precursor elements (Schmitt, H. et al., 2004; Migliore et al. 1997). More and more evidence is being collected to support the idea that the environment acts both as a reservoir for antibiotic resistance and a means by which this resistance can be broadly disseminated. Genes can move quickly through a bacterial population via vertical or horizontal transfer mechanisms and combined with the grand magnitude of the resistome, it is no wonder that bacteria can quickly adapt to resist new drugs soon after they are introduced for medical or agricultural use. Resistant bacteria, antibiotic resistance genes, degradative enzymes that work to inactivate antibiotics, and antibiotic molecules are present in the environment at all times and thus distinguishing naturally occurring resistance in organisms from resistance as a result of environmental pollution is a complicated task (Keen and Patrick, 2013; Migliore et al., 1997; Ebert et al., 2017).

Antibiotics used in therapeutic doses on farms for pigs and cows, together with their feces, are subsequently exported to fields with agricultural plants. Even in small doses, they can cause a toxic effect and form antibiotic resistance of microorganisms (Boxall et al., 2006; Christian et al., 2003; Diao et al., 2004; Diaz-Cruz et al., 2003; Chee-Sanford et al., 2001). The fluoroquinolones are one of the most used classes of antibiotics. Enrofloxacin belongs to the class of fluoroquinolone antibiotics that have been intensively used for the treatment of bacterial infections in veterinary medicine. Once antibiotics enter the ecosystems, they can be treated as an ecological factor, driving the evolution of the community structure (Migliore, 2003; Aminov, 2007; Symochko et al., 2018). Accordingly, the change of community structure influences the ecological function of soil ecosystems such as biomass production and nutrient transformation. Indirect effects from the antibiotic disturbance to the micro-ecosystem are largely unknown, and it is expected that such disturbance might have significant and long-term effects on the rate and stability of ecosystem functioning.

In the environment, enrofloxacin can undergo degradations by different processes including photolysis, biodegradation and oxidation by mineral oxides but it is not sensitive to hydrolysis. Despite these degradation mechanisms, environmental half life time of enrofloxacin is very long. This long environmental persistence of enrofloxacin can affect the growing of plant and the activity of the soil microbial communities. As final products of metabolism, enrofloxacin and its metabolite ciprofloxacin end up in excrement (Boxall et al., 2006; Symochko, Mariychuk, 2018). Livestock manure is commonly used as organic fertilizer. One of its uses is on the fields where food plants are grown. The manure includes the residue of fluoroquinolones in addition to other drug residue. Plants can also intake fluoroquinolones along with minerals. The intake of drugs in small amounts can lead to drug resistance in pathogenic microbes and cause allergies and liver damage. Manure is often contaminated with veterinary antibiotics which enter the soil together with antibiotic resistant bacteria. However, little information is available regarding the main responders of bacterial communities in soil affected by repeated inputs of antibiotics via manure (Cavigelli, M. A., & Robertson, G. P., 2000; Hammesfahr U, Heuer H, Manzke B, Smalla K, Thiele-Bruhn S., 2008; Heuer H, Smalla K., 2007; Heuer H, Schmitt H, Smalla K., 2011; Torsvik 2002). Nevertheless, some investigators have commented that antibiotic resistance transfer via vegetables represents a risk to human health. Investigation of terrestrial ecosystems as reservoirs of antibiotic-resistant microorganisms and screening of antibiotic-resistant bacteria-causative agents of human diseases is one of the important aspects in modern environmental microbiology. Our main idea is to study soil microbiome of terrestrial ecosystems and its screening for antibiotic resistance bacteria. As a model ecosystem we investigated primeval forest. The primeval forests as etalon ecosystems better combine above resistance and stability with high productivity biomass. In the

Transcarpathian region of Ukraine (south-west), the Uzhanskyi National Nature Park (Uzhanskyi NNP) offers a unique opportunity for studying the biodiversity and natural processes of primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity. For comparing we investigated agroecosystems and urboecosystems.

