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# Environmental Impacts of Antibiotic Use in the Animal Production Industry

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## Introduction

Antibiotics are routinely used in the livestock industry to treat and prevent disease. In addition, subtherapeutic concentrations of antimicrobials are commonly added to animal feed and/or drinking water sources as growth promoters, and have been a regular part of swine production since the early 1950s (Cromwell, 2001). When used in this manner, antibiotics can select for resistant bacteria in the gastrointestinal tract of production animals, providing a potential reservoir for dissemination of drug-resistant bacteria into other animals, humans and the environment (Andremont, 2003). Bacteria have been shown to readily exchange genetic information in nature, permitting the transfer of different resistance mechanisms already present in the environment from one bacterium to another (Stewart, 1989; Amábile-Cuevas and Chicurel, 1992; Salyers and Amábile-Cuevas, 1997). Transfer of resistance genes from faecal organisms to indigenous soil and water bacteria may occur (Lorenz and Wackernagel, 1994; Daane et al., 1996; DiGiovanni et al., 1996; Nielsen et al., 2000), and because native populations are generally

better adapted for survival in aquatic or terrestrial ecosystems, persistence of resistance traits may be likely in natural environments once they are acquired. Antibiotic resistance has received considerable attention due to the problem of emergence and rapid expansion of antibiotic-resistant pathogenic bacteria.

The potential for long-term, cumulative inputs of antibiotics and correspondingly, their potential effects on acquisition and maintenance of antibiotic resistance mechanisms in bacteria, collectively suggest a degree of impact on the occurrence, persistence and mobility of resistance genes in natural environments. A number of reviews, reports and opinion papers have emerged to address the possible link between antibiotic use and the impact on antibiotic resistance development (e.g. Gustafson and Bowen, 1997; Khachatourians, 1998; USGAO, 1999; Isaacson and Torrence, 2002; Séveno et al., 2002; Kümmerer, 2004). These papers have highlighted various issues related to antibiotic use in agriculture, often focusing on the link to emerging antibiotic-resistant bacteria, gene transfer mechanisms and consequent risks to human and animal health.

Table 29.1. Survey of the most commonly used antibiotics in animal production in the United States. Source: AHI, 2001.

Antibiotic class	Amount (millions of kg)
Ionophores/arsenicals	3.53
Tetracyclines	3.25
Other antibiotics - includes macrolides, lincosamides, polypeptides, streptogramins, cephalosporins	1.94
Penicillins	0.823
Sulphonamides	0.269
Aminoglycosides	0.117
Fluoroquinolones	0.016

In the following review, we provide an overview of antibiotic use and animal waste management in the United States, the dissemination and fate of antibiotic residues, and the environmental persistence, mobility and transferability of antibiotic resistance determinants and their bacterial hosts in the context of environmental conditions encountered during the course of manure storage and in natural soil and water environments following the practice of land application of animal waste. This description provides a background to determining the true ecological impact of antibiotics and antibiotic resistance genes in natural environments.

### Antibiotic Use in Animal Agriculture

In commercial livestock production, antibiotics are used: 1) therapeutically to treat existing disease conditions, 2) prophylactically at subtherapeutic doses to mitigate infection by bacterial pathogens of livestock animals undergoing high stress situations, and 3) subtherapeutically to enhance growth. A survey of members of the Animal Health Institute reported that overall, the ionophores/arsenicals and tetracycline classes of antibiotics were the most commonly used antimicrobials in animal production (AHI, 2001; Table 29.1). Among the antibiotics commonly used in swine, poultry and beef cattle, penicillins, macrolides, polypeptides, streptogramins and tetracyclines are used not only for purposes of disease treatment and disease prevention, but also for growth promotion (Table 29.2).

Table 29.2. Antibiotics commonly used in swine, poultry and beef cattle production industries in the United States. Source: USGAO, 1999.

Antibiotic class (examples)	Industry
Aminoglycosides (gentamicin, neomycin streptomycin)	Swine, Poultry, Beef Cattle
β-Lactams (penicillins, ceftiofur)	Swine, Poultry, Beef Cattle
Chloramphenicol (florfenicol)	Beef Cattle
Ionophores (monensin, salinomycin, semduramicin, lasalocid)	Poultry, Beef Cattle
Lincosamides (lincomycin)	Swine, Poultry
Macrolides (erythromycin, tilmicosin, tylosin)	Swine, Poultry, Beef Cattle
Polypeptides (bacitracin)	Swine, Poultry
Quinolones (Fluoroquinolones) (sarafloxacin, enrofloxacin)	Poultry, Beef Cattle
Streptogramins (virginiamycin)	Swine, Poultry, Beef Cattle
Sulphonamides (sulphadimethoxine, sulphamethazine, sulphisoxazole)	Swine, Poultry, Beef Cattle
Tetracyclines (chlortetracycline, oxytetracycline, tetracycline)	Swine, Poultry, Beef Cattle
<b>Others</b>	
Bambermycin	Swine, Poultry, Beef Cattle
Carbadox	Swine
Novobiocin	Poultry
Spectinomycin	Swine, Poultry

Other classes, such as quinolones, lincosamides and aminoglycosides, are primarily used only in disease treatment or prevention. The Animal Health Institute (AHI, 2001) and Union of Concerned Scientists (UCS, 2001) recently reported two different estimates of antibiotic usage in agriculture.

