

## DIRTY ENVIRONMENTS AND ANTIBIOTICS RESISTANCE

**CÎRSTEA Doina Maria, ȘTEFĂNESCU Mugur Cristian**

**Abstract.** Studies on the resistance to antibiotics are carried out mainly on bacterial strains from clinical environments. Resistance reservoirs from natural environments or polluted environments have not been sufficiently studied, as they are a genuine source of maintenance of the phenomenon of resistance as well as its origin. Antibiotic resistance is present in the world of microorganisms before antibiotics appear, this was stimulated by the presence in the environment of various substances that led to the emergence and development of resistance mechanisms. Such pollutants are the stress factors for microbiota area, leading to the development of defence strategies against pollutant, strategies that can be used as efficient mechanisms of antibiotic resistance phenomenon. The present study was aimed to signal the importance dirty environments of the antibiotic resistance phenomenon.

**Keywords:** antibiotic resistance, pollutant, microorganism.

**Rezumat. Mediile poluate și rezistența la antibiotic.** Studiile asupra rezistenței la antibiotice sunt frecvente, efectuate mai ales pe tulpini bacteriene provenite din medii clinice. Rezervoarele de rezistență din mediile naturale, respectiv poluate nu au fost suficient studiate, acestea constituind o reală sursă de întreținere a fenomenului de rezistență cât și originea acestuia. Antibioresistență este prezentă în lumea microorganismelor înainte de apariția antibioticelor, aceasta a fost stimulată de prezența în mediu a diferitelor substanțe ce au condus la apariția și dezvoltarea mecanismelor de rezistență. Astfel, poluanții sunt factorii de stres pentru microbiota zonală, ce conduc la dezvoltarea unor strategii de apărare contra poluantului, strategii ce pot fi utilizate ca mecanisme eficiente în fenomenul de antibioresistență. Prin prezentul studiu s-a urmărit semnalarea importanței mediilor murdare în fenomenul de rezistență la antibiotic.

**Cuvinte cheie:** antibioresistență, poluant, microorganism.

### INTRODUCTION

Antibiotic resistance represents the ability of microorganisms (bacteria, fungi, etc.) to survive and multiply in the presence of antibiotic doses, frequently a lethal dose (ZAMAN et al., 2017; DUGASSA & SHUKURI, 2017).

The uncontrolled use of antibiotics over the last 80 years and the increase in pollution have exerted a major impact on bacterial communities, leading to the selection of antibiotic-resistant bacteria, which are a major health problem (LEVY, 1992), so studying the phenomenon of antibiotic resistance is important for both human health and the environment.

Much of the specialty studies focus on discovering and understanding of the resistance mechanisms, which are carried out on bacterial strains in clinical and veterinary isolated, whereas other environmental reservoirs of antibiotic resistance are not well characterized (NWOSU, 2001; SEVENO et al., 2002; RIESENFIELD et al., 2004).

### ORIGIN OF ANTIBIOTIC RESISTANCE

The origin of antibiotic resistance has not been established and will most likely not be, but antibiotic resistance is known to have occurred before Penicillin was discovered by Alexander Fleming in 1928. The assertion that antibiotic resistance existed prior to the therapeutic use of antibiotics, as the first patient was treated with penicillin in 1942, supports the discovery of penicillinas (β-lactamases) in 1940 (DAVIES et al., 2010). Moreover, in the history of antibiotics, one can notice the discovery of resistance mechanisms specific to certain antibiotics almost simultaneously with their introduction into the clinical environment. Thus, in 1944 streptomycin was introduced for the treatment of Tuberculosis, during the treatment of the first patients, a bacterial strain of *Mycobacterium tuberculosis* proving to be resistant to the antibiotic concentrations used (DAVIES & DAVIES, 2010).

Resistance mechanisms are present in natural environments prior to the emergence and use of antibiotics, they have emerged as a result of the selective pressure of some favoured agents, which probably evolved in bacterial populations in water and soil in millions of years. (MARTINEZ, 2012). Resistance has emerged as a way of defending against toxic compounds present in the environment, such as plant metabolites and microbiota from soil or pollutants.

