FULL REPORT

Non-medical uses of antibacterial compounds (antibiotics): time to restrict their use?

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Summary

The global crisis of antibacterial drug, antibiotic, resistance has reached a point where, if no action is taken now, human medicine will be exposed to a post-antibiotic world, where simple injuries could once again be life threatening. With growing numbers of antibiotic resistant bacteria now outcompeting the ability to innovate new products, the problem needs tackling on two fronts: investment in discovery, research and development of new treatments for bacterial infections and optimising the use of existing antibiotics. Whilst the use of antibiotics in human medicine is identified as a strong risk factor in the development of antibiotic resistance, use outside of human medicine is also responsible and is often over-looked. Antibiotics are commonly used outside of human medicine around the world, including in animal husbandry, apiculture, aquaculture, ethanol production, horticulture, anti-fouling paints, food preservation and domestic uses. This use drives both transfer of antibiotic resistance genes between bacteria but also selection of antibiotic resistance mutations. In several instances, the use of antibiotic compounds is inadequately justified, especially where simple, cost-effective alternatives are available. In addition, in many countries the sale of antibiotics for use outside of human medicine is much greater than that used in people. The restriction of antibiotic use outside of human medicine is now a priority so as to delay the selection and spread of antibiotic resistant bacteria.

1. Introduction

1.1 The Extent of the Problem

The discovery of penicillin by Alexander Fleming in 1928 marked the beginning of the golden age of antibiotics (Kardos and Demain, 2011). However, since their discovery, man has been on the losing side of an "arms race" against bacterial infections (WHO 2013). Humans have had the drive and knowledge to discover, research and develop an arsenal of novel anti-bacterial compounds (hereafter referred to as antibiotics) that target bacteria in numerous different ways. Despite this, evolution has ensured that once antibiotic susceptible bacteria have now developed resistance to current antibiotic therapies and these resistant bacteria have spread throughout the world. Antibiotic use underpins many disciplines of medicine, each of which is now becoming compromised by increasing numbers of antibiotic resistant bacteria and a lack of new drug development (Piddock 2012). Resistance not only complicates treatment of common infections but also undermines the success of many surgical procedures and cancer treatments (Piddock 2012).

For over a decade, numerous groups have reported on increasing numbers of antibiotic-resistant bacteria, and more recently on the lack of new drugs (Figure. 1). In fact, only two new antibiotics (ceftolozane/tazobactam and ceftazidime/avibactam) active against Gram-negative bacteria have been marketed in the 12 months before March 2015 (The Pew Charitable Trusts). These drugs have only had limited indications and are combinations agents (inhibitor of resistance mechanism with antibiotic). Most newly discovered antibiotics are targeted only at Gram-positive bacteria.

In 2011, the WHO identified antibiotic resistance as one of the greatest threats to human health and this led to the WHO publishing a strategic action plan on antimicrobial resistance (WHO Regional Office for Europe, 2011). Since then many other reports have conveyed the same level of urgency. In 2013 and 2014, the World Economic Forum published its global risks report highlighting antibiotic resistance as an emerging risk to human health (Howell 2013; World Economic Forum 2014). The rising problem of antimicrobial resistance led to discussions by finance ministers and policy-makers at the London June 2013 G8 summit where the issue was highlighted as a global concern (Department for Business, Innovation and Skills and Prime Minister's Office 10 Downing Street, 2013). In 2009, the Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) was founded in the hope of improving cooperation between the EU and US on antimicrobial resistance (CDC, 2014). Recently the WHO report, 'Antimicrobial Resistance: Global Report on Surveillance', published in May 2014, amassed global antimicrobial resistance data, and in doing so, further emphasised the need for action on this global crisis (WHO, 2014a). Issues concerning a lack of new antibacterial drugs to tackle antibiotic resistant bacteria were also highlighted in Volume 2 of the 2011 Annual Report of the UK Chief Medical Officer (Davies 2013), as well as numerous other reports (e.g. Buckland 2013) and publications in medical journals. Several national strategic action plans have been put in place or are under development and the World Health Assembly has emphasised the need to implement a global action plan for which a draft has been prepared (WHO, 2014b; WHO, 2014c). Most recently, the UK Antimicrobial resistance review, chaired by Lord Jim O'Neill, has revealed that resolving this crisis will be costly and is vastly underfunded (AMR Review, 2014). Nevertheless, despite the awareness amongst the scientific community, infectious diseases physicians and

clinical microbiologists, actions taken so far by international and national agencies have been slight in proportion to the problem faced. It is clear that little real action has been taken, and that there are few new alternatives to the treatment options available to human medicine. Alternatives may involve the development of new antibiotics or innovative ways to prevent and treat bacterial infections including changes to the way antibiotics are prescribed such as using collateral sensitivity cycling (Imamovic and Sommer, 2013).

1.2 The Source of the Problem

The focus of many reports on healthcare acquired infections appears to have led to the misconception that bacterial resistance is a problem that must be dealt with primarily in a human and hospital based setting. Antibiotic use in humans, notably, their over-prescription and misuse is commonly blamed as a prominent factor for the rise of antibiotic resistant bacterial strains (Harbarth et al 2001; Vernaz et al 2011; Wertheim et al 2004). However, as this report reveals, antibiotics are used widely in numerous areas outside of human medicine from animal husbandry to ethanol production. Whilst not all antibiotics are used as drugs in human medicine, all have the capacity to select bacteria resistant to drugs used for human treatment. This is because resistance mechanisms developed against one antibacterial compound may allow that bacterium to withstand another antibacterial compound using the same mechanism e.g. through efflux pumps (Piddock 2006a). The widespread use of antibiotics, coupled with the use of other antibacterial chemicals that promote co-selection of resistance, in any environment can select antibiotic resistant bacteria, which proliferate and become shared between ecosystems. As a result of the sheer volume of antibiotics used, it is important to realise that the task of reducing antibacterial resistance must be tackled on a global scale and in all areas of use.

With an ever-depleting number of effective antibiotics for treatment, it has become apparent that antibiotic stewardship needs to be taught and practiced; this includes the prevention of their indiscriminate use worldwide, especially in situations where alternatives are available. This article shows the scale of non-human uses of antibacterial compounds globally and in so doing poses the question: should antibacterial compounds be restricted to human use?

1.3 How bacteria become resistant to antibiotics

The development of antibiotic resistance is the consequence of Darwinian evolution. One important evolutionary characteristic of bacteria is that they are able to share genetic information (DNA) between one another via mobile (transmissible) genetic elements such as plasmids, transposons and integrons. These elements may contain genes that are advantageous for bacteria in specific environments such that an antibiotic resistance gene may be transferred between bacteria of different species. This is important as it means that non-pathogenic bacteria can potentially pass on antibiotic resistance to pathogenic bacteria if they co-localise in the same environment. These methods of resistance gene transfer between different species of bacteria are proposed to be the main driving forces for the development of most antibiotic resistance in human pathogens. For example, *Kluyvera* spp., bacteria that infrequently cause human infections and are commonly associated with the environment, possess *bla* genes which have been proposed to be the progenitor of transmissible CTX-M Extended-Spectrum β-Lactamases (ESBLs), which confer resistance to third generation cephalosporin antibiotics (Oliver et al., 2001). *Kluyvera* spp.. CTX-M ESBLs are now one of the most prominent ESBLs and are associated with numerous human bacterial pathogens (Cantón et al 2012). The ESBL genes

arose in the pathogenic bacterial species through mobilization of *Kluyvera* spp. chromosomal genetic material via mobile genetic elements (Cantón et al 2012).

Another mode of evolution is via mutation on the bacterial chromosome. A population of bacteria of the same species share an almost identical gene pool, however random mutations can occasionally occur due to incorrect copying of genetic information and sometimes these mutations can confer a favourable trait such as antibiotic resistance. The short generation time of bacteria is partly responsible for their rapid adaption. For example, the generation time of *Escherichia coli* (*E. coli*) is typically 20 minutes with spontaneous mutations being that of an estimated 1×10^{-3} per genome per generation, with rates known to alter under stressful selective conditions (Lee et al 2012). As a consequence of this rapid growth, bacterial populations can increase exponentially in a matter of hours. Upon exposure to antibiotic only the antibiotic resistant bacteria will survive whilst the antibiotic-susceptible bacteria will be destroyed. Resistant strains will then dominate the population, irrespective of whether colonising or infecting a human, an animal or another environment. There have been multiple examples of this *de novo* resistance arising in both humans and animals due to antibiotic treatment (e.g. Blair et al 2015; Humphrey et al 2005).

A specific concentration of antibiotic that inhibits the visible growth of targeted bacteria is known as the minimum inhibitory concentration (MIC). An antibiotic concentration that is needed to kill a bacterium is known as the minimum bactericidal concentration (MBC). Consequently, the MIC and MBC of an antibiotic will be higher for an antibiotic resistant bacterium than for a susceptible strain. In order for an antibiotic to completely inhibit growth, or kill resistant bacterial strains, it is necessary to ensure that the concentration of the antibiotic is higher than that of the

MIC/MBC of antibiotic for the resistant strain in order to prevent their dissemination (Gullberg et al 2011). At the MIC of the antibiotic susceptible strains, the susceptible bacteria cannot replicate, whereas the resistant strains can; this antibiotic concentration range is known as the 'traditional selection window' and will cause the selection of resistant strains from the antibiotic exposed population (Figure 2). When an antibiotic is present in the environment but not equal to or above the MIC of the susceptible strain, a sub-therapeutic window is seen, which fails to halt growth of the susceptible bacterium. However, this sub-therapeutic window will facilitate the selection and enrichment of resistant strains from the bacterial population, due to very small quantities of antibiotic hindering (not preventing) susceptible strain growth and thus presenting a selective advantage to the resistant strains (Gullberg et al 2011; Figure 2). This mutant selection concentration (MSC) is dependent on the bacterial species and resistance mechanism, but the MSC has been found up to 230 times lower than the MIC (Gullberg et al 2011). In addition, resistance can emerge in bacteria when there are no resistant bacteria present initially. This was shown in a study where wildtype bacteria were grown for 600 generations in sub-MIC conditions, after which a sub-population of high-level resistant bacteria emerged (Gullberg et al 2011). Each traditional selection window is mobile; as antibiotic resistant bacteria emerge, higher concentrations of antibiotic are required to inhibit growth, this selects for strains with higher levels of resistance causing the mutant prevention concentration to shift further to the right of the scale until the antibiotic is rendered clinically ineffective (Figure 2). When humans or animals are treated, the dose has to be high enough to eradicate the infection; sub-MIC values can present themselves in treatment if the dose is too low or the treatment is not completed. The concept of using an *in vitro* MIC as a measure for human and animal treatment in not foolproof since the translation of these

quantities into an *in vivo* system makes the dose relatively imprecise (Andersson and Hughes, 2014). Another concern is that the antibiotics used for treatment are subsequently diluted into the environment after drug courses where sub-MIC values are easily achieved and selection of resistant strains can occur. In fact, sub-inhibitory concentrations are much more likely to facilitate the step-wise development of resistant bacteria since high concentrations of antibiotics are withstood by those bacteria with a pre-disposed ability to survive (Andersson and Hughes, 2014). Not only this, but at sub-MICs bacteria with increased mutation rates can occur; such sub-MICs can also affect the rate of horizontal gene transfer, since there is a smaller 'jump' between wild-type and slightly resistant under low antibiotic concentrations than wild-type and highly resistant under high antibiotic concentrations (Andersson and Hughes, 2014). Furthermore, horizontal gene transfer can be stimulated by antibiotic stress responses, such as the SOS response leading to increased transfer of antibiotic resistance genes (Andersson and Hughes, 2014). In addition, antibiotic stresses can increase recombination events (Andersson and Hughes, 2014).

