ENVIRONMENTAL SPREAD OF ANTIBIOTIC MOLECULES, ANTIBIOTIC RESISTANT BACTERIA AND GENES: JIGSAW PIECES OF A PUBLIC HEALTH PROBLEM

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RESUMO

O uso massivo de antibióticos na medicina humana e no ambiente de produção animal é responsável pela libertação no ambiente de resíduos contaminados por moléculas de antibióticos, bactérias resistentes e seus respectivos genes. Condições particulares encontradas no ambiente podem promover a selecção e a persistência, por longos períodos de tempo, de microrganismos com uma capacidade extraordinária de participar em eventos genéticos de disseminação horizontal e recombinação, que melhoram as suas competências de adaptação e/ou colonização a vários hospedeiros, incluindo o Homem. A contaminação de diferentes nichos ambientais em várias regiões do globo pode colocar em risco o sucesso de medidas restritivas à disseminação de bactérias resistentes.

PALAVRAS-CHAVE

Bactérias resistentes a antibióticos, Genes que conferem resistência a antibióticos, Moléculas de antibióticos, Ambiente

ABSTRACT

A massive use of antibiotics in human medicine and in animal production setting is responsible for the release to the environment of waste products contaminated by antibiotic molecules, antibiotic resistant bacteria and/or their genes. Particular conditions found in the environment might promote the selection and a long time persistence of microorganisms with an extraordinary ability to participate in genetic horizontal dissemination and recombination events, which might improve their adaptation and colonization skills to diverse settings, including the human one. The contamination of different environmental niches in several regions worldwide may undermine the success of the resistant bacteria containment measures.

KEYWORDS

Antibiotic resistant bacteria, Antibiotic resistance genes, Antibiotic molecules, Environment

1. INTRODUCTION

Bacteria inhabit our planet for three and a half billion years during which have been defied by numerous adverse conditions. Their adaption to different environmental circumstances is extraordinary and the use of antibiotics was only one more challenge to overcome. We know that bacterial resistance to these molecules did not suddenly appear with the use of penicillin or streptomycin fifty years ago, since it has been shown that microorganisms from glacier water with over two thousand years were already resistant to antimicrobial compounds (Dancer *et al*., 1997). Many of the known resistance mechanisms had primary physiological functions and evolved to overcome the presence of biocide substances (Linares *et al*., 2006). This evolution is related to the bacteria ability to mutate and to acquire foreign DNA harbouring specific genes or being involved in recombination events that favour their survival in particular environments.

Although antibiotic resistant bacteria have been detected for a long time, the massive use of antibiotics during the last decades in human and animal therapy for prophylaxis, as growth promoters in animal production or in agriculture, led to the selection of a high number of multidrug resistant microorganisms in different ecological niches (del Campo *et al*., 2003; Phillips *et al*., 2004; Kümmerer, 2005). These microorganisms can be easily disseminated to humans by the different routes shown in Figure 1 and, when involved in human infections, might challenge medicine worldwide due to the scarce therapeutic options. In this review we will focus on several human activities that might promote selection and/or dissemination of antibiotic molecules, antibiotic resistant microorganisms or their associated genes in non-hospital settings.

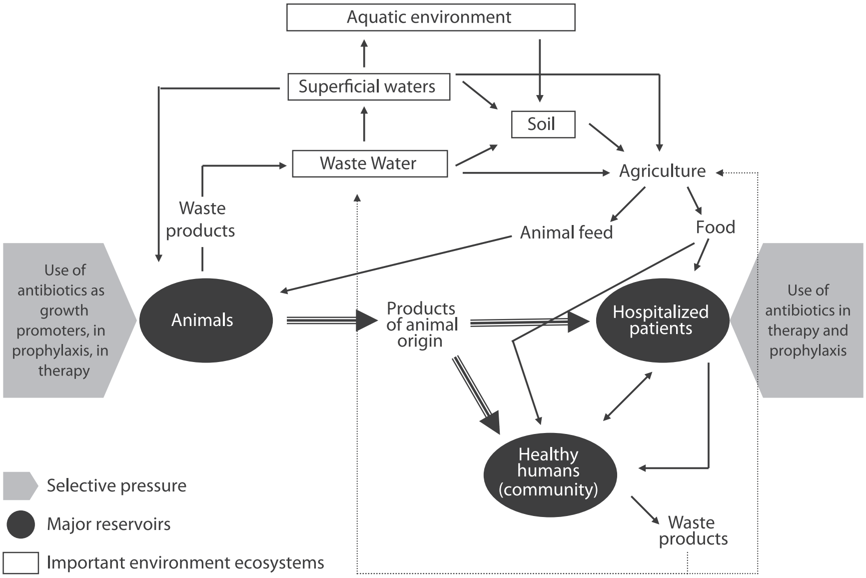

Figure 1. Dissemination paths of antibiotic molecules, antibiotic resistant bacteria or their genes between different ecological niches (Adapted from Witte *et al*., 2000).

