

A Review: Improper Antibiotic Utilization evokes the Dissemination of Resistances in Biotic Environments - A High Risk of Health Hazards

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Abstract

Antibiotics are used irresponsibly at every opportunity to treat mild or even viral infections. Accordingly antibiotics have been used in massive amounts in agriculture as a preventive provision against bacterial diseases and to promote the growth of animal feedstock such as poultry, beef and pigs, respectively. Consequently many reports of the past decades contained numerous treatises on bacteria's ability to become resistant to antibiotics what in turn is a growing issue in health care. For example a diverse set of clinical pathogens which includes multi-drug-resistant strains of *Mycobacterium tuberculosis*, *Staphylococcus aureus* and various Enterococci species are now nearly untreatable with standard antibiotics and pose a growing threat to patients in hospitals and the community at large.

Furthermore, antibiotic resistance genes (ARGs) are prevalent in environments resulting in enhanced health hazard risks. Moreover, conjugative transfers of ARGs help to disseminate multiple antibiotic resistant pathogens which pose a serious threat to humans if allowed to enter the food chain. Thus, better knowledge and more information on the fate of antibiotics as well as the development and spread of antibiotic resistance bacteria and genes in the environment are required to understand underlying processes.

This review is an effort to emphasize how biotic environments become polluted initiated by several antibiotic applications via human beings.

Keywords: Antibiotics; Antibiotic resistance genes; Environment; Health hazardous; Antibiotic pollution

Introduction

Antibiotic resistances existed long before people discovered how to use antibiotics as medicine. The discovery and use of antibiotics in medicine in the 20th century, better hygienic conditions and vaccinations can rid bacteria and cure humans so that infectious diseases have declined markedly [1,2]. However, antibiotics are often used carelessly on every opportunity to treat mild or even viral infections. In addition to that antibiotics have been used in massive amounts in agriculture as a preventive measure against bacterial diseases, and to promote the growth of poultry, beef and pigs in animal farms [3-5]. Yet often we hear about multi resistant bacteria which cannot be eliminated effectively by antibiotics. These bacteria are known as antibiotic resistant, and they have become a major clinical and public health problem in the life of most people living today [6-8].

The continual use of antibiotics over a long period of time evokes an intense selective pressure on bacteria and triggers antibiotic resistance dissemination among bacteria [2,9,10]. Although most of the bacteria are killed due to the use of antibiotics, sometimes, some bacteria with a vantage are viable as a consequence of conjugative transfer of resistance genes among bacterial communities and high antibiotic resistance competence, respectively [1,10-13].

During the past few decades, it became very difficult to treat some specific bacterial infections due to the fact of high multiple-antibiotic resistances in bacteria. Many multi-drug resistant bacteria exist (e.g., *Mycobacterium tuberculosis*, *Staphylococcus aureus*), which have become nearly untreatable by standard antibiotics [4]. However, patterns of multiple-antibiotic resistance in gram-negative bacilli are also increasing alarmingly world-wide [14]. These multi-drug resistance bacteria cause threat to patients in hospitals. Also live stocks have been identified as a serious source for the spread of pathogenic antibiotic resistant bacteria in the environment [3-5,10].

Since beginning of the antibiotic era, the dissemination of ARGs was introduced to almost all ecosystems. Especially agricultural soils,

water surfaces and wastewaters receive a notably input of antibiotics which promote the amplification of ARGs [15,16]. The dissemination of antibiotic-resistant bacteria and ARGs from wastewater irrigation to soil and aquatic habitats contributes also to antibiotic resistances world-wide [17].

Another important aspect is the co-selection of antibiotic resistances provoked by the presence of heavy metals or xenobiotics, anthropogenically introduced into the environment [10-12,18]. This co-resistance could occur due to the fact that the presence of resistance genes of heavy metals, xenobiotics and antibiotics are often located on plasmids which are either transferable or mobilizable. Especially plasmid-mediated horizontal gene transfer (HGT) plays an important role in the emergence of new pathogens [1,9,10,16,19].