2. Animal Use

The use of antibiotics in animals is divided between therapeutic and non-therapeutic use. Therapeutic use includes prophylaxis (treatment to prevent infection), metaphylaxis (treatment of a whole group because individuals show infection) and individual treatment of diseased animals whereas non-therapeutic uses mainly describes the use of the antibiotic as a growth promoter, to rapidly improve the weight of the animals reared for food production.

2.1 Growth Promotion in animals reared for food production

After the establishment of the benefits of antibiotic use for therapy, mass drug production was initiated, and as a result waste products were often sold for animal feed. It was soon discovered that the waste products of chlortetracycline production provided growth-promoting effects on poultry. This discovery led to several different antibiotics being included in animal feed or water to increase the size of the animal produced for food; this decreased the time required for its rearing and provided an improved return on investment (Jukes et al 1953; Butaye et al 2003). It is unclear how antibiotics promote growth. Several hypotheses have been proposed, all relating to the antibacterial properties of the drugs and include nutrient protection (antibiotic mediated reduction in gut flora (microbiome) restricts the breakdown of nutrients by bacteria); increased absorption (to thinner intestinal linings due to reduced bacterial numbers); fewer bacterial toxins (less energy expenditure on raising an immune response) and a reduction in sub-clinical infections (Butaye et al 2003). It is likely that the true mode of action by which antibiotics deliver growth-promoting properties is through a combination of these mechanisms.

The concern with the addition of antibiotics to animal feed for the purposes of growth promotion is that dosing of antibiotic is not controlled, and some animals will receive sub-therapeutic doses (Ungemach 2006). This will allow the selection of antibiotic resistant bacteria, most likely in the digestive system of the exposed animal. These same bacteria will be present in the animals' faeces thereby contaminating the local environment. In addition, some countries use animal waste as fertilizer causing spread of antibiotic resistance genes onto food crops; this practice is also true for human waste in some countries.

In 1969, the Swann report recommended that antibiotics used in humans should not be used in animals as growth promoters (Swann et al 1969). Many countries and

companies adhered to this recommendation; nonetheless very similar compounds were used for treatment of people and animals (whether in therapy, prophylaxis or growth promotion). Use of antibiotics in animals has been shown to select for bacteria resistant to compounds used in human medicine; examples include the use of avoparcin which gave rise to vancomycin resistance, virginiamycin that promoted resistance to other streptogramins and fluroquinolones (not used for growth promotion) such as enrofloxacin which gave rise to ciprofloxacin resistance (Endtz et al 1990; Klare et al 1995; Piddock 1995; Schouten et al 1997; Werner et al 1998; Witte et al 1999; Piddock 2002). Reduction in the use of antibiotics in animals came in 2006, when following recommendations from the WHO meeting on the medical impact of the use of antimicrobials in food animals in 1997, the EU regulation No 1831/2003 was implemented, which prevented the administration of antibiotics, other than coccidiostats and histomonostats, to animals as growth promoters (WHO 1997; European Union, 2003). However, prior to the introduction of this regulation, several antimicrobial compounds had already been withdrawn from the EU market, including avoparcin, tylosin, spiramycin and virginiamycin, over concerns of producing cross-resistance to treatments used in human medicine (Schwarz et al., 2001). Despite this EU regulation, some countries outside of the EU continue to use antibiotics for the growth promotion of animals, including the USA. Furthermore, recent data suggests that the amount of antibiotics administered to animals in the USA is now much greater then that used in humans, with 2011 data showing human use to be 3.29 million kg a year compared to 13.5 million kg for food producing animals (FDA, 2012; FDA, 2013). It is important to realise that, although there is a greater volume of antibiotics sold for animal use, it is likely that these high figures also reflect the large livestock and poultry populations of the USA. Globally antibiotic consumption in

food animals is set to rise 67% by 2030 due largely to increased large-scale intensive farming (Van Boeckel et al 2015).

In Denmark, concerns were raised regarding the antibiotic resistance developed by *Enterococcus faecium* and *Enterococcus faecalis* to avoparcin, a drug similar to vancomycin used in humans (Aarestrup et al 1996). Many have argued that avoparcin use did not give rise to vancomycin resistance because of strain differences between humans and animals; however, molecular typing and genetic analysis reveal that the genetic elements encoding resistance move between animal and human strains of bacteria (Klare, 1995; Sletvold et al 2010). Glycopeptide-resistant *E. faecium* harbour plasmids encoding the *vanA* gene, which confers vancomycin and avoparcin resistance. An 18kb sequence, including the entire Tn*1546* transposon (which is associated with the *van*A gene cluster) is conserved with a >99% identity in both human isolated *E. faecium* and animal isolated *E. faecium* strains (Sletvold et al 2010). As a result of consumer pressure and concern over the presence of antibiotic resistant bacteria on food items Denmark discontinued the use of avoparcin as a promoter in 1995 and discontinued all growth promoters for cattle, broiler chickens and finisher pigs by 1998 (weaner pig use was stopped the following year) (WHO 2002). The Danish Poultry Council has shown that this did not decrease the total mass of produce delivered by the farms nor did it cause an increase in death rate of broiler chickens overall for the four years following antibiotic growth promoter discontinuation (Figure 3; Emborg et al 2002). However, there were reports that the withdrawal of antibiotics may have had detrimental impacts on the health of some animals. For example, as of the beginning of 2005, in Denmark, pigs between the weights of 7-30kg (weaners) had experienced both increased mortality and decreased weight gains as a result of the withdrawal of antibiotic growth promoters.

Furthermore, within this period, in an effort to treat diarrhoea in weaners, therapeutic antibiotic use increased as antibiotic growth promoter use decreased (Kjeldsen and Callesen, 2005). Farmers also modified some of their practices to minimise losses, which included the addition of organic acids into the pig feed (Kjeldsen and Callesen, 2005). While use of therapeutic drugs in weaners was still relatively high as of 2012, this number was also reflective of increases in the numbers of pigs produced and exported since the banning of growth promoters (DANMAP 2012). Conversely, therapeutic antibiotic use in Norway decreased by 39% from 1993 to 2003, even with a 10% increase in pig production (Norway banned growth promoters in 1995)(Grave et al 2006). Finland also did not experience any significant increases in the use of therapeutic antibiotics to treat weaners following an antibiotic growth promoter ban (Laine et al 2004). In Sweden, the consequences of the growth promoter ban were initially mixed, with therapeutic antibiotic use increasing by 21% in the first two years, staying at this quantity for 6 years before declining by 47% between 1994 and 2003. The number of pigs raised for slaughter also decreased by 16% during this period (Grave et al 2006). During the first two years, there were fears of necrotic enteritis outbreaks in broilers, subsequently prescription in feed antibiotics were given (Grave et al 2006). The steep decline in therapeutic antibiotic use between 1994 and 2003 was assumed to be due to increased animal welfare, husbandry standards and disease control management. Initially, studies on the growth promoting benefits of sub-therapeutic levels of antibiotics were considered significant. However, as time has progressed it appears as though the benefits may not be as great nowadays, particularly in high-income countries. This is possibly due to modern day hygiene, vaccination and nutritional intake improvements within animals.

In 1999, the US National Academy of Sciences estimated the economic costs of the banning of antibiotic growth promoters in the US (National Research Council, 1999). The report suggested that meat producers would not be greatly affected by a ban because animals no longer live in stressful and sub-optimal sanitary conditions. They estimated at that time the average annual per capita cost to the consumer in the USA would range from $4.84-$9.72 for a total ban on sub-therapeutic drug use, equating to $1.2 - $2.5 billon per year for the industry (National Research Council, 1999). A WHO (2002) report suggested that the overall consequence (on all animals) of withdrawal of antibiotic growth promoter in Denmark resulted in an overall drop in Danish GDP of 0.03%. This included a 1.04€ loss for each pig produced (1.4% lower) and no loss for poultry (0.4% higher since poultry production benefited from decreased pig production). The WHO report concluded that countries with similar farming practices to Denmark could discontinue the use of antibiotic growth promoters without negative effects occurring on the economy (WHO, 2002).

Advocates of the unrestricted use of antibiotics in animals argue that production of animals for food must be a balance between the economic efficiency of the farm and production of cheap food versus the safety of antibiotic use. Numerous august bodies and individuals have argued that caution should be taken about continued use of antibiotics in animals. Notably, if use in animals poses a risk to human health, policies and controls should be put in place by governments to reduce the risk. It is evident that alternatives to using antibiotics in animals for growth production are urgently required in all countries. Current evidence suggests that greater use of vaccination, improved hygiene and controlling of infections and improved animal feeds will confer many of the benefits of antibiotic growth promotion.