MATERIALS AND METHODS

Materials of research were soil samples, which had been collected by envelope method from different type of ecosystems: virgin forest, agroecosystems, urboecosystems. Materials of research were soil samples, which had been collected by envelope method from the virgin forests of Uzhanskyi National Nature Park at the deep 0-25cm. Uzhanskyi National Nature Park is located in the western part of Transcarpathia in the basin of the river Uzh and extends from the southwest of the village Zabrod (226 m above sea level) to north-east to Uzhotskyi pass (852 m above sea level). In 2007 primeval beech forests of Uzhanskyi National Nature Park were included into UNESCO World Heritage List "Primeval Beech Forests of the Carpathians and the Ancient Beech Forests of Germany". The Uzhanskyi NNP is located in the southwest sub-region of the Atlantic-continent climatic region of the temperate zone, in the low-mountainous zone of moderate relative humidity. Absolute temperature maxima occur most often in July and August, and make up + 34 ° C - + 37 ° C, in some years fall on April - May. The absolute minimum temperatures are January-February and are characterized by -28 ° C -32 ° C. The total vegetation period in the region lasts 195 days. The average annual rainfall is 856 - 909 mm. There are mainly brown mountain-forest soils (brown-soils) in the park among them are two subtypes: dark brown and light-brown mountain-forest soils. The reaction of the forest soil is slightly acidic. Humus is dominated by full-fatty acids, which are closely linked to the one and a half oxides of iron and aluminum. Sampling was carried out seasonally at different altitudes from 450 m to 650 m. Studies of soils were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes (Uzhhorod National University), Research Laboratory Monitoring of Water and Terrestrial Ecosystems of department entomology and biodiversity conservation (Uzhhorod National University) and at Laboratory of Soil microorganisms, (Institute of Agroecology and Environmental Management, Kyiv Agrarian Academy of Sciences of Ukraine). The research was carried out within the framework of the complex theme „Eco-microbiological monitoring of various types ecosystems of the Carpathian region” №0116U003331 (state registration number), following the standard protocol (Shyrobokov, 2011; Goldman, Green, 2015) All soil samples were analyzed within 24 hours. Microbiological study of soil was performed in sterile conditions. The method of serial dilution was used to obtain the suspension where microorganisms titre were 10-3 CFU/ml. - 10-5 CFU/ml. 100 µl of the soil suspension was evenly distributed on the surface of the medium with a sterile spatula. For the study we used the following media: Endos agar, Meat peptone agar, Strepto agar and Entero agar, Agar-Agar, Eshbi agar, Soil agar, Chapek agar, Starch agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 37°C for 48 hours in aerobic conditions.

All isolated microorganisms were identified by applying of appropriate biochemical test-systems LACHEMA according to the instructions. Antibiotic resistance of the identified microorganisms was analysed by Kirby-Bauer method with the aim to find antibiotic resistant strains of pathogenic microorganisms. All isolates from the soil were examined for resistance to 12 antibiotics of the main pharmacological groups: TE₃₀ Tetracycline; VA₃₀ Vancomycin; L₁₀ Lincomycin; CXM₃₀ Cefuroxime; AMP₁₀ Ampicillin; CIP₅ Ciprofloxacin; GEN₁₀ Gentamicin; DO₃₀ Doxycycline; AK₃₀ Amikacin; AMX₁₀ Amoxicillin; E₁₅ Erythromycin; OL₁₅ Oleandomycin. Anaerobic microbiota were additionally tested to Metronidazole MT₅; Rifampicin RIF₅; Clindamycin CD₂. The amount of enrofloxacin in the soil was determined in triplicate on each sample by High Performance Liquid Chromatography (HPLC).

Results were expressed as means (±) standard deviation (SD) and (SSD₀₅) smallest significant differences of experiments conducted in quadruplicating. Data were evaluated using the software Statistica 10.0. An assessment of the integrability of soil microbial community in different edaphotops of ecosystems was carried out using correlation-regression analysis.