However, different routes for the introduction of antibiotic spreads into the environment especially in agriculture exist [10]. Currently the application of livestock manure in agriculture is occurring at large scale, causing serious threats of antibiotic disseminations in the environment as excreta of farm animals may contain high doses of antibiotics [5,20-22]. Consequently, different sources of contaminations are causing an uncontrolled spread of antibiotic resistant bacteria in the environment [9,10,23,24]. Also aquatic environments are contaminated by land application of antibiotics in agriculture responsible for the spread of antibiotic resistant bacteria [25].

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Hence, this review is an effort to emphasize how biotic environments are polluted by an improper use of antibiotics by human beings. Furthermore, antibiotic resistance genes are prevalent in the environment with high risk of health hazards. In this review, mechanisms of antibiotic resistances are also discussed.

Pollution by antibiotics

The utilization of antibiotics in clinical sectors or farming purposes selects resistant microorganisms [5,26]. Thus, it is predictable that residues from hospitals or farms will contain both types of pollutants; antibiotics and resistance genes. Nevertheless, the fate of both types of pollutants is likely different. Several antibiotics are natural compounds that have been in contact with environmental microbiota for millions of years and thus are biodegradable, and even serve as a food resource for several microorganisms [27]. Synthetic antibiotics can be more refractory to biodegradation. However, synthetic antibiotics naturally degrade in the environment at different rates, depending on the housing environmental matrix. In the wastewater treatment some antibiotics are efficiently removed by photodegradation [28]. Accordingly, reports exist describing that in ground water samples the concentrations of antibiotics were usually below their detection limits, whereas sediment samples accumulated higher concentration of antibiotics [25,29]. Nevertheless, antibiotics naturally degraded (e.g. by photodegradation) are not necessarily proper pollutants.

Also other sources of ecosystems (hospital, farms, and livestock) are constantly releasing antibiotics in the environment and pollute them (Figure 1). Currently the use of antibiotics is becoming more common for the prevention of infectious diseases in humans or animals as well as in livestock to guarantee fast growth. Therefore, antibiotics are discharged in the environment through excreta from sewage treatment plants and also by, e.g., fish farming [22,26]. According to World Health Organization, specified limits of antimicrobial quantities in animal husbandries are maintained by only a few countries (WHO 2002). This issue is an important drawback to assess the effect of antibiotic application in animal husbandries and to the release of antibiotics in the environment.

Several studies have reported that the application of specific antibiotics in animal production leads to an accumulation of antibiotics in fresh manure, manure storage tanks, soil, surface and underground water [5,22,26,30,31]. Jacobsen and Halling-Sørensen detected tetracycline and sulphonamides in swine manure, whereas tylosin was not detected because of its poor recovery from manure. De Liguoro et al. [32] detected 0.11 mg kg⁻¹ of tylosin and 10 mg kg⁻¹ of oxytetracycline in fresh calf manure, whereas negligible concentrations were detected in soil and water. Dolliver and Gupta found that 1.2% to

1.8% chlortetracycline, monensin and tylosin were lost from manure stockpile by runoff water. Campagnolo et al. [33] detected a significant quantity of macrolides, sulphonamides and fluoroquinolones in the nearby surface water.

However, taking into consideration that 20–75% of the antibiotics administered to feedlot animals are excreted unaltered in faeces (Table 1), it is clear that the antimicrobial use in livestock is an important source of antibiotics release into the environment [26]. Although some effects of antibiotics on plant's growth have been reported [34], the main impact of these pollutants will be on the environmental microbiota. This impact has different levels and consequences. First, the utilization of antibiotics can select for antibiotic-resistant bacteria within the treated host. In the case of antibiotics used for farming purposes, the selection of resistance can be important for both the treatment of animal infections and human health. Several evidences support an association between the use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans [4,14]. According to a report of Massé et al. [5] the quantitative use of livestock antibiotic increased over a time period of 54 years (1950–2004) to 108-fold in the USA, triggered by a recommendation to increase the limits of growth-promoting antibiotics in poultry and pig diets from 3 ppm to 200 ppm. About 91% of livestock industries in US are using 11.2 million kg antibiotics annually sold “over-the-counter” as growth promoters. Antibiotics fed to animals end up in manure and eventually in the environment. Schmieider and Edwards [35] reported that approximately 30% of these antibiotics are metabolized in the bodies of animals. The majority of antibiotics and metabolites are released into environment by animal faeces and urine (Table 2). At sub toxic levels, these antibiotics can lead to an increase in antibiotic resistant bacteria within receiving environments exerted by selection pressures on bacterial communities and, consequently, accelerating their resistances. Excreta from domestic animals and wastewater from cleaning stables disembody in manure storage tanks or lagoons. These manures are used as fertilizer or as a substrate for methane production in biogas plants. The digested residues are also used as fertilizer. According to a report of Berkner et al. [36] several studies have demonstrated that fluorochinolones accumulated in sewage sludge entered rivers and lakes since the sludge was used as fertilizer or filling material. In addition, antibiotics also enter aquatic environments directly from pharmaceutical industries. Antibiotics are also used in culture medium for the production of biological pharmaceuticals.

