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Effect of Heavy Metals on the Biofilm Formed by Microorganisms from Impacted Aquatic Environments

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Abstract

The aquatic environment is highly complex and diverse, consisting of several types of ecosystems that are dynamic products of complex interactions between biotic and abiotic components. Changes in the physical and chemical properties of these ecosystems can significantly affect the balance of life forms present, especially in their microbiota. Among the main pollutants present in these environments are heavy metals. Several studies demonstrate the effects of these minerals on the structure and function of microbial communities, which may develop adaptation mechanisms for survival and permanence in these sites. In addition, the resistance to heavy metals may contribute to the evolution of resistance genes to the different types of antimicrobials due to the increase of the selective pressure in the environment, becoming a public health problem. One of the adaptive mechanisms present in bacteria from impacted environments that has been frequently investigated is the formation of biofilms. Recent studies have reported significant changes in the structure and amount of biofilm formed in the presence of different metals, and consequently, an increase in the tolerance to these pollutants and antimicrobials. This review will discuss the effects of some metals on bacterial biofilms and their consequences for the marine environment.

Keywords: chemical pollution, toxicity, mechanisms of adaptation, metals, antimicrobials

1. Introduction

The aquatic environment is highly complex and diverse, comprising various types of ecosystems that are dynamic products of complex interactions between biological and abiotic components. Changes in physical properties and ecosystems may affect the balance of life forms present there [1, 2].

In recent decades, these ecosystems have been significantly altered due to multiple environmental impacts from the release of large amounts of effluent without adequate prior treatment, resulting in the scarcity of existing natural resources [3, 4]. Among the main pollutants that generate negative impacts on life

forms are heavy metals. The presence of these contaminants may cause changes in the structure and function of microbial communities [5], which can develop various resistance mechanisms that enable their survival [6]. In addition, heavy metal resistance may contribute to the evolution of resistance genes to different types of antimicrobials due to increased selective pressure in the environment [7].

Adaptability as well as metabolic and physiological differences are essential characteristics for microorganisms to remain in these locations. One of the adaptive mechanisms present in bacteria that has been frequently investigated is biofilm formation [8]. Biofilms are structures composed mainly of microbial cells and a matrix formed by a cluster of extracellular polymeric substances (EPSs) [9]. Biofilm-grown cells have some distinct properties from planktonic cells, one of which is increased resistance to antimicrobials and heavy metals [10]. In this review, we propose to report the latest findings on the survival strategies of microorganisms in impacted aquatic environments, more precisely on the influence of heavy metals on biofilm formation.

2. Microorganisms in contaminated aquatic environments

Water is an indispensable natural resource for the survival of man and other living beings [11, 12]. According to Raucci and Polette [13], 97% of the planet's water is found in the oceans, and of the remaining 3%, only 0.3% is available for human consumption and is stored in springs, lakes, rivers, and groundwater.

According to the United Nations (UN), access to water supply and sanitation is a human right and vital to the dignity and health of all people. However, there are still about 1.1 billion people without access to clean water and 2.4 billion people without access to basic sanitation services [14].

The decline in water quality has become one of the most serious problems worldwide, a fact that has been intensified by the increase in population and the absence of public policies aimed at the preservation of water resources. According to the World Health Organization—WHO [15], approximately half of the world's developing population will be affected by diseases that are directly related to poor-quality water and/or lack of adequate or even no sanitation.

Contamination of natural waters represents one of the main risks to public health, a fact that is directly related to the discharge of untreated domestic, hospital, and industrial effluents, which cause contamination of aquatic bodies by pathogenic microorganisms such as bacteria, viruses, protozoa, and helminth eggs [16].

Among the bacteria can be highlighted those belonging to the Enterobacteriaceae family, represented by species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* and *Providencia rettgeri*. Most of these species are commonly found in the intestinal tract of humans and animals, and their presence in aquatic environments indicates fecal contamination [4, 17].

Another problem found in the aquatic environment is the contamination by resistant bacteria from humans and animals exposed to antimicrobials [18, 19], as well as the disposal of antimicrobial waste from domestic and hospital effluents. Water is not only a means of spreading resistant microorganisms, but also the pathway through which resistance genes are introduced into the ecosystem, altering the environmental microbiota [20].