RESULTS

Environment surrounding is a huge bacterial reservoir, and antibiotic resistance can be passed between bacteria in the environment, including in the soil. Soil microorganisms as a part of forest ecosystems plays an important role in sustainable development of forestry. They are highly sensitive to anthropogenic pressure, so changes of qualitative and quantitative composition of the soil microbiota – is an indicator of the environmental changing. Due to the high sensitivity to changes in environment microorganisms serve as a convenient object of observation. They are in a close contact with habitat and they are characterized by high rate of growth and reproduction. Biocenotic relations of trophic and topical types are decisive in edaphotope shaping of different type of ecosystems [20]. Due to this fact, the purpose of the research was to determine the number of different ecological-functional groups of soil microorganisms. Studies of the soil were taken from primeval ecosystems revealed general regularities of distribution of main ecological-functional groups of microorganisms, their population dynamics in different habitats. The most favourable conditions for the development and functioning of microorganisms were recorded in an edaphotop which was located at an altitude of 450 meters above sea level. It is highly connected to local temperature and water regime, as well as reserves of nutrients (organic origin) in the soil (Table 1).

The number of ammonifiers at an altitude of 450 m amounted 5.33 million CFU/gr.ab.d.s. and at an altitude of 650 m – 3.01 million CFU/gr.ab.d.s., which indicate a significant enrichment of soil by organic matter of plant origin. Anthropogenic impact, namely soil compaction, was negatively influenced on the structure of soil microbiota. The content of oligotrophic microbiota significantly increased in the samples of this soil, but the number of ammonifiers and pedotrophes microbiota was minimal compared to the other surveyed edaphotopes.

Table 1. Microbial community composition in soils of primeval forest ecosystems (CFU/gr.d.s.)

№	Biotope, altitude above msl, m	Ammonifiers *10 ⁶	Spore forming bacteria *10 ⁶	Micromycetes*10 ³	Actinomyces*10 ³	Bacteria which are using mineral forms of nitrogen*10 ⁴	Anaerobic bacteria*10 ³	Aerobic nitrogen fixing bacteria, %	Anaerobic nitrogen fixing bacteria *10 ³	Oligotrophic bacteria *10 ⁶	Oligonitrophic bacteria*10 ⁴	Pedotrophic bacteria*10 ⁶
1	450	5,33	2,35	430	10,78	3,35	35,20	66,51	6,70	3,45	4,45	2,34
2	500	3,89	2,23	540	12,03	3,26	41,22	49,60	9,56	3,97	3,81	2,89
3	650	3,54	3,07	573	16,89	2,67	50,22	40,22	10,67	4,10	3,65	3,44
4	650.1	1,32	4,66	240	32,96	1,24	94,68	17,21	16,25	7,45	1,35	1,89
5	650.2	4,89	3,78	380	20,34	2,66	73,82	29,73	12,78	3,24	2,97	4,78
SSD05		0,24	0,16	6,28	0,37	0,44	0,21	2,18	0,64	1,20	1,77	0,72

With the creation of favourable conditions for competitive species of microbiota we can see changes in microbial cenoses, owing to the active competition of microorganisms. In edaphotopes that were not changed by direct human impact the dominate type of microorganisms was organotrophic microbiota. It should be noted that their percentage in the structure of groups was reduced by 36% with increasing of the height. At the same time the number of oligotrophes also increased with altitude. Significant negative changes in the structure of microbiocenosis of the soil can be the effect of anthropogenic influence. Violation of the integrity of the phytocenosis as a result of deforestation has led to the increase in the content of oligotrophes and pedotrophes, which indicates disruption of the normal flow of microbiological processes in the soil. It also influenced the decline in biodiversity of soil

