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Hospital effluent: impact on the microbial environment and risk to human health

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Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

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ENVIRONMENTAL PROTECTION AGENCY

The Environmental Protection Agency (EPA) is responsible for protecting and improving the environment as a valuable asset for the people of Ireland. We are committed to protecting people and the environment from the harmful effects of radiation and pollution.

The work of the EPA can be divided into three main areas:

Regulation: *We implement effective regulation and environmental compliance systems to deliver good environmental outcomes and target those who don't comply.*

Knowledge: *We provide high quality, targeted and timely environmental data, information and assessment to inform decision making at all levels.*

Advocacy: *We work with others to advocate for a clean, productive and well protected environment and for sustainable environmental behaviour.*

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We regulate the following activities so that they do not endanger human health or harm the environment:

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- large scale industrial activities *(e.g. pharmaceutical, cement manufacturing, power plants)*;
- intensive agriculture *(e.g. pigs, poultry)*;
- the contained use and controlled release of Genetically Modified Organisms *(GMOs)*;
- sources of ionising radiation *(e.g. x-ray and radiotherapy equipment, industrial sources)*;
- large petrol storage facilities;
- waste water discharges;
- dumping at sea activities.

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- Working with local authorities and other agencies to tackle environmental crime by co-ordinating a national enforcement network, targeting offenders and overseeing remediation.
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- Prosecuting those who flout environmental law and damage the environment.

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- National coordination and oversight of the Water Framework Directive.
- Monitoring and reporting on Bathing Water Quality.

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- Preparing Ireland's greenhouse gas inventories and projections.
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- Funding environmental research to identify pressures, inform policy and provide solutions in the areas of climate, water and sustainability.

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- Assessing the impact of proposed plans and programmes on the Irish environment *(e.g. major development plans)*.

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- Monitoring radiation levels, assessing exposure of people in Ireland to ionising radiation.
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- Monitoring developments abroad relating to nuclear installations and radiological safety.
- Providing, or overseeing the provision of, specialist radiation protection services.

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- Providing advice and guidance to industry and the public on environmental and radiological protection topics.
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- Advising Government on matters relating to radiological safety and emergency response.
- Developing a National Hazardous Waste Management Plan to prevent and manage hazardous waste.

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- Generating greater environmental awareness and influencing positive behavioural change by supporting businesses, communities and householders to become more resource efficient.
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- Office of Climate, Licensing and Resource Use
- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Radiological Protection
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet regularly to discuss issues of concern and provide advice to the Board.

EPA Research Programme 2014-2020

Hospital effluent: impact on the microbial environment and risk to human health

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EPA Research Report

Prepared for the Environmental Protection Agency

by

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The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive summary

Water is one of the most critical environmental resources that people depend on. This in reflected in the recognition of fresh water use as one of nine "planetary boundaries" that identify a safe operating space for humanity with respect to the functioning of the Earth system. Good access to water supports health in many ways by, for example, providing water for drinking, food production, hygiene and healthcare. Although Ireland has abundant water resources, problems with the quality of water can cause illnesses in Ireland, as they do elsewhere in the world. Problems with water quality may be caused by contamination with chemicals or microbes. The contamination may be natural in some cases, but there are growing concerns regarding contamination caused by human activity.

In the last few decades, there has been widespread and increased usage of antibiotics (over 100 tonnes per year are now used in Ireland for people and animals). As antibiotics have been used more widely, microbes have become increasingly resistant to them. It is now commonplace for antibiotics, which could be relied upon 20 years ago, to fail. Antibiotic resistance is a worldwide public health emergency. The increased use of antibiotics is influenced by an increasing and ageing population, longer survival of people with complex illnesses, changes in food production systems, and other social and economic factors. Some of the antibiotics used find their way into water sources. Antibiotics that have been found in water in Europe include penicillins, cephalosporins, macrolides (e.g. erythromycin) and quinolones (e.g. ciprofloxacin). Antibiotics in water are a potential problem for two reasons. First, antibiotics as chemical contaminants have the potential for direct effects on human health. Second, there is potential for indirect harm if they change the microbes in the water. Antibiotics can have obvious effects on bacteria at concentrations in the µg/l range, and they have been detected in water at concentrations between 0.004 and 201 µg/l. The immediate public health concern, regarding the effect of antibiotics in water, is that microbes in water change to become more antibiotic resistant. There is also concern that antibiotics could change the natural balance of the microbial ecosystem. Changes in microbial cells and populations can last long after an antibiotic has been degraded or removed. If people drink the affected water, or swallow it during recreation, this may spread antibiotic-resistant microbes over a large population very quickly.

The goal of this research was to learn more about how antibiotics get into the environment and to look at the possible effects of antibiotics in the environment on human health and antibiotic resistance. The research combined new laboratory measurements with reanalysis of previously published research and modelling to estimate the quantities of antibiotics and antibiotic-resistant bacteria in water in order to assess the likely impacts on human health and antibiotic resistance. This study represents the first of its kind in Ireland. Key findings from this project are as follows: (1) there are high levels of antibiotic-resistant *Escherichia coli* in urban wastewater; (2) dealing with hospital effluent in isolation will not substantially address the overall issue of antibiotic-resistant bacteria in urban wastewater; (3) the effect of wastewater treatment plants (WWTPs) on the removal of antibiotic-resistant bacteria is variable, but it is clear that, at best, such plants do not remove or inactivate all antibiotic-resistant bacteria; (4) the overall risk of human exposure to antibiotic-resistant *E. coli* related to swimming in seawater that receives treated urban wastewater is low; (5) predicted discharges of antimicrobial agents into the environment from hospitals are substantial, although diluted because of the mixing of hospital wastewater with the general wastewater stream; (6) toxic effects from direct human exposure to discharged antibiotics are very unlikely given the large dilution effects; (7) some antimicrobial agents (notably fluoroquinolones) may persist in the environment for extended periods after discharge; and (8) the predicted levels of antimicrobial agents in the environment are such that they may plausibly contribute to the development and maintenance of antibiotic resistance in the environment, at least in some settings.

On any given day, about one in every three patients in a major hospital is taking antibiotics. In many cases, a patient may be on several different antibiotics simultaneously. A significant quantity of the antibiotic given to the patient is shed into the toilet in urine or faeces, in a form that is still biologically active. Furthermore, and related in part to the use of antibiotics in hospitals,

a high proportion of patients in major hospitals have antibiotic-resistant bacteria resident in their gut. Large numbers of these bacteria are also passed into the toilet every day. *E. coli* is a very common gut bacteria. *E. coli* is a very common cause of infection (such as urinary tract infections and life-threatening infections, including blood stream infections) and has become increasingly resistant to antibiotics in recent decades. In Ireland and most of Europe, hospital effluent is commonly released into the urban wastewater system without any specific measurement of antibiotic levels or antibiotic-resistant bacteria, and without any pre-treatment. There are concerns that the release of these contaminants into the urban wastewater may result in downstream exposure to antibiotics and contribute to the growing problem of antibiotic resistance. To study this problem, the number of *E. coli* resistant to antibiotics in two wastewater treatment systems was measured. One of the systems receives and treats effluent that includes effluent from a major hospital and the other does not include a major hospital. Antimicrobial-resistant (AMR) *E. coli* were present in the wastewater going into and out of both plants. Overall, the measurements did not show a clear difference between the two WWTPs in terms of the frequency with which antibiotic-resistant *E. coli* were detected in the treated wastewater.

A computer model (Monte Carlo simulation) was developed to estimate the release of antibiotics into water and to identify the factors that influence the level of antibiotics in the environment. In some cases (penicillins), the total amount of antibiotic used is the key factor that influences the level in the environment at any given time, whereas for other antibiotics (fluoroquinolones), the stability of the antibiotic in the body and its excretion (metabolism) are more important. The computer model identified quinolones/fluoroquinolones as a group of antibiotics with very high potential to drive the development of resistance in the environment. Not only does the metabolism of fluoroquinolones lead to the shedding of a high proportion of the dose from the body, but, in addition, fluoroquinolones have a very low rate of break down in the environment (50% after 100 days for quinolones/fluoroquinolones, compared with 99.8% for penicillins after 100 days). Another computer model (Monte Carlo simulation) was developed to predict the levels of a specific fluoroquinolone, ciprofloxacin, in wastewater from a hospital through to the WWTP. Computer modelling for this type of study is particularly helpful because accurate laboratory measurement of antibiotic levels in the wastewater is challenging and expensive. The mean predicted concentration of ciprofloxacin was 579 mg/m^3 in hospital effluent, compared with 0.15 mg/m^3 in seawater receiving wastewater from a treatment plant. This model was also used to estimate how much a person swimming in the sea receiving treated wastewater may be exposed to ciprofloxacin. It seems that it is highly unlikely that a swimmer in such seawater would be exposed to levels that exceed the acceptable daily intake (12 µg/kgBW/day).

Wastewater is considered to be the main source of entry of antimicrobials/antibiotics and antibiotic-resistant bacteria into the environment. In Ireland, as in most European countries, urban wastewater is treated in WWTPs before discharge to the environment. The value of wastewater treatment in reducing AMR bacterial contamination has been disputed. Some researchers report that wastewater treatment helps to reduce the concentration of antibiotic-resistant bacteria, whereas others suggest that the treatment process may increase the concentration. The differences in the findings may be because WWTPs differ in the effluent they receive and in the treatment process used, and because the season and rainfall may also impact on results. The results from 161 different research projects, previously published in this area, were analysed together (in a meta-analysis) to address this question. The results suggest that, overall, WWTP processing appears to increase the proportion of resistant bacteria [odds ratios (ORs) of 1.60, 1.33 and 1.19 for multiple AMR bacteria, single AMR *E. coli* and quinolone- or fluoroquinolone-resistant bacteria, respectively]. These ORs are a measure of the likelihood of an outcome occurring given a particular exposure, compared with the likelihood of the outcome in the absence of that exposure. This may suggest that antibiotic-resistant bacteria are better able to survive the wastewater treatment process. There is a need for further research to understand how the secondary wastewater treatment process may impact on the development of antimicrobial resistance; in particular, further research is needed to understand what drives the development of resistance in effluent and what helps to maintain it. However, it is important to note that analytical data from this project indicate unequivocally that the total concentration of antibiotic-resistant *E. coli* is greatly reduced by wastewater treatment, even if the proportion is somewhat increased.

It is important, however, to consider that the situation may differ with respect to newer antibiotics, which are used, almost exclusively, in hospitals. In such cases, resistance has become established in some hospitals but resistance is not yet widely established in the community (e.g. carbapenemease-producing *Enterobacteriaceae*). The measured levels of antibiotic-resistant bacteria also helped to develop a computer model to estimate levels of *E. coli* in water receiving discharge from a WWTP. The AMR *E. coli* level was predicted to be between 6 and 193 *E.* coli/100 ml seawater. There are currently no guidelines for levels of AMR *E. coli* in coastal waters. However, the European Union Bathing Water Directive specifies 250 *E.coli*/100 ml as the upper limit for excellent-quality bathing water. Considering the volumes of water likely to be swallowed by swimmers, the model predicted low-level exposure to AMR *E. coli* associated with swimming in receiving waters.

1 Introduction

1.1 The problem of antimicrobial resistance

Antimicrobial resistance is a major public health problem and has been acknowledged in a series of authoritative reports in Ireland and elsewhere (SARI, 2001; CDC, 2013; World Economic Forum, 2013). Such is the level of concern, the World Health Organization is in the process of drafting a Global Action Plan for combating antimicrobial resistance and has invited submissions from Member States. The Centre for Health from Environment, National University of Ireland Galway, has contributed to this process.

Infection associated with antimicrobial-resistant (AMR) bacteria results in significant increases in healthcare costs, morbidity and mortality. The European Centre for Disease Prevention and Control (ECDC) estimates that antimicrobial resistance results in 25,000 deaths and related costs, resulting from healthcare expenses and productivity losses, of over **€1.5 billion** annually (ECDC/ EMA, 2009). In 2011, the European Commission published an "Action plan against the rising threats from Antimicrobial Resistance" (EC, 2011). In 2013, the Centers for Disease Control and Prevention (CDC) published a report on the threats associated with antimicrobial resistance in the USA (CDC, 2013) and estimated that at least 2,049,442 cases of illness and 23,000 deaths were caused annually in the USA by AMR bacteria. They also grouped these organisms into categories (urgent, serious and concerning) based on the severity of illness they cause, how often infections occur and how quickly they spread. Carbapenemase-producing *Enterobacteriaceae* (CPE), extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-PE) and vancomycin-resistant enterococci (VRE), which are ranked as urgent (CPE) and serious (ESBL-PE and VRE), respectively, under the CDC categorisation, are significant public health concerns in Ireland and elsewhere. Treatment for infection associated with these organisms can be very limited as they are frequently co-resistant to multiple antimicrobial classes. The increasing problem of antimicrobial resistance in Ireland is highlighted by European Antimicrobial Resistance Surveillance Network data. The proportion of *Escherichia coli*, causing invasive infection in Ireland, that are ESBL producers has been increasing annually, from 1.2% in 2002 to 10.1% in 2014. Most recent data also indicate that 15% of *E. coli* were multidrug-resistant and 26.8% were resistant to the fluoroquinolones, the highest annual proportions recorded to date (EARS-Net, 2014). Bacteria may be intrinsically resistant to antimicrobial agents or may acquire resistance as a consequence of genetic change. The emergence and dissemination of acquired antimicrobial resistance is related to the use of antimicrobial agents. Exposure of microorganisms to sub-lethal concentrations of antimicrobial agents over extended periods increases the frequency with which AMR bacteria are selected.

1.1.1 Antimicrobial resistance and the environment

The presence of antimicrobial residues in the environment can be difficult to detect because of their low concentration, but this does not mean that they are not having an adverse impact on microbial biodiversity, and potentially on human health, through resistance formation. Therefore, it is of interest to examine, firstly, the environmental risks that antimicrobials pose, both to humans and to the environment, and, secondly, the occurrence of resistance formation within target bacterial populations. Human and animal bacteria are continuously being released into the environment, commonly via wastewater. Many of these organisms possess antimicrobial resistance genes. Simultaneously, antimicrobials may enter the waste stream via the same routes. Their presence can potentially alter microbial ecosystems. Municipal wastewater treatment was developed to reduce the amount of bacteria and other chemical and physical contaminants that enter the receiving environment. However, wastewater treatment plants (WWTPs) were never designed to specifically reduce antimicrobial residues or resistant bacteria. It is assumed that wastewater treatment processing does not exert a different effect on susceptible or resistant bacteria, but the conditions to which the bacteria are exposed may preserve or even promote the dissemination of resistance. The accumulation of bacterial

populations allow for the exchange of a wide variety of resistance genes (Boon *et al.*, 2001). Units in WWTPs with high bacterial densities (biofilm and floc formations, high metabolic activities, aerators and clarifiers) allow, and may even promote, resistance formation (Mancini *et al.*, 1987). WWTPs may also allow the exchange of bacterial genes from different environmental compartments, such as hospital effluent and surface waters (Schluter *et al.*, 2007). The importance of individual case studies is emphasised because antimicrobial resistance does not always correlate with the amount of drugs used or with the concentration of resistant residues identified in the environment (Kümmerer, 2008).

Although Guardabassi *et al.* (2002) concluded that tertiary wastewater treatment processes did not result in selection of AMR bacteria, their data show an increase in the mean proportion of AMR *Acinetobacter* spp. in treated sewage compared with raw sewage (Guardabassi *et al.*, 2002). Zhang *et al.* (2009) examined the effect of WWTPs on the resistance of *Acinetobacter* isolates to eight antimicrobials and, although there was a consistent decline in total *Acinetobacter* isolates through the WWTP, the prevalence of AMR *Acinetobacter* isolates significantly increased (Zhang *et al.*, 2009). Diehl and LaPara (2010) investigated the effectiveness of aerobic and anaerobic digestion on AMR bacterial removal from wastewater. Aerobic digesters did not significantly remove the quantity of resistant genes. In contrast, anaerobic digestion significantly reduced the quantity of antimicrobial genes, with increased removal as temperature increased (Diehl and LaPara, 2010). Galvin *et al.* (2010) identified an increase in AMR *E. coli* after the introduction of hospital effluent into the wastewater stream, which were not totally eliminated after secondary wastewater treatment. Whether this means that resistant bacteria can withstand WWTP processes (by means of underlying resistance affecting the natural WWTP bacterial-removal processes) or whether WWTP processes increase the level of resistance development among susceptible bacteria is, as yet, unknown (Galvin *et al.*, 2010).

1.2 Antimicrobial agents

Antimicrobial agents have been used for decades in human and veterinary medicine and for other applications. Wise (2002) has estimated that the total annual worldwide antimicrobial market consumption is between 100,000 and 200,000 tonnes. A worldwide population increase, coupled with longer life spans, has resulted in an increase in the use of antimicrobial agents for therapeutic purposes. Governance and consumption of antimicrobial agents in human medicine, veterinary medicine and agriculture vary throughout the world and have impacted significantly on the emergence and dissemination of AMR bacteria.

1.2.1 Antimicrobial classes

Antimicrobial agents kill (bactericidal) or inhibit (bacteriostatic) the growth of microorganisms and are also referred to as antibacterials, antifungals, antivirals or antiparasitics depending on the group of microorganisms they target. Antibacterials may be classified into groups or classes of agents based on their chemical structure and mechanisms of action. The principal targets of antibacterial agents within a bacterial cell are (1) protein synthesis/function (tetracyclines and aminoglycosides); (2) nucleic acid synthesis (sulphonamides and quinolones); (3) cell membrane integrity (gramidicin); and (4) cell wall (β-lactams and glycopeptides). The most widely used classes of antimicrobial agents in human and veterinary medicine are the β-lactams, the quinolones/fluoroquinolones, the macrolides, the tetracyclines and the sulphonamides/trimethoprim. Frequently, analogues of antimicrobial agents licensed for use in human medicine are also licensed for use in veterinary medicine (e.g. ciprofloxacin, which is used in human medicine, and its analogue enrofloxacin, which is used in veterinary medicine).

1.2.2 Antimicrobial use in humans

The European Surveillance of Antimicrobial Consumption Network (ESAC-Net) collates data relating to human consumption of antimicrobial agents in the community and hospital sectors. Most recent ESAC-Net data indicate that the consumption of antibacterial agents, for systemic use in the community sectors in Ireland, is, on average, 23 defined daily doses (DDDs) per 1000 inhabitants per day, which is above the European Union (EU) average of 21.5 DDDs per 1000 inhabitants per day (ESAC-Net, 2014). Data suggest that the most frequently used class of antibacterial agents, in both the hospital and community sectors across Europe, are the penicillins, followed by other beta-lactam agents (e.g. cephalosporins) and the quinolones/fluoroquinolones for the hospital sector, and the macrolides and tetracyclines for the community sector.

1.2.3 *Antimicrobial use in animals*

The European Surveillance of Veterinary Antimicrobial Consumption project collects data on the use of antimicrobial agents in animals in Europe. Most recent data reveal that 101.2 tonnes of antimicrobials agents were used in veterinary medicine in Ireland in 2012, compared with 88.5 tonnes in 2011 and 96.7 tonnes in 2010 (IMB, 2011; ESVAC, 2012). The most commonly used antimicrobial agents in veterinary medicine in Ireland in 2012 were the tetracyclines, representing 36.7% of total sales, followed by the penicillins (22.1%) and the sulphonamides/trimethoprim (21.4%) (IMB, 2011). In an effort to control the emergence and dissemination of antimicrobial resistance, a number of EU-wide risk management decisions were implemented in recent years. In line with these, the Health Products Regulatory Authority has updated the labelling of newer antibiotic classes (e.g. fluoroquinolones and third- and fourth-generation cephalosporins) to include so-called "responsible use" warnings (HPRA, 2014).

1.3 Antimicrobial agents and the environment

Stockholm County Council (SCC) has determined a mechanism for assessing the impact of a pharmaceutical agent on the environment. Each compound is classified using a PBT (persistence, bioaccumulation and toxicity) index, which can range between 0 and 9; the higher the PBT index, the higher the risk to the environment.

1.3.1 *β-lactams*

Benzylpenicillin has a PBT index of 4 (SCC, 2009). The excretion of un-metabolised penicillins can range from 18% to 87% (Bryskier, 2005). Backhaus and Grimme (1999) documented the acute toxicity value [concentration at which a drug is 50% effective (EC_{50})] to be 163 mg/l. Although penicillins can have acute adverse effects, they are of reduced environmental concern as a result of their susceptibility to multiple modes of degradation (Table 1.1) and are, thus, short lived within the aqueous environment (Hirsch *et al.*, 1999). The broad-spectrum cephalosporins can be excreted 30–90% unchanged. The EC_{50} value for the cephalosporin cephalexin was documented to be 0.01 mg/l (Turkdogan and Yetilmezsoy, 2009). The SCC (2009) has assigned a PBT index of 3 to the cephalosporins cefepime, ceftriaxone, cefuroxime and cefotaxime. In contrast, the cephalosporin ceftazidime has a PBT index of 6. Cephalosporins are relatively resistant to degradation (Table 1.1); hence, they may persist in the environment.

1.3.2 *Macrolides*

Macrolides are hydrophobic molecules and thus can be assumed to adhere to particles (Kümmerer, 2008). Lampen *et al.* (1998) documented that 60% of macrolides are metabolised in the small intestine. The Ecological Structure Activity Relationships (ECOSAR) [United States Environmental Protection Agency (USEPA)] software that estimates toxicity, determined the EC_{50} value for erythromycin to be 71.4 mg/l (ECOSAR, 2009). The macrolides erythromycin and

Table 1.1. Antimicrobial susceptibility to modes of removal or degradation from the environment (Breslow *et al.*,1976; Graafland *et al.*, 1979; Fasani *et al.*, 1998; Ingersley and Halling-Sorenson, 2000; Deshpande *et al.*, 2004; Kümmerer, 2008; Park and Choi, 2008; Mohring *et al.*, 2009)

Removal/degradation	Penicillins	Macrolides	Cephalosporins	Tetracyclines	Sulphonamides	Quinolone/ fluoroquinolones
Photolysis	X	X	X	Y	Y	Y
Hydrolysis	Y	–	Y	Y	Y	X
Thermolysis	Y	–	–	–	Y	X
Sorption[a]	Y	Y	–	Y*	Y	Y
Biodegradation	Y	X	X	X	X	X
Anaerobic conditions	–	–	–	–	Y	X

'–' indicates that data were not available.

a Removes antimicrobials from the water by attachment to particles, but this does not mean it has been degraded.

X, not susceptible; Y, susceptible; Y*, susceptible but weak.

clarythromycin have a PBT index of 6 (SCC, 2009). Erythromycin A is the most common macrolide, as a result of its spectrum of application and biological activity, which is significantly higher than that of other macrolides. Macrolides are of concern because they are not susceptible to biodegradation (Table 1.1) and can therefore persist in the environment.

1.3.3 Tetracyclines

Tetracyclines were one of the first groups of broad-spectrum antimicrobials to be described (Bryskier, 2005). Lalumera *et al.* (2004) determined the EC_{50} value for oxytetracycline to be 121–139 mg/L (Lalumera *et al.*, 2004). Tetracycline and oxytetracycline have a PBT index of 5 and 6, respectively (SCC, 2009). It is estimated that approximately 50% of tetracyclines are excreted via urine, with 5% consisting of metabolites (Agwuh and MacGowan, 2006). Tetracyclines are susceptible to degradation within the environment (Table 1.1).

1.3.4 Sulphonamides

Sulphonamide antimicrobials are synthetic compounds which contain a sulphonamide group. They are often used in conjunction with trimethoprim. Although their metabolism is high, once released they can persist for more than 90 days in soil and for more than 21 days in surface waters (Kümmerer, 2008). Jung *et al.* (2008) determined the EC_{50} values of sulfamethazine, sulfamethoxazole and sulfathiazole to be 185, 205 and 136 mg/l, respectively, after 48 hours, and 148, 178 and 79 mg/l, respectively, after 96 hours (Jung *et al.*, 2008). It was also determined that these toxicity values were several orders of magnitude higher than those detected in ambient water systems (Jung *et al.*, 2008). The SCC (2009) has assigned sulfamethoxazole a PBT index of 6. Sulphonamides are susceptible to many forms of degradation (Tables 1.1 and 1.2).

1.3.4 Quinolones/fluoroquinolones

Quinolones/fluoroquinolones are a family of broad-spectrum synthetic antimicrobial agents that includes nalidixic acid, ciprofloxacin, levofloxacin, flumequine and enrofloxacin. Flumequine has a documented EC_{50} value of between 12 and 15 mg/l (Lalumera *et al.*, 2004). Levofloxacin and ofloxacin have a PBT index of 8 and 9, respectively, whereas moxifloxacin has a PBT index of 4, and ciprofloxacin and norfloxacin have a PBT index of 5 (SCC, 2009). Approximately 76% of excreted ciprofloxacin consists of the un-metabolised parent compound (Harder *et al.*, 1990). Quinolones/fluoroquinolones are of particular concern because of their persistence in the environment and low susceptibility to biodegradation (see Section 1.5, and Tables 1.1 and 1.2). Ciprofloxacin has been detected at extremely high concentrations in effluent from pharmaceutical manufacturers, for example 28,000–31,000 µg/l in Hyberdad, India (Larsson *et al.*, 2007), and in hospital effluent samples, for example 39.84 µg/l in hospital effluent in Norway (Thomas *et al.*, 2007) and 9.3 µg/l in hospital effluent in Sweden (Zorita *et al.*, 2009). The

Table 1.2. Pharmaceutical levels reported in waterways (mg/ml) and lowest acute toxicity

Antimicrobial agent	Antimicrobial class	USA (Guerrero-Preston and Brandt-Rauf, 2008; Kolpin *et al.*, 2002)	Germany (Hirsch *et al.*, 1999)	Switzerland (Hartmann, 1998)	France (Feitosa-Felizzola *et al.*, 2009)	Italy (Zuccato *et al.*, 2005)	LAC (Kümmerer, 2008)
Amoxicillin	β-lactam	–	–	201	–	0.004	3.7
Chloramphenicol	Phenicol	–	0.06	–	–	–	–
Sulfamethoxazole	Sulphonamide	1.9	0.48	–	0.15	0.13	30
Trimethoprim	Folate pathway inhibitor	0.71	0.2	–	–	–	–
Clarithromycin	Macrolide	–	0.26	–	2.33	0.2	–
Roxithomycin	Macrolide	0.18	0.56	–	–	–	–
Erythromycin	Macrolide	1.7	1.7	–	–	0.02	–
Ciprofloxacin	Fluoroquinolone	0.03	87	14.5	9.66	0.3	9.3
Norfloxacin	Fluoroquinolone	0.12	–	6.2	–	–	–

'–' indicates that data were not available.
LAC, lowest acute toxicity.

fluoroquinolone ciprofloxacin is neutral at pH 7.04 and many of its chemical properties, including solubility, hydrophobicity and hydrophilicity, are pH dependent (Kümmerer, 2008) (Table 1.2).

1.4 Mode of entry of antimicrobial agents into the environment

The increased usage of antimicrobial agents in human medicine, veterinary medicine and agriculture has resulted in an increase in residues entering the environment through direct or indirect routes (Figure 1.1). Antimicrobial agent residues [e.g. ciprofloxacin, sulfamethoxazole, tetracycline and trimethoprim have environmental levels ranging from 0.20 to 1.4 µg/l, 0.21 to 2.8 µg/l, 0.061 to 1.1 µg/l and 0.21 to 7.9 µg/l, respectively (Batt *et al.*, 2007)], metabolites and degradation products [e.g. the penicillin degradation products penilloic acid, penicilloic acid and isopenillic acid in river water (Li *et al.*, 2008)] may reach terrestrial and aquatic environments as a result of human and animal use (Kemper, 2008). Ciprofloxacin is one of the most frequently detected antimicrobials in waterways (Table 1.2). The leading source of antimicrobial contamination in the environment appears to be as a result of human and animal consumption and subsequent excretion. Antimicrobial residues in water are attributed to many sources, but it is postulated that the common route of release of antimicrobials into the environment is through wastewater treatment processes (EMEA, 2006).