### SELECTIVE PRESSURE OF EXTERNAL FACTORS ON THE OCCURRENCE, MAINTENANCE AND TRANSMISSION OF ANTIBIOTIC RESISTANCE PHENOMENON

Both pathogenic and non-pathogenic microorganisms are pressured by the selective exercise of natural, but especially anthropogenic factors (Fig. 1), we can talk about a wide variety of toxins or inhibitory molecules found in the environment.

Stress factors can be representations of many sources, such as: herbal products, natural and synthetic antibiotics, produced as a result of organic degradation. Biocides secreted by insect plants and fungal compounds that inhibit the growth of bacteria in the rhizosphere. A selective pressure factor is also lysozyme from the nasal secretion, which favoured the selection of antibiotic-resistant strains.

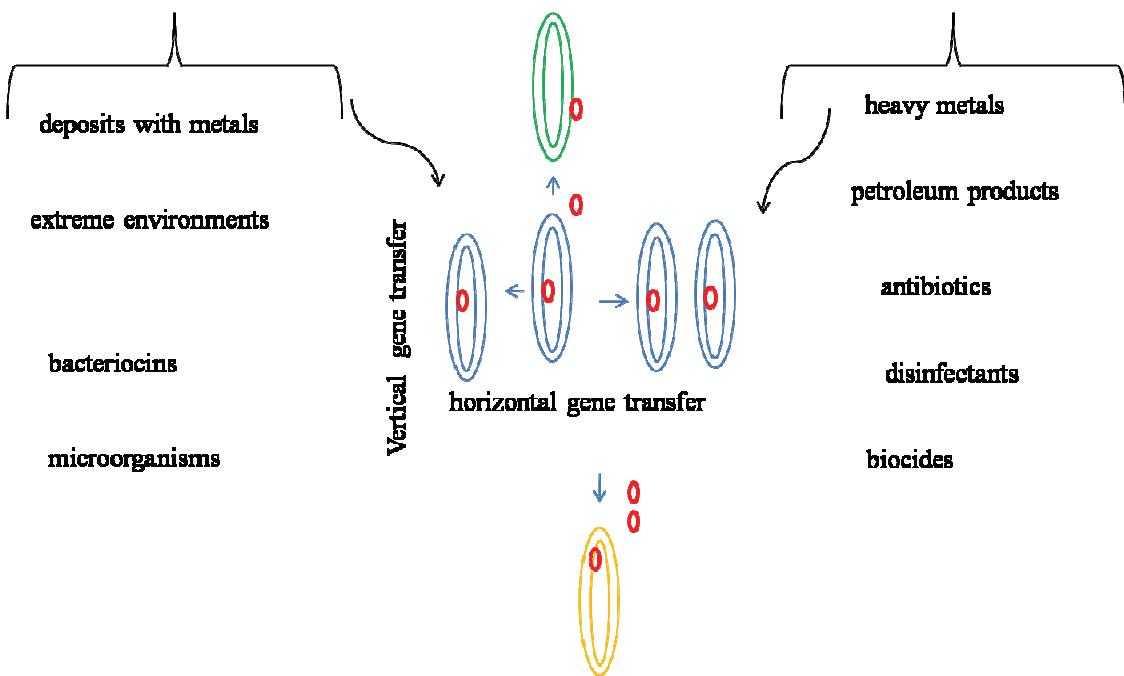


Figure 1. Factors for the occurrence, maintenance and transmission of antibiotic resistance phenomenon (original).

These factors can lead to mutations (GULLBERG et al., 2014; LEEKITCHAROENPHON et al., 2016; DIARMAID & ANDERSSON, 2017), with effect on the resistance to different antibiotics. These mutations can be maintained within the bacterial line that appears, horizontal gene transfer, but may be transmitted to other species by vertical gene transfer.

Thus, antibiotic resistance genes are ubiquitous in natural environments (AMINOV & MACKIE, 2007; MARTINEZ, 2009; ALLEN et al., 2010), and soil bacteria are a reservoir of new antibiotic resistance genes (RIESENfeld et al., 2004). These stress factors cause the emergence of resistance mechanisms, implicitly resistance genes.

#### POLLUTED MEDIA AND ANTIBIOTIC RESISTANCE

Antibiotic resistance is due to chromosomal genes, mobile DNA segments (transposons, integrated by genetic recombination or resulting by mutation of normal, integron genes, but most often given by the presence of the plasmids. These genetic determinants can lead to multiple resistance to antibiotics, which facilitates the spread of resistance in natural bacterial populations. In some cases, plasmid-induced resistance has specific molecular mechanisms, which are fundamentally different from those of chromosomal-induced resistance. In most cases, multiple antibiotic resistance is provided by efflux systems (RAMOS et al., 2002).