2.2 Antibiotic use in veterinary medicine

The veterinary use of antibiotics in animals has been divided into three areas: therapy, control and prevention of infection (prophylaxis). Therapy is classified as the use of antibiotics for the purposes of treatment of animals showing clinical disease. In contrast, control is defined as the use of antibiotics in animals that show sub-clinical symptoms of disease in the group, such as sub-clinical mastitis and bovine respiratory disease complex. Prophylaxis is the use of antibiotics prior to the onset of any symptoms in animals, as to minimise the risk of developing a bacterial infection during a period of specific risk (Kemper, 2008).

The therapeutic use of antibiotics in animals varies depending on the number of animals that need to be treated and whether they are farmed animals or domestic pets. Individual treatment usually only occurs in pets, horses, cows and calves (Schwarz et al 2001). Cattle often require treatment for mastitis, scours (diarrhoea) and respiratory infections. The ideal situation is the individual treatment of animals should follow identification of the pathogen. However, as in human medicine, due to the lack of rapid tests, the veterinarian is unable to obtain quick laboratory results and must therefore rely on prior experience and veterinary knowledge to prescribe the appropriate antibiotics to treat animals based on the symptoms of illness that they exhibit. Many antibiotics used in animals are of the same antibiotic class to those in human healthcare (Table 1). There are few families of antibiotics that have exclusive use for human or animal treatment and therefore bacterial resistance arising from antibiotic use in humans or animals creates the risk of selecting resistant bacteria able to withstand a whole family of antibiotic drugs, thus, compromising both human and animal healthcare. It is necessary for the veterinarian to consider these risks when prescribing antibiotics. The recommendations for the individual treatment of these animals is very similar to those in human medicine in that it is guided by initial

diagnosis of pathogen and antibiotic sensitivity testing (if applicable or available). However, due to the time required for laboratory confirmation of bacterial species and test results, this is rarely carried out. Antibiotics are given to those animals that show the symptoms of bacterial infection or are within an infected group and may be incubating the infection, administration may be oral or injected and the dose will be reflective of the severity of the morbidity, where the infection is localised, the organism suspected of causing the infection and the size of the animal. Individual treatment of animals is the ideal scenario, however, it is impractical for herds or flocks of animals, and so these are often treated together where some of the treated animals are ill, some are incubating the infection and some are healthy. This is known as metaphylaxis. In this case, antibiotics are administered via feed or water and should be commenced after identification of the microorganism causing the infection in those animals showing symptoms. Herd metaphylaxis brings with it a number of risks that may lead to the development of antibiotic resistant bacteria. The most worrying is the inability of the veterinarian to control the variation of the dose of antibiotic administered to each animal. The dose administered will vary for each animal depending on individual appetite, the level of homogenization in feed or water and distribution of the antibiotic in the feed or water (Ungemach 2006). These can lead to sub-optimal levels of antibiotic ingested by the animal, and this can provide positive selection of antibiotic resistant bacteria. Additional risks are that of interactions between feed and antibiotics or the drug poorly penetrates target tissues, increase the likelihood of livestock receiving sub-therapeutic doses and so reduce the effectiveness of an antibiotic. Antibiotics are commonly used in the prophylaxis of dairy cows to prevent mastitis at the end of their lactation period. To prevent introduction of

infections such as leptospirosis, antibiotics may also be given prior to the mixing of herds and flocks.

Implications for human health of antibiotic resistant bacteria emerging in animals and transmission to humans.

Livestock and pets also have the potential to act as reservoirs of antibiotic resistant bacterial strains. Transfer to and from humans is possible through skin-skin contact, contact with saliva, faeces, contaminated food or even air-borne transfer. The development of resistance and the events leading to its transmission are likely to be due to a combination of factors, which molecular and epidemiology studies are now helping to decipher (Paterson et al 2015). There have been documented case-reports and studies, which have demonstrated domesticated animals can host MRSA and this may facilitate the spread of MRSA to humans by pets and vice versa (Loeffier et al 2010). An increasing number of studies have demonstrated a link between antibiotic resistance in bacteria isolated from animals and humans, with increasing numbers of antibiotic resistant bacteria isolated in both (Overdevest et al 2011). Transfer of specific strains of *Staphylococcus aureus* (*S. aureus*), including ST398, has also been demonstrated to occur between pigs and pig farmers (Armand-Lefevre et al., 2005). In addition, an extensive list of strains of livestock-acquired MRSA has been found and these have also been successfully isolated from human clinical samples (Casey et al 2014).

Genomic analysis of the *S. aureus* stain S0385, a member of the ST398 lineage, revealed a number of key differences compared to other *S. aureus* strains, importantly and of most interest was the identification of novel variants of mobile genetic elements (Schijffelen et al 2010). Many convey virulence and antibiotic resistance including tetracycline, copper and methicillin resistance (Schijffelen et al 2010). The

latter being partly caused by a 38kb type V Staphylococcal Cassette Chromosome *mec* (SCCmec). Interestingly, copper is regularly used as a growth promoter in some livestock and flocks, and this could explain selection of this resistance trait, since there is a *cop*A gene in the SCCmec element, which could provide copper resistance (Hasman and Aarestrup, 2002). Copper has also been associated with the development of macrolide and glycopeptide resistance (Hasman and Aarestrup, 2002). Whether copper resistance genes were present in human *E. faecium* isolates has subsequently been investigated, since humans do not have a high copper supplemented diet and such resistance should not have developed. Copper resistant bacteria were found in 10% of human isolates suggesting that these copper-resistant bacteria were from animal origins (Hasman, and Aarestrup, 2002). Further evidence of these transfer events was provided when a novel SCCmec was linked to dairy cattle. This SCCmec was isolated from both bulk milk and human clinical samples (Garcia-Álvarez et al 2011). The strain types carrying this novel element were thought to be restricted to animals, strongly suggesting the origin of the genetic element to be bovine (Garcia-Álvarez et al 2011).

Analysis of antibiotic use in pig farming in China found some antibiotic resistance genes to be enriched by up to 28,000 fold in farm samples compared to soil controls (Zhu et al 2013). Arsenic, zinc and copper were also found in Chinese farm samples, with the latter being found up to 1,700 mg per kg in manure. It has been suggested that this could be directly responsible for the co-selection of antibiotic resistance genes (Zhu et al 2013). Zinc used in animal feed has also been linked with the emergence of MRSA (Cavaco et al 2011).

One possible reason for the exchange of bacteria between humans and animals is the ingestion of meat and aquaculture products (such as fish and shellfish) containing

antibiotic resistant bacteria, thus providing a pathway for resistant strains to transfer through the food chain (Vivekanandhan et al 2002; Yucel et al 2005; Van et al 2007; Hammerum and Heuer 2009; Overdevest et al 2011). Shellfish are problematic as they can concentrate pathogenic bacteria from human and animal sewage discharges (Potasman *et al.*, 2002). In addition, resistant strains may transfer resistant genes to commensal human bacteria during infection (Hammerum and Heuer 2009). It is also becoming apparent that resistant bacteria, such as ESBL-producing Enterobacteriaceae, can colonise the human flora. These colonisation events are frequent among travellers, with food and water being likely sources for the acquisition of antibiotic resistant bacteria; in addition, the likelihood of colonisation and infection is also very dependent on the geographical area that has been visited (Östholm-Balkhed et al 2013). Furthermore, numerous articles highlight the transfer of antibiotic resistant bacteria from poultry to humans. This includes a study that shows the transfer of ESBL genes, strains and plasmid transfer from poultry to humans (Leverstein-van Hall et al 2011). While additional evidence has shown the transfer, between poultry and humans, of fluoroquinolone resistant *Campylobacter* spp. (Humphrey et al 2005; Smith et al 1999). Notably, the number of quinolone-resistant bacteria acquired by people domestically increased, even there had been no use of quinolones in patients prior to sampling for testing for Campylobacter. The same study found a high incidence of quinolone-resistant bacteria in poultry, isolated from the same geographic location as the above patients (Smith et al 1999). Furthermore, there was an association between the molecular subtypes of resistant *Campylobacter* isolated from humans and poultry (Smith et al 1999). When poultry flocks underwent quinolone treatment, almost 100% of all *Campylobacter* became antibiotic resistant

and a large proportion of these bacteria were still present four weeks post-treatment (Humphrey et al 2005).

2.3 Antibiotics in beekeeping

Antibiotics are also used in in apiculture; beekeepers make use of these drugs to control disease outbreaks without the cost associated with burning infected hives. Foulbrood is a disease that affects very young bee larvae under the age of 48 hours. It is caused by the bacterial species *Paenibacillus larvae* (American foulbrood) and *Melissococcus plutonius* (European foulbrood), which cause death of larvae and eventually the destruction of a whole bee colony. In the USA, Argentina and Canada, oxytetracycline has been applied to bee colonies to treat and prevent this infection. In all three countries there has been widespread resistance to oxytetracycline resistance detected in *P. larvae* (Evans 2003; Alippi et al 2007). The prolonged use of oxytetracycline in apiculture raises concerns over the development of resistance amongst bacteria pathogenic to humans. The gut microbiota of honeybees has been shown to form a reservoir for eight different tetracycline resistance genes (Tian et al 2012). These include *tetB, tetC, tetD, tetH* and *tetY,* which are genes that encode efflux pumps, as well as *tetM* and *tetW,* which are ribosomal protection genes (Tian et al 2012). The *tetW* and *tet*M resistance genes have also been detected in human *Bifidobacterium* isolates (Aires et al 2007). It has been suggested that *tet* resistance genes have arisen in the bee population either through multiple genome mutations in a short period of time or horizontal transfer such as that conveyed by transposons or plasmids (Evans 2003). Many of these *tet* resistance genes are associated with plasmid-mediated transfer between bacteria (Chopra and Roberts 2001). This has been confirmed by isolation of plasmids from *P. larvae* containing *tet*L (Murray et al 2007). In addition conjugation experiments with *Bacillus subtilis* suggest *tet*K is also

plasmid mediated in *P. larvae* (Alippi et al 2007). *Lactobacillus sakei*, a bacteria found in cheese, meats, smoked fish and other raw fermented foods, has been shown to harbour a plasmid, containing a *tet*L gene, that has an almost 100% identity to that found in *P. larvae* (Ammor et al 2008). How this plasmid arose in both species in unknown, however, it strongly suggests horizontal gene transfer between these species either directly or indirectly (such as through intermediate bacteria). If this is the case honeybees harbouring resistance genes on plasmids may be aiding the rise of antibiotic resistance in medicine by indirect methods, for example, disseminating the resistance plasmid to bacteria that have the potential to directly affect the human microbiota such as *L. sakei*.

