Chapter 3. Socio-economical perspectives and impact of AR

3.1. Sources of antibiotic resistance – zoonotic, human, environment

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Abstract

Antibiotic resistance is a global problem that must be managed under the one-health perspective. Retrospectively, it is assumed that microbial populations able to cope with compounds with antimicrobial activity and susceptible bacteria lived in equilibrium for thousand years. This situation would change in the middle 40´s of the twentieth century when one of the most important revolutions of modern medicine started - the use of a natural antimicrobial compound, the penicillin, to treat infectious bacterial diseases. Over the years, the massive use of antibiotics in human and animal medicine, as well as in animal production for both growth promotion and infection prophylaxis/metaphylaxis accelerated and shaped one of the most successful evolutionary case-studies. As a result of an impressive combination of genome and community dynamics, bacteria with acquired antibiotic resistance are nowadays widespread across different environmental compartments (water, soil, wild life) as well as in the human food-chain (poultry, livestock, aquaculture, produce). Hence, the evolutionary success of these bacteria turned to represent a major threat to the human health. This chapter discusses some the drivers and paths of antibiotic resistance dissemination across zoonotic, human, and environmental sources.

Keywords: wastewater, soil, commensal bacteria, antibiotic usage, human-health threat;

3.1.1. The antibiotic era

The industrial revolution nurtured the need to combat infectious diseases and encouraged the efforts of scientists and pharmaceutical companies to find drugs with antimicrobial activity capable of overcoming infection, the main cause of mortality until the middle of the twentieth century (Cohen, 2000, Aminov, 2010). Therefore, antibiotherapy processes that were successful on the control of humans and animals infectious diseases became one of the most important revolutions of the modern human and veterinary medicine. The recognition that penicillin, a natural fungal metabolite, was able to control the development of pathogenic bacteria set a mark in the mankind history, and started what can be called the antibiotic era (Aminov, 2010). The search for antibiotics able to control pathogenic bacteria led to the recognition that the production of molecules with antibacterial activity is widespread in the microbial world, being produced by both fungi and bacteria. The effectiveness of these compounds explains why most of the antibiotics used nowadays in human and veterinary medicine are natural (biosynthetic) or semi-synthetic derivatives of these natural products (Butler and Buss, 2006).

Regardless of being biosynthetic, semi-synthetic or fully synthetic, the therapeutic action of antibiotics is due to their capacity to interfere with key structural components and/or functions of the bacterial cell and which are not present in the host's cells. Most of antibiotic classes with clinical relevance interfere with three major types of target in the bacterial cell – the cell wall synthesis, proteins synthesis, and DNA access, mainly during replication. Hence, although secondary toxic effects may occur, antibiotics selectively target bacteria, whose cells are destroyed or inhibited to divide, while no harm is anticipated in the host´s cells.

Unfortunately, the enthusiasm put on antibiotics as therapeutic agents would not last. Resistance, meaning the ability to survive and proliferate in the presence of antibiotics at concentrations used for therapeutic purposes, is found for all antibiotics, sooner or later after their commercialization (Alanis, 2005). When bacteria acquired the capability of recurrently grow in the presence of a clinically-used dose of antibiotic, they cause the failure of the antibiotic as therapeutic agent and are, hence, named as antibiotic resistant bacteria (ARB). The mechanisms used by ARB can be summarized as the ability to i) alter the antibiotic molecule (degradation, transformation), ii) control the antibiotic intracellular concentration (efflux, cell impermeabilization), or iii) modify the cellular antibiotic target (Blair et al., 2015).

3.1.2. Intrinsic and acquired antibiotic resistance

Some bacteria harbor ancestral traits that confer intrinsic resistance to one or more classes of antibiotics. Intrinsic resistance can result from specific cell properties, such as the absence of cell wall or presence of an external membrane or it can result from some specific chromosomal genes. In these cases, the genes encoding for such properties are part of the core genome of a given species or genus (Davies and Davies, 2010, EUCAST). Consequently, all members of a given taxonomic group (species, genus, family) share the same resistance phenotype. In contrast, when the genes encoding for antibiotic resistance (ARGs) are part of the accessory genome of a bacterial strain, which includes genetic information that was acquired, antibiotic resistance is only observed in some representatives of a given species (EUCAST). Acquired antibiotic resistance may result from gene mutation or genetic recombination (Martinez and Baquero, 2000, Davies and Davies, 2010, Zhang et al., 2009a). Gene mutations occur randomly in the genome, often potentiated by mutagenic agents, and when they represent an evolutionary advantage to the cell, they may become dominant through dissemination by vertical transmission (from one generation to the next). Genetic recombination, frequently referred to as horizontal gene transfer (HGT), is believed to be common among bacteria, representing one of the major driving forces for bacterial evolution (Ochman et al., 2000, Wiedenbeck and Cohan, 2011, Davies and Davies, 2010). The HGT may occur by: 1) conjugation among bacteria, that involves the transfer of genetic material from a donor to a recipient cell, requiring that both share the same space, but not necessarily the same species; 2) transformation, consisting on the uptake of naked DNA released by dead cells, and 3) transduction, mediated by bacteriophages (Andersson and Hughes, 2010).

3.1.3. The natural antibiotic resistome

Antimicrobial biosynthesis and the associated evasion mechanisms are common in bacteria that coexist in a given microbial habitat (D'Costa et al., 2007). Antibiotic resistance is a natural property of bacteria, hypothetically favored in nature to cope with microbial community members that naturally produce antibiotic residues. Hence, resistance mechanisms may be regarded as survival traits in bacteria thriving in natural communities, being observed in bacteria that were never exposed to

antibiotics of anthropogenic origin (Allen et al., 2010). The natural antibiotic resistome, i.e. the whole set of genes that contribute to cope with the presence of antibiotics, encodes possibly a wide panoply of functions that can span from microbial cell defense, inhibition of competitors growth, to biochemical signals, modulators of metabolic activity or even natural substrates (Davies et al., 2006, Martinez, 2009, Dantas et al., 2008). Remarkably, the soil natural resistome has been one of the most studied, with genetic determinants of resistance evidencing either high or low resemblance with the resistance determinants observed nowadays in clinically-relevant bacteria (Riesenfeld et al., 2004, D'Costa et al., 2006, D'Costa et al., 2011, Forsberg et al., 2012). The diversity of the natural resistome is also evidenced by the fact that it is probably spread over the whole domain *Bacteria*, with frequent descriptions of occurrence in members of the phyla *Actinobacteria*, *Proteobacteria*, or *Bacteroidetes* (Riesenfeld et al., 2004, D'Costa et al., 2011, D'Costa et al., 2006, Forsberg et al., 2012).

3.1.4. The contaminant resistome

Due to the widespread dissemination in the environment, ARB and ARG are nowadays considered environmental contaminants (Berendonk et al., 2015, Pruden et al., 2006). Contaminant ARB and ARG are emitted by a wide diversity of sources, mainly human and animal excreta. Hence, domestic and animal farm effluents as well as animal manure are the major sources for ARB and ARG, which continuously enrich what has been called the contaminant resistome (Vaz-Moreira et al., 2014, Manaia, 2017).

The human impact on the dissemination of antibiotic resistance is demonstrated by the observation of a direct correlation between human intervention and abundance and diversity of ARB and ARG. For example, studies with wildlife species, such as small mammals, gulls and birds of the prey, iguanas, or permafrost soils, show the wide dissemination of antibiotic resistance and, eventually, the effects of the continuous exposure of the biota to antibiotics and some pollutants (Thaller et al., 2010, D'Costa et al., 2011, Vredenburg et al., 2014, Furness et al., 2017). The risks that new resistance determinants jump from the natural resistome to clinically-relevant bacteria, jeopardizing the efficiency of antibiotics still regarded as valuable therapeutic tools, exist and have been deeply discussed (Wright, 2010, Perry and Wright, 2013, Manaia, 2017, Martinez et al., 2015). However, it is the continuous and

wide dissemination of the contaminant antibiotic resistome that represents nowadays a major threat for human health. Since the acquisition of ARGs represents an additional cost for the bacterial cell, which may be justified only if it brings a survival advantage, it is believed that it is stimulated by external selective pressures (Allen et al., 2010, Goh et al., 2002, Andersson and Hughes, 2014). Among the different selective pressures that are identified (e.g. metals or biocides), antibiotics are those considered the most important selectors for resistance acquisition and maintenance. Indeed, it is impossible to dissociate the evolution of the contaminant resistome from that of antibiotic usage, to which is dedicated the next section.