The AHI reported a total of 9.2 million kg of antibiotics sold for all animal use in 1999. Of these 9.2 million kg, 8 million kg were used for treatment and prevention of disease and only 1.3 million kg for improving feed efficiency and enhancing growth. In contrast, the UCS reported that 11.2 million kg of antibiotics were used for non-therapeutic purposes alone in the swine, poultry and cattle industries. According to the UCS report, livestock use accounts for the major share of total antimicrobials used in the United States, estimated at 22.7 million kg annually, based on extrapolation from a 1989 Institute of Medicine report (IOM, 1989).

### Management of Animal Waste from Production Agriculture

Historically, until the mid- to late 1970s, livestock operations were usually part of larger integrated farming operations that produced crops. Swine production, in particular, has seen a trend towards specialised large production facilities. Over the last 25 years, swine production has largely shifted from such integrated farming systems to concentrated animal feeding operations (CAFOs) that may house thousands of animals. In 1984, there were approximately 690,000 US producers producing 9.1 billion kg of pork. By 2000, about 95,000 producers were producing 11.8 billion kg of pork (USDA NASS, 2002). Due to geographical patterns of feed grain production and other market forces, CAFOs have become concentrated in certain geographical regions in the US, primarily North Carolina and the Midwest. US Department of Agriculture surveys performed in 2000 found that 28.3% of swine facilities were located within half a mile of another swine production site and 53.9% were within one mile of another site (USDA, 2001a, 2001b).

Under the earlier integrated system of production, producers typically owned large tracts of land necessary for agronomic activity. Waste and effluent from a modest number of animals was applied rotationally over different fields, effectively diluting nutrients and recycling waste for fertiliser use. Swine each typically produce approx. 1.5 tonnes of fresh manure in the 5-6 months it takes to grow them to a market weight of 114 kg (Richert et al., 1995). The National Agricultural Statistics Service (NASS) estimated that in 2002, 185 million head of swine were sold in the US, generating approx.  $2.8 \times 10^8$  tonnes of fresh manure annually. With the advent of CAFOs, large quantities of waste are concentrated in a single location and/or region, and producers may only own sufficient land to site their facilities.

The most common method to dispose of swine effluent in the United States is through land application, where application of liquid manure at agronomic rates can produce crop yields that equal those obtained with chemical fertilisers (Schmitt et al., 1995). In order to utilise and dispose of the manure effluent, CAFO operators often contract with neighbouring growers to apply effluent to their land or apply it to land surrounding the facility. Because it is

costly to transport liquid effluent any great distance, there is an incentive to apply effluent as close to the source as possible. In the US the crop cycles coincide with seasonal cycles, with the application of manure occurring between crop cycles. For many locations, manure is stored for six months to one year before being applied to crop fields as fertiliser. Effluent differs from fresh manure in that it has a much greater water volume. Fresh swine waste contains approximately 10% solids, while deep pit effluents are 4-8% solids and lagoon effluents less than 0.5-1% (Fulhage and Post, 2005). O'Dell et al. (1995) found that the solids content ranged from 4-10 g/L in 18 separate tank loads of swine effluent that had been agitated for 24 h prior to application, suggesting effluent application rates can be highly variable.

Confinement livestock production, especially large animal facilities, is increasingly a source of surface water and groundwater contamination. The widespread practice of land application prompted the Environmental Protection Agency (EPA) in the 1990s to require nutrient management plans for CAFOs. Initially, nutrient management plans were nitrogen-based, requiring manure to be applied at a rate that would not exceed crop nitrogen requirements. Swine manure, however, has a high phosphorus content relative to nitrogen content; as excreted, swine manure contains a  $P_2O_5:N$  ratio of approximately 0.86:1 (LPES, 2005). Applying effluent to meet the nitrogen requirements of a crop often leads to a build-up of phosphorus in the soil, in some instances to values in excess of 2,000 mg/kg of total soil phosphorus (Lehmann et al., 2005).

The three primary methods used to apply effluent are: 1) surface application, 2) surface application followed by incorporation, and 3) direct soil injection. One primary reason to incorporate surface-applied effluent is to limit the loss of nitrogen by at least 50% compared with surface application alone (Rotz, 2004). Other reasons include odour reduction and minimisation of surface runoff. The preferred method of application from a nutrient management standpoint is deep injection into the soil, which eliminates the nitrogen losses associated with other methods, reduces odour and virtually eliminates the possibility of surface runoff.

Because surface application has been associated with nitrogen losses, it is often considered 'environmentally

unfriendly', yet it has merits as a method of managing pathogen loads. Hutchison et al. (2004) reported that the mean D-value, or time needed to reduce the variable being measured by one order of magnitude, for four zoonotic pathogens, *Salmonella* sp., *E. coli* 0157, *Listeria* sp. and *Campylobacter* sp., was 1.42 days for unincorporated pig slurry and 2.48 days for slurry incorporated immediately after application. These pathogens also declined at similar rates regardless of season (summer versus winter). Desiccation may be an important factor in population decline because more intense UV radiation in the summer would be expected to accelerate cell mortality (Booth et al., 2001; Hoerter et al., 2005). A significant rainfall event immediately following surface application of effluent would likely result in vertical movement of bacteria and mobile compounds into the soil profile as well as off-site movement due to surface runoff (Saini et al., 2003). Surface applications to frozen soil are usually avoided because of the likelihood of significant runoff.

### Entry of Antibiotics into the Environment

Antibiotics used in animal agriculture can enter the environment via a number of routes, including the drug manufacturing process, disposal of unused drugs and containers, and through the use and application of waste material containing the drugs. The excretion of waste products by grazing animals, atmospheric dispersal of feed and manure dust containing antibiotics, and the incidental release of products from spills or discharges are also potential pathways of antibiotic residue entry into the environment.