Antibiotics, other than tetracyclines, have also been used in the treatment of bee infections; these include: streptomycin, sulphonamides, tylosin, erythromycin, chloramphenicol, nitrofurans, nitroimidazoles, fluoroquinolones and fumagillin (Reybroeck et al 2012). Worryingly, many of these antibiotics are banned and so their detection is surprising, especially finding chloramphenicol in honey (Reybroeck et al 2012). Unlike antibiotic use in other food-producing organisms, honey production has the additional problem that honeybees themselves do not actively metabolise these antibiotic compounds, leading to high levels of antibiotics being present in honey. For example, lincomycin, nitrofurans, chloramphenicol, streptomycin, sulfamethazine have been detected in honey $\geq$ 290 days after dosing the hive with antibiotics, with the latter four being found 1 year after dosing (Reybroeck et al 2012). Thus, inadequate testing against all these antibiotics before selling these products could lead to unknown consumption of these compounds, which in itself could facilitate selective pressure for antibiotic resistance in the human microflora. Furthermore, fluoroquinolones have been associated with prophylactic treatment of beehives, the

former being heavily associated with use in Asia (Reybroeck et al 2012). To prevent further dissemination of resistant bacteria in bees, management systems involving sterilization and burning of the hives, as is common in the EU, instead of the use of antibiotics would seem a good precautionary measure against resistance emergence.

2.4 Use of antibiotics in aquaculture

Aquaculture is a term used to describe the farming of aquatic organisms such as fish. Aquaculture in developing countries, particularly those in Asia, is a growing industry, which is set to grow further as the leading global fisheries are depleted (Liao and Chao 2009). In industrial aquaculture, fish are raised in a stressful environment, being exposed to a number of stressors such as human handling (Barton et al 1991). This increase in stress has been shown to dampen the natural immune response in fish and make it easier for pathogenic bacterial infections to establish and contaminate fish stocks (Barton et al 1991). Through this argument prophylactic use of antibiotics in aquaculture has been justified (Cabello, 2006). The availability of antibiotics for this purpose has raised concern over poor living conditions for the fish on the farms, similar to concerns seen in animal husbandry. Issues include increased fish population densities, closely compartmentalised farms, poor sanitary conditions and inability to isolate diseased fish. All of these can increase the progression of bacterial infection and the development of antibiotic resistance amongst these populations when associated with high rates of antibiotic use. Poor sanitary conditions encourage the growth of pathogenic bacteria such as *Aeromonas salmonicida*, *Flavobacterium spp.* and *Lactococcus garvieae* (Noble and Summerfelt 1996; Vendrell et al 2006). In addition, the use of antibiotics in this environment has led to antibiotic resistance in these bacterial pathogens (Schmidt et al 2000). Furthermore, a high density of fish in aquaculture is a likely reason for the increased spread of antibiotic resistant bacteria

within the farm (Krkošek 2010). Fish farms, sharing the same body of water have the potential to transfer bacteria from one farm to another and subsequently between different species of fish (Krkošek 2010). The dissemination of microbes can occur over large distances due to currents and wild fish movements (Krkošek 2010).

The poor fish living conditions and associated high frequency of infections further increase the demand for prophylactic antibiotic use within the industry. Antibiotics are given as part of the feed added to the inhabiting waters of the fish. Much of the feed is not consumed and so sinks to the base of the water system alongside unmetabolised antibiotics present in the excreted faeces of the fish. The antibiotics and antibiotic resistant bacteria can then be leeched from fish farms and washed away to distant sites by the prevailing currents in the area (Guardabassi et al 2000; Cabello 2006; Krkošek 2010; Buschmann et al 2012). As a consequence, selective pressure in microenvironments at sites distant to the aquaculture farm results in the development of increased numbers of antibiotic resistant bacteria (Guardabassi et al 2000; Buschmann et al 2012). Furthermore, antibiotics administered directly to the water systems diffuse and rapidly fall in concentration, especially at aquaculture sites located on free running rivers or natural open water systems. These sub-therapeutic levels of antibiotics may result in selection of antibiotic resistant bacteria. Furthermore, horizontal gene transfer means that these resistance genes can pass to terrestrial bacteria or human commensal bacteria that are known to be pathogenic or potentially pathogenic to humans (Rhodes et al 2000; Cabello 2006; Poirel et al 2012).

Mobile genetic elements containing genes for resistance have been suggested to have crossed the boundary between aquaculture and human medicine. Florfenicol is a prophylactic antibiotic that has been used extensively in aquaculture in Eastern

industrial countries such as Japan since the 1980s (Angulo and Griffin, 2000). The gene pp-*flo* confers resistance to florfenicol. It was first identified in *Pasteurella piscicida*, a bacterial pathogen of fish (Kim and Aoki, 1996). Furthermore, the tetracycline-resistance class G resistance gene was originally identified in the fish pathogen, *Vibrio anguillarum* (Zhao and Aoki, 1992). Both the *flo*R gene (a gene with a high similarity (97%) to pp-*flo*) and the class G resistance gene have been identified in *Salmonella enterica* (*S. enterica*) serotype Typhimurium DT104, which causes disease in humans (Bolton et al 1999; Angulo and Griffin 2000; Cloeckaert et al 2001). The *flo*R gene and the *tet*G gene are found on a genetic fragment called the salmonella genomic island 1 (SGI1) which is common among many isolates of *Salmonella* including Typhimurium DT104 and *S*. Enteritidis (Boyd et al 2001). SGI1 can be transferred via horizontal gene transfer (Doublet et al 2005). Since the gene is heavily disseminated it is a prime example of how rapidly antibiotic resistance can appear. Combined, these studies suggest the existence of horizontal transfer between bacteria in aquaculture and human pathogenic bacteria. Another example of horizontal transfer of antibiotic resistance genes was identified in 1992 when an outbreak of antibiotic resistant cholera in Latin America was due to horizontal transfer of genes from bacteria selected by the extensive use of antibiotic in the Ecuadorian shrimp industry (Weber et al 1994; Angulo 2000). Seafood is a particular concern for disease outbreaks since it is often eaten lightly cooked or raw.

There is potential for transfer of aquatic bacteria, which may have already been exposed to the effects of antibiotics, to a terrestrial environment through fishing and the mixing of freshwater and seawater with wastewater at treatment plants. Further overlap in terrestrial and aquatic environments may occur when aquafarms and traditional agriculture are located close to each other. Aquaculture antibiotic run-off

coupled with human and animal wastes have also been shown to be a potential contributor to large-scale ecological disturbances and a theoretical driving force for antibiotic resistance development in China (Zou et al 2011). Therefore, although policy and industrial restrictions in most Asian countries ban the use of some antibiotics in aquaculture, such as chloramphenicol and nitrofurans, those that have not been banned may still find their way into water systems, such as tetracyclines (Zou et al 2011; Rico et al 2012).

Recently, ten antibiotic resistance genes have been isolated from *Aeromonas* spp. in ornamental fish, three of which are plasmid-mediated quinolone resistance genes (Dobiasova et al 2014). The majority of *Aeromonas* spp. isolated, that showed quinolone resistance genes, were from Thailand and Vietnam, where norfloxacin, the first synthetic fluroquinolone, is commonly used in aquaculture (Sapkota et al 2008; Dobiasova et al 2014). *Aeromonas* spp. has been highlighted as an important aquatic source of antibiotic resistance genes due to its ability to form a biofilm (micro-organisms which stick together and grow as a community) and because it possesses multiple antibiotic resistance plasmids such as IncU and ones which bear the CTX-M-15 ESBL (Amos et al 2014b; Dobiasova et al 2014). Interestingly, it has been shown that some antibiotics can aid the transmission, via conjugation of the IncU plasmid, if the antibiotic is administered at a low concentration or the antibiotic is not effective at treatment, thus, facilitating more rapid dissemination of resistance genes (Cantas et al 2012). *Aeromonas spp*. possesses the IncU R plasmid, pRAS1, that is a close, if not, identical copy of the pIE420 plasmid hosted by human commensal *E. coli*; this gives strong evidence for an interspecies transmission event between the two species (Rhodes et al 2000). Both these plasmids have the *tet*A resistance determinant. The plasmid pRA1 from *Aeromanas hydrophila* was the first member of the IncA/C

plasmid family identified. The composition of pRA1 is highly similar to IncA/C plasmids isolated from *S. enterica*, *E. coli, Yersinia pestis* (bacterium that causes bubonic plague) and *Vibrio cholerae* (causes cholera) (Fricke et al 2009). Of a particular concern for healthcare is the IncA/C plasmid in *Y. pestis*, which contains 18 antibiotic resistance genes. It is suspected that all IncA/C plasmids originated from the pRA1 plasmid of *A. hydrophila* due to the pRA1 plasmid having the fewest antibiotic resistance genes and is therefore the least developed (Fricke et al 2009). The pRA1 plasmid confers resistance to sulphonamides and tetracyclines, which were two of the first antibiotics used in human and animal medicine (Fricke et al 2009). *Aeromonas* spp. appears an effective agent for the transfer of antibiotic resistance plasmids and subsequently use of antibiotics in aquaculture is likely to increase antibiotic resistance in human pathogens. It is also worth noting that *Aeromonas* spp. have been associated with causing human infection directly in both immunocompetent and immunocompromised individuals (Krovacek et al 1992; Janda and Abbott, 2010). Therefore, if antibiotic resistant *Aeromonas* spp. is selected for in the aquaculture environment, this could severely compromise our ability to treat these infections.

Antibiotics are not the only option available for disease control in aquaculture. Other alternatives are already either in use or under investigation including vaccination, health management, phage therapy, probiotics and quorum sensing disruption (Defoirdt et al 2007). These methods may be able to reduce the reliance on antibiotics by aquaculture industries. Vaccination programs are widely used in some countries such as Norway, where mass vaccination was introduced primarily to effectively reduce furunculosis, a condition in fish caused by *Aeromonas* spp. (Midtlyng et al 2011).

3. Use of antibiotics in horticulture

Horticulture is defined as the cultivation of plants for human use. Although not used in horticulture as widely as their use in animal farming, antibiotics have proven to be useful in the routine control of some plant pathogens (Vidaver 2002; McManus et al 2002).