3.1.5. Evolution of antibiotics usage

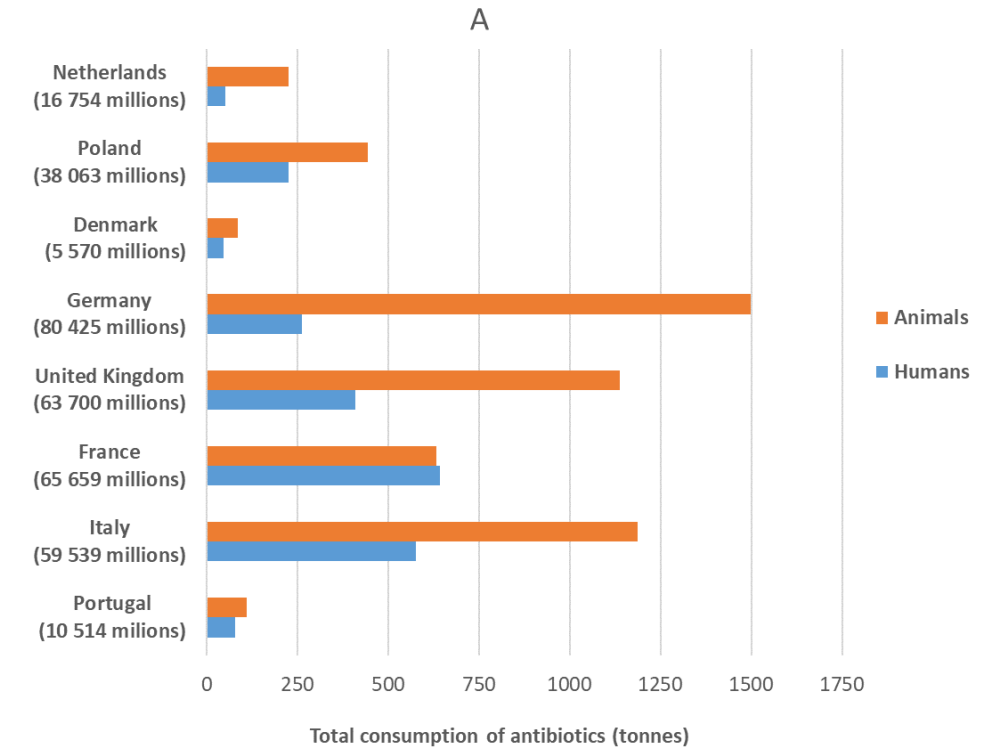
Since the 1940's the global production and consumption of antibiotics has increased not only due to the rise of the human population but also to prosperity (CDDEP, 2015). It is estimated that during the first decade of the XXI century, occurred an overall increase of 36% on antibiotic use for human consumption. This percentage corresponds to an average increase from 5×10^{10} to 7×10^{10} standard units, i.e., the number of doses sold, in 71 countries with different incomes (Van Boeckel et al., 2014). In general, the consumption of antibiotics *per capita* is higher in high-income than in middle-income countries. However, in middle-income countries the increase in human antibiotic consumption has been higher than in high-income countries, where, in general, the consumption stabilized or decreased (Van Boeckel et al., 2014). Brazil, Russia, India, China and South Africa account for 76% of increase in antibiotic sails for human consumption (Van Boeckel et al., 2014).

The absence of adequate measures to prevent infectious diseases and the inappropriate antibiotic consumption have probably a significant contribution to the excessive antibiotic use. Indeed, it has been argued that in some low-income countries, antibiotic consumption increase is manly a compensation of the lack of efficient programs of vaccination and sanitation (Laxminarayan et al., 2016). In turn, inadequate antibiotics prescription is associated with the uncertainty in diagnosis (e.g. frequently to treat upper respiratory tract infections caused by viruses), motivating the prescription of an unnecessary broad spectrum antibiotic, in an incorrect dosage or duration (Starrels et al., 2009, Om et al., 2016). Notoriously, these situations are not necessarily related with the country development index. In hospitals, the excessive use of broad-spectrum antibiotics is leading to dangerous and almost difficult to treat

infections. However, the lack of or delayed access to antibiotics still kills more people than resistant infections (CDDEP, 2015).

The simultaneous increase of the *per capita* income and population growth have been driving pressure for availability of animal protein, and consequently, the need to use antibiotics to optimize intensive animal/aquaculture farming. Antibiotics and other antimicrobials have been extensively used in livestock animals and aquaculture not only to treat diseases, but mainly to improve growth or to prevent infections (FAO, 2016, Liu et al., 2017). As a consequence, the utilization of antibiotics in animal farming (e.g. poultry, swine and cattle) is higher than the used for human consumption (**Figure 3.1A**), constituting up to 70% of the annual consumption of antibiotics in each country (FDA, 2015). Although with a smaller impact, antibiotics are used also to control diseases and pests in household pets and agriculture crops, respectively (CDDEP, 2015, Prescott, 2008, Lloyd, 2007). The utilization of antimicrobials in intensive farming, some times without the supervision of veterinary professionals, raises a major concern for human health since many compounds used in food animals belong to the same antimicrobial classes as those used in humans (**Figure 3.1B**) (FAO, 2016).

Although the worldwide data on the antimicrobial sales for animal production is limited, Van Boeckel and collaborators (2015) conducted a first study assessing the antimicrobial consumption in food-producing animals around the world. The authors estimated a total consumption of more than 63 000 tonnes of antibiotics in livestock in 2010, and predicted a rise to more than 105,000 tons by 2030. Among the countries with highest antibiotics consumption in 2010 were China (23%), United States of America (13%), Brazil (9%), India (3%), and Germany (3%). The Van Boeckel *et al*. (2015) predictions for food animal production in 2030 identify China (30%), United States of America (10%), Brazil (8%), India (4%), and Mexico (2%) as the five major consumers.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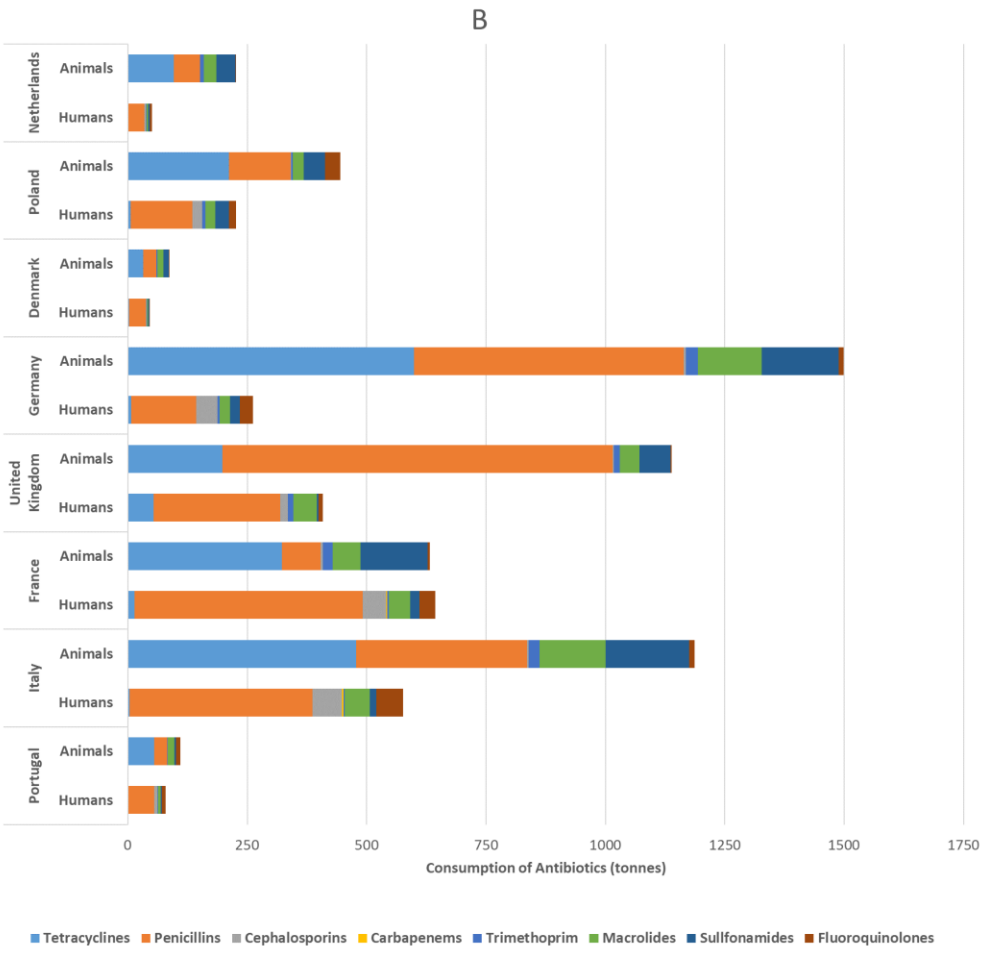
Total consumption of antibiotics (tonnes)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Consumption of Antibiotics (tonnes)
Tetracyclines
Penicillins
Cephalosporins
Carbapenems
Trimethoprim
Macrolides
Sullfonamides
Fluoroquinolones

Figure 3.1. Consumption of antibiotics for use in humans and food-producing animals A) by selected European Countries in 2012 and B) by selected classes of antibiotics for each European country. Data of consumption in tonnes was recovered from ECDC, EFSA and EMA report (ECDC/EFSA/EMA, 2015). Data from human consumption includes the antibiotics used in the community and hospitals. Data from food-producing animal's consumption includes the use in pigs, cattle and broilers. The countries population size data are estimates from the World Bank for the year 2012 (http://data.worldbank.org/indicator/SP.POP.TOTL).