Many antibiotics are not completely absorbed in the gut, resulting in the excretion of the parent compound and its breakdown metabolites (Feinman and Matheson, 1978; Halling-Sørensen et al., 1998; Boxall et al., 2004). Elmund et al. (1971) estimated that as much as 75% of the antibiotics administered to feedlot animals could be excreted into the environment. Feinman and Matheson (1978) suggested that about 25% of the oral dose of tetracycline is excreted in faeces and another 50-60% is excreted unchanged or as an active metabolite in urine.

Oral administration of the macrolide tylosin resulted in a maximum of 67% of the antibiotic excreted, mainly in the faeces.

The practice of land application of livestock manure provides large-scale areas for introduction of antibiotics into the environment. Once released into the environment, antibiotics can be transported either in a dissolved phase or (ad)sorbed to colloids or soil particles into surface water and groundwater (Campagnolo et al., 2002; Kolpin et al., 2002; Yang and Carlson, 2003; Krapac et al., 2004). Manure and waste slurries potentially contain significant amounts of antibiotics and their presence can persist in soil after land application (Donohoe, 1984; Gavalchin and Katz, 1994).

### Chemical Characteristics of Antibiotics and Behaviour in Soil and Water

Veterinary antibiotics comprise a group of organic compounds that have a wide variety of functional groups that affect their chemical properties. The octanol-water partition coefficient ( $K_{ow}$ ) is used as a general measure of hydrophobicity, and most antibiotics have  $\log K_{ow}$  values less than 5, indicating that they are relatively non-hydrophobic (Tolls, 2001). In addition, the water solubility for many antibiotics exceeds 1 g/L, suggesting that they are relatively hydrophilic. Tolls (2001) and Boxall et al. (2004) compiled sorption coefficients ( $K_d$ ) for a variety of antibiotics, soils and soil components measured over the course of many studies. Based on  $K_d$  values, antibiotics exhibit a range of affinities for the solid phase ( $K_d$  0.2-6,000 L/kg), with consequent effects on their mobility in the environment. Estimations of antibiotic organic carbon-normalised sorption coefficients ( $K_{oc}$ ) made by using a compound's octanol-water partition coefficient ( $K_{ow}$ ) generally results in underestimates of the  $K_{oc}$  value, suggesting that mechanisms other than hydrophobic partitioning occur. Cation exchange, surface complexation and hydrogen bonding are included as likely mechanisms for antibiotic sorption to soils. Many of the acid dissociation constants ( $pK_a$ ) for antibiotics are in the range of soil pH values, such that the protonation state of these compounds depends on the pH of the soil solution (Tolls, 2001).

Studies have shown that under a broad range of environmental conditions, tetracyclines (tetracycline, chlortetracycline and oxytetracycline) can adsorb strongly to clays (Pinck et al., 1961a, 1961b; Sithole and Guy, 1987a, 1987b; Allaire et al., 2006), soil (Krapac et al., 2004) and sediments (Rabolle and Spliid, 2000). Sorption of chlortetracycline also occurs rapidly in sandy loam soil (Allaire et al., 2006). Macrolides such as tylosin have a weaker tendency to sorb to soil materials (Rabolle and Spliid, 2000), although a sorption kinetic study showed that 95% of tylosin was sorbed within 3 h in both sandy loam and clay soils (Allaire et al., 2006). Sulphonamides exhibit weak sorption to soil, and are probably the most mobile of the antibiotics (Tolls, 2001). Pinck et al. (1962) determined that two macrolide antibiotics (carbomycin and erythromycin) sorbed significantly (231-263 mg/g) to montmorillonite and to a much lesser extent (0-39 mg/g) to vermiculite, illite and kaolinite. In a review on the fate of antibiotics in the environment Huang et al. (2001) concluded that there was little information on the sorption of aminoglycoside and beta-lactam antibiotics. Because aminoglycosides can be protonated under acidic conditions, they could be sorbed to clay minerals under certain conditions, while  $\beta$ -lactams are highly polar compounds and would not be expected to sorb readily to soil components. Because of the strong sorption of the tetracycline and macrolide antibiotics, their mobility in the environment may be facilitated by transport with manure and soil colloidal material (Kolz et al., 2005a). Interestingly, although most antibiotics do not require metal ion coordination to exert biological action, other compounds such as bacitracin, streptonigrin, bleomycin and tetracycline have prerequisites for binding of metals ions to function properly (Ming, 2003). Sorption of these drug compounds in clays, where intercalation of metal complexes occur, may provide suitable conditions for the drug to exert a biological effect.

### Mechanisms of Antibiotic Degradation

Because antibiotics are generally introduced from livestock operations via water (effluent) into the environment, hydrolysis can be an important degradation path-

way.  $\beta$ -Lactams, macrolides, and sulphonamides appear to be the most susceptible classes of antibiotics to hydrolysis (Huang et al., 2001). At near neutral pH, tylosin A was found to have a hydrolysis half-life of 300 to 500 hours at 60°C (Paesen, 1995). At more environmentally relevant temperatures, these half-lives are expected to be longer. Doi and Stoskopf (2000) determined that under relatively high temperatures (43°C) the half-life of oxytetracycline in deionised water was 0.26 days, but was relatively stable at 4°C.  $\beta$ -Lactams are rapidly hydrolysed under mild acidic and basic conditions (Hou and Poole, 1969; Huang et al., 2001). Photolysis can be another abiotic transformation process affecting antibiotics introduced into the environment. The photodegradation of antibiotics in soil can occur at the soil-atmosphere interface and at the surface of liquid manure. Soils can provide a much different photodegradation environment than aqueous solutions and transformation rates can vary significantly in soils compared with those in water (Balmer et al., 2000). Quinolones and tetracyclines are susceptible to photodegradation (Huang et al., 2001), and photodegradation of oxytetracycline is three times more rapid under light than dark conditions (Doi and Stoskopf, 2000). Halling-Sørensen (2000) suggested that tylosin might be resistant to photolysis because it has only limited light absorbance in the visible spectrum, and Boxall et al. (2004) determined that sulphonamides would not be readily photodegraded. Beausse (2004) concluded that photodecomposition of antibiotics under field conditions was negligible when compared with other abiotic processes.

