REVIEW ARTICLE

The Scourge of Antibiotic Resistance: The Important Role of the Environment

Rita L. Finley,[1] Peter Collignon,[2] D. G. Joakim Larsson,[3] Scott A. McEwen,[4] Xian-Zhi Li,[5] William H. Gaze,[6] Richard Reid-Smith,[7] Mohammed Timinouni,[8] David W. Graham,[9] and Edward Topp[10]

[1]Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, Guelph, Ontario; [2]Infectious Diseases Unit and Microbiology Department, The Canberra Hospital and Canberra Clinical School, Australian National University; [3]Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Sweden; [4]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Canada, [5]Veterinary Drugs Directorate, Health Canada, Ottawa, Ontario; [6]European Centre for Environment and Human Health, Exeter University Medical School, Knowledge Spa, Royal Cornwall Hospital, Truro, United Kingdom; [7]Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario; [8]Molecular Bacteriology Laboratory, Pasteur Institute of Morocco, Casablanca; [9]School of Civil Engineering and Geosciences, Newcastle University, United Kingdom; and [10]Agriculture and Agri-Food Canada, London, Ontario

Antibiotic resistance and associated genes are ubiquitous and ancient, with most genes that encode resistance in human pathogens having originated in bacteria from the natural environment (eg, β-lactamases and fluoroquinolones resistance genes, such as *qnr*). The rapid evolution and spread of "new" antibiotic resistance genes has been enhanced by modern human activity and its influence on the environmental resistome. This highlights the importance of including the role of the environmental vectors, such as bacterial genetic diversity within soil and water, in resistance risk management. We need to take more steps to decrease the spread of resistance genes in environmental bacteria into human pathogens, to decrease the spread of resistant bacteria to people and animals via foodstuffs, wastes and water, and to minimize the levels of antibiotics and antibiotic-resistant bacteria introduced into the environment. Reducing this risk must include improved management of waste containing antibiotic residues and antibiotic-resistant microorganisms.

Keywords. antibiotic resistance; environment; human resistant infections.

Resistant infections are becoming more difficult or even impossible to treat with current antibiotics, leading to infections causing higher morbidity and mortality, imposing huge costs on our society [1, 2]. This increasing resistance involves many common human pathogens, including *Enterococcus faecium*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa*, and other *Enterobacter* species [2, 3]. However, many of these bacteria and/or their modes of resistance came from the natural environment, including bacteria within soils and water. Antibiotic resistance development is not just a local public health issue but includes broader environmental influences, which are amplified by international travel and global trade in foodstuffs.

The World Health Organization (WHO) recently announced a suite of policies that, if implemented, should mitigate the emergence and further dissemination of antibiotic-resistant organisms [4]. These initiatives have focused on antibiotic stewardship in the hospital and community settings, and reducing antibiotic use in livestock production. However, if we are to better manage antibiotic resistance, it is also vital that we consider the broader environment. Therefore, an improved understanding of the impacts of human activities on antibiotic resistance development is needed, such as nonhuman antibiotic use, pharmaceutical manufacturing waste, domestic and agricultural waste releases into the environment, and the influence of poor sanitation and unsafe water supplies.

Received 18 January 2013; accepted 15 May 2013; electronically published 30 May 2013.

Correspondence: Rita Finley, MSc, Centre for Food-borne, Environmental and Zoonotic Infectious Diseases, Public Health Agency of Canada, 255 Woodlawn Road Unit 120, Guelph, ON, N1H 8J1, Canada (rita.finley@phac-aspc.gc.ca).

Clinical Infectious Diseases 2013;57(5):704–10

© The Author 2013. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/cit355

There are emerging concerns that anthropogenic impacts are changing environmental reservoirs of resistance genes, "the resistome" [5], which will increase the probability of recruitment of resistance genes into clinically relevant pathogens [6]. For example, wastewater treatment, drug manufacturing, and agricultural effluents release massive quantities of antibiotic residues and resistant bacteria, selected in the digestive tracts of people or animals by antibiotic use [7].