3.1.6. Antibiotic resistance evolution

It is assumed that the presence of antimicrobials in a given ecological niche will lead to the successive elimination of susceptible bacteria with the simultaneous Darwinian selection of ARB (Founou et al., 2016). Hence, any host, human or animal, consuming antibiotics or environmental compartment contaminated with antibiotics, are potential reservoirs of ARB and ARG. In parallel with selection, the stress imposed by the presence of antibiotic residues may favor the acquisition of ARG by susceptible bacteria, making of antibiotic residues important evolution drivers (Andersson and Hughes, 2011). Although antibiotic resistance acquisition is common in nature these processes are facilitated by the massive and intensive use of antibiotics (Sørensen et al., 2005). A high number of reports and studies produced over the last decades show not only an increase of resistance prevalence but also of the diversity and distribution of ARG (EARS-Net, ESAC, NARMS, Knapp et al., 2010). The exposure of the bacterial communities to antibiotics has been suggested as a major driver for the emergence and spread of resistance. It is believed that mechanisms such as the disturbance of the microbial communities, the interference with gene expression or biofilm formation can trigger or act in combination with the horizontal transfer of ARG (Andersson and Hughes, 2012, Andersson and Hughes, 2014, Martinez, 2008, You and Silbergeld, 2014). In addition, it has been argued that the levels of resistance observed at any time and place are not only the result of the recent conditions but also of the global history of antibiotic resistance acquisition (O'Brien, 2002). This can be explained based on the fact that when the carriage of resistance determinants do not impose a fitness cost they will not be lost by their host, even in

the absence of selective pressures (Andersson and Hughes, 2011, Andersson and Hughes, 2010).

3.1.7. Stressors for antibiotic resistance

Besides the antibiotic residues or metabolites thereof, some other chemical compounds have been described to contribute to ARB and ARG enrichment due to processes of co- or cross-resistance. Co-resistance to antibiotics and other chemicals occurs when the genes specifying the resistance phenotypes are located together in the same genetic element. In turn, cross-resistance arises from a given mechanism that confer resistance to two or more antimicrobials from different classes. The best example of co-resistance is the genetic linkage of ARG and metal translocation genes (e.g. Hg, Cu, Cd, Zn) (Seiler and Berendonk, 2012, Pal et al., 2015). Examples of cross-resistance mechanisms are the broad substrate spectrum efflux pumps or neutralizing enzymes, reduced cell envelop permeability or alteration of the target site, with the consequent invulnerability to antimicrobial agents belonging to different classes (Seiler and Berendonk, 2012, Wales and Davies, 2015).

The potential of metals as antibiotic resistance stressors is relevant, given their widespread distribution. Metals supplementation is used in animal and aquaculture feed, and are found in the composition of organic and inorganic fertilizers, in pesticides, and in anti-fouling products. In addition, some metals are also used as biocides, controlling diseases, mainly in the pig and poultry sectors (Seiler and Berendonk, 2012, Wales and Davies, 2015). Consequently, they are found not only in agricultural soils and sediments but also in aquaculture and in domestic and industrial wastewater, through which they reach the wastewater treatment plants (WWTPs) (Nicholson et al., 2003, Gall et al., 2015). Besides metals, many other compounds are used as biocides. These antimicrobial compounds have a wide application, being used as preservatives of pharmaceuticals, cosmetics or feed/food products or as disinfectants or antiseptics in diverse human activities (agricultural, industrial and health care settings and at community level). Like metals, biocides residues are found in different ecological niches, as domiciliary, healthcare or aquatic environments (Antizar-Ladislao, 2008, Bloomfield, 2002, Meyer and Cookson, 2010, Maillard, 2005, Chen et al., 2014), where they are pointed out as favoring the emergence of antibiotic resistance through co-selection. Such conclusions are based on the fact that some bacterial strains have low susceptibility (i.e. high MICs) to simultaneously

metals and/or biocides and antibiotics (Wales and Davies, 2015).

Co-resistance of antibiotics and metals such as Hg, Cd, Cu, and Zn, has been described (Seiler and Berendonk, 2012, Pal et al., 2015). Mercury (Hg) has been detected in fish feed and is frequently found in sewage and activated sludge (Choi and Cech, 1998, Olson et al., 1991). Genetic linkage of multiple antibiotic resistance (sulfonamides, tetracyclines, beta-lactams, streptomycin and florfenicol) and Hg was found in an *Aeromonas salmonicida* subsp. *salmonicida* strain isolated from salmon from aquaculture facilities (Seiler and Berendonk, 2012). Co-resistance of macrolide/aminoglycoside antibiotics and Cu, Cd, and Zn used as animal growth promoters and frequently found in different environments, has also been described (Seiler and Berendonk, 2012, Pal et al., 2015). Hence, even at very low concentrations, metals have the potential to select bacteria with multidrug-resistance plasmids, contributing to the emergence, maintenance, and transmission of antibiotic-resistant bacteria (Gullberg et al., 2011). Co-resistance of Hg and quaternary ammonium compounds (QAC) (Pal et al., 2015) as well as QAC and sulphonamides has been described (Wales and Davies, 2015). Cross-resistance of fluoroquinolone or tetracycline and phenolic biocides is also reported in *Escherichia* and *Salmonella* strains (Wales and Davies, 2015).

Besides the reduced fitness costs explained above, other mechanisms are believed to contribute to antibiotic resistance persistence even in the absence of selective pressures. The toxin-antitoxin system has been suggested as an important factor for the stabilization of ARGs and metal or biocide resistance genes that are co-transferred by conjugative plasmids. It is supposed that these plasmids would be more persistent when vertically transferred from one generation to the next, even in the absence of selective pressure, since the toxin-antitoxin systems stabilize plasmids in their hosts by killing daughter cells that do not inherit the plasmid (Pal et al., 2015). The toxin-antitoxin system is responsible for the production of a toxin and of an antidote for that toxin (antitoxin). If the plasmid is absent in a daughter cell, the unstable antitoxin is degraded and the stable toxic protein kills the new cell; this is known as post-segregation killing (Van Melderen and De Bast, 2009).

Beside exposure to antimicrobials, many other adaptive stress responses may influence the susceptibility to antibiotics since they may impact many of the same components and processes that are targeted by antimicrobials. Some non-antimicrobial agents have been described as potential selectors for antibiotic

resistance. Some examples are feed or food preservation agents, such as phenazopyridine or sepiolite, or sub-lethal concentrations of salt and acidic pH as well as herbicides, such as glyphosate, widely used in agriculture and gardening (McGowan et al., 2006, Rodriguez-Beltran et al., 2013, Amabile-Cuevas and Arredondo-Garcia, 2013, Kurenbach et al., 2015). In addition, nutrient starvation, oxidative stress, thermal shock, or cell envelope damage are among the factors compromising the cell growth by stimulating protective changes in cell physiology or in lifestyle (biofilm formation) or inducing mutations (Poole, 2012).

In summary, a wide range of conditions, including those promoted by micropollutants, prevailing in the different ecological niches (animal and human hosts, feed and food products, anthropogenic impacted environments) may contribute to the overall increase and dissemination of antibiotic resistance.

3.1.8. Paths of antibiotic resistance dissemination

The human and animal intestinal tracts favor the occurrence of antibiotic resistance selection and/or horizontal transfer (Shoemaker et al., 2001, Sommer et al., 2010), being considered important reservoirs of ARB and ARG (Salyers et al., 2004). Also livestock and aquaculture are recognized reservoirs of ARB and ARG into the environment (Landers et al., 2012, CDC, 2013, Woolhouse et al., 2015). All these sources have the potential to supply ARB and ARG to the environment, where they can accumulate and spread through aquatic systems and soils (**Figure 3.2**). The use of animal manure or compost to amend agriculture soils, a practice that is in line with the nowadays so appreciated organic farming, is another potential source of contamination of soil, surface and groundwater with ARB and ARGs from animal origin. The point source contamination will diffuse through processes such as water runoff or percolation, invading the aquatic systems (**Figure 3.2**). While these diffuse paths of ARB dissemination may be associated with the transmission from the environment to humans, some point-source contamination can also be recognized. For example, humans with a high degree of exposure to reservoirs of resistance such as health care facilities, animal farms and abattoirs as well as food-handlers, are major receptors and potential disseminators of ARB and ARG into the community and health care settings (Marshall and Levy, 2011). Also humans living in contact with livestock and pets are important links in the antibiotic resistance dissemination network (Smith et al., 2009, Guardabassi et al., 2004, Lloyd, 2007).

Domestic wastewater is another relevant source of ARB and ARG, which, in world regions with adequate sanitation network, are collected in sewage systems and treated at urban wastewater treatment plants (UWTP). Because most of these plants use conventional treatments designed mainly to reduce the organic and microbial loads of wastewater, part of the ARB and ARG of the incoming sewage is discharged into the natural water body receptors that include rivers, lakes or the sea in coastal areas (Michael et al., 2013, Czekalski et al., 2014, Rizzo et al., 2013). Studies conducted in different world regions show that every minute a well-functioning domestic UWTP can release more than 10^9 antibiotic-resistant enteric bacteria and more than 10^{14} copies of antibiotic resistance genes (Vaz-Moreira et al., 2014, Manaia et al., 2016). This is a very high microbial load that will result in the contamination of rivers or lakes, widely demonstrated in the literature (Novais et al., 2005, Tacão et al., 2014, LaPara et al., 2011), as well as their sediments (Czekalski et al., 2014, LaPara et al., 2011) or soils (Jones-Dias et al., 2016a). The conditions prevailing in the biological reactors of the UWTPs (high load of readily metabolizable nutrients and of bacteria together with micropollutants) may not favor the elimination of ARB, or even enhance the selection of ARB and/or the occurrence of HGT of ARG. Hence, the implementation of tertiary treatments including disinfection are desirable (Manaia et al., 2016). These processes may reduce the microbial load of the treated wastewater (up to 4 log units) to lower values than those with conventional treatments (up to 2 log units) (Sousa et al., 2017, Michael et al., 2013). Nevertheless, disinfected treated wastewater still contains ARB and ARG, regardless the method employed (e.g. UV 254 nm, chlorination, ozonation) (Moreira et al., 2016, Sousa et al., 2017, LaPara et al., 2011). Moreover, some authors have suggested that disinfection may increase the prevalence of ARB and ARG in the treated wastewater (Hu et al., 2016, Chen and Zhang, 2013, Alexander et al., 2016).