Studies have shown bacterial resistance in various aquatic environments including rivers and coastal areas, domestic sewage, hospital sewage, sediment, surface water, lakes, oceans, and drinking water [4, 21–24].

Among the main pollutants found in this environment, we highlight the heavy metals that when introduced into the environment can cause changes in

Figure 1.
Impacted aquatic environment and survival strategy of the present microorganisms.

the structure and function of microbial communities [25]. Aquatic systems may be introduced as a result of natural processes such as weathering, erosion, and volcanic eruptions [26]. However, in recent decades, the increase in urbanization and industrialization has contributed to the large increase of these environmental contaminants worldwide [27].

Thus, microorganisms have been developing various resistance mechanisms that allow their survival [6]. Among the various mechanisms, intra and extra-cellular, are bioaccumulation [28], biosorption [29], biomineralization and precipitation [30, 31], oxidation and enzymatic reduction of the metal to the less toxic form [32], production of siderophores [33], and biofilm formation [34]. **Figure 1** shows an impacted aquatic environment and a survival strategy for the microorganisms present there.

3. General characteristics of heavy metals

The term “heavy metals” is used to identify a group of chemical elements that have atomic density greater than 5 g cm^{-3} or have atomic number greater than 20 [35]. Some of these elements, such as sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), zinc (Zn), and copper (Cu), are essential microelements for various life forms, as they are necessary for the functioning of some metabolic pathways [36]. However, the excess or lack of these elements can lead to disturbances in organisms, and in extreme cases, even death [37]. Other elements such as mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) are highly toxic even when present in low concentrations, and account for most health problems due to environmental pollution [38].

Heavy metals participate in the global ecobiological cycle, derived from numerous sources and are dynamically transported through the atmosphere, soil, and

water; also, because they are not biodegradable, they can remain in the environment for long periods [39].

Among the various metals, mercury, cadmium, and lead stand out for being associated with contamination of the aquatic environment, which can cause problems of poisoning to man and other organisms. These elements are capable of reacting with molecules and ligands present in cell membranes, conferring them with the properties of bioaccumulation, food chain biomagnification, persistence in the environment, and metabolic disturbances of living beings [40].

4. Effects of heavy metals on biofilm

Biofilm is a porous and complex structure formed by one or more species of microorganisms, organized in several layers irreversibly adhered to a biotic or abiotic surface and enclosed in a matrix composed of extracellular polymeric substances (EPS) [9].

They are formed dynamically and gradually, involving several stages. The first is reversible bacterial adhesion that can occur on biotic surfaces mediated by molecular interactions or abiotic surfaces through physicochemical interactions. The second is irreversible adhesion, where the adhesion process is consolidated through the production of EPS. After the establishment and maturation of the protective matrix in the irreversible phase, the cycle ends with the rupture of the biofilm and the release of bacterial cells (**Figure 1**) [9, 41].

Bacteria in the form of free (planktonic) cells are not often found in nature; most of them live in communities or attached to various biotic or abiotic surfaces, such as clinical and industrial equipment. Several factors may contribute to bacterial adhesion such as flagella, fimbriae, adhesin, and polymers, as well as adhesion forces such as electrostatic and hydrophobic attraction, van der Waals interactions, hydrogen bridges, and covalent bond [10].

Biofilm formation is an effective strategy for microbial survival and persistence under stress conditions, such as in the presence of antimicrobials and heavy metals [42]. The biofilm structure may be associated with a protective mechanism that allows the bacteria to survive and persist in environments with high metal concentrations [43]. Studies have shown that subinhibitory heavy metal concentrations can induce biofilm formation [44, 45], like lead [46], cadmium [47], and nickel [48] among others.

Giovanella et al. [46] evidenced the increase in formation by an isolate of *Pseudomonas* sp. in the presence of mercury (Hg^{2+}). Similarly, Araújo et al. [49] verified an increase in biofilm formation in *Klebsiella pneumoniae* isolates obtained from an impacted urban stream. However, other studies show that depending on the metal and its concentration, biofilm formation may be reduced [50, 51]. These differences may be related to the fact that the effects of metals depend on their concentration and speciation [47, 51, 52], growth conditions, and especially the bacterial isolate that is being exposed [53, 54].

