Mobility of antibiotic resistance genes in the environment and potential threats for drinking water

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Voorwoord

Dit rapport beschrijft de bevindingen van een literatuur studie naar de risico's van resistentieontwikkeling t.a.v. antibiotica in grondwater door diergeneesmiddelengebruik. Het rapport is het product voor de taak: 'Verify whether resistance genes can end up in groundwater and drinking water' binnen het BTO project "Veterinary pharmaceuticals in the water cycle" (B111754). Dit onderzoek heeft en nauw verband met de rapporten risico's van 'emerging pathogens' voor de microbiologiche veiligheid van drinkwater (BTO 2012.002) [1] waarin de betekenis van allerlei pathogenen, zoals resistente bacteriën en hun genen, voor de veiligheid van drinkwater wordt besproken. Tevens wordt de ontwikkeling van antibioticaresistentie en de relevantie toegelicht in de 'trend-alert' van de Dutch Water Sector Intelligence [2].

Summary

Antimicrobial resistance genes are of all ages, however as a result of the consumption of antimicrobial agents, the occurrence of antimicrobial resistance (genes) increase in clinical environments (hospitals) but also in the environment (i.e. soil, surface water, and groundwater). The current study describes the occurrence and fate of resistance genes in a soil environment. Additionally, some implications for drinking water are shortly discussed. It was found that the amount and diversity of resistance genes increase in soils as a result of (veterinary) consumption of antimicrobials. Furthermore, resistance genes are rather hydrophilic and therefore mobile in a soil environment, but their persistence (as free DNA or in plasmids) is rather short. Nevertheless, these genes remain in soils even years after exposure to antibiotics or manure application. This suggests that these genes are incorporated in the soil microbial gene pool. The preliminary interpretation of the literature suggests that risks of antibiotic resistance in groundwater and produced drinking water is small compared to more obvious risks of infections in healthcare or via animal products. However, there is hardly any data on the occurrence of resistance genes in drinking water sources, their recalcitrance in drinking water treatment, let alone potential human health effects need to be studied in more detail.

1 Introduction

The development in pharmacology over the past century has led to a vast increase in the use of antimicrobial agents in human and veterinary medicine. In Europe, the total consumption of antimicrobial agents in human medicine and veterinary medicine is of similar magnitude. However, in the Netherlands, the veterinary consumption of antibiotics exceeds human consumption of antibiotics by more than an order of magnitude due to intensive life stock farming and relatively low consumption of antimicrobial agents in human practice [1, 2].

The consumption of antibiotics can lead to the development of resistance in microorganisms. Currently, there is a political debate on the development of antimicrobial resistance as a result of the large consumption of antimicrobial agents in both human and veterinary medicine. A decade ago, the 'Gezondheidsraad (Dutch Health Council) studied the risks of resistance development in the Netherlands and in Europe (http://www.gezondheidsraad.nl/nl/adviezen/antibioticaresistentie). It was concluded that correct hygiene-measures in hospital settings and epidemiologic research prevent the spread of resistant bacteria. However, it was also concluded that there was insufficient international harmonization of measures to prevent the spread of resistant bacteria, and at that time the import of resistant bacteria from other countries was considered a serious threat. Over the last years an increase in the occurrence of antibiotic resistant pathogens has been observed. Part of this increase might be related to the consumption of antibiotics in veterinary practice and aquacultures, as epidemiologic studies showed correlation between the increase in the occurrence of resistant strains in veterinary practice and humans. Consumption of meat is considered to be the most important vector for the transport of resistance genes between live stock and humans.

Resistance genes that enable the resistance towards various classes of antibiotics can potentially be transferred between microorganisms [3]. The potential spread of resistance genes in the environment and transfer towards human pathogens is a human health risk. The multi-resistant bacteria that currently occur in hospitals are evidence of adaptation and/or gene transfer in a clinical setting. A well known example is the Meticilline-resistant Staphylococcus aureus (MRSA) that frequently occurs in hospitals. Additionally, transport of antibiotic resistance via the food chain is observed as meat, dairy products and even vegetables contain resistant extended-spectrum beta-lactamase (ESBL) producing bacteria [4] and the occurrence of veterinary-MRSA and quinolone-resistant Campylobacter jejuni in live stock and humans seem to coincide. Generally food (meat, dairy products) is considered to be the main vector for the exposure to ARB. Resistant bacteria and genes have not only in hospital wastewaters and manure, but also in sewage, wastewater treatment plants, surface water, groundwater, and even in drinking water [3]. Furthermore, an increase in resistance genes was observed in Dutch soils that were exposed to animal manure for decades [4]. There is, however, limited information on the human exposure to resistance genes or resistant bacteria via the environment (e.g. groundwater, surface water and drinking water) and its relevance for human health. Very recently, Walsch and coworkers [5] illustrated that resistance genes (NDM-1) could be transferred between different environmental bacterial species in a laboratory setting. Additionally these researchers observed these resistance genes in various bacterial strains obtained from New Delhi surface water and even tap water. The occurrence of bacteria carrying these genes is a serious threat to human health of especially vulnerable human populations (e.g. elderly, children, patients suffering from injuries or immune system disorders) because infected humans can not be effectively treated by antimicrobial agents.