Exposure of environmental bacteria to antibiotics as well as to large numbers of resistant bacteria may accelerate the evolution of resistance, increase the abundance and distribution of resistance genes within the resistome that is critical to the development of clinical resistance, and increase exchange of antibiotic resistance genes between bacteria [8, 9]. People and animals are connected to each other through the environment, and it is important to consider antibiotic resistance within the "One Health" concept, which provides a global strategy for expanding interdisciplinary collaboration and communication.

ANTIBIOTIC RESISTANCE GENES ARE UBIQUITOUS AND ANCIENT

Our world is inhabited by approximately 5×10^{30} bacteria, the vast majority of which are not pathogenic. Through evolutionary time, microorganisms developed capabilities for the biosynthesis of chemicals toxic to bacteria, "antibiotics," which vary widely in chemical structures, mode of action, and spectrum of activity. This was paralleled by the development of strategies to defeat antibiotics. Environmental bacteria, which predate the modern antibiotic era by billions of years, carry genes encoding resistance to antibiotics that have become critically important in medicine [10]. However, because only approximately 1% of environmental strains are culturable [11, 12], our knowledge of the true diversity and composition of the environmental resistome is limited.

The ability to quantitatively link the transfer of specific resistance genes from environmental strains to human pathogens has been difficult and, grossly underappreciated, although the ancient nature of environmental resistance is clear [5]. For example, viable multidrug-resistant bacteria were cultured from the Lechuguilla Cave in New Mexico even though it has been totally isolated for >4 million years [12]. These bacteria were resistant to at least 1 antibiotic and often 7–8 antibiotics, including β-lactams, aminoglycosides, and macrolides, as well as newer drugs such as daptomycin, linezolid, telithromycin, and tigecycline. Two distinct new macrolide inactivation mechanisms were identified, suggesting that the utilization of the environmental microbiome could be used to help combat resistance through the development of novel antibiotics designed not to be inactivated by these mechanisms.

Likewise, DNA extracted from 30 000-year-old Beringian permafrost contained genes coding for resistance to β-lactams, tetracyclines, and glycopeptides, confirming that resistance predates antibiotic use in medicine and agriculture [10]. Furthermore, major β-lactamase classes predate the existence of humans. Class A β-lactamases evolved approximately 2.4 billion years ago and were horizontally transferred into the gram-positive bacteria about 800 million years ago. The family of genes, including the progenitors of CTX-Ms, diverged 200–300 million years ago [13]. Overall, these studies provide compelling evidence of the breadth of the resistome in environmental strains and the intrinsic capacity for all bacteria to gain resistance.

Why are genes that confer resistance to antibiotics at clinically relevant concentrations ubiquitous? One explanation is that bacteria that produce antibiotics must be resistant to them to avoid self-destruction. In a highly diverse and competitive microbial environment such as soil, antibiotic-resistant bacteria will have a competitive advantage against susceptible bacteria. In addition, antibiotics are products of secondary metabolism, and some have important physiological functions at different concentrations, including the regulation of gene expression and communication between bacteria [14]. Antibiotics at sublethal concentrations can promote genetic exchanges through multiple pathways involving various stress responses [15]. Frequency of transfer of tetracycline-resistance plasmids in *S. aureus* was increased by up to 1000-fold in the presence of subinhibitory concentrations of β-lactams [14, 16]. Also, antibiotics in animal feed induced prophages in swine fecal microbiomes and contributed to phage-mediated resistance gene transfer [17], highlighting multiple environmental vectors for the horizontal transfer of resistance genes. Finally, many bacteria, while also resistant to multiple antibiotics, can actually use antibiotics as their sole carbon source [18]. Overall, the ancient origin of resistance genes highlights the need to take effective measures to control antibiotic usage in people and animals, the major drivers for the modern emergence of resistance. Indeed, in Australia, low levels of resistance to fluoroquinolones in key pathogens have resulted from restricted quinolone use in humans and absent use in food animals [19].

