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Origins, transfer and accumulation of antibiotic resistance genes in the aquatic environment

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ABSTRACT

The development of antibiotic resistance has relevant implications for its impact on bacteria that can infect humans as well as for the impact on environmental microbiota. Uncontrolled and widespread use of antibiotics in human, veterinary medicine and farming had a major impact on bacterial communities, by selecting some antibiotic-resistant bacteria and dissemination of antibiotic resistance determinants in pathogenic bacterial populations. Resistance determinants are widely distributed in commensal and pathogenic bacteria and can be disseminated by one or more distinct gene transfer mechanisms. Considering that the emergence of bacterial strains resistant to antibiotics used in clinics is associated to the existence of resistant bacteria in the environment, it is important to increase the knowledge of the environmental reservoirs of resistance in order to identify risk factors for the diffusion of a resistance mechanism in a particular community, and thus to prepare for problems in the clinic in a proactive manner. This article summarizes literature information relating to types, distribution and mechanisms transfer of antibiotic resistance genes in different aquatic ecosystems and also describe the origins and the mechanisms of antibiotic resistance.

Keywords: *antibiotic resistance gene, aquatic environment, horizontal gene transfer, heavy metals*

1. INTRODUCTION

Studying the antibiotic resistance phenomenon has relevant implications for both human health and from ecological point of view. Uncontrolled and widespread use of antibiotics in human and veterinary medicine and farming had a major impact on bacterial communities, by selecting some antibiotic-resistant bacteria, which is a major problem in the effective treatment of bacterial infections [1].

The presence of antibiotic-resistant bacteria in aquatic ecosystems has been shown by numerous studies [2, 3, 4, 5, 6] as being a result of uncontrolled urban and farming wastewater discharge [7, 8, 9, 10, 11]. Even if these bacteria do not always present a direct risk factor for public health, they are added to the environmental resistance gene fund; these genes can be transferred to other bacterial species and genera, including potential human pathogens [12].

The emergence of antibiotic resistance is an evolutionary phenomenon, caused by selective pressure of environmental factors [13]. Genes encoding resistance factors have pre-existed at the moment of introducing antibiotics in human and veterinary medicine, and the use of antibiotics exerted a selection pressure for bacterial strains that had genetic antibiotic resistance determinants and natural

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ability to resist the antibiotic action. Many of these factors can be transferred and spread from one type of resistant bacteria to other bacteria, without genus and species limitations [12].

Resistance is the ability of an organism to grow in the presence of high levels of an antimicrobial agent. Bacteria have shown a remarkable ability to develop resistance to each new antibiotic used, often by surprising mechanisms that became functional very soon. Antibiotic resistant bacteria can survive and even multiply in the presence of therapeutic antibiotic concentrations [14].

During the stress, saprobiontic and pathogenic bacteria increase their mutation rate, i.e. become hyper-mutable. They express and duplicate their survival information, including antibiotic resistance genes located in plasmids, transposons and integrons [15]. On the other hand, bacteria have a high ability to retain their genetic material that confers a selective-evolutionary advantage and preserves the convenient mutations even in the presence of DNA repairing mechanisms, which tend to correct them. At international level, most studies betaken to the rising problem of antibiotic resistance are performed on strains isolated from clinics. Intensive use of antibiotics in human clinics and animal farming resulted in the emergence of a growing number of bacterial strains resistant to a higher number of antibiotics [16]. Considering that the emergence of bacterial strains resistant to antibiotics used in clinics is associated to the existence of resistant bacteria in the environment [17], it is important for research studies on antibiotic resistance to include environmental microorganisms.

2. ORIGINS OF ANTIBIOTIC RESISTANCE GENES

At the level of bacterial saprobiontic and pathogenic communities, there is a significant reserve of resistance genes for which two assumptions were made: - natural existing resistance genes, which probably evolved in thousands of millions of years within bacterial populations from soil and water, as protection means against the action of their own product or nonspecific defense mechanisms against existing toxic compounds in environment, such as metabolites in plants and soil microbiota. Thus, the actinomycetes that produce most aminoglycoside molecules used in clinics have resistance genes that confer them self-defence. Comparative molecular analysis of these resistance genes and their products to antibiotic-producing strains has shown their common origin. Confirmation of gene transfer from producers to pathogenic microorganisms was demonstrated by the fact that tetracycline-resistant mycobacteria have the same resistance genes as the tetracycline-producing *Streptomyces rimosus* [18]. Antibiotic resistance genes are often located witin the same restriction fragment together with genes for antibiotic production; - by mutations in constitutive genes that encode for specific enzymes in bacterial metabolism and have evolved by changing the substrate on which they work, modifying and inactivating antibiotics (e.g kinases and acetyltransferases that can modify aminoglycosides by phosphorylation and adenylylation processes).

The acquisition of antibiotic resistance is a stage process. The full resistance is not always conferred by an immediate change in the bacterial genome. The first stage is the tolerance or the bacterial ability to survive in the presence of the antibiotic, without following the growth and division. Resistance is installed when bacteria not only survive but multiply within the antibiotic environment. Tolerance increases the resistance development and tolerant strains often become resistant under selective pressure conditions [14].