The reduction of the organic load of the raw wastewater is accompanied with the increase of the biomass of the degrading microorganisms. The spent activated sludge is further stabilized, digested or eventually used for composting (Kelessidis and Stasinakis, 2012). ARB and ARG surviving these processes may reach soils (farms and gardens) through the direct application of activated sludge, digested sludge or compost as soil amendments (Zhang et al., 2011, Ma et al., 2011). Wastewater reuse is another key issue regarding antibiotic resistance dissemination. The human population growth and prosperity combined with the climate changes is increasingly

pressing for the reuse of treated wastewater (Becerra-Castro et al., 2015). The consequent contamination of soils and of the human food-chain is a major concern (Jones-Dias et al., 2016b, Campos et al., 2013).

ARB and ARG have been detected in produce and food products (e.g. vegetables, meat, poultry, milk, eggs), which can function as vehicles of ARG transfer to humans, mainly if consumed raw (van den Bogaard and Stobberingh, 2000, Mena et al., 2008, Leverstein-van Hall et al., 2011, Sunde and Norstrom, 2006, Aarestrup et al., 2000, Marti et al., 2013, Bezanson et al., 2008). Indeed, ARGs that encode resistance against antibiotics only used in animals (e.g. nourseothricin, apramycin) were detected in commensal bacteria of both human and animal origin, in zoonotic pathogens like *Salmonella* spp., and in strictly human pathogens, like *Shigella* spp. (van den Bogaard and Stobberingh, 2000).

Soils and water contamination may also affect different forms of wildlife such as the lynx Iberian or seagulls that fly over our cities (Vredenburg et al., 2014, Sousa et al., 2014). In these cases, the migratory routes of these wild animals can represent important forms of dispersion of resistance between distant zones. The widespread environmental contamination with ARB is also detected indoor mainly in urban areas, as have been shown in studies conducted on bus lines (Mendes et al., 2015) or with domestic animals that cohabit with their owners (Leite-Martins et al., 2014). In fact, from what is known nowadays, one can wonder which places are antibiotic-resistance-free. This contamination originates essentially from animals and humans. If a part of this contamination returns to humans is an issue yet to be clarified (Manaia, 2017).

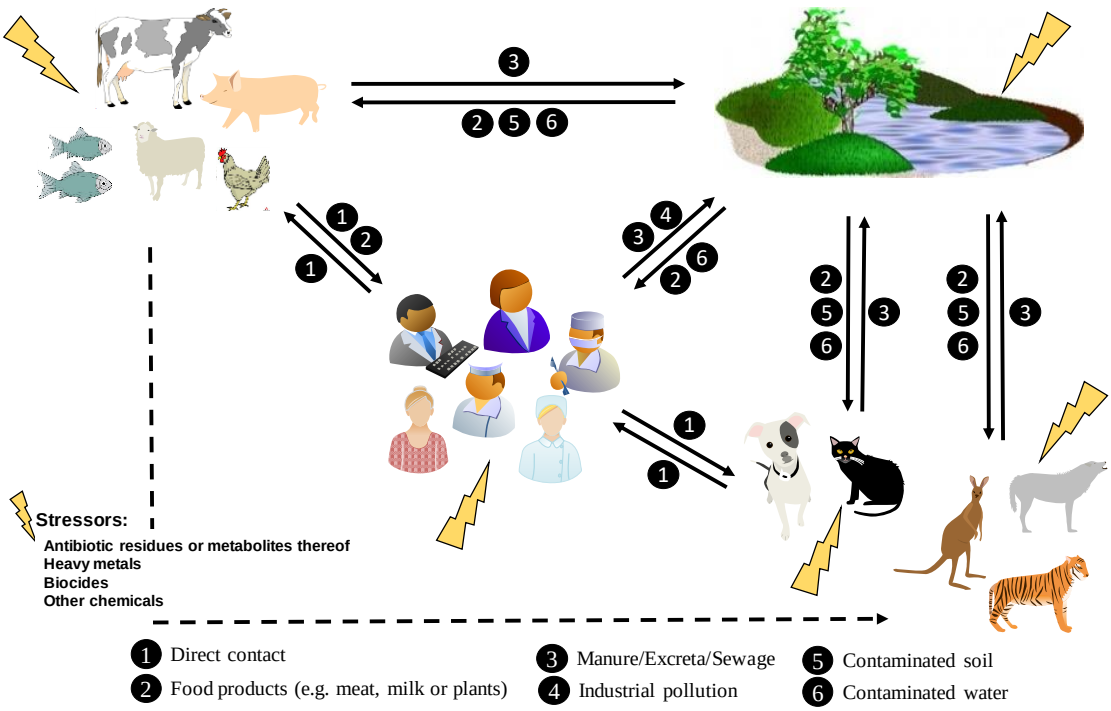

Figure 3.2. Antibiotic resistance dissemination pathways.

3.1.9. Antibiotic resistance in humans and animals

Prophylaxis and metaphylaxis, i.e. the use of therapeutic or sub-therapeutic doses of antibiotics in healthy animals to prevent infectious diseases, or to both healthy and infected animals are relevant contributions for the contaminant resistome. Although some antibiotics are restricted to the animal use (e.g. tylosin, apramycin, avoparcin), others are used in both food animals and the treatment of humans' infections (e.g. gentamycin, tetracyclines, penicillins and sulfonamides) (Landers et al., 2012, Kemper, 2008). In addition, although some antibiotics are restricted to the veterinary use, they belong to the same general classes of those used in humans, thus, even if they are not the same exact compound their mode of action is the same or similar (Phillips et al., 2004). The major fraction of antibiotic consumption is associated with animal production, not only because more individual doses are used, but also because the therapeutic doses, which are proportional to the body weight, are higher for some animals than for humans. The influence of the antibiotic use on the enrichment of ARB in animals has been demonstrated. For example, in Denmark it was observed a

reduction, from 80% to 3%, in the levels of vancomycin resistant enterococci in poultry after the banning of avoparcin as growth promoter (Singer et al., 2003). Although the association of avoparcin, and other glycopeptides, with the prevalence of vancomycin resistance in livestock is one of the most studied, other associations have been demonstrated for other classes of antimicrobials, such as virginiamycin use and quinupristin-dalfopristin resistance; tylosin use and erythromycin resistance; avilamycin use and avilamycin resistance (Wegener, 2003, Cogliani et al., 2011, Bengtsson and Wierup, 2006). Some examples of co-selection were also documented, as the genetic linkage of the genes *van*A and the macrolide resistance gene *erm*B in glycopeptide-resistant enterococci from porcine origin. Thus, the ban of avoparcin was not enough to decrease the prevalence of glycopeptide-resistant enterococci, being this decrease observed only after the ban of macrolide growth-promoters (Boerlin et al., 2001, Aarestrup, 2000).

Recently the World Health Organization published a list of antibiotic-resistant "priority pathogens", including 12 groups of bacteria that pose the greatest threat to human health (WHO, 2017). The most critical groups given their potential to develop multidrug resistance phenotypes, include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and different *Enterobacteriaeae* genera (including *Klebsiella pneumoniae*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Providencia* spp. and *Morganella* spp.). In the same list, and considered to be of high or medium risk, are *Enterococcus faecium*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter*, *Haemophilus influenzae*, *Salmonella* spp., *Shigella* spp. and *Neisseria gonorrhoeae.* Some of these bacteria are also important commensals or pathogens in animals. Indeed, animals can be vehicles of *Acinetobacter baumannii*, *Enterobacteriaceae* (e.g. *Klebsiella pneumoniae*), *Enterococcus*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter*, *Salmonella* spp., and *Shigella* spp. (Cantas and Suer, 2014, Lahuerta et al., 2011, EFSA and ECDC, 2015, Damborg et al., 2016, Abdel-Raouf et al., 2014). Among these, *Salmonella* and *Campylobacter* are considered as the most frequent food-borne bacterial agents in Europe (EFSA and ECDC, 2015), while *Acinetobacter baumannii* have been more associated with companion animals (Damborg et al., 2016, Lupo et al., 2017, Ewers et al., 2017). In general, farm animals are recognized as important antimicrobial resistance reservoirs (Bywater et al., 2004, EFSA and ECDC, 2017), including resistance to some recent drugs as carbapenems (Fischer et al., 2013), that

are detected both in animals and humans (Gupta et al., 2003). Nevertheless, there are few studies establishing the direction of movement of resistance between human and livestock populations (Woolhouse et al., 2015). Indeed, the demonstration of transmission of ARB from animals to humans may be not straightforward.

In 1976, Levy *et al*. (1976), maybe in one of the first reports, published a study demonstrating the dissemination of bacteria from animals to humans, observing the same tetracycline resistant *E. coli* strain in chickens and in the workers of the farm. Later studies, based on the whole genome sequencing (WGS), showed that the lineage CC97 of methicillin resistant *Staphylococcus aureus* (MRSA) was observed to enter the human populations from a livestock source on more than one occasion over the past 100 years (Spoor et al., 2013). In contrast, for the lineage ST5, globally disseminated in poultry, it was concluded that it was transmitted from humans (Lowder et al., 2009). However, other authors quantifying the occurrence of cross-species transmission for the MRSA strain CC398, concluded that the transmissions from livestock-to-human were more frequent than from human-to-livestock over the evolutionary history of the strain (Ward et al., 2014). Similarly, Sørensen *et al*. (2001) studying the transmission of glycopeptide-resistant *Enterococcus faecium*, confirmed the risk associated with the consumption of meat products contaminated with resistant bacteria, showing that these bacteria ingested via chicken or pork meat lasted in human stool for up to 14 days.