microorganisms. The most negative changes in the structure of soil microbial community were observed due to compaction. Nearly 80% of the studied ecological-functional groups of microorganisms were oligotrophes, which shows a significant deterioration of the ecological state of the soil. Soil microbial communities are intricately linked to ecosystem functioning because they play important roles in carbon and nitrogen cycling, and feedback to plant communities as mutualists and pathogens (Symochko L. Hamuda H.B., 2015). Although much research has been done to study the impacts of a range of disturbances on soil microbial communities and their functioning (Van der Heijden M.G.A., Bardgett R.D., Van Straalen N.M., 2008), many uncertainties remain about the controls on soil microbial community stability, and the consequences of disturbance-induced changes in microbial communities for their capacity to withstand further disturbances. The influence of biodiversity on ecosystem stability is complex and depends not only on species richness but also on the evenness or composition of the soil microbial community. Resistance and resilience to disturbance might also vary between functional microbial guilds dependent on their levels of functional redundancy. Our results indicate high stability of the studied soil systems and suggest the existence of functional redundancy among soil microorganisms, leading to ecosystem resistance and resilience (Table 2). A high level of functional redundancy, within a functional community, that is, a high number species performing the same function, might act as a buffer against the effect of biodiversity loss on functioning. However, functional redundancy is likely more limited within specialised rather than global processes. The results of our studies have shown that there are strong correlation relations between the majorities of functional groups of soil microorganisms in non-disturbed edaphotopes (Table 2).

Table 2. Integrity of soil microbial community in non-disturbed edaphotopes

	Ammonifiers	Spore forming bacteria	Micromycetes	Actinomyces	Bacteria which are using mineral forms of nitrogen	Anaerobic bacteria	Aerobic nitrogen fixing bacteria	Anaerobic nitrogen fixing bacteria	Oligotrophic bacteria	Oligonitrophic bacteria	Pedotrophic bacteria
Ammonifiers	-	-0,55	-1,00	-1,00	0,74	-0,90	0,98	-1,00	-1,00	1,00	-0,94
Spore forming bacteria	-0,55	-	0,58	0,95	-0,97	0,86	-0,68	0,62	0,55	-0,55	0,79
Micromycetes	-1,00	0,58	-	0,81	-0,76	0,91	-0,99	1,00	1,00	-1,00	0,95
Actinomyces	-1,00	0,95	0,81	-	-1,00	0,98	-0,88	0,84	0,79	-0,79	0,95
Bacteria which are using mineral forms of nitrogen	0,74	-0,97	-0,76	-1,00	-	-0,96	0,84	-0,80	-0,74	0,74	-0,92
Anaerobic bacteria	-0,90	0,86	0,91	0,98	-0,96	-	-0,96	0,93	0,90	-0,90	0,99
Aerobic nitrogen fixing bacteria	0,98	-0,68	-0,99	-0,88	0,84	-0,96	-	-1,00	-0,99	0,99	-0,99

Anaerobic nitrogen fixing bacteria	-1,00	0,62	1,00	0,84	-0,80	0,93	-1,00	–	1,00	–	1,00	0,97
Oligotrophic bacteria	-1,00	0,55	1,00	0,79	-0,74	0,90	-0,99	1,00	–	–	1,00	0,94
Oligonitrophic bacteria	1,00	-0,55	-1,00	-0,79	0,74	-0,90	0,99	-1,00	-1,00	–	–	-0,94
Pedotrophic bacteria	-0,94	0,79	0,95	0,95	-0,92	0,99	-0,99	0,97	0,94	–	–	–

Oligotrophic bacteria and pedotrophic bacteria correlated with micromycetes and actinomycetes, a correlation coefficients (CC) were 1,0; 0,79 and 0,95; 0,95.

Aerobic nitrogen fixing bacteria positive correlated with ammonifiers (CC=0,98) and bacteria which are using mineral forms of nitrogen (CC=0,84). anaerobic bacteria high correlated with spore forming bacteria (CC=0,86), micromycetes (CC=0,91) and actinomycetes (CC=0,98).

In general, should be noted a high level integrity of soil microbiome, 46 bonds were identified between different groups of microorganisms, which characterized a high level of correlation (CC > 0,75).

Microbiological soil control is necessary for assessing and predicting the possibility of spreading antibiotic-resistant microorganisms.