The origin of antibiotic resistance genes of pathogenic bacteria is not clear. The time period, from the beginning of antibiotic therapy (50-60 years ago), up to the emergence of bacteria that express efficient resistance mechanisms is too short to explain the development of resistance factors in cellular proteins by spontaneous mutation. If a resistance mechanism requires a cooperative action of

several proteins (e.g, vancomycin resistance), de novo generation of a complex resistance mechanism of pathogenic bacteria is unlikely.

Most antibiotics are derived from metabolites of soil organisms, especially fungi and actinomycetes. All known resistance mechanisms to bacterial pathogens (RNA methylases, ABC transporters = ATP binding cassette, aminoglycoside phosphotransferases and β-lactamases) already exist in antibiotic producers. It was assumed that bacteria forming normal microbiota of the digestive tract can act as a reservoir of resistance genes that can be transferred to bacteria such as *Enterococcus faecalis*, a component of the normal colon microbiota and the *Staphylococcus aureus* and *Streptococcus* that colonize the oropharynx. The *tetO* gene was identified in some *Lactobacillus* strains that colonize the vaginal tract. These are the arguments that favour the hypothesis of horizontal transfer between members of normal microbiota and pathogenic bacteria, which can occur in certain body micro-niches [14].

Genes for resistance to β-lactam antibiotics were discovered before the discovery of antibiotics themselves. For example, it was found that a strain of *S. aureus* isolated before the discovery of antibiotics and kept within the collection, synthesizes the β-lactamase. It is considered that the lysozyme in nasal mucus exerted a selective pressure that favoured the selection of antibiotic-resistant cells [19].

Resistance may be natural (intrinsic) or acquired through resistance gene mutation or by acquisition of exogenous genes.

Natural resistance (intrinsic, constitutive) - is found in all strains of a particular species and its genetic support is the bacterial chromosome, being genetically carried to its offspring, by vertical transfer. For example, mycoplasma, bacteria lacking the cell wall, are naturally resistant to antibiotics that act at this level: (β-lactam); microorganisms with deficient transport systems (anaerobic bacteria) are intrinsically resistant to aminoglycosides [20].

Natural resistance of these species is usually due to low permeability of the cell membrane to antibiotics. Coating structures of the bacterial cell form natural permeability barriers that severely limit the free diffusion of molecules. Absence of cellular target for antibiotic action is a natural resistance cause. *Chlamydia* g. bacteria are not sensitive to the action of cell wall synthesis inhibitors (penicillins) because they do not have peptidoglycan, although they have the penicillin-binding enzymes (PBP) required for peptidoglycan synthesis [14].

Regarding Gram positive bacteria, the thick and relatively stiff peptidoglycan wall, does not confer protection against antibiotics. Antibiotics thet have smaller molecules than 1200 Da diffuse freely by the mureinic wall with a porosity of 1.1 nm. Most antibiotics can freely diffuse through the membrane because they are at least partially hydrophobic.

Outer membrane of Gram-negative bacteria is an effective protection barrier against small molecules such as antibiotics. Water molecules, O_2, CO_2 (smaller molecules than 0.8 nm), fat-soluble molecules (which are dissolved in lipids of the cell membrane, as tetracycline) and free-load organic molecules, up to glycerol size, get through passive diffusion.

Target inaccessibility due to some structure impermeability, as the external membrane of Gram-negative bacteria, is a passive resistance. But target inaccessibility may be an active process: for example, the activity of energy dependent efflux pumps, which remove the drug against a concentration gradient [14].

Acquired resistance – is translated by decrease or complete loss of antibiotic susceptibility of a bacterial strain.

Acquired antibiotic resistance can be determined by: - action of chromosomal genetic determinants; - excess DNA segments (transposons), integrated by recombination; - presence of antibiotic and multidrug resistance R plasmids.

Chromosome-mediated resistance is due to a mutation of either gene encoding antibiotic target, or the transportation system at membrane level, that controls antibiotic penetration [12].

It was initially assumed that resistance was acquired by spontaneous mutation. Mutation resistance is called primary resistance. Errors of replicative DNA synthesis and system inability for DNA repair lead to a spontaneous mutation frequency of a pair of bases/10^7- 10^{10}-cells, which means that for each of the 10^7- 10^{10} cells, only one base undergoes a change. But the rate of spontaneous mutation generating resistance is lower because multiple mutations have to occur for the emergence of primary resistance. For this reason, it was considered that the occurrence of antibiotic-resistant strains through mutational processes during therapy is unlikely. Although mutation is a rare event, the very fast rate of bacterial growth promotes relatively quickly, the resistance expression in a cell population. Once the mutational resistance gene was stabilized, it can be transferred directly to all the descending cells (vertical gene transfer).

Microevolutionary genetic changes (point mutations) can alter organism sensitivity to antibiotics by structural modification of the target. For example, some microorganisms change their β-lactamase enzyme by point mutation and thus the enzyme activity spectrum expands. Sulfonamide resistance is the consequence of a single amino acid change in the sequence of pteridine-synthetase enzyme. Similarly, a point mutation changes a ribosomal protein that confers resistance to streptomycin.