Recent studies have shown that metals can affect various stages in biofilm formation and development [55]. Metals can impact cell surface adhesion and/or cell-to-cell aggregation process, promoting biofilm formation and, consequently, its resistance. Harrison et al. [56] verified that the increase in cadmium concentration induces cell adhesion and biofilm formation in *Rhizobium alarii* YAS34. Subinhibitory concentrations of manganese (Mn) and zinc (Zn) affected cell aggregation in *Xylella fastidiosa* isolates. Mn increased the process of biofilm formation in this bacterium, while Zn impaired this process probably by reducing cell adhesion on the surface [50, 57]. Perrin et al. [48] observed that some isolates of *Escherichia coli* K-12 formed biofilm

in response to subinhibitory nickel (Ni) concentrations and that cells embedded in the biofilm were less affected by metal exposure than planktonic cells. These studies show that bacterial cells exposed to metals generally respond by inducing adhesion processes, and consequently, biofilm formation and maintenance [55].

In addition to changes in cell adhesion, exposure to heavy metals may cause structural changes in the biofilm extracellular polymeric substance (EPS) matrix. Araújo et al. [49] verified by scanning microscopy, the increase of EPS in *K. pneumoniae* biofilms formed when exposed to subinhibitory mercury concentrations (Hg^{2+}). Sheng et al. [58] also demonstrated that heavy metals stimulate EPS production in *Rhodopseudomonas acidophila*. Schue et al. [59] observed in *R. alarii* isolates the formation of a more condensed biofilm in the presence of subinhibitory concentrations of Cd when compared to isolates not exposed to this metal. The increase of extracellular matrix in *Thiomonas* sp. subinhibitory concentrations of arsenic (III) possibly contributed to biofilm integrity and physiological heterogeneity of immobilized cell subpopulations [60].

In stabilized biofilm, the presence of metals impacts cells via passive processes by the influence of gene expression, resulting in mechanisms of resistance or tolerance to these pollutants [55]. Extracellular polymeric matrix (EPS) acts as a barrier to toxic metals, which can be sequestered, immobilized, mineralized, and precipitated, diminishing their effect on bacteria [61]. In *Pseudomonas putida* ATCC 33015, sugars present in the biofilm matrix exposed to chromium (Cr) probably facilitated the immobilization process of this metal [62]. The biomineralization process was described in *Cupriavidus metallidurans* CH34, which was able to form gold (Au) nanoparticles in biofilm through the reduction and precipitation mechanism of the toxic gold complex (Au III) [63].

5. Heavy metal resistance

Environmental contamination by heavy metals has been increasing in recent years, due to various anthropogenic activities. Heavy metals, because they are not biodegradable, have a tendency for biomagnification and bioaccumulation and are extremely toxic to various biological functions, causing serious impacts on the environment and human health [64].

Microorganisms present in contaminated environments have developed different resistance mechanisms to adapt to stress caused by heavy metals. The ability to survive under these extreme conditions depends on acquired biochemical and physiological attributes, as well as genetic adaptations [65].

Several studies suggest that metal contamination in the natural environment may play an important role in maintaining and proliferating antimicrobial resistance (**Table 1**) [67–69]. In the environment, selective pressure exerted by metals may select resistant isolates similar to antibiotics, since both resistance genes are often located on the same moving elements [70, 71].

Bacteria develop some mechanisms to neutralize mercury toxicity, the most common being enzymatic reduction of the highly toxic mercuric ion (Hg^{2+}) to the volatile and less toxic elemental mercury (Hg^0). This reduction is catalyzed by the cytosolic mercury reductase (MerA) enzyme encoded by a gene belonging to the operon mer. Studies have shown the frequent association between operon mer and antimicrobial resistance [66, 72]. Péres-Valdespino et al. [73] demonstrated that several clinical isolates of *Aeromonas* sp. that presented the merA gene were resistant to different antibiotics such as tetracycline, trimethoprim, nalixidic acid, and streptomycin. Araújo et al. [49] verified, when comparing isolates of *K. pneumoniae*, that the isolate that presented the merA gene was resistant to the highest number of antimicrobials and presented the minimum inhibitory concentration

Resistance mechanisms	Heavy metals	Antibiotics	References
Reduction in permeability	As, Cu, Zn, Mn, Co, Ag	Cip, Tet, Cholr, β -lactâmicos	[32, 74]
Drug and metal alteration	As, Hg	β -lactâmicos, Chlor	[75, 76]
Drug and metal outflow	Cu, Co, Zn, Cd, Ni, As	Tet, Chlor, β -lactâmicos	[77, 78]
Cell signaling change	Hg, Zn, Cu	Cip, β -lactâmicos, Trim, Rif	[79, 80]

Abbreviations: Cholr, chloramphenicol; Cip, ciprofloxacin; Rif, rifampicin; Tet, tetracycline; Trim, trimetropim. Adapted from Baker-Austin et al. [66].