Resistance genes and antibiotics are of all ages. Various (micro) organisms are capable of producing natural antimicrobial agents to compete for energy sources or fight off infections. Penicillin is, for example, produced by fungi (http://en.wikipedia.org/wiki/Penicillin). Additionally, tetracycline antibiotics, the most commonly used class of veterinary pharmaceuticals, are actually produced by bacterial strain Streptomyces (http://nl.wikipedia.org/wiki/Tetracycline). In response to these 'chemical weapons' resistance genes naturally occur in bacterial populations. The tetracycline-producing bacteria even have their own resistance genes to be able to survive while using their 'chemical weapons' in competition with other microorganisms. However, the increasing consumption of antibiotics since the discovery of penicillin in 1928 generated a strong selective pressure on bacteria and led to the spread of antibiotics and increase of microorganisms containing resistance genes in the environment. There are currently no effect-based guidelines for the presence of (resistance) genes in the environment or drinking water, and the (human health) risks of the occurrence of antibiotic residues and resistance genes in the environment is unknown.

Resistant bacteria and resistance genes can enter the water cycle. Communal and hospital waste water effluents are discharged to surface water, while and veterinary manure is generally applied to soil and groundwater. The current report focuses on the latter topic, and considers the fate of antibiotics, antibiotic resistance genes and antibiotic resistant bacteria in the (ground)water cycle and during drinking water production. A better understanding of the fate of resistance genes in the (soil) environment provides information on the exposure and potential threats for the environment and human health. Additionally, this route of exposure is compared to more obvious routes such as the spread of these bacteria (or genes) within a hospital setting and via the food chain.

2 Fate of resistance genes in the soil environment

2.1 Resistance genes in the environment

Figure 1 illustrates the potential route of resistance genes (and antibiotics) towards groundwater, surface water and drinking water.

