

## Emergence and Dispersion of Resistance Genes by the Aquatic Environment: a review

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**ABSTRACT:** The association of the emergence of bacterial resistance to clinical environments is common; however, aquatic environments, especially the polluted ones, also play a key role in this regard. Aquatic environments can act as facilitator for the exchange of mobile elements, responsible for resisting antibiotics. They even may stimulate the emergence and selection of these elements through contaminants or the natural competition between bacterial phyla. Currently there is a large number of highly-reliable resistance genes, which is selected in aquatic environments, mostly due to several types of pollution, such as the *mcr-1* gene that causes resistance to one of the antibiotics, available in the market, namely colistin. Thus, the present review aims to show a range of impacts capable of selecting bacterial resistance in the environment, thus clarifying this environment's role in dispersion of resistance.

**Keywords:** Antibiotic resistance, Dispersal genes, pollution, *mcr-1*

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### INTRODUCTION

When thinking about the interaction between bacteria and water, it is common to talk about contamination, since it is responsible for turning a great part of world's drinking water inappropriate for consumption; however, there is another problem associated with that interaction, to wit the appearance and facilitation of horizontal transference of resistance genes (Baquero et al., 2008; Hsu et al., 2014; Wintersdorff, von et al., 2016; Xu, J. et al., 2015).

It is common to associate emergence of bacterial resistance with the selective pressure they undergo in a hospital

environment, wherein there is a constant use of antibacterial drugs in treatment of different infectious processes (Hsueh et al., 2005). Nevertheless, many studies have already proven that a lot of resistance genes are also present in environments outside the hospitals, such as aquatic ecosystems (Finley et al., 2013; Wellington et al., 2013; Xiong et al., 2015). As a result, bacteria responsible for carrying these genes may be directly connected with the resistance occurring frequently in hospitals (Czekalski et al., 2014; Huerta et al., 2013).

### Emergence of Resistance in Aquatic Environment

In aquatic environments, resistance can occur through selective pressure imposed

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many times by human beings when contaminating these bodies of water with wastes that mainly contain heavy metals as well as remnants of antibacterial drugs (Furukawa et al., 2015; Gullberg et al., 2014). These drugs can reach the environments via various routes such as flow of rain water with remnants of drugs, used by agriculture, to rivers, lakes, and wells; absence of any treatment of human wastes containing antibacterial drugs along with its direct disposal into bodies of water; and the use of antibiotics in agriculture. This selective pressure, though not as intense as that of a hospital environment, may also exert its effects (Forsberg et al., 2014; Gibson et al., 2014).

### **Aquaculture**

Aquaculture is the rearing of aquatic animals, used for human consumption, in controlled environments, such as lakes, rivers, and seas. The use of antibiotics has become unruly and frequent in this type of practice, as manipulation of animals causes a high level of stress in them, leading to weaker immune system and, consequently, higher sensibility to bacterial infection. To prevent this problem, many aquaculture owners use prophylactic antibiotics in order that the bacteria could struggle to grow in the treated water (Huang, Y. et al., 2015; Shah et al., 2014).

Many aquaculture practices are found in connection to large bodies of water, which facilitates the dispersion of the drugs used in excess along with the resistance-carrying bacteria, leading to a situation in which a resistance gene that occurs in one specific place can spread for kilometers (Cabello et al., 2016; Muziasari et al., 2016; Tomova et al., 2015).

Such excessive use of antibiotics has already collected its toll. There are already reports of pathogens in many species of fish and other aquatic animals, resistant to lots of medications meant to combat them (Muziasari et al., 2016). The appearance of

resistance among these pathogens is preoccupying, for it makes rearing aquatic animals more difficult, due to both infections and the possibility of occurrence of these genes' horizontal transference in bacteria, pathogenic to humans (Muñoz-Atienza et al., 2013; Xiong et al., 2015).

As the population grows, new forms of food production tend to grow as well, one of which is aquaculture which needs a better way of regulating the use of antibiotics (Rico & Brink, Van den, 2014).