Macroevolutionary genetic changes involve the rearrangement of large DNA segments by transposition. If these changes involve movement and transfer of resistance genes and their passing under the control of a new promoter, the body might become resistant.

Cross-resistance means a phenomenon by which acquiring a single resistance mechanism confers resistance to an entire class of antibiotics. For example, resistance of S. aureus to meticillin confers resistance to all β-lactam antibiotics and cephalosporins. But cross-resistance is not always a rule: kanamycin-resistant *Pseudomonas aeruginosa* remains susceptible to similar antibiotics (gentamicin). If the resistance mechanism is a nonspecific one, such as efflux pumps, it may confer cross-resistance to a wide range of drugs.

Co-resistance means the simultaneous activity of several resistance mechanisms to the same organism [21].

Co-selection refers to the selection of multiple antibiotic resistance genes and it occurs when resistance genes are part of the same operon and therefore under the control of the same promoter [14].

3. TRANSFER MECHANISMS OF ANTIBIOTIC RESISTANCE GENES IN AQUATIC ENVIRONMENT

Antibiotic-resistant bacteria can reach the aquatic ecosystems through untreated sewage, effluent of wastewater treatment plants and leakage/infiltrations from agricultural areas on which animal manure was stored/applied [22, 23, 24, 25]. These antibiotic resistance genes may also contaminate water resources used as sources for drinking water production and they can be transferred to drinking water or human food chain bacteria [26, 27].

Antibiotic resistance can be propagated by vertical and horizontal gene transfer [28,29]. Vertical gene transfer means transmitting the genetic information, including resistance genes, in successive cell generations, together with the division. This mechanism is considered "slow" evolutionary in the

context of antibiotic resistance genes' propagation compared to horizontal gene transfer. Horizontal gene transfer means the transfer of genetic information between bacterial cells, free from barriers of gender and species, by other pathways ways than division. Horizontal gene exchange is the primary mechanism of antibiotic resistance evolution and it is achieved by conjugation, transformation or transduction. This continuous flow of genes seems to be a defining characteristic of the bacterial world and to have contributed substantially to the extreme metabolic versatility of bacteria in almost all natural environments [30].

3.1. Horizontal transfer of antibiotic resistance genes in aquatic environment. Numerous studies demonstrate that horizontal transfer of resistance genes between bacteria takes place in a variety of aquatic habitats [31, 32, 33, 34]: river water, lake water, wastewater, sediment, activated sludge from wastewater treatment plants.

In oligotrophic and eutrophic aquatic environments, bacteria tend to increase the surface contact and adhere to substrates or surfaces, forming multi-sepcific bacterial biofilms [35] and high densities of bacterial populations. The genetic transfer is possible among the biofilm cells (including R and virulence plasmids); it is facilitated by the proximity of cells, a process that leads to resistance occurrence in an initial cell population which is sensitive to certain antibiotics, within a multi-clone or multi-specific community and thus the resistance phenomenon is amplified [36]. In addition, cells contained in biofilms have a high rate of mutations (*hipermutators* cells), influenced by stress conditions with an adaptative role [35]. The extra-cellular biofilm matrix in aquatic habitats also produces favorable conditions for both plasmid transfer and transformation process [37]. It has a netlike structure and contains water (95-99% of biofilm weight) and different matters: polyosides, some of them with an adhesive role, capsular polysaccharides, DNA fragments, proteins such as fimbriae and acids involved in bacterial conjugation [35].

Wiedenbeck and Cohan, 2011 [38] mention that bacterial adaptation to the selective pressure of antibiotics accelerates the acquisition of antibiotic resistance genes by horizontal transfer from the donor to the recipient bacterial cells.

Horizontal transfer of antibiotic resistance genes among bacteria in the aquatic environment can be achieved by: (a) conjugation, by means of plasmids and transposons, as well as integrons from plasmid and transposon structure; (b) genetic transformation - if bacteria are naturally able to take up the naked DNA from the outer environment or for which the environmental conditions (presence of calcium) induce these skills [39]; (c) transduction - resistance genes are transferred by bacteriophages.

a**) Conjugation** is the DNA transfer from a donor cell to the recipient, by direct cell contact mediated by a special multiprotein complex called *conjugation device*. In Gram-negative bacteria, the physical contact is achieved through extra-cellular filamentous structures called *pili* [14].

Two types of conjugative genetic structures have been described:

- conjugative transposons;
- conjugative plasmids.

Conjugative transposons are mobile DNA molecules that encode for all the functions required for intracellular transposition, but also for intercellular conjugation. They include in their composition, a series of structural genes, bounded by IS (insertion sequences) on their extremities, all being repeated in a reverse order.

From the genetic structure point of view, simple transposons are similar to IS, but differ from them in that, in the central sequence, they carry genes whose phenotypic expression is identifiable and confers new properties to the carrying cell (e.g kanamycin or tetracycline resistance genes, etc.).