PUTMAN et al., 2000 described two major classes of transporters that are involved in multiple antibiotic resistance: the ABC transporter that uses energy released by ATP hydrolysis, to pump antibiotics from inside the cell to the outside, and the secondary transporter that uses the gradient electrochemical transmembrane of H<sup>+</sup> or Na<sup>+</sup> ions, to pump antibiotics from inside the cell to the outside.

Some mechanisms of multiple antibiotic resistance have been observed in some bacteria, functioning as hydrocarbon excretion systems, used to maintain the intracellular concentration of hydrocarbons below the equilibrium level. These systems involve an efflux pump, which also contributes to tolerance to n-peptane, cyclohexane, toluene, phenanthrene, anthracene (RAMOS et al., 2002; HEARN et al., 2003).

Since 1999, KOBAIASHI et al. have demonstrated the connection between toluene efflux pumps and antibiotic resistance efflux pumps. They obtained a mutant of the *Pseudomonas putida* strain IH-2000, a toluene-tolerant strain. In the mutant strain, some of the outer membrane proteins were lost, including the Srp protein, which is a channel protein of the toluene efflux pump. Thus, the decrease of the tolerance to hydrocarbons, but also the reduction of the minimum inhibitory concentration (CMI) to antibiotics was found, from which it was concluded that the efflux pump systems are linked or shared with the efflux pump system involved in antibiotic resistance.

MARTINEZ, 2009; 2012 considers that antibiotic resistance is similar in many aspects to the contamination with heavy metals.

WRIGHT et al., 2008, believes that bacteria isolated from polluted environments with heavy metals have high antibiotic resistance.

In 2009 THOMPSONET et al., highlights the presence of the *tet A* gene, a gene that confers tetracycline resistance to a strain of *Sratia marcasicens*, a strain isolated from heavy metal water.

Thus it is possible to speak of the selective pressure of the pollutants on the zone microbiota (DERORE et al., 1994; D'COSTA et al., 2006), conferring a simultaneous resistance to both the pollutant and the antibiotic.

## CONCLUSIONS

The genetic support underlying the phenomenon of antibiotic resistance has been present in nature long before the finding of this phenomenon in the clinical environments.

The mechanisms involved in resistance may be common or similar to the mechanisms involved in pollutant removal.

Pollutants can be considered as selective pressure factors in the phenomenon of resistance.

## ACKNOWLEDGMENT

This work was funded by the *The antibioresistance and virulence phenomenon in some bacterial strains isolated from polluted environments* project.