### **Agriculture and Animal Waste**

Antibiotics are commonly used to aliment animals throughout the world, generally to prevent or treat diseases (Wang, L. et al., 2015). The major problem with this practice is that it goes unchecked, without a correct dosage control, which leads to the accumulation of these medications in these animals' urine and feces (Zhang et al., 2015; Zhu et al., 2013). Other elements, found in animal wastes, include the heavy metals, such as cadmium, arsenic, mercury, and zinc (Hölzel et al., 2012). When not correctly disposed, these metabolic residues end up in bodies of water, taking with them a great deal of antibiotics (Ji et al., 2012). Such a large volume of antibiotics and heavy metals stimulates the selection of resistant bacteria, which is followed by horizontal transference of the genes (Martins et al., 2014).

### **Hospital Effluents**

The residual water from hospitals is certainly an important source of pathogenic organisms and genes, resistant to antibiotics. It is then released into the environment, since these effluents transport chemical and biological substances, including the active principle of many medications and their metabolites, chemical products, nutrients, and bacteria with their resistant genes (Hocquet et al., 2016; Laffite et al., 2016; Stalder et al., 2014). In aquatic environments, a great part of the bacterial community is found aggregated, forming a biofilm on the surface. Such biofilms are considered an ideal place

for genes' horizontal transference to occur, resulting in the dissemination of resistance to antibiotics (Ory et al., 2016). Integrons, found in hospital effluents, codify the resistance to many different types of antibiotics. Present in bacterial communities, these Integrons increase the meta-genomic plasticity of the said communities, enhancing their evolution (Simo Tchuinte et al., 2016; Stalder et al., 2014; Vaz-Moreira et al., 2016). The Integrons may be involved in the dissemination of resistance in clinical environments, being a participant in the genetic exchange of resistant genes in different ecosystems (Gillings, 2014).

### **Natural Competition**

Another way for resistance to appear in the environment is through natural competition (Hibbing et al., 2010). Considering that great part of antimicrobials, utilized by man, is extracted from bacteria or based on substances, produced by them, it is possible for the emergence of resistance to begin as a kind of self-defense in the struggle for perpetuation of the species (Koch et al., 2014; Vargas-Bautista et al., 2014). In general, aquatic environments possess a very diverse bacterial community, resulting in a constant dispute, mainly for nutrients (Wanjugi & Harwood, 2013). It is during these disputes that antibiotics are produced with the aim of inhibiting the growth of other species (Audrain et al., 2015; Gerdt & Blackwell, 2014). This form of resistance appears regardless to the contamination of the locale in which the bacteria are found (Koch et al., 2014).

### **Heavy Metals and Industries**

There are many sources of heavy metals that contaminate the environment and bacterial resistance might be linked to contamination of these metals, which acts as indirect agents of selection, likely to play an important role in the proliferation of resistance to antibiotics. One of these is co-resistance (Pal et al., 2015; Yazdankhah et al., 2014). Co-resistance occurs when genes that codify

resistant phenotypes are located in the same genetic element, such as the plasmid (Baker-Austin et al., 2006; Hobman & Crossman, 2015). Genes of resistance to metals and antibiotics are specifically linked to plasmids, which can result in a co-selection of resistance to both antibiotics and metals. This co-selection might be derived from various contaminated environments with great quantities and varieties of metals, in which bacteria resistant to both metals and antibiotics could be present. There is no direct evidence for co-selection of resistance to antibiotics through exposure to metals; however, this hypothesis is studied thanks to the frequency that resistance to antibiotics and metals is found in the same bacteria (Bruins et al., 2000; Verma et al., 2001).

### **Dispersion of Resistance in Aquatic Environments**

Phenomena such as genetic exchange and natural selection can explain the increase in bacterial resistance (Hu et al., 2015). The exchange of genes between these organisms (via plasmids, transposons, and integrons) can occur by bacterial conjugation, transformation, or transduction. In these cases, such genes can confer different resistance mechanisms to these bacteria, including inactivation of antibiotic action, reduction of intracellular concentration of antibiotics due to efflux pumps, reduction of the cellular membrane's permeability, and alteration of the antibiotic target, making the association of the antimicrobial more difficult (Brown-Jaque et al., 2015; Stone et al., 2016).

