ANTIBIOTIC USE DURING AN INFLUENZA PANDEMIC: DOWNSTREAM ECOLOGICAL EFFECTS AND ANTIBIOTIC RESISTANCE

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The years 2009–2010 saw the first pandemic virus in several decades. Only in retrospect has the low pathogenicity of the virus been able to be confirmed. The pandemic saw as many deaths per capita as a seasonal influenza virus, but with the significant difference that the young (<18 years) were atypically impacted over those >18 years old (Kamigaki and Oshitani, 2009). Pharmaceuticals played an important part of health care during the influenza pandemic. Many nations implemented huge stockpiles of antivirals in response to the pandemic, but owing to the low pathogenicity of the virus, there was a negligible increase in existing antibiotic use over interpandemic usage. However, current estimates for antiviral and antibiotic use during a moderate and severe influenza pandemic are without historical precedent (Singer et al., submitted). Here we discuss the environmental and human health implications of a moderate or severe influenza pandemic with regard to Tamiflu itself and the use of antibiotics to treat secondary bacterial infections. Antibiotic use will be framed in the context of existing paradigms of antibiotic treatment and how these practices already contribute to human and environmental hazards and how these hazards might be minimized in the event of a moderate or severe influenza pandemic.

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26.1 PANDEMIC INFLUENZA

26.1.1 Introduction

The World Health Organization (WHO) has recently documented that infectious diseases are not only spreading faster but emerging at an unprecedented rate of one or more per year, resulting in the addition of ~40 infectious diseases that were unknown only a generation ago (WHO, 2007). As evident from the H1N1 pandemic of 2009, we cannot be certain whether highly pathogenic avian influenza A H5N1, H9N2, or another subtype will spark a human pandemic (Wan et al., 2008), but what does appear certain is that another influenza pandemic will occur at some point in the future in the absence of a universal influenza vaccine (Cabinet Office, 2008; U.S. Homeland Security Council, 2007). In response to this pressure, many countries worldwide have published pandemic preparedness plans (European Influenza Surveillance Scheme, 2008; Mounier-Jack and Coker, 2006). The aims of these plans are to maintain essential services, reduce disease transmission, minimize the socio-economic consequences of a pandemic, and reduce clinical cases, hospitalizations, and deaths (WHO, 2005). Key to the plans is slowing the spread of pandemic influenza through: (i) vaccine development, stockpiling, and distribution (Department of Health and Human Services and Department of Homeland Security, 2007), (ii) nonpharmaceutical measures (U.S. Centers for Disease Control and Prevention, 2007), and (iii) antiviral and antibiotic stockpiling and distribution (Hampson, 2008; NAS, 2008; U.S. Department of Health and Human Services, 2007). While the existence of preparedness plans in many countries highlights the attention that is given to the effects of a pandemic on society, possible implications of pharmaceutical therapies on the environment have been addressed much less. This chapter addresses how concentrations of antivirals and antibiotics occurring in wastewater treatment plants (WWTP) and surface water can be simulated, and whether these concentrations could have environmental effects or favor resistance development.

26.1.2 Influenza—Symptoms and Treatment

Influenza in adults is characterized by the abrupt onset of fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis. Otitis media, nausea, and vomiting are the more commonly reported symptoms of influenza in children. Pronounced elevation of proinflammatory cytokines during H5N1 influenza virus infection, known as the “cytokine storm,” is hypothesized to be the main cause of pathology and ultimately of death from uncomplicated influenza (Salomon et al., 2007). Although uncomplicated influenza typically resolves within 7 days, a cough and malaise can persist for >2 weeks. Influenza can cause viral pneumonia, exacerbate underlying medical conditions (e.g., pulmonary or cardiac disease), and lead to secondary bacterial pneumonia.

A vaccine is the first line of defense against influenza virus infections; however, its production is hampered by the inability to predict the antigenic details of the pandemic strain before it arrives. Moreover, a vaccine will only become available roughly 4–6 months after the outset of a pandemic as a result of an outdated vaccine production system (e.g., egg culture) and insufficient production capacity (WHO, 2006a). Owing to this delay, it is estimated that only 14% of the world's population

are predicted to have available to them a vaccine for the pandemic influenza strain within the first year (Osterholm, 2005; Uscher-Pines et al., 2006). Given these uncertainties, the WHO has recommended a number of mitigating strategies to help slow the spread of the pandemic, thereby providing additional time for a vaccine to be developed, distributed, and administered. Antivirals are to play a key role in this mitigation strategy.

Treatment for influenza using antivirals must be achieved within 48 hours of the onset of symptoms. Empirical antiviral therapy will be used in the absence of rapid diagnostic tests for influenza infection (Lim, 2007; U.S. Department of Health and Human Services, 2008). Currently, licensed antivirals for therapy and/or prophylaxis of influenza fall into two classes—the adamantanes (amantadine and rimantadine), M2 ion channel inhibitors effective against influenza A viruses only, and the neuraminidase inhibitors [NAI; oseltamivir ethylester phosphate (OE-P; Tamiflu) and zanamivir (Relenza)]—that are effective against both influenza A and B viruses. Although amantadine (Symmetrel) and rimantadine (Flumadine) are approved by the U.S. Food and Drug Administration (FDA) for the treatment or prevention of influenza, adamantane resistance is high and growing and is therefore not recommended for use during an influenza pandemic (Barr et al., in press; Hurt et al., 2007; Jefferson, 2007). Neuraminidase inhibitors (NAI), such as Tamiflu, Relenza, and Peramivir, are sialic acid analogs that inhibit the influenza neuraminidase enzyme, which is required for the release of progeny virions from infected cells. An inhibited neuraminidase ensures that the virions remain tethered to the infected cell surface, thereby limiting viral shedding (Ong and Hayden, 2007). Neuraminidase enzyme IC_{50} values for oseltamivir for clinically isolated influenza A ranged from 0.1 to 1.3 nM, and for influenza B was 2.6 nM (Roche, 2007).

The WHO has strongly recommended the use of Tamiflu (oseltamivir carboxylate; produced and distributed by Hoffmann–La Roche) as the primary choice for combating an influenza pandemic as: (i) it is easy to administer (capsule), (ii) it is systemically active, and (iii) it is effective against characterized influenza A and B viruses (Roche Group, 2006; WHO, 2006b).

26.1.3 Antiviral Stockpiling

Many countries have stockpiled antivirals as part of their preparedness plans. Even before the 2009 pandemic, Roche reported orders for Tamiflu from more than 80 countries, equating to approximately 215 million treatments (Tierney and Reddy, 2007). Many countries, including the United States and the United Kingdom, are supplementing their antiviral stockpiles (Reuters, 2007) to include Relenza, (Zanamivir, marketed and distributed by GlaxoSmithKline), with the expectation of further supplementation with drugs soon to be available, such as Peramivir (marketed and distributed by BioCryst Pharmaceuticals) (Morse, 2007; Pharmaceuticals, 2007) and A-315675 (produced by Abbott Laboratories) (Kati et al., 2002), among many others in development.