Limited numbers of studies have been conducted to assess the biodegradation of antibiotics. Biodegradation of organic compounds by microorganisms in soil is dependent in part on factors such as temperature, concentration, bioavailability, time of exposure, availability of other nutrients and the enzymatic capabilities of the extant microbial population. Aerobic processes have been the primary focus of such studies, and little attention has been devoted to anaerobic processes, the latter being of significance in the soil subsurface and microzones.

Depending on test conditions, biodegradation half-lives of organic compounds can widely vary. Studies using standard laboratory test assays have demonstrated limited or no degradation of antibiotics such as metro-

Table 29.3. Persistence of antibiotics in manure (modified from Boxall et al., 2004).

Antibiotic class	Half-life (d)
Aminoglycosides	30
β-Lactams	5
Macrolides	<2-21
Quinolones	100
Sulphonamides	<8-30
Tetracyclines	100

nidazole and oxytetracycline (Jacobsen and Berglund, 1988; Samuelsen et al., 1994; Kümmerer et al., 2000). In another study of 18 antibiotics tested, none were found to be readily biodegraded, although some activity occurred when additional nutrient supplement was made (Alexy et al., 2004). Penicillin G was found to be readily biodegradable along with some biodegradation of amoxicillin, imipenem and nystatin (Gartiser et al., 2007a; 2007b). A study of aquaculture sediments showed bacterial mineralisation of erythromycin A (Kim et al., 2004). Inherent to the process of biodegradation, the toxic effects of antibiotics on the resident bacteria have also been demonstrated. A range of antibiotic concentrations were found to inhibit activated sludge in wastewater treatment (Alexy et al., 2004; Gartiser et al., 2007a), but the exact effects of antibiotic entry in natural environments on microbial populations resident to these systems are not yet known.

Another biological mechanism of antibiotic fate, plant uptake and bioaccumulation of antibiotics, has received considerable interest due to issues of food safety and human health. A number of studies have shown this mechanism to occur with a variety of plant species (e.g. ; Kumar et al., 2005; Boxall et al., 2006; Dolliver et al., 2007), and while significant, discussion of these processes is outside of the scope of this review.

### Persistence of Antibiotics in Manure

Antibiotics excreted from animals are often concentrated in the solid phase because of sorption dynamics (Tolls, 2001; Loke et al., 2002; Kolz et al., 2005a, 2005b). Half-lives that have been reported for a variety of antibiotic

Table 29.4. Antibiotic concentrations detected in manure from swine and poultry lagoons.

Antibiotic	Concentration	Reference
Lincomycin	2.5-240 (µg/L)	Campagnolo et al., 2002
Chlortetracycline	68-1000 (µg/L) 0.1 (mg/kg) <0.5-1.0 (mg/kg)	Campagnolo et al., 2002 Hamscher et al., 2002 Hamscher et al., 2005
Tetracycline/ Oxytetracycline	25-410 (µg/L) 4.0 (mg/kg) 14.1-41.2 (mg/kg)	Campagnolo et al., 2002 Hamscher et al., 2002 Hamscher et al., 2005
Sulphamethazine	2.5-380 (µg/L) 0.13-8.7 (mg/kg) 0.2-7.2 (mg/kg)	Campagnolo et al., 2002 Haller et al., 2002 Hamscher et al., 2005
Sulphadimethoxine	2.5 (µg/L)	Campagnolo et al., 2002
Erythromycin	2.5 (µg/L)	Campagnolo et al., 2002
Penicillin G	2.1-3.5 (µg/L)	Campagnolo et al., 2002

classes in manure (Boxall et al., 2004) (Table 29.3) were less than the anticipated storage period of manure, suggesting the possibility that significant degradation of the parent compounds might occur prior to land application.

Quinolones and tetracyclines were the most persistent, with half-lives approaching 100 d. Kolz et al. (2005b) determined that 90% of tylosin, tylosin B and tylosin D was lost within 30 to 130 h in anaerobic manure slurries at 22°C. In aerobic manure slurries, 90% of tylosin was lost in 12 to 26 h. Although biodegradation and abiotic degradation occurred, the primary mechanism for tylosin loss was sorption to manure solids (Kolz et al., 2005a, 2005b). Residual tylosin and its breakdown product, dihydrodesmycosin, were also detected in the slurries after eight months. In several studies, tetracycline concentrations were found to be generally higher than those of macrolides, β-lactams and sulphonamides (Table 29.4). Tetracycline concentrations in some swine lagoons were as great as 1 mg/L (Campagnolo et al., 2002). Gavalchin and Katz (1994) determined the persistence of seven antibiotics in a soil-faeces matrix under laboratory conditions and found that the order of persistence was chlortetracycline > bacitracin > erythromycin > streptomycin > bambarmycin > tylosin > penicillin with regard to their detection in the soil. The application of manure to agricultural fields also likely introduces breakdown products into the environment along with the parent compound, but persistence data for degradation products were not found in the literature reviewed.