2. ENVIRONMENTAL CONTAMINATION BY ANTIBIOTIC MOLECULES

Antibiotic molecules are released to the environment mainly by human and animal excrement and by pharmaceutical industry even when they are submitted to sewage treatment plants (STP). A high amount of these molecules are, in fact, expected in residual waters if we review the numbers of official reports and studies concerning their consumption in human medicine and animal production. Only in Denmark, between 2006 and 2007, 115-121 tons of antibiotics in animal production were consumed (DANMAP, 2007) and in Portugal, during 2006, 155 tons of antibiotics were used for pets and animal production (INFARMED, 2006). Although high, these numbers have been decreasing among European countries in the last years, contrarily to the human antibiotic consumption in ambulatory care of some countries (Ferech *et al*., 2006).

The chronic exposure of ecosystems to the effects of antibiotics or their metabolites is of concern since they might behave as xenobiotics (Sanderson *et al*., 2003). Their accumulation in water, soil and food might trigger human adverse effects as allergies (Webb *et al*., 2003) and in other organisms, as *Daphnia magna*, might induce sexual alterations (Flaherty *et al*., 2005). Several molecules in different concentrations have been found in sewage, rivers, sea and other superficial waters all over the world during the last years. In an urban sewage, in the USA, and in the Seine river in France, 35 and 17 antibiotics were detected, respectively, which included molecules as tetracyclines, macrolides, sulphonamides and fluoroquinolones (Kolpin *et al*., 2002; Tamtam *et al*., 2008). Other examples can be revised in literature (Kümmerer *et al*., 2009).

The use of manure containing antibiotic molecules in agriculture might contaminate vegetables by absorption, and superficial and deep waters by infiltration and run-off phenomenon (Giger *et al*., 2003). However, a further source can release to the environment even bigger amounts of antibiotic molecules than human and animal settings: the pharmaceutical industry. Larsson *et al*. (2007) performed a study in a geographical area of Southern India, where medicine production is significant, and showed that the STP studied received effluents of 90 industries on a dairy basis, and antibiotics (e.g. ciprofloxacin) were detected in concentrations that corresponded to a dairy discharge of 45 Kg of active substance.

Besides the direct effect of antibiotics in the environment and humans, their presence in different ecological niches might also be responsible for the local selection of resistance bacteria and interfere with bacterial communication in a phenomenon called as *quorum sensing* (Bassler *et al*., 2006). In the latter process, antibiotics are one type of molecules that can work as autoinducers allowing bacteria to control their population density and gene expression (e.g. virulence and biofilm production) (Williams *et al*., 2007). For example, in the presence of sub-inhibitory concentrations of tetracycline, *Pseudomonas aeruginosa* increases the expression of piocianine (fenazinic compound that acts as a competitive virulence factor and signalling molecule of *quorum sensing*) (Liang *et al*., 2008). Additionally, *quorum sensing* might increase the competence state of bacteria or the ability to mate, promoting the acquisition of foreign DNA in transformation or conjugation events and, therefore, increasing the probability of incorporate biocide resistance genes available in local metagenomes.

3. ENVIRONMENTAL CONTAMINATION WITH ANTIBIOTIC RESISTANT BACTERIA AND GENES

Several studies have reported the presence of antibiotic resistant bacteria outside the hospital setting worldwide, namely in food, sewage, waters for recreational activities, soil, air, animal, healthy human faeces, restaurant flies, seagulls, among others (Kümmerer, 2004; Kühn *et al*., 2005; Kumar *et al*., 2005; Macovei *et al*., 2006; Sapkota *et al*., 2006; Poeta *et al*., 2008). Many of the microorganisms found presented multiple resistance phenotypes/genes to antibiotics, belonged to persistent clones with clinical interest and/or harboured mobile genetic elements similar to that found in hospital units (Novais *et al*., 2005a; Novais *et al*., 2005c). These studies included Gram positive and Gram negative environmental, pathogenic and commensal bacteria (Kühn *et al*., 2005; Antunes *et al*., 2006; Quinteira *et al*., 2006; Neela *et al*., 2007; Lewis *et al*., 2008; Machado *et al*., 2008).