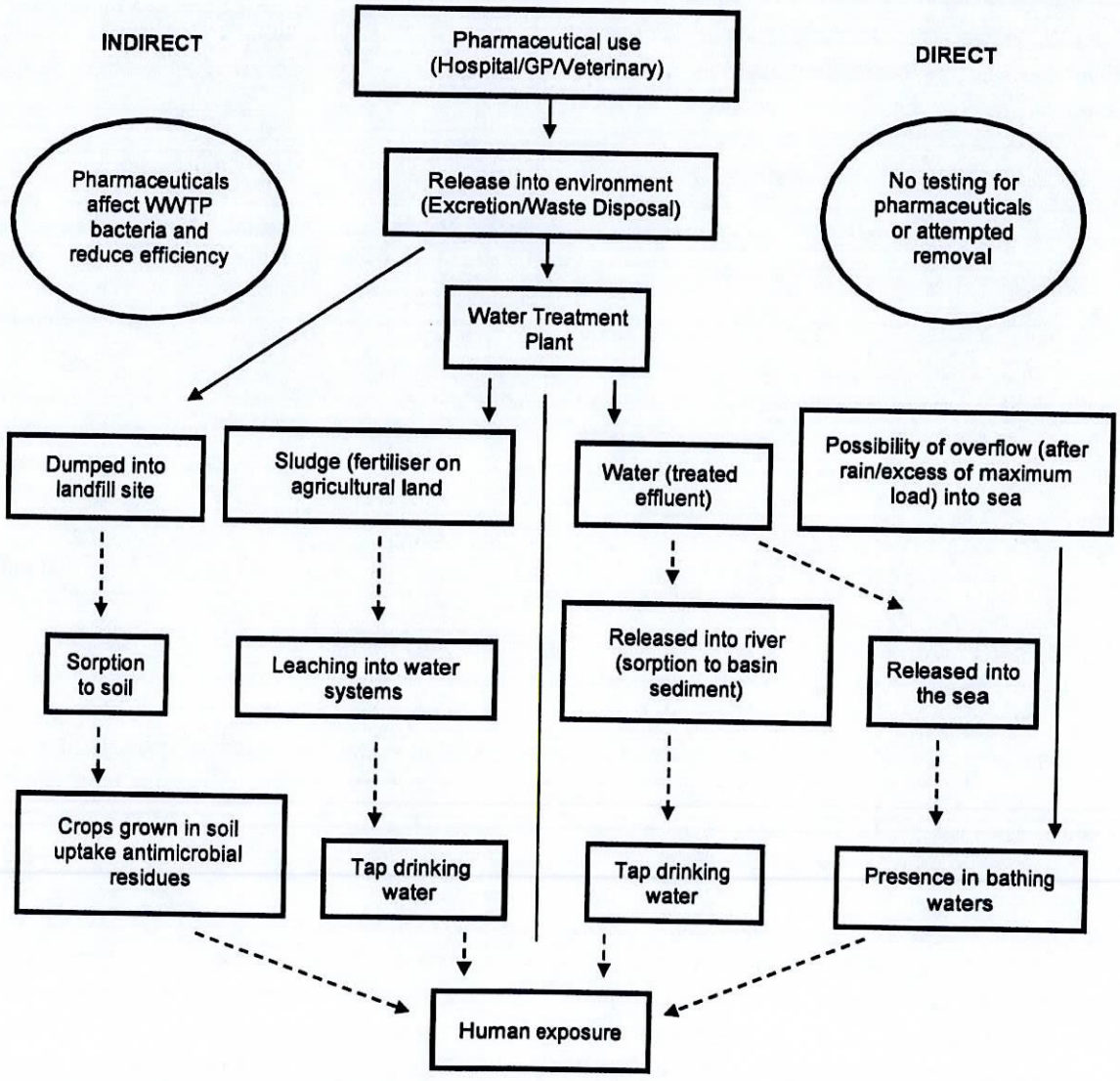

Figure 1.1. Potential routes for antimicrobial agent entry into the environment and modes of human exposure. Broken arrows (- - ►) indicate the possible stages at which antimicrobial residue entry into the environment could be limited, through either extended treatment or complete process removal.

1.4.1 Wastewater treatment processes

The range of sources of antimicrobials entering the wastewater stream varies, but whether from production company effluent, from excretion after therapeutic application at home or in hospital, or from direct deposition down the drain, a large proportion of antimicrobials ends up in the wastewater stream, resulting in the accumulation of the products in WWTPs. Zuccato *et al.* (2006) reported the mean level of antimicrobial residues in nine WWTPs in Italy. The antimicrobials for which monitoring and ecotoxicology studies are a priority, namely amoxycillin, clarithromysin, erythromycin, sulfamethoxazole and ofloxacin, were found at 4.7, 18.1, 47.4, 127.2 and 600 ng/l, respectively (Zuccato *et al.*, 2006). Watkinson *et al.* (2007) documented varying rates of removal of quinolone antimicrobials in a conventional WWTP. Norfloxacin and ciprofloxacin were reduced by 85% and 83%, respectively, the levels of enrofloxacin remained the same and, in contrast, the concentration of nalidixic acid increased from "not detected" to 55 ng/l (Watkinson *et al.*, 2007). A study carried out by Fick *et al.* (2009) investigated whether or not surface water, groundwater and drinking water, in the region of a WWTP in India, were contaminated by antimicrobials.

Exceptionally high levels of ciprofloxacin were found in the WWTP effluent (14,000 µg/l). Samples taken upstream of the plant contained much lower levels of ciprofloxacin (12 µg/l). Downstream sampling found a gradient decline, with the highest levels (10–2,500 µg/l) reported closest to the WWTP. Surrounding lakes and drinking wells were found to have ciprofloxacin levels of between 2,500 and 6,500 µg/l and between 4 and 14 µg/l, respectively. The study concluded that insufficient wastewater management, in an area of high-level pharmaceutical production, results in contamination of surface water, groundwater, and drinking water (Fick *et al.*, 2009). Fick *et al.* (2009) also demonstrated significant transport of fluoroquinolones by groundwater. The most urgent aspect of antimicrobial presence in the environment is the promotion of highly AMR organisms after exposure to high levels of broad-spectrum antimicrobials and the possibility of the horizontal transfer of resistance determinants to human pathogens (Fick *et al.*, 2009). Currently, there is little available information on the effect of individual WWTP processes on antimicrobial residues and AMR bacteria. To fully understand the effect of the contribution of WWTPs to antimicrobial presence in the environment, there is a need to investigate and understand the fate of both antimicrobial agents and AMR bacteria within a WWTP. This would allow for mitigation strategies to target specific areas of the treatment process. The use of biosolids (treated organic municipal or sewage sludge) as an agricultural fertiliser is common practice. Common sludge treatment processes include anaerobic or aerobic digestion, dewatering, liming, thermal drying and incineration (or a combination of these processes). As the concentrations of pharmaceuticals are not currently monitored or regulated, and the removal of antimicrobials by WWTP processes is incomplete (Watkinson *et al.*, 2007), it is not possible to say if these processes affect the prevalence of biologically active antimicrobials, but their presence is well documented within sludge samples (Golet *et al.*, 2003; Göbel *et al.*, 2005). Golet *et al.* (2003) found ciprofloxacin and norfloxacin in sewage sludge from several WWTPs at concentrations ranging from 1.4 to 2.4 mg/kg (Golet *et al.*, 2003). Göbel *et al.* (2005) examined a WWTP in Germany and documented levels of sulfapyridine, sulfamthoxazole, tripethoprim, azithromycin, clarythromycin and roxithromycin, measured using pressurised liquid extraction, in activated sewage sludge as 51, 100, 87, 158, 41 and 61 µg/kg, respectively. Göbel *et al.* (2005) also examined two Swiss WWTPs and found levels of sulfapyridine, sulfamthoxazole, tripethoprim, clarythromycin and roxithromycin in activated sewage sludge to be non-determinable 24, 34–51, 13 and 25–32 µg/kg, respectively. Antimicrobials can persist in soil for long periods. Christian *et al.* (2003) found mg/kg levels of some antimicrobials (including sulfadimidine) in manure and µg/kg levels in soil after sludge spreading at least 3 months before sampling. Antimicrobials, both parent compounds and metabolites, can remain in the sludge even after prolonged storage (Burkholder *et al.*, 2007). The application of sludge/liquid manure can lead to surface run-off (particularly after periods of heavy rain), leaching into deeper soils and groundwater, drift and direct input into the environment (Christian *et al.*, 2003). With knowledge of the potential dangers of pharmaceuticals in the environment, particularly the emergence of antimicrobial resistance, caution in their usage is required. Burkholder *et al.* (2007) recommend ecosystem monitoring, toxicology assessment of contaminants, research into fate and transport, surveillance programmes, wastewater and drinking water treatment monitoring (to ensure adequate removal or inactivation of emerging contaminants), direct pollution prevention or reduction, and education as the necessary steps for the prevention of the negative impacts of antimicrobial

agents in the environment. Boxall *et al.* (2006) examined the accumulation of veterinary medicines, including antimicrobial agents, in crops, as a result of their presence in the soil, and concluded that although the levels were large enough to be quantified, they were all lower than the acceptable daily intake (ADI) values (Boxall *et al.*, 2006). An ADI is a value that has been set by the US Food and Drug Administration (FDA) as a guideline for consumption of food contact substances (FDA, 2002). It is important to emphasise the danger of medicines that have a very low ADI, in addition to substances that elicit subtle effects over long periods or as a result of multiple exposures of various drugs from one or multiple sources. In addition, in the case of antimicrobial agents, it is not only ingestion that is a relevant concern, but also the selection effects on bacteria, which may also be ingested.

1.4.2 Direct deposition

Disposal of pharmaceuticals via household waste may be an important source of environmental contamination (Kümmerer, 2003). Bound and Voulvoulis (2005) reported that from 400 households surveyed in the south-east of England, only 20% of respondents returned pharmaceuticals to a dispenser (Bound and Voulvoulis, 2005). In a survey conducted in Ireland, findings suggest that there is little awareness among the public regarding the appropriate ways to dispose of unused medicines (Vellinga *et al.*, 2014). Of 398 respondents, 202 (51%) indicated that they had disposed of medicines in an environmentally inappropriate manner: via household waste [103 (26%)], down the sink (15%) or down the toilet (7%) (Vellinga *et al.*, 2014). The European Environment Agency (EEA, 2010) has highlighted the need for an EU-wide pharmaceutical take-back scheme. There is no well-defined process for the return of unwanted medicines in Ireland at present, although some pharmacies may accept returns on a voluntary basis. Such schemes will impact on drug safety and economics and will result in a reduction in the direct disposal of pharmaceuticals into the environment. A number of EU Member States already have pharmaceutical take-back systems in place with varying success.

1.4.3 Agriculture

Although the non-therapeutic use of antimicrobial agents in agriculture was banned in the EU in 2006, antimicrobial agents are still used as growth promoters in agriculture in many parts of the world (Loftin *et al.*, 2005). The same authors reported that the complete metabolism of antimicrobial agents is rare and therefore significant quantities of biologically active compounds can end up in livestock waste. Alexander *et al.* (2008) reported that low-level exposure to chlortetracycline in feed lot cattle increased the prevalence of shedding AMR *E. coli*. Dagan *et al.* (2008) demonstrated a link between reduced antimicrobial use and a rapid decrease in AMR bacteria. Karci and Balcioglu (2009) examined antimicrobial pollution of soil through the application of animal manure and found that all of the samples contained at least one antimicrobial compound. In addition to the accumulation of antimicrobial compounds in the soil, the use of animal manure containing antimicrobials as fertiliser can lead to the presence of antimicrobial resistance genes in the environment.

Knapp *et al.* (2010) explored the historical soil archives of the Netherlands to determine the relative abundance of antimicrobial resistance genes over time and found significant increases in the presence of genes encoding β-lactamases and tetracycline and erythromycin resistance, from marginal (close to zero) in 1940 soil samples to 16, 8 and 3 genes, respectively, in soil samples from 2008 (Knapp *et al.*, 2010). Knapp *et al.* (2010) speculate that this may be as a result of the increased application of antimicrobials in veterinary medicine; their examination of antimicrobial use since 1997 indicates that trends have mimicked gene abundance at some of the sample sites. They concluded that environmental factors promoting antimicrobial resistance proliferation may not yet be fully defined, which points to the requirement for further research into antimicrobial resistance and its environmental/anthropogenic causes.

1.5 Mode of degradation

The metabolism or biotransformation of an antimicrobial is the process by which antimicrobials are conjugated and, in some cases, deactivated. Glucose is commonly found in biological systems (e.g. within the body) and thus glucuronide formation is a common route of drug metabolism in humans. Glucuronide is one of the forms in which antimicrobials can be present in the environment (along with the unchanged parent compound, individual metabolites or as a complex mixture of many metabolites) (Kümmerer, 2008). Many possible degradation or removal routes

can also occur in the environment (Table 1.1). There is evidence that conjugated compounds can become deconjugated (i.e. revert to the parent compound) during WWTP processing, and, consequently, parent drug metabolites are sometimes assumed to react in the same way as the parent compounds (as a precaution in risk analysis) (Kümmerer, 2008). Metabolites are known to be more soluble in water than the parent compound and, therefore, their mobility potential is increased (Diaz-Cruz and Barcelo, 2006). Many risk assessment studies consider only the antimicrobial parent compound, with very little consideration given to the metabolites. Hilton and Thomas (2003) investigated N4-acetylsumlfametgoxazole, a metabolite of the sulfonamide sulfamethoxazole, along with other parent compounds. N4-acetylsumlfametgoxazole was found in all samples at levels of between 50 and 2,200 ng/l, whereas sulfamethoxazole could not be detected in any of the samples. Similarly, Hirsch *et al.* (1999) examined antimicrobials in surface water and detected only metabolites. Analytical methods and reference substances are limited for antimicrobial metabolites and there is a need for an international standard method of testing and reporting (Diaz-Cruz and Barcelo, 2006).

Natural biodegradation within the aquatic environment varies, depending greatly on the individual compound (Table 1.1.) (Gartiser *et al.*, 2007). Vasconcelos *et al.* (2009) showed that ciprofloxacin has very high rates of photo-degradation (with a half-life of 0.15, 0.77 or 0.38 hours at pH 7, 5 or 9, respectively) (Vasconcelos *et al.*, 2009). Li *et al.* (2008) assessed the hydrolysis of Penicillin G and its metabolites. Penicillin G was reduced by 41% by hydrolysis. In contrast, the metabolite penillic acid was reduced by only 0.1% by hydrolysis and remained almost constant throughout the test. Junker *et al.* (2006) examined the biodegradability of antimicrobials within WWTPs and reported an approximate 25% mineralisation of benzylpenicillin and 0% mineralisation of ceftriaxone and trimethoprim. These examples highlight the importance of complete compound mineralisation. Mineralisation is considered to be the complete elimination of antimicrobials and their metabolites from the contaminated source [i.e. the total conversion of such contaminants into carbon dioxide, water and inorganic ions (Sires *et al.*, 2007)], whereas processes including photodegradation, hydrolysis and thermolysis are considered primary mineralisation and do not completely remove the compound or, in particular, its toxic effects, from the environment.

Xu *et al.* (2007) analysed the elimination of antimicrobials in sewage treatment plants in China and showed that fluoroquinolones were mostly eliminated from the WWTP effluent. The inability to detect fluoroquinolones in high concentrations in WWTPs can result in the assumption that they are being biodegraded. However, high concentrations of the compounds were found in the sludge, suggesting that they are not biodegraded but concentrated in the sludge. It is important to realise the potential of particle sorption when examining antimicrobial presence in the environment (Xu *et al.*, 2007). Antimicrobial sorption depends greatly on pH, lipophilicity, redox potential and the stereo chemical structure of the antimicrobial agent (Kümmerer, 2008). It is currently unknown how strongly antimicrobials are sorbed to particular matter and under what conditions they are bio-available and active after sorption (Kümmerer, 2008).

Other methods of antimicrobial degradation include hydrolysis, thermolysis and photolysis. Antimicrobials designed for oral intake are generally relatively insensitive to hydrolysis (Andreozzi *et al.*, 2003). Antimicrobials that are sensitive to heat can undergo thermal decomposition (Mendez *et al.*, 2008). Photolysis and photo-degradation are the absorption of solar and/or artificial light (Zepp *et al.*, 1977), which causes molecules to become unstable and degrade resulting in the formation of stable by-products (Doll and Frimmel, 2003). Incomplete photo-degradation can lead to the production of toxic compounds (Gonzalez *et al.*, 2007).

The effectiveness of photolysis, and most methods of pharmaceutical degradation, depends on environmental conditions, such as light intensity and frequency (Hu and Coats, 2007), pH, water hardness (Werner *et al.*, 2006), location, season and shading. Hence, in certain conditions degradation may not occur (Kümmerer, 2008).

Loftin *et al.* (2008) concluded that ionic strength did not significantly affect the degradation of any of the examined antimicrobials and that, in general, hydrolysis rates increased as temperature and pH increased (Loftin *et al.*, 2008). Light exposure can also vary the rate of antimicrobial degradation. Doi and Stoskopf (2000) reported a threefold increase in oxytetracycline photolysis compared with samples tested under dark conditions. Kim and Aga (2007) highlighted the importance of analysing antimicrobials and their degradation products when assessing human health impacts from

antimicrobial residues in the environment (Aldick *et al.*, 2007). The by-products, chemically distinct from the parent compound, potentially exhibit the same, or related, antibacterial activity/toxicity as the parent compound. Under such circumstances, removal or transformation of a parent compound does not equate to loss of potential for toxicity (Kim and Aga, 2007; Kümmerer, 2008). Kim and Aga (2007) also emphasise the importance of analysing the chronic effects of micro-pollutant mixtures. Examination of single compounds may result in a low-risk outcome, but this is not an accurate comparison with actual environmental conditions (Lindberg *et al.*, 2007).

The effectiveness of catalyst addition to improve degradation rates has been assessed by many authors. Chatzitakis *et al.* (2008) investigated the effectiveness of photo-degradation of chloramphenicol in the presence of the catalyst titanium dioxide (TiO_2). Photocatalysis resulted in the complete mineralisation of the compound. Chatazitakis *et al.* (2008) concluded that the fast reduction of the drug activity shows that photocatalysis could be employed to improve degradation of antimicrobials in wastewaters. The use of solar- or ultraviolet (UV)-A-activated TiO_2 is an economic and practical solution to process wastewater containing antimicrobials. However, as other authors have shown, the level of degradation and mineralisation can vary for each antimicrobial. The comparison of photocatalysis and photolysis of sulfamethoxazole and trimethoprim by Abellán *et al.* (2009) highlights this point. Although sulfamethoxazole degradation was improved by the presence of the catalyst TiO_2, the two photo-degradation processes resulted in similar end products for trimethoprim. Giraldo *et al.* (2010) investigated the effects of photocatalysis on oxolinic acid. Under optimal conditions, the substrate and antimicrobial activity were eliminated and the toxicity of the solution was reduced by 60%. However, the compound was not fully mineralised, but only transformed into more highly oxidised by-products. Elmolla and Chaudhuri (2010) investigated the effects of UV/TiO_2 and UV/hydrogen peroxide (H_2O_2)/TiO_2 photocatalysis on amoxicillin, ampicillin and cloxacillin. Although no significant degradation occurred using 300-minute UV irradiation, the addition of H_2O_2 and TiO_2 resulted in complete degradation of all three antimicrobials within 30 minutes, including the mineralisation of organic carbon, nitrogen and sulphur.

There is a need for investigation into the modes of antimicrobial degradation for risk assessment. It is necessary to create very specific guidelines on how to sample and how to report antimicrobials and their metabolites in the environment. Finite acceptable limits are needed for both parent compounds and their metabolites, and toxicity/hazard testing should, if possible, always include the metabolites of the examined compounds. Improved specific classification and limits urgently need to be identified. It is currently not possible to draw conclusions on the effect of multiple antimicrobials degrading at the same time. There may be combined or synergistic effects, with each antimicrobial and its metabolites having different susceptibilities to the modes of degradation. Each environment is different and has a varied composition of antimicrobials (e.g. hospital effluent and general household wastewater).

Investigation into the toxicities of mixtures and different environments is necessary. It is not possible to provide an exact representation of actual environmental conditions using *in vitro* studies. But, by investigating likely environmental conditions, it may be possible to develop precautionary measures and improved methods to encourage the complete mineralisation of antimicrobials in a specific environment.

1.6 Antimicrobial residue removal in wastewater treatment plants

Antimicrobial removal from the wastewater stream is vital to prevent exposure and potential resistance within bacterial populations. Antimicrobial compounds amass in WWTPs and their removal efficiency and degradation times vary enormously. Removal can depend on the nature of the compound, treatment process, age of activated sludge and environmental conditions, such as seasonality (O'Brien and Dietrich, 2004). High levels of rainfall can cause a surge in WWTP influent volume and reduce the WWTP efficiency, or even result in untreated sewage being released directly into the environment (Rauch and Harremoes, 1996). Golet *et al.* (2003) suggested specialised treatment of hospital waste as a possible method of reducing antimicrobial levels in the aquatic environment (Golet *et al.*, 2003). Sedlak and Pinkston (2001) showed that advanced methods for treatment of hospital wastewaters, such as by reverse osmosis, using activated carbon or by ozonation, reduce or eliminate antimicrobials. Reducing sludge spreading is another possible method for decreasing the level of antimicrobials entering the waterways. The disposal of sewage sludge into agricultural fields has

been forbidden in Switzerland since January 2003. Adams *et al.* (2002) have credited oxidation with ozone or chlorine species as having the highest capacity for the removal of antimicrobial agents from surface and spiked distilled waters (Table 1.3). These authors also identified that minimal antimicrobial removal occurs through coagulation, flocculation, sedimentation or excess lime softening, a chemical process that converts calcium and magnesium in water to calcium carbonate and magnesium hydroxide (Adams *et al.*, 2002). The compounds are less soluble and settle by forming flocs, allowing antimicrobial sorption or coprecipitation (Adams *et al.*, 2002). DeWitte *et al.* (2008) provide data on ozonation of ciprofloxacin, which are similar to Adams *et al.* (2002). By the injection of ozone through multiple air streams, DeWitte *et al.* (2008) showed a 95% reduction in ciprofloxacin concentration, through the evaluation of degradation products. Although these methods are deemed substantially successful, for sufficient degradation of the pharmaceuticals (>90%) from wastewater to occur, the ozone concentration has to be equal to the dissolved organic carbon value (Zwiener *et al.*, 2000). Wu *et al.* (2010) examined the effects of ozonation on tetracycline degradation in a WWTP. Tetracycline was hydroxylated after 5 minutes, but the results suggest that ozonation cannot mineralise tetracyclines (Wu *et al.*, 2010). Furthermore, Wu *et al.* (2010) showed that the degradation products of tetracycline had an increased risk of toxicity. Ternes (1998) highlighted the importance of choosing a suitable removal method, as removal efficiency for different methods may vary for different antimicrobials (Tables 1.3 and 1.4). Kümmerer (2008) believes that membranes are one of the most constructive methods for removing antimicrobial residues in WWTPs (Kümmerer, 2008). Membrane filtration techniques include microfiltration, ultra-filtration, membrane bioreactors and nano-filtration using reverse osmosis. Although studies have found that the use of membranes in WWTPs results in both marginal (Clara *et al.*, 2004) and substantial removal (Zuehlke *et al.*, 2006) of antimicrobials (compared with conventional WWTPs), the efficiency of treatment processes seem to depend greatly on the compound in question (Table 1.4). Membrane filtration could be considered as a possible treatment method for wastewaters with large quantities of antimicrobial residues, such as hospital effluent.

1.7 Regulation

Although antimicrobials have been identified in the environment (Zuccato *et al.*, 2005; Feitosa-Felizzola *et al.*, 2009; Galvin *et al.*, 2010), current EU legislation (including the European Union Sewage Sludge Directive 86/278/EEC, the Bathing Water Directive 2006/7/EC and the Dangerous Substances Directive 2006/11/EC) does not include specific regulations on either antimicrobial residues or AMR bacteria. A detailed risk assessment of antimicrobial residues and AMR bacteria is a priority in order to determine if there are major risks to the environment and human health. Specifically, the effect of antimicrobial residues present at levels

Table 1.3. Removal rates of common antimicrobials[a] (present in surface water and groundwater), using conventional drinking water treatment processes from surface and spiked distilled water

Method	Reduction efficiency (%)	Reference
Carbon sorption	57–97	Adams *et al.*, 2002
Chlorination	50–90	Adams *et al.*, 2002
Ozonation	>95	Adams *et al.*, 2002; DeWitte *et al.*, 2008
Ion exchange	21–58	Adams *et al.*, 2002
Ultraviolet photolysis	50–80	Adams *et al.*, 2002
Reverse osmosis	>90	Adams *et al.*, 200
Coagulation	NSR	Adams *et al.*, 2002
Flocculation	NSR	Adams *et al.*, 2002
Sedimentation	NSR	Adams *et al.*, 2002
Combined processes	<10 to >90	Ternes, 1998; Joss *et al.*, 2005

a Carbadox, sulfachlorpyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfathiazole, trimethoprim, ciprofloxacin (ozonation), roxithromycin, sulfamethoxazole and N-acetyl-sulfamethazole, and the pharmaceuticals carbamazepine, diclfenac, ibuprofen and naproxen.
NSR, no significant removal.

Table 1.4. Removal efficiencies of membrane filtration and standard WWTP processes on pharmaceuticals

Compound	MBR removal (%)	STP removal (%)	MBR reference	STP reference
Erythromycin (antimicrobial)	9	0	Kim and Aga, 2007	Castigilioni *et al.*, 2005
Sulfamethoxazole (antimicrobial)	64	24	Kim and Aga, 2007	Castigilioni *et al.*, 2005
Ibuprofen (antiphlogistic)	99	55	Kim and Aga, 2007	Castigilioni *et al.*, 2005
Carbamazepine (anti-epileptic agent)	0	0	Kim and Aga, 2007	Castigilioni *et al.*, 2005
Naproxen (antiphlogistic)	36	0–80	Kim and Aga, 2007	Nakada *et al.*, 2006
Triclosan (antimicrobial)	66	45–90	Kim and Aga, 2007	Nakada *et al.*, 2006
Estradiol (hormone)	90	99	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006
Estrone (hormone)	90	99	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006
Ethinyl estradiol (hormone)	80	95	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006
Phenazone (analgesic)	<15	60–70	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006
Propyphenazone (analgesic)	<15	60–70	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006
Formylaminoantipyrine (analgesic)	<15	60–70	Zuehlke *et al.*, 2006	Zuehlke *et al.*, 2006

MBR, membrane filtration; STP, sewage treatment plant.

less than the clinical breakpoint for resistance and the effect of WWTP processing and possible enrichment of antimicrobial resistance (prevalence) require careful consideration. In-depth analyses of the effect of specific treatment processes on the presence of antimicrobial residues and AMR bacteria are warranted. The probability of human exposure to antimicrobial residues and AMR bacteria as a result also requires further study in order to prepare adequate guidelines or possible legislation.

1.8 Risk assessment

There are many risk assessment models currently in use (Table 1.5). However, the suitability of a specific model for a particular application is difficult to identify (Keller, 2006). Given global concern surrounding antimicrobial resistance and the impact that the presence of antimicrobials in the environment could have on the emergence and dissemination of AMR bacteria, there is an urgent need to evaluate the risks that antimicrobial residues and AMR bacteria present in the environment pose. Risk assessment is a very important tool used to assess the potential risk of antimicrobials to human health.

1.8.1 Environmental risk assessment

The USEPA has listed models that are commonly in use today for the examination of pesticides in groundwater, surface water, bathing water, drinking water and soil. Keller (2006) has identified the models used to assess "down-the-drain" chemicals, with the Mackay models and the European Union System for the Evaluation

Table 1.5. Models used for the risk assessment of chemicals in the environment (from consumer use/ consumption)

Model	Examines	Advantage	Disadvantage	Reference
Mackay	Chemicals	Can be used as a basis for other models	Requires extensive data	Keller, 2006
EUSES	Chemicals	High accuracy; endorsed by the European Commission	Comprises three individual models; requires extensive data	Keller, 2006; Kümmerer, 2008
PhATE	Pharmaceuticals	High accuracy	Requires extensive data, data quality and input data; examines individual catchments	Murphy *et al.*, 2007; Kümmerer, 2008
GREAT-ER	Pharmaceuticals	High accuracy; examines entire catchment areas	Require extensive data, data quality and input data	Schowanek and Webb, 2002; Keller, 2006; Kümmerer, 2008

GREAT-ER, Geo-referenced Regional Exposure Assessment Tool for European Rivers; PhATE: *Pharmaceutical* Assessment and Transport Evaluation

of Substances (EUSES) model being most commonly used (Keller, 2006). The Mackay models identify the fate of a chemical within a particular media, such as air, soil or water, and estimate concentrations within each environmental zone (Keller, 2006). EUSES, created by the European Commission, is a risk assessment model that performs a "worst case" quantitative assessment using reduced data sets. As with the Mackay models, EUSES analyses environmental compartments and identifies chemical concentrations within those sections. As indicated earlier, the SCC have also developed a qualitative risk assessment strategy where each compound is classified using a PBT index.

1.8.2 European Medicines Agency (EMEA) guidelines

Risk assessment is required for both new drugs and existing drugs that require further analysis. The European Medicines Agency (EMEA) provides guidelines on the environmental risk assessment of medicinal products for human use (EMEA, 2006). The guidelines are based on a tiered system with acceptable environmental concentration levels of pharmaceuticals that determine entry into the next phase of assessment. Certain pharmaceuticals, regardless of the quantity released into the environment, will be assessed if they are deemed dangerous to the environment, such as lipophilic substances or endocrine disrupters. However, as Bound and Voulvoulis (2005) discussed, the calculations do not take into account the amount of active ingredient in the pharmaceutical product or the contribution to the overall levels by other pharmaceuticals with the same active ingredient; this leads to an "underestimation of the overall environmental burden" (Swayne, 2004). The standard bioassays used for toxicity are not specific and do not target the mode of action of the active ingredient, which may also lead to dangerous pharmaceutical products being deemed safe (Swayne, 2004). Each pharmaceutical is individually tested, whereas in the environment it is likely that organisms will be exposed to many compounds simultaneously. Therefore, the actual environmental effect could be unknown, even if the EMEA-recommended methodology is used.