Fire blight, a disease caused by *Erwinia amylovora* (*E. amylovora*), is a disease that affects apple and pear orchards. Traditionally, in many parts of the world streptomycin has been used to treat this plant infection because of its high efficacy and resistance against degradation by light. However, in the early 1960s streptomycin-resistant strains of plant pathogens were discovered in Florida followed by identification of streptomycin resistant *E. amylovora* in California in 1971 (McManus et al 2002). Since then resistant *E. amylovora* has spread along the whole of the Western USA and British Columbia in Canada (McManus et al 2002). Resistant *E. amylovora* strains have also been isolated throughout Europe, Israel and parts of New Zealand (McManus et al 2002). Two types of resistant bacteria have been isolated; one highly resistant strain (with MICs of 2000µg ml^{-1} streptomycin) and a moderately resistant strain (with MICs of between 500-750µg ml^{-1}) suggesting more than one mechanism of resistance (McManus and Jones, 1994; McManus et al 2002). The presence of resistant strains is not limited to areas of streptomycin use, streptomycin-resistant strains have been found in areas of Lebanon where no antibiotics are used on the crops (Saad et al 2000). This may show that streptomycin resistant bacteria have been spread on imported fruit or trees or that there is a high rate of obtaining resistance genes from naturally resistant species from the environment. The pEL60 plasmid in *E. amylovora* has been found in isolates only from Israel and Lebanon, suggesting that the resistant strain in Lebanon may have

originated from Israel (Rezzonico et al 2011). The transposon, Tn*5393* originally found in *E. amylovora*, was identified to carry two streptomycin resistance genes, which may have arisen and spread due to extensive use of streptomycin on crops (Sköld, 2001). Another gene, *dfr*9, found on a plasmid in *E. coli* from pigs was found inserted within one of these streptomycin genes that was localized on a genetic structure closely related to Tn*5393* (Sköld, 2001). Since *dfr*9 is located on a genetically mobile element it can be strongly considered that there has been direct transfer, or a common mediator for the movement of this gene, between the plant pathogen, *E. amylovora* and *E. coli* from pigs. In addition this *dfr*9 gene, with a genetic fragment of Tn*5393*, has been found in the human pathogen *Campylobacter jejuni*, which is also a known commensal bacteria of pigs (Gibreel and Sköld, 1998). In areas where streptomycin resistant bacteria preclude use of this agent, oxytetracycline is used instead. One of the key differences in features of the two drugs is that streptomycin kills *E. amylovora* whereas oxytetracycline merely inhibits growth (i.e. it is bacteriostatic) (McManus et al 2002) Therefore, when levels of oxytetracycline become diluted, bacteria can flourish.

Although the USA licences only streptomycin, oxytetracycline and more recently the aminoglycoside, kasugamycin in special cases for use in plant agriculture, other countries use several other antibiotics. The most worrying is the use of gentamicin in Latin America (Vidaver 2002). This is of concern as gentamicin is very important in human medicine and is used to treat serious bacterial infections in humans. Based on the "precautionary principle" the US Environmental Protection Agency has banned the import of plant agricultural produce treated with gentamicin as sub-therapeutic residues of the drug on food may have the potential to compromise the effectiveness of the drug in human medicine (Vidaver 2002). The consumption of fruits, salad items

and herbs may confer a direct route for antibiotic resistant bacteria, transmissible resistance genes and antibiotic residues to reach the human microflora.

Issues have also arisen concerning the selection or transfer of resistant bacteria on food crops due to manure containing antibiotic residues or antibiotic resistant bacteria being directly used as fertilizer. Recently, culinary herbs imported from countries in South East Asia, notably, Vietnam, Thailand and Malaysia, have hosted multi-drug resistant bacteria containing up to ten different types of antibiotic, including those conferring resistance to third generation cephalosporins, quinolones and aminoglycosides (Veldman et al 2014).

4. Industrial uses of anti-bacterials

4.1 Food preservation

Lactic acid producing bacteria (LAB) are found commonly and have a preservative role in some foods. Bacteria produce antimicrobial compounds such as lactic acid, acetic acid and hydrogen peroxide as well as bacteriocins. Bacteriocins are antimicrobial proteins produced by bacteria to limit the growth and spread of other bacterial species (Cleveland et al 2001). Bacteriocins have been distinguished from antibiotics on the basis that they are ribosomally derived, i.e. proteins made within the bacteria, whereas historically antibiotics tend to be secondary metabolites that are excreted by microorganisms. The mode of action of bacteriocins is unclear, but evidence suggests LAB bactericins function through interference with the target bacterial cell wall or membrane structure (Cotter et al 2005). Currently, there is interest in the use of bacteriocins in food preservation against spoilage by bacterial species such as *Listeria monocytogenes* (Cotter et al 2005).

The widespread colonisation of LAB in foods already means that bacteriocins derived from them are likely to be safe for human consumption. Currently, nisin is the only bacteriocin that has widespread commercial success in food preservation (Cotter et al 2005). Nisin is produced by *Lactococcus lactis* and is commonly added to dairy products and canned foods because of its ability to prevent the growth of pathogens such as *L. monocytogenes* and other Gram-positive species.

Although some bacteriocins have proven effective, *L. monocytogenes* can become bacteriocin-resistant and so concerns have been raised over the development of such resistant strains in preserved foods (Bouttefroy and Millière 2000). The development of bacteriocin resistant bacteria compromises food preservation methods and could potentially lead to food spoilage and even food poisoning. The mechanism of bacteriocin resistance is under investigation but studies suggest that resistance is due to changes in bacterial cell surface properties (Hassan et al 2012).

As the number of effective antibiotics for human medicine dwindle, there has been interest in the development of new treatments. Interestingly, bacteriocins have proven to be a realistic alternative to antibiotics in a clinical setting with activity already seen against *Staphylococcus aureus*, *Staphylococcus pneumoniae* and *in vitro* against *Mycobacteria* spp (Goldstein et al 1998; De Kwaasteniet et al 2009; Carroll et al 2010). Although bacteriocins have been used in food preservation for the last 50 years, it cannot be assumed resistance to them will not arise through this method (Collins et al 2010b). In addition, it is known that several mechanisms in bacteria, which provide innate nisin resistance, also provide antibiotic resistance to numerous β-lactam antibiotics (Collins et al 2010a; Collins et al 2010b). There is currently insufficient evidence on whether widespread use of bacteriocins may promote cross-resistance to clinically significant antibiotics.

4.2 Ethanol Production

In theory, production of ethanol requires the use of a starch-based grain, water and yeast. However, in practice the fermentation process often gets contaminated by LABs, which convert sugars to lactic acid instead of ethanol. As a result antibiotics, such as erythromycin, have been added to the mix by the distilling industry (Luther, 2014). There were claims by ethanol producers that the antibiotics were rendered inactive after production and pose no threat. However, in 2008 the FDA tested wet and dry grains produced by distillers and confirmed the presence of two antibiotics, erythromycin and virginiamycin (Luther, 2014). Erythromycin is commonly used to treat several infections amongst human patients who are allergic to penicillin. Both antibiotics have also been used in livestock and poultry as a prophylactic and for treatment purposes (Olmsted 2009).

The grains left over from the production process form a nourishing feed called dried distillers grains with soluble (DDGS), which consists of protein, fibre and oil. This is sold as feed to farm animals, thus, presenting a path for unregulated exposure of animals to antibiotic residues. Therefore, the legislation to prevent the use of prophylactic antibiotics in animals in the EU may be bypassed by the unknowing administration of antibiotics to animals via imported DDGS feed. Antibiotics important to human health do not need to be used in ethanol production, as alternatives to using antibiotics exist, which are economical and effective. Buffered sodium chlorite can be used as well as enzymes isolated from hop extract, which show antimicrobial properties (Olmsted 2009).

4.3 Boat hull and oil paint

Fouling is the accumulation of unwanted material on a solid surface, which has detrimental effects on function. Boat hulls suffer from biofouling, a process by which an initial biofilm develops around the hull of a boat allowing for the attachment of larger organisms such as barnacles (Almeida et al 2007). The larger organisms reduce the efficiency of the boat to traverse through water swiftly leading to increased fuel costs. Anti-fouling paints were developed using the biocide tributyltin and this proved highly effective. However, this biocide was linked with shell deformations and reduced tissue growth in oysters, and the development of male sex organs in female *Nucella sp* (Peterson et al 1993; Evans et al 1995). Subsequently, a ban on the use of tributyltin on boats with hulls less than 25m in length was put in place (Champ, 2000). As a consequence boat owners began using the antibiotic tetracycline, which they obtained from a veterinarian, and began mixing it into anti-fouling paint; although the total scale of this misuse is not known (Peterson et al 1993). They hypothesised that antibiotics would reduce the build up of bacterial biofilms and in turn prevent the attachment of barnacles. The major concern with this is similar to that of aquaculture, whereby the presense of antibiotics may select resistant bacteria in the aquatic environment (e.g. tetracycline and chlortetracycline). In addition, fish are also treated for diseases such vibriosis (a disease caused by bacteria from the *Vibrio* genus) using tetracycline. Therefore, concern about this practice has been raised amongst the aquaculture industry. There may also be other ecological impacts such as in aquatic food chains, which could be disturbed by the use of these antibiotics.

It has been suggested that as tetracycline is a reactive compound, it will bind to magnesium and calcium in seawater rendering it biologically inactive (Peterson et al 1993). Furthermore, studies on antibiotic leaching and activation using seawater and high-pressure liquid chromatography have demonstrated that the concentrations of

active tetracycline leached are unlikely to cause toxicity to sea life in the marine environment (Peterson et al 1993). Active compounds also have a half-life of approximately 130 hours and so they were therefore deemed safe for use. Nonetheless, these studies do not take into account that leeched concentrations may select antibiotic-resistant bacteria. Some *tet* genes have proved transmissible in the aquatic environment and as such the advantage of acquiring these genes would seem a strong selective force (Zhang et al 2009). Before these agents can be classified as safe, studies need to be carried out on the effects of antibiotics on the hull surface prior to and post leaching and the development of antibiotic-resistant bacteria making up ship hull biofilms. This is particularly difficult to assess since the bacterial species that are responsible for the construction of the initial biofilm may drastically vary between areas due to local differences in pH, temperature and water composition. It is evident that with the ecosystem being so vast and varied, it is difficult to predict how even minute quantities of tetracycline will affect the environment. To assess its relative impact on the development of antibiotic resistance more information is required concerning the extent of tetracycline use on boat hulls.