### Persistence of Antibiotics in Soil and Water

Until recently, information regarding the occurrence, fate and transport of antibiotics under field conditions has been limited. Spatiotemporal ‘hotspots’ of bacteria and antibiotic residues are likely to occur in effluent-applied soil due to the variable solids content of the waste effluent, soil characteristics and the frequency and timing of application. In a sandy soil that had repeated manure applications, tetracycline and chlortetracycline were detected down to a depth of 30 cm (Hamscher et al., 2002, 2005). The highest tetracycline and chlortetracycline concentrations, 198 and 7.3 µg/kg, respectively, were detected at soil depths of 10-20 cm and 20-30 cm, respectively. Sulphamethazine was generally not detected in soil samples, but was detected in groundwater collected at a depth of 1.4 m. Oxytetracycline, sulphadiazine, sulphathiazole, sulphamerazine, sulphamethoxypyridazine, sulphamethoxazole, sulphadimethoxine and tylosin were not detected in any soil or groundwater samples. While it appeared some of the tetracyclines could accumulate in soil, none of the antibiotics from the study were detected at soil depths greater than 30 cm and only sulphamethazine was detected in groundwater, suggesting limited transport even in highly porous sandy soils.

In a field study with clay loam soil that received swine manure spiked with the sulphonamide sulphachlorpyridazine (SCP), the antibiotic was found to be mobile and readily entered the field drain, with a maximum concentration of 590 µg/L detected seven days after manure application (Boxall et al., 2002). In the same study conducted with sandy loam field soil, SCP concentrations in soil pore water were significantly lower (max. concentration 0.78 µg/L) than for the field with clay loam, and contrasted with laboratory sorption studies that predicted larger soil water concentrations. The lower concentrations detected in the field samples were hypothesised to be the result of SCP degradation. In another soil transport study, SCP and oxytetracycline (OTC) were detected in soil at concentrations up to 365 and 1691 µg/kg, respectively (Kay et al., 2004). Similar to other investigations, these compounds were not detected below a depth of about 37 cm. SCP and OTC were detected in tile drainage at peak concentrations of 613 and 36 µg/L, respectively. Only 0.004% of the OTC

that was applied was in the particulate phase, and 23% of OTC moved to tile drainage. The investigators concluded that the antibiotics behaved similarly to pesticides under field conditions, and that tile drainage may be a significant route for these compounds to migrate to surface waters. The manure in this study was surface-applied without incorporation into the soil and the authors suggested that tillage prior to or during manure application might limit transport of antibiotics. In a later study by the same authors, swine manure spiked with SCP, OTC and tylosin was surface-applied to wheat stubble in a clay loam soil and mass recovery of SCP and OTC lost in surface runoff was 0.42 and 0.07%, respectively (Kay et al., 2005). While surface runoff did not appear to be a significant transport loss, the authors suggested that incorporation of manure into the soil would further limit loss from the soil. Tylosin was not detected in any samples, suggesting its rapid degradation in the manure and supporting previous evidence that macrolides may more readily undergo microbial degradation processes. In a study where swine manure was spiked with sulphadiazine and sulphathiazone and irrigated on to grassland, less than 5% of sulphonamide applied was lost to runoff (Burkhardt et al., 2005). The sulphonamide losses were 10 to 40 times greater on the manured plots when compared with control plots, the latter receiving only aqueous solutions of the compounds. The authors concluded that the manure formed a seal at the soil surface, creating conditions for more runoff. In addition, the high manure pH may have caused deprotonation of the sulphonamides, resulting in decreased sorption to the soil. These results suggest that repeated surface application of manure may yield a higher likelihood of runoff situations.

While detection of antibiotic residues poses a challenge in any environmental matrix, detection of low levels of compounds, particularly in natural waterways, is highly challenging. The US Geological Survey (USGS) has a comprehensive stream-monitoring network throughout the US and has improved detection of compounds by developing state-of-the-art analytical techniques such as LC-MS-MS. A recent study by the USGS (Kolpin et al., 2002) conducted a reconnaissance of the occurrence of pharmaceuticals, hormones and other organic wastewater contaminants in water resources. In 139 streams sampled across 30 states during 1999 and 2000, a number of antibiotics occurred in appreciable amounts (Table 29.5).

Carbodox, doxycycline, enrofloxacin, sarafloxacin, sulphachlorpyridazine, sulphamerazine, sulphathiazole and virginiamycin were not detected in any samples. Many of the compounds that were not detected are commonly used in livestock operations, suggesting limited transport of these compounds to surface waters in the aqueous phase. As analytical technologies improve, detection of compounds can provide a more accurate characterisation of the quantities and occurrence of antibiotics in natural soil and water systems.

In a study to investigate the occurrence of five tetracyclines and six sulphonamides in water collected along the Cache la Poudre River, Colorado, no antibiotics were detected in a pristine mountain stretch of the river (Yang and Carlson, 2003). Few sulphonamides were detected along the entire river, but the frequency of detection and concentration of tetracyclines increased as the river water quality became affected by urban and agricultural sources. Tetracycline concentrations in filtered samples ranged from 0.08 to 0.30 µg/L. Photolysis, biodegradation and sorption of the tetracyclines could have occurred in various reaches of the stream but the authors concluded that proximate agricultural activity influenced tetracycline occurrence in the river. In a study to detect antibiotics in surface waters and groundwater, Campagnolo et al. (2002) found 31% and 67% of the samples collected near swine and poultry confinement facilities, respectively, had detectable quantities, albeit low, with less than 10 µg/L.