As model terrestrial ecosystem of soil resistome we analysed virgin forest. A study of soil samples from the primeval forests showed that the microbial community characterized by a low content of antibiotic-resistant (AR) microorganisms (Table 3). Among 78 isolated bacteria only two of them *Bacillus cereus*, and *Pantoea agglomerans* demonstrated high level of resistance to antibiotics.

Table 3. Percentage of AR bacteria in the different ecosystems

№	Type of ecosystem	Total number of dominant bacteria with level of AR to tested antibiotics more than 30% (468)	Total number of dominant bacteria with level of AR to tested antibiotics more than 70% (79)
1	Natural ecosystem	78	2
2	Agroecosystem of medicinal plant	106	13
3	Agroecosystem contaminated by antibiotic	148	42
4	Urboecosystem	136	22

The fluoroquinolones are one of the most used classes of antibiotics. Enrofloxacin belongs to the class of fluoroquinolone antibiotics that have been intensively used for the treatment of bacterial infections in veterinary medicine. The effect of enrofloxacin on the function and structure of soil microbial communities were evaluated (Table 4).

It should be noted, the soil with a high concentration of antibiotic was characterized by a low content of nitrogen-fixing microorganisms and a high number of oligotrophic and sporeforming microbiota. Among AR microorganisms were anaerobic bacteria: *Clostridium difficile*, *Clostridium perfringens* and aerobic bacteria: *Enterococcus faecalis*, *Yersinia enterocolitica*, *Enterobacter cloacae*. Other dominant bacteria were characterized by a high or moderate level of antibiotic resistance. From the soil, were isolated bacteria resistant to all tested antibiotics. They were representatives of aerobic microbiota: *Bacillus licheniformis*, *Serratia fonticola*, *Hafnia alvei*, *Bacillus cereus*, *Pantoea agglomerans*, *Bacillus megaterium* and anaerobic bacteria - *Clostridium difficile*. In natural conditions, from the soil of model ecosystems were isolated mostly bacteria of the genus *Bacillus*. All of them are antibiotic resistant and are the causative agents of foodborne infections and pose a threat not only to the environment but also to human health.

Table 4 Microbial community composition (CFU/gr.d.s.) in soils of agroecosystems contaminated by antibiotic (1000mg*kg-1).

No	Agroecosystems	Ammonifiers*10 ⁶	Spore forming bacteria *10 ⁶	Micromycetes*10 ³	Actinomycetes*10 ³	Bacteria wich are using mineral forms of nitrogen *10 ⁴	Anaerobic bacteria*10 ³	Aerobic nitrogen fixing bacteria, %	Anaerobic nitrogen fixing bacteria *10 ³	Oligotrophic bacteria *10 ⁶	Oligonitrophic bacteria *10 ⁴	Pedotrophic bacteria *10 ⁶
1	<i>Mentha piperita</i>	4,07	3,88	11	7,21	4,32	35,20	19,22	3,77	2,63	4,56	3,68
2	<i>Calendula officinalis</i>	8,30	1,88	20	10,33	3,64	41,22	28,56	5,96	1,61	2,17	1,88
3	<i>Thymus Serpillum</i>	5,46	4,45	15	14,11	3,22	50,22	22,34	8,22	2,87	3,27	3,52
4	<i>Anethum graveolens</i>	7,93	1,74	28	21,22	3,14	94,68	38,23	11,35	1,24	1,68	2,26
5	<i>Lactuca sativa var. crispa</i>	8,66	2,23	25	12,38	2,18	73,82	29,67	9,23	1,70	2,95	2,96
	SSD ₀₅	0,48	0,23	0,36	0,37	0,32	0,21	1,12	1,34	0,41	0,55	0,18

The level of integration of soil microbiome in contaminated edaphotopes was lower than in etalon ecosystem (Table 3). It was recorded 29 correlation connections between different ecological groups of soil microorganisms with high level of correlation (CC > 0,75). Strong correlation connections have been observed between anaerobic bacteria and micromycetes (CC=0,85); anaerobic bacteria and actinomycetes (CC=0,98). Oligotrophic bacteria strong correlated with micromycetes, actinomycetes and anaerobic nitrogen fixing bacteria.

