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# SUSTAINABILITY AND ENVIRONMENTAL RESISTOME: CHALLENGES AND WAYS OF SOLUTIONS

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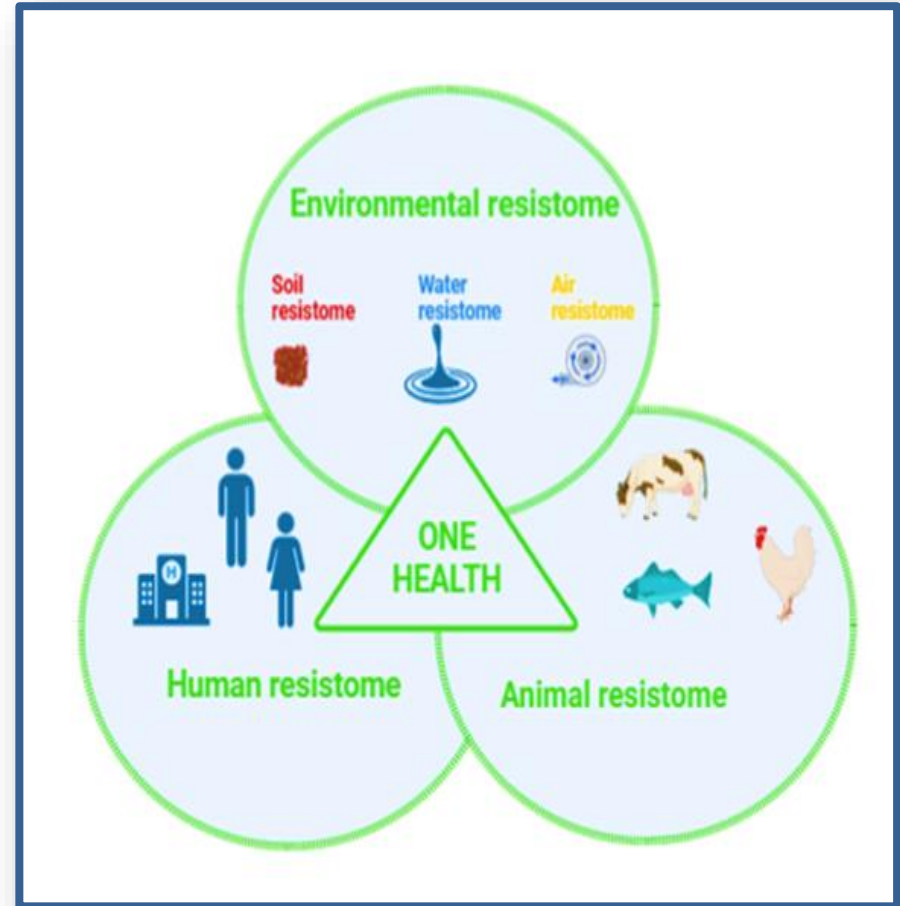


**June 9-10, 2023, Chicago, Illinois, USA  
(online)**

**Essays on Ecosystem  
and Environmental Research**



- The "resistome" concept, introduced in 2006, and refers to the collection of all antibiotic resistance genes (ARGs) present in a particular environment, such as soil, water, or the human body. It encompasses both the genetic potential for resistance and the actual expression of resistance in microbial communities.
- The resistome is primarily shaped by the microbial community structure in the natural environment, but anthropogenic activities play a significant role also in this process.
- These findings have led to the development of the "One-Health" approach.





- Food security is associated with the social determinants of health, which are the conditions in which people are born, grow, live, work.
- Food security in the context of social determinants of health is interconnected with political instability, economic development, urbanization, agricultural policy, and environmental factors such as land use and climate change.
- At the same time, food security is one of the important factors that can determine nutritional outcomes.
- **Antibiotic resistant microorganisms in agricultural production systems and food chain are major public health challenge.**