26.1.4 Secondary Bacterial Infections

The main complication from influenza is secondary bacterial infection, particularly pneumonia (Brundage, 2006; Brundage and Shanks, 2008; Gupta et al., 2008;

Morens et al., 2008; New England Journal of Medicine Editors, 2009; Peltola et al., 2005; Rainsford, 2006; Schwarzmans and Sullivan, 1971; UK Department of Health, 2007b). The most common bacterial etiologies for influenza-associated community-acquired pneumonia (IA-CAP) are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* (Lim, 2007; Schwarzmans and Sullivan, 1971; Siquier et al., 2006; UK Department of Health, 2007b; Lim, 2007; Schwarzmans and Sullivan, 1971; Siquier et al., 2006; UK Department of Health, 2007b). IA-CAP is characterized by fever (97% of cases), malaise (80%), respiratory symptoms, for example, cough, chest pain, shortness of breath (80–85%), headache (65%), and others (Bannister et al., 2006; Lim, 2007; Louria et al., 1959). Mortality of viral or combined viral-bacterial pneumonia is ~10–12% (Public Health Agency of Canada, 2004).

Chemotherapy guidelines for treating IA-CAP have been recently published with the backing of the British Infection Society, British Thorax Society, and the Health Protection Agency (Lim, 2007). This guidance is tailored toward pandemic IA-CAP, which distinguishes itself from previously published CAP guidelines, for example, American Thoracic Society (ATS)(ATS, 2005). Antibiotic therapy for IA-CAP will be empirical as a result of the anticipated overwhelming surge of patients during a pandemic (Gupta et al., 2008; Nap et al., 2007; UK Department of Health, 2007a).

Patients with IA-CAP infections receive antibiotic therapy and a range of over-the-counter (OTC) medications, for example antipyretics, antiphlogistics, cough medicine, and decongestants, used to relieve some of the discomfort of the illness (U.S. Department of Health and Human Services, 2005). In addition to treating the influenza virus infection, there will be considerable use of OTC drugs including analgesics and antiphlogistics. Personal stockpiles of these drugs have been recommended for all families within the U. S. Pandemic Flu Preparedness Checklist for Individuals & Families (Meltzer et al., 1999; U.S. Department of Health and Human Services, 2006).

The availability of antibiotics within a country is considerably less defined than it is for antivirals. Recently, the UK Department of Health announced the intention to purchase 14.7 million courses of antibiotics, to cover at-risk groups totaling 25% of the population (Department of Health, 2007). However, to date, there was no indication of the nature of the stockpile. Owing to the threat of bioterrorism, many countries maintain a stockpile of antibiotics, primarily ciprofloxacin, for postexposure anthrax treatment (Gupta et al., 2008). The United States stockpiles sufficient antibiotics (ciprofloxacin and doxycycline) to treat 60 million people. Both of these antibiotics could be mobilized to treat some, but not all, of the causes of IA-CAP (Fowler et al., 2005; Gupta et al., 2008).

26.2 EXPOSURE ASSESSMENT OF ANTIBIOTICS AND ANTIVIRALS DURING A PANDEMIC

In order to determine risks of pandemic antiviral and antibiotic loads in the aquatic environment, knowledge on the concentrations in WWTPs and surface water as well as on possible effects on WWTP bacterial communities and occurrence of antibiotic resistance is needed. In this section, we summarize how concentrations can be predicted from coupling a spatially structured global epidemic model with a GIS-

based watershed model. our knowledge, the modeling approaches described in (Singer et al., submitted) and in this chapter are the first investigations of possible antibiotic concentrations during an influenza pandemic.

The spatiotemporal pattern of the pandemic and its concurrent secondary infections were simulated by use of the global epidemic and mobility (GLEaM) model (Balcan et al., 2009a), which maps 6 billion individuals and integrates their mobility data. Parameters of the infection dynamics consisted of transmission of influenza under seasonal variation and included secondary infections according to the UK Pandemic Assumptions for complication, hospitalization, and intensive care unit admission rates (Balcan et al., 2009b) (UK Department of Health, 2009). In order to incorporate a wide range of possible influenza transmission potentials, we explored a mild pandemic ($R_0=1.65$), a moderate ($R_0=1.9$), as well as a severe ($R_0=2.3$) scenario. R_0 , the basic reproductive number, is the average number of secondary infections produced by a single infected individual while he or she is infectious in an entirely susceptible population. This is a measure of the degree of transmissibility of an infection (Anderson and May, 1991). No large-scale antiviral treatment was included in the mild scenario. For the other two scenarios, Tamiflu antiviral treatment was foreseen, with the assumption that 30% of the cases were detected and antiviral treated. It was assumed that antiviral treatment reduced the infectious period and the rate of development of secondary infections (Kaiser et al., 2003; Nicholson et al., 2000; Treanor et al., 2000; Whitley et al., 2001).

Within each scenario, we explored the effect of antiviral prophylaxis for 2 or 4 weeks from the start of the outbreak per country, as well as the effect of no prophylaxis. Antibiotics were assumed to be used according to guidelines for treating influenza-associated pneumonia sanctioned by the British Infection Society, British Thoracic Society, and the UK Health Protection Agency (Lim, 2007) (Table 26.1). It should be noted that this is only a small subset of the drugs that have been proposed to be used during a pandemic. Table 26.2 presents data on the environmental fate of a wide range of antibiotics that may be used during a pandemic, reflecting the considerable variation in antibiotic prescribing that exists regionally, nationally, and globally (Coenen et al., 2006; de Neeling et al., 2001; Ferech et al., 2006; García-Rey et al., 2002; Muller et al., 2007; Priest et al., 2001); and the possibility that, dependent on the extent of any stockpile, shortages are likely to occur and a range of antibiotics might be used (Gupta et al., 2008). Table 26.3 summarizes data on concentrations in WWTPs and surface water for pharmaceuticals that have been proposed for use during an influenza pandemic.

Simulations of an influenza pandemic (R_0) resulted in estimates of the cases of influenza and pneumonia at each stage of disease progression, together with the quantities of drugs used. Simulations were able to project cases and drugs down to the spatial resolution scale of $1/4^\circ$ and a time resolution of one day. A water quality model, Low Flows 2000—Water Quality Extension (LF2000-WQX) (Rowney et al., 2009), then predicted the environmental release of antivirals and antibiotics. Values for excretion of active substances within the feces and/or urine were taken from the literature (see Table 26.4). Dilution processes in the river were modeled using the annual mean flow from each river stretch, while a worst-case scenario of no pharmaceutical degradation or loss to sorption in WWTPs and rivers was applied (see Fig. 26.1). The Thames catchment in the southern United Kingdom was chosen as a model test case.

TABLE 26.1 Preferred and Alternative Empirical Antibiotic Treatment Regimens for Pneumonic Influenza-Associated Complications

CURB-65 Score	Compartment	Preferred Treatment Regimen	Alternative Treatment Regimen	Duration
0–2	P^I, P^{II}	Co-amoxiclav 625 mg tds PO or doxycycline 200 mg stat and 100 mg od PO	Macrolide (erythromycin 500 mg qds PO or clarithromycin 500 mg bd PO) or fluoroquinolone (e.g., levofloxacin 500 mg od PO or moxifloxacin 400 mg od PO)	7 days
3–5	P^{III}	Co-amoxiclav 1.2 g tds IV or cefuroxime 1.5 g tds IV or cefotaxime 1 g tds IV plus macrolide (erythromycin 500 mg qds IV or clarithromycin 500 mg bd IV)	Fluoroquinolone with some enhanced pneumococcal activity (e.g., levofloxacin 500 mg od IV or moxifloxacin 400 mg od PO) plus, either macrolide (erythromycin 500 mg qds IV or clarithromycin 500 mg bd IV) or β -lactamase stable antibiotic (i.e., co-amoxiclav 1.2 g tds IV or cefuroxime 1.5 g tds IV or cefotaxime 1 g tds IV)	10 days

Source: From Lim (2007).