Antibiotic resistances in the environment

Also antibiotic resistances are a public health concern of great urgency as the inefficacy of antimicrobial agents to treat infectious diseases conspicuously increased. The propagation of ARGs released from anthropogenic sources plays a decisive part in that set off by an overuse of antimicrobials in humans and intense use of antibiotics in animals and agriculture [8,37,38].

Several studies have reported the presence of antibiotic resistant bacteria outside hospitals worldwide, namely in food, sewage, waters for recreational activities, soil, air, animals, healthy human feces and others [39,40].

The presence of antibiotic resistant bacteria in water sources throughout the world has also been well documented. Bacteria are able to adapt rapidly to new environmental conditions and the presence of antimicrobial molecules, respectively, and as a consequences, resistances are increasing with an frivolous use of antimicrobials [41-43]. Several water-borne disease outbreaks occur due to the presence

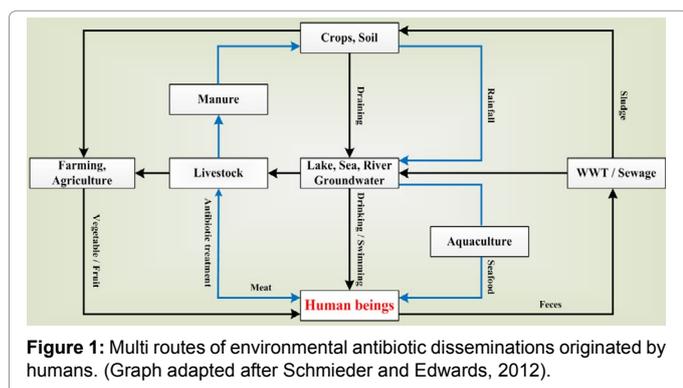


Figure 1: Multi routes of environmental antibiotic disseminations originated by humans. (Graph adapted after Schmieider and Edwards, 2012).

Antibiotics	Manure source	Excretion level (%)	Reference
Chlortetracycline	Steers faeces	75	Elmund et al., 1971 [77]
Tetracycline	Animal faeces	25	Feinman and Matheson, 1978 [78]
Tylosin	Urine	50-60	Feinman and Matheson, 1978 [78]
Oxytetracycline	Castrate sheep	21	Montforts et al., 1999 [84]
Chlortetracycline	Young bulls	17-75	Montforts et al., 1999 [84]
Tylosin	Pigs	40	Kolz et al., 2005 [80]
Monensin	Beef cattle faeces	40	Donoho et al., 1978 [76]
Virginiamycin	Piggeries liquid manure	20	Cocito et al., 1979 [75]
Oxytetracycline	Calves manure (faeces, urine and bedding)	23	Arikan, 2008 [73]

Table 1: Sources and excretion levels of different antibiotics.