Inverted sequences gives a higher stability to transposon structure and play the role of perceiving sites for the transposase, an enzyme that catalyzes the transposition process.

Most transposons are resident in the plasmid DNA or phage DNA. Few transposons are resident in bacterial chromosome. They are found in a wide variety of Gram-positive bacteria (Tn 916 in *Enterococcus faecalis*) and Gram negative ones (Tn 455 – with tetracycline resistance gene in *Bacteroides*) and they are important for the dissemination of antibiotic resistance genes. The transfer frequency of conjugative transposons ranges between 10^{-4} - 10^{-9} [14].

Transposons are mobile genetic sequences: - move between plasmid and chromosome; - go from a plasmid to another; - are trasnposing between different sites of the same plasmid. Transposons are major factors of genetic information reorganization and therefore, for the agglomeration of several resistance genes within a single plasmid, due to their transposition and insertion ability. Genetic structure and mobility make multiple the antibiotic resistance very predictably. Transposition is an easy and effective way for simultaneous resistance transfer to several antibiotics.

Integrons are frequently part of some *transposons*' structure. They are DNA sequences that contain genetic determinants of a system for site-specific recombination. Integrons recognize, integrate and thus mediate the movement of short mobile DNA sequences called *gene cassettes* [40]. Most gene cassettes contain a single antibiotic resistance gene. An integron groups and integrates multiple gene cassettes. Integration of a gene cassette in a replicon is reversible. The excided cassette is integrated in another replicon. The resistance gene counterpart can always be rearranged and moved from one replicon to another, through *gene cassettes.*

Several studies have highlighted the important role of integrons, particularly those in Class 1, in the evolution of multi-antibiotic resistance in clinics [15]. They are true platforms for grouping multiple gene cassettes, favoring the simultaneous resistance dissemination in several antibiotics in a single transfer event, through their integration into mobile genetic elements such as plasmids and transposons.

Recent studies of environmental microbial communities have demonstrated the presence of Class 1 integrons in water samples, their prevalence reflecting the degree of water pollution with heavy metals [41]. Water and sediment pollution with nitrogen (quaternary ammonium salts), detergents and / or antibiotics also favours the selection of bacteria that possess Class 1 integrons and qac gene, responsible for bacterial resistance to ammonium ion, by the activity of efflux resistance systems [42, 43].

Resistance plasmids (R) are extra-chromosomal circular double-stranded DNA molecules, which physically replicate independently of the chromosome and carry genes that confer adaptive characters that are beneficial to bacterial hosts, allowing their survival under certain environmental conditions. Plasmid genes coding for antibiotic resistance and tolerance to heavy metals (such genes are present in many strains of pathogenic bacteria) are listed in this category.

Broad-host range plasmids identified in streptococci and enterococci, are transferred by conjugation, with a variable frequency of $1/10^3$-10^6 (a conjugation event to 10^3-10^6 cells), depending on the plasmid and the genotype of the two plasmids forming the couple. They confer resistance to macrolides, lincosamides and streptogramin B.

Spreading multi-antibiotic resistance from one cell to another is assigned to resistance plasmids (R). A bacterial cell can carry several resistance genes, that can confer it a multidrug-resistance or super-resistance and even resistance to all commonly used antibiotics. Antibiotic resistance genes on R plasmids might be considered as virulence genes because acquiring resistance to antibacterial agents determines the increase of incidence and continuous spread of bacterial infections [12].

The occurrence of R plasmids confers to pathogenic bacteria the property of resisting to normally lethal antibiotic concentrations, creating difficulty in the treatment of bacterial infections. The situation is worsened by the possibility R plasmids transfer from indigenous carrying bacteria, to allochthonous pathogenic bacteria, even if they belong to other species and genera [12].

R plasmids have a complex genetic structure and consist of the following categories of genetic determinants: - genes that confer antibiotic resistance ("*r* " genes); - genes that confer the conjugon function (transferon) to plasmid R. They are grouped into a transposon, forming the resistance transfer factor (FTR). They are called *tra* genes and encode for protein synthesis required for conjugation plasmid transfer; - insertion sequences; - initiation sequence of the replication process ("ori"); - genes that provide a physical autonomous replication of the plasmid. The "r" genes are part of the transposon structure (being bounded by insertion sequences) and have a very pronounced mobility, i.e they are moving from one site to another within the plasmid structure or between plasmid and chromosome. The number of resistance genes in a plasmid is variable. Unlike F plasmids, R plasmids do not integrate into the bacterial chromosome. If R plasmid does not contain tra genetic determinants (FTR), the transfer is made by transduction mediated by a large size phage or by conjugation initiated by other conjugative plasmids.

b) **Genetic transformation** consists in taking by the bacterial cell, of an exogenous DNA fragment. DNA must cross the cell layers and be integrated into the host cell chromosome, by homologous recombination. In the homologous recombination process, a part of the chromosome is replaced by a homologous DNA.