A survey of the literature as well as an examination of the STPWIN model (U.S. Environmental Protection Agency, 2007) within the Estimation Program Interface (EPI) SuiteTM 4.0 indicates low (<20%) removal for most antibiotics in WWTPs, inclusive of loss due to sorption and biodegradation (Fig. 26.1). A literature search revealed that most antibiotics, particularly those not containing a β -lactam moiety, are resistant to metabolism in vivo as well as in the environment (Al-Ahmad et al., 1999; Alexy et al., 2004; Benotti and Brownawell, 2009; Brain et al., 2004a; 2004b; Gartiser et al., 2007; Junker et al., 2006; Kümmerer et al., 2000), with half-lives of days to weeks (Benotti and Brownawell, 2009; Christensen, 1998). For these reasons, we feel there was justification in assuming a conservative pharmaceutical biodegradation model of zero degradation/sorption.

26.2.1 Interpandemic Antibiotic and Antiviral Use

A very wide range of pharmaceuticals are in constant use in a population and thus will be present in the wastewater during a pandemic (Kümmerer, 2009a, 2009b). While the relative contribution of single antibiotic classes to overall use does not greatly vary between countries (Fig. 26.2), the absolute amount of defined daily

TABLE 26.2 List of Pharmaceuticals Proposed or Likely to Be Used During an Influenza Pandemic^a

ATC Code	Common Name	CAS	D ₅₀ Daily (g)	Total Excretion (%) ^b	Total STW Removal (%)	STW Biodegradation Removal (%)	STW Adsorption Removal (%)
<i>β-Lactam Antibacterials</i>							
J01AA07	Tetracycline	60-54-8	1	91	8.78	7.13	1.65
J01AA02	Doxycycline	564-25-0	0.1	80	8.78	7.13	1.65
J01AA06	Oxytetracycline	2058-46-0	1	35	8.78	7.13	1.65
J01AA03	Chlortetracycline	57-62-5	1	20	1.85	0.09	1.76
J01AA01	Demeclocycline	127-33-3	0.6	85	8.78	7.13	1.65
J01BA01	Chloramphenicol	56-75-7	3	11	8.86	7.16	1.70
J01CE01	Benzylopenicillin	61-33-6	3.6	89	22.60	20.90	1.68
J01CE02	Phenoxymethyl-penicillin	87-08-1	2	35	23.10	21.22	1.88
J01CF05	Floxacinil (Flucloxacillin)	5250-39-5	2	80	3.34	0.10	3.24
J01CA04	Amoxicillin	26787-78-0	1	75	22.04	20.57	1.47
c	Clavulanic acid	58001-44-8	0.375	38	92.06	91.72	0.33
	Cefalexin	15686-71-2	2	100	22.02	20.56	1.46
J01DB01	Cefuroxime	55268-75-2	0.5	95	21.98	20.53	1.45
J01DC02	Cefuroxime	53994-73-3	1	85	21.99	20.54	1.45
J01DC04	Cefaclor	63527-52-6	4	61	22.01	20.55	1.46
J01DD01	Cefotaxime	73384-59-5	2	100	8.78	7.13	1.65
J01DD04	Ceftriaxone	74431-23-5	2	70	75.06	74.44	0.62
J01DH51	Imipenem						
<i>Sulfonamides and Trimethoprim Antibacterials</i>							
J01EA01	Trimethoprim	738-70-5	0.4	100	8.83	7.15	1.68
J01EC01	Sulfamethoxazole	723-46-6	2	100	22.05	20.57	1.47
<i>Imidazole Antibacterial</i>							
J01XD01	Metronidazole	443-48-1	1.5	100	21.98	20.53	1.45

(Continued)

TABLE 26.2 (Continued)

ATC Code	Common Name	CAS	Daily Dose (g)	Total Excretion (%) ^b	Total STW Removal (%)	STW Biodegradation Removal (%)	STW Adsorption Removal (%)
<i>Macrolide Antibacterials</i>							
J01FA01	Erythromycin	114-07-8	1	100	6.23	0.13	6.11
J01FA06	Roxithromycin	80214-83-1	0.3	60	4.05	0.11	3.94
J01FA10	Azithromycin	83905-01-5	0.3	85	30.99	0.33	30.66
J01FA09	Clarithromycin	81103-11-9	0.5	55	7.30	0.14	7.17
J01FF01	Clindamycin	18323-44-9	1.2	14	9.66	7.46	2.20
<i>Aminoglycoside Antibacterial</i>							
J01GB03	Gentamicin	1403-66-3	0.24	80	21.97	20.53	1.44
<i>Fluoroquinolone Antibacterials</i>							
J01MA02	Ciprofloxacin	85721-33-1	1	100	8.79	7.13	1.66
J01MA12	Levofloxacin	100986-85-4	0.5	96	1.88	0.09	1.79
J01MA06	Norfloxacin	70458-96-7	0.8	40	8.78	7.13	1.65
J01MA02	Ofloxacin	82419-36-1	0.4	85	1.85	0.09	1.76
J01MA14	Moxifloxacin	354812-41-2	0.4	100	1.88	0.09	1.79
<i>Glycopeptide Antibacterial</i>							
J01XA01	Vancomycin	1404-90-6	2	75	1.85	0.09	1.76
<i>Analgesic/Antiphlogistics</i>							
M01AB01	Indometacin	53-86-1	0.1	95	78.45	46.71	31.74
M01AB05	Diclofenac	15307-86-5	0.1	95	86.57	46.94	39.63
M01AE01	Ibuprofen	15687-27-1	1.2	100	94.93	80.36	14.57
M01AE02	Naproxen	22204-53-1	0.5	90	83.68	79.66	4.02
M01AE03	Ketoprofen	22071-15-4	0.15	100	82.89	79.26	3.63
N02BE01	Acetaminophen	103-90-2	3	94	75.09	74.46	0.63
N02BA01	Acetylsalicylic acid	50-78-2	3	100	92.11	91.74	0.37
R05DA04	Codeine	76-57-3	0.1	100	8.87	7.16	1.71

J05AH02	Oseltamivir ethylester-P	204255-11-8	Antivirals	20	22.06	20.58	1.48
^d	Oseltamivir carboxylate	187227-45-8	0.15	80	75.07	74.45	0.62
J05AH01	Zanamivir	139110-80-8	0	100	92.06	91.72	0.33
N04BB01	Amantadine	768-94-5	0.2	100	48.58	46.41	1.91
J05AC02	Rimantadine	13392-28-4	0.2	100	38.81	30.11	8.17
C10AA01	Simvastatin	79902-63-9	<i>Statins (HMG CoA Reductase Inhibitors)</i>				
C10AA02	Lovastatin	75330-75-5	0.015	73	96.49	58.76	37.73
C10AA05	Atorvastatin	134523-00-5	0.03	70	90.7	64.4	26.3
C10AA03	Pravastatin	81093-37-0	0.01	88	97.29	25.72	71.57
			0.02	78	94.93	92.26	2.67
M04AB01	Probenecid	57-66-9	<i>Uricosurics (Transporting Inhibitors)</i>				
			1	88	34.8	28.2	6.6
R01BA02	Pseudoephedrine	90-82-4	<i>Decongestants</i>				
R01AA04	Phenylephrine	59-42-7	0.24	90	75.2	74.5	0.65
R01BA01	Phenylpropanolamine	14838-15-4	0.004	3	92.1	91.7	0.4
			0.1	90	75.1	74.5	0.64