Antibiotics	Environment	Concentrations	References
Oxytetracycline	Manure	136 mg/L	Martinez-Carballo et al., 2007 [82]
Chlortetracycline	-	46 mg/L	-
Tetracycline	Swine manure	98 mg/L	Chen et al., 2012 [74]
Oxytetracycline	-	354 mg/L	-
Chlortetracycline	-	139 mg/L	-
Doxycycline	-	37 mg/L	-
Sulfadiazine	-	7.1 mg/L	-
Tetracycline	Swine manure	30 mg/kg	Jacobsen and Halling, 2006 [29]
Sulphonamide	-	2 mg/kg	-
Tylosin	Fresh calf manure	0.11 mg/kg	De Liguoro et al., 2003 [32]
Oxytetracycline	-	10 mg/kg	-
Chlortetracycline	Beef manure	6.6 mg/kg	Dolliver and Gupta, 2008 [26]
Monensin	-	120 mg/kg	-
Tylosin	-	8.1 mg/kg	-
Oxytetracycline	Cow manure	0.5-200 mg/L	Ince et al., 2013 [79]
Chlortetracycline	Swine manure	764.4 mg/L	Pan et al., 2011 [85]
Chlortetracycline	Swine manure storage	1 mg/L	-
Oxytetracycline	Lagoon	0.41 mg/L	Campagnolo et al., 2002 [33]

Table 2: Overview of antibiotic concentrations in manures.

of drug resistant enteric pathogens which survive due to the failure of patients to respond to antibiotics treatments [44,45]. For instance, *E. coli* serve as a crucial reservoir for transmissible resistances to a broad spectrum of antibiotics and is therefore considered to be potentially very dangerous not least because this organism has developed a number of mechanisms for acquiring and disseminating plasmids to some other pathogenic bacterial strains. Thus rendering the treatment of infectious diseases is very difficult [14]. Such selective processes leading to the emergence and maintenance of bacteria resistant to antibiotics are mainly brought about by an incorrect or abusive utilization of these drugs [21]. According to many reports [4,9,10,14,24], bacteria living in contaminated environments develop high multiple antibiotic resistances.

Nonetheless, proliferation of resistance genes can take place by vertical transfer (multiplication of cells harboring a resistance gene), and by three recognized mechanisms of prokaryotic gene transfer (transformation, conjugation, and transduction). HGT by conjugation seems to be of particular importance under environmental conditions because resistance genes are often found on plasmids that are either transferable or mobilizable [46,47]. Conjugative plasmids have been identified in bacterial populations inhabiting, for instance, the phytosphere of sugar beet, the rhizosphere of wheat, contaminated soils, river epilithon, marine sediments, marine air water interfaces, marine water, marine biofilm communities, sewage and activated sludge [7,11,18]. Because of the fact that soil can continuously be enriched with antibiotic resistant genes, it is important to investigate the interplay of ecosystems and the transmission of ARGs. Soils are

conquered by enteric bacteria from the intestinal flora of humans and animals through wastewaters and the use of manure as fertiliser [8,9,22,48,49]. Furthermore, the natural production of antibiotics in soil by the micro-biota can be another potential source of selection for antibiotic resistance genes within earthy environments [46,50,51].

The expanded application of antibiotics has caused an increased incidence of resistances to these antimicrobial compounds, even within bacterial species that are not directly subject to antibiotic control mechanisms. Numerous genes conferring resistance to antibiotics are presently circulating not only in host range but also in many bacterial genera in nature what was not the case prior the selective pressure produced by an increased use of antibiotics [52,53]. Therefore, it is not surprising that contemporary strains of gram negative bacteria frequently display resistance to high levels of commercially administered antibiotics, and such cases have been documented repeatedly with these and many other bacteria [14].

Antibiotic resistance genes in bacteria

Pollution by ARGs can increase the chances of survival of human pathogens by acquiring resistances. The release of residues with human microbiota into environments containing bacteria stimulates conjugative resistance elements and consequently leads to increased possibilities of acquiring novel resistance determinants by human-linked bacteria. For this reason, it has been proposed that the release of residues from hospitals that contain human commensal and infective bacteria (resistant and susceptible) as well as antibiotics should be

reduced to a minimum to avoid interchange of genetic material (even in wastewater treatment plants [54]). It also has been reported that ARGs are present in pristine environments as well as in human and animal populations that have not been in contact with antibiotics what indicates that, once they are integrated in successful gene-transmission elements, antibiotic resistance genes can persist and spread even in the absence of antibiotics. Reports supply the notion that the use of antibiotics for non-therapy purposes can enrich the population of resistant bacteria capable to infect humans [55].