HUMAN ACTIVITY IS ENHANCING THE ENVIRONMENTAL RESISTOME

Human activity since the industrialization of antibiotic production after World War II has changed the distribution and increased the abundance of resistance genes. Genes encoding resistance were 2–15 times more abundant in 2008 compared to the 1970s in DNA extracted from archived soil samples collected between 1940 and 2008 in the Netherlands [20]. In particular, genes encoding resistance to β-lactams and tetracyclines were enriched. Worrisomely, an increase in extended spectrum β-lactamases (ESBLs) of the CTX-M family was observed, which appears to predate any clinical detection of these

enzymes. Furthermore, since industrialization, millions of tons of antibiotics have been released into the environment, including via wastewater effluents, land application of animal wastes, treatment of crop diseases, aquaculture, and many other activities. For example, 71% of total Danish antibiotic consumption (kg) in 2010 was for animal production [21]. A similar trend of antibiotic use in humans vs animals was also observed in Canada [22].

Public health impacts from antibiotic use in agriculture and aquaculture have already drawn much attention in the last decade [23–26]. Importantly, antibiotics used in humans and animals often belong to the same classes. The WHO has established a list of "critically important" antibiotics in humans to ensure prudent drug use in both human and veterinary medicine [26]. The third- and fourth-generation cephalosporins, fluoroquinolones, and macrolides are considered the drugs most urgently requiring risk management of their use in food animals. The use of extra-label third-generation cephalosporins poses an important challenge [27–30]. A comparison of ESBL-producing *E. coli* from retail chicken meat and humans has shown significant genetic similarities with respect to mobile resistance elements, virulence genes, and genomic backbone [31, 32]. The relationship between antibiotic use and resistance is exemplified in a novel manner by recent work on the long-term exposure of tetracyclines on honeybees, which showed the accumulation of mobile tetracycline resistance genes closely related to those from human pathogens in the gut microbiota of bees [33].

Antibiotic use in large-scale industrial agricultural facilities, which raise food animals at high-density, highlight many public health impacts including increased resistance and decreased water quality [34–36]. Similarly, impacts from large-scale and widespread antibiotic use in aquaculture need to be addressed [20, 37]. Specifically, fish infections are treated through the administration of antibiotics directly into the water, avoiding any kind of purification processes [38]. Aquaculture is increasingly important because fish production has increased substantially over the last 50 years with 52.5 million tons processed in 2008 [39].

Many antibiotics are excreted unchanged, are environmentally persistent, and can be detected downstream of wastewater treatment plants and adjacent to fields receiving animal manures [40]. In treated effluents and sewage sludge, antibiotic residues of several classes range in concentrations from nanograms per liter up to low micrograms per liter [41]. Although these are well below minimum inhibitory concentrations (MICs), even low concentrations provide selective advantages for certain resistant strains [42].

Concentrations well above MICs (milligram per liter range) have been found in treated wastewater from drug manufacturing [43, 44]. Environments contaminated with such high concentrations of antibiotics lead to selection for antibiotic resistance [45]. Antibiotic concentrations in untreated hospital effluents are lower, but still in the microgram per liter range [46]. Wastewater treatment and hospital effluents are therefore potential "hot spots" for the enrichment and transmission of resistant bacteria [42].

We also release large numbers of resistant bacteria that have multiplied exponentially in the gastrointestinal tracts of people and animals treated with antibiotics. These bacteria, in agricultural and wastewater effluents, harbor resistance genes and genetic elements that promote their exchange between bacteria [47, 48]. Commensals as well as pathogens are important sources of resistance genes that can be shared, eventually leading to human infections and disease [49]. Indirect selection for antibiotic resistance also needs to be considered. Resistance mechanisms to biocides or heavy metals may be present on the same genetic elements as those conferring resistance to antibiotics [50], causing cross-resistance.

Antibiotic resistance can be acquired through mutation of existing DNA, uptake of foreign DNA by means of transformation or phage-mediated transduction, and/or by conjugation (DNA exchange directly from other bacteria). Transposition of DNA within genomes also plays an important role in the mobilization of resistance determinants. Horizontal gene transfer is highly important in the evolution and transmission of resistance genes between species and includes the movement of resistance genes from fecal bacteria to environmental bacteria, as well as the reverse; that is, emergence of novel mechanisms of acquired resistance in pathogens, genes that originally were present in harmless bacteria [51]. Transduction has been identified to be important in the exchange of these genes with other organisms, particularly in freshwater [52]. Taken together, these anthropogenic inputs have increased reservoirs of resistant bacteria, including significant acquired resistance in pathogenic strains [51].