The short-term nature of the toxicity screening is another concern relating to the significance of the overall data (Koschorreck *et al.*, 2002). In the environment, antimicrobials may be discharged continuously into the waterways and, without the examination of the effects of long-term toxicity, the actual effect on the environment will be unidentified (Hernando *et al.*, 2006). Bound and Voulvoulis (2005) have proposed a framework to overcome the problems associated with the EMEA guidelines; however, as they have stated, retaining some of the standardised tests is important in order to maintain uniformity and reproducibility (Swayne, 2004). This would enable the comparison of results obtained either from different pharmaceutical products or by different authors. Hernando *et al.* (2006) assessed a risk quotient method as a novel approach to estimate the environmental risk of the pharmaceuticals that are most frequently detected in wastewater effluents, surface waters and sediments. Their major concern was antimicrobials at low concentrations (ng/l or µg/l levels) potentially causing resistance in natural bacterial populations and inducing toxic effects in humans. The predicted environmental concentrations (PECs) were compared with the predicted no effect concentrations (PNECs) for risk assessment analysis. The PNEC is calculated based on the mean EC_{50} value, which is obtained from a set of acute toxicity tests (on algae, daphnia and fish). The resulting PNEC value is compared with a PEC or measured environmental concentration (MEC) value to estimate risk. The PEC/PNEC value determines the risk factor, which is classified as low (>0.01 to<0.1), medium (>0.1 to<1.0) or high (>1.0), and determines the need for further examination. Antimicrobials were analysed using this method and identified as having a high environmental risk in WWTP effluent and a medium risk in sediments (i.e. the PEC/PNEC value was >1.0 and between 0.1 and 1.0, respectively). Hernando *et al.* (2006) deem that further research into the potential toxicity of drug residues is a major area of importance and they believe that there is a lack of data on the effects of drug residues on the environment (Hernando *et al.*, 2006). Schwab *et al.* (2005) used a similar method to Hernando *et al.* (2006). They estimated the PNEC value and compared it with MEC and PEC values. Based on the PNEC/MEC ratio, Schwab *et al.* (2005) concluded that there was no significant risk to human health as a result of the presence of antimicrobial residues in surface or drinking water. However, they did find that selection for resistance may occur at lower levels, resulting in direct toxic effects. In considering antimicrobial agents and toxicity testing, the recommended PNEC/PEC analysis may be of limited relevance for many antimicrobial agents. PNECs are based on EC_{50} values for cyanobacteria (algae). Cyanobacteria are oxygenic phototrophic organisms.

They are phylogenetically distinct from the heterotrophic bacteria that are affected by antimicrobials and can differ in their reproduction, growth rate and metabolism (Madigan *et al.*, 2008). Although some cyanobacteria produce toxins that can have negative human impacts (skin infections and occasionally bursitis and peritonitis), they are not recognised as significant human pathogens. Hence, the toxicity assessment could be deemed inappropriate.

Currently, there are two complex catchment scale models [P*h*ATE™ (Pharmaceutical Assessment and Transport Evaluation) and GREAT-ER (Geo-referenced Regional Exposure Assessment Tool for European Rivers)] used to assess pharmaceuticals in the environment. They are aimed at evaluating water catchments. The only limiting factors in their use are the extensive data requirements, the level of data quality required and the availability of appropriate input data on environmental fate (Kümmerer, 2008).

The P*h*ATE model was created to predict concentrations of active pharmaceuticals, which result from patient consumption of medicines, in surface waters in the USA (Murphy *et al.*, 2007). It is based on particular watersheds in the USA, chosen to be representative of most watersheds in the USA. P*h*ATE uses a mass balance to model PECs in water catchment areas. It estimates the levels entering the environment based on population data and pharmaceutical compound use per capita (i.e. P*h*ATE does not account for veterinary pharmaceuticals, septic tank discharges or leaching as a result of sludge spreading). P*h*ATE accounts for loss of pharmaceuticals by means of human metabolism and through reduction in a WWTP. Natural biodegradation processes are not currently included, but the format is available (Kümmerer, 2008). The P*h*ATE model is suitable for assessing environmental exposure during an environmental risk assessment, such as levels of ciprofloxacin in WWTP sludge. The main drawback associated with using P*h*ATE is the need for separate evaluation of individual water catchments. Because of this, resulting PEC values are expected to vary substantially (as a result of variability among catchments). GREAT-ER is an exposure assessment model, developed as an aquatic chemical exposure prediction tool for use within environmental risk assessment schemes and river basin management (Schowanek and Webb, 2002). The software is used to calculate the distribution of PECs of consumer chemicals in surface waters, for both individual river stretches and for entire catchment areas (from hospital effluent to drinking waters), unlike the P*h*ATE model, which can analyse only individual catchments (Schowanek and Webb, 2002). Better predictions and a higher tiered risk assessment can be obtained by using the GREAT-ER model, as it assesses geographically referenced "real" data sets instead of average or generic values (Keller, 2006). GREAT-ER generates distribution concentrations using multiple Monte Carlo simulations; this is used for each zone of the catchment (Kümmerer, 2008). The results from individual simulations (e.g. concentration data) are collected and assessed to form a distribution. This can be used to calculate various percentiles (e.g. 95% of the time, water catchments will have less than 10 µg/l ciprofloxacin). Probability distributions can then be created from the collected data for single catchments (as in the P*h*ATE model), combinations of catchments or the total catchment area (Kümmerer, 2008). GREAT-ER has been used for exposure assessments of pharmaceuticals across Europe (Schowanek and Webb, 2002). Both P*h*ATE and GREAT-ER can be altered or refined to include specific parameters related to individual areas of study, such as biodegradation rate or WWTP removal rates. The parameters can be assessed as either single values or as ranges of values within an uncertainty analysis (i.e. Monte Carlo simulations for GREAT-ER) (Kümmerer, 2008).

Cunningham *et al.* (2009) used P*h*ATE and GREAT-ER to evaluate the risk of pharmaceutical compounds to human health through potential environmental exposure via drinking water and fish consumption. They calculated PECs and PNECs and assumed that if the PEC/PNEC value was less than 1, there was no risk to human health from consuming drinking water or fish containing trace levels of antimicrobial compounds. Although Cunningham *et al.* (2009) identified no appreciable risk, they believe that water will continue to be a concern (as a direct route for human exposure to pharmaceutical residues), specifically because of uncertainties in the ability to assess the effects of continuous exposure to a mixture of compounds (including the effect on immuno-compromised individuals) (Cunningham *et al.*, 2009).

Although there is limited available literature on the assessment of antimicrobial residues in specific circumstances [e.g. in drinking water or WWTPs, levels resulting from down the drain disposal (Ternes *et al.*, 2002), and levels in bathing water after release through WWTPs], there is a common method used to examine

the potential risk of a substance to the environment. This method consists of comparison of the PEC and PNEC values (Fent *et al.*, 2006), which has been used to assess many environmental zones (Kemper, 2008; Kümmerer, 2008; Cunningham *et al.*, 2009). Because of the lack of experimental data on pharmaceutical ecotoxicology, this method of predicting the environmental concentrations has become very important, but also leads to uncertainties (Kümmerer, 2008). Through the evaluation of current risk assessment studies, qualitative, quantitative, deterministic and stochastic methods have been used to assess the effects of wastewater contaminants (antimicrobials) and their risk on the environment and human health (Table 1.6). Focazio *et al.* (2008), during their examination of pharmaceuticals and other organic wastewater contaminants in the USA, demonstrated that qualitative analysis can be helpful in the first stages of analysis. They used this method to graphically represent their first findings, assigning un-quantified concentration indicators to their data (i.e. detected concentrations were not quantified but were reported). Quantitative analysis requires more in-depth analysis but, consequently, provides more statistically stable findings. The selection of the correct risk assessment strategy depends greatly on government requirements and the desired level of detail (e.g. whether or not long-term monitoring is required).

1.8.3 Human health risks risk assessment

The FDA recommends a qualitative approach for evaluating the safety of antimicrobials in relation to the impact of bacteria on human health (FDA, 2002). The recommended assessment is based on World Organisation for Animal Health guidelines and, at each stage defined in the guidelines, an assessment is carried out to determine whether or not the risk is low, medium or high; the stages are release assessment, exposure assessment, consequence assessment and risk estimation. The EMEA (2006) and Irish EPA (2006) recommend applying this method to most risk assessment cases (i.e. using single values rather than an array of data or probability distributions for the input). Hurd *et al.* (2004) chose a deterministic quantitative model to analyse the risk of two macrolide antimicrobials. They developed a deterministic model as it creates precise calculations and, in addition, it allows for the modification of the analysis as more information or improved data become available. They produced a unique farm-to-patient deterministic analysis using extensive available scientific and governmental numerical data. The model allows probability analysis to occur at each stage of the process (provided that the necessary data are available). Their final result was an estimation of the hazard (the expected illness per capita per year in the USA for which human antimicrobial treatment is presumed to fail or be compromised by the presence of resistant bacteria resulting from the administration of antimicrobials to food animals). They successfully identified a low risk for human treatment failure from the administration of the macrolide antimicrobials tylosin and tilmicosin to animals, with an annual probability of human treatment failure of less than 1 in 10 million and less than 1 in 3 billion, respectively.

The Irish EPA (2006) guidance on environmental liability risk assessments examines a proposed hazard and assigns a risk category. Depending on the severity of the risk, further assessment, and ultimately restoration/necessary action, is instigated (EPA, 2006). The recommended procedure includes risk identification (including the identification of the process, hazard and environmental receptors), assessment of risks, risk ranking (EPA financial provision depends on severity), risk prevention, and mitigation and ongoing risk management. The application of a more a comprehensive risk analysis (such as Monte Carlo simulation) may be necessary to obtain a more accurate assessment of health and

Table 1.6. Model type and risk assessment method used for assessment of human and environmental exposure to pharmaceutical residues

Model	Risk assessment	Compound	Exposure	Compartments	Reference
Quantitative	Deterministic	Macrolides	Human health	Use in food animals	Hurd *et al.*, 2004
Risk quotient	Deterministic	Pharmaceutical residues	Environment	WWTP effluent, surface waters and sediments	Hernando *et al.*, 2006
Qualitative and quantitative	Stochastic	Pharmaceuticals	Human health	WWTP effluent and groundwater, surface water and drinking water	Focazio *et al.*, 2008
Qualitative	Deterministic	New antimicrobials	Human health	Drug use/application	FDA, 2002

ecological risks associated with antimicrobials in the environment (Kim and Aga, 2007).

At present, there are no regulations governing the surveillance of antimicrobial agents and AMR organisms in hospital effluent, effluent from WWTPs or biosolids used in agriculture. It is evident from studies conducted worldwide to date that antimicrobial agents and AMR bacteria are being emitted in hospital effluent, in effluent from WWTPs and in sewage sludge. Given the serious public health threat posed by antimicrobial resistance, it is important to investigate the potential role of hospital effluent in amplifying the emergence and spread of antimicrobial resistance and to assess the potential associated risk to human health. Research into the occurrence, fate, effect and risks associated with the presence of antimicrobial agents and AMR bacteria in such environments and the impact on human health is needed to inform policymakers.

The findings of the literature review presented in this section have been published by Harris *et al.* (2012b).

2 Project aims and objectives

The overall objective of this project was to provide information on the contribution of hospital effluent to levels of quinolones/fluoroquinolones and AMR *E. coli* in urban wastewater, and the potential for these substances/organisms to persist through the steps in the treatment of wastewater and the land application of biosolids. The results of the quantification steps informed the development of a risk assessment approach for sanitary authorities/regulatory authorities to assess human exposure to quinolones/fluoroquinolones and AMR *E. coli* in recreational water and from contact with biosolids spread on agricultural land. This will inform decisions on requirements for specific monitoring or treatment of hospital effluent and general urban effluent to manage the risks of AMR bacteria in effluent.

Specific aims include:

1. To quantify the impact of hospital discharge on the number of AMR organisms (*E. coli*) and the concentration of quinolones/fluoroquinolones in urban wastewater.
2. To quantify/estimate the survival of AMR *E. coli* in each step of the wastewater treatment process up to the discharge stage.
3. To quantify/estimate the persistence/removal of antimicrobial agents (specifically quinolones/fluoroquinolones) in each step of the wastewater treatment process up to the discharge stage.
4. To use the knowledge gained, from specific aims 1, 2 and 3, to develop a risk assessment model of human exposure to antimicrobial agents (quinolones/fluoroquinolones) and AMR organisms (*E. coli*) in recreational water exposed to discharges of hospital effluent.
5. To quantify/estimate the survival of AMR *E. coli* in sewage sludge and treated sludge (biosolids).
6. To quantify/estimate the persistence/removal of antimicrobial agents (specifically quinolones/fluoroquinolones) in sewage sludge and treated sludge (biosolids).
7. To use the knowledge gained, from specific aims 4 and 5, to develop a risk assessment model of human exposure to AMR organisms (*E. coli*) and antimicrobial agents (quinolones/fluoroquinolones) as a result of landspreading of biosolids produced in a WWTP that treats wastewater streams that include effluent from a hospital.

3 Quantification of antimicrobial-resistant bacteria and antimicrobial residues

3.1 Overview

A previous EPA-funded project detected antimicrobials and AMR *E. coli* in effluent samples from various water sources (Cormican *et al.*, 2012). Quinolone-like activity was detected in hospital effluent and in urban effluent collected downstream from a hospital. During this previous project, the Colilert® (IDEXX, Technopath, Limerick, Ireland) method was successfully adapted with the inclusion of antimicrobial agents (Cormican *et al.*, 2012) to detect AMR *E. coli* in aqueous samples. The levels of *E. coli* and *E. coli* resistant to seven different antimicrobial agents in all the effluent, seawater and sludge samples collected as part of this project were examined using this adapted Colilert system.

This section will outline the work completed to quantify AMR *E. coli* and antimicrobial residues in hospital effluent, municipal wastewater effluent, sludge generated by the wastewater treatment process and biosolids generated after sludge treatment. The fate of AMR *E. coli* and antimicrobial residues through the various steps of the secondary wastewater treatment process was also assessed. Data generated in this work were used to validate the risk assessment models described in Sections 4 to 8.

3.2 Methods

3.2.1 Sample collection

3.2.1.1 Hospital effluent

Hospital effluent samples were collected from two hospitals (Hospital 1 and Hospital 2). Hospital 1 is a 639-bed hospital with the capacity to treat 27,000 patients annually. Effluent samples were collected from the foul pump house of the hospital which receives wastewater from only the hospital. The hospital effluent is released into the main municipal sewerage system and is treated with all of the other city wastewater in the local WWTP. Fifteen effluent samples were collected in total from this hospital sewer on a weekly basis over a period of 9 months. Hospital 2 is a smaller hospital with a 136-bed capacity. Hospital 2 is located in a smaller town approximately 60 km from Hospital 1. Effluent samples were collected from the main sewerage pipe leaving the hospital on five sample dates.

3.2.1.2 Municipal effluent

Municipal effluent samples were collected from the closest available points in the municipal sewerage system, upstream and downstream from both hospitals. Effluent samples collected upstream and downstream of Hospital 1 were sampled within 10 minutes of each other on 15 separate occasions. Effluent samples collected upstream and downstream of Hospital 2 were collected within 10 minutes of each other on five separate occasions.

3.2.1.3 Wastewater treatment plant effluent

Effluent samples were taken from two WWTPs (WWTP 1 and WWTP 2). WWTP 1 receives municipal wastewater from a large city which includes effluent released from Hospital 1. The plant is designed to treat effluent for 91,600 population equivalent (PE), but generally operates above capacity and has been granted an upgrade licence from the EPA set at 170,000 PE. This facility treats effluent from a city with a population of 72,000 people and includes four major hospitals. Effluent and sludge samples were collected at four points, dispersed throughout the plant, at various stages of the treatment process. Fifteen effluent samples were collected, on separate sampling dates, at four points in the treatment plant in order to collect the following: (1) raw influent, (2) post-return effluent, (3) primary effluent and (4) final effluent. Raw influent samples were composite samples taken over a 24-hour period; at this point, effluent point has been screened to remove large debris. Post-return effluent samples were collected as grab samples; the effluent at this stage has undergone biological aeration treatment and is being recycled back through the plant to undergo primary biological treatment again. Primary effluent samples were collected as grab samples; the effluent at this stage has undergone primary biological

treatment twice and is pumped to the last/secondary stage of treatment. Final effluent samples were collected as composite samples over a 24-hour period. The final effluent has undergone secondary treatment/final settlement and was sampled just before being pumped out to the receiving water body. WWTP 2 serves a large town with a population of approximately 6000. The plant operates at an average treatment rate of 21,000 PE. The effluent entering WWTP 2 is not influenced by hospital effluent. Fifteen effluent samples were taken from this treatment plant on separate sampling occasions. The sample types (composite or grab) and sample points were the same for WWTP 1 as for WWTP 2, with the exception of post-return effluent which was not sampled from WWTP 2.

3.2.1.4 Wastewater treatment plant sludge

Sludge generated from the wastewater treatment process was sampled from WWTP 1 and WWTP 2, as described above. Fifteen sludge samples (5 g each) were collected, on separate sample dates, from WWTP 1 at two points, after different stages of treatment: (1) primary sludge after primary treatment and (2) dried sludge after anaerobic digestion and drying.

Five sludge samples were collected, on separate sampling dates, at two points from WWTP 2: (1) primary sludge after primary treatment and (2) dried sludge after aerobic digestion and drying.

3.2.1.5 Seawater

Seawater samples were collected across the receiving water body of treated effluent from WWTP 1, which treats effluent from Hospital 1. Five points were sampled on five occasions. Four of the samples, SW 1–4, were collected offshore. Seawater was also sampled at WWTP 1 (SW 5). Grab samples (1 l each) were collected from each sampling point, with the exception of the inflow and outflow points of WWTP 1, at which composite samples were collected. All samples (water, effluent and sludge) were immediately transported to the laboratory and stored at 4°C before processing.

3.2.2 Enumeration of E. coli *and antimicrobial-resistant* E. coli

Stock solutions of antimicrobial agents were prepared (Table 3.1). The Colilert test returns results within defined limits [$< 1 \times 10^5$ to 2.4×10^5 MPN (most probable number)/100 ml]. A range study was carried out on all sample types to determine the dilution factor of best fit to return results within the detectable limits of the Colilert system. The test sample dilution factors used for all effluent and sludge samples to detect *E. coli* within readable ranges were grouped depending on the antimicrobial being assessed, as follows: 1 in 10,000 for ampicillin, 1 in 10,000 for streptomycin, 1 in 100 for cefoxitin, 1 in 100 for cefotaxime, 1 in 1000 for tetracycline, 1 in 10,000 for sulphonamides and 1 in 1000 for ciprofloxacin (Table 3.1). Seawater samples were diluted 1 in 10 due to salinity. Sludge samples were prepared by adding 1 g of sludge to 99 ml of sterile water and mixed by vigorous shaking for 2 minutes; shaking was repeated three times. The modified Colilert method for detection of AMR bacteria was carried out as previously described (Galvin *et al.*, 2010).

3.2.3 Detection of antimicrobial residues

The presence of penicillins and cephalosporins, macrolides, quinolones, tetracyclines and aminoglycosides was determined using a biological assay as previously described (Galvin *et al.*, 2010).

Table 3.1. Concentrations of antimicrobial agents used

Antimicrobial agent	Antimicrobial stock concentration (mg/ml)	Volume of stock used (ml)	Final concentration (µg/ml)	Volume of test sample (µl)	Dilution factor
Ampicillin	1	1.6	16	10	1 in 10,000
Streptomycin	1	1.6	16	10	1 in 10,000
Cefoxitin	1	1.6	16	1000	1 in 100
Cefotaxime	0.1	1	2	1000	1 in 100
Tetracycline	1	0.8	8	100	1 in 1000
Sulphamethoxazole	10	2.56	256	10	1 in 10,000
Ciprofloxacin	200	1	2	100	1 in 1000

3.3 Results

3.3.1 *Enumeration of* E. coli *and antimicrobial-resistant* E. coli

3.3.1.1 *Hospital effluent*

Although there is a significant difference in the size of the two hospitals, the mean number of *E. coli* per 100 ml recovered from both hospitals was of the same order of magnitude ($\times 10^6$) (Table 3.2). *E. coli* resistant to ampicillin, streptomycin, sulphonamides and ciprofloxacin were detected in all hospital effluent samples collected. *E. coli* resistant to ampicillin were present in the highest proportions in effluent from both hospitals, reaching 80% on one occasion in effluent from Hospital 1 and 25% on one occasion in effluent from Hospital 2. In contrast, *E. coli* resistant to cefoxitin were not detected on any occasion in effluent from Hospital 2 and were the lowest proportion (Table 3.2) of AMR *E. coli* detected in effluent from Hospital 1. Overall, the proportions of *E. coli* resistant to all antimicrobials were considerably higher in effluent from Hospital 1 than from Hospital 2 (Table 3.2).

3.3.1.2 *Municipal effluent*

The mean MPN/100 ml of *E. coli* was higher in effluent upstream of Hospital 1 than in effluent upstream of Hospital 2 (1.7×10^6 MPN/100 ml vs 8.7×10^5 MPN/100 ml). Ampicillin-resistant *E. coli* accounted for the highest mean percentage of AMR

Table 3.2. The mean *E. coli* MPN/100 ml and mean proportion of AMR *E. coli* in effluent from Hospitals 1 and 2

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Hospital 1	HE1-1	1.43×10^6	14.07	35.96	0.01	0.02	0	66.27	15.38
	HE1-2	1.85×10^6	80.04	5.41	5.41	0	2.23	39.91	39.20
	HE1-3	1.42×10^6	29.08	21.41	7.04	6.97	14.15	51.97	19.54
	HE1-4	1.43×10^6	14.08	35.96	14.08	21.18	0	66.27	0.91
	HE1-5	1.48×10^6	9.87	15.74	0.27	0.36	4.31	46.45	11.34
	HE1-6	5.79×10^6	7.85	7.57	0.18	0.75	5.94	13.10	5.95
	HE1-7	1.48×10^6	9.87	15.74	0.27	0.36	4.31	46.45	8.77
	HE1-8	4.88×10^6	12.54	11.34	0.26	1.33	14.06	14.61	7.48
	HE1-9	2.25×10^6	48.29	8.96	22.88	23.10	6.94	13.69	5.32
	HE1-10	3.04×10^6	2.08	3.26	0.13	0.07	6.17	11.53	4.07
	HE1-11	1.73×10^6	70.65	62.91	0.10	0.07	5.78	6.29	2.31
	HE1-12	1.19×10^6	26.96	24.90	0.12	0.07	6.03	9.63	3.44
	HE1-13	1.60×10^6	75.88	19.05	0.53	0.26	2.09	6.13	0.84
	HE1-14	3.98×10^5	26.96	24.90	0.12	0.07	6.03	9.63	3.44
	HE1-15	1.73×10^6	70.65	6.29	0.16	0.70	5.78	6.29	0.26
	Mean	–	33.26	19.96	3.44	3.95	6.45	27.22	8.55
Hospital 2	HE2-1	3.65×10^6	19.63	4.88	0	2.37	1.57	2.24	11.30
	HE2-2	2.76×10^7	25.07	0.86	0	0.22	0.12	0.25	0.73
	HE2-3	5.17×10^6	14.91	2.87	0	1.33	0.67	2.21	1.93
	HE2-4	4.61×10^6	14.24	2.38	0		0.71	1.70	4.38
	HE2-5	6.87×10^6	10.59	1.11	0	0.39	1.00	1.10	2.94
	Mean	–	16.89	2.42	0.00	1.08	0.81	1.50	4.26

AMP, ampicillin; CEF, cefotaxime; CIP, ciprofloxacin; FOX, cefoxitin; HE1, effluent from Hospital 1; HE2, effluent from Hospital 2; STR, streptomycin; SUL, sulphonamides; TET, tetracycline.

E. coli upstream of both hospitals. Cefoxitin- and cefotaxime-resistant *E. coli* accounted for the lowest proportions, 1% and 2%, respectively, upstream of Hospital 1 and upstream of Hospital 2; cefotaxime-resistant *E. coli* accounted for 0.01% and cefoxitin resistant *E. coli* were not detected on any sample date. Upstream of Hospital 2, 15% of *E. coli* were resistant to ciprofloxacin whereas upstream of Hospital 1 only 9% were resistant to ciprofloxacin. This is the only incidence of a higher proportion of *E. coli* resistant to any of the antimicrobials in effluent upstream of Hospital 2 than upstream of Hospital 1. Overall, effluent downstream from Hospital 2 contained lower levels of total *E. coli* than effluent downstream of Hospital 1 (orders of magnitude of $\times 10^5$ MPN/100 ml vs $\times 10^6$ MPN/100 ml). As observed with upstream samples, ampicillin-resistant *E. coli* accounted for the highest mean proportions (30% for effluent downstream of Hospital 1 and 27% for effluent downstream of Hospital 2) of AMR *E. coli* in the effluent downstream from both hospitals. Cefoxitin- and cefotaxime-resistant *E. coli* accounted for the smallest mean proportions of AMR *E. coli* (1% and 3%, respectively) in effluent downstream from Hospital 1 and were not detected in effluent downstream of Hospital 2 on any sample occasion. Interestingly, tetracycline-resistant *E. coli,* which accounted for a mean proportion of 13% of AMR *E. coli* in effluent downstream of Hospital 1, were not detected in effluent downstream of Hospital 2 on any sample date. A higher proportion of AMR *E. coli* resistant to cefotaxime and ciprofloxacin in effluent downstream of Hospital 1 than in effluent upstream of Hospital 1 was observed (Table 3.3).

An increase in the proportion of *E. coli* resistant to ampicillin was observed between effluent from upstream of Hospital 2 and effluent from downstream of Hospital 2; cefoxitin- and cefotaxime-resistant *E. coli* proportions remained the same; and decreases were observed for mean proportions of streptomycin-, tetracycline-, sulphonamide- and ciprofloxacin-resistant *E. coli.*

3.3.1.3 Wastewater treatment

The mean *E. coli* MPN/100 ml and mean proportion of AMR *E. coli* in raw influent from WWTPs 1 and 2, post-return effluent from WWTP 1, primary effluent from WWTPs 1 and 2, and final effluent from WWTPs 1 and 2 are outlined in Table 3.4. Overall, the total number of *E. coli* in raw influent from WWTP 2 was higher than the total number of *E. coli* in raw influent from WWTP 1 (in the order of $\times 10^6$ MPN/100 ml vs $\times 10^7$ MPN/100 ml). The proportions of *E. coli* resistant to ampicillin and streptomycin were higher in raw influent from WWTP 2, the proportions of ciprofloxacin were the same in both treatment plants and raw influent from WWTP 1 had higher proportions of sulphonamide- and tetracycline-resistant *E. coli. E. coli* resistant to cefoxitin and cefotaxime were detected in only raw influent from WWTP 2 on five sample dates. Post-return effluent was sampled only from WWTP 1. *E. coli* resistant to all antimicrobials were detected with means ranging from 24% for tetracycline to 1% for cefoxitin. The overall mean *E. coli* MPN/100 ml in post-return effluent was one order of magnitude higher than the overall mean *E. coli* MPN/100 ml in the raw influent. Primary effluent samples were taken at both treatment plants; the mean *E. coli* MPN/100 ml in primary effluent from WWTP 1 was 10-fold higher than in primary effluent from WWTP 2. In contrast, the mean *E. coli* MPN/100 ml was higher in raw influent from WWTP 2 than in raw influent from WWTP 1. Ampicillin- and tetracycline-resistant *E. coli* were present in the higher mean proportions in primary effluent from WWTP 2 than from WWTP 1: 27% versus 17% and 19% versus 14%, respectively. Consistent with previous effluent samples from WWTP 2, cefoxitin- and cefotaxime-resistant *E. coli* accounted for the lowest proportions of AMR *E. coli* in primary effluent from WWTP 1 (Table 3.4). The total number of *E. coli* in the final effluent from both treatment plants was similar: 6.4×10^5 MPN/100 ml for WWTP 1 and 3.0×10^5 MPN/100 ml for WWTP 2. The highest proportions of AMR *E. coli* were in final effluent from WWTP 2: 28% of AMR *E. coli* from this treatment plant were ampicillin resistant and 25% were streptomycin resistant. Higher proportions of *E. coli* resistant to cefoxitin (9% vs 0%) and tetracycline (5% vs 3%) were found in final effluent from WWTP 2 than in final effluent from WWTP 1. This is a significant finding as no cefoxitin-resistant *E.* coli were observed in raw influent or primary effluent at either treatment plant. The proportions of cefotaxime-resistant *E. coli* were the same in the final effluent from both treatment plants (1%), but the proportions of sulphonamide- and ciprofloxacin-resistant *E. coli* were higher in final effluent from WWTP 1 (Table 3.2). The proportion of ciprofloxacin-resistant *E. coli* in final effluent from WWTP 2 was 1%, which represents a significant decrease from previous stages of effluent treatment (e.g. 4% in primary effluent from WWTP 2).