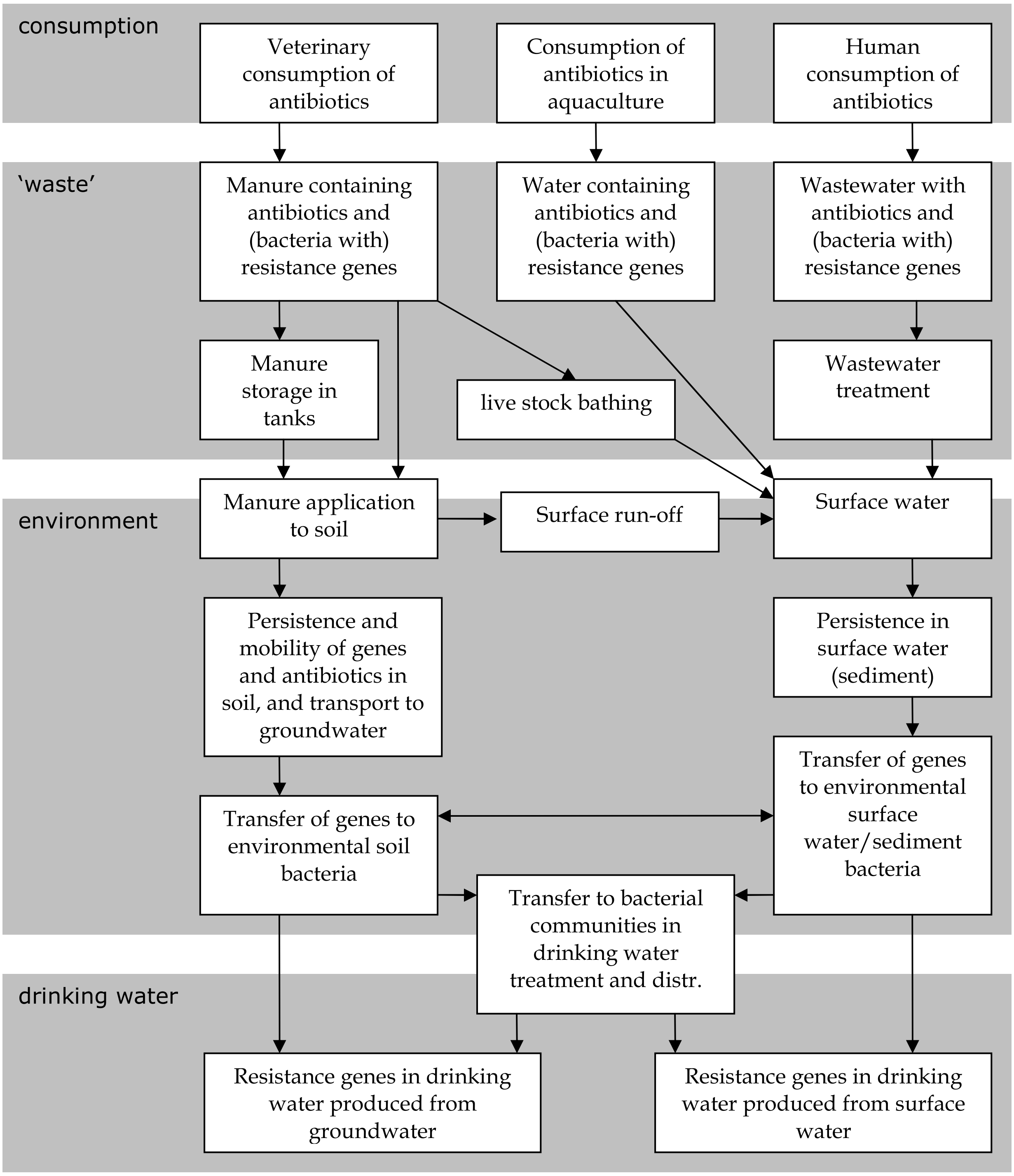

Figure 1: antibiotics, resistance genes and resistant bacteria in the water cycle

This study focuses on the transfer of resistance from veterinary practice to groundwater and produced drinking water, thereby covering the left side of Figure 1. Routes via food irrigation of contaminated surface waters are ignored in this figure as this is not part of the water cycle.

2.2 Mobility of genes in the soil environment

Studies with soil columns illustrated that DNA of resistance genes can be transported over considerable distances in water saturated soil and groundwater [5]. It was observed that plasmids (small protein-covered DNA segments of bacteria) leached out of the soil almost uniformly with the tracer, suggesting that the sorption to the soil was very low and the mobility high. This was supported by a sorption study that showed that soil sorption coefficients of DNA are rather low (log K_D ranged from 0 to 2.3) for different soils [6].

2.3 Persistence of genes in the soil environment

The persistence of plasmids in batch systems with soil is variable. Batch sorption studies with soil suspensions revealed half lives of several hours [5] and initial degradation rates in soil were also rather short (hours to days, see [7] and references therein). However, various other studies illustrated that substantial residues of plasmids could be obtained after several months of incubation (see also [7] and references therein). In some cases a two phase degradation of plasmids was observed (initial fast degradation and subsequent slow degradation). This phenomenon is well known for persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons [8], and is considered a result of sequestration of the chemicals (slow diffusion into locations that are difficult to access for microorganisms). However, whether sequestration is also relevant for DNA fragments that generally have much lower sorption coefficients than most POPs, remains to be studied.

2.4 Occurrence of resistance genes in the (soil) environment

Various studies have shown the presence of resistance genes for various classes of antimicrobial agents in the environment [9]. Resistance genes have been detected in the water of 'livestock waste lagoons' [10, 11], and elevated levels of resistance genes have been observed in surface waters impacted by aquaculture (i.e. fish farming) [12]. Furthermore, tetracycline and sulfonamide resistance genes have been found in manure from veterinary practice and in agricultural soils [13, 14] in groundwater underlying swine farms [15], and in New Delhi (India) , Germany and U.S., resistance genes have even been observed in tap water [16] [3].

As mentioned before, resistance genes are of all ages, so the question rises whether their observed occurrence is related to consumption of antibiotics. Knapp et al. [4] showed that amounts of resistance genes for tetracyclins, β-lactams and erythromycins significantly increased in various Dutch soils since the 1940s. These observations provide evidence that the increasing consumption of veterinary antibiotics leads to changes in the gene reservoirs of the soil. Additionally, Schmitt et al. [17] showed that elevated levels of resistance genes remained present in field soils even when the soil was not exposed to manure containing antibiotics and resistance genes for several years. They also showed that bacterial communities in soils, pre-exposed to veterinary antibiotics, revealed higher tolerance towards antibiotics than communities that were not pre-exposed. If we consider that DNA is not strongly sorbed to soil [6] and rather degradable, these observations suggest that resistance genes are included and maintained within the gene pool of the bacteriological community [4]. Additionally, the presence of tetracycline resistance genes in groundwater [15], suggests that these genes have leached from the top soil as tetracycline strongly sorbs to soil and (log K_D ranging from 3 to 5)[18], and have not be detected in groundwater [19].