# GFSI – AN EFFECTIVE WAY OF ASSESSING THE COUNTRY'S POTENTIAL

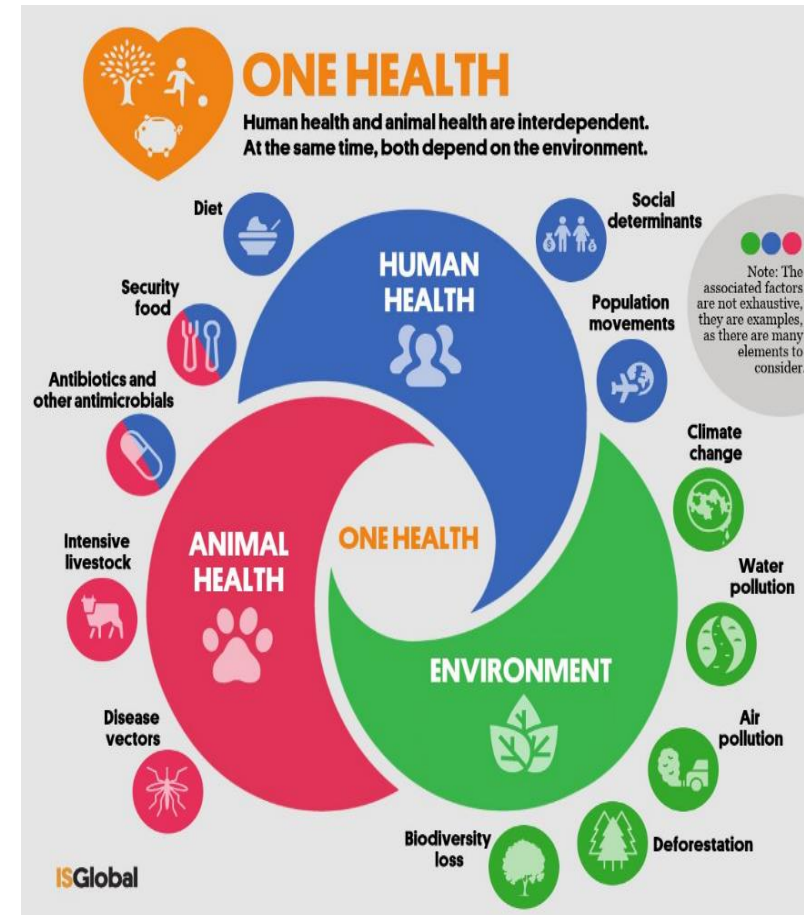
- Several factors influence the food security of the country:
- production volumes,
- weather conditions,
- geographical location.
- In 2012 The Economist Intelligence Unit introduced the Global Food Security Index which until recently consisted of 4 main parameters:
- quality,
- safety,
- financial and physical accessibility of food,
- natural resources and sustainability.

In the general rating Ukraine was ranked (71; 2022) (63; 2021) among 113 countries.

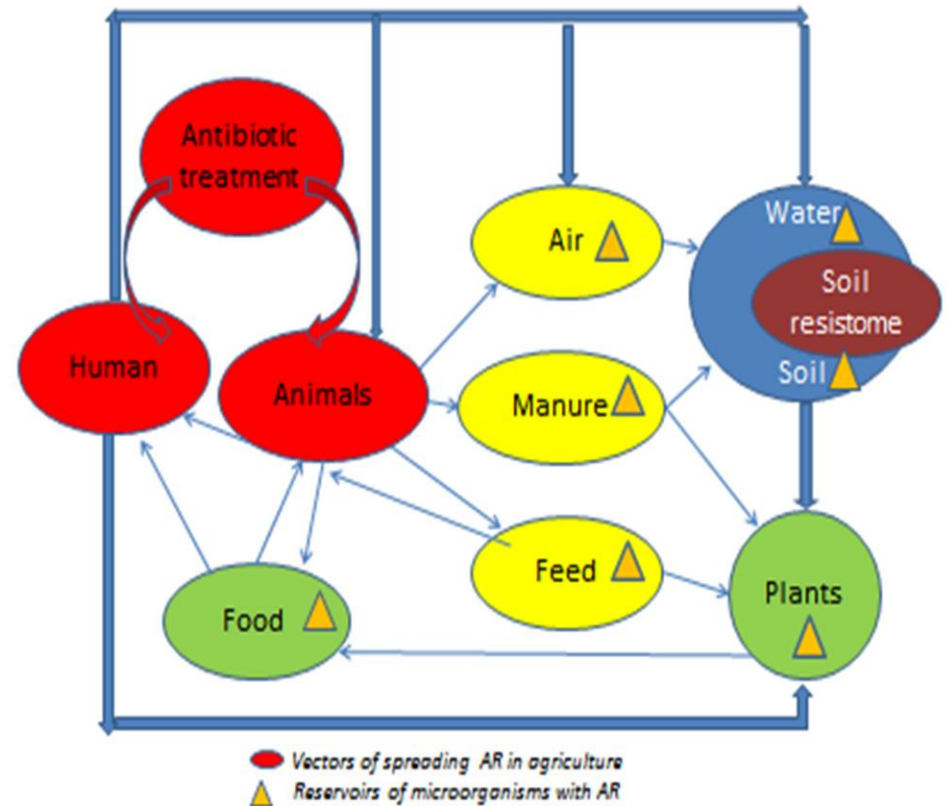
Portugal (10th; 2022);

Spain (15th; 2022).