Table 3.3. The mean *E. coli* MPN/100 ml and mean proportion of AMR *E. coli* in effluent from upstream and downstream of Hospitals 1 and 2

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Upstream of Hospital 1	UH1-1	1.21×10^6	80.84	8.26	0	0.01	0	0	19.64
	UH1-2	5.16×10^5	39.15	39.15	0	0	5.93	10.00	5.08
	UH1-3	7.24×10^5	87.29	27.76	0	0	13.67	13.81	1.67
	UH1-4	1.18×10^5	53.67	71.67	0.70	1.72	43.82	25.99	0
	UH1-5	2.33×10^5	36.16	4.28	0.04	5.42	36.95	47.13	10.19
	UH1-6	2.56×10^5	38.26	47.32	0.08	1.58	39.55	33.61	4.78
	UH1-7	2.18×10^5	18.74	18.74	0.23	3.71	37.72	39.41	12.01
	UH1-8	3.05×10^5	31.76	5.23	0.03	1.31	39.70	32.09	3.61
	UH1-9	2.62×10^5	40.75	11.67	7.59	5.46	3.56	5.10	10.13
	UH1-10	7.24×10^5	13.81	7.20	0.01	0.28	0.47	11.88	1.52
	UH1-11	1.66×10^6	67.30	57.61	0	0	14.54	14.54	8.53
	UH1-12	4.87×10^5	17.32	4.14	0.21	0.17	1.72	22.57	29.13
	UH1-13	1.66×10^6	30.71	4.15	0.02	0.06	0.41	0.45	10.13
	UH1-14	5.73×10^5	19.00	19.00	3.52	0.02	0.15	1.46	24.78
	UH1-15	1.66×10^7	6.73	6.73	5.76	0	0	1.45	14.54
	Mean	1.71×10^6	38.77	21.10	0.89	1.80	17.21	21.13	8.53
Upstream of Hospital 2	UH2-1	8.26×10^5	2.16		0.02	12.67	15.51		12.11
	UH2-2	7.62×10^5	3.11		0.02	2.95	15.46		26.53
	UH2-3	8.78×10^5	1.69		0.23	5.59	13.31	11.39	11.39
	UH2-4	8.55×10^5	1.29		0.02	2.61	2.61		
	UH2-5	1.06×10^6	0.72		0.01	8.15	1.21	19.01	
	Mean	8.77×10^5	16.67	1.79		0.06	6.39	9.62	15.20
Downstream of Hospital 1	DH1-1	2.43×10^6		4.11	0.01	0.00	8.30	8.30	25.59
	DH1-2	5.21×10^5	19.19	19.19			19.19	79.27	23.59
	DH1-3	1.31×10^6	56.34	7.63	0.08	0.01	7.63	23.36	2.75
	DH1-4	1.09×10^5	78.24	7.90	1.39	2.50	3.76	47.38	1.85
	DH1-5	1.21×10^5	43.02	25.27	0.43	8.16	41.59	51.69	57.54
	DH1-6	9.79×10^4	20.63	20.53	1.41	3.87	6.46	41.78	12.37
	DH1-7	3.36×10^5	25.63	15.26	0.22	3.49	11.72	22.21	7.81
	DH1-8	1.09×10^5	18.55	9.09	1.16	3.96	7.90	58.03	14.35
	DH1-9	1.45×10^5	74.33	35.61	0.50	0.82	3.78	2.11	2.48
	DH1-10	2.02×10^4				11.64	74.26	97.38	
	DH1-11	3.00×10^7	2.11	1.74	0.00	1.02	0.67	0.67	1.07
	DH1-12	2.72×10^6	58.12	0.37	4.46	0.23	0.16	13.93	1.66
	DH1-13	3.56×10^5	14.35	4.94	0.08	0.24	1.07	5.41	21.88
	DH1-14	1.09×10^6	11.91	12.79	0.92	1.03	0.79	1.00	2.56
	DH1-15	3.00×10^7	2.11	1.74	0.00	1.02	0.67	0.67	0.11
	Mean	4.69×10^6	30.32	11.87	0.82	2.71	12.53	30.21	12.54
Downstream of Hospital 2		6.57×10^5	12.98		0.02		0.67	11.08	1.52
	DH2-1	9.32×10^5	10.73				0.30	4.88	4.39
	DH2-2	9.59×10^5	31.91	1.04			0.28	6.11	10.43
	DH2-3	8.52×10^5	60.57			0.02	0.25	7.47	35.68
	DH2-4	9.59×10^5	21.06	1.04		0.01	0.15	7.14	10.43
	Mean	8.72×10^5	27.45	1.04	0.02	0.02	0.33	7.34	12.49

AMP, ampicillin; CEF, cefotaxime; CIP, ciprofloxacin; DH1, effluent from downstream of Hospital 1; FOX, cefoxitin; STR, streptomycin; SUL, sulphonamides; TET, tetracycline; UH1, effluent from upstream of Hospital 2; UH2, effluent from upstream of Hospital 2.

Table 3.4. The mean *E. coli* MPN/100 ml and mean proportion of AMR *E. coli* from WWTPs 1 and 2

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Raw effluent from WWTP 1	TP1-RI-1	7.49×10^{6}	16.33	9.85	0	0.00	2.70	13.76	15.01
	TP1-RI-2	1.04×10^{7}	7.08	0	0	0.96	0.49	27.64	24.39
	TP1-RI-3	8.05×10^{6}	10.68	1.24	0	0	2.51	30.22	0.20
	TP1-RI-4	2.03×10^{6}	92.92	5.95	0.04	0.49	3.88	0	0.49
	TP1-RI-5	3.36×10^{5}	18.84	22.21	0.44	3.13	8.12	51.59	20.77
	TP1-RI-6	3.45×10^{6}	6.54	5.90	0.03	0.18	7.58	1.32	0.35
	TP1-RI-7	2.88×10^{6}	55.38	6.90	0.07	0.31	10.72	13.51	0.64
	TP1-RI-8	2.14×10^{6}	12.84	12.23	0.04	0.41	9.55	22.79	0.80
	TP1-RI-9	3.55×10^{5}	21.01	24.25	0.05	0.33	10.30	5.61	2.13
	TP1-RI-10	8.60×10^{5}	0	0	0	0	0	1.67	23.49
	TP1-RI-11	1.37×10^{7}	3.28	1.96	0.01	0.06	0.55	11.74	0.02
	TP1-RI-12	3.36×10^{5}	28.88	18.66	22.00	2.17	3.98	4.81	14.50
	TP1-RI-13	2.32×10^{5}	43.10	19.18	0.43	0.35	10.00	5.11	2.32
	TP1-RI-14	2.49×10^{6}	19.00	8.11	8.56	3.45	0.28	0.49	0.54
	TP1-RI-15	1.37×10^{7}	6.73	3.28	1.96	0.01	0.06	0.55	11.74
	Mean	4.57×10^{6}	23.45	10.68	2.41	0.67	5.10	14.43	7.13
Raw influent from WWTP 2	TP2-RI-1	4.64×10^{6}	16.06	26.11	0	0	1.36	14.01	2.82
	TP2-RI-2	5.21×10^{6}	30.65	20.91	0	0	14.30	21.12	1.00
	TP2-RI-3	5.12×10^{6}	25.83	26.08	0	0	10.17	21.26	2.36
	TP2-RI-4	1.17×10^{7}	8.22	7.36	0	0	5.41	3.53	1.04
	TP2-RI-5	5.38×10^{6}	29.34	22.51	0	0	18.01	22.51	2.97
	TP2-RI-6	5.29×10^{6}	20.37	27.67	0	0	13.82	22.66	4.21
	TP2-RI-7	6.22×10^{6}	19.51	19.21	0	0	9.81	15.17	2.16
	TP2-RI-8	7.20×10^{6}	21.92	16.36	0	0	6.60	10.47	2.20
	TP2-RI-9	3.87×10^{6}	36.18	15.53	0.05	0.02	1.34	3.90	7.53
	TP2-RI-10	5.12×10^{6}	25.83	26.08	0	0	10.17	21.26	2.36
	TP2-RI-11	1.73×10^{6}	33.92	33.92	0.04	0.04	0.65	0	1.00
	TP2-RI-12	4.08×10^{6}	18.08	11.14	0.00	0.03	0.25	0.43	26.94
	TP2-RI-13	2.46×10^{7}	39.51	1.74	0.00	0.00	0.14	0.46	7.53
	TP2-RI-14	3.46×10^{6}	31.77	13.16	0.03	0.07	0.04	0.35	63.14
	TP2-RI-15	1.73×10^{6}	33.92	3.39	0.04	0.04	0.65	0	1.00
	Mean	6.36×10^{6}	26.07	18.08	0.03	0.03	6.18	12.09	8.55
Post-return effluent from WWTP 1	TP1-PR-1	1.39×10^{7}	7.91	6.96	0	0	1.45	18.61	2.08
	TP1-PR-2	7.12×10^{6}	11.97	7.32	0	0	1.41	18.59	7.28
	TP1-PR-3	7.71×10^{6}	8.12	6.76	0	0	1.30	12.56	6.88
	TP1-PR-4	2.02×10^{6}	25.79	2.04	4.80	0.01	0.02	0.50	4.95
	TP1-PR-5	4.17×10^{5}	29.36	23.77	0.17	1.81	19.05	2.40	3.21
	TP1-PR-6	8.16×10^{6}	6.78	5.37	0.09	0.35	2.62	6.27	0.74
	TP1-PR-7	7.12×10^{5}	38.66	7.25	0.14	1.20	11.15	29.64	1.70
	TP1-PR-8	3.28×10^{6}	19.24	16.93	0.27	1.87	7.96	23.77	2.08
	TP1-PR-9	4.55×10^{5}	29.68	4.42	0.18	0.17	1.84	3.19	3.25
	TP1-PR-10	1.00×10^{4}	0	0	0	0	3.06	0	0
	TP1-PR-11	4.11×10^{7}	0.71	1.93	0	0	0.45	7.43	0.15
	TP1-PR-12	3.95×10^{5}	84.94	24.53	0.32	1.61	1.12	3.07	34.18
	TP1-PR-13	3.07×10^{5}	25.38	0.82	11.88	1.85	1.94	2.54	27.83
	TP1-PR-14	3.95×10^{7}	6.72	0.27	0.37	0.01	0.02	0.05	4.20
	TP1-PR-15	4.11×10^{7}	0.71	1.93	0	0	0.45	7.43	0.29
	Mean	1.11×10^{7}	21.14	7.88	2.02	0.99	3.59	9.72	7.06

Table 3.4. Continued

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Primary effluent from WWTP 1	TP1-PE-1	4.64×10^6	16.06	16.06	0	0	0	23.24	5.71
	TP1-PR-2	9.60×10^6	1.04	5.43	0	0	6.58	2.53	1.59
	TP1-PE-3	7.61×10^6	4.02	4.02	0	0	1.31	11.08	1.55
	TP1-PE-4	1.00×10^5	10.00	0	20.20	1.20	1.09	0	0
	TP1-PE-5	1.81×10^5	28.51	28.29	0.54	3.10	4.08	29.16	2.28
	TP1-PE-6	2.41×10^6	10.32	14.49	0.08	0.38	8.47	10.48	1.56
	TP1-PE-7	2.81×10^5	52.04	39.10	0.34	1.85	6.22	3.22	3.03
	TP1-PE-8	1.62×10^6	19.95	21.93	0.13	0.68	15.35	13.20	2.65
	TP1-PE-9	2.11×10^5	29.97	2.47	0.06	0.30	0.88	27.87	2.47
	TP1-PE-10	2.02×10^4	0	0	0	0	12.04	2.58	15.15
	TP1-PE-11	2.42×10^6	0.15	0.11	0	0	7.13	0.26	0.09
	TP1-PE-12	2.49×10^5	29.91	20.92	0.49	2.40	0.30	15.86	25.37
	TP1-PE-13	1.26×10^5	23.09	18.23	0.52	3.56	0.31	33.36	182.32
	TP1-PE-14	3.36×10^5	22.21	2.56	0.05	0.26	0.33	1.18	22.21
	TP1-PE-15	2.42×10^6	15.10	10.97	0	0	0.07	0.26	9.46
	Mean	1.81×10^7	18.74	14.20	2.49	1.52	4.58	12.45	19.68
Primary effluent from WWTP 2	TP2-PE-1	2.33×10^6	73.52	31.92	0	0	1.75	4.28	2.22
	TP2-PE-2	4.57×10^6	18.83	13.84	0	0	19.57	1.51	0.46
	TP2-PE-3	3.40×10^6	28.46	21.88	0	0	8.93	8.99	0.25
	TP2-PE-4	1.73×10^6	29.81	17.68	0	0.01	3.99	5.78	0.18
	TP2-PE-5	4.13×10^6	23.43	18.02	0	0	7.40	9.99	0.48
	TP2-PE-6	3.05×10^6	31.76	28.19	0	0.00	10.03	10.03	0.32
	TP2-PE-7	3.20×10^6	31.21	20.98	0	0.00	9.99	6.73	0.59
	TP2-PE-8	2.37×10^6	44.98	26.85	0	0.00	5.84	7.11	0.91
	TP2-PE-9	3.26×10^6	25.86	10.32	0.03	0.60	1.68	2.12	0.52
	TP2-PE-10	3.40×10^6	28.46	21.88	0	0	8.93	8.99	0.25
	TP2-PE-11	3.08×105	18.96	27.37	0.30	0.04	12.90	0	5.51
	TP2-PE-12	9.34×10^6	10.71	3.09	0	0.09	0.01	0.01	18.14
	TP2-PE-13	2.07×10^7	4.51	1.06	0	0.00	0.00	0.24	7.77
	TP2-PE-14	1.29×10^7	8.10	2.16	0	0.10	0.09	0.35	16.21
	TP2-PE-15	3.08×10^6	18.97	2.74	0.03	0.00	1.29	0	5.51
	Mean	5.18×10^6	26.51	16.53	0.09	0.09	6.16	5.09	3.96
Final effluent from WWTP 1	TP1-FE-1	3.05×10^5	3.28	9.96	0	3.28	0	27.66	5.79
	TP1-FE-2	4.64×10^4	26.11	11.23	0	0	6.60	23.24	2.16
	TP1-FE-3	4.04×10^6	10.11	5.00	0	0	0	15.48	0.85
	TP1-FE-4	8.44×10^4	2.39	6.76	0.32	0.62	4.89	11.60	1.18
	TP1-FE-5	6.26×10^4	0	0	0	0.16	3.21	0	1.60
	TP1-FE-6	9.69×10^4	31.37	20.85	0	0.31	1.03	0	1.03
	TP1-FE-7	4.13×10^4	0	0	0.24	0.24	2.42	0	2.42
	TP1-FE-8	6.32×10^4	15.82	15.82	0	0.65	6.41	0	3.20
	TP1-FE-9	4.04×10^6	0	0	0.25	0.00	0.00	0	2.47
	TP1-FE-10	1.00×10^4	0	0	0	0	0	0	
	TP1-FE-11	2.46×10^5	5.95	2.54	0.07	0.24	1.98	0	0.70
	TP1-FE-12	6.26×10^4	0	0	1.60	0.49	0	0	
	TP1-FE-13	1.46×10^5	0	0	0.34	0.18	0.02	0	3.42
	TP1-FE-14	1.46×10^5	0	0	0	0	0	0	3.56
	TP1-FE-15	2.46×10^5	5.95	2.54	0.07	0.24	1.98	0	0.70
	Mean	6.43×10^5	12.62	9.34	0.41	0.58	2.86	19.50	2.24

Table 3.4. Continued

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Final effluent from WWTP 2	TP2-FE-1	2.02×10^5	49.50	49.50	0	0	0	0	0
	TP2-FE-2	2.02×10^5	0	0	0	0	0	0	0
	TP2-FE-3	1.00×10^5	0	0	0	0	0	0	0
	TP2-FE-4	6.26×10^5	0	0	0	0	0	0	0.16
	TP2-FE-5	2.02×10^5	49.50	0	0	0	0	0	0.50
	TP2-FE-6	2.00×10^5	50.00	0	0	0	0	0	0.50
	TP2-FE-7	2.39×10^5	41.90	41.90	0	0	0	0	0.42
	TP2-FE-8	3.09×10^5	32.33	32.33	0	0	0	0.00	0.32
	TP2-FE-9	6.32×10^4	15.82	0	15.82	0.48	1.58	0	0
	TP2-FE-10	1.00×10^5	0	0	0	0	0	0	0
	TP2-FE-11	6.26×10^5	3.23	0.46	0	0	0.32	7.41	0
	TP2-FE-12	9.90×10^5	20.40		0	0	0	0	0
	TP2-FE-13	2.90×10^4	17.24	0	1.72	2.38	17.24	0	0
	TP2-FE-14	3.06×10^4	0	0	0	0.33	3.27	0	0
	TP2-FE-15	6.26×10^5	3.23	0.46	0	0	0.32	7.41	0
	Mean	3.03×10^5	28.32	24.93	8.77	1.06	4.55	4.94	0.38

AMP, ampicillin; CEF, cefotaxime; CIP, ciprofloxacin; FOX, cefoxitin; STR, streptomycin; SUL, sulphonamides; TET, tetracycline; TP1-FE, final effluent from WWTP 1; TP2-FE, final effluent from WWTP 2; TP1-PE, primary effluent from WWTP 1; TP2-PE, primary effluent from WWTP 2; TP1-PR, post-return effluent from WWTP 1; TP1-RI, raw influent from WWTP 1; TP2-RI, raw influent from WWTP 2.

3.3.1.4 Seawater

E. coli were detected in all sample sites on all sample dates; however, no AMR *E. coli* were detected in any of the seawater samples tested. The total *E. coli* present ranged from 1×10^4 to 1.75×10^5 MPN/100 ml in the seawater tested closest to the receiving point of treated effluent from the outflow point of WWTP1. The highest number (1.75×10^5 MPN/100 ml) was detected in a sample taken after a period of heavy rainfall. Although we saw the highest number of total *E. coli* MPN/100 ml after the rainfall event, there was no impact on the incidence of AMR *E. coli* in the seawater.

3.3.1.5 Sludge

The mean *E. coli* MPN/g total dissolved solids (TDS) and the mean percentages of AMR *E. coli* in primary sludge from WWTPs 1 and 2, in dried sludge from WWTPs 1 and 2, and in lime-treated sludge from WWTP 2 are outlined in Table 3.5. *E. coli* and AMR *E. coli* were found in every sample of the primary sludge tested from both treatment plants. The mean *E. coli* MPN/g TDS was in the same order of magnitude for both treatment plants; however, the mean AMR *E. coli* MPN/g TDS from WWTP 2 was consistently lower, by one or two orders of magnitude, than from WWTP 1 (Table 3.3). The mean percentage of *E. coli* resistant to all antimicrobials was higher in samples from WWTP 1 than from WWTP 2. *E. coli* and AMR *E. coli* were detected on all sample dates and in every sample of dried sludge from both treatment plants. The mean *E. coli* MPN/g TDS was 10-fold lower in samples from WWTP 1 than from WWTP 2; in addition, all proportions of AMR *E. coli,* excluding cefotaxime- and cefoxitin-resistant *E. coli*, were lower in samples from WWTP 1 than from WWTP 2 (Table 3.3). Resistant proportions of *E. coli* were approximately twofold higher in samples from WWTP 2 than in samples from WWTP 1 with regard to ampicillin (49% vs 25%), sulphonamide (54% vs 23%), tetracycline (9% vs 4%) and ciprofloxacin (2% vs 1%) resistance, and 2.5-fold higher with regard to streptomycin resistance (46% vs 18%). Lime treatment is carried out on site in WWTP 2; however, in WWTP 1 the sludge is lime treated off site by an organics company that then spreads the treated biosolids. Because of this, we were unable to access and test the lime-treated sludge from WWTP 1. *E. coli* were detected on all of the sample dates in the lime-treated sludge

Table 3.5. The mean *E. coli* MPN/100 ml and mean proportion of AMR *E. coli* in sludge from WWTPs 1 and 2

Location	Sample code	*E. coli* MPN/100 ml	AMR *E. coli* (%)						
			AMP	STR	FOX	CEF	TET	SUL	CIP
Primary sludge from WWTP 1	TP1-PS-1	3.92×10^6	17.43	5.44	0.19	0.74	9.00	16.12	1.61
	TP1-PS-2	3.92×10^6	54.07	2.66	0.16	0.50	7.08	14.49	1.43
	TP1-PS-3	5.72×10^6	10.84	4.67	0.11	0.47	5.60	14.28	1.44
	TP1-PS-4	5.92×10^6	13.66	5.37	0.16	0.59	4.63	19.06	1.51
	TP1-PS-5	5.72×10^6	54.38	14.43	17.96	3.71	10.00	27.58	2.06
	TP1-PS-6	5.92×10^6	42.58	9.42	6.60	0.84	8.15	24.03	1.84
	TP1-PS-7	5.72×10^6	61.88	6.74	10.12	0.52	2.46	24.57	1.56
	TP1-PS-8	5.92×10^6	76.73	10.58	3.69	0.49	12.30	10.58	1.62
	TP1-PS-9	3.92×10^6	55.28	17.60	1.68	0.81	28.70	11.23	1.60
	TP1-PS-10	3.92×10^6	75.52	17.60	1.97	1.30	63.06	37.41	2.76
	TP1-PS-11	3.92×10^6	64.22	12.65	1.58	9.96	31.88	30.40	1.13
	TP1-PS-12	5.92×10^6	54.74	7.51	2.34	3.82	11.83	26.81	0.65
	TP1-PS-13	5.72×10^6	58.73	10.95	2.79	2.87	19.03	23.70	1.21
	TP1-PS-14	5.92×10^6	74.91	17.19	4.63	1.29	9.66	22.63	0.64
	TP1-PS-15	5.92×10^6	54.74	7.51	2.34	3.82	11.83	26.81	0.65
	Mean	5.20×10^6	51.31	10.02	3.76	2.11	15.68	21.98	1.45
Primary sludge from WWTP 2	TP2-PS-1	1.04×10^6	49.77	5.88	0.55	0.50	6.38	12.03	0.58
	TP2-PS-2	1.10×10^6	42.14	1.83	0.56	0.45	4.96	7.54	0.18
	TP2-PS-3	9.45×10^6	52.93	8.74	0.55	0.45	4.26	22.60	0.21
	TP2-PS-4	1.63×10^6	32.65	6.41	0.28	0.29	2.99	7.78	0.25
	TP2-PS-5	1.01×10^6	48.86	1.98	0.45	0.56	2.40	8.20	0.20
	Mean	1.14×10^6	45.27	4.97	0.48	0.45	4.20	11.63	0.29
Dried sludge from WWTP 1	TP1-DS-1	4.16×10^7	32.09	16.87	0.38	1.59	8.97	32.10	1.41
	TP1-DS-2	3.81×10^7	29.25	15.21	0.26	0.74	6.47	23.57	1.32
	TP1-DS-3	4.16×10^7	26.76	15.56	0.28	1.00	9.62	28.51	1.19
	TP1-DS-4	7.00×10^7	10.17	5.66	0.10	0.28	5.72	11.16	0.35
	TP1-DS-5	7.00×10^7	15.64	10.73	0.22	0.55	5.67	17.59	1.13
	TP1-DS-6	4.16×10^7	30.95	20.50	0.28	1.59	4.82	33.76	1.13
	TP1-DS-7	7.00×10^7	15.91	15.83	0.17	0.22	3.46	20.18	0.75
	TP1-DS-8	3.81×10^7	23.57	26.90	0.20	0.42	3.69	35.42	2.36
	TP1-DS-9	7.00×10^7	11.16	16.43	0.12	0.13	2.36	17.59	1.39
	TP1-DS-10	4.16×10^7	26.21	27.74	0.21	0.41	2.46	22.23	2.63
	TP1-DS-11	7.00×10^7	14.16	8.58	0.19	0.26	1.91	9.75	1.75
	TP1-DS-12	3.81×10^7	33.83	23.44	0.47	4.04	2.16	13.00	3.30
	TP1-DS-13	3.81×10^7	38.85	11.66	0.53	2.67	1.40	27.36	4.87
	TP1-DS-14	4.16×10^7	26.30	27.74	0.43	0.39	3.38	33.95	2.16
	TP1-DS-15	3.81×10^7	33.83	23.44	0.47	4.04	2.16	13.00	3.30
	Mean	4.99×10^7	24.58	17.75	0.29	1.22	4.28	22.61	1.93
Dried sludge from WWTP 2	TP2-DS-1	9.10×10^6	46.89	18.91	0.61	0.67	5.76	26.62	1.89
	TP2-DS-2	8.82×10^6	30.28	38.40	0.41	0.81	7.40	32.84	1.69
	TP2-DS-3	5.50×10^6	39.99	73.10	0.27	0.95	10.48	68.70	1.11
	TP2-DS-4	5.91×10^6	69.87	61.27	0.29	1.00	11.26	86.63	0.34
	TP2-DS-5	6.81×10^6	57.77	38.47	0.32	0.90	8.08	56.95	1.86
	Mean	7.23×10^6	48.96	46.03	0.38	0.87	8.60	54.35	1.38

AMP, ampicillin; CEF, cefotaxime; CIP, ciprofloxacin; FOX, cefoxitin; STR, streptomycin; SUL, sulphonamides; TET, tetracycline; TP1-DS, dried sludge from WWTP 1; TP2-DS, dried sludge from WWTP 2; TP1-PS, primary sludge from WWTP 1; TP2-PS, primary sludge from WWTP 1.

from WWTP 2 and ranged from 2.4×10^2 *E. coli*/g TDS to 3.2×10^3 *E. coli*/g TDS. These levels are considerably lower than the levels of *E. coli*/g TDS detected in the dried sludge, indicating the effectiveness of lime treatment at reducing microbial loads in dried sludge. No AMR *E. coli* were detected in any sample; this again indicates the effectiveness of lime treatment at reducing microbial load in dried sludge.

3.3.2 Detection of antimicrobial residues

Quinolone- and penicillin-like activities were detected in the effluent from Hospital 1 and in the municipal effluent downstream of Hospital 1. No other antimicrobial residues tested for were detected at these or other sample locations. In hospital effluent, penicillin-like activity ranged from 0.72 µg/l to 3.44 µg/l and quinolone-like activity ranged from 0.5 µg/l to 7.27 µg/l. In effluent downstream of the hospital, penicillin-like activity ranged from 0.03 µg/l to 0.07 µg/l and quinolone-like activity ranged from 0.05 µg/l to 0.54 µg/l. No traces of antimicrobial residues were detected in the raw influent entering WWTP 1, which treats effluent from Hospital 1, thus indicating that, although antimicrobial residues are emitted in hospital effluent and can be detected downstream from the hospital, the residues are not detectable in the municipal wastewater system because of dilution, biotransformation or a combination of these effects.

3.4 Conclusions

Hospital effluent contains high levels of *E. coli* and AMR *E. coli* which impact on downstream municipal effluent. Although the numbers of *E. coli* and AMR *E. coli* are reduced by wastewater treatment, significant numbers of *E. coli* and AMR *E. coli* are released into the environment in treated effluent. *E. coli* resistant to older classes of antimicrobials are present in high proportions throughout municipal wastewater systems and in WWTPs. *E. coli* resistant to newer classes of antimicrobials, such as quinolones (ciprofloxacin), second-generation cephalosporins (cefoxitin) and third-generation cephalosporins (cefotaxime), are present in low proportions in hospital effluent but can survive the wastewater treatment process and are released into the environment in treated effluent.

Residues exhibiting antimicrobial activity are released into municipal wastewater systems in hospital effluent. The correlation, if any, that exists between the presence of sub-inhibitory levels of antimicrobials in the environment and the selection of *E. coli* resistant to them needs to be assessed.

4 Risk ranking of antimicrobials in the aquatic environment from human consumption: an Irish case study

4.1 Overview

The presence of antimicrobials in the environment can lead to resistance and present the potential for direct toxicity. The literature review detailed in Section 1 identified penicillins, β-lactams, tetracyclines, macrolides, quinolones/fluoroquinolones and sulphonamides/trimethoprim as residues of interest, as they are some of the most commonly used antimicrobials in Europe. Although their presence is well documented, it is uncertain what factors contribute most significantly to their presence in the environment, hence a risk ranking analysis was carried out to determine the predicted concentrations and leading contributing factors. The model-predicted values were ranked to assess each antimicrobial group with regard to the possibilities of resistance and toxic effects on the receiving environment. The findings of this section have been published (Harris *et al.*, 2013a); details of model development and the human and environmental risk assessment approaches used can be found in this publication.

4.2 Results and discussion

The PECs for each antimicrobial group were calculated (Table 4.1). The results suggest that the use of antimicrobials for systemic treatment can result in appreciable quantities being found in the environment. Penicillins had the highest mean PEC (1.05 mg/m^3); however, penicillin use in Ireland is higher than that of other antimicrobials and this may, therefore, account for the higher PEC. Although the PECs are low, it is important to note that these compounds are continuously released into the environment and that they may not be homogenously distributed, which may result in environmental reservoirs. Using Spearman's rank-order correlation coefficient, it was possible to evaluate which input influenced each PEC for penicillin, β-lactam, tetracycline, macrolide, quinolone/fluoroquinolone and sulphonamide/trimethoprim antimicrobial groups (Table 4.2). Interestingly, compound degradation had the least significant effect on each antimicrobial PEC, excluding penicillin. This suggests that the degradation of penicillins within the soil or aqueous environment is less influential on their environmental concentration when compared with use or excretion. The hazard quotient (HQ) values for all antimicrobial groups were also simulated (Table 4.2). The quinolone/fluoroquinolone HQ of 1.51 indicates that there is a moderate risk of direct toxicity. As the predicted HQs for penicillins, β-lactams, tetracyclines, macrolides and sulphonamides/trimethoprims were < 1, the toxicity risk is low. The results from the scenario analysis can be seen in Table 4.3, which highlights the effect of retention time in the WWTP. The PEC for penicillin was greatly affected, reducing

Table 4.1. Mean (and 95th percentile) simulation results for the six main antimicrobial groups used in Ireland (De = 1)

Antimicrobial	Mean PEC (95th percentile), mg/m^3	Leading contributing factor	Correlation coefficient	Hazard quotient	Toxicity (%)	
					Acute	Chronic
PEN	1.05 (4.21)	Use	0.61	0.10	0.00	0.44
BET	0.19 (0.52)	Use	0.52	0.10	0.00	0.00
TET	0.06 (0.18)	Metabolism	0.65	0.01	0.00	0.95
MAC	0.07 (0.25)	Metabolism	0.69	0.00	0.00	0.62
Q/F	0.02 (0.08)	Metabolism	0.63	1.51	0.00	0.00
S/T	0.08 (0.26)	Use	0.79	0.05	0.00	0.15

BET, β-lactams; De, degradation; MAC, macrolides; PEN, penicillins; Q/F, quinolones/fluoroquinolones; S/T, sulfonamides/trimethoprims; TET, tetracyclines.