There is an interrelationship between humans, animals, and the environment. Both methicillin-resistant *S. aureus* (MRSA) and ESBL-producing *E. coli* can be used as indicators to evaluate the movement of resistant bacteria in the environment [53]. ESBL-producing bacteria cause serious infections around the world and can be recovered from foods for human consumption as well as in wildlife [53–55]. However, only after ESBL-producing *E. coli* appeared in livestock was this organism then identified in wildlife, suggesting that ESBL-producing *E. coli* is likely disseminated via manure applications [53]. Similarly, genes responsible for MRSA (*mec*A) have rarely been reported in isolates from aquatic environments. However, a *mec*A gene was recently identified from phage DNA isolated from waste and natural water, although its presence did not correlate with fecal contamination [56]. The emergence of novel MRSA strains in animals and the multiple methicillin resistance gene transfer events that

have occurred in these strains point to the powerful selective pressure exerted by antibiotic use in farming [57].

The rapid emergence of infections associated with multidrug resistance in *Acinetobacter* species has been increasingly observed globally. In the 1970s–1980s, *Acinetobacter*, a gram-negative organism commonly found in soil and water, was often susceptible to antibiotics. Today, *Acinetobacter* is one of the most difficult resistant gram-negative bacteria to control and treat [58]. Outbreaks have been associated with contamination of the hospital environment and equipment with multidrug-resistant strains introduced into hospitals by returning soldiers [59] and earthquake survivors [60]. Multidrug-resistant *A. baumannii* possesses almost all typical mechanisms of resistance (eg, multiple β-lactamases including carbapenamases, aminoglycoside-modifying enzymes, and drug efflux pumps) that render the organism resistant to almost all classes of antibiotics. Resistance islands in the chromosome of *A. baumannii* have large numbers of resistance genes and mobile genetic elements, and explains the sophisticated mechanisms of resistance in this species [61]. Most of these resistance genes have likely been acquired from *Pseudomonas*, *Salmonella*, or *E. coli* [61], probably mediated by environmental reservoirs.

EVIDENCE THAT HUMAN PATHOGENS HAVE ACQUIRED RESISTANCE GENES FROM ENVIRONMENTAL BACTERIA

There are 2 types of evidence that show human pathogens have acquired resistance genes from environmental bacteria. These are phylogenetic evidence (analyses of gene sequences and their context, revealing historic signatures of resistance determinants), and direct epidemiologic evidence from locations with less than adequate sanitation, especially poor drinking water quality.

The origins for some of the most common and problematic resistance genes are aquatic organisms such as *Shewanella* species, which carries a gene encoding quinolone resistance genes (*qnr*) on its chromosome [62]. In humans, plasmid borne *qnr* genes are more commonly identified in strains from Enterobacteriaceae infections, particularly *E. coli* and *Salmonella.* However, in the natural environment, this gene is mainly found in waterborne species, such as *Aeromonas* species and *Citrobacter* species, and in the Vibrionaceae family [62].

The origin of CTX-Ms was in *Kluyvera* species although it is unknown whether mobilization of the progenitor genes occurred in the environment or within the human microbiome [63]. *Kluyvera* has been recovered from water, soil, sewage, hospital sinks, and food of animal origin [64], but rarely from human clinical infections. However, even more resistance genes have now been identified, such as KLUC-1, a chromosomal β-lactamase found in *Kluyvera cryocrescens*. Although many of these have yet to be identified in clinical cases, they represent a reservoir for new potential clinical ESBLs [52].