The presence of enrofloxacin in the soil, especially in high concentrations, cause negative changes in the microbial community, significantly increasing number of antibiotic-resistant bacteria loses stability and integrity of soil microbiome. Contamination by antibiotics is one of the important factors in the formation of soil resistome. The urban environment the main habitat for human population is a potential harmful substance source, with high risk for resident's health. Indeed, harmful substance concentration and distribution are related to traffic intensity, distance from roads, local topography, and heating. Industrial emissions also contribute to the release of toxic elements. Sustainable management and microbiological control of urban soils and gardens in relation to human health are necessary for estimation and improvement of ecological situation in metabolises. The effects of most trace metals on human health are not yet fully understood. Uncertainty is still prevailing, particularly with non-essential elements that are "suspected" to be harmful to humans, causing serious health problems as intoxication, neurological disturbances and also cancer. Contamination of urban soils causes changes in the structure of soil microbiome, increase the number of antibiotic resistance bacteria (Table 3), most of them are causative agents of foodborne diseases.

Soil microbial communities are heterogeneous entities with distinct components that are each capable of responding differently to environmental characteristics. Microbial composition was shown dynamics and differences in the structure of microbial communities, depending on the level of anthropogenic impact.

One of the important indicators of the ecological and sanitary state of the soil and the whole ecosystem is the presence of conditionally pathogenic and pathogenic microorganisms. Particularly dangerous are the antibiotic-resistant microorganisms, which, together with the bioproduction, can enter to he human and animal organisms from the terrestrial ecosystems.

Table 5. Integrity of soil microbial community in edaphotopes of agroecosystem contaminated by antibiotic

	Ammonifiers	Spore forming bacteria	Micromycetes	Actinomyces	Bacteria which are using mineral forms of nitrogen	Anaerobic bacteria	Aerobic nitrogen fixing bacteria	Anaerobic nitrogen fixing bacteria	Oligotrophic bacteria	Oligonitrophic bacteria	Pedotrophic bacteria
Ammonifiers	–	-0,58	-1,00	-0,33	0,72	-0,55	0,46	-0,77	-0,25	1,00	-0,92
Spore forming bacteria	-0,58	–	0,55	0,95	-0,97	0,86	-0,68	0,62	0,55	-0,50	0,79
Micromycetes	-1,00	0,55	–	0,81	-0,76	0,85	-0,99	1,00	1,00	-1,00	0,95
Actinomyces	-0,33	0,95	0,81	–	-1,00	0,98	-0,88	0,84	0,79	-0,77	0,35
Bacteria which are using mineral forms of nitrogen	0,72	-0,97	-0,76	-1,00	–	-0,56	0,84	-0,80	-0,74	0,68	-0,62
Anaerobic bacteria	-0,55	0,86	0,85	0,98	-0,56	–	-0,96	0,34	0,60	-0,90	0,35
Aerobic nitrogen fixing bacteria	0,46	-0,68	-0,99	-0,88	0,84	-0,96	–	-1,00	-0,48	0,99	-0,49
Anaerobic nitrogen fixing bacteria	-0,77	0,62	1,00	0,84	-0,80	0,34	-1,00	–	1,00	-1,00	0,97
Oligotrophic bacteria	-0,25	0,55	1,00	0,79	-0,74	0,60	-0,48	1,00	–	-0,44	0,52
Oligonitrophic bacteria	1,00	-0,50	-1,00	-0,77	0,68	-0,90	0,99	-1,00	-0,44	–	-0,64
Pedotrophic bacteria	-0,92	0,79	0,95	0,35	-0,62	0,35	-0,49	0,97	0,52	-0,64	–

The structure of microbial communities of the soil is interrelated with the presence of antibiotic-resistant pathogenic microorganisms. In the soil of agroecosystems where the number of pedotrophes and oligotrophes was higher, a greater number of antibiotic-resistant microorganisms were isolated. *Clostridium perfringens* (resistant to erythromycin, clindamycin, tetracycline, rifampicin, amoxicillin, moderately sensitive to metronidazole and vancomycin sensitive), *Clostridium oedematiens* (moderately susceptible to amoxicillin and vancomycin), *Clostridium difficile* (sensitive to metronidazole). Nevertheless, the enrichments of *Clostridium* in soil which was continually treated with manure containing can be dangerous for public health. The enrichment of these bacteria, which are phylogenetically closely related to human pathogens, may improve the chance of transferring antibiotic resistance genes to human pathogens, since horizontal gene transfer is more prevalent between closely related organisms than between those distantly related (Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, et al., 2012; Symochko L., 2019).