Few studies have determined the occurrence of veterinary antibiotics in groundwater. Krapac et al. (2004) collected shallow (<8 m) groundwater samples near two swine confinement facilities. Fewer than 5% of the samples contained any of the tetracyclines at either of the facilities. Parent tetracycline compounds were detected in a small number of groundwater samples collected from wells that had also been significantly impacted by manure seepage as evident by elevated chloride, ammonium and potassium concentrations. Tetracycline breakdown products were detected in some groundwater samples even when the parent compound was not detected. When detected, antibiotic concentrations were less than 0.5 µg/L. Hirsch et al. (1999) collected more than 30 groundwater samples from agricultural areas in Germany containing large numbers of animal confinement facilities. Of the 18 antibiotics representing macrolides, sulphona-

Table 29.5. Detection frequency and maximum concentrations of selected antibiotics in 139 filtered stream samples from 30 U.S. states (modified from Kolpin et al., 2002).

Antibiotic	Frequency of Detection (%)	Maximum Concentration (µg/L)
Trimethoprim	27.4	0.30
Erythromycin-H	20.2	1.5-1.7
Lincomycin	21.5	1.7
Sulphamethoxazole	19.0	0.52
Tylosin	13.5	0.28
Roxithromycin	4.8	0.18
Ciprofloxacin	2.6	0.03
Chlortetracycline	2.4	0.69
Oxytetracycline	1.2	0.34

mides, penicillins and tetracyclines, only sulphonamide residues were detected in four samples, and none of the other antibiotics were detected in the groundwater samples. The authors concluded that sulphonamides in two of the samples were the result of sewage irrigation and sulphamethazine detected in the other samples was likely from veterinary use.

### Occurrence of Bacteria and Development of Antibiotic Resistance in Animal Guts

Antibiotic resistance among commensal bacteria represents a major avenue for the development of resistance in bacterial pathogens, since resistances increase first in commensals and are then transferred to pathogens later. First, commensal gut bacteria are likely to be highly efficient contributors to antibiotic resistance because the numbers of commensal bacteria in the intestinal ecosystem are large, often more than  $10^{14}$  bacteria comprising several hundred species (Andremont, 2003). Anaerobic bacteria dominate this ecosystem and number  $10^{11}$ - $10^{12}$  cells/g of intestinal content, whereas enterobacteria and enterococci are relatively minor players ranging from  $10^6$  to  $10^8$  cells/g of intestinal content. Second, the commensal genetic pool is large and encompasses the potential for many different mechanisms conferring antibiotic

resistance. Third, antibiotic-resistant commensal bacteria may be selected each time an antibiotic is administered regardless of the health status of the animal. This microbial population is excreted in faeces and stored as manure where it undergoes changes in the numbers and proportions of the dominant bacterial species. An analysis of stored swine manure indicated that the predominant culturable microorganisms from these environments were obligately anaerobic, low mol% G + C Gram positive bacteria (Firmicutes) comprising members of the Clostridial, Eubacterial and Lactobacillus/Streptococcus phylogenetic groups (Cotta et al., 2003).

Although reports of the percentage of viable, culturable antibiotic-resistant bacteria in swine effluent vary, it is clear that antibiotic resistance is a common phenomenon. A study conducted in the 1980s of coliforms in swine waste found that 97% of *E. coli* were resistant to at least one of the following antibiotics: ampicillin, furazolidone, chloramphenicol, kanamycin, streptomycin, sulphonamides or tetracycline (Hanzawa et al., 1984). Haack and Andrews (2000) found that 71% of *Enterococcus faecalis* isolates from swine farrowing house effluent were resistant to tetracycline. Cotta et al. (2003) found that between 4 and 32% of the bacteria in swine manure were resistant to tylosin, depending on the depth from which the sample was collected in the manure holding pits.

### Persistence of Bacteria Introduced to Soil

Land application of animal manure, with its high concentration of microbial biomass, is a significant route for the introduction of new bacteria into the terrestrial environment, including potential pathogens (e.g. *E. coli* O157:H7) and some human enteric viruses (e.g. rotavirus). The persistence and transport of these organisms in the environment continues to be a concern for environmental quality and food safety, as well as human and animal health. Gavalchin and Katz (1994) concluded that the longer an antibiotic persists in the soil in an active form, the greater the potential for native soil bacterial populations to be affected. Nutrient amendment via the application of animal waste to soil has been hypothesised to promote faster ad-

aptation of the soil microbial community to antibiotic effects (Schmitt et al. 2005). In addition, biologically active antibiotics (or antibiotic breakdown products) introduced to the soil may confer a selective advantage for soil commensal bacteria carrying resistance genes, or exert selective pressure for acquisition of resistance genes in soil commensal populations.

It has been well documented over the years that many microorganisms survive the transition from effluent pit or lagoon into soil (Kibbey et al., 1978; Chandler et al., 1981; Stoddard et al., 1998; Bolton et al., 1999; Lee and Stotzky, 1999; Jiang et al., 2002; Guan and Holley, 2003; Boes et al., 2005). However, most investigations have focused on pathogens of clinical interest. The length of time that introduced organisms can persist in the soil varies with temperature, moisture, pH and the indigenous community present. The wide range of persistence times of four well-studied pathogens in different environments and at different temperatures has been reported (Table 29.6).