^a including their defined daily dose (DDD); % excretion as parent or active metabolite; % loss in sewage treatment plant (STW) owing to biodegradation and adsorption.
^b Total excretion is the sum of the % parent chemical released in the urine and feces. Metabolites were only included in the sum if the metabolite has a known bioactivity.
^c There is no official DDD for clavulanic acid. Clavulanic acid is administered in conjunction with amoxicillin in the form of Co-amoxiclav. In this case we used a typical adult dose of 0.125 g and assumed 3 doses per day, equating to a DDD equivalent of 0.375 g.
^d Oseltamivir carboxylate (OC) is the active metabolite of the prodrug oseltamivir ethylester phosphate (OE-P); 20% of OE-P is excreted in feces, 80% of OE-P is excreted in urine as OC (He et al. 1999).

TABLE 26.3 Pharmaceuticals Proposed for Use During an Influenza Pandemic^{a,b}

Drug	Influent (mean–high) (µg/L)	Ref.	Effluent (mean–high) (µg/L)	Ref.	River Water (mean–high) (µg/L)	Ref.
Tetracycline	0.738–1.41	25, 26, 48, 56	0.337–0.977	25, 34, 37, 48, 51, 56	0.11 ^c	4, 8
Doxycycline	0.157–0.22	25, 26	0.073–0.046	22, 26, 34	^c	4, 8, 22
Oxytetracycline	7.76–23.62	25, 26	0.66 ^a	26	0.34 ^c	4, 8
Chlortetracycline	52.63–171	25, 26	0.28 ^a	26	0.69 ^a	4, 8
Demeclocycline	1.45–3.15	25, 26	0.65–1.12	26	—	
Chloramphenicol	0.19	14	0.396–0.56	8, 14, 30	0.124–0.266 ×10 ⁻³	8, 55
Benzylpenicillin	29 ^a	48	^c	48	^c	8
Phenoxymethylpenicillin					^a	8
Amoxicillin	1.73–2900	35, 48	16.3–78.2	35, 48	*0.104–0.187	55
Cefalexin					0.021–0.026	35
Ceftriaxone					^c	10
Cefotaxime	0.006–0.024	48	0.009–0.034	48		
Trimethoprim	1.44–7.9 ^c	14, 24, 39, 48, 56, 59, 60	0.025.5–2.5 ^c	8, 14, 22, 24, 28, 30, 37, 39, 48, 51, 53, 56, 57, 59, 60, 61	0.073–0.71 ^c	4, 8, 22, 28, 52, 57, 59, 60
Sulfamethoxazole	0.304–1	14, 24, 26, 25, 39, 56	0.702–2.0	8, 14, 22, 24, 28, 30, 37, 39, 51, 53, 56, 57	0.077–1.9 ^c	4, 8, 17, 27, 28, 33, 37, 39, 52, 57
Metronidazole			0.050–0.080 ^a	8, 14, 22, 24, 28, 30, 37, 39, 51, 53, 56, 57	0.0143–0.043 ^c	22
Erythromycin	0.215–0.810	14, 24, 59, 48	0.645–0.85 ^c	8, 14, 22, 24, 28, 30, 34, 45, 48, 49, 57, 59	1.955–67.0 ^a	2, 4, 8, 10, 22, 27, 28, 33, 52, 55, 57, 59
Roxithromycin	0.056	14	2.743–1.0	14, 30, 34, 45	0.080–0.18 ^c	4, 8, 27, 55
Azithromycin			0.059–0.077	63	0.022–0.047 ^c	63
Clarithromycin	^c	14	0.180–0.536	8, 14, 30, 34, 45	0.059–0.26 ^a	8, 27
Clindamycin	^c	14	0.050–0.11 ^c	14, 37	0.034–0.14	10, 37
Ciprofloxacin	0.558–1.4	1, 3, 35, 56	0.340–0.97 ^c	1, 3, 9, 9, 22, 34, 35, 37, 51, 56, 61	0.107–0.36 ^c	1, 4, 35, 37, 52
Levofloxacin					^a	50
Norfloxacin	0.32–0.6	1, 3, 48	0.121–0.367 ^a	1, 3, 9, 9, 22, 34, 35, 48, 61	0.06–0.251 ^c	1, 4, 22, 35, 55

Ofloxacin	0.421–1	1, 14, 39	0.205–0.58 ^a	1, 14, 22, 34, 39, 61	0.047–0.109 ^c	1, 22, 52, 55
Indometacin			0.27–0.6	7	0.04–0.2	7
Diclofenac	0.757–1.92	20, 23, 24, 32, 41, 59, 60	0.480–5.45	19, 21, 23, 24, 28, 30, 32, 40, 41, 42, 44, 53, 57, 59, 60, 61	0.061–0.360 ^a	21, 27, 28, 29, 32, 53, 57, 60
Ibuprofen	25.56–68,700	5, 20, 23, 24, 36, 38, 41, 59, 60	3.42–28 ^c	5, 6, 7, 19, 20, 21, 23, 24, 28, 30, 36, 38, 40, 41, 42, 44, 53, 57, 59, 60, 61	1.01–3.1 ^a	4, 5, 6, 10, 21, 27, 28, 33, 38, 43, 53, 57, 59, 60
Naproxen	33.49–280	5, 7, 20, 24, 36, 37, 41, 60	33–3.3 ^c	5, 6, 8, 19, 20, 21, 24, 30, 36, 40, 41, 44, 53, 57, 60, 61	0.75–0.85 ^c	5, 6, 21, 27, 29, 53, 57, 60
Ketoprofen	0.959–2.9	5, 7, 20, 41, 60	1.39–36 ^c	5, 7, 19, 20, 21, 30, 40, 41, 44, 53, 60, 61	0.050–0.290 ^a	5, 21, 53, 60
Acetaminophen	117.6–281	23, 24, 38	1.5–11.5 ^c	6, 19, 23, 24, 38, 40, 57	0.025–10 ^c	4, 6, 27, 28, 33, 37, 38, 57
Acetylsalicylic acid						
Codeine	5.2–11	23	0.037–0.057	19	0.025–0.037 ^c	43
Simvastatin	0.004	47	3.7–8.1	23	0.004–1.0 ^c	4, 33, 43
Lovastatin	0.049	47	0.001	47	ND	47
Atorvastatin	0.076	47	0.014	47	0.0183 ^c	47, 62
Pravastatin	0.117	47	0.037	47	ND	47
		47	0.059	47	ND	47

^a The absence of a pharmaceutical from this table should not be interpreted as its absence from the environment. The mean is from all the reported concentrations from the noted references and the max is the highest reported concentration from the references.