Homologous ARGs identified in humans and farm animals isolates provided evidences for the possible cross transfer of ARB between animals and humans. For example, the gene responsible for methicillin resistance (*mec*A) in *S. aureus* found in animal isolates (e.g. cattle, pigs, chickens) was also detected in isolates from farmers (Lee, 2003). Also in farms where apramycin was used as growth promoter, the gene *aac(3)-IV*, encoding resistance to aminoglycosides, was detected in *E. coli* isolates of swine, poultry and of farm workers (Li et al., 2015, Zhang et al., 2009b, Chaslus-Dancla et al., 1991).

Cephalosporins are extensively used for production of food animals and beta-lactam resistance has been used in the literature to illustrate antibiotic resistance dissemination between both potential reservoirs. For example, there are some evidences of possible cross-contamination of bacteria carrying enzymes conferring resistance against this type of antibiotic, such as the beta-lactamase CTX-M-14, across humans, pets and poultry in Asian countries (Ewers et al., 2012). In addition,

the CTX-M-15 subtype, observed to be spread by different *Enterobacteriaeae*, including a *K. pneumoniae* clone in humans, is also found in pets and horses (Ewers et al., 2014). Contrary to cephalosporins, carbapenems (a class of last resort antibiotics) are not used in livestock. However, carbapenemase encoding genes (e.g. the bla_{NDM}, bla_{IMP}, bla_{VIM} and bla_{KPC} genes) were already detected in isolates of livestock animals (Mollenkopf et al., 2017). Woodford *et al*. (2014) demonstrated that the ARB from swine and poultry animals carrying the bla_{VIM} carbapenemase were also found in other farm animals (e.g. insects and rodents) being the transmission carried out through manure. Yet, studies on evidencing the transmission of carbapenem resistance from humans to animals are scarce the possible (Mollenkopf et al., 2017). Similarly, for the beta-lactamase NDM-1, which emerged recently as a major clinical threat that was spread via inter-continental dissemination, was never found an indication of animal-human transmission (Kumarasamy et al., 2010, Nordmann, 2011, Cabanes et al., 2012). Though, the gene bla_{NDM-1} was already found in *E. coli* isolates of companion animals, suggesting the possibility of inter-species transmission (Shaheen et al., 2013).

Colistin is considered a last resort antibiotic, namely for NDM-1 producing bacteria. Originally described as being a chromosomally encoded resistance type, recently colistin resistance became transferable through the gene *mcr-1*, identified in human and animal *E. coli* isolates from China (Schwarz and Johnson, 2016, Liu et al., 2016). The higher prevalence of *mcr-1*-carrying bacteria in animals over humans suggests that this resistance gene has animal origin and spread to humans (Liu et al., 2016). Supporting this hypothesis is the extended use of polymyxins as animal growth promoters, for prophylaxis and therapy, which may have selected for plasmid-mediated colistin resistance (Al-Tawfiq et al., 2017). Notoriously, recent reports highlighted the continuous spread of plasmid-mediated *mcr-1* gene to imported food, urban rivers and to humans (do Monte et al., 2017, Arcilla et al., 2016, Al-Tawfiq et al., 2017). Another MCR subtype, the *mcr-2* gene, has been identified only in livestock *E. coli* isolates, reinforcing the animal origin for plasmid-mediated resistance to colistin (Xavier et al., 2016).

In a screen for the presence of 260 ARGs in different habitats, including wastewater, soil, human and livestock samples Li *et al*. (2015) observed that the most abundant and commonly distributed ARGs were the ones associated with antibiotics extensively used in human and veterinary medicine. These included the growth

promotors (e.g. aminoglycoside, bacitracin, β-lactam, chloramphenicol, macrolide-lincosamide-streptogramin, quinolone, sulphonamide and tetracycline). Among the 99 of out of 260 ARGs shared between human and young livestock faeces, it was possible to observed that some were more abundant in humans than in animals (e.g. bacitracin resistance genes) while the opposite was observed for others (e.g. macrolide-lincosamide-streptogramin, tetracycline, multidrug, sulphonamide and aminoglycoside resistance genes) (Li et al., 2015). The association between humans and domestic wastewater was also reported by Li *et al*. (2015), who found that 68 ARGs (out of 260) encoding resistance to tetracycline, beta-lactam and aminoglycosides detected in human faeces samples and domestic raw wastewater showed high similarity. While it is clear that most of the ARGs detected in humans can reach the wastewater treatment plants and even persist after treatment, there are evidences that the dissemination in the environment may favour some, probably harboured by stable mobile genetic elements and/or ubiquitous bacteria (Munck et al., 2015, Czekalski et al., 2014). Some ARGs, despite the low general prevalence, are good exemples of high persistence or dissemination potentail. For example, the gene $bla_{\text{KPC-2}}$ gene carried by carbapenem resistant bacteria have been detected in the rivers receiving wastewater treatement plants dischargs (Yang et al., 2017, Picão et al., 2013). In India, New Delhi, the $bla_{\text{NDM-1}}$ gene was detected in drinking water bacteria (*Achromobacter* spp, *Kingella denitrificans* and *Pseudomonas aeruginosa*), after the observation of some cases of human contamination with *Enterobacteriaceae* and *Acinetobacter baumannii* isolates carrying this gene (Walsh et al., 2011). In summary, although some evidences are available, the impact of animal and environmental reservoirs onto the human health is still an issue deserving thorough research (Woolhouse et al., 2015, Phillips et al., 2004, Martinez, 2008).

3.1.10. Final considerations

This overview reinforces the need for an urgent implementation of the One-Health perspective, in which the health and well-being of the citizen, the safety of the food chain and the protection of the environment are comprehensively surveilled and controlled. Along with other contaminants and human-health threats, antibiotic resistance is a key issue in the One-Health priorities for the next decades.

References:

Aarestrup, F. M. 2000. Characterization of glycopeptide-resistant *Enterococcus faecium* (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides. *Journal of Clinical Microbiology,* 38**,** 2774-2777.

Aarestrup, F. M., Kruse, H., Tast, E., Hammerum, A. M. & Jensen, L. B. 2000. Associations between the use of antimicrobial agents for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers and pigs in Denmark, Finland, and Norway. *Microbial Drug Resistance-Mechanisms Epidemiology and Disease,* 6**,** 63-70.

Abdel-Raouf, M., Abdel-Gleel, Y. & Enab, A. 2014. Study on the role of pet animals for *Helicobacter pylori* transmission. *Journal of American Science* 10**,** 20-28.

Al-Tawfiq, J. A., Laxminarayan, R. & Mendelson, M. 2017. How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *International Journal of Infectious Diseases,* 54**,** 77-84.

Alanis, A. J. 2005. Resistance to antibiotics: are we in the post-antibiotic era? *Archives of Medical Research,* 36**,** 697-705.

Alexander, J., Knopp, G., Dotsch, A., Wieland, A. & Schwartz, T. 2016. Ozone treatment of conditioned wastewater selects antibiotic resistance genes, opportunistic bacteria, and induce strong population shifts. *Science of the Total Environment,* 559**,** 103-12.

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J. & Handelsman, J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology,* 8**,** 251-9.

Amabile-Cuevas, C. F. & Arredondo-Garcia, J. L. 2013. Nitrofurantoin, phenazopyridine, and the superoxide-response regulon soxRS of *Escherichia coli*. *Journal of Infection and Chemotherapy,* 19**,** 1135-40.

Aminov, R. I. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. *Frontiers in Microbiology,* 1**,** 134.

Andersson, D. I. & Hughes, D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nature Reviews Microbiology,* 8**,** 260-71.

Andersson, D. I. & Hughes, D. 2011. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiology Reviews,* 35**,** 901-11.

Andersson, D. I. & Hughes, D. 2012. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resistance Updates,* 15**,** 162-72.

Andersson, D. I. & Hughes, D. 2014. Microbiological effects of sublethal levels of antibiotics. *Nature Reviews Microbiology,* 12**,** 465-78.

Antizar-Ladislao, B. 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environment International,* 34**,** 292-308.

Arcilla, M. S., Van Hattem, J. M., Matamoros, S., Melles, D. C., Penders, J., De Jong, M. D. & Schultsz, C. 2016. Dissemination of the mcr-1 colistin resistance gene. *The Lancet Infectious Diseases,* 16**,** 147-149.

Becerra-Castro, C., Lopes, A. R., Vaz-Moreira, I., Silva, E. F., Manaia, C. M. & Nunes, O. C. 2015. Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health. *Environment International,* 75**,** 117-35.

Bengtsson, B. & Wierup, M. 2006. Antimicrobial resistance in Scandinavia after a ban of antimicrobial growth promoters. *Animal biotechnology,* 17**,** 147-156.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Burgmann, H., Sorum, H., Norstrom, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F. & Martinez, J. L. 2015. Tackling antibiotic resistance: the environmental framework. *Nature Reviews Microbiology,* 13**,** 310-7.

Bezanson, G. S., Macinnis, R., Potter, G. & Hughes, T. 2008. Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. *Int J Food Microbiol,* 127**,** 37-42.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O. & Piddock, L. J. 2015. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol,* 13**,** 42-51.