Table 1.
Examples of characteristics and negative effects on metal and antibiotic resistance mechanisms.

(MIC) value up to four times higher than the others, suggesting a co-resistance mechanism for mercury and antimicrobials tested.

Martins et al. [81] observed that isolates of *P. aeruginosa*, obtained from a contaminated river in southeastern Brazil, had a conjugative plasmid with co-resistance to tetracycline and copper, reinforcing that resistance to antibiotics may be induced by selective pressure of heavy metals in the environment. Caille et al. [82] demonstrated that in *P. aeruginosa*, copper can induce imipenem resistance by the CopR-CopS two-component regulatory system mechanism. Ghosh et al. [83] verified resistance to ampicillin, arsenic, chromium, cadmium, and mercury in *Salmonella abortus equi* isolates and observed that after removal of the plasmids, isolates became sensitive to these compounds.

In order to corroborate the evidence of co-resistance of metals and antibiotics, some studies compared the resistance profiles of bacteria collected in contaminated and uncontaminated environments. Rasmussen and Sørensen [84] demonstrated an increase in the occurrence of conjugative plasmids at contaminated sites and found that the mercury and tetracycline resistance genes were located on the same plasmid. Mcarthur and Tuckfield [85] examined metal and antibiotic resistance profiles in contaminated and uncontaminated stream sediments and found that isolates obtained from the contaminated sediment were more resistant to kanamycin and streptomycin than the others.

Thus, not only the indiscriminate use of antibiotics but also environmental contamination by heavy metals can pose risks and harm to human health, as resistance genes can be transferred horizontally from environmental microorganisms to human diners [66].

6. Conclusions

Increased urbanization and industrialization have contributed to heavy metal contamination in aquatic ecosystems, modifying the structure and function of microbial communities. The ability of microorganisms to survive under stress conditions, such as in the presence of heavy metals, depends on structural and biochemical attributes, as well as physiological and/or genetic adaptations. The studies cited demonstrated that the presence of heavy metals influences at different stages of biofilm formation. Additionally, the correlation between resistance to metals and antimicrobials was demonstrated, showing the environmental impact that these contaminants can cause in aquatic environments.