Small DNA embedded fragments from the external environment favour the intra-gene recombination, thus resulting mosaic genes that contain DNA from the original allele, but even DNA from other genes or organisms. Most mosaic genes are lost, but some may express a phenotype that favours the organism survival because they encode proteins with new properties: for example, the gene that encodes an altered PBP with low affinity for β-lactam antibiotics. Such a gene promotes tolerance to the environmental antibiotic and the carrier cell is favored by selection.

c) **Transduction** consists in DNA segment transfer from one bacterium to another via bacteriophages by generalized or special transduction. In generalized transduction, bacterial genes (from any part of the genome) are taken and transferred in successive infection cycles - lysis (*lytic cycle*). In special transduction, gene transfer is made with temperate phages which are able to become prophages by including their genetic material into the bacterial chromosome. Prophages are replicated simultaneously with the bacterial chromosome, so all bacteria resulting from this division are lysogenes, having prophages (lysogenic cycle). In the separation process of prophages from the bacterial chromosome, the viral genome may take one or some genes from it. For a new cycle, genes are transferred from one cell to another, by infecting another bacterial cell and including the temperate phage possessing bacterial genes in the chromosome of the bacteria-host cell. Temperate bacteriophages, due to their lysogene and prophage status, conduct a specialized transduction in that they carry a specific fragment of the bacterial chromosome, in their genome [44].

Bacteriophages are important constituents of aquatic ecosystems [45, 46] having an important role in the horizontal gene transfer in aquatic environment [47].

Recent studies [48, 49] have shown the presence of genes coding for the synthesis of β-lactamases (bla_{TEM}, $bla_{CTX\text{-}M}$) and the gene encoding meticillin resistance (*mec*A) in the viral metagenome of activated sludge and wastewater from treatment plants, as well as in the wastewater receiving tank, thus confirming that transduction may be responsible for the dissemination of resistance genes in these aquatic habitats. Moreover, the presence of the *mec*A gene in phage DNA from aquatic

environments represents a major problem for public health, due to the threat of infections with meticillin-resistant *Staphylococcus aureus* (MRSA), both in hospitals and in the community [50].

4. ANTIBIOTIC RESISTANCE GENES IN AQUATIC ENVIRONMENTS

Uncontrolled and widespread use of antibiotics in human, veterinary medicine and farming had a major impact on bacterial communities, by selecting some antibiotic-resistant bacteria and dissemination of antibiotic resistance determinants in pathogenic bacterial populations. Therefore, it was found an increased incidence of antibiotic-resistant bacteria and numerous genes that encode antibiotic resistance were detected in different aquatic environments [39].

Antibiotic resistance induced by genes isolated from environmental bacterial strains is achieved through different molecular mechanisms: - target bypass (*dfrA1, A5, A7, A12, A15, A17, A18; sulI, II, III* and *A*), inaccessibility of the antibiotics to their target enzyme by mutational changes or loss on the enzyme gene; - efflux pumps (*cmlA1* and *A5; flo$_R$; otrB; tetA, A(41), B, C, D, E, G, J, Y, Z, 33* and *39*), reduction of intracellular concentrations of antibiotics by structural alteration of cellular membrane; - antibiotics inactivation (*aacC1, C2, C3* and *C4*; *aadA1, A2, A5, A13* and *B*; *ampC; aphA1, D; mphA; sat1* and *2; strA* and *B*); - target modification (*ermA, B, C, E, F, T, V* and *X; mecA; penA; otrA; tetB(P), M, O,Q,S, T* and *W; vanA* and *B)*

Environmental factors which can influence the dissemination of antibiotic resistance genes in aquatic environments may include: - spatial distribution and density of donor and recipient bacterial strains; - temperature and pH; - nutrient availability; - selective pressure determined by the presence of antibiotics and/or heavy metals.

The direct contact between the donor and recipient cells must occur for the transfer of antibiotic resistance genes to be carried out through conjugation. Therefore, the spatial distribution of strains and density of antibiotic-resistant microorganisms play an important role in gene transfer frequencies with an increased dissemination of genes observed for high densities of donor strain [51].

Sediments and clay-rich soils lead to higher frequencies of gene transfer due to large colonization areas, thus creating a favourable environment to horizontal gene transfer by conjugation between the bacterial cells [52, 53]. It has been shown that horizontal gene transfer by conjugation process is frequently present between bacteria forming a biofilm, the process being frequent in aquatic sediments and it is favourable to environmental cell adaptation, as well as to the diversity of microbial populations, but also having a negative connotation, namely the emergence of bacteria that are multiresistant to antibiotics and heavy metals [54].

The influence of temperature and pH on the horizontal gene transfer is related to the optimum field of these parameters. Although laboratory studies have shown that the optimum temperature for maximum transfer frequencies ranges from 22ºC to 30ºC, with an optimum pH between 6.15 to 7.45, indigenous soil microorganisms showed higher frequencies in the range 15ºC - 25ºC, suggesting that by adaptation to specific environmental conditions, bacteria can increase the horizontal gene transfer frequencies [55].