However, a recent study examining the survival of *E. coli* and *Salmonella typhimurium* applied to a clay soil with swine effluent found considerably shorter persistence times (21 days for *E. coli* and 7 days for *Salmonella typhimurium*) (Boes et al., 2005), highlighting the variation in survival times under different environmental conditions. Sengelov et al. (2003) studied the persistence of culturable aerobic, heterotrophic, tetracycline-resistant bacteria in four Danish farm soils following variable rates of pig slurry application. An increase in numbers of resistant bacteria was seen following application, with greater increases occurring in the more heavily manured soils. Five months following application, the proportion of tetracycline-resistant bacteria in all of the treated soils had returned to levels within the range of the non-manured control samples. Andrews et al. (2004) found enterococci declined from  $4.8 \times 10^5$  colony forming units (CFU)/g soil to  $< 10$  CFU/g in soil microcosms over a five-week period. These studies suggest that although a decline in numbers occurs with time, there may be sufficient time and opportunity for mechanisms of resistance selection and gene transfer to occur.

Table 29.6. Persistence times of pathogenic bacteria in different environments.

Environment	Temp (°C)	Estimation of survival time			
		<sup>1</sup> Salmonella	<sup>2</sup> Campylobacter	<sup>3</sup> Yersinia enterocolitica	<sup>4</sup> E. coli 0157:H7
Water	<0	~6 mo	<8 wks	>1 yr	>300 d
	~5	~6 mo	1 wk - 4 mo	>1 yr m	>300 d
	~30	~6 mo	~4 d	~10 d	~84 d
Soil	<0	>6 mo	<28 wks	>1 yr	>300 d
	~5	<28 wks	~2 wks	>1 yr	~100 d
	~30	~4 wks	~1 wk	~10 d	~2 d
Slurry		<75 d	<112 d	>28 d	100 d
Dry surfaces		<7 d	~1 d	~1 d	~1 d

References cited:

<sup>1</sup>Guo et al., 2002; Santo Domingo et al., 2000; Mitscherlich and Marth, 1984; Zibilske and Weaver, 1978.

<sup>2</sup>Buswell et al., 1998; Rollins and Colwell, 1986; Mitscherlich and Marth, 1984; Blaser et al. 1980.

<sup>3</sup>Karapinar and Gonul, 1991; Chao et al., 1988.

<sup>4</sup>Wang and Doyle, 1998; Tauxe 1997; Zhao et al. 1995; Cieslak et al., 1993.

## Detection of Antibiotic Resistance Genes in the Environment

Accurate and meaningful information on the persistence and dissemination of antibiotic resistance genes in bacteria is of fundamental importance in assessing potential health risks and environmental quality. The detection of specific genes and their bacterial hosts are important components, and recently developed techniques have been applied for detection of specific resistance genes and bacteria in natural environments. In particular, the use of molecular techniques provides rapid, sensitive and specific detection without the requirement for bacterial growth and isolation, which often poses a major challenge given the vast unknown of environmental microbial species. Commonly used molecular microbial techniques are based on unique sequence features of genes to allow detection and identification of microorganisms. Gene probes and the use of polymerase chain reaction (PCR) amplification of nucleic acids is now widely used to enable detection and quantification of low levels of target sequences, and has become a key procedure in the detection and identification of bacteria and genes from a variety of environments including soil, water and faecal material (Josephson et al., 1993; Karch et al., 1995; Wang et al., 1996; Malik et al. 2008). New approaches such as microarray technology have already being developed specifically to detect and

identify antimicrobial resistances in clinical and environmental bacteria (Call et al., 2003; Volokhov et al., 2003). A recent study using a gene array approach simultaneously screened for the presence of 23 tetracycline resistance genes and 10 erythromycin resistance genes in soil and faecal samples from swine to find the most prevalent genes (Patterson et al., 2007). Molecular fingerprinting tools and robotic technology have facilitated more accurate and sensitive microbial characterisation of complex environmental samples and have proven to be essential in providing more informative data in environmental monitoring studies. The recent development of a number of probes that target specific antibiotic resistance genes has increased the number of studies investigating the occurrence of these genes in natural environments. Such studies include detection of genes from antibiotic-producing bacteria, as well as genes resident in the background of natural populations. The following section highlights the application of molecular-based methods for detection and quantification of antibiotic resistance genes in bacteria and environmental samples.

Specific classes of antibiotics can be characteristic of the industry in which they are used, and multiple antibiotic resistance phenotype profiles of bacteria have been used to identify sources of faecal pollution (e.g. human, poultry, cattle, swine) in environmental samples (Kaspar et al., 1990; Pillai et al., 1997; Wiggins et al., 1999). Many

of these studies focus on bacterial strains of clinical importance and do not fully address the characterisation of populations that have acquired resistance genes in natural environments. To circumvent issues related to cultivation of bacteria, analysis of antibiotic resistance genes can be used to characterise the genetic pool from an environment, with the possibility of tracking the source of faecal contamination in surface waters and groundwater. Similar to the strategy used in microbial diversity studies, the starting point in the design of probes and primers for detection of antibiotic resistance genes is a robust phylogenetic analysis. Specific gene sequences can be targeted for detection, and such an approach has been used to demonstrate the diversity of antibiotic-resistant genes present in swine lagoon and pit effluent. For example, Aminov et al. (2001, 2002) and Chee-Sanford et al. (2001) found that the tetracycline resistance efflux genes (tet B, C, E, H, Y, Z) and the ribosomal protection protein (RPP) genes (tet W, O, Q, M, S, T, B(P), and otr A) were all present in a single swine waste lagoon. Koike et al. (2007) detected tet (M), (O), (Q), (W), (C), (H) and (Z) continually over a three-year period in groundwater underlying two swine farms. Furthermore, tet (W) sequences detected in the groundwater were nearly identical (99.8%) to those found in the corresponding lagoon. In the same study, the application of the same PCR primers further allowed the detection of unique and novel tetracycline resistance gene sequences. Using molecular-based detection, agricultural soils were found to be a rich reservoir of genes closely related to the glycopeptide resistance gene *vanA* in enterococci (Guardabassi and Agersø, 2006).