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References

- [1] Rand GM, Wells PG, Mccarty LS. Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. 2nd ed. Washington: Taylor & Francis; 1995
- [2] Zhang X-X, Zhang T, Fang HHP. Antibiotic resistance genes in water environment. Applied Microbiology and Biotechnology. 2009;**82**(3):397-414
- [3] Freitas JHES, Santana KV, Nascimento ACC, et al. Evaluation of using aluminum sulfate and water-soluble *Moringa oleifera* seed lectin to reduce turbidity and toxicity of polluted stream water. Chemosphere. 2016;**163**:133-141
- [4] Purificação-Júnior AF, Araújo LCA, Lopes ACS, et al. Microbiota sampled from a polluted stream in Recife-PE, Brazil and its importance to public health. African Journal of Microbiology Research. 2017; **11**:1142-1149
- [5] Dixit G, Singh AP, Kumar A, Mishra S, Dwivedi S, Kumar S, et al. Reduced arsenic accumulation in rice (*Oryza sativa* L.) shoot involves sulfur mediated improved thiol metabolism, antioxidant system and altered arsenic transporters. Plant Physiology and Biochemistry. 2015;**99**:86-96
- [6] Adarsh VK, Mishra M, Chowdhury S, Sudarshan M, Thakur AR, Ray CS. Studies on metal microbe interaction of three bacterial isolates from east Calcutta wetland. Journal of Biological Sciences. 2007;**7**:80-88
- [7] Sarma B, Axharya C, Joshi SR. Pseudomonas: A versatile bacterial group exhibiting dual resistance to metals and antibiotics. African Journal of Microbiology Research. 2010;**4**:2828-2835
- [8] Chouduri AU, Wadud A. Twitching motility, biofilm communities in cephalosporin resistant *Proteus* spp and the best in vitro amoxicillin susceptibility to isolates. American Journal of Microbiological Research. 2014;**2**(1):8-15
- [9] Trentin DS, Giordani RB, Macedo AJ. Biofilmes bacterianos patogênicos: Aspectos gerais, importância clínica e estratégias de combate. Revista Liberato. 2013;**14**(22):113-238
- [10] Flemming HC, Wingender J. The biofilm matrix. Nature Reviews Microbiology. 2010;**8**:623-633
- [11] Agência Nacional das Águas. Panorama da qualidade das águas superficiais do Brasil, 2012; Agência Nacional de Águas. Brasília: ANA; 2012
- [12] Paz VPS, Teodoro REF, Mendonça FF. Recursos hídricos, agricultura irrigada e meio ambiente. Revista Brasileira de Engenharia Agrícola e Ambiental. 2000;**4**(3):465-473
- [13] Raucci GD, Polette M. Subsídios para análise da capacidade de suporte da praia central de balneário Camboriú-SC. Perfil do Usuário. In: XIV Semana Nacional de Oceanografia Rio Grande-RS Livro de síntese do evento citado: Furg. 2001. pp. 117-118
- [14] WHO. Water Sanitation and Health. 2014. Available from: http://www.who.int/water_sanitation_health/diseases [Accessed: 24 September 2017]
- [15] WHO. Sanitation Safety Planning: Manual for Safe Use and Disposal of Wastewater, Greywater and Excreta. Geneva: World Health Organization; 2016
- [16] Geldreich EE. The bacteriology of water. In: Microbiology and Microbial Infections. London: Arnold; 1998

- [17] Liu SY, Zhangs SN, Geng TY, Li CM, Yed D, Zhang F, et al. High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. *Applied and Environmental Microbiology*. 2010;**76**:5972-5976
- [18] Shakibaie MR, Jalilzadeh KA, Yamakanamardi SM. 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. *Journal of Environmental Biology*. 2009;**30**(1):45-49
- [19] Al-Bahry SN, Mahmood IY, Al-Khaifi A, Elshafie AE, Al-Harthy A. Hability of multiple antibiotic resistant bacteria in distribution lines of treated sewage effluent used for irrigation. *Water Science and Technology*. 2009;**60**(11):2939-2948
- [20] Caumo KS, Duarte M, Cargin ST, Ribeiro VB, Tasca T, Macedo AJ. *Revista Liberato: Revista de divulgação de educação, ciência e tecnologia*. Novo Hamburgo, RS. 2010;**11**(16):89-188
- [21] Maal-Bared R, Bartlett KH, Bowie WR, Hall ER. Phenotypic antibiotic resistance of *Escherichia coli* and *E. coli* O157 isolated from water, sediment and biofilms in an agricultural watershed in British Columbia. *Science of the Total Environment*. 2013;**443**:315-323
- [22] Middleton JH, Salierno JD. Antibiotic resistance in triclosan tolerant fecal coliforms isolated from surface waters near wastewater treatment plant outflows (Morris County, NJ, USA). *Ecotoxicology and Environmental Safety*. 2013;**88**:79-88
- [23] Zhang X, Li Y, Liu B, Wang J, Feng C, Gao M, et al. Prevalence of veterinary antibiotics and antibiotic-resistant *Escherichia coli* in the surface water of a livestock production region in northern China. *PLoS One*. 2014;**9**:e111026
- [24] Chen Z, Yu D, He S, Ye H, Zhang L, Wen Y, et al. Prevalence of antibiotic-resistant *Escherichia coli* in drinking water sources in Hangzhou City. *Frontiers in Microbiology*. 2017;**8**:1133
- [25] Doelman P, Jansen E, Michels M, Van TM. Effects of heavy metals in soil on microbial diversity and activity as shown by sensitivity-resistance index, an ecologically relevant parameter. *Biology and Fertility of Soils*. 1994;**17**:177-184
- [26] Foster IDL, Charlesworth SM. Heavy metals in the hydrological cycle: Trends and explanation. *Hydrological Processes*. 1996;**10**:227-261
- [27] Fashola MO, Ngole-Jeme VM, Babalola OO. Heavy metal pollution from gold mines: Environmental effects and bacterial strategies for resistance. *International Journal of Environmental Research and Public Health*. 2016;**13**:1047
- [28] Blindauer CA, Harrison MD, Robinson AK, et al. Multiple bacteria encode metallothioneins and Smt A-like zinc fingers. *Molecular Microbiology*. 2002;**45**:1421-1432
- [29] Quintelas C, Rocha Z, Silva B, Fonseca B, Figueiredo H, Tavares T. Biosorptive performance of *Escherichia coli* biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II). *Chemical Engineering Journal*. 2009;**152**:110-115
- [30] Podda FP, Zuddas A, Minacci M, Baldi F. Heavy metal coprecipitation with hydrozincite $[Zn_5(CO_3)_2(OH)_6]$ from mine waters caused by photosynthetic micro organisms. *Applied and Environmental Microbiology*. 2000;**66**:5092-5098