Bloomfield, S. F. 2002. Significance of biocide usage and antimicrobial resistance in domiciliary environments. *Journal of Applied Microbiology,* 92**,** 144s-157s.

Boerlin, P., Wissing, A., Aarestrup, F. M., Frey, J. & Nicolet, J. 2001. Antimicrobial growth promoter ban and resistance to macrolides and vancomycin in enterococci from pigs. *Journal of clinical microbiology,* 39**,** 4193-4195.

Butler, M. S. & Buss, A. D. 2006. Natural products - the future scaffolds for novel antibiotics? *Biochem Pharmacol,* 71**,** 919-29.

Bywater, R., Deluyker, H., Deroover, E., De Jong, A., Marion, H., Mcconville, M., Rowan, T., Shryock, T., Shuster, D. & Thomas, V. 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *Journal of Antimicrobial Chemotherapy,* 54**,** 744-754.

Cabanes, F., Lemant, J., Picot, S., Simac, C., Cousty, J., Jalin, L., Naze, F., Boisson, V., Cresta, M.-P. & André, H. 2012. Emergence of Klebsiella pneumoniae and Salmonella metallo-beta-lactamase (NDM-1) producers on Reunion Island. *Journal of clinical microbiology,* 50**,** 3812-3812.

Campos, J., Mourao, J., Pestana, N., Peixe, L., Novais, C. & Antunes, P. 2013. Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. *Int J Food Microbiol,* 166**,** 464-70.

Cantas, L. & Suer, K. 2014. Review: the important bacterial zoonoses in “one health” concept. *Frontiers in public health,* 2**,** 144.

Cdc 2013. Antibiotic Resistance Threats in the United States. USA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

Cddep 2015. The State of the World's Antibiotics, 2015. *In:* CENTER FOR DISEASE DYNAMICS, E. P. (ed.) *CDDEP*. Washington, D.C.

Chaslus-Dancla, E., Pohl, P., Meurisse, M., Marin, M. & Lafont, J. 1991. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. *Antimicrobial agents and chemotherapy,* 35**,** 590-593.

Chen, H. & Zhang, M. 2013. Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China. *Environ Sci Technol,* 47**,** 8157-63.

Chen, Z. F., Ying, G. G., Liu, Y. S., Zhang, Q. Q., Zhao, J. L., Liu, S. S., Chen, J., Peng, F. J., Lai, H. J. & Pan, C. G. 2014. Triclosan as a surrogate for household biocides: An investigation into biocides in aquatic environments of a highly urbanized region. *Water Research,* 58**,** 269-279.

Choi, M. H. & Cech, J. J. 1998. Unexpectedly high mercury level in pelleted commercial fish feed. *Environmental Toxicology and Chemistry,* 17**,** 1979-1981.

Cogliani, C., Goossens, H. & Greko, C. 2011. Restricting antimicrobial use in food animals: lessons from Europe. *Microbe,* 6**,** 274.

Cohen, M. L. 2000. Changing patterns of infectious disease. *Nature,* 406**,** 762-7.

Czekalski, N., Gascon Diez, E. & Burgmann, H. 2014. Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. *ISME J,* 8**,** 1381-90.

D'costa, V. M., Griffiths, E. & Wright, G. D. 2007. Expanding the soil antibiotic resistome: exploring environmental diversity. *Curr Opin Microbiol,* 10**,** 481-9.

D'costa, V. M., King, C. E., Kalan, L., Morar, M., Sung, W. W., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G. B., Poinar, H. N. & Wright, G. D. 2011. Antibiotic resistance is ancient. *Nature,* 477**,** 457-61.

D'costa, V. M., Mcgrann, K. M., Hughes, D. W. & Wright, G. D. 2006. Sampling the Antibiotic Resistome. *Science,* 311**,** 374-377.

Damborg, P., Broens, E. M., Chomel, B. B., Guenther, S., Pasmans, F., Wagenaar, J. A., Weese, J. S., Wieler, L. H., Windahl, U. & Vanrompay, D. 2016. Bacterial zoonoses transmitted by household pets: state-of-the-art and future perspectives for targeted research and policy actions. *Journal of comparative pathology,* 155**,** S27-S40.

Dantas, G., Sommer, M. O., Oluwasegun, R. D. & Church, G. M. 2008. Bacteria subsisting on antibiotics. *Science,* 320**,** 100-3.

Davies, J. & Davies, D. 2010. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev,* 74**,** 417-33.

Davies, J., Spiegelman, G. B. & Yim, G. 2006. The world of subinhibitory antibiotic concentrations. *Curr Opin Microbiol,* 9**,** 445-53.

Do Monte, D. F., Mem, A., Fernandes, M. R., Cerdeira, L., Esposito, F., Galvao, J. A., Franco, B. D., Lincopan, N. & Landgraf, M. 2017. Chicken Meat as Reservoir of Colistin-Resistant Escherichia coli Carrying mcr-1 Genes in South America. *Antimicrob Agents Chemother*.

Ears-Net. *European Antimicrobial Resistance Surveillance Network (http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx)* [Online]. Available: http://www.ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx [Accessed 10 February 2012.

Ecdc/Efsa/Ema 2015. ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. *EFSA Journal,* 13**,** 114.

Efsa & Ecdc 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014.

Efsa & Ecdc 2017. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA Journal,* 15.

Esac. *European Surveillance of Antimicrobial Consumption (http://app.esac.ua.ac.be/public/index.php/en_gb/home)* [Online]. Available: http://app.esac.ua.ac.be/public/index.php/en_gb/home [Accessed 10 February 2012.

Eucast. *European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/)* [Online]. Available: http://www.eucast.org/ [Accessed 10 February 2012.

Ewers, C., Bethe, A., Semmler, T., Guenther, S. & Wieler, L. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. *Clinical Microbiology and Infection,* 18**,** 646-655.

Ewers, C., Klotz, P., Leidner, U., Stamm, I., Prenger-Berninghoff, E., Göttig, S., Semmler, T. & Scheufen, S. 2017. OXA-23 and ISAba1–OXA-66 class D β-lactamases in Acinetobacter baumannii isolates from companion animals. *International Journal of Antimicrobial Agents,* 49**,** 37-44.

Ewers, C., Stamm, I., Pfeifer, Y., Wieler, L. H., Kopp, P. A., Schønning, K., Prenger-Berninghoff, E., Scheufen, S., Stolle, I. & Günther, S. 2014. Clonal spread of highly successful ST15-CTX-M-15 Klebsiella pneumoniae in companion animals and horses. *Journal of Antimicrobial Chemotherapy***,** dku217.

Fao 2016. Drivers, dynamics and epidemiology of antimicrobial resistance in animal production. *In:* NATIONS, F. A. A. O. O. T. U. (ed.).

Fda 2015. Summary report on Antimicrobials sold or distributed for use in food-producing animals. *In:* SERVICES, F. A. D. A. D. O. H. A. H. (ed.).

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R. & Guerra, B. 2013. Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. *J Antimicrob Chemother,* 68**,** 478-80.

Forsberg, K. J., Reyes, A., Wang, B., Selleck, E. M., Sommer, M. O. & Dantas, G. 2012. The shared antibiotic resistome of soil bacteria and human pathogens. *Science,* 337**,** 1107-11.

Founou, L. L., Founou, R. C. & Essack, S. Y. 2016. Antibiotic Resistance in the Food Chain: A Developing Country-Perspective. *Frontiers in Microbiology,* 7.

Furness, L. E., Campbell, A., Zhang, L., Gaze, W. H. & Mcdonald, R. A. 2017. Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. *Environ Res,* 154**,** 28-34.

Gall, J. E., Boyd, R. S. & Rajakaruna, N. 2015. Transfer of heavy metals through terrestrial food webs: a review. *Environmental Monitoring and Assessment,* 187**,** 201.

Goh, E. B., Yim, G., Tsui, W., Mcclure, J., Surette, M. G. & Davies, J. 2002. Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. *Proc Natl Acad Sci U S A,* 99**,** 17025-30.

Guardabassi, L., Schwarz, S. & Lloyd, D. H. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother,* 54**,** 321-32.

Gullberg, E., Cao, S., Berg, O. G., Ilback, C., Sandegren, L., Hughes, D. & Andersson, D. I. 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog,* 7**,** e1002158.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hoekstra, M. P., Whichard, J. M., Barrett, T. J. & Group, N. a. R. M. S. P. W. 2003. Emergence of multidrug-resistant Salmonella enterica serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *Journal of Infectious Diseases,* 188**,** 1707-1716.

Hu, Q., Zhang, X. X., Jia, S., Huang, K., Tang, J., Shi, P., Ye, L. & Ren, H. 2016. Metagenomic insights into ultraviolet disinfection effects on antibiotic resistome in biologically treated wastewater. *Water Res,* 101**,** 309-17.

Jones-Dias, D., Manageiro, V. & Canica, M. 2016a. Influence of agricultural practice on mobile bla genes: IncI1-bearing CTX-M, SHV, CMY and TEM in Escherichia coli from intensive farming soils. *Environ Microbiol,* 18**,** 260-72.

Jones-Dias, D., Manageiro, V., Ferreira, E., Barreiro, P., Vieira, L., Moura, I. B. & Canica, M. 2016b. Architecture of Class 1, 2, and 3 Integrons from Gram Negative Bacteria Recovered among Fruits and Vegetables. *Front Microbiol,* 7**,** 1400.

Kelessidis, A. & Stasinakis, A. S. 2012. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. *Waste Manag,* 32**,** 1186-95.