^b Including their occurrence in STW influent and effluent and river water.

^c Denotes a study that looked for the drug but did not detect it.; ¹ (Vieno et al. 2006); ² (Sacher et al. 2001); ³ (Giger et al. 2003); ⁴ (Kolpin et al. 2002); ⁵ (Lindqvist et al. 2005); ⁶ (Ternes 1998); ⁷ (Heberer 2002); ⁸ (Hirsch et al. 1999); ⁹ (Golet et al. 2001); ¹⁰ (Zuccato et al. 2000); ¹¹ (Alexy et al. 2004); ¹² (Gartiser et al. 2007); ¹³ (Junker et al. 2006); ¹⁴ (Alexy et al. 2006); ¹⁵ (Ternes et al. 2002); ¹⁶ (Lindberg et al. 2007); ¹⁷ (Liebig et al. 2006); ¹⁸ (Kümmerer et al. 2000); ¹⁹ (Rabiet et al. 2006); ²⁰ (Vieno et al. 2005); ²¹ (Tixier et al. 2003); ²² (Wenmalm 2005); ²³ (Gomez et al. 2007); ²⁴ (Trenholm et al. 2006); ²⁵ (Choi et al. 2007); ²⁶ (Yang and Carlson 2003); ²⁷ (Wiegel et al. 2004); ²⁸ (Ashton et al. 2004); ²⁹ (Ahrer et al. 2001); ³⁰ (Ternes 2001); ³¹ (Carlsson et al. 2006); ³² (Buser et al. 1998); ³³ (Stackelberg et al. 2004); ³⁴ (Miao et al. 2004); ³⁵ (Costanzo et al. 2005); ³⁶ (Carballa et al. 2004); ³⁷ (Batt et al. 2006); ³⁸ (Bound and Voulvoulis 2006); ³⁹ (Brown et al. 2006); ⁴⁰ (Brun et al. 2006); ⁴¹ (Brun et al. 2006); ⁴² (Reemtsma et al. 2006); ⁴³ (Moldovan 2006); ⁴⁴ (Dreves et al. 2003); ⁴⁵ (McArdell et al. 2003); ⁴⁶ (Joss et al. 2006); ⁴⁷ (Miao and Metcalfe 2003); ⁴⁸ (Gulkowska et al. 2008); ⁴⁹ (Cordy et al. 2004); ⁵⁰ (Conleya et al. 2008); ⁵¹ (Sacher et al. 2001); ⁵² (Batt and Aga 2005); ⁵³ (Haggard et al. 2006); ⁵⁴ (Metcalfe et al. 2003); ⁵⁵ (Al-Ahmad et al. 1999); ⁵⁶ (Batt et al. 2007); ⁵⁷ (Kim et al. 2007); ⁵⁸ (EA 2003); ⁵⁹ (Roberts and Thomas 2006); ⁶⁰ (Bendz et al. 2005); ⁶¹ (Andreozzi et al. 2003); ⁶² (Conlea et al., 2008); ⁶³ (Jones-Lepp 2006).

TABLE 26.4 Percentage of Parent Pharmaceuticals Investigated in This Study Excreted in the Feces and Urine unchanged and/or as a Bioactive Metabolite

Pharmaceutical	% Excreted as Parent or Bioactive Metabolite
Tamiflu	100
Amoxicillin	75
Clavulanate	38
Doxycycline	80
Cefuroxime	95
Cefotaxime	61
Erythromycin	100
Clarithromycin	55
Levofloxacin	96
Moxifloxacin	100

Source: From Dollery (1999) and Wishart et al. (2006).

dosages per 1000 inhabitants per day ranges between 10 and >20 in Europe (Coenen et al., 2006; Ferech et al., 2006; Muller et al., 2007). If one was to use the average annual antibiotic usage within England during an interpandemic period (NHS BSA, 2008) as a first approximation, 62 µg antibiotics/L would be present in the UK WWTPs, which was assumed to be diluted by the median volume of wastewater in WWTP within the Thames catchment area, 230 L/head/day (Table 26.5).

Table 26.3 and Figure 26.3 give an extensive overview on the concentrations of antibiotics that have been found in WWTP and surface water globally as well as in the United Kingdom. When comparing predicted and actual concentrations of antibiotics in WWTPs (Table 26.5 vs Table 26.3), it should be noted that veterinary use significantly contributes to concentrations in surface water (Hurd and Raef, 2010; Smith et al., 2002) such that WWTP effluent is not the only source of antibiotic residues in the aquatic environment. Over- and underestimations of predicted concentrations of antibiotics in the environment can also be caused by unforeseen losses from sorption and/or degradation, the impact of climate on degradation, difference in antibiotic use between different regions within a country, as well as country-specific differences, seasonal fluctuations in drug use, as well as seasonal fluctuations on WWTPs and river dilution (ter Laak et al., 2010). Hence, it is very difficult to project the concentration of antibiotics in use during interpandemic periods for any one location at any particular time. For this study we employed annual usage statistics for England (2007) with no further modification or adjustment.

Unlike antibiotics, interpandemic usage of Tamiflu in the United Kingdom has been reported to be negligible (Kramarz et al., 2009), implying that any substantial increase will be as a result of the influenza pandemic.

26.2.2 Projected Concentrations of Antibiotics and Tamiflu in WWTP and Rivers During a Pandemic

In WWTPs, a mild pandemic scenario was projected to increase interpandemic antibiotic use of the same antibiotics (see Section) by only approximately 1% (95%

FIGURE 26.1 Percent removal of a range of antibiotics in WWTPs, from biodegradation.

FIGURE 26.2 Outpatient antibiotic use in 10 European countries in 2005 for each of seven major classes of antibacterials and the total DDD per 1000 inhabitants per day.

TABLE 26.5 Estimated Concentration of Antibiotics in English WWTP During an Interpandemic period^a

	Combined µg/head/d	% of Total Antibiotics in Use (mass basis)	Estimated Concentration in WWTP (µg/L)
Floxacillin + co-fluampicil	4068	28.0	17.5
Amoxicillin + co- amoxiclav	3698	25.4	15.9
Cefalexin	2023	13.9	8.68
Erythromycin	1391	9.6	5.97
Ampicillin	840	5.8	3.61
Ciprofloxacin	582	4.0	2.50
Penicillin V	531	3.6	2.28
Trimethoprim	387	2.7	1.66
Cefradine	253	1.7	1.09
Clarithromycin	156	1.1	0.671
Cefaclor	129	0.9	0.553
Cefadroxil	126	0.9	0.543
Clavulanate	108	0.7	0.464
Oxytetracycline	66	0.5	0.285
Lymecycline	47	0.3	0.202
Sulfamethoxazole	36	0.3	0.156
Cefuroxime	24	0.2	0.103
Minocycline	30	0.2	0.128
Doxycycline	12	0.1	0.052
Azithromycin	16	0.1	0.069
Ofloxacin	9	0.1	0.041
Norfloxacin	8	0.1	0.035
Levofloxacin	7	0.1	0.031
Moxifloxacin	5	<0.1	0.022

^aDrug use was as reported by the National Health Service Business Services Authority (NHS BSA, 2008). Where the ADQ (average daily quantity) was unknown, the DDD (defined daily dose) (World Health Organization, 2004) was used to calculate the mass of drug used per head per day (population of England served by the NHS: 54,180,000).

reference range, RR, of the stochastic epidemic model: 0.4–23%). An increase by 13% (95% RR, 1–83%) and 252% (95% RR, 158–279%) was determined for the moderate and severe scenarios. In absolute concentrations, a moderate pandemic would increase interpandemic concentrations by 1.2–3 µg/L (total of all antibiotics; mean concentration of all 135 WWTP in the Thames catchment area for the median epidemic scenario, Table 26.6). A severe pandemic would cause total antibiotic concentrations to reach 55–60 µg/L on average, with a maximum of 800 µg/L for the WWTP with the lowest dilution. Amoxicillin and erythromycin have the greatest share of the total antibiotic load (59 and 18%, respectively). Tamiflu concentrations greatly vary with the epidemic scenario for mild and moderate R_0 and are highest if antiviral prophylaxis is assumed for 10% of the population (around 45 µg/L in the mild and moderate scenario). A severe pandemic would increase Tamiflu concentrations to >80 µg/L on average (Table 26.6).