As stated by Baquero et al. [24] the contact of human microbiota with other types of microbiota from different ecosystems will increase the possibility of genetic variations and the emergence of novel resistance mechanisms which are re-introduced in human environments [24].

Apart from chemical pollutions caused by antibiotics themselves, the use of antibiotics may also accelerate the development of ARGs in bacteria, which shade health risks to humans and animals [8,56]. These bacteria might be transmitted from environment to human via direct or indirect contact [20,51]. Bacterial communities are mainly impacted by an improper clinically and veterinary use of antibiotics as various multiple resistances against antibiotics rapidly develop what is genetically controlled by ARGs. It is also noteworthy that the resistance of certain antibiotics is associated with different ARGs based on multiple mechanisms and environments.

For example, tetracycline-resistant bacteria were found to emerge in environments by the introduction of tetracycline [10,43]. The efflux genes of tet A, B, C, D, and E frequently appeared in various environmental compartments including activated sludge of sewage treatment plants (STPs) [57,58], fish farming ponds [59], contaminated soil [10], wastewater treatment plants and surface water [58], and poultry litter [60]. Several *erm* genes have been detected in *Enterococcus* spp. isolated from poultry, raising wastewaters and from livestock manures [3]. Six classes of *erm* genes (A, B, C, F, T, and X) have been detected and quantified in samples taken from animal production, lagoons, and a biofilter system treating hog house effluents [3]. Among the macrolide resistance determinants, *ermB* is considered as the most prevalent gene in environmental microorganisms, especially in the strains of *Enterococcus* [61] and *Streptococcus* spp. [62].

According to Makowska et al. [58] WWTPs are considered as hot spots for both multiplication and dissemination of antibiotic-resistant bacteria and resistance genes. They reported that treatment processes (WWTP) fostered both the frequency of tetracycline- and sulfonamide-resistant bacteria and *intI1*-positive strains as well as the relative abundance of all quantified ARGs and *intI1* gene. But also *tetA* and *sul2* were increasing significantly. The discharge of treated wastewater raised the number of *intI1*, *tet* and *sul* genes in the receiving river water both in terms of copy number ml⁻¹ and relative abundance. A recent study by Anjum and Krakat (2015) [10] revealed the presence of multiple ARGs (*ampC*, *ermB*, *ermD*, *ermG*, *mecA*, *tetM*) in different combinations in bacteria isolated from contaminated alluvial soils. Hedayatianfard et al. [59] investigated bacterial strains isolated from fishes collected from different fish farms and the genes detected were *tetA*, *tetB*, *tetM*, *tetO* and *tetS*. Most of the bacteria were either intermediate or fully resistant to tetracycline. Thereby *tetA* resistance genes were most widely distributed while the *tetM* gene was of minor importance.

Zhang et al. [63] investigated activated sludge and 14 tetracycline resistance (*tet*) genes, 9 genes encompassing efflux pumps (*tetA*, *tetC*, *tetE*, and *tetG*), ribosomal protection proteins (*tetM*, *tetO*, *tetQ*, and

tetS), and enzymatic modification (*tetX*) were commonly detected, whereas 5 genes *tetB*, *tetD*, *tetL*, *tetK*, and *tetA* were not detected. Additionally, 109 lactose-fermenting Enterobacteriaceae (LFE) strains were isolated from the activated sludge. Tetracycline-resistant LFE accounted for 32% of the total 109 LFE strains. The detected frequencies of *tet* genes among all TR-LFE strains varied from 0 to 91% [63].

Böckelmann et al. [43] recorded a frequent presence of *tetO*, *mecA* and *ermB* genes in reclaimed water in Sabadell (Spain) while solely *tetA* was not detected within investigated waste waters in Nardò (Italy).

Graham et al. (2009) [60] characterized the survival of antimicrobial-resistant Enterococci and Staphylococci as well as resistance genes in poultry litter. The *ermB* – and *ermA* resistance genes were most commonly detected in Enterococci and Staphylococci, respectively, whereas three isolates of *E. faecium* were found to carry resistance genes *ermB* or *ermA*. However, Gupta et al. [64] declared that particularly *erm* genes encoding for DNA modifying methyltransferase enzymes reduce the ribosomal binding of corresponding antibiotics and consequently confer resistances to microorganisms.