Kemper, N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological indicators,* 8**,** 1-13.

Knapp, C. W., Dolfing, J., Ehlert, P. A. & Graham, D. W. 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environ Sci Technol,* 44**,** 580-7.

Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C. G. & Irfan, S. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet infectious diseases,* 10**,** 597-602.

Kurenbach, B., Marjoshi, D., Amabile-Cuevas, C. F., Ferguson, G. C., Godsoe, W., Gibson, P. & Heinemann, J. A. 2015. Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in Escherichia coli and Salmonella enterica serovar Typhimurium. *MBio,* 6.

Lahuerta, A., Westrell, T., Takkinen, J., Boelaert, F., Rizzi, V., Helwigh, B., Borck Høg, B., Korsgaard, H., Ammon, A. & Mäkelä, P. 2011. Zoonoses in the European Union: origin, distribution and dynamics: the EFSA-ECDC summary report 2009. *Eurosurveillance (Online Edition),* 16**,** 5-8.

Landers, T. F., Cohen, B., Wittum, T. E. & Larson, E. L. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep,* 127**,** 4-22.

Lapara, T. M., Burch, T. R., Mcnamara, P. J., Tan, D. T., Yan, M. & Eichmiller, J. J. 2011. Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environ Sci Technol,* 45**,** 9543-9.

Laxminarayan, R., Sridhar, D., Blaser, M., Wang, M. & Woolhouse, M. 2016. Achieving global targets for antimicrobial resistance. *Science*.

Lee, J. H. 2003. Methicillin (oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. *Applied and environmental microbiology,* 69**,** 6489-6494.

Leite-Martins, L., Meireles, D., Bessa, L. J., Mendes, A., De Matos, A. J. & Da Costa, P. M. 2014. Spread of multidrug-resistant Enterococcus faecalis within the household setting. *Microb Drug Resist,* 20**,** 501-7.

Leverstein-Van Hall, M. A., Dierikx, C. M., Stuart, J. C., Voets, G. M., Van Den Munckhof, M. P., Van Essen-Zandbergen, A., Platteel, T., Fluit, A. C., Van De Sande-Bruinsma, N., Scharinga, J., Bonten, M. J. M. & Mevius, D. J. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical Microbiology and Infection,* 17**,** 873-880.

Levy, S. B., Fitzgerald, G. B. & Macone, A. B. 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature,* 260**,** 40-42.

Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J. M. & Zhang, T. 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The ISME journal,* 9**,** 2490-2502.

Liu, X., Steele, J. C. & Meng, X.-Z. 2017. Usage, residue, and human health risk of antibiotics in Chinese aquaculture: A review. *Environmental Pollution*.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H. & Shen, J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis,* 16**,** 161-8.

Lloyd, D. H. 2007. Reservoirs of antimicrobial resistance in pet animals. *Clin Infect Dis,* 45 Suppl 2**,** S148-52.

Lowder, B. V., Guinane, C. M., Ben Zakour, N. L., Weinert, L. A., Conway-Morris, A., Cartwright, R. A., Simpson, A. J., Rambaut, A., Nubel, U. & Fitzgerald, J. R. 2009. Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus. *Proc Natl Acad Sci U S A,* 106**,** 19545-50.

Lupo, A., Châtre, P., Ponsin, C., Saras, E., Boulouis, H.-J., Keck, N., Haenni, M. & Madec, J.-Y. 2017. Clonal Spread of Acinetobacter baumannii Sequence Type 25 Carrying blaOXA-23 in Companion Animals in France. *Antimicrobial Agents and Chemotherapy,* 61**,** e01881-16.

Ma, Y. J., Wilson, C. A., Novak, J. T., Riffat, R., Aynur, S., Murthy, S. & Prudens, A. 2011. Effect of Various Sludge Digestion Conditions on Sulfonamide, Macrolide, and Tetracycline Resistance Genes and Class I Integrons. *Environmental Science & Technology,* 45**,** 7855-7861.

Maillard, J. Y. 2005. Antimicrobial biocides in the healthcare environment: efficacy, usage, policies, and perceived problems. *Ther Clin Risk Manag,* 1**,** 307-20.

Manaia, C. M. 2017. Assessing the Risk of Antibiotic Resistance Transmission from the Environment to Humans: Non-Direct Proportionality between Abundance and Risk. *Trends in Microbiology.*

Manaia, C. M., Macedo, G., Fatta-Kassinos, D. & Nunes, O. C. 2016. Antibiotic resistance in urban aquatic environments: can it be controlled? *Appl Microbiol Biotechnol,* 100**,** 1543-57.

Marshall, B. M. & Levy, S. B. 2011. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev,* 24**,** 718-33.

Marti, R., Scott, A., Tien, Y. C., Murray, R., Sabourin, L., Zhang, Y. & Topp, E. 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Appl Environ Microbiol,* 79**,** 5701-9.

Martinez, J. L. 2008. Antibiotics and antibiotic resistance genes in natural environments. *Science,* 321**,** 365-7.

Martinez, J. L. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ Pollut,* 157**,** 2893-902.

Martinez, J. L. & Baquero, F. 2000. Mutation frequencies and antibiotic resistance. *Antimicrob Agents Chemother,* 44**,** 1771-7.

Martinez, J. L., Coque, T. M. & Baquero, F. 2015. What is a resistance gene? Ranking risk in resistomes. *Nature Reviews Microbiology,* 13**,** 116-123.

Mcgowan, L. L., Jackson, C. R., Barrett, J. B., Hiott, L. M. & Fedorka-Cray, P. J. 2006. Prevalence and antimicrobial resistance of enterococci isolated from retail fruits, vegetables, and meats. *J Food Prot,* 69**,** 2976-82.

Mena, C., Rodrigues, D., Silva, J., Gibbs, P. & Teixeira, P. 2008. Occurrence, identification, and characterization of Campylobacter species isolated from portuguese poultry samples collected from retail establishments. *Poult Sci,* 87**,** 187-90.

Mendes, A., Martins Da Costa, P., Rego, D., Beca, N., Alves, C., Moreira, T., Conceicao, T. & Aires-De-Sousa, M. 2015. Contamination of public transports by Staphylococcus aureus and its carriage by biomedical students: point-prevalence, related risk factors and molecular characterization of methicillin-resistant strains. *Public Health,* 129**,** 1125-31.

Meyer, B. & Cookson, B. 2010. Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control? *Journal of Hospital Infection,* 76**,** 200-205.

Michael, I., Rizzo, L., Mcardell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C. & Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Res,* 47**,** 957-95.

Mollenkopf, D. F., Stull, J. W., Mathys, D. A., Bowman, A. S., Feicht, S. M., Grooters, S. V., Daniels, J. B. & Wittum, T. E. 2017. Carbapenemase-Producing Enterobacteriaceae Recovered from the Environment of a Swine Farrow-to-Finish Operation in the United States. *Antimicrob Agents Chemother,* 61.

Moreira, N. F. F., Sousa, J. M., Macedo, G., Ribeiro, A. R., Barreiros, L., Pedrosa, M., Faria, J. L., Pereira, M. F. R., Castro-Silva, S., Segundo, M. A., Manaia, C. M., Nunes, O. C. & Silva, A. M. T. 2016. Photocatalytic ozonation of urban wastewater and surface water using immobilized TiO2 with LEDs: Micropollutants, antibiotic resistance genes and estrogenic activity. *Water Research,* 94**,** 10-22.

Munck, C., Albertsen, M., Telke, A., Ellabaan, M., Nielsen, P. H. & Sommer, M. O. 2015. Limited dissemination of the wastewater treatment plant core resistome. *Nature communications,* 6.

Narms. *National Antimicrobial Resistance Monitoring System (http://www.cdc.gov/narms/)* [Online]. Available: http://www.cdc.gov/narms/ [Accessed 10 February 2012.

Nicholson, F. A., Smith, S. R., Alloway, B. J., Carlton-Smith, C. & Chambers, B. J. 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. *Science of The Total Environment,* 311**,** 205-219.

Nordmann, P. 2011. Global Spread of Carbapenemase-producing Enterobacteriaceae-Volume 17, Number 10—October 2011-Emerging Infectious Disease journal-CDC.

Novais, C., Coque, T. M., Ferreira, H., Sousa, J. C. & Peixe, L. 2005. Environmental contamination with vancomycin-resistant enterococci from hospital sewage in Portugal. *Appl Environ Microbiol,* 71**,** 3364-8.

O'brien, T. F. 2002. Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clin Infect Dis,* 34 Suppl 3**,** S78-84.

Ochman, H., Lawrence, J. G. & Groisman, E. A. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature,* 405**,** 299-304.

Olson, B. H., Cayless, S. M., Ford, S. & Lester, J. N. 1991. Toxic element contamination and the occurrence of mercury-resistant bacteria in Hg-Contaminated soil, sediments, and sludges. *Archives of Environmental Contamination and Toxicology,* 20**,** 226-233.

Om, C., Daily, F., Vlieghe, E., Mclaughlin, J. C. & Mclaws, M.-L. 2016. "If it's a broad spectrum, it can shoot better": inappropriate antibiotic prescribing in Cambodia

Antimicrobial resistance and infection control, 5.

Pal, C., Bengtsson-Palme, J., Kristiansson, E. & Larsson, D. G. 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics,* 16**,** 964.

Perry, J. A. & Wright, G. D. 2013. The antibiotic resistance "mobilome": searching for the link between environment and clinic. *Front Microbiol,* 4**,** 138.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R. & Waddell, J. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy,* 53**,** 28-52.