FIGURE 26.3 Pre-pandemic river water concentration of pharmaceuticals that have been examined in STW effluent and/or river water.

Mean concentrations of total antibiotics in the Thames catchment were projected to be <0.09 and <0.8 $\mu\text{g/L}$ for a mild and moderate pandemic, respectively (Table 26.7). On the other hand, a severe pandemic was projected to achieve <15 $\mu\text{g/L}$ total antibiotics, with a maximum of 80 $\mu\text{g/L}$. A mild and moderate pandemic with AVP >0 was projected to generate mean concentrations of Tamiflu in the Thames catchment between 1.1 and 11.5 $\mu\text{g/L}$ (Table 26.7). A more severe pandemic, regardless of AVP, was projected to result in mean concentrations of Tamiflu in the Thames catchment in excess of 100 $\mu\text{g/L}$, consistent with previous projections of a severe pandemic in southern England (Singer et al., 2007).

26.3 EFFECTS ASSESSMENT OF ANTIBIOTICS DURING A PANDEMIC

Elevated concentrations of antibiotics during a pandemic will, in the first instance, affect microbial consortia in WWTPs and surface waters. In order to evaluate such potential effects of antibiotics, we determined the fraction of bacteria that were potentially affected by a given antibiotic exposure [(potentially affected fraction, PAF). This approach projects effects on whole communities from toxicities of a substance to single members of the community compiled in species-sensitivity distributions (SSD) (Newman et al., 2002)]. As experimental data on the toxicity of the chosen antibiotics is almost entirely lacking, we based our analyses on minimum inhibitory concentrations (MIC) of human pathogens from the EUCAST database (EUCAST, 2009). Through the application of models for mixture toxicity (De Zwart and Posthuma, 2005), we accounted for the presence of all eight antibiotics simultaneously (clavulanate was omitted because no MICs were present for this

TABLE 26.6 Projected Mean Concentrations of Antibiotics and Tamiflu in WWTP in the Thames Basin^a

Scenario ^b	Antibiotics (µg/L)		Tamiflu (µg/L)	
	Mean ± Stdev	Maximum	Mean ± Stdev	Maximum
<i>R</i> ₀ 1.65				
s1	0.36 ± 0.4	4.76	0.78 ± 0.87	10.41
s2	0.34 ± 0.37	4.49	4.63 ± 5.14	61.55
s3	0.34 ± 0.37	4.46	4.86 ± 5.4	64.65
s4	0.3 ± 0.33	3.97	45.35 ± 50.37	603.42
s5	0.29 ± 0.32	3.83	45.43 ± 50.46	604.4
s6	0.05 ± 0.06	0.73	0.11 ± 0.12	1.49
<i>R</i> ₀ 1.9				
s1	2.99 ± 3.32	39.8	4.02 ± 4.46	53.44
s2	2.85 ± 3.17	38	4.79 ± 5.32	63.67
s3	2.97 ± 3.3	39.5	6.05 ± 6.72	80.53
s4	2.27 ± 2.52	30.2	45.3 ± 50.4	603.3
s5	1.71 ± 1.9	22.8	47 ± 52.3	625.9
s6	1.2 ± 1.33	15.9	1.52 ± 1.69	20.22
<i>R</i> ₀ 2.1				
s1	60.2 ± 66.8	800.6	87.9 ± 97.6	1169.5
s2	59.7 ± 66.3	793.6	87.8 ± 97.5	1167.6
s3	59.3 ± 65.9	789.6	87.6 ± 97.3	1166.1
s4	57 ± 63.3	757.8	85.5 ± 94.9	1137.2
s5	53.7 ± 59.6	714	80.1 ± 89	1065.6
s6	54.2 ± 60.2	721.3	80.7 ± 89.6	1073.6

^aMean values are inclusive of all excreted antibiotics. Values reflect the median epidemic scenario for each condition and reflect the mean concentration for all 461 river stretches utilized within LF2000-WQX of parent pharmaceuticals investigated in this study excreted in the feces and urine unchanged and/or as a bioactive metabolite.

^bs1 = where AVP = 0, rate of AVT = 30%, limited supply of Tamiflu.

s2 = where 2 wk AVP, AVP = 1%, rate of AVT = 30%, limited supply of Tamiflu.

s3 = where 4 wk AVP, AVP = 1%, rate of AVT = 30%, limited supply of Tamiflu.

s4 = where 2 wk AVP, AVP = 10%, rate of AVT = 30%, limited supply of Tamiflu.

s5 = where 4 wk AVP, AVP = 10%, rate of AVT = 30%, limited supply of Tamiflu.

s6 = where AVP = 0, rate of AVT = 30%, unlimited supply of Tamiflu.

substance on its own). Toxicities were determined for WWTP and river stretches in the Thames catchment area from antibiotic concentrations predicted through exposure modeling. The results of this determination of the potentially affected fraction of bacteria in the community based on simulated antibiotic concentrations are shown in Figures 26.4 and 26.5. For a mild pandemic, projected toxicity in WWTP was well below 1% PAF (less than 1% of the community might be growth inhibited, Fig. 26.4a). During a moderate pandemic, toxicities >5% were predicted in 74% of the WWTP for concentrations at the upper bound of the 95% RR of the stochastic model, while no toxicity was predicted for the lower bound (Fig. 26.4a). A proportion of growth-inhibited species >5% was chosen as a pragmatic threshold for possible effects on community functioning (European Chemicals Agency, 2008). The severe pandemic was projected to affect between 8 and 32% of the microbial species in WWTP (Figs. 26.4b and 26.5c).