2.5 Gene transfer between microorganisms

Various studies have illustrated that (resistance) genes can occur in soil and in sources of drinking water and in drinking water, but the mere presence of these genes is no threat for human health. Gene fragments can be transported via plasmids, viruses or as bare DNA fragments (Figure 2, in Dutch). Risks occur when these genes are able to be incorporated in human pathogens. This means that resistance needs to be transferred to these bacteria. In general, bacteria can incorporate resistance genes from other bacteria or even plants (horizontal inter- and intra-kingdom transfer), or become resistant by activating mutations in genes [9, 20]. Horizontal gene transfer is considered to play a key role in evolution of bacterial populations and the adaptation to (changing) environments. A lab study of Amina et al. [21] showed the occurrence of horizontal gene transfer of antimicrobial resistance genes between various classes of bacteria. However, in a field study of Andersen et al. [22] no horizontal gene transfer to soil bacteria was observed for genes from genetically modified sterilized microbial biomass, that was applied as fertilizer 7 years after exposure. The sterilization might have prevented gene transfer. However, the persistence of resistance genes in soils suggests that these genes are incorporated in the bacterial soil community [17], because manure is not sterilized before application.

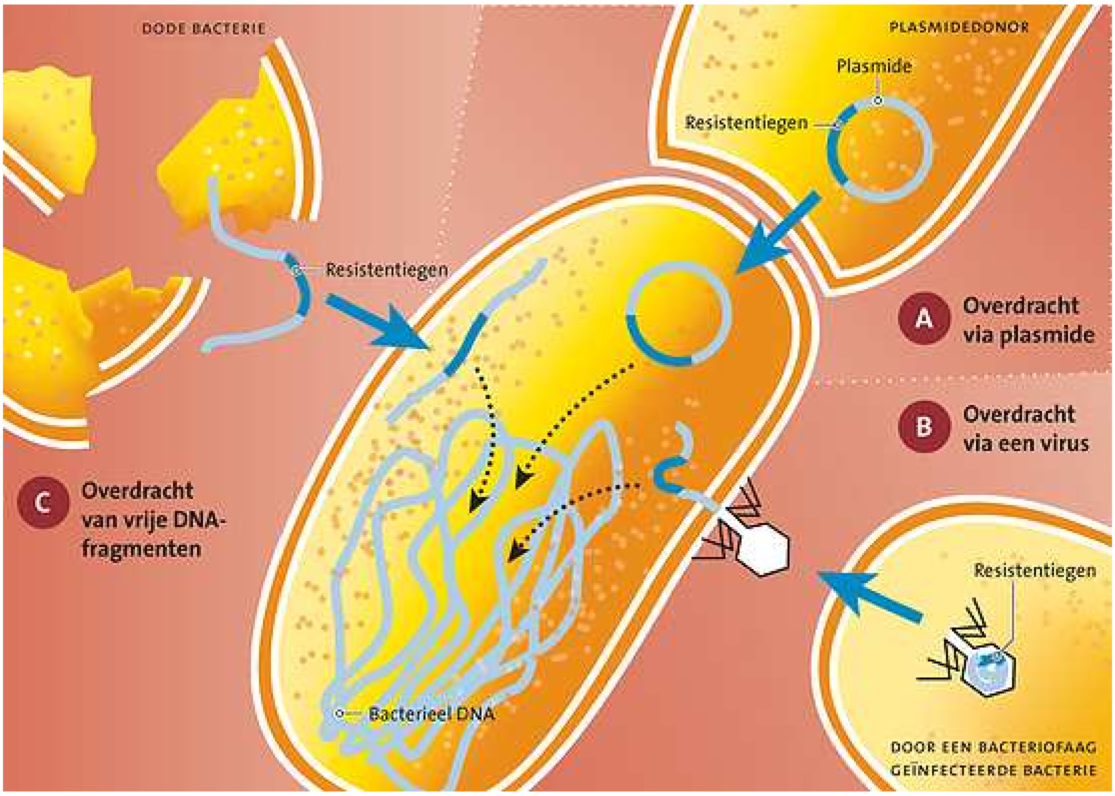

Figure 2: Transport of genes between bacteria (Figure taken from www.kennislink.nl)