The main factor contributing to the horizontal transfer of antibiotic resistance genes is the selective pressure exerted by the intensive use of antibiotics in human and veterinary therapy. High selective pressure facilitates the acquisition of antibiotic resistance genes, which may lead to increased incidence of resistant bacteria, allowing fast evolution and dissemination on a global scale [56]. Furthermore, the presence of sub-inhibitory antibiotic concentrations in environment may accelerate horizontal transfer and dissemination of antibiotic resistance genes in environment [57, 58].

Bacteria in heavy metals polluted environments seem to develop more easily antibiotic resistance phenotypes comparable to those existing in control areas [21, 41]. A new gene coding for tetracycline resistance, *tet*A (41) was identified in strains of *Serratia marcescens* isolated from a heavy metals polluted stream [59] providing indirect evidence of co-resistance. Thus, the resistance plasmids may be responsible for conferring not only antibiotic resistance, but also the simultaneous resistance to heavy metals, favoring the maintenance and spread of antibiotic resistance due to selective pressure exerted by the presence of heavy metals [60, 21].

Investigating the resistance gene pools in the aquatic environment is important in order to identify risk factors for the diffusion of a particular resistance mechanism in a particular community, with negative effects on receiving waters and human health, highlighting the need for further measures of water quality monitoring.

6. REFERENCES

[1] Levy S.B., The antibiotic paradox: How miracle drugs are destroying the miracle, *New York, Plenum Press,* **1992.**

[2] Bell J.B., Elliott G.E., Smith D.W., Influence of sewage treatment and urbanization on selection of multiple resistance in fecal coliform populations, *Appl. Environ. Microbiol*., 46, 227-232, **1983.**

[3] Huys G., Rhodes G., McGann P., Denys R., Pickup R., Hiney M., Smith P., Swings J., Characterization of oxytetracycline-resistant heterotrophic bacteria originating from hospital and freshwater fishfarm environments in England and Ireland, *Syst. Appl. Microbiol.*, 23, 4, 599-606, **2000.**

[4] Balotescu C., Israil A., Radu R., Alexandru I., Dobre G., Aspects of constitutive and acquired antibioresistance in *Aeromonas hydrophila* strains isolated from water sources, *Roum Arch Microbiol Immunol*., 62,3-4,179-89, **2003.**

[5] Ram S, Vajpayee P, Shanker R., Prevalence of multi antimicrobial agent resistant, shiga toxin and enterotoxins producing *Escherichia coli* in surface water of the river Ganga, *Environ. Sci. Tech.*, 41, 7383-7388, **2007.**

[6] Tao R., Ying G.G., Su H.C., Zhou H.W., Sidhu J.P.S. Detection of antibiotic resistance and tetracycline resistance genes in *Enterobacteriaceae* isolated from the Pearl rivers in South China, *Environ. Pollut.*, 158, 2101-2109, **2010.**

[7] Goñi-Urriza M., Capdepuy M., Arpin C., Raymond N., Caumette P., Quentin C., Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp., Appl Environ Microbiol., 66, 1, 125-32, **2000.**

[8] Kim S., Aga D.S., Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants, J. *Toxicol. Environ. Health.*, 10, 559-573, **2007.**

[9] Auerbach E.A., Seyfried E.E., McMahon K.D., Tetracycline resistance genes in activated sludge wastewater treatment plants, *Water Res.*, 41, 1143–1151, **2007.**

[10] Garcia S., Wade B., Bauer C., Craig C., Nakaoka K., Lorowitz W., The effect of wastewater treatment on antibiotic resistance in *Escherichia coli* and *Enterococcus sp*. *Water Environ Res.*, 79, 12, 2387-95, **2007.**

[11] Gulkowska A., Leung H.W., So M.K., Taniyasu N., Yamashita N., Yeung L., Richardson B., Lei A.P., Giesy J.P., Lam P., Removal of antibiotics from waste water by sewage treatment facilities in Hong Kong and Shenzhen, China, *Water Research*, 42, 1-2, 395-403, **2008.**

[12] Lazar Veronica,. *Microbiologie medicala. Note de curs si principii de diagnostic microbiologic,* Editura Universitatii Bucuresti, Bucuresti, **2007.**

[13] Greenwood D., Resistance to antimicrobial agents: a personal view, *J. Med. Microbiol*., 47, 751-755, **1998.**

[14] Mihaescu G., Chifiriuc M.C., Ditu L.M., *Antibiotice si substante chimioterapeutice antimicrobiene*, Editura Academiei Romane, Bucuresti, **2008.**

[15] Cambray G., Guerout A.M., Mazel D., Integrons. *Annu. Rev. Genet*., 44, 141-166, **2010.**

[16] François J., Chemarat M., Weber M., Berard A., From The Antibiogram to Prescription, *2th Ed., bioMérieux, ISBN 973–86485–2-1,* **2004.**