WATER AS A DISSEMINATION ROUTE FOR RESISTANCE

Bacteria do not live in isolation, but are readily dispersed through the world by humans, animals, plants, soil, water, and air. An underappreciated exposure route for the dissemination of antibiotic resistance is water, and multidrug-resistant bacteria have been detected from various water sources, including drinking water. This is a major concern in developing countries and has been a major route for the transmission of pathogenic bacteria to people in developed countries in the past [48, 52, 65, 66]. Consumption or handling of water, whether treated or not, can lead to the colonization of the gastrointestinal tract in humans [67] and animals with bacteria containing resistance genes. This in turn, can result in exchange of genes with bacteria (commensal or pathogenic) already present in the human/animal gut. In addition, water is used for the irrigation of plants for animal and human consumption, contaminating products that could also lead to human/animal colonization with antibiotic-resistant organisms.

Freshwater is an important vehicle for the spread and emergence of antibiotic resistance [52]. Recently, the New Delhi metallo-β-lactamase-1 (NDM-1) genes were shown to be widely disseminated in many different bacterial species in water sources. Of more concern was the high prevalence of NDM-1 genes in bacteria in chlorinated municipal drinking water samples in India [68]. These NDM-1 genes were identified among 11 new species including *Shigella boydii*, *Vibrio cholera*, and *Aeromonas caviae*. The transfer of NDM-1 genes to *E. coli*, *Salmonella* Enteritidis, and *Shigella sonnei* was optimal at 30°C, the average daily peak temperature between April to October in that area of India. This specific gene is of great public health concern because it confers resistance to all β-lactams, including carbapenems, and coexists on gene transfer vectors with many other resistance determinants [68]. This means that with the rapid and simultaneous transmission of resistance can occur to almost all clinically important antibiotics [68].

Coastal waters also comprise a potential exposure route of resistant bacteria via contamination with wastewater. Based on WHO risk assessments, Shuval et al estimated that globally there are in excess of 120 million cases of gastrointestinal disease from exposure to coastal waters via recreation or by eating raw or lightly cooked shellfish [69]. This in itself is not proof of mobilization of resistant bacteria from environment to clinic, but it illustrates the scale of direct human exposure to contaminated water. Preliminary data from Gaze et al (written communication, November 2011) suggest that in some UK bathing waters, CTX-M carriage in enteric bacteria may be as high as 0.1%.

Another waterborne organism of major concern is resistant strains of *Salmonella* Typhi, which causes human infections often after exposure/consumption of contaminated drinking water or foods. Waterborne transmission of resistant *Salmonella* Typhi was initially reported in the early 1970s and further supported by epidemiologic data. Most infections occur in developing countries, where water supplies are often of poor quality, similar to developed countries of the 19th century, when poor water quality and *Salmonella* Typhi infections were very common. An epidemic of multidrug-resistant typhoid fever with 8901 cases and 95 associated deaths was attributable to the consumption of municipal water in Tajikistan [70]. Multidrug-resistant *Salmonella* Typhi infections, involving 6000 people in Nepal, were due to lack of chlorination processes in the local water plant facility [71]. Only when safe drinking water has been provided to the majority of the population have such infections been controlled or virtually eliminated [64].

CONCLUSIONS

The bacterial metagenome is vast and bacteria are promiscuous. Rare gene transfer events can be clinically significant, but this vastness makes it very difficult to pinpoint when and where gene transfer events have led to acquired resistance in human pathogens and, in turn, demonstrate causality. However, our expanding understanding of the ancient origin and modern evolution of antibiotic resistance genes have demonstrated the important role of the environment in both the emergence and spread of resistance. Various human activities have contributed to the rapid evolution of antibiotic resistance since the start of the antibiotic era.