2.6 Resistance genes in drinking water: some considerations

The main focus of the current report is the fate of resistance genes in soil and groundwater. Nevertheless, some issues considering the fate in drinking water treatment and the relevance of the drinking water cycle in transport, human exposure and risks is discussed below. Generally, surface water derived drinking water is produced using advanced sorptive (active carbon treatment), oxidative (ozonation UV/H_2O_2) and/or filtration (various membranes) techniques. Active carbon treatment is not expected be an effective barrier for DNA (plasmids) due to the rather hydrophilic character of DNA. Additionally, microcosms growing on sand filter beds and active carbon beds might be able to adopt these resistance genes, thereby becoming a source of these genes (see van der Wal 2012 for background information[1]). Contrastingly, oxidative techniques and (micro) filtration probably remove DNA more effectively, as DNA can be oxidized relatively easily and is too large to pass membranes with low molecular cut-offs. Groundwater treatment is generally less advanced. Oxidative and microfiltration techniques are often absent in groundwater treatment. This might enable the passage of resistance genes through drinking water treatment. However, further research is necessary to assess passage of genes in drinking water sources and passage of drinking water treatment.

2.7 Relevance of (drinking) water for resistance gene transport and human health risks

The human health risks of resistant bacteria have been clearly illustrated in hospitals and during food production, where multi-resistant bacteria occur and threaten human health. In hospitals the ‘cycle’ of bacteria and/or resistance genes is rather short (i.e. contamination between patients or via vectors as ‘contaminated' rooms, materials or personnel) and the consumption of antimicrobial agents is high (high selective pressure). Additionally, various researchers have indicated that consumption of antimicrobial agents in pet animals and live stock might pose a risk for the people near these animals and to consumers of products of live stock (farmers, pet owners, the general public) [23-25]. In these situations the ‘routes’ are rather short and the selective pressure (i.e. antibiotic consumption promoting these genes) is rather high. The situation differs for the drinking water cycle (Figure 1), as the routes from manure application and emissions of wastewater effluents to produced drinking water are long. This suggests that the human health risk of drinking tap water is negligible compared to other exposure routes such as the consumption of meat or dairy products or beeing treated in a hospital. The direct exposure to resistant bacteria via drinking water that come from the human and veterinary consumption of antibiotics is negligible [1].

Nevertheless, soil, or more general the environment, contains huge amounts of bacteria, and studies have shown that the continuous consumption of antimicrobial agents lead to increasing amounts of resistance genes for various antibiotic classes in environmental gene pools of soil, shallow groundwater, sediments and surface water [3, 4, 26]. Usually, antibiotic resistance bacteria and genes emerge in the environments (treated humans and live stock, wastewater treatment plants, manure tanks) under the selective pressure of some antibiotics, and often remain present (are ‘persistent’) even when the antibiotic pressure disappears [27, 28]. Even though no direct evidence of risks have been appointed to the increased occurrence of these genes in environmental matrices, the long-term risks of increasing amounts of resistance genes in these gene-pools is supposed to be a daunting public health risk [29]. This also might be relevant for the drinking water sector, since sources (especially surface waters) contain resistance genes, and these genes might be transferred to biofilms within sand filtration, active carbon filtration and distribution systems.

3 Conclusions

The increasing consumption of antimicrobial agents has led to an increase of resistance genes in the environment. Resistance genes have been observed in manure, soil, surface water, groundwater, and even in drinking water. Literature research on 'naked' resistance genes (plasmids) illustrates that these DNA fragments are rather mobile and degradable in the (soil) environment. It is therefore suggested that their persistence up to several years in (top) soils is related to the incorporation in the genes in the gene pool of the soil bacteria. The direct human health risks of increasing amounts of these genes in soil, surface waters and groundwater seems to be limited compared to more obvious risks of resistance genes and resistant bacteria in hospitals or meat and dairy products. However, the long-term human health and environmental risks of increasing resistance genes in the environment is daunting. Increasing levels of resistance genes in the large bacterial gene pool present in the environment might not pose a direct threat to human health as most bacteria in the environment are no human pathogens (in contrast to bacteria that occur in hospitals or live stock). However, since resistance genes can be transferred to pathogenic bacterial strains, an increasing pool of resistance genes in the environment and drinking water treatment is undesirable. For the water sector, the adoption of resistance genes to biofilms in water treatment and distribution might be studied in more detail.

4 Literature

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