Table 4.2. The effect of degradation on the mean (and 95th percentile) PECs (mg/m³) for each antimicrobial group

Antimicrobial	Mean PEC (95th percentile), mg/m³ Degradation (days)						Degraded after 100 days (%)
	1	5	10	15	20	100	
PEN	1.05 (4.20)	0.27 (1.15)	0.10 (0.44)	0.05 (0.21)	0.03 (0.11)	0.002 (0.00)	99.8
BET	0.19 (0.54)	0.17 (0.48)	0.15 (0.43)	0.13 (0.40)	0.12 (0.37)	0.04 (0.14)	78.9
TET	0.06 (0.19)	0.05 (0.17)	0.05 (0.16)	0.04 (0.15)	0.04 (0.14)	0.02 (0.08)	66.7
MAC	0.07 (0.25)	0.06 (0.22)	0.06 (0.20)	0.05 (0.18)	0.05 (0.17)	0.02 (0.07)	71.4
Q/F	0.02 (0.08)	0.02 (0.08)	0.02 (0.07)	0.02 (0.07)	0.02 (0.06)	0.01 (0.04)	50.0
S/T	0.09 (0.27)	0.08 (0.24)	0.07 (0.22)	0.06 (0.21)	0.06 (0.20)	0.02 (0.20)	77.8

BET, β-lactams; MAC, macrolides; PEN, penicillins; Q/F, quinolones/fluoroquinolones; S/T, sulfonamides/trimethoprims; TET, tetracyclines.

Table 4.3. Estimated environmental levels (mg/m³) of each antimicrobial group reported worldwide

Antimicrobial	Minimum	Maximum	*n*	References
PEN	0.00000048	0.8	8	Watkinson *et al.*, 2007; Li *et al.*, 2008
BET	Non-detectable	0.6	1	Watkinson *et al.*, 2007
TET	0.02	10.9	10	Miao *et al.*, 2004; Karthikeyan and Meyer, 2006; Watkinson *et al.*, 2007
MAC	0.008	3.29	19	Göbel *et al.*, 2004; Miao *et al.*, 2004; Haggard *et al.*, 2006; Karthikeyan and Meyer, 2006; Watkinson *et al.*, 2007
Q/F	0.010	0.72	13	Miao *et al.*, 2004; Haggard *et al.*, 2006; Karthikeyan and Meyer, 2006; Watkinson *et al.*, 2007
S/T	0.008	3.52	29	Miao *et al.*, 2004; Haggard *et al.*, 2006; Karthikeyan and Meyer, 2006; Watkinson *et al.*, 2007

BET, β-lactams; MAC, macrolides; PEN, penicillins; Q/F, quinolones/fluoroquinolones; S/T, sulfonamides/trimethoprims; TET, tetracyclines.

the PEC from 1.05 mg/m³ to 0.00 mg/m³ after 100 days (99.8% reduction). In contrast, the PEC of quinolones/fluoroquinolones remained unchanged from day 1 to day 20 (0.02 mg/m³) and had reduced by only 50% after 100 days. Literature sources were examined to compare the model's predictions with reported levels, which ranged from not detected to 11 mg/m³ throughout the world (Table 4.4).

The toxicity results changed with the application of assessment factors (AFs) (Tables 4.2 and 4.5). Tetracycline chronic toxicity increased from <1% to 81% with the application of an AF of 1000. Quinolone/fluoroquinolone chronic toxicity increased from 1% to 22% when AFs of 100 and 1000 were applied (i.e. to 22% of the time the PEC was at a concentration considered toxic to the environment).

The examination of resistance formation potential (Table 4.5) [at degradation (De) = 1, i.e. worst case scenario] indicates that for more than 90% of the time, conditions were conducive for resistance formation for all antimicrobials when the boundaries for resistance formation are not limited (Table 4.5) (i.e. when any level of antimicrobial agent is likely to select for resistance). Kohanski *et al.* (2010) have shown that prolonged exposure to low levels of antimicrobials can encourage antimicrobial-sensitive bacteria to become antimicrobial resistant. Kohanski *et al.* (2010) treated bacteria with low levels of ampicillin (1 mg/ml) and the majority of mutants that arose showed cross-resistance to antimicrobials that they had never been exposed to. In contrast, mutants that appeared after selection by high concentrations of antimicrobials without prior exposure to low levels of antimicrobials demonstrated little cross-resistance to other classes of antibiotics. The results presented by Kohanski *et al.* (2010) imply that environments, such as WWTP effluents, containing sub-lethal levels of antimicrobials, may be acting to enrich for resistant variants, thus promoting the development of resistance to an abundance of antimicrobials. When the margins

Table 4.4. Mean acute and chronic toxicity analysis (De=100) of the PEC (with the application of AFs) for the six main antimicrobial groups used in Ireland

Antimicrobial	Acute toxicity (%)		Chronic toxicity (%)	
	AF=100	AF=1000	AF=100	AF=1000
PEN	0	0	0	0
BET	0	0	0	4
TET	3	41	63	81
MAC	2	21	27	63
Q/F	0	2	1	22
S/T	0	5	15	53

BET, β-lactams; De, degradation; MAC, macrolides; PEN, penicillins; Q/F, quinolones/fluoroquinolones; S/T, sulfonamides/trimethoprims; TET, tetracyclines.

Table 4.5. The mean probability of resistance formation between assumed limits of the MIC for each antimicrobial at De=1

Resistance formation limit	Resistance formation potential (%)					
	PEN	BET	TET	MAC	Q/F	S/T
0.2 of MIC	0.35	0.30	0.00	0.00	1.66	0.23
0.4 of MIC	0.77	0.82	0.03	0.01	3.98	0.67
0.6 of MIC	1.79	1.59	0.08	0.03	8.50	1.34
0.8 of MIC	4.85	4.44	0.52	0.17	19.15	3.27
0.9 of MIC	10.27	10.36	1.98	0.54	33.42	7.31
1 of MIC	98.65	98.85	99.95	100.00	92.68	99.61

BET, β-lactams; De, degradation; MAC, macrolides; PEN, penicillins; Q/F, quinolones/fluoroquinolones; S/T, sulfonamides/trimethoprims; TET, tetracyclines.

for resistance formation are limited, we examined the probability that the PECs will occur between these set boundaries (i.e. the PEC may be less than the set boundary and so no resistance is assumed to occur). Quinolones/fluoroquinolones show the highest resistance formation potential in all bound tests. At 90% of the minimum inhibitory concentration (MIC), the potential for resistance formation was 33%. The model PEC is much lower than the concentration tested by Kohanski *et al.* (2010) and it remains unknown whether or not the PECs calculated here would have a comparable effect on the promotion of resistance. Although boundaries have been set to examine resistance formation potential, it is important to note that there is no evidence regarding the effects that compounds at this low concentration may have.

Murray-Smith *et al.* (2011) used the environmental reference concentration (ERC) method to assess safe levels for emission of pharmaceutically active compounds to the environment. They identified long-term and short-term concentrations that should not be exceeded in an aquatic environment receiving effluents from pharmaceutical manufacturing. They generated four ERC values for the protection of aquatic and freshwater life, for aquatic life in the marine environment, for fish eating predators and for humans. Murray-Smith *et al.* (2011) report that the ERC methodology is restricted to assessing direct impacts of pharmaceuticals and cannot be applied in the case of indirect impacts, such as formation and dissemination of antimicrobial resistance (Murray-Smith *et al.*, 2011). Currently, there are no government-recommended guidelines to assess or quantify antimicrobial resistance formation in the environment; consequently, the authors propose that the method presented here for antimicrobial resistance formation could be used in conjunction with the ERC methodology to create a mechanistic model to assess antimicrobial resistance formation potential.

4.3 Conclusions

Monte Carlo simulation models are useful tools for determining the PEC of toxic elements in the environment. Experimental data and an in-depth understanding

of the environmental stability and toxicity of pharmaceuticals and their removal by WWTPs are still lacking. With regard to low concentrations of antimicrobials selecting for resistance formation, knowledge is also lacking. The recommended toxicity reference values provided by ECOSAR may be unsuitable for antimicrobials and other pharmaceuticals but it is currently the best available method of assessment. Experimental measurements, such as field studies, are necessary to fully understand the extent of antimicrobial toxicity in the environment. With regard to lower limits of MICs selecting for resistance formation, knowledge is again lacking. Currently, there are a variety of methods that are used to test for both antimicrobial residue and AMR bacteria in the environment. As with all areas of environmental toxicity, the standardisation of methods for the sampling, testing and reporting of antimicrobial contamination in the environment are required. Each antimicrobial is different; hence, the challenge of standardising testing methods for all possible agents is enormous. Perhaps standardised methods may be possible for antimicrobial groups and, in the first instance, it may be appropriate to concentrate on a limited number of agents that are widely used and for which there is preliminary evidence of persistence in the environment. There is a need to develop finite acceptable limits, at least for major classes of antimicrobial agents. Difficulties arise with regard to varying environments, classes of antimicrobial agents (let alone individual antimicrobials), combined effects, synergistic effects and different sensitivities, highlighting the need for specific field studies.

The model identified quinolones/fluoroquinolones as the group of antimicrobials for which conditions were most likely to be optimum for resistance formation potential (when the resistance formation concentration boundaries were limited). The model also predicted quinolones/fluoroquinolones to have the lowest rate of degradation. Tetracyclines and macrolides were predicted to have an average degradation rate and the lowest resistance formation potential (when the resistance formation concentration boundaries were limited). As highlighted in this study, through the sensitivity analysis, the systemic use of antimicrobials can be a leading factor contributing to their presence in the environment. For already existing antimicrobials, risk management should focus on reducing the input of antimicrobials into the environment. The sensitivity analysis also indicated that antimicrobials that are highly metabolised within the body are less likely to be excreted into the environment. The rate of metabolism of new antimicrobials could be considered a risk factor for their presence in the environment. The development of highly metabolised antimicrobials with a low excretion rate should be considered. The model, presented here, could be used in other areas that require investigation by inputting the specific variables, and the simulated output could be used for remedial decision making. There are very particular difficulties in modelling the potential for resistance formation from the PEC, given the current gaps in knowledge. Additional experimental data on concentrations of antimicrobial agents in the environment, the factors influencing these concentrations and their effects on resistance formation in the environment should be integrated into more refined models to provide improved understanding of environmental impacts in this particularly complex field.

5 Simulation model to predict the fate of ciprofloxacin in the environment after wastewater treatment

5.1 Overview

The risk ranking model presented in Section 4 identified quinolones/fluoroquinolones as the antimicrobial group with the greatest toxicity (based on the HQ) and resistance formation potential, and the lowest degradation (De) rate. As a result of this, the fluoroquinolone ciprofloxacin was examined further to identify its predicted release within both the aquatic and soil environments, its toxicity, its resistance formation potential and the possible risk it presents to exposed swimmers. These findings have been published by Harris *et al.* (2013b) and details of model development and human and environmental risk assessment approaches can be found in this publication.

5.2 Results and discussion

The Monte Carlo simulation model produced probability density distributions of predicted ciprofloxacin concentrations for each sample point: hospital effluent, urban effluent, WWTP effluent and sludge (Figure 5.1). All of the samples, excluding hospital effluent, were below the limit of detection (LD) (10 mg/m³). Table 5.1 shows the mean and the 5th and 95th percentiles of the predicted concentrations for each sample point. The results shown in Table 5.1 suggest that ciprofloxacin residues enter the environment at low concentrations (means of 2.59 and 3.48 mg/m³ for WWTP effluent and sludge, respectively) after release in hospital effluent. Validation is important to determine whether or not

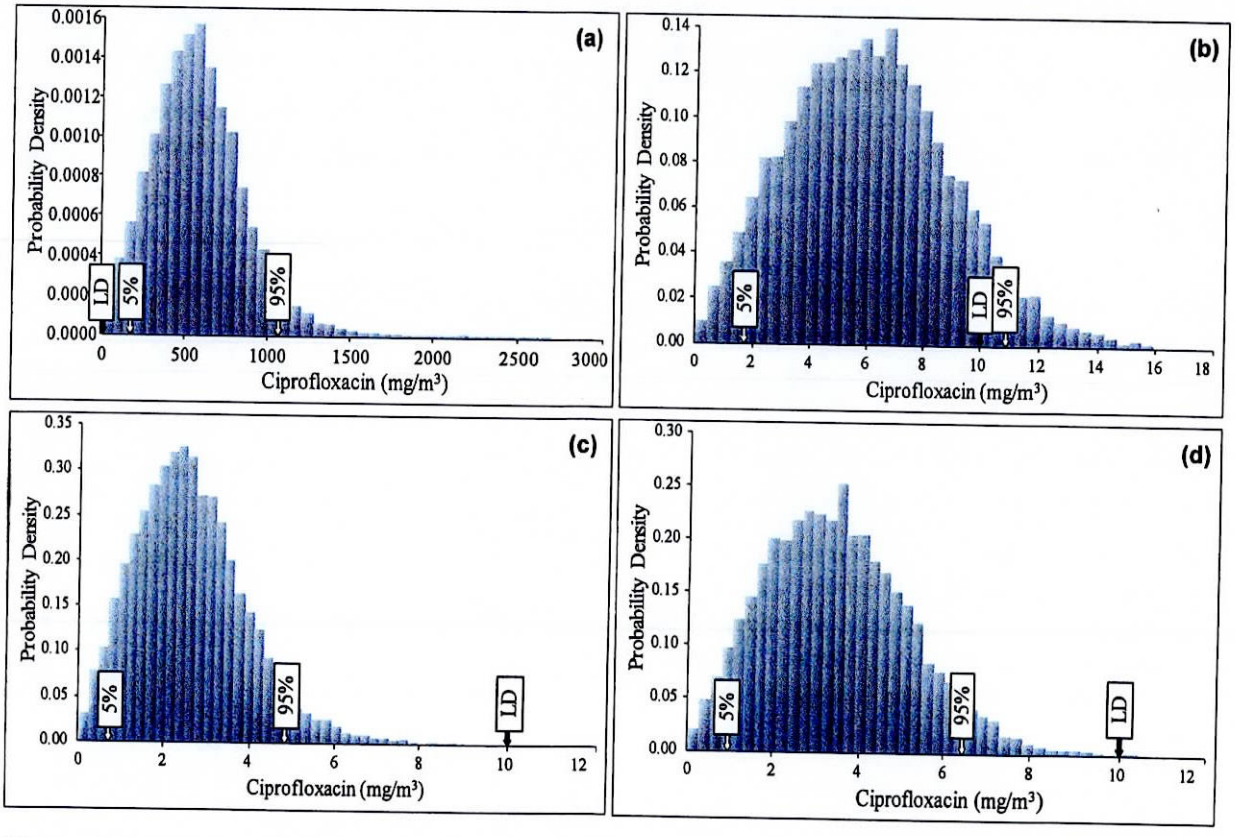

Figure 5.1. Simulated predicted concentrations of ciprofloxacin in (a) hospital effluent, (b) urban effluent, (c) WWTP effluent and (d) sludge. 5% indicates the 5th percentile and 95% indicates the 95th percentile.

Table 5.1. Simulation model output results including uncertainty for predicted concentrations of ciprofloxacin in the municipal wastewater treatment and subsequent toxicity

Output	Mean	5th percentile	95th percentile	Unit	Monitored concentration (n=15)
Hospital effluent PC	579.90	170.99	1062.61	mg/m³	All samples > 10
Urban effluent PC	6.06	1.72	10.95	mg/m³	All samples < 10
WWTP effluent PC	2.59	0.71	4.89	mg/m³	All samples < 10
Sludge PC	3.48	0.97	6.44	mg/m³	All samples < 10
Soil PC	0.006	0.002	0.012	mg/m³	–
Seawater PC	0.15	0.04	0.29	mg/m³/day	–
Seawater swallowed PC	0.26	0.07	0.48	µg/kgBW/day	–
HQ	0.31	0.07	0.72	–	–
Toxicity[a]	0	–	–	%	–

a The probability of the PC exceeding the toxicity reference value.
PC, predicted concentration.

the model is an accurate representation of the actual system under study (Law, 1981). Validation was carried out at each sample point (n=15). In all of the monitored samples, excluding hospital effluent, ciprofloxacin residues were below the LD. This corresponds with model predictions, as the simulated 95th percentile was found to be well below the LD (i.e. unlikely to be detected using this method of testing) for urban effluent, WWTP effluent and sludge concentrations and hence partially validates the study. The model predicted concentrations are likely to be present at levels which are conducive to *E. coli* resistance formation (Table 5.2). For example, if resistance is assumed to occur below the MIC and above 20% of the MIC value, it is predicted that 2% of the time, conditions (i.e. ciprofloxacin levels) in the WWTP effluent will be conducive to resistance formation. This increases to 65% for the scenario where resistance can occur between the MIC and 80% of the MIC value (Table 5.2).

5.3 Model assumptions and limitations

Model results must be viewed in the context of model assumptions and limitations (Cummins, 2008). These may influence the model outputs and conclusions; therefore, their consideration is important. The following modelling assumptions and limitations have been identified:

1. Hospital effluent is considered to be the only source of ciprofloxacin.
2. Ciprofloxacin used in outpatient departments was assumed to be excreted in hospital effluent.
3. Twenty-five per cent of patients attending accident and emergency are admitted to hospital. Hence, 25% of ciprofloxacin use in accident and emergency was assumed to be excreted in hospital waste.
4. Excretion of metabolites and parent compounds were considered the same, as metabolites can be more toxic than the parent compound and can de-conjugate and revert back to the parent compound.
5. Sorption is the only method of ciprofloxacin removal (from the water to solid phase) considered.
6. The antimicrobial ciprofloxacin is evenly distributed and the bacteria come in contact with it.
7. Only *de novo* resistance is considered.
8. No degradation of ciprofloxacin was considered during lime and heat treatment (common treatment method for sludge before land application).
9. No uptake of ciprofloxacin by plants after land application.
10. No release of ciprofloxacin into water after land application.

5.4 Conclusions

As there are currently no antimicrobial residue monitoring programmes, government-recommended testing methods or acceptable limits, simulation modelling can be useful for predicting the risk that antimicrobial residues may pose to human health or the environment. In this study, hospital usage and subsequent excretion of

Table 5.2. The probability of resistance formation of *E. coli* from WWTP effluent, sludge and the receiving soil between assumed limits of the MIC

Resistance formation limit	Resistance formation potential (%)			
	Effluent	Sludge	Soil	Seawater
0.2 of MIC	1.96	4.86	0.05	0.00
0.4 of MIC	7.63	15.63	0.07	0.00
0.6 of MIC	24.01	38.92	0.16	0.00
0.8 of MIC	65.66	75.76	0.38	0.00
1 of MIC	99.12	95.99	99.94	100.00

antimicrobials into the environment has been shown to result in concentrations that are of low toxicity concern (as determined by the HQs), but may be present at levels that are conducive to resistance formation. The potential for sub-MICs to select for resistance remains uncertain. Testing the boundaries of resistance and excluding lower limits of the MIC resulted in conditions that are conducive for resistance dissemination. At the lowest examined range (20% of the MIC), favourable conditions for *de novo* resistance formation remained for *E. coli* in all sites (occurred on<5% of occasions), excluding seawater. Although the model predicted no exposure risk to swimmers, it is unknown what effect, if any, long-term repeated exposure may have on the gut microbiota and what accumulation may occur. Most significantly, it remains uncertain what influences antimicrobial dissemination and maintenance within the effluent system and the environment. The release of hospital effluent into the municipal waste system appears to impact residue presence in the environment. The presence of antimicrobial residues in the environment has been linked to the maintenance and formation of resistance in the environment. There are some promising new antimicrobials currently being investigated, but if the new antimicrobial agents are not protected, resistance will accumulate just as fast and the situation will remain unchanged.

There is a need for further investigation into antimicrobials in the environment and the development of AMR strains and, in particular, what effect, if any, sub-MICs will have on the development and dissemination of resistance.

6 The effect of conventional wastewater treatment on the levels of antimicrobial-resistant bacteria in effluent: a meta-analysis of current studies

6.1 Overview

The literature review outlined in Section 1 identified that the effects of wastewater treatment on resistant bacteria is varied and a general view remains uncertain. In view of this uncertainty, a meta-analysis of current studies was carried out to determine what effect, if any, WWTP processing has on the prevalence of AMR bacteria. The findings of this section have been published (Harris *et al.*, 2012a). Details of approaches and assumptions adopted in completion of this meta-analyses can be found in this publication.

6.2 Results

A simple random-effects model was developed to characterise the effect of WWTP processing on the prevalence of resistant bacteria within the total population. By collecting data from published studies, it was possible to conduct a systematic meta-analysis. The data were assessed using Equation 1 to determine the odds ratio (OR) and the MIX software package was used for data analysis (version 1.7) and graphical representation (version 2.0).

$$OR = \frac{P_{Re}\left(P_{Sc}\right)}{P_{Se}\left(P_{Rc}\right)}$$

Where x refers to the control (c) or experimental (e) condition, P_{Rx} is the probability of resistant bacteria, P_{Sx} is the probability of susceptible bacteria, R_x is the frequency of resistant bacteria, N_e is the total number of bacteria and S_e is the frequency of susceptible bacteria ($S_x = N_x - R_x$). In this study, the "control" is represented by levels of bacteria in influent before wastewater treatment and "experimental" represents the levels in effluent after wastewater treatment.

The OR was simulated for fluoroquinolone-resistant (FR) bacteria (Table 6.1), single antimicrobial-resistant (SAR) *E. coli* and multiple antimicrobial-resistant (MAR) bacteria, resulting in graphical representations (Figure 6.1; box size represents study weighting in the analysis) of the population proportions of FR bacteria, SAR *E. coli* and MAR bacteria. The OR for FR bacteria, SAR *E. coli* and MAR bacteria was positive as a result of WWTP processing, with ORs of 1.19 [95% confidence interval (CI) 1.05–1.34, $p<0.01$], 1.33 (CI 1.19–1.50, $p<0.01$) and 1.60 (CI 1.39–1.84, $p<0.01$), respectively. The positive OR values indicate an increase in the proportion of resistant bacteria after WWTP processing. A sensitivity test for heterogeneity was assessed through an exclusion sensitivity assessment. The resulting ORs for FR bacteria, SAR *E. coli* and MAR bacteria varied from 1.15 to 1.23 ($p<0.05$), 1.32 to 1.35 ($p<0.01$) and 1.56 to 1.69 ($p<0.01$), respectively. The fact that the OR range variations are very small and are positive in each case indicates that no single study dominated the analysis and that the OR values are not sensitive to study variation.

6.3 Conclusions

Given the potential risks to human and animal health, a concerted approach is required to investigate sources of antimicrobial resistance. Meta-analysis was used as a tool to integrate experimental results and yield a combined evaluation of the impact of conventional wastewater treatment on the proportion of resistant bacteria. From the data analysed, it was concluded that the proportion of resistant bacteria within the total bacterial population increases as a result of WWTP processing. The results presented here highlight that, within WWTPs, there is a selective pressure on bacteria to become resistant. The factors that lead to the formation of resistance remain uncertain and thus further research is needed in this area to better understand the relationship between antimicrobial consumption, wastewater treatment and resistance within the environment. It is important to note that the process of resistance formation in the environment – and, specifically, in a WWTP – is very complex. The impact may be dependent on the precise nature of the WWTP, and it is possible that some treatment plants may increase the proportion of resistant bacteria, whereas others may reduce the proportion or leave it unchanged. The specific WWTP processes could also have implications

Table 6.1. FR bacteria eligible data

Study	Antimicrobial	Bacteria	N_c	R_c	N_e	R_e
1[a]	Ciprofloxacin	*E. coli*	152	0	158	2
2[a]	Ciprofloxacin	*E. coli*	152	0	158	1
3[a]	Ciprofloxacin	Enterococcus	88	12	55	10
4[a]	Ciprofloxacin	Enterococcus	88	37	55	39
5[a]	Ciprofloxacin	Enterococcus	132	24	126	15
6[a]	Ciprofloxacin	Enterococcus	132	82	126	56
7[b]	Nalidixic acid	*E. coli*	263	5	263	11
8[b]	Nalidixic acid	*E. coli*	290	29	290	12
9[c]	Ciprofloxacin	*Escherichia* spp.	159	4	115	11
10[c]	Ciprofloxacin	*Shigella* spp.	10	0	16	1
11[c]	Ciprofloxacin	*Klebsiella* spp.	20	0	23	1
12[d]	Ciprofloxacin	Heterotroph	4,060,505	105,573	25,750	669
13[d]	Ciprofloxacin	Heterotroph	5,074,968	86,274	5124	97
14[d]	Ciprofloxacin	Heterotroph	646,806	12,289	15,949	303
15[d]	Ciprofloxacin	Enterococcus	1,274,774	16,572	40,442	688
16[d]	Ciprofloxacin	Enterococcus	634,120	4439	253,184	2279
17[d]	Ciprofloxacin	Enterococcus	1,610,012	32,200	5112	143
18[d]	Ciprofloxacin	Enterococcus	825,951	28,908	1359	73
19[d]	Ciprofloxacin	Enterococcus	261,189	9142	332	17

a Garcia *et al.* (2007).
b Reinthaler *et al.* (2003).
c Ferreira da Silva *et al.* (2007).
d Manaia *et al.* (2010).
N_c, total of the control group (influent); N_e, total of the experiment group (effluent); R_c, mean of the control group (influent); R_e, mean of the experiment group (effluent).

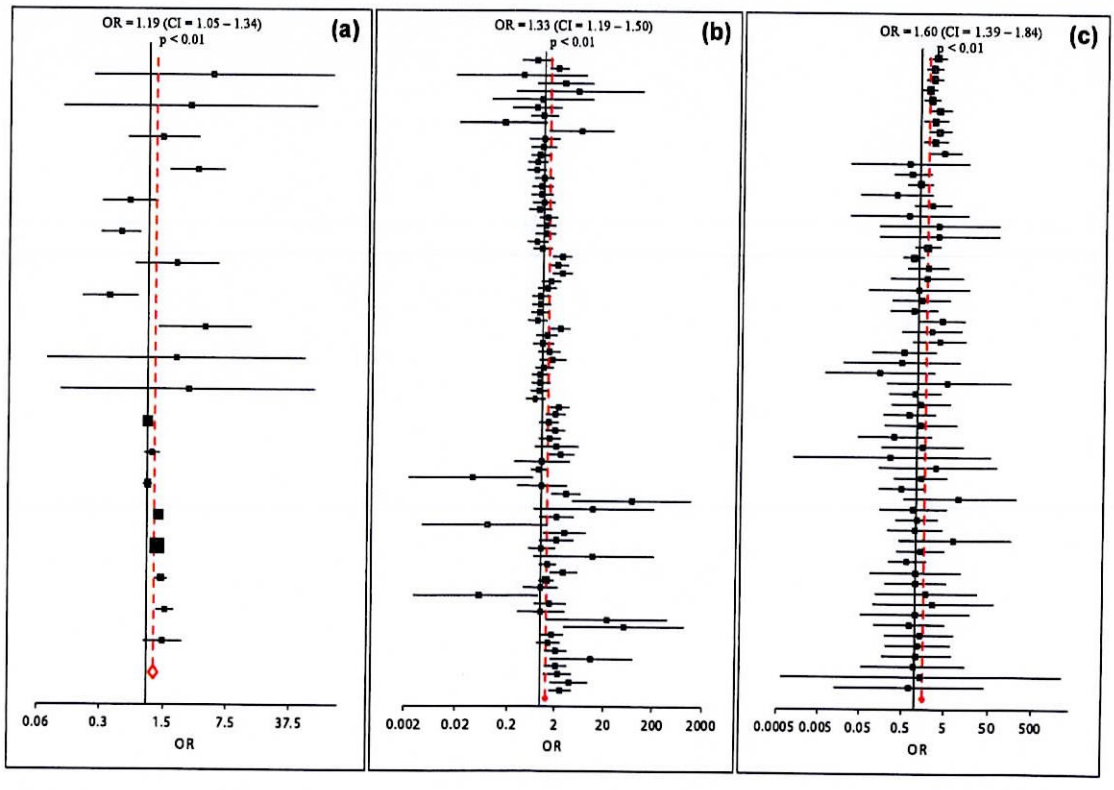

Figure 6.1. The effect of WWTP processing on the proportion of resistant bacteria as represented by the OR for (a) FR bacteria, (b) SAR *E. coli* and (c) MAR bacteria.

for effluent. More modern plants that employ technologies such as bio discs, which increase surface areas and hence improve treatment, should be considered. There is insufficient research in the area of antimicrobial resistance dissemination in the environment to fully understand the driving forces behind resistance dissemination and the differences between different antimicrobials and specific bacteria. Hence, there is an immediate need to develop a standard method of enumerating total and AMR bacteria to facilitate a better comparison of studies. This study has investigated one possible avenue for resistance formation, and highlights the possible role that antimicrobial agents and genetic determinants may play in resistance formation within a WWTP.

7 The effect of hospital effluent on antimicrobial-resistant *E. coli* within the municipal wastewater system

7.1 Overview

Analysis of current studies on the effect of WWTPs on antimicrobial resistance determined that there was a proportional increase in the population prevalence of antimicrobial resistance after WWTP processing for SAR, MAR and FR *E. coli*. To determine if the same effect was seen in WWTPs in Ireland and if the included treatment of hospital effluent further influenced the proportion of resistant bacteria, a site-specific analysis was carried out. The work presented in this section has been published by Harris *et al.* (2013c) and details of the approaches adopted can be found in this publication.