- <https://foodsecurityindex.eiu.com/Index>



- The World Health Organization has identified antibiotic resistance as a serious threat to human health across the world.
- Soils to providing humanity with 98.8% of its food. The soil microbiome plays an important role in the development and spreading of antibiotic resistance in humans.
- One of the biggest sources of ARM is modern agroecosystems.





the World Health Organization (WHO) published a list of pathogens for which new effective treatment was urgently needed in February 2017. This broad list contains a group of pathogenic bacteria under “priority status” and named “**ESKAPE**”

**ESKAPE** pathogens are differentiated from other pathogens due to their increased resistance to commonly used antibiotics such as penicillin, vancomycin, carbapenems and more.

**ESKAPE** is an acronym that stands for :

*E = Enterococcus faecium,*

*S = Staphylococcus aureus,*

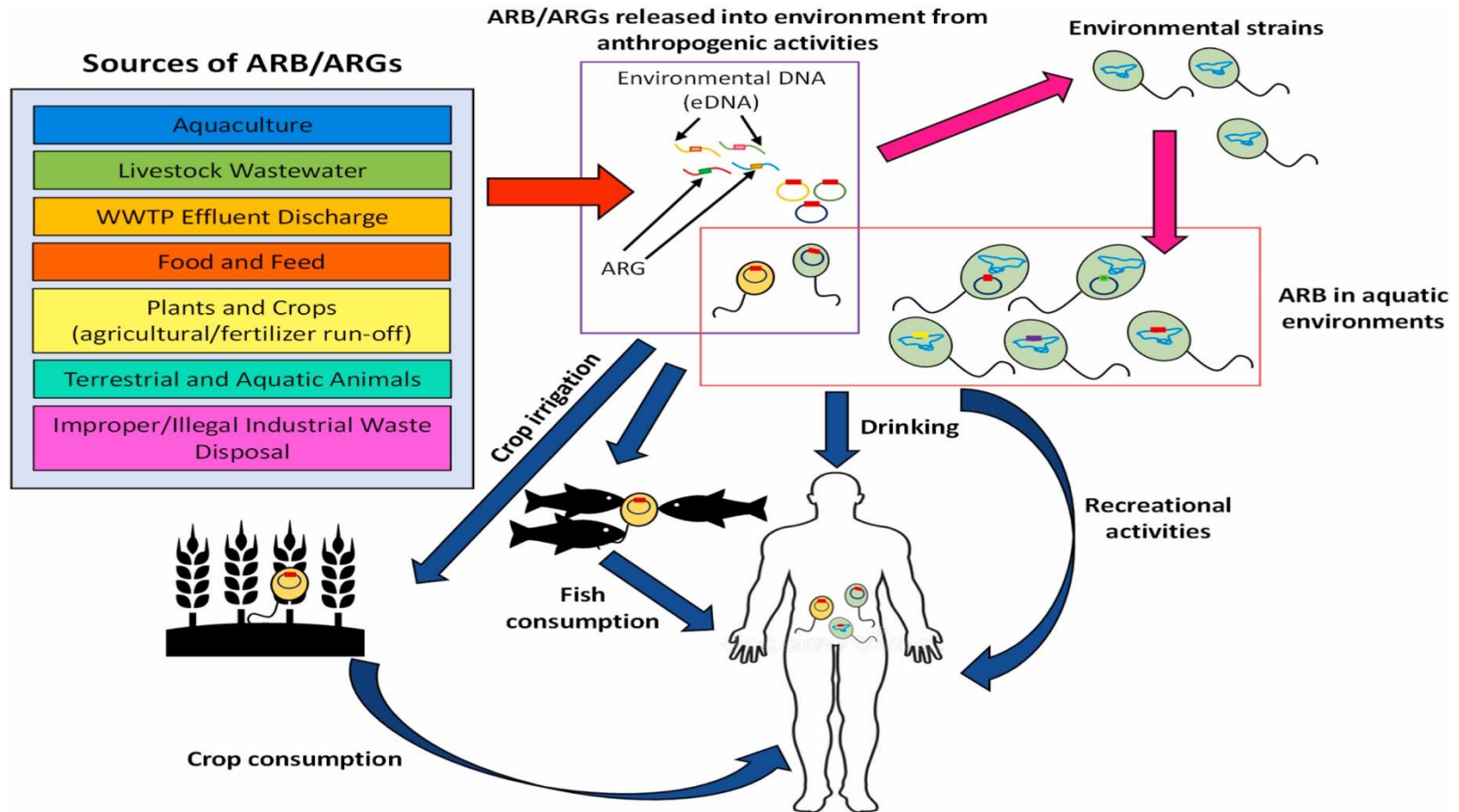
*K = Klebsiella pneumoniae,*

*A = Acinetobacter baumannii,*

*P = Pseudomonas aeruginosa, and*

*E = Enterobacter species.*

## ESKAPE pathogens in the environment



## Aims of the contribution:

The soil and water microbiome play an important role in the development and spread of antibiotic resistance in humans. From 2016 to 2021, various types of ecosystems were monitored for the presence of ARM including ESKAPE pathogens.