5. Domestic uses

Triclosan is a synthetic compound marketed with antibacterial, antifungal and antiviral properties. It is found in numerous domestic products such as soaps, dishwashing liquids, deodorants, skincare products and oral care products. In addition, it has been incorporated into a number of solid products such as fabrics, cutting boards and children's toys (Schweizer 2001).

Triclosan has activity against Gram-positive and Gram-negative bacteria, being bacteriostatic at low concentrations and bactericidal at higher concentrations. Bacterial resistance to triclosan can be via several mechanisms. Triclosan is a FabI

inhibitor and mutations in *fab*I allow resistance. FabI homologues are present in numerous bacteria, some such as, FabK in *Bacillus* spp. and FabL in *Staphyloccus aureus* are resistant to triclosan or reversibly inhibited by triclosan, respectively (Sivaraman et al 2004). The experimental antibiotic diazoborine is also capable of targeting the FabI protein. This is concerning since there is similarity in the mode of action between diazoborine and isoniazid (a leading tuberculosis drug), with isoniazid targeting a structurally related protein to FabI called InhA. The high similarity raises concerns over triclosan selecting antibiotic resistance to isoniazid (McMurry et al 1998b). Furthermore, FabI inhibitors (e.g. diazborine) are being actively sought for use in human medicine, so triclosan use in the domestic setting is of concern. Efflux pumps that export antibiotics out of the bacterial cell also confer triclosan resistance in *E. coli, Salmonella* Typhimurium and *Pseudomonas aeruginosa* (McMurry et al 1998a; Chuanchuen 2001; Webber et al 2008). The development and selection of triclosan-resistant bacteria is of particular concern since several efflux pumps, many associated with triclosan resistance, also provide antibiotic resistance (Chuanchuen 2001; Piddock 2006b). Even at low expression levels, efflux pumps provide a selective advantage when under stressful conditions; this facilitates an increase in the mutant selection window and subsequently causes increased antibiotic resistance within the bacterial population. Therefore, although resistance to triclosan will impact upon its everyday use, the main concern to human health arises from the possibility of selecting triclosan-resistant bacteria that are cross resistant to drugs used in human medicine.

Class 1 integrons are mobile genetic elements that can convey both antibiotic and quaternary ammonium compound (QAC) resistance. QACs are detergents and antimicrobial compounds, which are used in several settings, including facial

cleansers, sun creams, hand sanitisers, building materials (for anti-fouling), healthcare surface sterilises and mouthwashes (Buffet-Bataillion et al 2012). With such an extensive and varied range of uses, concern has been raised about the use of these chemicals and their ability to increase the prevalence of antibiotic resistance genes in an environment. Environments that had been exposed to antibiotics and/or QACs have significantly higher levels of class 1 integrons suggesting that use of QACs allowed co-selection of antibiotic resistance genes, as the antibiotic resistance gene and the QAC resistance gene were co-located in the transmissible element (Gaze et al 2011). They also estimated that there are >1.5 x 10^{19} bacteria with mobile genetic elements with the potential to pass antibiotic resistance within UK soil (Gaze et al 2011). The use of detergents, heavy metals, biocides and other biologically active compounds can facilitate the dissemination of antibiotic resistance genes by increasing and selecting for mobile genetic elements (Gaze et al 2011).

The sheer quantity of antibiotic resistant genes in the environment is daunting, with one study finding 2895 putative antibiotic resistance genes out of all 8882 (Forsberg et al 2014). Most of these were unique DNA sequences and lacked homology to other proteins in databases. Many of the putative antibiotic resistance genes lacked an association with mobile genetic elements and by proxy, the ability for horizontal gene transfer (Forsberg et al 2014). This suggests that the bacterial community composition is important to the antibiotic resistome of the soil as biologically active compounds could select for specific species of bacteria in the environment, making these genes more available for capture by transmissible elements.

6. Pollution of the water supply and the environment by antibiotics and antibiotic resistant bacteria

Antibiotics are bioactive substances that may not be fully metabolised by the mammalian body. Even after brief exposure to medication, animals and humans excrete a large proportion of antibiotic metabolites and active substances. The proportion of antibiotics excreted relative to the dose varies with the antibiotic, whether it is orally or intravenously administered, what condition it is being prescribed for (e.g. if the condition has associated diarrhoea) and how well the animal is able to metabolise that drug. For example in animals, 80% of an initial tetracycline dose can be excreted via urine and faeces, while only an estimated 40% is for metronidazole (Kumar et al 2005). As a result large amounts of active antibiotic may be present in the urine and faeces of animals and people exposed to these drugs and subsequently enter the environment. In addition, antibiotic resistant bacteria can be produced in animals and people and released directly into the environment via urine and faeces. Furthermore, due to the slow degradation of some un-metabolized antibiotics in soil, it is possible that the continual addition of sewage as fertilizer to fields will increase local antibiotic concentrations and so select antibiotic resistant bacteria (Kümmerer 2003). This is important since the ng per ml concentration range of antibiotic is a sufficient selection force to select for antibiotic resistant bacteria (Gullberg et al 2011).

Antibiotic resistant bacteria, antibiotic resistance genes and un-metabolised antibiotics can also be leached into surface water, ground water and possibly drinking water distribution systems (Kümmerer 2003; Schwartz et al 2003). Although water treatment, such as chlorination, vastly reduces the number of bacteria in drinking water, the process may increase the antibiotic resistance of those bacteria that have survived the treatment process (Xi et al 2009). In some instances, tap water (drinking water) has been shown to have increased numbers of some antibiotic resistant bacteria

and antibiotic resistance genes in comparison to samples taken from finished water suggesting resistant bacteria may be selected for by chlorination and then grow on route to tap water outlets (Xi et al 2009). The reason for this chlorine-induced antibiotic resistance selection may be due to forcing bacteria to up-regulate mechanisms, such as efflux pumps, that are also used for antibiotic removal. There is growing concern over the spread of antibiotics and antibiotic resistant bacteria into the environment through water supplies (Figure 4). Clean water supplies are a major concern in cities in some countries, for instance 2/50 samples of water taken from public taps and 13/171 samples from waste seepage sites in New Delhi, India contained the metallo-beta-lactamase 1 (NDM-1) gene. This encodes an enzyme that confers resistance to beta-lactam antibiotics including carbapenems (Walsh et al 2011). The spread of this gene is unlikely to be the result of agricultural run off, and most likely is due to the contamination of the water supply by human faeces. The study also found 11 new species of bacteria harbouring NDM-1, including *Vibrio cholerae*. Access to clean water and good public health and sewage systems will minimise the spread of resistance genes. Furthermore, safe disposal of antibiotic waste will prevent the further spread of antibiotic resistant pathogens.

Biofilms develop in water systems and these are less susceptible to antibiotics in part due to higher bacterial densities, higher rates of conjugation and development of persisters (bacteria which enter a dormant, non-dividing state) (Watnick and Kolter 2000; Lewis 2010; Szabo & Minamyer, 2014). Therefore, it has been suggested that biofilms can increase the development of antibiotic resistant bacteria when under the selective pressure of antibiotics (Watnick and Kolter 2000; Lewis 2010). The *van*A, *mec*A and *amp*C genes found in wastewater biofilms in Mainz, Germany, were isolated from *enterococci*, *staphylococci* and Enterobacteriaceae, respectively

(Schwartz et al 2003). Furthermore, *van*A and *amp*C were amplified from drinking water biofilms (Schwartz et al 2003). Thus, water treatment such as chlorination does not completely kill the bacterial population and may instead promote the development of resistant for these strains (Xi et al 2009). Water systems have the potential to act as antibiotic resistance gene reservoirs and potentially provide a direct route to the human microflora.

Effluent discharge from wastewater treatment plants (WWTPs) can also be a driving force behind the development of antibiotic resistance in the environment. For example, it has been suggested that the isolation of resistance genes from domestic and wild animals may be due to direct intake of effluent water (Amos et al 2014a). It has been shown recently that there are significantly increased numbers of neomycin, amikacin and ciprofloxacin resistant bacteria downstream of WWTPs compared to upstream of the treatment plant (Amos et al 2014a). Some of the resistance genes identified were identical or similar to those found in pathogenic bacteria isolated from people.

Since at least the 1970s, it has been shown that antibiotic resistant bacteria are in UK rivers (Hughes and Meynell 1974). Clinically important extended-spectrum β-lactamase (ESBL) genes have been isolated from a UK river from which wastewater from a water treatment plant is pumped (Amos et al 2014b) and from the River Thames in London (Dhanji et al 2011). The River Thames has a large quantity of sewage introduced into it when sewage systems are unable to cope with excess surface water caused by heavy rain (Dhanji et al 2011). A significant increase in the numbers of third-generation cephalosporin resistant bacteria in river sediment downstream of the wastewater treatment plant was found compared to upstream of the treatment plant in both 2009 and 2011 (Amos et al 2014b). This suggests that the

wastewater treatment plant promotes the dissemination of resistance genes between bacteria. A carbapenem-resistant *E.coli* was also recently isolated from a UK river (Amos et al 2014b). During periods of heavy rainfall (high flow), there is a large increase in the number of faecal indicator organisms (FIOs) disseminating from farms, notably improved pastures, into rivers and coastal waters compared to base FIO export (Kay et al 2008). This is a likely source for the spread of antibiotic resistant bacteria and genes. Furthermore, coastal receiving waters pose a risk for human exposure to antibiotic resistant bacteria either directly (abstraction of drinking water and use of water for bathing) or indirectly (introduction of FIOs to shellfish intended for harvesting).