- [31] Mire CE, Tourjee JA, O'brien WF, Ramanujachary KV, Hecht GB. Lead precipitation by *Vibrio harveyi*: Evidence for novel quorum-sensing interactions. *Applied and Environmental Microbiology*. 2004;**70**(2):855-864
- [32] Silver S. Bacterial heavy metal resistance: New surprises. *Annual Review of Microbiology*. 1996;**50**(69):753-789
- [33] Schalk IJ, Hannauer M, Braud A. New roles for bacterial siderophores in metal transport and tolerance. *Environmental Microbiology*. 2011;**13**:2844-2854
- [34] Taga ME, Bassler BL. Chemical communication among bacteria. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;**100**(Suppl):14549-14554
- [35] Marques JJGSM, Curi N, Schulze DG. Trace elements in Cerrado soils. In: Alvarez VH, Schaffer CEGR, et al., editors. *Tópicos em Ciência do Solo (Topics in Soil Science)*. Viçosa: Sociedade Brasileira de Ciência do Solo; 2002. pp. 103-142
- [36] Aguiar MRMP, Novaes AC, Guarino AWS. Remoção de metais pesados de efluentes industriais por aluminossilicatos. *Química Nova*. 2002;**25**(6b):1145-1154
- [37] Virga RSP, Geraldo LP, Santos FH. Avaliação de contaminação por metais pesados em amostras de siris azuis. *Ciência Tecnologia e Alimentação, Campinas*. 2007;**27**(4):779-785
- [38] World Health Organization. *Trace Elements in Human Nutrition and Health*. Geneva: WHO; 1996
- [39] Linde AR, Arribas P, Sanchez-Galan S, Garcia-Vazquez E. Eel (*Anguilla anguilla*) and Brown Trout (*Salmo trutta*) target species to assess the biological impact of trace metal pollution in freshwater ecosystems. *Archives of Environmental Contamination and Toxicology*. 1996;**31**:297-302
- [40] Tavares TM, Carvalho FM. Avaliação de exposição de populações humanas a metais pesados no ambiente: Exemplos do Recôncavo Baiano. *Revista Química Nova*. 1992;**15**(2):147-154
- [41] Monroe D. Looking for chinks in the armor of bacterial biofilms. *PLoS Biology*. 2007;**5**(11):307
- [42] Azevedo NF, Cerca N. *Biofilmes: Na Saúde, no Ambiente, na Indústria*. 1st ed. Portugal: Publindústria Edições Técnicas; 2012
- [43] Muller D, Médigue C, Koechler SA. A tale of two oxidation states: Bacterial colonization of arsenic-rich environments. *PLoS Genetics*. 2007;**3**:e53
- [44] Kaplan JB, Izano EA, Gopal P, et al. Low levels of β -lactam antibiotics induce extracellular DNA release and biofilm formation in *Staphylococcus aureus*. *MBio*. 2012;**3**(4):1-14
- [45] Hennequin C, Aumeran C, Robin F, Traore O, Forestier C. Antibiotic resistance and plasmid transfer capacity in biofilm formed with a CTX-M-15-producing *Klebsiella pneumoniae* isolate. *The Journal of Antimicrobial Chemotherapy*. 2012;**67**(9):2123-2130
- [46] Giovanella P, Cabral L, Costa AP, et al. Metal resistance mechanisms in Gram-negative bacteria and their potential to remove Hg in the presence of other metals. *Ecotoxicology and Environmental Safety*. 2017;**140**:162-169
- [47] Wu X, Santos RS, Fink-Gremmels J. Cadmium modulates biofilm formation by *Staphylococcus epidermidis*. *International Journal of Environmental*