Resistant organisms disseminate from humans to animals, and vice versa, often through various environmental pathways, including foodstuffs, animal wastes, and water sources. However, although food products may have the established maximum antibiotic residue limits, there is no threshold guidance regarding the presence of resistant bacteria or resistance determinants in water sources. Current water quality guidelines tend to focus only on specific bacteria, but do not have appropriate guidance for the presence of antibiotics introduced by manufacturers, domestic disposal, agriculture, and/or the medical sector. In addition, other environmental sources of antibiotics and resistance genes, such as human and agricultural wastes, lack strong guidance, particularly for risk management. Therefore, new guidance is needed and actions taken to reduce selection pressures in natural and farmed/aquaculture environments and also to reduce human exposure rates to resistant strains. A priority should include risk management to minimize antibiotic residues and resistant bacteria in intensive animal facilities as well as from aquaculture. Pruden et al have recommended the use of composting and manure digestion for the degradation of any residual antibiotic present in animal manure, as well as the need for better rearing methods for fish to decrease the levels of disease and the need for regulations and monitoring for antibiotic use in aquaculture [72]. In addition, several recommendations were made for the removal of antibiotics or antibiotic resistance genes present at wastewater treatment plants, looking at different components of the water cleaning process as critical control points. Nevertheless, increasing antibiotic resistance will not be reversed only by removing selective pressure. The rate of resistance acquisition from intrinsic sources must be reduced, especially to human pathogens, and this can only be done through much greater consideration of the natural environment in resistance transmission. A One Health approach is clearly needed to address all the different contributions that assist in the development and dissemination of antimicrobial-resistant organisms. Having all the sectors working independently is not sufficient; communications and collaborations must be strengthened to be effective and have an impact.

Notes

Financial support. This manuscript was conceived at the Antimicrobial Resistance in the Environment: Assessing and Managing Effects of Anthropogenic Activities workshop, held in Montebello, Québec, Canada, on 4–8 March 2012. The workshop was sponsored by the Canadian Society of Microbiologists with financial support from AstraZeneca, Pfizer Animal Health, F. Hoffman-La Roche, GlaxoSmithKline, Unilever, Huvepharma, the American Cleaning Institute, the Canadian Animal Health Institute, the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety, Health Canada, and the Public Health Agency of Canada. D. G. J. L. has received institutional grant funding from the Swedish Research Council, and W. H. G. received institutional funding to travel to the meeting from the Canadian Society of Microbiology.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. de Kraker ME, Davey PG, Grundmann H, et al. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Med **2011**; 8:e1001104.
2. Carlet J, Collignon P, Goldmann D, et al. Society's failure to protect a precious resource: antibiotics. Lancet **2011**; 378:369–71.
3. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis **2009**; 48:1–12.
4. Leung E, Weil DE, Raviglione M, et al. The WHO policy package to combat antimicrobial resistance. Bull World Health Organ **2011**; 89:390–2.
5. D'Costa VM, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. Science **2006**; 311:374–77.
6. Knapp CW, McCluskey SM, Singh BK, Campbell CD, Hudson G, Graham DW. Basal antibiotic resistance gene abundances correlate with metal and geochemical conditions in Scottish soils. PLoS One **2011**; 6:e27300.
7. Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, et al. Abundance of six tetracycline resistance genes in wastewater lagoons at