The widespread use of antibiotics in food animal production systems has resulted in the emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain, by direct contact with live and death animals (veterinarians, farmers, food manipulators) or indirectly throughout soil and contaminated waters (Fig.1). Development of resistance can result from chromosomal mutations, but is more commonly associated with the horizontal transfer of resistance determinants borne on mobile genetic elements (Agerso *et al*., 2005; Binh *et al*., 2008; Freitas *et al.,* 2009). The animal species that seem to be the main reservoir of such elements are swine and poultry. Moreover they have been frequently pointed out as source of antibiotic resistant pathogenic bacteria with a negative impact on public health, due to an increased incidence of treatment failure and severity of disease. One of the most enlighten cases is the increasing resistance of *Campylobacter* spp. and *Salmonella* spp. to fluoroquinolones since the 80´s, when ciprofloxacin resistance was infrequent in isolates from this genus. With the massive use of enrofloxacin as an animal growth promoter during the 90´s, *Campylobacter* spp. and *Salmonella* spp. not responding to fluoroquinolones were increasingly recovered from human infections (Phillips *et al*., 2004). Among this group, a particular *Salmonella* Typhimurium DT-104 clone was strongly selected, which acquired different antibiotic resistant genes and has been recovered from animal and humans worldwide, sometimes with fatal outcome (Mølbak *et al*., 1999). Studies conducted by Antunes *et al.* (2006) also support the evidence that resistance to multiple antibiotics observed in *Samonella* spp. isolated from human infections are originally acquired in the animal production setting and is frequently associated with mobile genetic elements.

Intensive animal production can also be a reservoir of non-pathogenic bacteria (e.g. *Enterococcus* spp., *Escherichia coli*) which frequently accumulate and share antibiotic resistant genetic elements. Therefore, they are considered good indicators of the local antibiotic resistance epidemiology and detain an important role in the evolution of antibiotic resistance in different ecological niches. An example was the identification, in piggery samples, namely in manure, of *Enterococcus* spp. belonging to high risk clonal complexes responsible for epidemic nosocomial outbreaks in different countries (Freitas *et al*., 2009). The pig isolates already harboured a set of antibiotic resistance and virulence features usually found in the nosocomial strains. Other evidences concerning the role of animals in the dissemination of antibiotic resistance bacteria and their mobile genetic elements to humans and environment might be found in literature (Agersø *et al*., 2005; Novais *et al*., 2005a; Novais *et al*., 2005b; Machado *et al*., 2008; Smith *et al*., 2008).

Commensal and pathogenic multidrug resistant bacteria have been not only isolated from poultry, swine, rabbits, bovine, fish and other animals of intensive production, but also in wild animals that enter in contact with contaminated environments (Phillips *et al*., 2004; Kozak *et al*., 2009). Even in the surrounding air of pig farms, *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp. carrying genes that confer resistance to tetracyclines, macrolides, lincosamides and streptogramins were observed (Sapkota *et al*., 2006), suggesting that air might also act as a dissemination vehicle of these bacteria in long distances.

Other activities associated to the animal setting that can contribute to the dissemination of antibiotic resistant bacteria and mobile genetic elements in the environment is the use of contaminated manure or biopesticides. Binh *et al*. (2008) observed in soil fertilized with pig manure plasmids carrying *bla*TEM, *sul1*, *sul2* or *sul3* genes, and Agersø *et al*. (2005) found several bacterial genus harbouring class I integrons with gene cassettes conferring resistance to tetracyclines and aminoglicosides in poultry faeces and in soil enriched with these samples. The biopesticide *Paenibacillus popilliae* used in the United States for more than 50 years for suppression of Japanese beetle populations harboured resistance genes with some homology to *van* genes found in clinical isolates of *Enterococcus* spp., one of the most worrying resistance patterns in the clinical setting (Patel *et al.,* 2000). The authors suggest that dissemination of their spores by birds, insects, mice and other animals might have an impact in the dissemination of this important cluster of genes to bacteria associated with human infections.

The food industry might also being accidently introducing antibiotic resistant bacteria in the gut of animals and humans since the use of probiotics with resistance to antibiotics has already been described (Perreten *et al*., 1997). For example, a *Lactococcus* spp. from cheese harboured a plasmid formed by DNA fragments from four different bacterial genus, showing the ability of probiotic bacteria to exchange DNA and creating/acquiring genetic platforms with multiple resistance.