Khan et al. [62] isolated macrolide-resistant Staphylococci from fresh poultry litter and found several macrolide resistance genes *ermC*, *ermA*, and *ermB*. In addition, researchers have found that erythromycin-resistant Staphylococci containing *ermA* and *ermC* in poultry isolates could be transferred to human strains of *S. aureus* [65]. The *ermA* and *ermC* genes have been reported to be common genes encoding macrolide resistance among Staphylococci of human origin.

Even so, β -Lactam antibiotics are the most widely used groups of antibiotics. The resistance to these antibiotics is a severe threat because they have low toxicity to humans and are used to treat a broad range of infections [58].

The mechanisms of β -lactam resistance include inaccessibility of antibiotics to their target enzymes, modifications of target enzymes, and/or direct deactivation of the antibiotics by β -lactamases [66,67]. In gram-negative bacteria, the primary resistance mechanism is enzymatic inactivation through the cleavage of the β -lactam ring by β -lactamases. More than 400 different β -lactamases encoded by hundreds of ARGs (*bla*) have been identified, and the enzymes are divided into four molecular classes, A–D, mediating resistances to a broad range of β -lactams including penicillins and cephalosporins [68].

A variety of *bla* genes have been identified in bacteria derived from faecal slurry and lagoon water of dairy farms [69] and surface water [58,70]. The environmental compartments may further serve as reservoirs for β -lactam resistance genes. The *bla* genes are often detected in animal-derived environmental pathogens including *Aeromonas*, *Enterobacter* [60], *Salmonella*, and *Staphylococcus* [60,62]. *AmpC* gene encoding β -lactamases have been detected in microbial isolates from wastewater, surface water, and even from drinking water biofilms [70]. *MecA* gene encoding for methicillin resistances in *Staphylococci* was observed to be prevalent in hospital wastewater biofilms [70].

Antibiotic resistance mechanisms in bacteria

Depending of the classes of antibiotics different defence mechanisms are activated by attacked bacterial cells (Figure 2). In general four main mechanisms of antibiotic resistances are described by Schmieder and Edwards [35]: a) The inactivation or modification the antibiotics; b) An alteration in the cellular target site of antibiotic that eliminates the drug/binding capacity c) The modification of metabolic pathways to circumvent the antibiotic effect; d) The reduced intracellular antibiotic

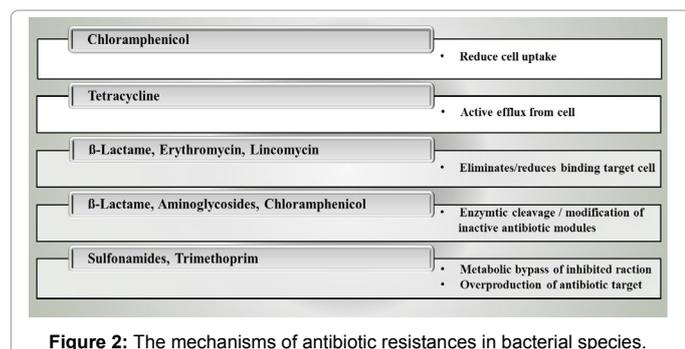


Figure 2: The mechanisms of antibiotic resistances in bacterial species.

accumulation by decreasing the permeability and/or increasing the active efflux of antibiotics..

Moreover, Bacteria can develop antibiotic resistances initiated by the mutation of corresponding antibiotic resistance genes [35,71,72]. When resistant genes are mutated, the interspecies transfer can be realized by conjugation, HGT and transformation and transduction among bacterial populations [10,18,19,43]. Mobile genetic elements, including phages, plasmids and transposons mediate this transfer, and under some circumstances the presence of low antibiotic levels in the environment is the key signal that promotes gene transfer, perhaps ensuring that the whole microbial community is resistance to the antibiotic [35,73-87].

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Conflict of Interests

The authors have declared no conflict of interests.

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