cattle feedlots with different antibiotic use strategies. Environ Microbiol **2007**; 9:143–51.
8. Gillings MR, Stokes HW. Are humans increasing bacterial evolvability? Trends Ecol Evol **2012**; 6:346–52.
9. Wright GD. Antibiotic resistance in the environment: a link to the clinic? Curr Opin Microbiol **2010**; 13:589–94.
10. D'Costa VM, King CE, Kalan L, et al. Antibiotic resistance is ancient. Nature **2011**; 477:457–61.
11. Monier JM, Demaneche S, Delmont TO, Mathieu A, Vogel TM, SImonet P. Metagenomic exploration of antibiotic resistance in soil. Curr Opin Microbiol **2011**; 14:229–35.
12. Bhullar K, Waglechner N, Pawlowski A, et al. Antibiotic resistance is prevalent in an isolated cave microbiome. PLoS One **2012**; 7:e34953.
13. Hall BG, Barlow M. Evolution of serine β-lactamases: past, present and future. Drug Resist Updat **2004**; 7:111–23.
14. Aminov RI. The role of antibiotics and antibiotic resistance in nature. Environ Microbiol **2009**; 11:2970–88.
15. Blázquez J, Couce A, Rodríguez-Beltrán J, Rodriguez-Rojas A. Antimicrobials as promoters of genetic variation. Curr Opin Microbiol **2012**; 15:561–9.
16. Barr V, Barr K, Millar MR, Lacey RW. β-Lactam antibiotics increase the frequency of plasmid transfer in *Staphylococcus aureus.* J Antimicrob Chemother **1986**; 17:409–13.
17. Allen HK, Looft T, Bayles DO, et al. Antibiotics in feed induce prophages in swine fecal microbiomes. MBio **2011**; 2:e00260–11.
18. Dantas G, Sommer MO, Oluwasegun RD, Church GM. Bacteria subsisting on antibiotics. Science **2008**; 320:100–3.
19. Chent AC, Turnidge J, Collignon P, et al. Control of fluoroquinolone resistance through successful regulation, Australia. Emerg Infect Dis **2012**; 18:1453–60.
20. Knapp CW, Dolfing J, Ehlert PAI, Graham DW. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol **2010**; 44:580–87.
21. DANMAP 2010. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 2011. ISSN 1600-2032. Available at: www.danmap.org.
22. Government of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2008. Guelph, ON: Public Health Agency of Canada, **2011**.
23. Heuer OE, Kruse H, Grave K, et al. Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis **2009**; 49:1248–53.
24. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev **2011**; 24:718–33.
25. Kuehn BM. FDA claims to curb farm use of antibiotics. JAMA **2012**; 307:2244–45.
26. World Health Organization. Critically important antimicrobials for human medicine. 3rd ed. Geneva, Switzerland: WHO, **2012**.
27. Dutil L, Irwin R, Finley R, et al. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis **2010**; 16:48–54.
28. Food and Drug Administration, Department of Human and Health Services. New animal drugs; cephalosporin drugs; extralabel animal drug use; order of prohibition. Final rule. Fed Reg **2012**; 77: 735–45.
29. Folster JP, Pecic G, Singh A, et al. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat and humans in the United States 2009. Antimicrob Agents Chemother **2012**; 9:638–45.
30. Wittum TE. The challenge of regulating agricultural ceftiofur use to slow the emergence of resistance to extended-spectrum cephalosporins. Appl Environ Microbiol **2012**; 78:7819–21.
31. Kluytmans JA, Overdevest IT, Willemsen I, et al. Extended-spectrum β-lactamase-producing *Escherichia coli* from retail chicken meat and humans: comparison of strains, plasmids, resistance genes, and virulence factors. Clin Infect Dis **2013**; 56:478–87.
32. Overdevest I, Willemsen I, Rijinsburger M, et al. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis **2011**; 17:1216–22.
33. Tian B, Fadhil NH, Powell JE, Kwong WK, Moran NA. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut of microbiota of honeybees. mBio **2012**; 3:e00377–12.
34. Burkholder J, Libra B, Weyer P, et al. Impacts of waste from concentrated animal feeding operations on water quality. Environ Health Perspect **2007**; 115:308–12.
35. Gilchrist MJ, Greko C, Wallinga DB, et al. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. Environ Health Perspect **2007**; 115:313–16.
36. Greger M, Koneswaran G. The public health impacts of concentrated animal feeding operations on local communities. Fam Community Health **2010**; 33:11–20.
37. Weir M, Rajic A, Dutil L, et al. Zoonotic bacteria and antimicrobial resistance in aquaculture: opportunities for surveillance in Canada. Can Vet J **2012**; 53:619–22.
38. Kümmerer K. Antibiotics in the aquatic environment—a review. Part II. Chemosphere **2009**; 75:435–41.
39. The state of world fisheries and aquaculture 2010. FAO Fisheries and Aquaculture Department. Rome, Italy: FAO, **2011**.
40. Zhang T, Li B. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. Crit Rev Environ Sci Technol **2011**; 41:951–98.
41. Kummerer K. Antibiotics in the aquatic environment—a review. Part I. Chemosphere **2009**; 75:417–34.
42. Andersson DI, Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist Updat **2012**; 15:162–72.
43. Larsson DGJ, de Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater **2007**; 148:751–55.
44. Sim W-J, Lee J-W, Lee E-S, Shin S-K, Hwang S-R, Oh J-E. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. Chemosphere **2011**; 82:179–86.
45. Kristiansson E, Fick J, Janzon A, et al. Pyrosequencing of antibiotic-contaminated river sediments revewals high levels of resistance and gene transfer elements. PLoS One **2011**; 6:e17038.
46. Verlicchi P, Al Aukidy M, Galletti A, Petrovic M, Barceló D. Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Sci Total Environ **2012**; 430:109–18.
47. Gaze WH, Zhang L, Abdouslam NA, et al. Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. ISME J **2011**; 5:1253–61.
48. Czekalski N, Berthold T, Caucci S, Egli A, Bürgmann H. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. Front Microbiol **2012**; 3:106.
49. Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother **2009**; 64 (suppl 1):i3–10.
50. Martinez JL. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ Pollut **2009**; 157:2893–902.
51. Baquero F, Martinez JL, Canton R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol **2008**; 19:260–65.
52. Lupo A, Coyne S, Ulrich Berendonk T. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front Microbiol **2012**; 3:18.
53. Guenther S, Ewers C, Wieler LH. Extended spectrum β-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front Microbiol **2011**; 2:246.
54. Li XZ, Mehrotra M, Ghimire S, Adewoye L. β-Lactam resistance and β-lactamases in bacteria of animal origin. Vet Microbiol **2007**; 121:197–214.
55. Smet A, Martel A, Persoons D, et al. Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: molecular aspects,