Soil particles carrying viable bacteria can be transported over long distances and might contribute to the spreading of antibiotic resistant bacteria over wide geographic ranges. A significant number of aerobic microorganisms with

multiple antibiotic resistances were isolated from the agroecosystem, all of them are the causative agents of foodborne infections. The soil microbiome plays an important role in the development and spread of antibiotic resistance in humans.

CONCLUSION

Structure of soil microbiome of terrestrial ecosystem depends from anthropogenic impact. Natural ecosystems characterized by high functional biodiversity and stability and low number of AR bacteria, humans pathogens. The taxonomic structure of the microbial community has been determined by biochemical markers and showed a significant difference between level of transformation and soil biodiversity of ecosystems. The screening of conditionally pathogenic and pathogenic microorganisms of soil microbiome has proved that terrestrial ecosystems are the source of the spread of pathogenic and opportunistic antibiotic-resistant microorganisms. In total from 468 bacteria were isolated 79 which characterized by high level of antibiotic resistance. All of them are pathogenic or conditionally pathogenic for human and can cause food borne diseases.

REFERENCES

1. Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., & Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, 8(4), pp. 251-259. doi: 10.1038/nrmicro2312;
2. Aminov R. (2007). Evolution and ecology of antibiotic resistance genes. *FEMS Microbiol Lett.*, Vol. 271, pp.147–161;
3. Boxall, A. B., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E., & Levy, L. S. (2006). Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry*, 54(6), pp. 2288-2297. doi: 10.1021/jf053041t;
4. Cavigelli, M. A., & Robertson, G. P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, Vol.81, no 5, pp. 1402-1414;
5. Cavigelli, M. A., & Robertson, G. P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, 81(5), pp. 1402-1414. doi: 10.2307/177217;
6. Chee-Sanford, J. C., Aminov, R. I., Krapac, I. J., Garrigues-Jeanjean, N., & Mackie, R. I. (2001). Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Applied and environmental microbiology*, 67(4), pp. 1494-1502. doi: 10.1128/aem.67.4.1494-1502.2001;
7. Christian, T., Schneider, R. J., Färber, H. A., Skutlarek, D., Meyer, M. T., & Goldbach, H. E. (2003). Determination of antibiotic residues in manure, soil, and surface waters. *CLEAN–Soil, Air, Water*, 31(1), pp. 36-44;
8. Diao, X., Sun, Y., Sun, Z., & Shen, J. (2004). Effects of Apramycin on microbial activity in different types of soil. *Ecology and Environment*, 13(4), pp. 565-568;
9. Díaz-Cruz, M. S., de Alda, M. J. L., & Barcelo, D. (2003). Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *TrAC Trends in Analytical Chemistry*, 22(6), pp. 340-351. doi:10.1016/s0165-9936(03)00603- 4;
10. Ebert, I., Bachmann, J., Kühnen, U., Küster, A., Kussatz, C., Maletzki, D., & Schlüter, C. (2011). Toxicity of the fluoroquinolone antibiotics enrofloxacin and ciprofloxacin to photoautotrophic aquatic organisms. *Environmental Toxicology and Chemistry*, 30(12), pp. 2786-2792. doi:10.1002/etc.678;
11. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MOA, et al. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. *Science*, Vol. 337, pp. 1107–1111;
12. Goldman, E., & Green, L. H. (Eds.). (2015). *Practical handbook of microbiology*, Third Edition. doi:10.1201/b17871;