Picão, R. C., Cardoso, J. P., Campana, E. H., Nicoletti, A. G., Petrolini, F. V., Assis, D. M., Juliano, L. & Gales, A. C. 2013. The route of antimicrobial resistance from the hospital effluent to the environment: focus on the occurrence of KPC-producing Aeromonas spp. and Enterobacteriaceae in sewage. *Diagnostic microbiology and infectious disease,* 76**,** 80-85.

Poole, K. 2012. Stress responses as determinants of antimicrobial resistance in Gram-negative bacteria. *Trends Microbiol,* 20**,** 227-34.

Prescott, J. F. 2008. Antimicrobial use in food and companion animals. *Animal Health Research Reviews,* 9**,** 127-133.

Pruden, A., Pei, R., Storteboom, H. & Carlson, K. H. 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environ Sci Technol,* 40**,** 7445-50.

Riesenfeld, C. S., Goodman, R. M. & Handelsman, J. 2004. Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environ Microbiol,* 6**,** 981-9.

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I. & Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Sci Total Environ,* 447**,** 345-60.

Rodriguez-Beltran, J., Rodriguez-Rojas, A., Yubero, E. & Blazquez, J. 2013. The animal food supplement sepiolite promotes a direct horizontal transfer of antibiotic resistance plasmids between bacterial species. *Antimicrob Agents Chemother,* 57**,** 2651-3.

Salyers, A. A., Gupta, A. & Wang, Y. 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol,* 12**,** 412-6.

Schwarz, S. & Johnson, A. P. 2016. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother,* 71**,** 2066-70.

Seiler, C. & Berendonk, T. U. 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front Microbiol,* 3**,** 399.

Shaheen, B. W., Nayak, R. & Boothe, D. M. 2013. Emergence of a New Delhi metallo-β-lactamase (NDM-1)-encoding gene in clinical Escherichia coli isolates recovered from companion animals in the United States. *Antimicrobial agents and chemotherapy,* 57**,** 2902-2903.

Shoemaker, N. B., Vlamakis, H., Hayes, K. & Salyers, A. A. 2001. Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon. *Appl Environ Microbiol,* 67**,** 561-8.

Singer, R. S., Finch, R., Wegener, H. C., Bywater, R., Walters, J. & Lipsitch, M. 2003. Antibiotic resistance - the interplay between antibiotic use in animals and human beings. *Lancet Infect Dis,* 3**,** 47-51.

Smith, T. C., Male, M. J., Harper, A. L., Kroeger, J. S., Tinkler, G. P., Moritz, E. D., Capuano, A. W., Herwaldt, L. A. & Diekema, D. J. 2009. Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. *PLoS One,* 4**,** e4258.

Sommer, M. O., Church, G. M. & Dantas, G. 2010. The human microbiome harbors a diverse reservoir of antibiotic resistance genes. *Virulence,* 1**,** 299-303.

Sørensen, S. J., Bailey, M., Hansen, L. H., Kroer, N. & Wuertz, S. 2005. Studying plasmid horizontal transfer in situ: a critical review. *Nat Rev Microbiol,* 3**,** 700-10.

Sørensen, T. L., Blom, M., Monnet, D. L., Frimodt-Møller, N., Poulsen, R. L. & Espersen, F. 2001. Transient intestinal carriage after ingestion of antibiotic-resistant Enterococcus faecium from chicken and pork. *New England Journal of Medicine,* 345**,** 1161-1166.

Sousa, J. M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M. F., Silva, A. M., Nunes, O. C. & Manaia, C. M. 2017. Ozonation and UV254nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. *J Hazard Mater,* 323**,** 434-441.

Sousa, M., Goncalves, A., Silva, N., Serra, R., Alcaide, E., Zorrilla, I., Torres, C., Canica, M., Igrejas, G. & Poeta, P. 2014. Acquired antibiotic resistance among wild animals: the case of Iberian Lynx (Lynx pardinus). *Vet Q,* 34**,** 105-12.

Spoor, L. E., Mcadam, P. R., Weinert, L. A., Rambaut, A., Hasman, H., Aarestrup, F. M., Kearns, A. M., Larsen, A. R., Skov, R. L. & Fitzgerald, J. R. 2013. Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus. *MBio,* 4**,** e00356-13.

Starrels, J. L., Barg, F. K. & Metlay, J. P. 2009. Patterns and Determinants of Inappropriate Antibiotic Use in Injection Drug Users. *Journal of General Internal Medicine,* 24**,** 263-269.

Sunde, M. & Norstrom, M. 2006. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products. *J Antimicrob Chemother,* 58**,** 741-7.

Tacão, M., Moura, A., Correia, A. & Henriques, I. 2014. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water Res,* 48**,** 100-7.

Thaller, M. C., Migliore, L., Marquez, C., Tapia, W., Cedeno, V., Rossolini, G. M. & Gentile, G. 2010. Tracking acquired antibiotic resistance in commensal bacteria of Galapagos land iguanas: no man, no resistance. *PLoS One,* 5**,** e8989.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A. & Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci U S A,* 112**,** 5649-54.

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A. & Laxminarayan, R. 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases,* 14**,** 742-750.

Van Den Bogaard, A. E. & Stobberingh, E. E. 2000. Epidemiology of resistance to antibiotics: Links between animals and humans. *International Journal of Antimicrobial Agents,* 14**,** 327-335.

Van Melderen, L. & De Bast, M. S. 2009. Bacterial toxin–antitoxin systems: more than selfish entities? *PLoS Genet,* 5**,** e1000437.

Vaz-Moreira, I., Nunes, O. C. & Manaia, C. M. 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS Microbiology Reviews,* 38**,** 761–778.

Vredenburg, J., Varela, A. R., Hasan, B., Bertilsson, S., Olsen, B., Narciso-Da-Rocha, C., Bonnedahl, J., Stedt, J., Da Costa, P. M. & Manaia, C. M. 2014. Quinolone-resistant Escherichia coli isolated from birds of prey in Portugal are genetically distinct from those isolated from water environments and gulls in Portugal, Spain and Sweden. *Environ Microbiol,* 16**,** 995-1004.

Wales, A. D. & Davies, R. H. 2015. Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens. *Antibiotics (Basel),* 4**,** 567-604.

Walsh, T. R., Weeks, J., Livermore, D. M. & Toleman, M. A. 2011. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis,* 11**,** 355-62.

Ward, M., Gibbons, C., Mcadam, P., Van Bunnik, B., Girvan, E., Edwards, G., Fitzgerald, J. R. & Woolhouse, M. 2014. Time-scaled evolutionary analysis of the transmission and antibiotic resistance dynamics of Staphylococcus aureus clonal complex 398. *Applied and environmental microbiology,* 80**,** 7275-7282.

Wegener, H. C. 2003. Antibiotics in animal feed and their role in resistance development. *Current opinion in microbiology,* 6**,** 439-445.

Who 2017. Global priority list of antibiotic-resistant bacteria to guide research, dicovery, and development of new antibiotics. Genéve: World Health Organization.

Wiedenbeck, J. & Cohan, F. M. 2011. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiol Rev,* 35**,** 957-76.

Woodford, N., Wareham, D. W., Guerra, B. & Teale, C. 2014. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *Journal of Antimicrobial Chemotherapy,* 69**,** 287-291.

Woolhouse, M., Ward, M., Van Bunnik, B. & Farrar, J. 2015. Antimicrobial resistance in humans, livestock and the wider environment. *Philos Trans R Soc Lond B Biol Sci,* 370**,** 20140083.

Wright, G. D. 2010. Antibiotic resistance in the environment: a link to the clinic? *Curr Opin Microbiol,* 13**,** 589-94.

Xavier, B. B., Lammens, C., Ruhal, R., Kumar-Singh, S., Butaye, P., Goossens, H. & Malhotra-Kumar, S. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. *Eurosurveillance,* 21.

Yang, F., Huang, L., Li, L., Yang, Y., Mao, D. & Luo, Y. 2017. Discharge of KPC-2 genes from the WWTPs contributed to their enriched abundance in the receiving river. *Science of The Total Environment*.

You, Y. & Silbergeld, E. K. 2014. Learning from agriculture: understanding low-dose antimicrobials as drivers of resistome expansion. *Front Microbiol,* 5**,** 284.

Zhang, T., Zhang, X. X. & Ye, L. 2011. Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. *PLoS One,* 6**,** e26041.

Zhang, X. X., Zhang, T. & Fang, H. H. 2009a. Antibiotic resistance genes in water environment. *Appl Microbiol Biotechnol,* 82**,** 397-414.

Zhang, X. Y., Ding, L. J. & Fan, M. Z. 2009b. Resistance patterns and detection of aac (3)-IV gene in apramycin-resistant Escherichia coli isolated from farm animals and farm workers in northeastern of China. *Research in veterinary science,* 87**,** 449-454.

Figures:

Figure 3.2. Consumption of antibiotics for use in humans and food-producing animals A) by selected European Countries in 2012 and B) by selected classes of antibiotics for each European country. Data of consumption in tonnes was recovered from ECDC, EFSA and EMA report (ECDC/EFSA/EMA, 2015). Data from human consumption includes the antibiotics used in the community and hospitals. Data from food-producing animal's consumption includes the use in pigs, cattle and broilers. The countries population size data are estimates from the World Bank for the year 2012 (http://data.worldbank.org/indicator/SP.POP.TOTL).

Figure 3.2. Antibiotic resistance dissemination pathways