mobility and impact on public health. FEMS Microbiol Rev **2010**; 34: 295–316.
56. Colomer-Lluch M, Jofre J, Muniesa M. Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. PLoS One **2011**; 6:e17549.
57. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. mBio **2012**; 3:300305–11.
58. Maragakis LL, Perl TM. *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis **2008**; 46:1254–63.
59. Whitman TJ, Qasba SS, Timpone JG, et al. Occupational transmission of *Acinetobacter baumannii* from a United States serviceman wounded in Iraq to a health care worker. Clin Infect Dis **2008**; 47:439–43.
60. Tao C, Kang M, Chen Z, et al. Microbiologic study of the pathogens isolated from wound culture among Wenchuan earthquake survivors. Diagn Microbiol Infect Dis **2009**; 63:268–70.
61. Fournier PE, Vallenet D, Barbe V, et al. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS Genet **2006**; 2:e7.
62. Poirel L, Cattoir V, Nordmann P. Plasmid-mediated quinolone resistance: interactions between human, animal and environmental ecologies. Front Microbiol **2012**; 3:24.
63. Sarria JC, Vidal AM, Kimbrough RC III. Infections caused by *Kluyvera* species in humans. Clin Infect Dis **2001**; 33:e69–74.
64. Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO, Dantas G. The shared antibiotic resistome of soil bacteria and human pathogens. Science **2012**; 337:1107–11.
65. Xi C, Zhang Y, Marrs CF, et al. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl Environ Microbiol **2009**; 75:5714–8.
66. Koczura R, Mokracka J, Jabłońska L, Gozdecka E, Kubek M, Kaznowski A. Antimicrobial resistance of integron-harboring *Escherichia coli* isolates from clinical samples, wastewater treatment plant and river water. Sci Total Environ **2012**; 414:680–5.
67. Coleman BL, Salvadori MI, McGeer AJ, et al. The role of drinking water in the transmission of antimicrobial-resistant *E. coli*. Epidemiol Infect **2012**; 140:633–42.
68. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis **2011**; 11:355–62.
69. Shuval H. Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. J Water Health **2003**; 1:53–64.
70. Mermin JH, Villar R, Carpenter J, et al. A massive epidemic of multidrug-resistant typhoid fever in Tajikistan associated with consumption of municipal water. J Infect Dis **1999**; 17:1416–22.
71. Lewis MD, Serichantalergs O, Pitaransi C, et al. Typhoid fever: a massive, single point source, multidrug-resistant outbreak in Nepal. Clin Infect Dis **2005**; 40:554–61.
72. Pruden A, Larsson DGJ, Amézquita A, et al. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ Health Perspect **2013**. Available at: http://ehp.niehs.nih.gov/1206446/.