13. Hammesfahr U, Heuer H, Manzke B, Smalla K, Thiele-Bruhn S. (2008). Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biol Biochem*, Vol.40, pp.1583–1591;
14. Heuer H, Schmitt H, Smalla K. (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol*, Vol.14, pp. 236–243;
15. Heuer H, Smalla K. (2007). Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environ Microbiol*, Vol. 9, pp. 657–666 ;
16. Keen, P. L., & Patrick, D. M. (2013). Tracking Change: A look at the ecological footprint of antibiotics and antimicrobial resistance. *Antibiotics*, 2(2), pp.191-205. doi:10.3390/antibiotics2020191;
17. Kümmerer, K. (2004). Resistance in the environment *J Antimicrob Chemother* 54 (2): pp. 311–320. doi:10.1093/jac/dkh325;
18. Lyudmyla Symochko (2019), “Food Safety in Agroecosystems-Soil Resistome”, 13th Paris International Conference (PACBES-19) Conference Proceedings, Paris, France, pp. 99-102;
19. Lyudmyla Symochko, Ruslan Mariychuk, Olena Demyanyuk, Vitaliy Symochko (2018). “Ecological effects of antibiotics on microbial community – soil resistome”. Abstract Book International Conference «Smart Bio» – 3-5 May, Kaunas, Lithuania, P. 43;
20. Martínez, J. L. (2008). Antibiotics and antibiotic resistance genes in natural environments. *Science*, 321(5887), pp. 365-367. doi:10.1126/science.1159483 ;
21. Migliore, L., Civitareale, C., Brambilla, G., & Di Delupis, G. D. (1997). Toxicity of several important agricultural antibiotics to *Artemia*. *Water Research*, 31(7), pp. 1801-1806. doi:10.1016/s0043-1354(96)00412-5;
22. Mikola, J., & Setälä, H. (1998). No evidence of trophic cascades in an experimental microbial based soil food web. *Ecology*, 79(1), pp.153-164. doi:10.2307/176871;
23. Murray, B. E. (1996). Antibiotic resistance. *Advances in internal medicine*, 42, pp.339-367;
24. Pruden, A., Pei, R., Storteboom, H., & Carlson, K. H. (2006). Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environmental Science & Technology*, 40(23), pp.7445-7450. doi:10.1021/es060413l;
25. Schmitt, H., Van Beelen, P., Tolls, J., & Van Leeuwen, C. L. (2004). Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine. *Environmental science & technology*, 38(4), pp.1148-1153. doi:10.1021/es034685p;
26. Shyrobokov V. P. (2011). *Medical Microbiology, Virology, and Immunology*. Nova Knyha, Vinnytsia (in Ukrainian);
27. Symochko L. (2017) Assessment of sorption and toxicity of fluoroquinolone antibiotic in agroecosystems. *Agroecological journal*, (3), pp.147–151;
28. Symochko L. Hamuda H.B. (2015). Microbial monitoring of soil as additional tools for conservation biology. *Obuda University e-Bulletin*, 5(1), pp.177–185;
29. Symochko L., Meleshko T., Symochko V., Boyko N. (2018). Microbiological control of soil-borne antibiotic resistance human pathogens in agroecosystems. *International Journal of Ecosystems and Ecology Sciences*, Vol. 8 (3), pp. 591-598. <https://doi.org/10.31407/ijeecs8320>;
30. Thiele-Bruhn, S. (2003). Pharmaceutical antibiotic compounds in soils—a review. *Journal of Plant Nutrition and SoilScience*, 166(2), pp. 145-167;
31. Torsvik V, Qvreaås L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Curr Opin Microbiol*, Vol.5, pp. 240–245;
32. Van der Heijden M.G.A., Bardgett R.D., Van Straalen N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.*, 11, pp. 296–310;
33. Wright, G. D. (2007). The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, 5(3), pp. 175-186. doi:10.1038/nrmicro1614;
34. Wright, G. D. (2010). The antibiotic resistome. *Expert opinion on drug discovery*, 5(8), 779-788. doi:10.1517/17460441.2010.497535;