TABLE 26.7 Projected Mean Concentrations of Antibiotics and Tamiflu in Rivers in the Thames Basin^a

Scenario ^b	Antibiotics (µg/L)		Tamiflu (µg/L)	
	Mean ± Stdev	Maximum	Mean ± Stdev	Maximum
<i>R</i> ₀ 1.65				
s1	0.085 ± 0.088	0.476	0.186 ± 0.192	1.04
s2	0.082 ± 0.084	0.445	1.12 ± 1.15	6.09
s3	0.083 ± 0.084	0.447	1.20 ± 1.21	6.47
s4	0.073 ± 0.074	0.400	11.1 ± 11.2	60.8
s5	0.070 ± 0.072	0.384	11.1 ± 11.3	60.6
s6	0.013 ± 0.014	0.073	0.027 ± 0.027	0.149
<i>R</i> ₀ 1.9				
s1	0.741 ± 0.744	3.95	1.00 ± 1.00	5.31
s2	0.690 ± 0.706	3.77	1.16 ± 1.19	6.33
s3	0.719 ± 0.731	3.90	1.47 ± 1.49	7.96
s4	0.552 ± 0.563	3.01	11.0 ± 11.2	60.0
s5	0.418 ± 0.427	2.27	11.5 ± 11.7	62.4
s6	0.294 ± 0.298	1.59	0.37 ± 0.38	2.02
<i>R</i> ₀ 2.1				
s1	14.8 ± 15.0	80.5	21.3 ± 21.3	102
s2	14.5 ± 14.8	80.6	21.0 ± 21.3	103
s3	14.5 ± 14.8	79.9	21.1 ± 21.2	102
s4	14.0 ± 14.2	75.9	20.7 ± 20.8	99.1
s5	13.1 ± 13.3	69.3	19.6 ± 19.9	103
s6	13.2 ± 13.4	72.3	19.6 ± 20.0	108

^aMean values are inclusive of all excreted antibiotics. Values reflect the median epidemic scenario for each condition and reflect the mean concentration for all 461 river stretches utilized within LF2000-WQX of parent pharmaceuticals investigated in this study excreted in the feces and urine unchanged and/or as a bioactive metabolite.

^bs1 = where AVP = 0, rate of AVT = 30%, limited supply of Tamiflu.

s2 = where 2 wk AVP, AVP = 1%, rate of AVT = 30%, limited supply of Tamiflu.

s3 = where 4 wk AVP, AVP = 1%, rate of AVT = 30%, limited supply of Tamiflu.

s4 = where 2 wk AVP, AVP = 10%, rate of AVT = 30%, limited supply of Tamiflu.

s5 = where 4 wk AVP, AVP = 10%, rate of AVT = 30%, limited supply of Tamiflu.

s6 = where AVP = 0, rate of AVT = 30%, unlimited supply of Tamiflu.

Absolute toxicity in rivers was projected to be slightly lower than in WWTPs, with the maximum PAF for any river stretch in a moderate pandemic being ~15% (Fig. 26.4e). During a severe pandemic, the 5% threshold of toxicity would be exceeded in about half of the river stretches at the upper bound of the 95% *R* (Fig. 26.4c), corresponding to about one third of total river length (Figs. 26.4d and 26.5f). Maximum toxicity during a severe pandemic was simulated to reach 30% (Fig. 26.4e).

The same effect models, when applied to the background concentrations of the eight investigated antibiotics, yield toxicities (PAF) in Thames catchment WWTP of between 4 and 17%. At these concentrations, however, no major functional breakdowns have been recorded. Reasons for the tolerance of WWTP communities to chronic exposure to antibiotics might be: (1) reduction of bioavailable concentrations through degradation or sorption, (2) acquired community tolerance through

FIGURE 26.4 Predicted toxicity in WWTPs and river stretches.

FIGURE 26.5 Predicted toxicity maps.

selection for more tolerant or resistant bacteria, (3) differences between the antibiotic sensitivity of human pathogens and WWTP bacteria, and (4) overcapacity of WWTPs, thereby enabling some decline in function to occur with no significant loss in overall sewage treatment. As only the severe scenario increases the PAF substantially, we conclude that a mild or moderate pandemic would not be likely to affect WWTP consortia.

Notably, effects modeling in this study were based on MICs of human pathogens instead of effects observed in WWTP toxicity testing. Arguably, the toxicity of antibiotics to functionally active WWTP bacteria might differ from pathogen MICs. Also, the predominance of biofilms in WWTPs as flocs will influence antibiotic toxicity. There is little experimental data to verify whether modeled toxicity matches experimental data. Also, often, studies are based on test systems that differ from a full-scale WWTP, such as through the use of batch studies or through the use of synthetic sewage as medium.

Erythromycin is the only antibiotic investigated in this study for which substantial data on toxicity in WWTPs exists. For example, in batch studies with activated sludge inoculum from French WWTPs (Louvet et al., 2010a) and raw wastewater as medium, a range of effects of erythromycin were observed. After exposure to the antibiotic for less than one hour, direct toxicity was seen in cell staining experiments. In 24-hour batch experiments with inocula collected at nine different time points, 10 mg/L erythromycin (equivalent to a PAF of 80% according to our models) inhibited COD (chemical oxygen demand) removal by 79% on average. Inhibition strongly varied between different inocula, highlighting the dependence of batch toxicity studies on the initial inoculum. Inhibition of nitrification was 40% on average in similar experiments.

Inhibition was also found at lower concentrations of erythromycin (see Table 26.8). Further, erythromycin interfered with floc structure: flocs divided and biomass was transferred to the foam layer on top of the batch reactors (Louvet et al., 2010a). At longer exposure times of batch reactors (5 days), effects on soluble COD were observed at erythromycin concentrations as low as 4 $\mu\text{g/L}$ (Louvet et al., 2010b),

TABLE 26.8 Effects of Antibiotics on Wastewater Treatment Plant Bacterial Consortia and Processes

Class	Compound	Concen-tration (mg/L)	PAF (%)	PAF (%) of Group Member	Size of Effect Measured (%)	Toxicity Parameter	N	Refs.
	Erythromycin	0.1	22		10–35	Reduction in live bacteria in mixed liquor samples after 20–45 min	3	1
	Erythromycin	1	56		50–62	Reduction in live bacteria in mixed liquor samples after 25–45 min	3	1
	Erythromycin	0.004	0.3		13/31/0	Batch reactors with activated-sludge-fed raw wastewater: decreased NH ₄ reduction after 40/65/90 h	2	1
	Erythromycin	0.1	22		55/90 (activated sludge from two different STP)	Batch reactors with activated-sludge fed raw wastewater: inhibition of specific N-NH ₄ evolution rate after 4 h	2	2
	Erythromycin	0.1	22		6/89 (activated sludge from two different STP)	Batch reactors with activated-sludge-fed raw wastewater: inhibition of the specific COD evolution rate after 4 h	2	2
	Erythromycin	0.5	46		62/44/29	Batch reactors with activated-sludge-fed raw wastewater: decreased nitrification (nitrate evolution) after 40/65/90 h	2	1

(Continued)

TABLE 26.8 (Continued)

Class	Compound	Concentration (mg/L)	PAF (%)	PAF (%) of Group Member	Size of Effect Measured (%)	Toxicity Parameter	N	Refs.
	Erythromycin	1	56		36/92 (activated sludge from two different STP)	Batch reactors with activated-sludge-fed raw waste-water: inhibition of specific N-NH ₄ evolution rate after 4 h	3	2
	erythromycin	1	56		51/70 (activated sludge from two different STP)	Batch reactors with activated-sludge-fed raw wastewater: inhibition of the specific COD evolution rate after 4 h	3	2
	Erythromycin	5	75		32	Batch reactors with activated-sludge-fed raw wastewater: inhibition of the initial ammonia uptake rate over 24 h	Not stated	3
	erythromycin	10	80		46	Batch reactors with activated-sludge-fed raw wastewater: inhibition of nitrification over 48 h	Not stated	3
	erythromycin	10	80		79 (standard deviation: 34)	Batch reactors with activated-sludge-fed raw waste water: inhibition of the specific COD evolution rate after 4 h	13	2
	Erythromycin	10	80		40 (standard deviation: 25)	Batch reactors with activated-sludge-fed raw wastewater: inhibition of	13	2