Pharmaceutical production plants are a likely contributor to antibiotic residues in the environment. A study that investigated the amount of pharmaceuticals in wastewater treatment plant effluent that serviced 90 pharmaceutical plants in India found ciprofloxacin levels up to 31 mg per litre and enrofloxacin at 780-900 μg per litre (Larsson et al 2007). This ciprofloxacin level is above that of the maximum therapeutic human plasma levels and relates to the plants releasing about 45 kg per day (Larsson et al 2007). It is likely as this effluent travels away from the wastewater treatment plant the antibiotic will become diluted exposing a large area of environment to the selective pressure of sub-MIC drug concentrations. Of further concern was that this antibiotic effluent was mixed with human sewage leading to direct exposure of human pathogens to large quantities of antibiotics (Larsson et al 2007). It is highly likely that antibiotic resistant bacteria are likely to emerge in a population conditioned to pharmaceutical plant discharge. A study has suggested pharmaceutical discharge as the source of single- and multiple- antibiotic resistance, including resistance to chloramphenicol, amoxicillin, oxytetracycline,

sulfamethoxazole and gentamicin, in *Acinetobacter* spp found downstream of a pharmaceutical plant (Guardabassi et al 1998). In addition pharmaceutical production is likely to be the cause of antibiotic contamination of surface, ground and drinking water in India, where unauthorised dumping of industrial material is a serious problem (Fick et al 2009). This occurrence presents a direct link for dissemination of antibiotic resistance through the food chain. More research is needed in this area as to obtain the extent of the role that pharmaceutical plants play in the development of antibiotic resistance.

Although many antibiotics are naturally occurring compounds and biodegradable, several are semi-synthetic or synthetic. For example, the synthetic antibiotic oxolinic acid takes 5 months to degrade by 20% compared to the complete degradation of ciprofloxacin within 3 months (Turiel et al 2005). This raises concerns over the effects of antibiotic compounds within the environment because long-term antibiotic activity will increase numbers of antibiotic resistant bacteria. Water supply pollution is a multifactor problem, with most of the processes highlighted within this report having the means to contaminate water supplies and spread resistance. Therefore, management of water systems originating from both clinical and non-clinical settings must include removal of both antibiotics and antibiotic-resistant bacteria.

7. Conclusion

The discovery of antibiotics is one of the most influential moments in human history. This can be seen through their widespread use in animal health, agriculture, industry and, most importantly, human medicine. This widespread use threatens the future effectiveness of these drugs in human medicine. It is evident that antibiotics are being misused and over-used, even in cases where cost efficient alternatives are available or no benefit is seen. Industrial practices using antibiotics are not sustainable and

alternatives should be researched and implemented with urgency. For the preservation of clinically significant antibiotics the use of antibiotics outside of human medicine must be minimised. Although it cannot be denied that the use of these drugs in human medicine needs to be addressed, attention to non-human uses of these drugs and effects of this use must also be thoroughly investigated and reduced wherever possible. This aspect is often overlooked but it is of utmost importance that awareness of this issue is raised to allow for the changes necessary to secure a safe future free of the fear of antibiotic-resistant bacterial infections. Antibiotic resistance must be viewed from the "One Health" prospective, not as a process that originates from only one source.

Recommendations

Animal Husbandry and Aquaculture

- A global ban on the use of all antibiotic growth promoters in animals.
- Increased monitoring of aquaculture and animal husbandry welfare practices so as to minimise the need for therapeutic drugs.
- To limit the need for therapeutic drugs, in veterinary medicine, there needs to be increased investment into research into minimising stress and disease experienced in aquaculture and farm animals.
- Consideration of withdrawal of certain antibiotics such as the cephalosporin class from therapeutic treatment of animals and reserve specific classes of antibiotics for human use only. The antibiotics to be withdrawn from animal use should be those that are capable of treating diseases with the highest mortality in people.
- Not to use any new classes of antibiotic in both animals and humans.
- To prevent dissemination of resistance genes between farms, aquaculture facilities should be positioned distant from wild fish populations.
- To prevent overuse of antibiotics, there should be increased research funding into disease diagnostic techniques for bacterial infections in animals and fish.
- Vaccination to become a primary means of disease prevention in aquaculture and animal husbandry globally. To facilitate this there needs to be increased funding for research into the development of new vaccine treatments.

Apiculture (bee-keepimg)

- Apiculture globally to follow the same guidelines as the EU (no antibiotics to be used for treatment, other than under MUMs, while instead, burning of the hives and sterilization of equipment should be made common practice when *P.*

larvae infection occurs). Subsidies may need to be available to help beekeepers with losses. Burning is a necessity due to the pathogens' ability to form spores, which are resistant to other modes of destruction.

Horticulture

- To minimise spread of antibiotic resistant pathogens on plants, ban antibiotic use in horticulture. To facilitate this there should be research into better containment systems and other means of control of plant pathogens for when outbreaks do occur.

Food Preservation

- To evaluate whether cross-resistance between bacteriocins and antibiotics used in human medicine is a likely outcome of their use, increased research is needed. If proved to be the case, bacteriocins should not be used in any setting, unless further research provides an opportunity for their use in medical treatment.

Ethanol Production

- Increased monitoring of antibiotic residues in ethanol production and appropriate penalties instituted when antibiotics are found.

Boat and Hull Paint

- Increased monitoring of antibiotic residues on boat hull surfaces and if residues are found, appropriate penalties should be put in place to act as a deterrent.

Domestic Use

- Triclosan to be withdrawn globally from all domestic applications.
- Better disposal of triclosan waste so as to minimise its prevalence in the environment.

Water Supply and Pollution

- To prevent antibiotic-, and bacterial-, contaminated water leeching into the environment, greater investment into sewage and water distribution systems, especially in low income countries.
- To adequately remove antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes from wastewater treatment plant effluent and drinking water sources. This may require research into the best method by which this can be achieved.

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Legends to Figures

Figure 1. The number of new antibiotics that have entered the pharmaceutical market (blue bars) and the total UK and International publications on antibiotic resistance (red bars), reproduced courtesy of Bragginton and Piddock (2014).

Figure 2. Growth rates of susceptible (blue line) and resistant (red line) bacterial populations as a function of antibiotic concentration. MIC (minimum inhibitory concentration) marks the concentration at which a strain of bacteria will no longer grow. MICres is the MIC of antibiotic for a resistant bacterium and is the ideal therapeutic concentration. MSC (minimal selective concentration) is the concentration of antibiotic at which growth of resistant bacteria will be higher then that of susceptible strains, therefore causing preferential selection of antibiotic-resistant strains of bacteria (Gullberg et al 2011).

Figure 3. The economic impact of the ban of antibiotic growth promoters in Denmark. Graph A demonstrates the quantity of broilers produced per square metre of land before and after the ban took place. Graph B demonstrates death amongst broilers grown on farms in Denmark before and after the ban (Emborg et al 2002).

Figure 4 Adapted from Linton, 1977 and Irwin, 2011. The routes of transfer of antibiotic compounds and antibiotic resistant bacteria into the environment

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Table 1. Antibiotic classes and global uses (both past and present). Adapted and extended from Kemper, 2008.

CLASS	COMPOUNDS	USED IN
Aminoglycosides	Apramycin	Cattle, pigs, poultry, lambs, rabbits
	Gentamicin	Animals, fish, horticulture, humans
	Kanamycin	Animals, fish, humans
	Neomycin	Animals, fish, humans
	Sisomicin	Humans
	Spectinomycin	Cattle, pigs, poultry, humans, sheep
	Streptomycin	Animals, fish, horticulture, humans

B-lactams: Penicillins	Amoxicillin	Animals, fish, humans
	Ampicillin	Animals, fish, humans
	Azlocillin	Humans
	Benzylpenicillin	Animals, humans
	Cloxacillin	Animals, humans
	Dicloxacillin	Animals, humans
	Flucloxacillin	Humans
	Methicillin	Humans
	Mezlocillin	Humans
	Nafcillin	Cattle, humans
	Oxacillin	Humans
	Piperacillin	Humans
	Phenoxymethylpenicillin	Animals, humans
B-lactams: Cephalosporins	Cephalexin	Animals, humans
	Cephalothin	Humans
	Cephazolin	Animals, humans
	Ceftiofur	Animals
	Cephotaxime	Humans
	Cefotiam	Humans
	Cefquinome	Cattle, poultry, pigs, sheep
Amphenicol	Chloramphenicol	Animals, fish, humans
	Thiamphenicol	Animals, humans
	Florfenicol	Animals, fish
Fluoroquinolones	Ciprofloxacin	Animals, fish, humans
	Enrofloxacin	Animals, fish
	Marbofloxacin	Animals
	Flumequine	Animals, fish, humans
	Ofloxacin	Animals, fish, humans
Macrolides	Azithromycin	Humans
	Carbomycin	Humans
	Clarithromycin	Horses, humans
	Erythromycin	Animals, Ethanol Production, fish, humans
	Roxithromycin	Humans
	Spiramycin	Animals, humans
	Tylosin	Animals
	Vancomycin	Humans
	Avoparcin	Animals
Sulphonamides	Sulphanilamide	Humans

	Sulphadimethoxine	Animals, fish, humans
	Sulphadimidine	Animals, fish
	Sulphamethoxazole	Animals, fish, humans
	Sulphapyridine	Cattle, humans
	Sulphathiazole	Animals, fish, humans
Dihydrofolate Reductase Inhibitor	Trimethoprim	Animals, fish, humans
Tetracyclines	Chlortetracycline	Animals, fish
	Doxycycline	Animals, fish, humans
	Oxytetracycline	Animals, fish, bees, horticulture
	Tetracycline	Animals, fish, humans, anti-fouling paints
Steptogramin	Virginiamycin	Animals, ethanol production

A, Animals, category title for when several species of food-producing mammalian and poultry species and/or domestic pets are treated with the antibiotic.

Figure 1. The number of new antibiotics that have entered the pharmaceutical market (blue bars) and the total UK and International publications on antibiotic resistance (red bars). Reproduced courtesy of Bragginton and Piddock (2014).