Research and Public Health.
2015;**12**:2878-2894

[48] Perrin C, Briandet R, Jubelin G, Lejeune P. Nickel promotes biofilm formation by *Escherichia coli* K-12 strains that produce curli. Applied and Environmental Microbiology. 2009;**75**:1723-1733

[49] Araújo LCA, Purificação-Júnior AF, Silva SM, Lopes ACS, et al. In vitro evaluation of mercury (Hg²⁺) effects on biofilm formation by clinical and environmental isolates of *Klebsiella pneumoniae*. Ecotoxicology and Environmental Safety. 2019;**169**:669-677

[50] Navarrete F, De La Fuente L. Response of *Xylella fastidiosa* to zinc: Decreased culturability, increased exopolysaccharide production, and formation of resilient biofilms under flow conditions. Applied and Environmental Microbiology. 2014;**80**:97-107

[51] Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;**283**:65-87

[52] Lemire JA, Harrison JJ, Turner RJ. Antimicrobial activity of metals: Mechanisms, molecular targets and applications. Nature Reviews Microbiology. 2013;**11**:371-384

[53] Tremarolli V, Fedi S, Turner RJ, Ceri H, Zannoni D. *Pseudomonas pseudoalcaligenes* KF707 upon biofilm formation on a polystyrene surface acquire a strong antibiotic resistance with minor changes in their tolerance to metal cations and metalloids. Archives of Microbiology. 2008;**190**:29-39

[54] Booth SC, George IFS, Zannoni D, Cappelletti M, Duggan GE, Ceri H, et al. Effect of aluminium and copper on biofilm development of *Pseudomonas pseudoalcaligenes*

KF707 and *P. fluorescens* as a function of different media compositions. Metallomics. 2013;**5**:723-735

[55] Koechler S, Farasin J, Cleiss-Arnold J, Arsene-Ploetze F. Toxic metal resistance in biofilms: Diversity of microbial responses and their evolution. Research in Microbiology. 2015;**166**:764-773

[56] Harrison JJ, Ceri H, Stremick C, Turner RJ. Differences in biofilm and planktonic cell mediated reduction of metalloids. FEMS Microbiology Letters. 2004;**62**:235-357

[57] Conibe PA, Cruz LF, Navarrete F, Ducan D, Tygart M, De La Fuente L. *Xylella fastidiosa* differentially accumulates mineral elements in biofilm and planktonic cells. PLoS One. 2013;**8**:e54936

[58] Sheng GP, Yu HQ, Yu Z. Extraction of extracellular polymeric substances from the photosynthetic bacterium *Rhodospirillum rubrum*. Applied Microbiology and Biotechnology. 2005;**67**(1):125-130

[59] Schue M, Fekete A, Ortet P, Brutec C, Heulin T, Schmitt-Kopplin P, et al. Modulation of metabolism and switching to biofilm prevail over exopolysaccharide production in the response of *Rhizobium alamyi* to cadmium. PLoS One. 2011;**6**:e26771

[60] Marchal M, Briandet R, Halter D, Koechler S, Dubow MS, Lett MC, et al. Subinhibitory arsenite concentrations lead to population dispersal in *Thiomonas* sp. PLoS One. 2011;**6**(8):e23181

[61] Kazy SK, Sar P, Songh SP, Sem Asish K, D'Souza SF. Extracellular polysaccharides of a copper-sensitive and copper-resistant *Pseudomonas aeruginosa* strain: Synthesis, chemical nature and copper binding. World

Journal of Microbiology and Biotechnology. 2002;**18**:583-588

[62] Priester JH, Olson SG, Webb SM, Neu MP, Hersman LE, Holden PA. Enhanced exopolymer production and chromium stabilization in *Pseudomonas putida* unsaturated biofilms. Applied and Environmental Microbiology. 2006;**72**:1988-1996