7.2 Results and discussion

The mean and range of MPN values for total *E. coli* and for *E. coli* resistant to each antimicrobial for each site sampled are presented in Table 7.1. AMR *E. coli* were detected in both WWTP systems and in both the influent and effluent samples. Total numbers of *E. coli* are generally comparable between the two systems. Table 7.2 shows the output of the statistical analysis which determined the likelihood of significant differences between observations between influent to a WWTP that receives hospital effluent ($WWTP_{he}$) and influent to a WWTP that does not receive hospital effluent ($WWTP_c$) and between influent and effluent for both WWTPs. The main observations from the statistical analysis are as follows:

1. In both WWTPs, there were fewer total colony-forming units (CFU) of *E. coli* in effluent than in influent in all instances. This indicates that the WWTPs reduce the total number of *E. coli*.
2. There was no significant difference in the percentage of resistant *E. coli* in the effluent compared with the influent in all instances, excluding tetracycline-resistant *E. coli* in both WWTPs and sulphonamide-resistant *E. coli* in the $WWTP_c$. In these instances, the percentage of resistant *E. coli* decreased from influent to effluent.
3. There was no significant difference in the percentage of AMR *E. coli* in the $WWTP_{he}$ or the $WWTP_c$ effluent (i.e. the percentage of AMR *E. coli* was not affected by whether or not the WWTP treats hospital effluent), excluding tetracycline-, sulphonamide- and ciprofloxacin-resistant *E. coli*. In these instances, the percentage of resistant *E. coli* within the final effluent was significantly higher for the $WWTP_{he}$. Therefore, it appears that the rate of resistance formation to these antimicrobials is influenced by the presence of hospital effluent.

Cefoxitin- and cefotaxime-resistant *E. coli* levels in the influent and effluent were often below the limit of detection (LD), hence the mean level equivalent was applied (LD/2). This means that the output was likely to be within the same range. Statistical analysis of this data set is not possible, as it would not give an accurate representation of actual events. Hence, no further analysis was carried out on these data sets. The authors cannot determine if there is an effect from hospital effluent on the rate of cefoxitin- and cefotaxime-resistant *E. coli*, but the fact that their presence was recorded in the environment is a concern.

For ampicillin- and streptomycin-resistant *E. coli*, hospital effluent does not significantly influence their prevalence. The results presented here are in agreement with Hawkey (2008) and Kümmerer (2008); the effect of hospital effluent containing antimicrobial residues varies for each antimicrobial and resistant bacteria complex. For some antimicrobials, the release of hospital effluent does not significantly affect the rate of resistance, but for other antimicrobials, such as ciprofloxacin, tetracycline and sulphonamide, the release of hospital effluent significantly impacts on the prevalence of resistant bacteria.

7.3 Conclusions

WWTPs were not originally designed to have a specific impact on resistant bacteria or antimicrobial residues, and their effects on these contaminants remains largely unknown. Thus, water quality monitoring is

Table 7.1. Antimicrobial-resistant *E. coli* (log_{10} CFU/ml) in the $WWTP_{he}$ and $WWTP_{c}$ influent and effluent

E. coli type	Location	Sample														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
$WWTP_{he}$																
Ampicillin resistant	Influent	5.09	5.87	4.93	5.28	4.80	5.35	4.78	5.44	4.87	LD/2	5.27	4.99	5.00	5.96	5.65
Ampicillin resistant	Effluent	4.00	3.27	4.15	4.31	LD/2	4.02	LD/2	4.00	LD/2	LD/2	3.11	LD/2	LD/2	LD/2	4.17
Streptomycin resistant	Influent	4.87	LD/2	5.00	5.08	4.87	5.31	5.30	5.42	4.93	LD/2	4.94	4.80	4.65	5.33	5.43
Streptomycin resistant	Effluent	4.48	3.72	4.31	3.76	LD/2	4.31	LD/2	4.00	LD/2	LD/2	4.45	LD/2	LD/2	LD/2	4.21
Cefoxitin resistant	Influent	LD/2	LD/2	LD/2	2.86	3.17	3.04	3.29	3.93	2.23	LD/2	3.35	3.87	2.99	3.93	2.87
Cefoxitin resistant	Effluent	LD/2	LD/2	LD/2	2.41	LD/2	LD/2	2.00	LD/2	3.00	LD/2	3.08	3.00	2.70	LD/2	2.25
Cefotaxime resistant	Influent	2.31	4.00	LD/2	3.99	4.02	3.79	3.95	3.95	3.06	LD/2	3.73	3.86	2.91	3.85	3.91
Cefotaxime resistant	Effluent	3.00	LD/2	LD/2	2.72	2.00	2.48	2.00	2.62	2.31	LD/2	2.00	2.49	2.41	LD/2	2.76
Tetracycline resistant	Influent	5.31	4.71	5.31	4.90	4.44	5.42	5.49	5.31	4.56	LD/2	5.07	4.13	4.37	4.08	4.88
Tetracycline resistant	Effluent	LD/2	3.49	LD/2	3.62	3.30	3.00	3.00	3.61	3.31	LD/2	3.69	LD/2	3.41	LD/2	3.69
Sulphonamide resistant	Influent	5.01	6.46	5.39	LD/2	4.86	4.66	5.59	5.69	5.08	4.16	5.01	4.21	5.01	5.01	6.21
Sulphonamide resistant	Effluent	4.93	4.03	4.80	4.00	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.31	LD/2	LD/2	LD/2	LD/2
Ciprofloxacin resistant	Influent	5.05	5.40	4.20	4.00	4.84	4.08	4.27	4.23	3.88	4.31	4.35	4.69	4.73	4.64	4.38
Ciprofloxacin resistant	Effluent	4.25	3.00	3.54	3.00	3.00	3.00	3.00	3.31	3.00	LD/2	3.12	LD/2	3.70	3.72	3.24
Total	Influent	6.87	7.02	6.91	6.31	5.53	6.54	6.46	6.33	5.55	5.93	6.14	5.53	5.37	6.40	7.14
Total	Effluent	5.48	4.67	6.61	4.93	4.80	4.99	4.62	4.80	6.61	5.00	5.39	4.80	5.17	5.17	5.39

LD, limit of detection; LD/2, mean of the level of detection.

Table 7.1. Continued

E. coli type	Location	Sample														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
WWTP$_c$																
Ampicillin resistant	Influent	5.87	5.20	5.12	5.98	5.20	5.03	5.08	5.20	5.15	5.12	4.40	5.87	5.99	5.04	5.77
Ampicillin resistant	Effluent	4.00	LD/2	LD/2	LD/2	4.00	4.00	4.00	4.00	4.00	LD/2	4.01	4.31	3.18	LD/2	4.31
Streptomycin resistant	Influent	4.86	5.04	5.13	4.49	5.08	5.17	5.08	5.79	5.78	6.05	5.27	4.66	4.63	5.62	5.00
Streptomycin resistant	Effluent	5.00	LD/2	LD/2	LD/2	LD/2	LD/2	5.00	4.00	LD/2	LD/2	4.26	LD/2	LD/2	LD/2	4.46
Cefoxitin resistant	Influent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.26	LD/2	2.51	2.09	2.02	3.04	2.84
Cefoxitin resistant	Effluent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	4.00	LD/2	LD/2	LD/2	2.70	LD/2	LD/2
Cefotaxime resistant	Influent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	2.96	LD/2	2.79	3.03	2.79	3.40	2.79
Cefotaxime resistant	Effluent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	2.49	LD/2	2.34	LD/2	2.84	2.00	LD/2
Tetracycline resistant	Influent	4.80	5.87	5.72	5.80	5.99	5.86	5.79	5.68	5.71	5.72	5.63	5.01	5.13	4.05	4.05
Tetracycline resistant	Effluent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.00	LD/2	3.51	LD/2	3.70	3.00	3.30
Sulphonamide resistant	Influent	5.81	6.04	6.04	5.62	6.08	6.08	5.97	5.88	5.18	6.04	5.99	4.24	5.06	4.08	LD/2
Sulphonamide resistant	Effluent	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.31	LD/2	LD/2	LD/2	4.67
Ciprofloxacin resistant	Influent	4.12	3.72	4.08	4.08	4.20	4.35	4.13	4.20	4.46	4.08	4.79	4.00	4.46	4.34	4.24
Ciprofloxacin resistant	Effluent	LD/2	LD/2	LD/2	3.00	3.00	3.00	3.00	3.00	LD/2	LD/2	3.00	LD/2	LD/2	LD/2	LD/2
Total	Influent	6.67	6.72	6.71	7.07	6.73	6.72	6.79	6.86	6.59	6.71	6.24	6.61	7.39	6.54	6.24
Total	Effluent	5.31	5.31	5.00	5.80	5.31	5.30	5.38	5.49	4.80	5.00	5.80	6.00	5.46	5.49	5.80

LD, limit of detection; LD/2, mean of the level of detection.

Table 7.2. Significance test of WWTP samples ($n=15$)

E. coli type	Location	%	Count
Ampicillin resistant	$WWTP_{he}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Ampicillin resistant	$WWTP_{c}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Ampicillin resistant	$WWTP_{he}$ vs $WWTP_{c}$ effluent	NSD, $p>0.05$	NSD, $p>0.05$
Streptomycin resistant	$WWTP_{he}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Streptomycin resistant	$WWTP_{c}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Streptomycin resistant	$WWTP_{he}$ vs $WWTP_{c}$ effluent	NSD, $p>0.05$	NSD, $p>0.05$
Tetracycline resistant	$WWTP_{he}$ influent to effluent	SD, $p<0.05$	SD, $p<0.05$
Tetracycline resistant	$WWTP_{c}$ influent to effluent	SD, $p<0.05$	SD, $p<0.05$
Tetracycline resistant	$WWTP_{he}$ vs $WWTP_{c}$ effluent	SD, $p<0.05$	NSD, $p>0.05$
Sulphonamide resistant	$WWTP_{he}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Sulphonamide resistant	$WWTP_{c}$ influent to effluent	SD, $p<0.05$	SD, $p<0.05$
Sulphonamide resistant	$WWTP_{he}$ vs $WWTP_{c}$ effluent	SD, $p<0.05$	NSD, $p>0.05$
Ciprofloxacin resistant	$WWTP_{he}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Ciprofloxacin resistant	$WWTP_{c}$ influent to effluent	NSD, $p>0.05$	SD, $p<0.05$
Ciprofloxacin resistant	$WWTP_{he}$ vs $WWTP_{c}$ effluent	SD, $p<0.05$	NSD, $p>0.05$

NSD, not significantly different; SD, significant difference.

recommended to identify the possibility of emerging hazards, particularly as antimicrobial resistance is of major public health significance. The correlation between antimicrobial residues, resistant bacteria, genetic elements, and the dissemination and persistence of resistance within the municipal wastewater system also remains unknown. Hospital effluent, containing these components, is released into the waste system allowing for exchange of genes and release into the aquatic and soil environment after treatment. From the results presented here, it is possible to conclude that hospital effluent may not be the main driving force behind resistance dissemination and persistence in the environment for AMR *E. coli*. The removal of hospital effluent containing more commonly used antimicrobials (in the home) may not significantly reduce the rate of resistance or persistence within the environment because resistance may already be well developed among the bacterial population. However, for other, hospital-specific antimicrobials, the release of hospital effluent containing these residues may encourage and preserve resistance within the environment into which it is discharged. This result corroborates the hypothesis postulated by Kümmerer (2003). There is evidence that there should be regulations with regard to the release of hospital effluent into the municipal waste system. To prevent the encouragement of antimicrobial resistance dissemination, effluents containing new antimicrobials should be separately treated, or treated with specialised methods, such as ozonation. It is important to note that antimicrobials and AMR bacteria vary in their persistence in the environment, method of removal and resistance mechanism. Hence, recommendations and regulations would need to be specific for the antimicrobial and resistant bacteria of interest. Most importantly, it must be noted that most antimicrobial resistance is from antimicrobial use, and, therefore, prudent use along with successful WWTP processing is the optimum course of action for battling the challenges of antimicrobial resistance dissemination in the environment.

8 Antimicrobial-resistant *E. coli* in the municipal wastewater system: effect of hospital effluent and environmental fate

8.1 Overview

The mean difference analysis outlined in Section 7 indicated that the effects of WWTP processing from influent to effluent were variable among different antimicrobial groups. A more detailed analysis of two WWTPs (one which treats hospital effluent and one which does not treat hospital effluent), including the effect of individual treatment process, was carried out to predict the probable levels of ampicillin-, streptomycin-, cefoxitin-, cefotaxime-, tetracycline-, sulphonamide- and ciprofloxacin-resistant and total *E. coli* in WWTP effluent and the receiving environments. The predicted concentrations were analysed for possible resistance formation potential and bather exposure. The work presented in this section has been published by Harris *et al.* (2014) and details of the approaches adopted can be found in this publication.

8.2 Results and discussion

The results in Tables 8.1 and 8.2 (which are comparable with measured validation) demonstrate that, in all instances, the mean percentage of AMR *E. coli* is greater in $WWTP_{he}$ influent than in $WWTP_{c}$ influent, excluding tetracycline-resistant *E. coli*. Tetracyclines are a class of older, broad-spectrum antimicrobials. Long-term and widespread use has significantly impacted on the development of resistance. Tetracyclines are among the lowest used antimicrobials in Europe, yet resistance remains prevalent.

There appears to be no major trend between the two WWTPs. The development of resistance appears to be completely AMR *E. coli*-specific within the WWTPs. Hence, it was not possible to determine any within-WWTP effect from hospital effluent; however, as

Table 8.1. Predicted mean percentages of AMR *E. coli* in $WWTP_{he}$ influent, effluent and sludge and measured validation

E. coli type	Mean (%) AMR *E. coli* in WWTP influent *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in WWTP effluent *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in primary sludge *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in dried sludge *(5th, 95th percentiles)*	Mean (CFU) AMR *E. coli* in WWTP effluent *(minimum, maximum)*	Mean (CFU) AMR *E. coli* in dried sludge *(minimum, maximum)*
Ampicillin resistant	18.64 *(0.92, 67.37)*	9.84 *(0.00, 57.41)*	51.15 *(17.14, 74.87)*	21.93 *(0.27, 80.29)*	6.98 *(0.12, 23.93)*	24.58 *(10.17, 38.85)*
Streptomycin resistant	11.27 *(0.62, 46.57)*	12.18 *(0.00, 64.02)*	9.43 *(0.94, 17.92)*	11.57 *(0.01, 55.14)*	8.22 *(0.12, 20.85)*	17.75 *(5.66, 27.74)*
Cefoxitin resistant	0.16 *(0.00, 0.56)*	4.32 *(0.00, 28.27)*	5.09 *(0.12, 21.55)*	8.03 *(0.00, 51.16)*	0.23 *(0.00, 1.60)*	0.29 *(0.10, 0.53)*
Cefotaxime resistant	0.86 *(0.02, 3.29)*	4.32 *(0.00, 28.37)*	2.01 *(0.28, 6.06)*	8.08 *(0.00, 51.37)*	0.23 *(0.00, 0.65)*	1.22 *(0.13, 4.04)*
Tetracycline resistant	11.75 *(0.44, 48.00)*	6.88 *(0.00, 45.20)*	13.79 *(3.00, 36.11)*	11.12 *(0.05, 43.59)*	2.14 *(0.01, 6.60)*	4.28 *(1.40, 9.62)*
Sulphonamide resistant	17.30 *(1.04, 65.97)*	8.70 *(0.00, 54.10)*	21.58 *(7.02, 34.57)*	17.83 *(0.08, 59.31)*	8.02 *(0.12, 27.66)*	22.61 *(9.75, 35.42)*
Ciprofloxacin resistant	4.20 *(0.27, 17.36)*	8.48 *(0.00, 51.15)*	1.49 *(0.47, 2.49)*	10.11 *(0.01, 72.07)*	1.80 *(0.02, 5.79)*	1.93 *(0.35, 4.87)*

Table 8.2. Predicted mean percentage of AMR *E. coli* in $WWTP_c$ influent, effluent and sludge and measured validation

E. coli type	Mean (%) AMR *E. coli* in WWTP influent *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in WWTP effluent *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in primary sludge *(5th, 95th percentiles)*	Mean (%) AMR *E. coli* in dried sludge *(5th, 95th percentiles)*	Mean (CFU) AMR *E. coli* in WWTP effluent *(minimum, maximum)*	Mean (CFU) AMR *E. coli* in dried sludge *(minimum, maximum)*
Ampicillin resistant	8.04 *(0.60, 28.70)*	19.22 *(0.03, 79.70)*	35.29 *(11.83, 51.59)*	38.37 *(10.68, 78.66)*	4.03 *(0.52, 15.82)*	48.96 *(30.28, 69.87)*
Streptomycin resistant	5.50 *(0.36, 19.70)*	10.71 *(0.04, 55.73)*	5.83 *(1.95, 8.52)*	41.10 *(10.65, 88.77)*	8.81 *(0.51, 49.50)*	46.03 *(18.91, 73.10)*
Cefoxitin resistant	0.02 *(0.00, 0.05)*	1.05 *(0.00, 4.93)*	0.37 *(0.12, 0.54)*	12.60 *(0.08, 64.23)*	0.14 *(0.01, 1.58)*	12.48 *(0.27, 61.11)*
Cefotaxime resistant	0.02 *(0.00, 0.07)*	2.50 *(0.00, 12.28)*	0.37 *(0.12, 0.54)*	0.79 *(0.23, 1.65)*	0.07 *(0.01, 0.48)*	0.87 *(0.67, 1.00)*
Tetracycline resistant	12.27 *(0.85, 35.05)*	4.99 *(0.00, 29.85)*	3.99 *(0.40, 7.57)*	9.70 *(0.75, 25.76)*	0.49 *(0.08, 1.72)*	8.60 *(5.76, 11.26)*
Sulphonamide resistant	15.28 *(1.03, 43.42)*	11.91 *(0.04, 59.43)*	11.51 *(5.32, 20.92)*	47.71 *(15.98, 90.66)*	2.94 *(0.32, 7.91)*	54.35 *(26.62, 86.63)*
Ciprofloxacin resistant	0.44 *(0.08, 1.26)*	1.65 *(0.01, 6.95)*	0.28 *(0.13, 0.51)*	1.92 *(0.15, 5.46)*	0.32 *(0.05, 0.79)*	1.38 *(0.34, 1.89)*

seen in Tables 8.3 and 8.4, a selective pressure may exist in the influent of $WWTP_{he}$. The effluent results are too variable to detect a comprehensive trend. Again, the sludge from the two WWTPs appears to show that hospital effluent has no specific influence on the percentage of AMR *E. coli* in sludge, although it was observed that the mean percentage of resistance in $WWTP_c$ increased in all instances, whereas in $WWTP_{he}$ the results were variable. The AMR *E. coli* counts within the two WWTPs were also very variable (Tables 8.3 and 8.4). For effluent, the numbers of resistant bacteria were comparable between the plants, further confirming the negligible effect of hospital effluent. However, for the sludge analysis, CFU count was slightly lower in primary and final sludge from $WWTP_c$ than from $WWTP_{he}$.

In general, the results from the prediction model, percentage and counts are too variable to detect any significant influence from hospital effluent. This may imply that hospital effluent is not the main driving force behind resistance formation and prevalence within the municipal wastewater system. This may also imply that resistance is already well developed within the bacterial population and analysis of the effects of hospital effluent would be redundant at this late stage of development. Alternatively, the results may imply that AMR bacteria are the leading contributing factor affecting the presence of resistance within the environment, and the trend and effects of treatment will be completely AMR bacteria specific.

Table 8.5 shows the results from the human environmental risk assessment. As discussed above, a no dose–response model was carried out. The table shows the mean predicted concentrations in 100 ml of seawater. The model predicts that a swimmer could be exposed to levels of AMR *E. coli* which could lead to the spread of resistance genes within the gastrointestinal tract of the contaminated individual.

8.3 Conclusions

AMR *E. coli* are widespread and their potential risks are well known. What remains uncertain is the main driving force behind their development and maintenance within receiving environments. The impact that hospital effluent, containing high loads of AMR *E. coli* and drug residues, has on the prevalence of AMR *E. coli* within the municipal system is of particular interest. From the results presented here, it can be concluded that hospital effluent may not be the main driving force behind resistance patterns within a WWTP, but may play a significant role in resistance prevalence in WWTP influent. Although the model predicted that hospital effluent was not a significant contributor to AMR *E. coli* in WWTPs, they are nevertheless being released into the aquatic environment. Assuming that bathers consume 100 ml of seawater each time they swim, they could potentially be exposed to bacteria retaining AMR genes that may disseminate among the infected individuals' gastrointestinal bacteria.

Table 8.3. AMR *E. coli* and total *E. coli* in $WWTP_{he}$ as measured using the adapted Colilert method

E. coli type	Location	Sample															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ampicillin resistant	I	5.09	5.87	4.93	5.28	4.80	5.35	4.78	5.44	4.87	LD/2	5.27	4.99	5.00	5.96	5.65	5.38
Ampicillin resistant	PR	6.04	5.93	5.80	5.72	5.09	5.74	5.44	5.80	5.13	LD/2	5.38	4.55	4.89	5.81	5.46	5.61
Ampicillin resistant	PE	5.87	5.00	5.49	4.00	4.71	5.40	4.67	5.51	4.80	LD/2	4.17	4.39	4.46	4.16	5.56	5.19
Ampicillin resistant	FE	4.00	3.27	4.15	4.31	LD/2	4.02	LD/2	4.00	LD/2	LD/2	3.11	LD/2	LD/2	LD/2	4.17	3.89
Ampicillin resistant	PS	5.84	6.33	5.79	5.91	6.49	6.40	6.55	6.66	6.34	6.47	6.40	6.51	6.53	6.65	6.51	6.36
Ampicillin resistant	DS	7.13	7.05	7.05	6.85	7.04	7.11	7.05	6.95	6.89	7.04	7.00	7.11	7.17	7.04	7.11	7.04
Streptomycin resistant	I	4.87	LD/2	5.00	5.08	4.87	5.31	5.30	5.42	4.93	LD/2	4.94	4.80	4.65	5.33	5.43	5.08
Streptomycin resistant	PR	5.99	5.72	5.72	4.62	5.00	5.64	5.71	5.74	4.30	LD/2	5.01	4.99	4.40	5.03	5.90	5.51
Streptomycin resistant	PE	4.87	4.08	4.49	3.70	4.71	4.54	5.04	5.55	4.72	LD/2	4.79	4.72	4.36	4.93	5.42	4.91
Streptomycin resistant	FE	4.48	3.72	4.31	3.76	LD/2	4.31	LD/2	4.00	LD/2	LD/2	4.45	LD/2	LD/2	LD/2	4.21	4.06
Streptomycin resistant	PS	5.33	5.02	5.43	5.50	5.92	5.75	5.59	5.80	5.84	5.84	5.70	5.65	5.80	6.01	5.65	5.66
Streptomycin resistant	DS	6.85	6.76	6.81	6.60	6.88	6.93	7.04	7.01	7.06	7.06	6.78	6.95	6.65	7.06	6.95	6.89
Cefoxitin resistant	I	LD/2	LD/2	LD/2	2.86	3.17	3.04	3.29	3.93	2.23	LD/2	3.35	3.87	2.99	3.93	2.87	3.36
Cefoxitin resistant	PR	LD/2	LD/2	LD/2	3.99	2.86	3.88	2.99	3.95	2.92	LD/2	3.79	3.10	3.56	3.17	LD/2	3.44
Cefoxitin resistant	PE	LD/2	LD/2	LD/2	3.31	2.99	3.30	2.99	3.33	2.08	LD/2	3.51	3.09	2.81	2.24	LD/2	2.96
Cefoxitin resistant	FE	LD/2	LD/2	LD/2	2.41	LD/2	LD/2	2.00	LD/2	3.00	LD/2	3.08	3.00	2.70	LD/2	2.25	2.49
Cefoxitin resistant	PS	3.88	3.80	3.80	3.97	6.01	5.59	5.76	5.34	4.82	4.89	4.79	5.14	5.20	5.44	5.14	4.90
Cefoxitin resistant	DS	5.19	4.99	5.07	4.85	5.19	5.06	5.08	4.88	4.94	4.94	5.13	5.26	5.30	5.26	5.26	5.09
Cefotaxime resistant	I	2.31	4.00	LD/2	3.99	4.02	3.79	3.95	3.95	3.06	LD/2	3.73	3.86	2.91	3.85	3.91	3.75
Cefotaxime resistant	PR	LD/2	LD/2	LD/2	2.21	3.88	4.45	3.93	4.79	2.90	LD/2	4.40	3.80	3.75	3.67	LD/2	4.00
Cefotaxime resistant	PE	LD/2	LD/2	LD/2	3.08	3.75	3.96	3.72	4.04	2.80	LD/2	LD/2	3.78	3.65	2.95	LD/2	3.47
Cefotaxime resistant	FE	3.00	LD/2	LD/2	2.72	2.00	2.48	2.00	2.62	2.31	LD/2	2.00	2.49	2.41	LD/2	2.76	2.44
Cefotaxime resistant	PS	4.46	4.30	4.43	4.54	5.33	4.69	4.48	4.47	4.50	4.71	5.59	5.35	5.21	4.88	5.35	4.82
Cefotaxime resistant	DS	5.82	5.45	5.62	5.29	5.59	5.82	5.19	5.21	4.95	5.23	5.26	6.19	6.01	5.21	6.19	5.54
Tetracycline resistant	I	5.31	4.71	5.31	4.90	4.44	5.42	5.49	5.31	4.56	LD/2	5.07	4.13	4.37	4.08	4.88	5.03
Tetracycline resistant	PR	5.31	5.00	5.00	3.49	4.90	5.33	4.90	5.42	3.92	3.49	5.46	3.64	3.78	3.79	5.27	5.01

DS, dried sludge; FE, final effluent; I, influent; PE, primary effluent; PR, post-return effluent; PS, primary sludge.

Table 8.3. Continued

E. coli type	Location	Sample															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Tetracycline resistant	PE	LD/2	5.80	5.00	3.04	3.87	5.31	4.24	5.39	3.27	3.39	4.21	3.87	3.59	3.04	4.24	4.92
Tetracycline resistant	FE	LD/2	3.49	LD/2	3.62	3.30	3.00	3.00	3.61	3.31	LD/2	3.69	LD/2	3.41	LD/2	3.69	3.32
Tetracycline resistant	PS	5.55	5.44	5.51	5.44	5.76	5.68	5.15	5.86	6.05	6.39	7.10	5.85	6.04	5.76	5.85	5.83
Tetracycline resistant	DS	6.57	6.39	6.60	6.60	6.60	6.30	6.38	6.15	6.22	6.01	6.13	5.92	5.73	6.15	5.92	6.24
Sulphonamide resistant	I	5.01	6.46	5.39	LD/2	4.86	4.66	5.59	5.69	5.08	4.16	5.01	4.21	5.01	5.01	6.21	5.62
Sulphonamide resistant	PR	6.41	6.12	5.99	4.00	4.00	5.71	5.32	5.26	4.16	LD/2	5.04	4.08	5.89	5.04	6.02	5.72
Sulphonamide resistant	PE	6.03	5.39	5.93	LD/2	4.72	5.40	4.96	5.33	4.77	4.72	4.94	4.60	4.62	4.60	5.80	5.40
Sulphonamide resistant	FE	4.93	4.03	4.80	4.00	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.31	LD/2	LD/2	LD/2	LD/2	4.17
Sulphonamide resistant	PS	5.80	5.75	5.91	6.05	6.20	6.15	6.15	5.80	5.64	6.17	6.08	6.20	6.13	6.13	6.20	6.02
Sulphonamide resistant	DS	7.13	6.95	7.07	6.89	7.09	7.15	7.15	7.13	7.09	6.97	6.83	6.69	7.02	7.15	6.69	7.00
Ciprofloxacin resistant	I	5.05	5.40	4.20	4.00	4.84	4.08	4.27	4.23	3.88	4.31	4.35	4.69	4.73	4.64	4.38	4.69
Ciprofloxacin resistant	PR	5.46	5.71	5.72	5.00	4.13	4.78	4.08	4.83	4.17	LD/2	4.80	5.13	5.27	5.22	5.08	5.18
Ciprofloxacin resistant	PE	4.42	4.18	4.07	LD/2	3.62	4.58	3.93	4.15	3.72	3.49	4.11	3.80	4.09	3.88	3.36	4.05
Ciprofloxacin resistant	FE	4.25	3.00	3.54	3.00	3.00	3.00	3.00	3.31	3.00	LD/2	3.12	LD/2	3.70	3.72	3.24	3.46
Ciprofloxacin resistant	PS	4.80	4.75	4.92	4.95	5.07	5.04	4.95	4.98	4.80	5.03	4.65	4.59	4.84	4.58	4.59	4.84
Ciprofloxacin resistant	DS	5.77	5.70	5.69	5.39	5.90	5.67	5.72	5.95	5.99	6.04	6.09	6.10	6.27	5.95	6.10	5.89
Total	I	6.87	7.02	6.91	6.31	5.53	6.54	6.46	6.33	5.55	5.93	6.14	5.53	5.37	6.40	7.14	6.57
Total	PR	7.14	6.85	6.89	6.31	5.62	6.91	6.23	6.52	5.66	5.00	6.61	5.60	6.49	6.60	6.61	6.61
Total	PE	6.67	6.98	6.88	5.00	5.26	6.38	5.45	6.21	5.32	5.31	5.40	5.40	5.10	5.53	6.38	6.30
Total	FE	5.48	4.67	6.61	4.93	4.80	4.99	4.62	4.80	6.61	5.00	5.39	4.80	5.17	5.17	5.39	5.81
Total	PS	6.59	6.59	6.76	6.77	6.76	6.77	6.76	6.77	6.59	6.59	6.59	6.77	6.76	6.77	6.77	6.71
Total	DS	7.62	7.58	7.62	7.85	7.85	7.62	7.85	7.58	7.85	7.62	7.85	7.58	7.58	7.62	7.58	7.68

DS, dried sludge; FE, final effluent; I, influent; PE, primary effluent; PR, post-return effluent; PS, primary sludge.