## REFERENCES

- ALLEN H. K., DONATO J., WANG H. H., CLOUD-HANSEN K. A., DAVIES J. E., HANDELSMAN J.. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Natural Revue Microbiology*. Elsevier. Paris. **8**: 251-259.
- AMINOV R. I. & MACKIE R. I. 2007. Evolution and ecology of antibiotic resistance genes. *FEMS Microbiology Letter*. Elsevier. Paris. **271**:147-161.
- D'COSTA, V. M., MCGRANN K. M., HUGHES D. W., WRIGHT G. D. 2006. Sampling the antibiotic resistome. *Science*. Springer. Berlin. **311**: 374-377.
- DAVIES J. & DAVIES D. 2010. Origins and Evolution of Antibiotic Resistance. *Microbiology and molecular biology reviews*. Elsevier. Paris: 417-433.
- DERORE H., TOP E., HOUWEN F., MERGEAY M., VERSTRAEET W. 1994. Evolution of Heavy-Metal Resistant Transconjugants in a Soil Environment with a Concomitant Selective Pressure. *Fems Microbiology Ecology*. Elsevier. Paris. **14**(3): 263-273.
- DIARMAID H. & ANDERSSON D. I. 2017. Evolutionary Trajectories to Antibiotic Resistance. *Annual Review of Microbiology*. American Society for Microbiology Publisher. New York. **71**: 579-596.
- DUGASSA J. & SHUKURI N. 2017. Review on antibiotic resistance and its mechanism of development. *Journal of Health, Health, Medicine and Nursing*. Academic Publisher. London. **3**(1): 1-17.
- GULLBERG E., ALBRECHT L. M., KARLSSON C., SANDEGREN L., ANDERSSON D. I. 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *mBio*. American Society for Microbiology Publisher. New York. **5**: 918-924.
- HEARN E. M., DENNIS J. J., GRAY M. R., FOUGHT J. M. 2003. Identification and characterization of the emhABC efflux system for polycyclic hydrocarbons in *Pseudomonas fluorescens* cLP6a. *Journal of Bacteriology*. University Press. London. **185**: 6233-6240.
- KOBAIASHI H., TAKAMI H., HIRAYAMA H., KOBATA K., USAMI R., HORIKOSHI K. 1999. Outer membrane changes in a toluene-sensitive mutant of toluene-tolerant *Pseudomonas putida* IH-2000. *Journal of Bacteriology*. University Press. London. **181**: 4493-4498.
- LEEKITCHAROENPHON P., HENDRIKSEN R. S., LE HELLO S., WEILL F. X., BAGGESEN D. L. 2016. Global genomic epidemiology of *Salmonella enterica* serovar Typhimurium DT104. *Applied Environmetal Microbiologie*. Elsevier. Paris. **82**:2516-2526.
- LEVY S. B. 1992. *The antibiotic paradox: How miracle drugs are destroying the miracle*. Plenum Press. New York. 181 pp.
- MARTINEZ J. L. 2009. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceedings Biological Science*. University Press. London. **276**: 2521-2530.
- MARTÍNEZ J. L. 2012. Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials, frontiers in Microbiology. *Research of Microbiology*. Elsevier. Paris. **3**: 12-25.
- NWOSU V. C. 2001. Antibiotic resistance with particular reference to soil microorganisms. *Research of Microbiology*. Elsevier. Paris. **152**: 421-430.
- PUTMAN M., VAN VEEN H. W., KONING W. N. 2000. Molecular properties of bacterial multidrug transporters. *Microbiology Molleculare Revue*. University Press. London. **64**: 672-693.

- RAMOS J. L., DUQUE E., GALLEGOS M. T., GODOY P., RAMOS-GONZALEZ M. I., ROJAS A., TEREN W., SEGURA A. 2002. Mechanisms of solvent tolerance in Gram-negative bacteria. *Annals Revue Microbiology*. Elsevier. Paris. **56**: 743-768.
- RIESENFIELD CH. S., GOODMAN R. M., HANDELSMAN JO. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environmental Microbiology*. Elsevier. Paris. **6**(9): 981-989.
- SEVENO N. A., KALLIFIDAS D., SMALLA K., VAN ELSAS J. D., COLLARD J. M., KARAGOUNI A. D., WELLINGTON E. M. H. 2002. Occurrence and reservoirs of antibiotic resistance genes in the environment. *Revue Medicine Microbiology*. Springer. Paris. **13**: 15-27.
- THOMPSON S. A., MAANI E. V., LINDELL A. H., KING C. J., MCARTHUR J. V. 2007. Novel tetracycline resistance determinant isolated from an environmental strain of *Serratia marcescens*. *Applied Environmental Microbiology*. Springer. Paris. **73**: 2199-2206.
- WRIGHT M. S., BAKER-AUSTIN C., LINDELL A. H., STEPANAUSKAS R., STIKES H. W., MCARTHUR J. V. 2008. Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *ISME Journal*. University Press. Paris. **2**: 417-428.
- ZAMAN S., HUSSAIN M., NYE R. 2017. A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus Journal of Medical Science*. University Press. London. **9**(6): 1403-1412.
- \*\*\*. <https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html> (accessed February, 2019).

**Cîrstea Doina Maria**

The Research Institute of the University of Bucharest, ICUB, Bucharest, Romania.

Institute of Biology Bucharest of the Romanian Academy, Spl. Independentei no. 296, sect. 6, 060031, Bucharest, Romania.  
E-mails: maria\_2na@yahoo.com, doina.cirstea@ibiol.ro

**Ștefănescu Mugur Cristian**

Institute of Biology Bucharest of the Romanian Academy, Spl. Independentei no. 296, sect. 6, 060031, Bucharest, Romania.  
E-mail: mugur.stefanescu@ibiol.ro

Received: April 10, 2019

Accepted: September 02, 2019