Tetracycline resistance genes have been found in large numbers in lagoon effluent. In a study of a cattle feedlot lagoon, a real time PCR method was used to detect and quantify tet (O), (W), and (Q) genes, and correlated gene copy numbers to tetracycline levels (Smith et al., 2004). As noted earlier, effluent loading can have a significant effect on bacterial levels and the upshot of resistance genes. Fields receiving multiple, high-volume (190,000-280,000 liters/hectare) applications of swine effluent each year showed consistently greater diversity and occurrence of tetracycline resistance genes than fields that received moderate volume (90,000-140,000 liters/hectare) applications of effluent on a two- or three-year rotation (S. Maxwell, unpublished). Within field variations also

occurred following effluent application, as demonstrated by uneven distribution of tetracycline resistance genes. A recent study showed the persistent effects of manure and the presence of sulphadiazine on soil bacterial communities, where the numbers of culturable resistant bacteria and sulphonamide resistance genes increased (Heuer and Smalla, 2007).

Recent studies have reported isolation of a wide range of antibiotic-resistant bacteria recovered from soil and water environments (Chee-Sanford et al., 2001; Ash et al., 2002; Esiobu et al., 2002; Onan and LaPara, 2003; Dang et al., 2008). A number of soil samples used in these studies were directly exposed to animal waste. Furthermore, sequences of resistance genes detected in bacterial isolates were found to be identical to sequences found in lagoon or animal waste. Nikolakopoulou et al. (2005) screened tetracycline-resistant streptomycete isolates from a range of environmental samples for oxytetracycline resistance genes and found resistance genes in non-tetracycline producing isolates. Identical isolates from the same samples have also been found to host different homologues of tetracycline resistance genes, as well as findings of individual isolates harbouring multiple determinants conferring the same type of resistance mechanism (S. Maxwell, unpublished). It is also noteworthy that cultivation strategies, particularly for populations from environmental samples, have thus far only provided an underestimate of bacteria, suggesting the possibility that a much higher diversity of antibiotic resistant bacteria may exist but has not yet been accounted for. Furthermore, archaea are now thought to be ubiquitous in many soil environments, including agricultural soils (Gattinger et al., 2006; Leininger et al., 2006). Far less is known about soil archaea and the extent of their resistance mechanisms or their contributions to genetic exchange within the soil metagenome.

## Conclusions

The impacts resulting from agricultural use of antibiotics and the practice of land application of animal waste on environmental quality and health risk potential is not completely clear, albeit there are demonstrated links to increased and accelerated incidences of antibiotic resist-

ant bacteria. However, the phenomenon is not a simple relationship of cause and effect. What is evident is the myriad complexity of antibiotic and biological mechanisms, and the ecological interactions that can occur at numerous points along the course of antibiotic use and disposal of livestock waste in soil environments, beginning with entry of antibiotics into animal gut systems. Regulatory aspects related to continued use of land application for waste management in animal production have real current concerns for nutrient (nitrogen, phosphorus) loads in soil; the practical impact of loading antibiotic residues and resistance genes is not yet known. The collective examination of specific mechanisms that affect the fate of compounds, microorganisms and the genetic pool will provide a better understanding of the true impacts of land application of effluent, as well as the general nature of the microbial and molecular ecology of antibiotic resistance.

Field information on the fate and transport of antibiotics is still limited, but in general, low amounts have been detected in soil and water environments, including the presence of breakdown metabolites. Predictive measures for solute and bacteria transport in soil and water have relied on existing models, which do not adequately predict contamination, and indicate a clear need for a larger database to develop and better inform models. The physico-chemical characteristics of the soil environment are likely to influence compound persistence, bacterial survival and genetic mechanisms at work. Trace amounts of antibiotics or other compounds (e.g. heavy metals) could act as selection pressures for maintenance and (co-) transfer of antibiotic resistance genes.

While the half-lives of antibiotics in manure are relatively short, it remains possible that drug residues may exert effects on biological functions within bacterial populations present in soils. Studies have shown application of animal manure to soil can readily lead to groundwater contamination with faecal bacteria. The acquisition of antibiotic resistances, however, appears to span a diverse phylogenetic range of bacteria, including those native to soil and water environments. Phylogenetic analyses of genes involved in tetracycline and erythromycin resistance demonstrate the evolution of these genes over time, and suggest that obtaining resistance genes from antibiotic-producing bacteria is not a major mechanism

of resistance acquisition evident in a broad range of bacteria. Resistance genes have been maintained in bacteria prior to the modern antibiotic era, even though the origin and purpose of these genes is not yet clear. The exact mechanisms contributing to antibiotic resistance gene acquisition and maintenance in natural environments are not yet well established, although increasing numbers of studies support lateral gene transfer events. Acquisition of antibiotic resistance through mechanisms of selective mutations and lateral gene transfer may be acting in concert with other natural mechanisms of genetic adaptation among a diverse range of bacteria in natural soil and water environments.

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