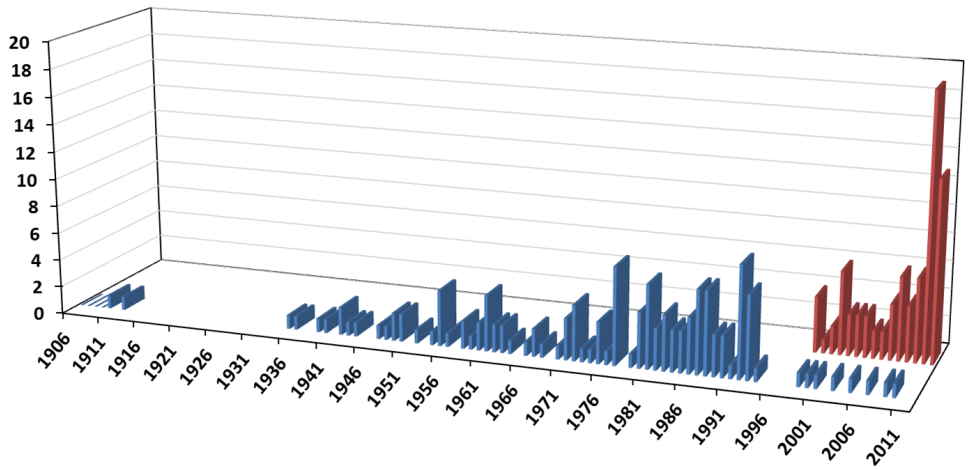


Figure 2. Growth rates of susceptible (blue line) and resistant (red line) bacterial populations as a function of antibiotic concentration. MIC (minimum inhibitory concentration) marks the concentration at which a strain of bacteria will no longer grow. MICres is the MIC of antibiotic for a resistant bacterium and is the ideal therapeutic concentration. MSC (minimal selective concentration) is the concentration of antibiotic at which growth of resistant bacteria will be higher then that of susceptible strains, therefore causing preferential selection of antibiotic-resistant strains of bacteria (reproduced from Gullberg et al 2011).

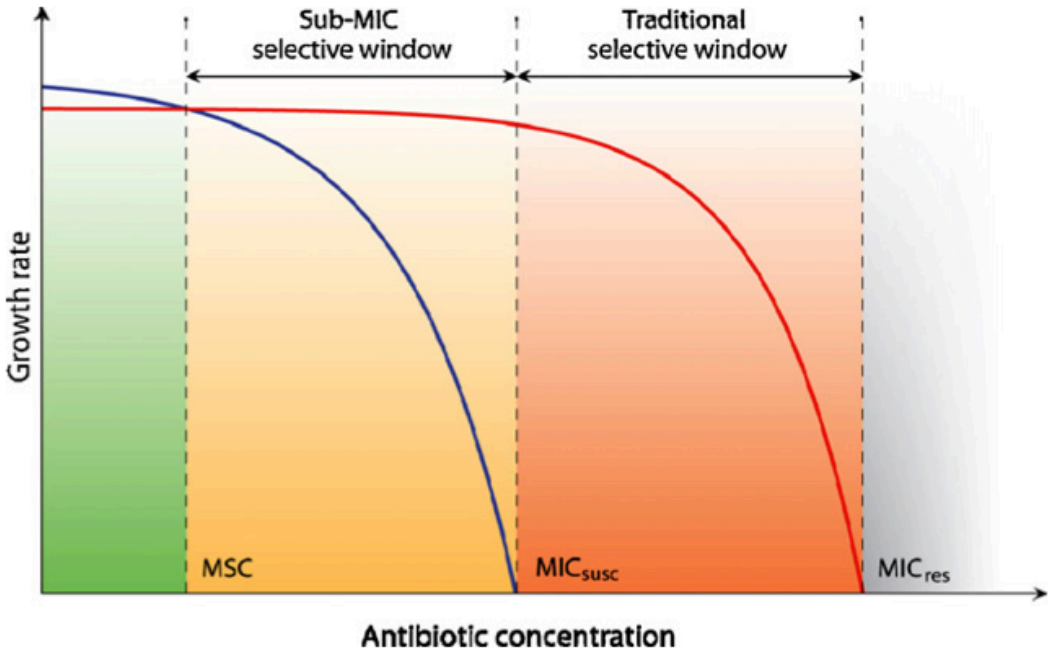
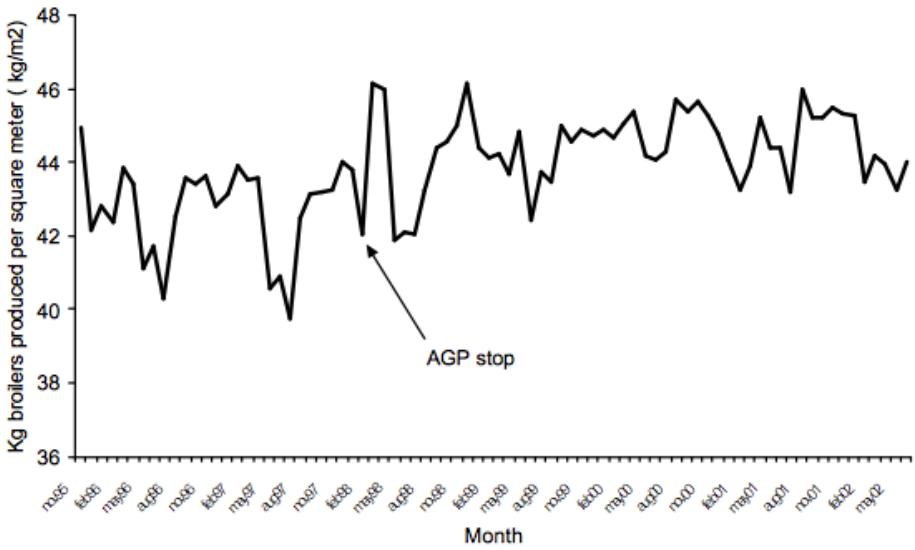


Figure 3. The economical impact of the ban of antibiotic growth promoters in Denmark. Graph A demonstrates the quantity of broilers produced per square metre of land before and after the ban took place. Graph B demonstrates the mean month percent death amongst broilers grown on farms in Denmark before and after the ban (reproduced from Emborg et al 2002)

A)



B)

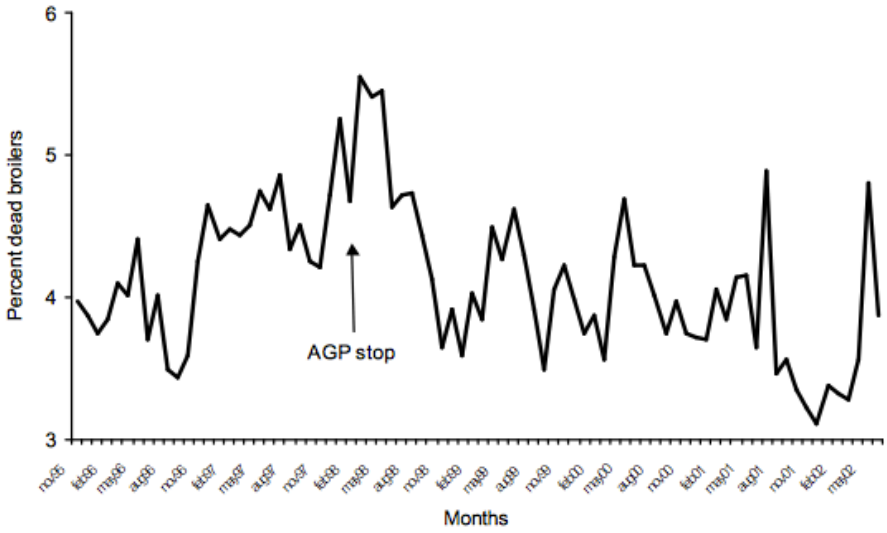
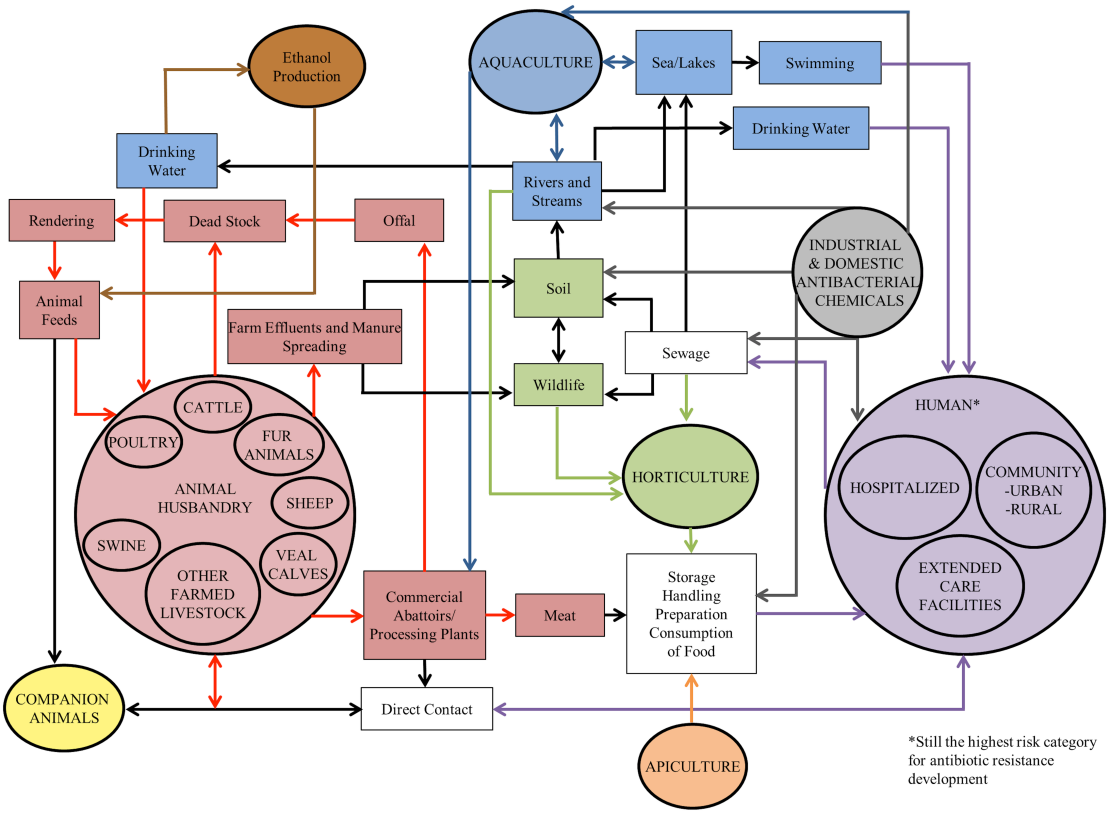



Figure 4. Adapted from Linton, 1977 and Irwin, 2011. The routes of transfer of antibiotic compounds and antibiotic resistant bacteria into the environment.