[63] Reith F, Etschmann B, Grosse C, Moors H, Benotmane MA, Monsieurs P, et al. Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. PNAS. 2009;**106**:17757-17762

[64] Li B, Zhao Y, Liu C, et al. Molecular pathogenesis of *Klebsiella pneumoniae*. Future Microbiology. 2013;**9**:1071-1081

[65] Abou-Shanab RAI, Van Berkum P, Angle JS. Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and the rhizosphere of *Alyssum murale*. Chemosphere. 2007;**68**:360-367

[66] Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. Trends in Microbiology. 2006;**14**(4):176-182

[67] Summers AO et al. Mercury released from dental silver fillings provokes an increase in mercury-resistant and antibiotic-resistant bacteria in oral and intestinal floras of primates. Antimicrobial Agents and Chemotherapy. 1993;**37**:825-834

[68] Summers AO. Generally overlooked fundamentals of bacterial genetics and ecology. Clinical Infectious Diseases. 2002;**34**:S85-S92

[69] Alonso A et al. Environmental selection of antibiotic resistance genes. Environmental Microbiology. 2001;**3**:1-9

[70] Fugimore H, Kiyono M, Nobuhara K, Pan-Hou H. Possible involvement of red pigments in defense against mercury in *Pseudomonas* K-62. FEMS Microbiology Letters. 1996;**135**(2-3):317-321

[71] McIntosh D, Cunningham M, Ji B, Fekete FA, et al. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of *Aeromonas salmonicida* subsp. *salmonicida* is associated with carriage of an IncA/C plasmid similar to the *Salmonella enterica* plasmid pSN254. The Journal of Antimicrobial Chemotherapy. 2008;**61**(6):1221-1228

[72] Mathema VB, Thakuri BC, Sillanpää M. Bacterial mer operon-mediated detoxification of mercurial compounds: A short review. Archives of Microbiology. 2011;**193**:837-844

[73] Pérez-Valdespino A, Celestino-Mancera M, Villegas-Rodriguez VL, Curiel-Quesada E. Characterization of mercury-resistant clinical *Aeromonas* species. Brazilian Journal of Microbiology. 2013;**44**(4):1279-1283

[74] Ruiz N. The role of *Serratia marcescens* porins in antibiotic resistance. Microbial Drug Resistance. 2003;**9**:257-264

[75] Mukhopadhyay R, Rosen BP. Arsenate reductases in prokaryotes and eukaryotes. Environmental Health Perspectives. 2002;**110**:745-748

[76] Wright JW, Natan MJ, Macdonnell FM, Ralston DM, O'Halloran TV. Mercury(II) Thiolate Chemistry and the Mechanism of the Heavy Metal Biosensor MerR. In: Lippard SJ, editor. Progress in Inorganic Chemistry. Lippard SJ, editor. 38 ed. New York: John Wiley & Sons; 1990. pp. 323-412

[77] Nies DH. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews. 2003;27:33-39

[78] Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. Journal of Applied Microbiology. 2002;92:65-71

[79] Barkay T, Miller SM, Summers AO. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Review. 2003;27:355-384

[80] Roberts MC. Update on acquired tetracycline resistance genes. FEMS Microbiology Letters. 2005;245:195-203

[81] Martins VV, Zanetti MOB, Pitondo-Silva A, Stehling EG. Aquatic environments polluted with antibiotics and heavy metals: A human health hazard. Environmental Science and Pollution Research. 2014;21:5873-5878

[82] Caille O, Rossier C, Perron K. A copper-activated two-component system interacts with zinc and imipenem resistance in *Pseudomonas aeruginosa*. Journal of Bacteriology. 2007;189:4561-4568

[83] Ghosh A et al. Characterization of large plasmids encoding resistance to toxic heavy metals in *Salmonella abortus equi*. Biochemical and Biophysical Research Communications. 2000;272:6-11

[84] Rasmussen LD, Sørensen SJ. Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil. FEMS Microbiology Ecology. 2001;36(1):1-9

[85] McArthur JV, Tuckfield RC. Spatial patterns in antibiotic resistance among stream bacteria: Effects of industrial pollution. Applied and Environmental Microbiology. 2000;66(9):3722-3726