[17] Abriouel H., Omar N.B., Molinos A.C., López R.L., Grande M.J., Martínez-Viedma P., Ortega E., Cañamero M.M., Galvez A., Comparative analysis of genetic diversity and incidence of virulence factors and

antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples, *Int. J. Food Microbiol.*, 123, 1-2, 38-49, **2008.**
[18] Lazar Veronica, Cernat Ramona, Balotescu Mariana Carmen, Herlea Victoria, Bulai Doina, Moraru Anca, *Microbiologie generala. Manual de lucrari practice*, Editura Universitatii din Bucuresti, Bucuresti, **2004.**
[19] Brock T.D., Medigan M.T., Martinco J.M., Parker J., Biology of Microorganisms Tenth Ed., *New Jersey, Prentice Hall Intern. Inc.*, **2003.**
[20] Chifiriuc C., Lazar V., Mihaescu G., *Microbiologie si virologie medicala*, Ed. Universitatii Bucuresti, **2011.**
[21] Baker-Austin C., Wright M.S., Stepanauskas R., McArthur J.V., Co-selection of antibiotic and metal resistance, *Trends Microbiol.*, 14,176–182, **2006.**
[22] Hayes J., English L., Carr L., Wagner D., Joseph S., Multiple-antibiotic resistance of *Enterococcus spp*. isolated from commercial poultry production environments, *Applied. and Environmental Microbiology*, **2004.**
[23] Mispagel H., Gray J.T., Antibiotic resistance from wastewater oxidation ponds, *Water environment Federation*, **77**, 7, 2996-3002, **2005.**
[24] Li D., Yang M., Hu J., Zhang J., Liu R., Gu X., Zhang Y., Wang Z., Antibiotic-resistance profile in environmental bacteria isolated from penicillin production wastewater treatment plant and receiving river, *Environmental Microbiology*, 11, 6, 1506-1517, **2009.**
[25] Haggard B., Bartsch L., Net changes in antibiotic concentrations downstream from an effluent discharge, *Journal Environmental Quality*, 38, 343-352, **2009.**
[26] Chee-Stanford J.C., Aminov R.I., Krapac I. J., Garrigues-Jeanjean N., Mackie R.I., Occurrence and diversity of tetracycline resistance genes in lagoon and groundwater underlying two swine production facilities, *Appl. Environmental Microbiology*, 67, **2001.**
[27] Shar A.H., Kazi Y.F., Soomro I.H., Antibiotic susceptibility of termo-tolerant *Escherichia coli* 2 isolated from drinking water of Khairpur City, Sindh, Pakistan, *Pakistan Journal of Biological Sciences*, 12, 8, 648-652, **2009.**
[28] Cotar A.I., Chifiriuc M.C., Banu O., Lazar V., Antibiotic resistance patterns of Staphylococcus aureus strains isolated from cardiovascular surgery associated infections, *Biointerface Research in Applied Chemistry*, 3, 1, 491-497, **2013**.
[29] Rysz M., Antibiotic resistance vector transport, reservoir amplification and attenuation, *Rice University, Houston, Texas*, **2007.**
[30] Jurcoane S., Cornea P., Stoica I., Vassu T., *Tratat de biotehnologie, Vol. II*, Editura Tehnica, Bucuresti, **2006.**
[31] Cernat R., Lazar V., Balotescu C., Cotar A., Coipan E., Cojocaru C., Plasmid-mediated transfer of antibiotic resistance into some antibioresistant enterobacterial strains isolated from various aquatic sources, *Bacteriol. Virusol. Parazitol. Epidemiol*., 47, 3-4, 179-84, **2002.**
[32] Guardabassi L., and Dalsgaard A., Occurrence, structure, and mobility of Tn1546-like elements in environmental isolates of vancomycin-resistant enterococci, *Appl. Environ. Microbiol.*, 70, 984-990, **2004.**
[33] Silva J., Castillo Gabriela, Callejas Lorena, Lopez H., Olmos Janet, Frequency of transferable multiple antibiotic resistance amongst coliform bacteria isolated from a treated sewage effluent in Antofagasta, Chile, *Electronic Journal of Biotehnology*, **2006.**
[34] Shakibaie M.R., Jalilzadeh K.A., Yamakanamardi S.M., Horizontal transfer of antibiotic resistance genes among gram negative bacteria in sewage and lake water and influence of some physico-chemical parameters of water on conjugation process., *J. Environ. Biol.*, 30, 1, 45-49, **2009.**
[35] Cernat Ramona, Lazar Veronica, *Taxonomia si Biodiversitatea Microorganismelor*, Editura Universitatii Bucuresti, Bucuresti, **2011.**
[36] Lazar Veronica, Chifiriuc Carmen, Architecture and physiology of microbial biofilms, *Rom. Arch. of Microb.& Immunol*., 69, 2, 95-107, **2010.**
[37] Molin S., Tolker-Nielsen T., Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure, *Curr. Opin. Biotechnol*., 14, 255–261, **2003.**
[38] Wiedenbeck J., Cohan F.M., Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiol. Rev.*, 35, 957–97, **2011.**
[39] Zhang Xu-Xiang, Zhang Tong, Fang Herbert H.P., Antibiotic resistance genes in water environment. *Appl. Microbiol. Biotechnol.*, 82, 397-414, **2009.**
[40] Mazel D., Integrons: agents of bacterial evolution., *Nat. Rev. Microbiol*., 4, 608-620, **2006.**