Table 8.4. AMR *E. coli* and total *E. coli* in $WWTP_c$ as measured using the adapted Colilert method

E. coli type	Location	Sample															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ampicillin resistant	I	5.87	5.20	5.12	5.98	5.20	5.03	5.08	5.20	5.15	5.12	4.40	5.87	5.99	5.04	5.77	5.54
Ampicillin resistant	PE	5.23	4.93	4.99	4.71	4.99	4.99	6.00	5.03	4.93	4.99	4.86	5.00	5.97	5.02	4.77	5.32
Ampicillin resistant	FE	4.00	LD/2	LD/2	LD/2	4.00	4.00	4.00	4.00	4.00	LD/2	4.01	4.31	3.18	LD/2	4.31	3.96
Ampicillin resistant	PS	5.71	5.66	5.70	5.72	5.69	–	–	–	–	–	–	–	–	–	–	5.70
Ampicillin resistant	DS	6.63	6.43	6.34	6.62	6.59	–	–	–	–	–	–	–	–	–	–	6.52
Streptomycin resistant	I	4.86	5.04	5.13	4.49	5.08	5.17	5.08	5.79	5.78	6.05	5.27	4.66	4.63	5.62	5.00	5.41
Streptomycin resistant	PE	4.16	4.79	4.87	4.03	4.87	4.03	4.83	4.14	4.53	4.87	4.64	4.46	4.34	4.44	4.93	4.63
Streptomycin resistant	FE	5.00	LD/2	LD/2	LD/2	LD/2	LD/2	5.00	4.00	LD/2	LD/2	4.26	LD/2	LD/2	LD/2	4.46	4.31
Streptomycin resistant	PS	4.79	4.30	4.92	5.02	4.30	–	–	–	–	–	–	–	–	–	–	4.67
Streptomycin resistant	DS	6.24	6.53	6.60	6.56	6.42	–	–	–	–	–	–	–	–	–	–	6.47
Cefoxitin resistant	I	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.26	LD/2	2.51	2.09	2.02	3.04	2.84	2.49
Cefoxitin resistant	PE	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	2.00	LD/2	2.99	LD/2	2.00	LD/2	LD/2	LD/2	2.97	2.25
Cefoxitin resistant	FE	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	4.00	LD/2	LD/2	LD/2	2.70	LD/2	LD/2	2.17
Cefoxitin resistant	PS	3.76	3.79	3.72	3.66	3.65	–	–	–	–	–	–	–	–	–	–	3.72
Cefoxitin resistant	DS	6.75	4.56	4.17	4.23	4.33	–	–	–	–	–	–	–	–	–	–	4.81
Cefotaxime resistant	I	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	2.96	LD/2	2.79	3.03	2.79	3.40	2.79	2.66
Cefotaxime resistant	PE	LD/2	LD/2	LD/2	2.00	LD/2	2.00	2.00	2.00	4.29	LD/2	3.70	3.92	2.10	4.12	2.05	3.50
Cefotaxime resistant	FE	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	2.49	LD/2	2.34	LD/2	2.84	2.00	LD/2	2.09
Cefotaxime resistant	PS	3.72	3.69	3.63	3.68	3.75	–	–	–	–	–	–	–	–	–	–	3.69
Cefotaxime resistant	DS	4.79	4.86	4.72	4.77	4.79	–	–	–	–	–	–	–	–	–	–	4.79
Tetracycline resistant	I	4.80	5.87	5.72	5.80	5.99	5.86	5.79	5.68	5.71	5.72	5.63	5.01	5.13	4.05	4.05	5.63
Tetracycline resistant	PE	4.61	4.95	5.48	4.84	5.49	5.49	5.51	5.14	4.74	5.48	4.66	4.93	3.55	4.04	4.60	5.15
Tetracycline resistant	FE	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.00	LD/2	3.51	LD/2	3.70	3.00	3.30	3.16

DS, dried sludge; FE, final effluent; I, influent; PE, primary effluent; PR, post-return effluent; PS, primary sludge.

Table 8.4. Continued

E. coli type	Location	Sample															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Tetracycline resistant	PS	4.82	4.74	4.60	4.69	4.38	–	–	–	–	–	–	–	–	–	–	4.65
Tetracycline resistant	DS	5.72	5.82	5.76	5.82	5.74	–	–	–	–	–	–	–	–	–	–	5.77
Sulphonamide resistant	I	5.81	6.04	6.04	5.62	6.08	6.08	5.97	5.88	5.18	6.04	5.99	4.24	5.06	4.08	LD/2	5.81
Sulphonamide resistant	PE	5.00	4.84	5.49	5.00	4.62	4.49	4.33	5.23	4.84	5.49	4.40	2.76	4.70	4.66	LD/2	4.95
Sulphonamide resistant	FE	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	LD/2	3.31	LD/2	LD/2	LD/2	4.67	4.31
Sulphonamide resistant	PS	5.10	4.92	5.33	5.10	4.92	–	–	–	–	–	–	–	–	–	–	5.07
Sulphonamide resistant	DS	6.38	6.46	6.58	6.71	6.59	–	–	–	–	–	–	–	–	–	–	6.54
Ciprofloxacin resistant	I	4.12	3.72	4.08	4.08	4.20	4.35	4.13	4.20	4.46	4.08	4.79	4.00	4.46	4.34	4.24	4.29
Ciprofloxacin resistant	PE	4.72	4.32	3.93	3.49	4.30	3.99	4.28	4.33	4.23	3.93	3.89	4.23	4.21	4.32	4.23	4.24
Ciprofloxacin resistant	FE	LD/2	LD/2	LD/2	3.00	3.00	3.00	3.00	3.00	LD/2	LD/2	3.00	LD/2	LD/2	LD/2	LD/2	2.85
Ciprofloxacin resistant	PS	3.78	3.3.	3.30	3.61	3.30	–	–	–	–	–	–	–	–	–	–	3.50
Ciprofloxacin resistant	DS	5.24	5.17	4.79	4.30	5.10	–	–	–	–	–	–	–	–	–	–	4.92
Total	I	6.37	6.66	6.53	6.24	6.62	6.48	6.51	6.38	6.51	6.53	6.12	6.97	7.32	7.11	6.49	6.80
Total	PE	6.37	6.66	6.53	6.24	6.62	6.48	6.51	6.38	6.51	6.53	6.12	6.97	7.32	7.11	6.49	6.72
Total	FE	5.31	5.31	5.00	5.80	5.31	5.30	5.38	5.49	4.80	5.00	5.80	6.00	5.46	5.49	5.80	5.53
Total	PS	6.02	6.04	5.98	6.21	6.00	–	–	–	–	–	–	–	–	–	–	6.05
Total	DS	6.96	6.95	6.74	6.77	6.83	–	–	–	–	–	–	–	–	–	–	6.85

DS, dried sludge; FE, final effluent; I, influent; PE, primary effluent; PR, post-return effluent; PS, primary sludge.

Table 8.5. Predicted average levels of AMR *E. coli* in final effluent and in seawater after effluent release

AMR *E. coli*	Average PC_{FE} (CFU/100 ml)	Average PC_{sw} (CFU/100 ml)
Ampicillin-resistant *E. coli*	29,361	58
Streptomycin-resistant *E. coli*	97,502	193
Cefoxitin-resistant *E. coli*	3285	6
Cefotaxime-resistant *E. coli*	15,086	30
Tetracycline-resistant *E. coli*	9702	19
Sulphonamide-resistant *E. coli*	84,770	168
Ciprofloxacin-resistant *E. coli*	4204	8

PC_{FE}, predicted concentration of AMR *E. coli* in final effluent; PC_{sw}, predicted concentration of AMR *E. coli* in seawater.

It is well known that the effects of antimicrobial drug residues and AMR genes are specific to each AMR bacteria complex and the results from this study substantiate this theory. This highlights the importance of site-specific examination of AMR bacteria. By adapting the model inputs presented here, this model could be applied elsewhere to examine the effects of hospital effluent and of WWTP processing on AMR prevalence and persistence.

9 Discussion

Antimicrobials are vital to many aspects of human, animal and plant disease treatment and prophylaxis. Their discovery drastically reduced the human mortality and morbidity resulting from bacterial infections. They are also used in common medical procedures, including organ transplantation, cancer treatment and orthopaedic surgery. The imprudent use of antimicrobial agents has significantly contributed to the development and spread of resistance worldwide in multiple environmental compartments.

Infection from AMR bacteria can lead to therapeutic failure and antimicrobial redundancy. The ECDC estimates that antimicrobial resistance results in 25,000 deaths and related costs, resulting from healthcare expenses and productivity losses, of over €1.5 billion annually. Regardless of these documented detrimental effects of long-term over use of antimicrobials, it continues. Antimicrobials are also released into the environment in large quantities in industrial, nursing-home and hospital effluents. In many countries, there is no monitoring or regulation of the release of antimicrobials or resistant genetic determinants into the municipal wastewater system.

Antimicrobial residues are present and can persist in the environment. Regardless of high antimicrobial consumption, continued reporting of the global spread of resistance, and the known human and environmental risks, monitoring or regulation is not a legal requirement in many countries. In addition to the absence of standardised testing and reporting, this has led to a lack of knowledge on the fate of antimicrobials in the environment. One of the main limitations when it comes to assessing the risks that antimicrobial residues have on the environment and human health, is the variability among antimicrobial groups. The methods of degradation and removal, as well as the methods of resistance formation, differ between antimicrobial groups. Without in-depth knowledge of individual antimicrobials and antimicrobial groups it is not possible to make appropriate decisions regarding mitigation.

Wastewater treatment plants are considered the main source of antimicrobial entry into the environment, but it remains uncertain what effect specific treatment processes have on antimicrobial residues and resistant determinants. It is unclear whether or not WWTP processing is less effective at removing resistant bacteria and whether or not the process promotes the development of resistance among susceptible bacteria. Verlicchi *et al.* (2012) identified hospital effluent as a significant source of environmental pollutants. They recommended an environmental risk assessment which identified antimicrobials as a significant pollutant in hospital effluent. The application of a more comprehensive risk assessment is recommended to obtain a more accurate assessment of health and ecological risks associated with antimicrobials in the environment (Verlicchi *et al.*, 2012). Along with the limitations and knowledge gaps discovered in the literature review (see Section 1), risk assessment has been identified as a vital tool in assessing antimicrobials in the environment. Risk assessment models incorporate inherent uncertainty and variability among input parameters; therefore, risk assessment modelling is ideal for assessing the possible risk posed to humans and the environment. Section 4 outlines the application of risk assessment strategies to assess antimicrobial residues in the environment. A Monte Carlo simulation model was developed to calculate the PECs for each antimicrobial group and to assess resistance formation potential along with environmental toxicity and the leading parameter which contributes to their presence in the environment.

It was concluded that the systemic use and subsequent release of antimicrobials resulted in their presence in the environment. Compound degradation contributed minimally to the presence of most of the antimicrobial groups in the environment, suggesting that degradation within the soil or aqueous environment is less influential on their environmental concentration than use or excretion. This is confounded by the fact that antimicrobials are continuously released into the environment. Metabolism or rate of excretion was identified as the leading contributing factor resulting in contamination of the environment by some antimicrobial groups. Therefore, it is recommended that risk management for existing antimicrobials should focus on reducing their input, whereas for antimicrobials that are currently being developed, the rate of metabolism should be considered. Although this discovery is important to prevent the contamination of the final receiving environment, a

significant lack in the supply of new antimicrobials and a failure to discover or develop any new antimicrobials remains, deeming the possible regulation of the metabolism of new antimicrobials currently unlikely.

The potential for lower limits of antimicrobial concentration to act as selectors for resistance formation is widely unknown. There are also many difficulties associated with modelling such a complex mechanism, which is influenced by many factors. The model presented in Section 4 assessed varying scenarios in which resistance formation may occur. The margins for resistance formation were limited, and the probability that the PECs would occur between these set boundaries was examined. Quinolones/fluoroquinolones showed the highest resistance formation potential in all bound tests. It is important to note that this scenario is unlikely to occur naturally, but there is also no evidence to suggest what effect, if any, compounds at this low concentration could have on resistance formation. Nevertheless, examining PECs with bound resistance limits is a useful method of assessment to test possible scenarios while other methods remain unavailable and we continue to further our understanding of the lower limits of inhibitory concentrations. In addition to the effects resulting from lower limits of antimicrobial residues, baseline creep, whereby the average MIC increases steadily, without limit, widening the possible concentrations at which resistance formation may occur, is another phenomenon that impacts on the ability to assess resistance formation potential. The model also predicted that quinolones/fluoroquinolones have the lowest rate of degradation. Hence, further investigation into this antimicrobial group is recommended. This discovery resulted in the development of the risk ranking model detailed in Section 4. The model was designed to specifically assess the fluoroquinolone ciprofloxacin. Specific hospital usage, excretion and dilution were incorporated to determine the PEC of ciprofloxacin in the environment. Along with the parameters analysed in the risk ranking model, including resistance formation potential, toxicity and HQ (Section 4), two further PEC endpoints were examined, namely seawater and soil, and the exposure risk to swimmers was also assessed. Ciprofloxacin was predicted to enter the environment at low concentrations with a low risk of toxicity, but the levels were, nonetheless, conducive for resistance formation. When the boundaries for resistance were set, the resistant formation potential significantly reduced; in particular, seawater samples were predicted to have negligible risk. Although for the model endpoints, namely seawater and soil, the concentration of ciprofloxacin had a very low resistance formation potential (when bound), the already resistant bacteria released within the effluent would have the ability to spread and preserve resistance through horizontal gene transfer.

Three risk assessment strategies were used to investigate the effects and contribution of AMR bacteria within the municipal system with regard to resistance development. Firstly, a meta-analysis was conducted to assess current literature and to identify general opinions on the effects of WWTP processing on the prevalence of resistance. An analysis of publications relating to MAR bacteria, SAR *E. coli* and quinolone-/fluoroquinolone-resistant bacteria was carried out. WWTPs that receive and treat various water types, including domestic, hospital, nursing-home, industry, dairy-farm and landfill effluent, were examined. The meta-analysis results suggest an association of increased resistance after WWTP processing. As the proportion of resistant bacteria was higher within the final effluent than within the influent, it was postulated that a selective pressure exists within the WWTP. Thus, it was hypothesised that WWTPs encourage the selection of AMR bacteria. Nevertheless, uncertainties remained regarding the origin of bacterial resistance within the environment. It has not yet been determined whether antimicrobial residues or genetic resistance determinants were the leading contributor of antimicrobial resistance development. It is not known whether resistant bacteria have a better rate of survival through WWTPs or whether resistance is spreading among the susceptible population opportunistically. Whether or not effluent containing high quantities of antimicrobial residues should be treated separately is still disputed, and there are no recommended government guidelines or mandatory practices in this regard.

Based on the results and conclusions presented in Section 7, further investigation is needed to understand the effects and causes of resistance formation within WWTPs, and to determine what the leading source of resistance is. The results support the need for further research into the development of AMR strains and possible selective pressures operating in WWTPs. As a result of the findings of this initial investigation presented in Section 7, the second risk assessment strategy, used to identify the effect of WWTP processing on antimicrobial resistance, was carried out, as presented in Section 8. A mean difference analysis

was carried out to assess the effects of WWTP processing on AMR *E. coli* in Ireland. This included an assessment of the effects of hospital effluent, which potentially contains antimicrobial residues and high loads of AMR bacteria and resistant determinants. By assessing water from two WWTPs (one of which does and one of which does not receive and treat hospital effluent) it was possible to assess the effects of WWTP processing on *E. coli* resistant to ampicillin, streptomycin, cefoxitin, cefotaxime, tetracycline, sulphonamide and ciprofloxacin. The treatment of hospital effluent within a municipal WWTP does not appear to influence the prevalence of all types of AMR *E. coli*. However, the inclusion of hospital effluent within a WWTP did significantly impact on the prevalence of *E. coli* resistant to the antimicrobials ciprofloxacin, tetracycline and sulphonamide. For clinical isolates, antimicrobial exposure is considered the main risk factor for fluoroquinolone resistance development (Carmeli *et al.*, 1999; Harris *et al.*, 1999). This may explain the trend seen in the model presented in Section 8; however, as no genetic investigation has been carried out on isolates from the sample points, it was not possible to determine if this is a valid conclusion. The model determined that the effect of hospital effluent containing antimicrobial residues and AMR *E. coli* is highly variable and specific. Resistant bacteria and genes within the WWTP may be the leading promoter of resistance dissemination and maintenance, or it may be too late to assess antimicrobial resistance from hospital effluent containing commonly used antimicrobials. Resistance may have already been well developed in the environment and the impact of hospital effluent may have already occurred (i.e. the impact has previously happened and hence cannot be subsequently identified). From the results presented in Section 8, it is possible to conclude that the separate treatment of hospital effluent containing these antimicrobials, for which environmental resistance is already well developed, would not reduce the rate of resistance or persistence. The genes have already spread among the bacterial communities. In contrast, for antimicrobial resistance that is not widely developed in the environment or for less commonly used antimicrobials, the release of hospital effluent containing these residues may encourage and preserve resistance. The regulation and separate treatment of hospital effluent containing these hospital-specific antimicrobials is, therefore, recommended.

As a result of the discoveries in Section 8, relating to the comparison of WWTP influent and effluent, a third risk assessment strategy was used to investigate the effects and contribution of AMR bacteria within the municipal system on resistance by examining the individual treatment stages. Along with examining the effect of hospital effluent on AMR *E. coli* presence and release within the WWTP and the receiving environments, the extended and more detailed model examined the possible exposure of bathers in seawater.

There appear to be no consistent effects on the prevalence of resistance as a result of the treatment of hospital effluent within a municipal WWTP. The model results mirrored the results presented in Section 8, implying that hospital effluent is not the main factor that influences the development and maintenance of AMR *E. coli* within WWTPs. Perhaps the already persistent AMR *E. coli* may be the leading factor or perhaps resistance may be too well developed to detect any trend resulting from hospital effluent at this late stage. It is noteworthy to report that the trends and effects of treatment, including hospital effluent, appear to be entirely AMR bacteria specific, highlighting the importance of the incidence-specific examination of AMR bacteria with a specific focus on the particular antimicrobial and bacterial species of interest.

Although hospital effluent does not seem to be the main factor influencing resistance prevalence, it is continuously being released into the environment and contains both antimicrobial residues and AMR bacteria. After WWTP processing and environmental release, there is potential for human exposure to these contaminants. This exposure may result in the dissemination of resistance genes among the gastrointestinal bacteria of exposed individuals and may, therefore, introduce resistance to the human population, thereby contributing to the cycle discussed in Section 1 and illustrated in Figure 1.1.

10 Conclusions

Antimicrobial residues and antimicrobial resistance determinants are present in the environment undoubtedly as a result of the systemic use of antimicrobial agents in human and veterinary medicine and in agriculture. WWTPs and effluents containing high loads of these contaminants contribute to their presence and further the dissemination of resistance in the receiving environment. Although resistance may increase during WWTP processing, it is highly variable and antimicrobial and bacteria specific. It is evident from the results presented here that there is substantial resistance in the examined environments, to the extent that the release of hospital effluent does not affect the prevalence of resistance (as it is already well developed and maintained within the bacterial community). Nevertheless, for some AMR bacteria, hospital effluent does significantly affect the prevalence of resistance. Antimicrobial resistance is a serious medical concern and some of the risks have been discussed here. This study identified the detrimental effects of releasing antimicrobial residues and genetic determinants of resistance into the environment. The implications for the future with regard to antimicrobial resistance are currently bleak. It appears that it may be too late to amend the spread of resistance for commonly used antimicrobials. For new antimicrobials, licensing regulations should consider metabolism within the body, as this contributes significantly to the release of antimicrobials into the environment.

11 Recommendations

The focus of this study was to provide information on the contribution of hospital effluent to the levels of quinolones/fluoroquinolones and AMR *E. coli* in urban wastewater, and the potential for these substances/ organisms to persist through the various steps of wastewater treatment and the land application of biosolids. These results informed the development of a risk assessment approach for sanitary authorities/ regulatory authorities to assess human exposure to quinolones/fluoroquinolones and AMR *E. coli* as a result of recreational water use and biosolids that are spread on agricultural land. The recommendations presented here are based on the research and conclusions presented in this report:

1. The development of international guidelines regarding regulation, monitoring and reporting of antimicrobial presence and antimicrobial resistance in the municipal wastewater system and receiving environments is recommended.

2. The models developed in this study predict that new antimicrobial agents, such as the quinolones/ fluoroquinolones, have the lowest rate of degradation and the highest resistance formation potential. Currently, there are no government-recommended guidelines to assess or quantify antimicrobial resistance formation in the environment. The authors recommend that the method presented here could be used in conjunction with ERC methodology to create a mechanistic model that can assess antimicrobial resistance formation potential.

3. As with all areas of environmental toxicity, standardisation of methods used to sample, test and report antimicrobial contamination in the environment is required.

4. The systemic use of antimicrobials is a leading factor contributing to their presence in the environment. For older antimicrobial agents, risk management should focus on reducing the input of these into the environment. Data demonstrate that antimicrobials that are highly metabolised within the body are less likely to be excreted into the environment. The rate of metabolism of new antimicrobials should be considered a risk factor for their regulation and, hence, their presence in the environment.

5. The study concludes that the proportion of resistant bacteria within the total bacterial population increases as a result of WWTP processing. Further research is recommended to understand the processes underlying this and identify risk management strategies.

6. Hospital effluent is not a significant contributor to the emergence, dissemination and persistence of *E. coli* resistant to antimicrobial agents that have been used for many years; however, caution is recommended for more recently developed antimicrobial agents, such as the quinolones/fluoroquinolones and carbapenems. Hence, recommendations and regulations would need to be specific for the antimicrobial and resistant bacteria of interest.

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Abbreviations

ADI	Acceptable daily intake
AF	Assessment factor
AMR	Antimicrobial-resistant
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CI	Confidence interval
DDD	Defined daily dose
De	Degradation
EC_{50}	Concentration at which a drug is 50% effective
ECDC	European Centre for Disease Prevention and Control
ECOSAR	Ecological Structure Activity Relationships
EMEA	European Medicines Agency
ERC	Environmental reference concentration
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
ESBL	Extended spectrum β-lactamase
EU	European Union
EUSES	European Union System for the Evaluation of Substances
FDA	US Food and Drug Administration
FR	Fluoroquinolone-resistant
GREAT-ER	Geo-referenced Regional Exposure Assessment Tool for European Rivers
H_2O_2	Hydrogen peroxide
HQ	Hazard quotient
LD	Limit of detection
MAR	Multiple antimicrobial-resistant
MEC	Measured environmental concentration
MIC	Minimum inhibitory concentration
MPN	Most probable number
OR	Odds ratio
PBT	Persistence, bioaccumulation and toxicity
PE	Population equivalent
PEC	Predicted environmental concentration
P*h*ATE	Pharmaceutical Assessment and Transport Evaluation (model)
PNEC	Predicted no effect concentration
SAR	Single antimicrobial resistant
SCC	Stockholm County Council
TDS	Total dissolved solids
TiO_2	Titanium dioxide
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WWTP	Wastewater treatment plant
$WWTP_c$	Wastewater treatment plant that does not receive hospital effluent
$WWTP_{he}$	Wastewater treatment plant that does receive hospital effluent

AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL

Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

Eolas: *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

Tacaíocht: *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

Ár bhFreagrachtaí

Ceadúnú

- Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:
- saoráidí dramhaíola (m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- an diantalmhaíocht (m.sh. muca, éanlaith);
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe (OGM);
- foinsí radaíochta ianúcháin (m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha);
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Uisce

- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol

- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil (m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Tháscairí).

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn

- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn

Taighde agus Forbairt Comhshaoil

- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta

- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn (m.sh. mórphleananna forbartha).

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas

- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol (m.sh. Timpeall an Tí, léarscáileanna radóin).
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

Múscailt Feasachta agus Athrú Iompraíochta

- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Measúnú Comhshaoil
- An Oifig um Cosaint Raideolaíoch
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

EPA Research Report 162

Hospital effluent: impact on the microbial environment and risk to human health

Authors: Dearbháile Morris, Suvi Harris, Carol Morris, Enda Commins and Martin Cormican

epaResearch
Climate - Water - Sustainability
Identifying pressures • Informing policy • Developing solutions

Antibiotic resistance is a major public health problem. In Ireland, and most of Europe, hospital effluent is released into the urban wastewater system without any specific measurement of antibiotic levels or antibiotic-resistant bacteria, and without any pre-treatment.

There are concerns that the release of these contaminants into the urban wastewater may result in downstream exposure to antibiotics and contribute to the growing problem of antibiotic resistance. In this report, a three year study was undertaken to 1) quantify the impact of hospital discharge on the number of antibiotic resistant *E. coli* and concentration of antibiotics in urban waste water, 2) estimate the survival of antibiotic resistant *E. coli* in each step of the waste water treatment process to discharge, 3) estimate the persistence/removal of antibiotics in each step of the waste water treatment process to discharge, 4) develop a risk assessment model of human exposure to antibiotics and antibiotic resistant *E. coli* in recreational water related to discharge in hospital effluent.

Identifying Pressures

This report demonstrates that there are high levels of antibiotic-resistant *E. coli* in urban wastewater, and dealing with hospital effluent in isolation will not substantially address the overall issue of antibiotic-resistant bacteria in urban wastewater. The report identifies that, at best, wastewater treatment plants (WWTPs) do not remove or inactivate all antibiotic-resistant bacteria and that further research is required to understand the processes underlying this and identify risk management strategies. This research also reveals that some antibiotics may persist in the environment for extended periods after discharge and that the predicted levels of antibiotics in the environment are such that they may plausibly contribute to the development and maintenance of antibiotic resistance.

Informing Policy

The research informs current policies on protection of public health from water borne contaminants and the necessity for development of additional indicators or monitoring strategies. The research builds capacity in the area of Environment and Health. The research is relevant in the context of the EU Water Framework Directive, the European Commission's 'A Blueprint to Safeguard Europe's Water Resources' and to the 7th EU Environment Action Programme which aims "*to safeguard the Union's citizens from environment-related pressures and risks to health and wellbeing*". The research also informs the following national regulations and EU directives, the EU Water Framework Directive, the Urban Wastewater Directive (1991); the Bathing Water Directive (2008); and the Drinking Water Directive (2014).

Developing Solutions

The research identifies that new antibiotics, such as the quinolones/fluoroquinolones, have the lowest rate of degradation and the highest resistance formation potential and provides a method which could be used to create a mechanistic model that can assess antimicrobial resistance formation potential. This study provides valuable evidence that high levels of antibiotic resistant *E. coli* are present in urban wastewater, and highlights the need for the development of international guidelines regarding regulation, monitoring and reporting of antibiotics and antibiotic resistant bacteria in the urban wastewater and receiving environments.

Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

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Fact sheet 2 of 6

Subject: CPE general information and background

For:

Patients, relatives and healthcare workers

Seirbhís Sláinte Nios Fearr á Forbairt | Building a Better Health Service

Version 1; January 2018 Developed by the HSE HCAI/AMR Implementation Team

CPE or CRE?

Over the years the letters "CPE" and "CRE" have both been used quite a bit and often people use them to mean more or less the same thing. However, they are not exactly the same thing and we now talk more about CPE as the main problem.

What is CPE?

CPE is the newest in a long line of what people sometimes call "superbugs". When we talk about "superbugs' we mean bacteria that are hard to kill with antibiotics. Of all the superbugs we have had so far CPE is the hardest to kill with antibiotics. We think the number of people who carry CPE in Ireland is still fairly small (probably hundreds of people). This means that if we take very good care of people who carry CPE over the next couple of years there is still time to stop CPE becoming very common.

What do the letters CPE stand for? Carbapenemase Producing *Enterobacteriaceae*

E stands for Enterobacteriaceae. *Enterobacteriaceae* means a larger family of bugs that live in the gut. You may have heard of one of these bugs called *E. coli*. *E. coli* is one of this family of gut bugs but there are many others.

C stands for Carbapenemase. The carbapenems are a very important group of antibiotics. The best known example in Ireland is an antibiotic called meropenem. A carbapenemase is an enzyme (a type of protein) that destroys meropenem and other antibiotics like meropenem.

P stands for Producer. So CPE is a gut bug that produces a protein/enzyme that destroys meropenem.

What do the letters CRE stand for? Carbapenem Resistant *Enterobacteriaceae*

E stands for *Enterobacteriaceae*. *Enterobacteriaceae* means a large family of bugs that live in the gut. You may have heard of one of these bugs called *E. coli*. *E. coli* is one of this family of gut bugs but there are many others.

C stands for Carbapenem. The carbapenems are a very important group of antibiotics. The best known example in Ireland is an antibiotic called meropenem. Until a few years ago meropenem killed pretty much all gut bacteria. We can say that gut bacteria are normally sensitive to meropenem.

R stands for Resistant. In the last few years there are more and more gut bugs that are **not** killed by meropenem – we say these are resistant to meropenem and to other members of this carbapenem family of antibiotics. So a CRE Carbapenem Resistant *Enterobacteriaceae* is a gut bug that is not killed by meropenem.

How did CPE appear?

In most ways a CPE is like an ordinary gut bug. The difference between an ordinary gut bug and CPE is that a CPE has picked up a gene (a piece of genetic code) that tells it how to make something (an enzyme) that destroys the carbapenem antibiotics. These pieces of genetic code have always been out there in the natural environment but until about 20 years ago we never saw this code in gut bugs that can cause infection. After hospitals started to use a lot of meropenem to treat infection we started to see ordinary gut bugs turn into CPE.

There are a few different pieces of genetic code that can turn a normal gut bug into a CPE. Each piece

of code makes a gut bug into a different type of CPE. The pieces of code spread like a virus from one bug to another. This makes controlling the CPE problem much harder to manage. The speed of spread of this piece of code for the enzyme also makes it much harder to track the spread of CPE because the code can turn up in the same hospital but in many different types of bug.