"Ecological and microbiological monitoring of different types ecosystems in Carpathian region" NSR No. 0116U003331, (2016-2020);

"Biomonitoring of terrestrial and aquatic ecosystems in conditions of climate change NSR No. 0119U102623, (2020-2023).

## Materials and methods:

- Materials of research were soil samples, which had been collected from different agroecosystems: *Capsicum annuum*, *Vitis vinifera*, *Rubus idaeus L.*, *Petroselinum crispum* where used only organic manure. Water samples from the river Uzh.
- **Microbiological studies** of soil were carried out at the Scientific Research and Educational Center of Molecular Microbiology and the Immunology of Mucous Membranes following the standard protocols.
- 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 following EUCAST recommendations with the aim to find antibiotic resistant strains of pathogenic microorganisms.

To determine the antibiotic resistance of microorganisms, we selected 12 antibiotics of the main pharmacological groups. (Metronidazole MT5 Rifampicin RIF5 Clindamycin CD2 - additionally tested antibiotics to anaerobic microbiota).

**Results were expressed** as means ( $\pm$ ) standard deviation (SD) and ( $SSD_{05}$ ) smallest significant differences of experiments conducted in quadruplication. Data were evaluated using the software Statistica 10.0.





# ARM isolated from the soil 2016

№	<i>Plant.sp</i>	<b>ARM</b> <i>(Total 153/73 ESKAPE)</i>
1	<i>Capsicum annuum</i> (22)	<i>Enterococcus faecalis, Yersinia enterocolitica, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa.</i>
2	<i>Vitis vinifera</i> (46)	<i>Clostridium perfringens, Clostridium difficile, Escherichia coli, Enterococcus faecalis, Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Enterobacter cloacae, Serratia fonticola, Enterococcus faecium, Pantoea agglomerans, Hafnia alvei, Acinetobacter baumannii, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae.</i>
3	<i>Rubus idaeus L.</i> (54)	<i>Clostridium perfringens, Clostridium oedematiens, Clostridium difficile, Staphylococcus aureus, Enterobacter cloacae, Enterococcus faecalis, Pseudomonas aeruginosa, Hafnia Alvei, Bacillus megaterium, Enterococcus faecium Bacillus mycoides, Bacillus subtilis, Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Acinetobacter baumannii, Staphylococcus aureus.</i>
4	<i>Petroselinum crispum</i> (31)	<i>Bacillus megaterium, Bacillus licheniformis, Enterococcus faecium, Hafnia alvei, Bacillus cereus, Enterococcus faecalis, Staphylococcus aureus.</i>



# ARM isolated from the soil 2021



№	<i>Plant.sp</i>	<i>ARM</i> (Total 341/162 ESKAPE)
1	<i>Capsicum annum</i> (42)	<i>Enterococcus faecalis, Yersinia enterocolitica, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Acinetobacter baumannii, Enterococcus faecium, Bacillus mycoides.</i>
2	<i>Vitis vinifera</i> (120)	<i>Clostridium oedematiens, Clostridium perfringens, Clostridium difficile, Escherichia coli, Enterococcus faecalis, Bacillus megaterium, Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Klebsiella pneumoniae, Enterobacter cloacae, Serratia fonticola, Enterococcus faecium, Pantoea agglomerans, Hafnia Alvei, Pseudomonas aeruginosa, Staphylococcus aureus.</i>
3	<i>Rubus idaeus L.</i> (128)	<i>Clostridium perfringens, Clostridium oedematiens, Clostridium difficile, Enterobacter cloacae, Enterococcus faecalis, Hafnia Alvei, Acinetobacter baumannii, Bacillus megaterium, Bacillus mycoides, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus, Escherichia coli, Enterococcus faecium.</i>
4	<i>Petroselinum crispum</i> (51)	<i>Escherichia coli, Bacillus megaterium, Bacillus licheniformis, Hafnia alvei, Bacillus cereus, Acinetobacter baumannii, Enterococcus faecalis, Enterococcus faecium</i>