The aquatic environment is a facilitator of the exchange of mobile genetic elements, because the movement of bacteria is easier when compared with a dry environment (Berglund et al., 2014; Mao et al., 2014). The presence of an elevated number of microorganisms with those bacteria that present some sort of resistance enables quite frequent exchanges of genetic elements that codify resistance to antibiotics (Yang, C.-W. et al., 2014). Through such contact,

many bacteria, not previously resistant, may come to exist through a phenomenon called “acquired resistance”. Events such as these are important for the diffusion of antimicrobial resistance (Gibson et al., 2015; Marathe et al., 2013).

### **Risks and Costs of Bacterial Resistance**

According to the data from World Health Organization (WHO), divulged in May 2014, infections are the main cause of death in low-income countries and the third leading cause worldwide. Majority of such deaths is caused by bacteria with some sort of resistance to antibiotics (World Health Organization, 2015).

Bacterial resistance is considered a public health issue throughout the world, and for that, studies investigating resistance mechanisms are necessary since they contribute to a better understanding of the said mechanisms and their consequence on human health (Nathan & Cunningham-bussel, 2013; Roca et al., 2015), because the complex system of bacterial resistance and its wide dissemination increasingly reduces available therapeutic options, leading to increased mortality (Molton et al., 2013; Sorlozano et al., 2014).

Infections, caused by resistant bacteria, impose high costs when treating the infected patient. A good example of this is the 2013 report by the Center for Disease Control (CDC) in the United States, which indicates that the expenses of treatments, related to resistant bacteria, are approximately USD 35 billion annually, and the total cost, considering the loss of productivity due to the time period of patient internment, is USD 55 billion each year (Dantas & Sommer, 2014; Manning et al., 2016). According to data from Economic Forum of Global Risks, in the European Union 25,000 people die each year due to complications, caused by resistant bacteria, with the relevant costs reaching up to EUR 1,5 billion annually (World Economic Forum (WEF) 2013; Jørgensen, 2016). These numbers estimate

that resistance to antibiotics will be one of the greatest social and economic problems that humanity will face in the coming years, due to number of deaths and elevated treatment costs (Barriere, 2015).

Studies that evaluate the prevalence of multi-resistant bacterial infections in hospitals are common. However, there have been increasing researches on resistant bacteria in community infections, which shows that the spread of these organisms to other environments are related to human healthcare (Economou & Gousia, 2015; Tschudin-Sutter et al., 2013). A number of these studies show that there might be a close relation between resistant genes that appear in the environment with those, present in hospitals, making them important for healthcare (Shah et al., 2014; Stalder et al., 2014). Even though the number of patients, infected with resistant pathogens in the community, is not as high as that of hospitals, they demand attention, because these patients can contribute to the dissemination of resistance to healthcare environments, contributing to its propagation (He, L.-Y. et al., 2016).

### **Main Resistance Genes**

Table 1 demonstrates that there is an already documented relation between resistance genes, found in aquatic environments, and those, found in medical environments. Various genes are of high importance, since they codify resistance to a variety of antibiotics, relevant in clinical treatment, such as Vancomycin (*vanA*), beta-lactams (*bla<sub>TEM</sub>*, *bla<sub>CTX-M</sub>* e *bla<sub>GES-5</sub>*), and e colistin (*mcr-1*). It is worth noting that *mcr-1* can already be found in the environment, given that it is responsible for resistance to Colistin, a potent antibiotic, used in cases of multi-resistant bacteria that do not respond to other antimicrobial therapies (Levin et al., 1999; Shiban et al., 2014).

Given the potency of Colistin, for many years it was considered that resistance to it would hardly appear, though in 2016, the

gene for resistance to Colistin was found among pigs in China, its horizontal dissemination being described for the first time via plasmid (Liu, Y.-Y. et al., 2016). The fact that this gene is already found in

aquatic environments shows two things: first, that it is easily disseminated, and second, that aquatic environments may have a relevant role in future dispersion (Liu, Y.-Y. et al., 2016; Ovejero et al., 2017).