How does CPE spread?

CPE lives in the gut along with billions of other gut bugs; most gut bugs are good for you. When you go to the toilet about half of the faeces (poo) that you pass is made up of these gut bugs. The bugs are very, very small. Look at the dot on this letter i. - it would take millions of gut bugs to cover that dot. This means that even the tiniest trace of poo, even on things that look clean; hands, clothing, furniture can be enough to pass on the CPE bug to another person.

For example, maybe you touch something that looks clean – there is a CPE there that gets on the tip of your fingers. You put your hand to your mouth and you put the CPE in your mouth. Maybe someone else gets CPE on their fingers and then they give you food or medicine and they put the CPE into your mouth. The CPE then goes down into your gut and makes itself at home. CPE is even more likely to make itself at home and multiply quickly if you are already on antibiotics.

Although antibiotics are very useful when you need them one of the unwanted downsides of using any antibiotic is that it will kill off a lot of the normal "good" gut bugs. As the normal good bugs die this makes your gut a better home for antibiotic resistant bugs like CPE.

How do we stop CPE from spreading in hospitals and nursing homes?

The biggest danger for spread of CPE right now in 2018 is in hospitals and nursing homes. This is because people in hospitals and nursing homes are more likely to carry CPE. People in hospital and nursing homes are also more likely to catch CPE because a lot of them are already sick and may be taking antibiotics. Clean hands (hand hygiene) are the most important thing in stopping the spread of CPE.

In hospitals and nursing homes, hand hygiene means using alcohol gel or soap and water and carefully following all the steps needed to kill or take away bugs on all parts of the hands. Hospital staff and nursing home staff receive training on when they need to carry out hand hygiene when caring for patients (see the hand hygiene section on www.hse.ie/hcai) hyperlink to new hand hygiene pages) This not only helps to stop CPE from spreading it stops a lot of other "superbugs" as well. The other big thing is to make sure that when people pass faeces (poo) that it is not allowed to spread. If there are tiny traces of poo on the toilet seat, on the commode, on the toilet roll or on hands this can be enough to spread the bugs. So cleaning the toilet seat and other things that people might put their hands on is very important.

Some countries have done a good job of stopping CPE from spreading and others have not managed to control it so well. We may still be able to stop CPE from getting out of control in Ireland if everyone works together but it will not be easy, it will not be fast and it will not be cheap. When we put better CPE controls in place we will need to keep them for good because there is always the risk of CPE coming back into a hospital from Ireland or from anywhere in the world. The risk of picking up CPE in hospital is very high in hospitals in some parts of the world.

When did CPE become a problem?

CPEs were found in different parts of the world in the last 15 to 20 years. Some were first found in Asia some in America. No one knows for sure where they started. The first CPE that was found in Ireland in

was in 2009 and we have found more every year since 2009. In 2017 more than 400 new people carrying CPE were found.

Why not use a new antibiotic to treat CPEs?

There are very few new antibiotics that work against gut bugs. Some drug companies have managed to re-jig some old antibiotics to help a bit. Doctors and scientists are trying to find new antibiotics that work but so far no one has found any completely new antibiotic that is ready to use yet. For CPE infection we often end up using some old antibiotics that we have known about for years but we tried to avoid using them because they are difficult to use and may have side effects for patients.

For more information on antimicrobial resistance and healthcare acquired infection or to view CPE guidance check www.hse.ie/hcai

Fact sheet 3 of 6

Subject: CPE information for healthcare workers

For:

Healthcare workers

Seirbhís Sláinte Nios Fearr a Forbairt | Building a Better Health Service

What can be done to prevent healthcare associated infection?

It is really difficult to completely stop bugs from spreading in hospitals and clinics and to prevent all healthcare associated infection. There is no country in the world that has a perfect system for doing this but some countries manage better than we do. It is possible to do better than we do now but it is not easy and it will not be fast. We will need to invest a lot and to change a lot about how we organise health care and how we behave. This is slow and demanding work that needs very strong leadership over a long period to time.

The following are some things that can reduce healthcare associated infection:

1. Make sure that people spend as little time as possible in hospitals, nursing homes and other residential healthcare facilities.

2. Help patients and residents to be less vulnerable to infection by:
    a. keeping people mobile
    b. helping people maintain a good diet and take plenty of fluids
    c. giving vaccines like flu and pneumococcal vaccine to reduce illness
    d. avoiding antibiotics that are not needed (they do more harm than good if you don't need them)
    e. keeping people free of tubes and needles (urinary catheters, intravenous drips) as much as possible

3. Make it harder for bugs that cause disease to spread from one person to another. Stopping bugs from spreading in a hospital or clinic is very hard to do. The most important idea here is **"Standard Precautions".** You cannot tell by looking at someone if they have an AMR bug. No system of lab testing for AMR bugs is perfect. This means we have to think that any patient we see might carry an AMR bug and so **standard precautions** means the basic care we take with **EVERY** patient to prevent spread of bugs between patients and between patients and staff. The most important part of standard precautions is hand hygiene. We know that hand hygiene works very well most of the time. Every time you perform hand hygiene you are protecting patients and protecting yourself. But it is really hard to remember to perform hand hygiene 100% right all of the time. Just one mistake – forgetting to perform hand hygiene when you rush from one patient to help another patient can put weeks of good work at risk. "Standard Precautions" also includes using personal protective equipment (PPE) when needed (clean gloves, plastic aprons and eye protection) and cough etiquette (cover up when coughing or sneezing). Gloves are always **as well as not instead of** performing hand hygiene so you must change gloves and perform hand hygiene between tasks.

4. Patients/residents and their visitors can also help to prevent spread of infection by washing hands or using alcohol based hand rub especially after visiting the toilet. Disposable hand wipes may be helpful for some patients. Remember alcohol hand rub is only suitable for

hands that look clean. If there is any dirt on the hands or under the fingernails, then cleaning with soap and water is needed.

Why are there so many different terms CPE, CRE, NDM, OXA
Most of the time the different terms do not matter for most people but the differences can be important for specialists who are dealing with the spread of CPE. This is a short explanation of the differences.

What do the letters CPE stand for?
E stands for Enterobacteriaceae. *Enterobacteriaceae* means a larger family of bugs that live in the gut. You may have heard of one of these bugs called *E. coli*. *E. coli* is one of this family of gut bugs but there are many others.

C stands for Carbapenemase. The carbapenems are a very important group of antibiotics. The best known example in Ireland is an antibiotic called meropenem. A carbapenemase is an enzyme (a type of protein) that destroys meropenem and other antibiotics like meropenem.

P stands for Producer.

So CPE is a gut bug that produces a protein/enzyme that destroys meropenem.

What do the letters CRE stand for?
E stands for *Enterobacteriaceae*. *Enterobacteriaceae* means a large family of bugs that live in the gut. You may have heard of one of these bugs called *E. coli*. *E. coli* is one of this family of gut bugs but there are many others.

C stands for Carbapenem. The carbapenems are a very important group of antibiotics. The best known example in Ireland is an antibiotic called meropenem. Until a few years ago meropenem killed pretty much all gut bacteria. We can say that gut bacteria are normally sensitive to meropenem.

R stands for Resistant. In the last few years there are more and more gut bugs that are **not** killed by meropenem – we say these are resistant to meropenem and to other members of this carbapenem family of antibiotics. So a CRE Carbapenem Resistant *Enterobacteriaceae* is a gut bug that is not killed by meropenem.

CRE stands for Carbapenem Resistant *Enterobacteriaceae*.

When a lab finds a Carbapenem Resistant *Enterobacteriaceae* (a CRE) in a patient sample the next question to ask in is what is it about the bug that makes it resistant to meropenem. There are a few different things that can make a bug resistant to meropenem. So the CRE bugs can be divided into different groups. One group of CRE is called CPE (Carbapenemase Producing *Enterobacteriaceae*). This CPE group is the group of CRE that we are most worried about because it is these CPE that are spreading rapidly all over the world.

You might also find the following leaflets helpful. There is a patient information leaflet available at this link https://www.hpsc.ie/publications/informationleafletsforthegeneralpublic/File,12779,en.pdf

Why does it matter if CPE can destroy meropenem?
Over the past 30 years or more gut bugs (*Enterobacteriaceae*) have become harder and harder to kill with antibiotics. This means that we have less choice when it comes to antibiotics to treat infection caused by these bugs. Until about 10 years ago one safe antibiotic we could nearly always

count on for people with very bad infection with these gut bugs was meropenem. CPE means that we can't count on meropenem anymore and so sometimes we have no safe, easy to use antibiotic to treat infection caused by CPE.

What is OXA, KPC and NDM?

This is another piece of the puzzle. CPE is a gut bug that makes a protein/enzyme that destroys meropenem. But all CPE are not the same. There are a few different enzymes. The three enzymes that destroy meropenem and that are most common in Ireland in 2017 are called (1) OXA, (2) KPC and (3) NDM. The difference between different CPEs can be important to a team trying to stop CPE from spreading. Suppose there are two patients in a ward called James and Peter. If James and Peter both carry CPE but James has the OXA type of CPE and Peter has the KPC type of CPE then we know that they have different kinds of CPE and the bug did not spread from James to Peter or from Peter to James.

If James and Peter both have a KPC type of CPE then we have to wonder if the bug spread from one of them to the other of if they both got it from someone else. Knowing how the bug is spreading can be important in making plans to stop spread.

What extra steps are needed to prevent spread go CPE bugs from patients who are known to carry CPE?

Remember **standard precautions** apply to all patients all the time and will reduce the risk of spread of CPE. You must expect that there will be patients in your care who have undetected CPE (or other superbugs) so do not let your guard down because you do not know someone has CPE or because they have had a laboratory test and CPE was not detected. CPE is spread by contact so standard precautions go a long way to reduce the risk of spread of CPE. Additional "**contact precautions**" should be applied with patients with known CPE colonisation or infection. Check your hospital policy for details but the additional measures will in so far as possible include single en-suite room with the door closed, signage on the door and use of long sleeve gowns for close contact.

Why not test staff to see if they are CPE carriers?

In relation to protecting patient or residents it is not necessary to know if staff are carrying CPE in their gut. All staff members at all times have to be careful about hygiene, especially about carrying out good hand hygiene after going to the toilet. Good hand hygiene deals with the risk of spreading gut bugs from a staff member. If a staff member did carry CPE there might be a greater risk of spread from the staff member to patients and other staff if they have diarrhoea. However, all health care workers with diarrhoea should stay away from care of patients/resident until at least 2 days after the diarrhoea has stopped. The basic staff rules about hand hygiene and staying out of work with diarrhoea are the ways we manage any risk to patients/residents from CPE or any other bugs that might be carried in the gut of the healthcare worker. There is no good reason or evidence to show that testing health care workers would make patients any safer.

Should health care workers know if they are carrying CPE?

Carrying CPE in the gut does not make a health care worker sick. There is no treatment for clearing it but it may go away on its own. So if a health care worker finds out that they are carrying CPE they may worry about it but there is nothing to do about it. Provided they follow standard good practice in the clinical area there is no reason to believe that they are putting patients at risk.

In relation to their own health we do not have any information that says health care workers who look after people who carry CPE are more likely to get sick with CPE than anyone else. Even for health care workers with health problems like diabetes or high blood pressure we have no reason to believe that they are more like to get sick with CPE because of their work. We do not have any information that says the family members of health care workers are more likely to carry CPE or to get sick with CPE.

We can never rule out a risk of infection to a health care worker. Health care workers have contact with sick people. Some of those sick people have infections that can make a health care worker sick than CPE (influenza, tuberculosis, salmonella and so on). The risk of infection at work is are small (probably a lot smaller than driving to work) for most health care workers especially if they following **standard precautions** at work – most especially carrying out hand hygiene properly when needed during the day and before going home from work. Remember standard precautions is about protecting healthcare workers as well as protecting patients.

Do we need to do anything special with the body of a person colonised with CPE after they die?

No; good routine practice should prevent the spread of bacteria from the dead body. With the dead as with the living carrying out hand hygiene by the proper method is the most important safeguard against spread of infection.

I want to know the technical details about antibiotic resistance?

Technically antibiotic resistance is assessed by growing a bug in the lab in a tube that has no antibiotic (the control tube) and in a set of tubes with gradually higher concentrations of antibiotic. We look to see how much antibiotic is needed to stop the bug from growing. If a small amount of antibiotic stops the bug growing it is sensitive and if the bug grows in a high concentration of antibiotic, it is resistant.

We divide antibiotic resistance into two categories. Intrinsic resistance and acquired resistance. Intrinsic resistance means that on the day the antibiotic was discovered it did not work for this particular type of bacteria. An example is the original penicillin (benzylpenicillin) never worked against the bug that most commonly causes cystitis (called *E. coli*). So the day it was discovered the first penicillin was not much use for treating cystitis caused by *E. coli*.

Acquired resistance means that on the day the antibiotic was discovered it worked for this particular type of bacteria but it does not work anymore. An example is the original penicillin (benzylpenicillin) was like magic for treating the bug that is the commonest cause of boils and wound infections (*Staphylococcus aureus*). In 1944 almost all *Staphylococcus aureus* were killed by tiny concentrations of benzylpenicillin today it is completely useless for 9 out of 10 *Staphylococcus aureus* that we find on people. This is acquired antibiotic resistance and most of the time that people talk about antibiotic resistance as a big problem it is this acquired antibiotic resistance that we are talking about – antibiotics that used to work but do not work anymore.

For more information on antimicrobial resistance and healthcare acquired infection or to view CPE guidance check www.hse.ie/hcai

Assessing Evidence of Transmission and End of Transmission of Carbapenemase Producing Enterobacterales[1] (CPE)

CPE Expert Group

National Guidance Document, Version 1.0

Scope of this Guidance

This guidance is intended for infection control specialists working in the acute hospital sector. Additional guidance or to confirm that you are using the most current version of this guidance, please go to www.hse.ie/hcai and www.hpsc.ie

Next review of this guidance document

This guidance document will be reviewed in 12 months (May, 2019).

[1] Recent changes in microbial nomenclature have altered the meaning of the term "*Enterobacteriaceae*" and mean that the term "Enterobacterales" now corresponds more closely to the former meaning of "*Enterobacteriaceae*".

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Glossary of Terms

CPE = Carbapenemase Producing *Enterobacterales*

The following in alphabetical order are some of the more common carbapenemase enzymes. There are a number of other carbapenemase enzymes.

IMP: Imipenemase

KPC: *Klebsiella pneumoniae* carbapenemase

NDM: New Delhi metallo-beta-lactamase

OXA: Oxacillinase-type carbapenemase (OXA-48 is the common variant)

VIM: Verona Integron-encoded metallo-beta-lactamase

ED = Emergency Department

IPC = Infection Prevention and Control

NCPERL = National CPE Reference Laboratory

OCT = Outbreak control team

Scope

In some cases it is readily apparent that CPE transmission has occurred in a hospital because two or more patients clearly linked in space and time, for example patients in adjacent beds in the same ward, are identified as having the same type of CPE. However the nature of CPE is such that transmission is not always easy to recognise. This document is intended to support Infection Prevention and Control (IPC) and Public Health practitioners in assessing evidence of possible CPE transmission in an acute hospital or other healthcare facility providing a similar intensity of care.

What follows in each section applies equally to hospitals and to other healthcare facilities providing a similar intensity of care.

Haemodialysis facilities should be considered as providing an intensity of care similar to an acute hospital.

This document is also intended to assist in determining when transmission can be considered to have ceased.

Definition of CPE

For the purpose of this document CPE is a member of the family *Enterobacterales* in which one of the recognised carbapenemase genes or enzymes, such as IMP, KPC, OXA-48, NDM or VIM, has been confirmed by a validated laboratory method.

Isolates of Enterobacterales with resistance to a member of the carbapenem family of antimicrobial agents **by other mechanisms or by an unconfirmed mechanism** do **NOT** meet the definition of CPE for this purpose. Such organisms may however require transmission based precautions as multi-drug resistant organisms.

The document should be considered in association with related HSE policy documents concerning CPE specifically "*Requirements for Screening of Patients for Carbapenemase Producing Enterobacterales (CPE) in the acute hospital sector, Version 1.0, February 2018*"; "*Acute Hospital Carbapenemase Producing Enterobacterales (CPE) Outbreak Control Checklist, Version 1.0, March 2018*"; and "*Interventions for Control of Transmission of CPE in the Acute Hospital Sector, Version 1.0, April 2018*".

The documents are available at the following link:

http://www.hpsc.ie/a-z/microbiologyantimicrobialresistance/strategyforthecontrolofantimicrobialresistanceinirelandsari/carbapenemresistantenterobacteriaceaecre/guidanceandpublications/

Introduction

The term Enterobacterales is used to describe families of bacteria normally found in the human bowel/enteric tract. The sharing of mobile genes (generally on plasmids) between Enterobacterales enables them to make enzymes, called carbapenemases such as IMP, KPC, OXA-48, NDM and VIM. When this happens, they are called carbapenemase producing Enterobacterales (CPE). CPE are generally resistant not only to a critical family of antimicrobials, known as carbapenems, but usually to many other antimicrobial agents also.

It is important to note that the mobile genes that convert bacteria into CPE can spread not only between bacteria of the same species (for example, from one *Escherichia coli* to another *Escherichia coli*) but also between different species of Enterobacterales (for example, from *E. coli* to *Klebsiella pneumoniae*). This is the basis of a fundamental difference between CPE outbreaks and most other outbreaks. In most outbreaks all the affected patients have the same species of bacteria or virus (for example, all patients involved in an outbreak of methicillin-resistant *Staphylococcus aureus*) but in CPE outbreaks multiple different species of bacteria can be involved.

The challenges in identifying potential CPE transmission in an acute hospital

Screening

Because CPE are mostly carried asymptomatically in the bowel, it may not be known that a person is carrying CPE unless a rectal swab or faeces specimen is taken to screen or check for CPE (See HSE policy "Requirements for Screening of Patients for carbapenemase producing Enterobacterales (CPE) in the Acute Hospital Sector, Version 1.0, February 2018"). If the national CPE screening policy is not implemented in an acute hospital, this increases the risk that CPE carriers will not be detected and that CPE may therefore be more likely to spread and evidence of transmission more difficult to detect.

Patient movement

For many reasons, patients will move from place to place when they are admitted to a hospital and may spend varying amounts of time in each place, from hours to days or weeks. Journeys include: From the emergency department (ED) to a ward; from ward to ward; between rooms on a ward; and from the ward to other departments such as the operating theatre, radiology, endoscopy etc.. Therefore, the epidemiological links between CPE cases may be difficult to identify even with careful review of patient journeys. In many cases, association with the hospital or haemodialysis unit alone is sufficient to declare a potential CPE outbreak.

Hospital length of stay

The length of stay in hospital is getting shorter. Some patients may have been discharged before a potential contact with a CPE case or link to a CPE outbreak is identifiable.

Undetected CPE

Transmission of CPE from patients with undetected CPE colonisation or from contamination of the patient environment may explain cases of CPE in settings where the source of CPE is not apparent.

First case not necessarily the first patient

The first CPE case identified may not be the first patient with CPE in the hospital. Without careful review of a patient's journey prior to CPE detection, other cases might be missed.

Different microorganisms with the same type of carbapenemase

A highly transmissible plasmid is often involved, for example, a plasmid carrying OXA-48, which is readily exchanged between different Enterobacterales. This means that two patients with different species of Enterobacterales producing the same type of carbapenemase (for example, one OXA-48 producing *E. coli* and one OXA-48 producing *Klebsiella pneumoniae*) and with different antimicrobial susceptibility patterns might indicate a CPE outbreak.

Robust laboratory testing protocol required

A robust local microbiology laboratory testing protocol is essential to ensure that CPE can be picked up from screening and clinical specimens and reported rapidly to the infection prevention and control team and confirmed as quickly as possible (either local confirmation or confirmation by the national reference laboratory service). Timely confirmation of a suspected CPE case is important, because the carbapenemase type, such as IMP, KPC, OXA-48, NDM or VIM, may be the key to identifying a potential link between CPE cases.

Robust local surveillance system required

A robust local surveillance system is vital to ensure that all new suspected and confirmed CPE cases are carefully reviewed by the Infection Prevention and Control Team (IPCT) in a timely manner. This allows the team to determine quickly any potential association with the hospital or haemodialysis unit. This process is vital to early identification of potential increased CPE incidence, which may warrant further investigation.

Comparison of isolates required

Patients in whom CPE is detected by validated direct molecular methods should be considered in the same way for infection prevention and control purposes as patients from whom CPE has been detected by conventional culture. Laboratories should attempt to culture the organism from such patients to facilitate comparison of isolates.

The challenges in associating a newly-detected CPE patient with the acute hospital

Many of the challenges in identifying potential CPE transmission outlined in the previous section are also challenges to identifying potential association between a newly detected patient with CPE and a particular hospital.

First case not necessarily the first patient

Sometimes a microbiology specimen taken from a patient in the context of investigating suspected infection (for example, urine, wound swab or blood culture) might be the first specimen found to contain CPE. If there are no previous microbiology specimens from that patient during the hospital stay, it may be difficult to determine exactly where the patient acquired the CPE.

Low levels of CPE (CPE not detected)

A patient could be admitted to hospital carrying CPE at very low levels, which might be below the threshold of detection of the CPE screening test in use and therefore be reported as "CPE not detected" on admission screen. Subsequent antimicrobial exposure confers a survival advantage for CPE, enabling them to multiply and become more readily detectable on a subsequent CPE screen. Thus a patient may appear to have acquired CPE recently whereas in fact they have carried CPE for some time but it has only recently become apparent.

Time lag before CPE becomes detectable

It may be very difficult to determine when a person picked up CPE, because it can take several weeks after contact before CPE becomes detectable from a screening specimen.

No antimicrobial susceptibility testing performed

A diagnostic specimen with mixed growth (and including Enterobacterales) may not have antimicrobial susceptibility testing performed in the absence of relevant clinical information to support infection. If CPE is detected from a screening or diagnostic specimen taken from the same patient at a later date during the same admission, it may be impossible to determine if one of the bacteria in the earlier sample was CPE.

CPE contacts

A patient who is identified as a CPE contact of a newly-confirmed CPE case may be screened for the first time as a CPE contact. In the event that the CPE contact is subsequently confirmed to also be a CPE case, a careful review is recommended. The possibility that the CPE contact was actually the source of transmission for the index CPE case needs to be considered.

The quality of the sample

The quality of the diagnostic or CPE screening specimen will influence the likelihood of recovery of CPE from that specimen and in turn the validity of the test results. If an initial sample is not of good quality, a subsequent correctly-taken specimen that tests positive for CPE may appear to be a newly acquired CPE associated with the specific hospital.

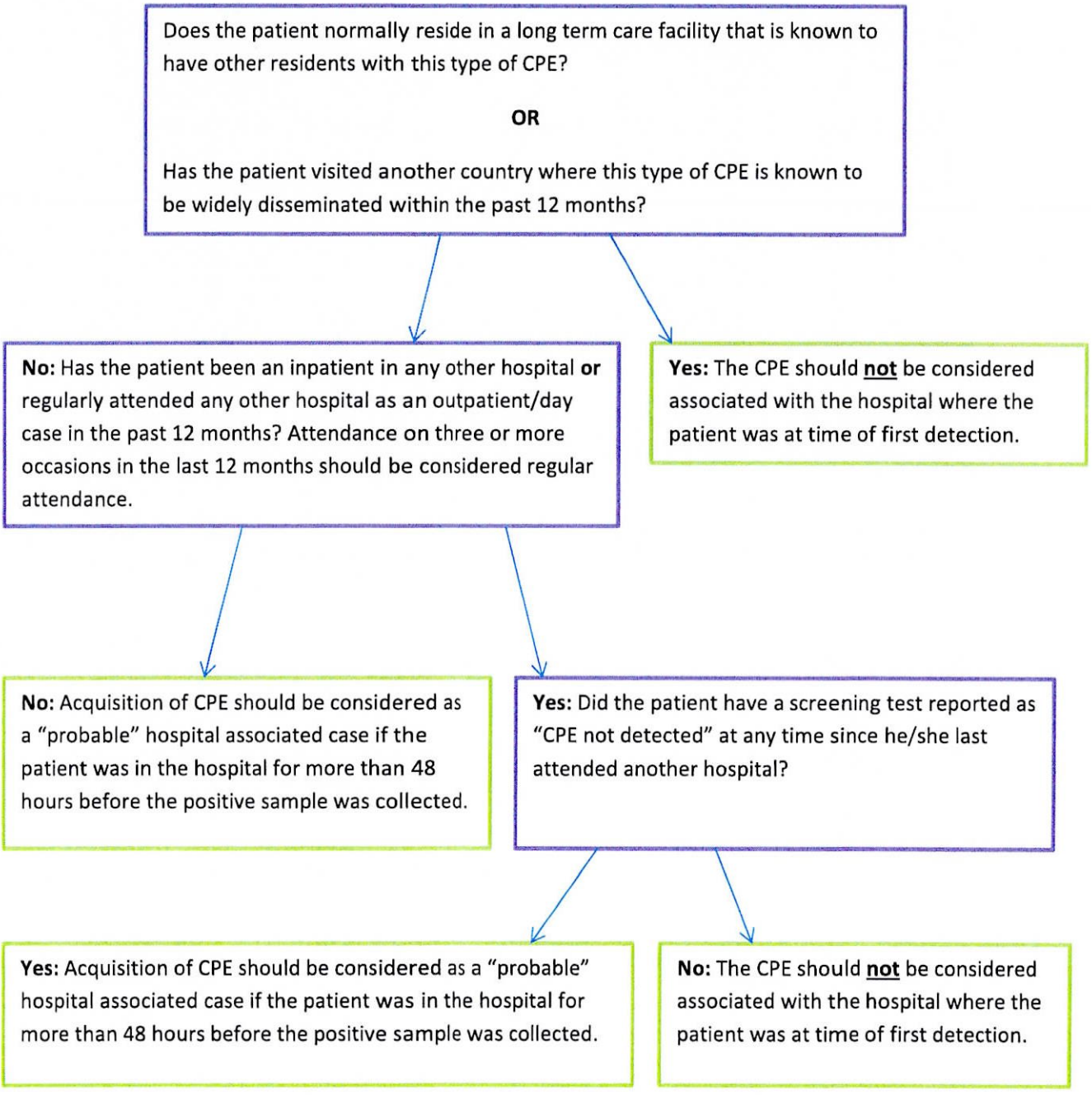
Given these limitations it is very often impossible to say with confidence where or when CPE was acquired. In the context of this uncertainty this document is intended to provide a consistent approach to making a determination about where CPE was acquired to support recognition of transmission (an outbreak).

How is association of a CPE isolate with the hospital defined?

1. Have you identified **two or more** patients who have been an inpatient in your hospital or attended your facility in the three months prior to CPE detection with "the same type of CPE"? For this purpose "the same type of CPE" refers to the specific type of CPE such as IMP, KPC, OXA-48, NDM and VIM. Organisms of different species with the same genetic mechanism of resistance should be considered as "the same type of CPE".

2. To establish for each individual patient whether they should be considered as probably associated with a specific hospital or facility, apply the algorithm on the next page.

Algorithm to determine if CPE is probably associated with your facility



Bear in mind that other patients who do not fulfil these criteria may possibly be associated with the same hospital. In the context of established evidence of transmission (an outbreak), it is generally preferable to assume that any patient with newly-detected CPE of the outbreak type from a screening or diagnostic specimen taken on day three or later after admission to the hospital/first dialysis is considered as probably associated with the hospital unless there is persuasive evidence to the contrary.

In some cases molecular typing of isolates and or plasmids may provide important evidence that makes a link between particular CPE isolates and particular hospitals or haemodialysis facilities more or less likely and should be discussed with colleagues at the National CPE Reference Laboratory Service (NCPERLS).

The criteria for possible/probable CPE transmission

Possible CPE transmission: Two newly-confirmed CPE cases of the same type, such as IMP, KPC, OXA-48, NDM, VIM, etc., detected within a three month period and deemed to be probably associated with the hospital or haemodialysis unit is considered possible CPE transmission (outbreak).

Probable CPE transmission: Three or more newly-confirmed CPE cases of the same type, such as IMP, KPC, OXA-48, NDM, VIM, etc., detected within a three month period and deemed to be probably associated with the hospital or haemodialysis unit is probable CPE transmission (outbreak).

Next steps when the criteria for possible/probable CPE transmission are not fulfilled

When latest available evidence does not fulfil criteria for possible or probable CPE transmission, the situation should be kept under close review as outlined above.

Next steps when the criteria for possible/probable CPE transmission are fulfilled

An outbreak control team (OCT) should be convened by the Hospital Manager to assess the evidence and to consider what further action is required. The Medical Officer of Health must be informed in accordance with legislation. Please also inform the National Lead for HCAI/AMR and the Consultant Microbiologist at HPSC and refer to HSE documents guiding the control of transmission as outline above.

Determining whether CPE transmission in a hospital has abated or ceased

In addition to a generic epidemic curve of all newly-detected CPE patients, a curve limited only to cases assessed as probably associated with the hospital should also be prepared along with measurement of the interval since the last case assessed as "probably" associated with the hospital.

Progress is assessed as a decline in the number of newly-detected CPE patients "probably" associated with the hospital. Given that the interval from CPE exposure to detection may be up to four weeks (or longer), one should anticipate that it may take four weeks from the time of making an intervention to observing a change in detection of new "probable" hospital associated cases.

A period of 90 consecutive days without a newly detected CPE patient assessed as a "probable" hospital associated case should be considered as reasonable evidence that transmission has ceased.

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Revision number		Responsibility for implementation	All HSE funded acute hospitals
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Draft or Final document	Final document		