[41] Wright M.S., Baker-Austin C., Lindell A.H., Stepanauskas R., Stikes H.W., McArthur J.V., Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities, *ISME J.*, 2, 417–428, **2008.**
[42] Gaze W.H., Abdouslam N., Hawkey P.M., Wellington E.M., Incidence of class1 integrons in a quaternary ammonium compound-polluted environment, *Antimicrob. Agents Chemother.*, 49, 1802–1807, **2005.**
[43] Gaze W.H., Zhang L., Abdouslam N.A., Hawkey P.M., Calvo-Bado L., Royle J., Brown H., Davis S., Kay P., Boxall A.B., Wellington E.M., Impacts of anthropogenic activity on the ecology of class1 integrons and integron-associated genes in the environment, *ISME J.*, 5, 1253–1261, **2011.**
[44] Madigan, M.T., Martinko, J.M., Brock Biology of Microorganisms, *11th Ed., Pearson Prentice Hall*, **2005.**
[45] Weinbauer M.G., Ecology of prokaryotic viruses, *FEMS Microbiol. Rev.*, 28, 127–181, **2004.**
[46] Srinivasiah S., Bhavsar J., Thapar K., Liles M., Schoenfeld T., Wommack K.E., Phages across the biosphere: contrasts of viruses in soil and aquatic environments, *Res. Microbiol.*, 159, 349–357, **2008.**
[47] Lupo A., Coyne S., Berendonk T.U., Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies, *Front. Microbio*., 3, 18, doi:10.3389/fmicb.2012.00018, **2012**.
[48] Parsley L.C., Consuegra E.J., Kakirde K.S., Land A.M., Harper W.F.Jr., Liles M.R.,. Identification of diverse antimicrobial resistance determinants carried on bacterial, plasmid, or viral metagenomes from an activated sludge microbial assemblage, *Appl. Environ. Microbiol.*, 76, 3753–3757, **2010.**
[49] Colomer-Lluch M., Jofre J., Muniesa M., Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples, *PloS ONE 6*, e17549, doi,10.1371/journal.pone.0017549, **2011.**
[50] Campanile F., Bongiorno D., Falcone M., Vailati F., Pasticci M.B., Perez M., Raglio A., Rumpianesi F., Scuderi C., Suter F.,Venditti M.,Venturelli C., Ravasio V., Codeluppi M., Stefani S., Changing Italian nosocomial-community trends and heteroresistance in *Staphylococcus aureus* from bacteremia and endocarditis, *Eur. J. Clin. Microbiol. Infect. Dis.*, PMID, 21822974, **2011.**
[51] Valnelsas J.D., Trevors J.T., Starodub M.E., Vanoverbeek L.S., Transfer of Plasmid Rp4 between *Pseudomonads* after Introduction into Soil – Influence of Spatial and Temporal Aspects of Inoculation, *Fems Microbiology Ecology*, 73, 1, 1-11, **1990.**
[52] Trevors J.T., Starodub M.E., R-Plasmid Transfer in Nonsterile Agricultural Soil, *Systematic and Applied Microbiology.*, 9, 3, 312-315, **1987.**
[53] Trevors J.T., Berg G., Conjugal Rp4–Transfer between *Pseudomonads* on Soil and Recovery of Rp4-Plasmid DNA from Soil, *Systematic and Applied. Micrbiology.*, 11, 2, 223-227, **1989.**
[54] Davey M.E., O'Toole G.A., Microbial biofilms: from ecology to molecular genetics, *Microbiol. Mol. Biol. Rev*., 64, 4, 847-867, **2000.**
[55] Fry J.C., Day M.J., Plasmid transfer in the epilithon, Bacterial genetics in natural environments, *J C Fry and M J Day. London, Chapman and Hall*, **1990.**
[56] Luo N., Pereira S., Sahin O., Lin J., Huang S., Michel L., Zhang Q., Enhanced in vivo fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure, *Proc. Natl. Acad. Sci. USA*, 102, 541–546, **2005.**
[57] Kümmerer K., Resistance in the environment, *J. Antimicrob. Chemother.*, 54, 311–320, **2004.**
[58] Hecht D.W., Kos I.M., Knopf S.E., Vedantam G., Characterization of BctA, a mating apparatus protein required for transfer of the *Bacteroides fragilis* conjugal element BTF-37, *Res Microbiol*., 158, 600–607, **2007.**
[59] Thompson S.A., Maani E.V., Lindell A.H., King C.J., McArthur J.V., Novel tetracycline resistance determinant isolated from an environmental strain of *Serratia marcescens, Appl. Environ. Microbiol.*, 73, 2199–2206, **2007.**
[60] Alonso A., Sanchez P., Martinez J.L., *Stenotrophomonas maltophila* D457R contains a cluster of genes from gram-positive bacteria involved in antibiotic and heavy metal resistance, *Antimicrobial Agents and Chemotherapy*, 44, 7, 1778-1782, **2000.**