## ESKAPE pathogens in the water ecosystem 2016/2021

ESKAPE Pathogen	Environmental Reservoir of Isolation	Number of Isolates		Antibiotic Resistance Profile (Percentage of resistant isolates)
		Total 35	Total 58	
<i>Enterococcus faecium</i>	River Uzh	$n = 7$	$n = 12$	Resistance to gentamicin (90.3%), penicillin (78.3%), erythromycin (64.4%), amoxicillin (60.5%), nitrofurantoin (55.5%), ampicillin (53.7%), and tetracycline (49.7%)
<i>Klebsiella pneumonia</i>	River Uzh	$n = 4$	$n = 5$	Resistance to ampicillin (77,1%), amoxicillin (66,4%), ciprofloxacin (63,5%), gentamicin (60,4%), amikacin (58.5%).
<i>Enterobacter spp.</i> <i>Enterobacter cloacea</i>	River Uzh	$n = 9$	$n = 12$	Resistance to $\beta$ -lactam antibiotics (82,3%), amikacin (75,4%), levofloxacin (70,4%), gentamicin (68,2%)
<i>Escherichia coli</i>	River Uzh	$n = 15$	$n = 29$	quinolones (59,3%). All isolates (100%) susceptible to imipenem and meropenem.



## ESKAPE pathogens in the agroecosystems 2016/2021

ESKAPE Pathogen	Environmental Reservoir of Isolation	Number of Isolates Total 73	Isolates Total 162	Antibiotic Resistance Profile (Percentage of resistant isolates)
<i>Enterococcus faecium</i>	agroecosystem	$n = 21$	$n = 42$	Resistance to gentamicin (89.3%), penicillin (86.9%), erythromycin (71.4%), amoxicillin (63.5%), nitrofurantoin (61.7%), ampicillin (58.9%), and tetracycline (47,9%)
<i>Staphylococcus aureus</i>	agroecosystem	$n = 18$	$n = 35$	Methicillin resistant isolates (4 VV), resistance to clindamycin (98,1%), erythromycin (88,2%), rifampin (64,2%), and vancomycin (58,9).
<i>Klebsiella pneumonia</i>	agroecosystem	$n = 4$	$n = 7$	Resistance to ampicillin (79,1%), amoxicillin (71,5%), ciprofloxacin (68, 3%), gentamicin (63,7%), amikacin (62.8%).
<i>Acinetobacter baumannii</i>	agroecosystem	$n = 4$	$n = 9$	Resistance to cefepime (93,6%), ceftazidime (88,4%), amikacin (81,2%), imipenem (77,3%), ciprofloxacin (75,4%), gentamicin (74,1%), levofloxacin (70,2%), ampicillin (66,7%).
<i>Enterobacter spp.</i> <i>Enterobacter cloacea</i>	agroecosystem	$n = 10$	$n = 23$	Resistance to $\beta$ -lactam antibiotics (88,9%), amikacin (76,4%), levofloxacin (73,3%), gentamicin (65,2%) quinolones (60,9%). All isolates (100%) susceptible to imipenem and meropenem.
<i>Escherichia coli</i>	agroecosystem	$n = 16$	$n = 46$	



## CONCLUSIONS

- The wide spreading of antibiotic resistant microorganisms (ARM) connected to increased amount of infectious and no-communicable human diseases and this is a multidisciplinary problem.
- The results of monitoring studies have shown that exogenous factors cause formation of environmental resistome and the spread of antibiotic-resistant microorganisms. Number of antibiotic resistant bacteria during 5 years increased in 2,28 times, and ESKAPE pathogens in 2,21 times.
- Modern agroecosystems are the source of spreading of pathogenic and opportunistic microorganisms including ESKAPE pathogens with multiple antibiotic resistances and endangering human health.
- This reserch highlight the urgent need for better monitoring and management of ESKAPE pathogens in various ecosystems. These pathogens can persist in different environmental settings, increasing the risk of transmission and exposure to humans and animals.





- To prevent the spread of these bacteria, proper agricultural practices, the correct disposal of medical waste, and effective water treatment are essential. It is also crucial to reduce the overuse of antibiotics in healthcare and agriculture.
- Management of soil microbiota is vitally important for safeguarding public health. The soil and water microbiome plays an important role in the development and spread of antibiotic resistance in humans.
- The risk to antibiotic resistance exposure via products should be better managed. It's necessary to implement tools for the protection of bioproduction from contamination by pathogenic microorganisms.
- Monitoring of antibiotic resistance in ecosystems is an additional tool for controlling and managing biosecurity which is very important for the implementation «One Health» concept.



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Thank you for your attention!

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