The challenge that some zoonotic bacteria impose to human health or the amazing ability of other to accumulate genetic elements with clinical interest led the European Union to gradually ban antibiotics as growth promoters until January 2006. Although some resistant microorganisms have been reduced in the animal setting after growth promoter ban, other still persist in some geographical regions with variable rates (Phillips *et al*., 2004). The continued use of antibiotics in veterinary medicine, the presence of other biocide agents, the consumption of metals as growth promoters and the presence of other selective molecules in the animal production setting, among other factors, might co-select multidrug resistant bacteria that can persist for long periods of time (Phillips *et al*., 2004). Therefore, the decrease of antibiotic resistance in the animal setting should also involve other changes in the production systems beside growth promoters' ban.

Our concerns should also be extended to other type of animals, the pets, frequently neglected as potential reservoirs of antibiotic resistant bacteria or genes. In 2004, Guardabassi *et al*. published a review pointing out an increase of antibiotic consumption by pets. Butaye *et al*. (2001) observed in pets a higher number of *Enterococcus* spp. resistant to ampicillin than in animals from intensive production farms, and Damborg *et al.* (2009) detected that dogs can be a reservoir of *Enterococcus faecium* CC17, a globally considered high-risk clonal complex.

Antibiotic resistant bacteria are not exclusively exchanged by animals and humans. The aquatic environment is largely affected by both settings and can also constitute a reservoir or a return path of microorganisms and genes that can reach different mammals, including humans.

Sewage, with their bacterial diversity, nutrients and different molecules as antibiotics, gather the conditions to be classified as an excellent place for the development of superbugs. For example, hospital residual waters are frequently enriched with a diversity of nosocomial adapted bacteria willing to exchange a variety of evolved and stable genetic elements harbouring a multiplicity of genes that confer resistant to antibiotics, antiseptics or virulence factors (Kümmerer, 2004). It is frequent to isolate from this niche bacteria as vancomycin resistant *Enterococcus* spp., methicillin resistant *Staphylococcus aureus*, Gram negative bacteria producing extended-spectrum beta-lactamases (ESBL) or carbapenemases, among others (Kümmerer, 2004; Quinteira *et al*., 2006; Novais *et al*., 2005a; Schwartz *et al*., 2003; Machado *et al*., 2009). The partially effective or ineffective treatment of STP is responsible for the dissemination of such microorganisms to superficial and deep waters, to land and marine animals. In arid regions, residual waters might be used for land irrigation and the sediments of sewage treatment as fertilizers, allowing antibiotic resistant bacteria to enter directly in human food chain (Kümmerer, 2004). Several reports of resistant microorganisms from superficial waters are also available, indicating a possible transmition to humans through recreational activities. Arvanitidou *et al*. (2001) detected, in Greece, *Enterococcus* spp. resistant to ciprofloxacin, erythromycin, rifampicin, streptomycin and kanamycin in coastal waters. In Portugal, within the estuary of the Douro river, isolates of *Pseudomonas aeruginosa* carrying the In*58* with a gene cassette encoding for resistance to carbapenems and *Enterococcus* spp. carrying the Tn*1546* with similar structure to those found in Portuguese hospitals were detected (Novais *et al*., 2005b; Quinteira *et al*., 2006). Marine animals have also been affected by antibiotic resistant microorganisms. Poeta *et al.* (2008) observed *E. coli* producing ESBL in seagulls and Foti *et al.* (2009) found different genus of Gram negative bacteria resistant to antibiotics (beta-lactams, tetracyclines) in turtles from the Mediterranean Sea. We might suppose that contamination of aquatic environment is restricted to areas with a high population density. However, although in small numbers, antibiotic resistant bacteria were already found in nonpolluted areas as the Himalaya glacial waters (Pathak *et al*., 2007).

Besides the worrisome environmental contamination by resistant bacteria, the role of autochthone bacterial flora in the evolution of antibiotic resistance in the environment should also be considered, since it can accumulate a diversity of genetic elements with clinical interest and work as an important reservoir, amplifier and disseminator of such elements. Moreover, in the presence of selective agents, a greater expression of these genes or their transfer to other more pathogenic species can occur.

Antibiotic molecules, antibiotic resistant bacteria and mobile genetic elements containing antibiotic resistance genes are linkable jigsaw pieces that contribute to the evolution of antibiotic resistance in different ecological niches. Particular conditions found in environment might promote the selection and a long time persistence of microorganisms with an extraordinary ability of participating in horizontal gene dissemination and recombination events, which improve their adaption and colonization skills to diverse settings, including the human one.

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