TABLE 26.8 (Continued)

Class	Compound	Concen-tration (mg/L)	PAF (%)	PAF (%) of Group Member	Size of Effect Measured (%)	Toxicity Parameter	N	Refs.
Third-generation cephalosporins	Ceftriaxone disodium	0.005		11 (cefotaxime)	Not stated	Reduction in CFU, development during CBT	Not stated	6
	Chlortetracycline HCl	0.003		0.7	Not stated	Reduction in CFU, development during CBT	11	4
	Clarithromycin	3		78	Not stated	Reduction in CFU, development during CBT	11	4
Tetra-cyclines	Tetracycline	16.8		88 (doxycycline)	100%	Inhibition of degradation of control substance in closed bottle test (OECD 301 B) over 31 days	1	7
	Tetracycline	37		93 (doxycycline)	Weak	COD removal in semiindustrial bioreactor	1	7
Tetra-cyclines	Tetracycline	11		84 (doxycycline)	41	Inhibition of degradation of control substance in closed bottle test (OECD 301 B) over 31 days	2	8
	Ofloxacin	12		93 (levofloxacin) / 96 (moxifloxacin)	Non-competitive inhibition constant	Inhibition of nitrification in batch reactors with nitrifying communities	1	9

Sources: ¹(Louvvet et al. 2010b); ²(Louvvet et al. 2010a); ³(Alighardashi et al. 2009); ⁴(Alexy et al. 2004); ⁵(Bundschuh et al. 2009); ⁶(Al-Ahmad et al. 1999); ⁷(Prado et al. 2009); ⁸(Prado et al. 2010); ⁹(Dokianakis et al. 2004).

which is equivalent to a PAF of 0.2%. Concentrations higher than 500 µg/L also inhibited nitrification. At 4 µg/L, erythromycin-treated reactors showed higher nitrification as compared to control reactors. This was explained through the extra source of nitrogen provided by dead bacteria.

In contrast, Fan et al. (2009) found that erythromycin (at concentrations of 500 µg/L or a single-substance PAF of 46% according to our calculations) did not have pronounced effects on nitrogen and phosphorus removal in sequencing batch reactors fed synthetic wastewater during long incubations (Fan et al., 2009). Still, phylogenetic investigations showed that the diversity of ammonium-oxidizing bacteria and of nitrifying bacteria had declined by up to 80%, highlighting effects of the antibiotic on the composition of the functional community. Further, short-term toxicity measurements with higher concentrations of erythromycin and H₂O–erythromycin showed that while the adapted nitrifier and ammonium oxidizer communities were more tolerant to high doses of antibiotics, functional parameters were still affected at concentrations equal or higher than the long-term concentration (Fan et al., 2009). Thus, shock concentrations of antibiotics during the onset of a pandemic might affect WWTP functioning, although almost all treatment plants will be acclimated to lower antibiotic concentrations during the predominant interpan-demic period. Further, while relatively low concentrations of single antibiotics are able to select for equally functional, but less diverse, antibiotic-tolerant communities, the question remains as to whether joint effects of several antibiotics might further reduce diversity and therefore functioning.

As seen above, investigations with full-scale treatment plants sometimes show a high functional stability also for other antibiotics. Most relevant to this study, Al-Ahmad et al. (1999) found that a mixture of 12 antibiotics tested in laboratory-scale treatment plants did not have effects on DOC elimination during 84 days of operation, even at concentrations that were 100 times higher than average influent concentrations. In contrast, effects of a sudden change in wastewater composition were apparent in two WWTPs purifying waste water from a pharmaceutical production site. Failure in ammonium oxidation and changes in the composition of ammonium-oxidizing bacterial communities were found when the plant started producing imidazoles, and problems continued for a few weeks (Wittebolle et al., 2005).

To conclude, congruence of modeled toxicity with experimental data on toxicity of shock loads of antibiotics suggests that a severe pandemic might indeed provide antibiotic concentrations capable of affecting microbial WWTP consortia, at least for a short time. This could compromise vital and obligate microbial functions such as nitrification, phosphorus, and COD removal. With longer exposure duration, changes in community composition, together with an increase in genetically encoded antibiotic resistance (see Section 1.3.2), might preserve WWTP functioning.

26.3.1 Effects on Antibiotic Resistance

The projected widespread use of antibacterials during an influenza pandemic introduces an opportunity for the rapid spread of antibiotic resistance. Bacteria can become resistant to antibiotics by mutations or the acquisition of appropriate genes from other microorganisms (Verhoef and Fluit, 2006). Many publications have shown that WWTPs are a hot spot for the occurrence of resistance genes (Schluter

et al., 2003; Szczepanowski et al., 2009), showing that the human community discharges bacteria and mobile genetic elements carrying resistance genes to the WWTP in interpandemic times. Further, WWTPs have been discussed as environments for gene transfer between bacteria (Geisenberger et al., 1999), and the presence of residual antibiotics could speed such genetic exchange. In any case, massive use of antibiotics during the pandemic will further resistance development in human microbiota (Barlow, 2009), and a significant proportion of these will reach WWTPs and be discharged with WWTP effluent after incomplete purification.

An additional question is whether elevated concentrations of antibiotics during a pandemic might be high enough for selection and recombination of resistant bacteria in the WWTP. There is little experimental evidence to prove or disprove this idea, as most studies so far have investigated treatment plants that also received human waste, presumably containing resistant bacteria. However, recent investigations in WWTPs treating waste from antibiotic production sites showed that high concentrations of antibiotics can indeed select for resistant populations that can subsequently be discharged to the receiving rivers (Li et al., 2009; 2010). In the first production plant, concentrations of penicillin G were around 150 µg/L (PAF of amoxicillin: 34%). Extremely high MICs for β-lactam antibiotics were observed in isolates from the WWTP, while resistance to unrelated antibiotics was also elevated. In the second plant, which produced oxytetracycline (30.5 mg/L in the sludge, equivalent to a doxycycline PAF of 92%), extremely high MICs were again observed in isolates tested for resistance to different tetracyclines. Also, many isolates showed multiresistance to unrelated antibiotics. Isolates often contained class I integrons, genetic elements that can integrate a multitude of resistance genes into bacterial genomes. From these two studies, it indeed appears that antibiotics can select for resistance in WWTPs.

26.4 CONCLUSIONS

Through epidemiological simulations and environmental and toxicity modeling, the concentrations and effects of antibiotics during an influenza pandemic were determined. It was shown that a severe pandemic might affect WWTP consortia and lead to increased nitrogen and COD release, at least for short periods. Pandemic concentrations are also likely to increase antibiotic resistance beyond levels already observed in WWTP. In line with modeling results obtained for a mild pandemic, no widespread WWTP failures were observed during the 2009/2010 influenza pandemic. Still, current experimental data is insufficient to disprove possible effects during an influenza pandemic characterized by a higher infectivity.

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