**Table 1. Relationship between the aquatic and clinical environment**

Genes	Research in aquatic environment	Research in clinical environment
	REFERENCE	REFERENCE
<i>aadA1</i>	(Canal et al., 2016)	(Lindstedt et al., 2003)
<i>ampC</i>	(Capkin et al., 2015)	(Paltansing et al., 2015)
<i>tetM</i>	(Chen, B. et al., 2015)	(Croucher et al., 2011)
<i>tetA</i>	(Cesare, Di et al., 2015)	(Ahn et al., 2016)
<i>bla<sub>CTX-M</sub></i>	(Conte et al., 2017)	(Abdi et al., 2014)
<i>sul1</i>	(Huang, L. et al., 2017)	(Manyahi et al., 2017)
<i>qnrS</i>	(Proia et al., 2016)	(Yugendran & Harish, 2016)
<i>sul2</i>	(Rowe et al., 2016)	(Teichmann et al., 2014)
<i>mecA</i>	(Seyedmonir et al., 2015)	(Carlesse et al., 2016)
<i>bla<sub>TEM</sub></i>	(Stange et al., 2016)	(Leverstein–van Hall et al., 2002)
<i>vanA</i>	(Young, S. et al., 2016)	(Phukan et al., 2016)
<i>bla<sub>GES-5</sub></i>	(Manageiro et al., 2014)	(Ribeiro et al., 2014)
<i>qnrA</i>	(Rafraf et al., 2016)	(Wu, J.-J. et al., 2007)
<i>ermB</i>	(Proia et al., 2016)	(Chu et al., 2015)
<i>mcr-1</i>	(Zurfuh et al., 2016)	(McGann et al., 2016)
<i>aadA1</i>	(Canal et al., 2016)	(Lindstedt et al., 2003)
<i>ampC</i>	(Capkin et al., 2015)	(Paltansing et al., 2015)
<i>tetM</i>	(Chen, B. et al., 2015)	(Croucher et al., 2011)
<i>tetA</i>	(Cesare, Di et al., 2015)	(Ahn et al., 2016)
<i>bla<sub>CTX-M</sub></i>	(Conte et al., 2017)	(Abdi et al., 2014)
<i>sul1</i>	(Huang, L. et al., 2017)	(Manyahi et al., 2017)
<i>qnrS</i>	(Proia et al., 2016)	(Yugendran & Harish, 2016)
<i>sul2</i>	(Rowe et al., 2016)	(Teichmann et al., 2014)
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<i>bla<sub>TEM</sub></i>	(Stange et al., 2016)	(Leverstein–van Hall et al., 2002)
<i>vanA</i>	(Young, S. et al., 2016)	(Phukan et al., 2016)
<i>bla<sub>GES-5</sub></i>	(Manageiro et al., 2014)	(Ribeiro et al., 2014)
<i>qnrA</i>	(Rafraf et al., 2016)	(Wu, J.-J. et al., 2007)
<i>ermB</i>	(Proia et al., 2016)	(Chu et al., 2015)
<i>mcr-1</i>	(Zurfuh et al., 2016)	(McGann et al., 2016)

## CONCLUSION

A better understanding of the ecologic role, portrayed by that aquatic environments, in the appearance of antibiotic resistance may help comprehending the evolution and dissemination of these mechanisms and contribute to the elaboration of policies that orient an appropriate management of hospital and industrial waste disposal. Every study that aims at increasing the knowledge and/or reducing the dispersion of bacterial resistance is commendable, given that at the moment it is not possible

to gain a better perspective of this grave problem in world public health.

One way to mitigate these problems would be investment in sanitation policies, aimed at collecting and treating more efficient sewage. As a result the amount of antimicrobials to reach the aquatic bodies would be vigorously decreased.

## Conflict of Interest

The authors declare no conflict of interest.

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