Global Emerging Microbial Issues in the Anthropocene Era

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The anthropocene can be defined as the influence of human activities on the global ecosystem consisting of the lithosphere, the hydrosphere and the atmosphere, all of which potentially contain microorganisms. In this chapter we examine some of the major global microbial issues that have been impacted by human activities. Some of these issues are directly caused by adverse human activities that result in pollution, as in the case of the recent BP *Deepwater Horizon* spill, off the Gulf of Mexico. Other issues result indirectly, such as problems related to global warming, or antibiotic-resistant bacteria.

31.1 MICROBIAL CONTRIBUTIONS TO CLIMATE CHANGE

31.1.1 Nitrous Oxide Emissions from Soil

Microbial activities can influence the formation of greenhouse gases including N_2O, CH_4 and CO_2, and are a major cause of global warming (Table 31.1). The driving force for CO_2 emissions in the age of the anthropocene is thought to be fossil fuel use and land-use change (Inselsbacher *et al.*, 2011). In contrast, major N_2O and

CH_4 emissions may be due to agricultural practices (IPCC, 2007).

The agricultural practice of adding nitrogen, either as chemical or manure fertilizer, to soil is a major contributor to the gradual increase in nitrous oxide (N_2O) emissions to the atmosphere, although other sources of N_2O in the atmosphere include burning of biomass, combustion of fossil fuels and chemical manufacturing of nylon (Mosier *et al.*, 1996). The source of nitrogen in fertilizers is mainly ammonia or ammonium-producing compounds such as urea. However, normally only about half the total nitrogen applied to a field as fertilizer or manure is assimilated by the crop (Delgado and Mosier, 1996). The remaining nitrogen is lost through leaching, erosion, gaseous emissions and microbial activity. Due to microbial activity, about 75% of the total global anthropogenic emissions of N_2O are due to agricultural fertilizers (Jackson *et al.*, 2009). This activity includes two mechanisms, both of which have the potential to produce N_2O. The first is nitrification, a process in which ammonium is chemoautotrophically oxidized first to nitrite and then to nitrate (Chapter 2). It has been suggested that N_2O production is associated with low-oxygen conditions, in which nitrifying bacteria utilize nitrite as an electron

TABLE 31.1 Global Atmospheric Concentrations of Selected Greenhouse Gases[1]

	Microbially mediated/Anthropogenic (parts per million)			Anthropogenic only (parts per trillion)	
	CO_2	CH_4	N_2O	SF_6[a]	CFC[b]
Preindustrial	278	0.700	0.275	0	0
2004	377	1.789	0.319	5.22[c]	794
Atmospheric lifetime (years)	50–200	12	114	3200	45–100

Data used in this table are from the Carbon Dioxide Information Analysis Center, which is supported by the U.S. Department of Energy Climate Change Research Division, http://cdiac.ornl.gov/.
[a]SF_6 = sulfur hexafluoride.
[b]CFC = CFC-11 (trichlorofluoromethane) and CFC-12 (dichlorodifluoromethane).
[c]Value is from 2001.

acceptor in place of oxygen, as a result reducing NO_2^- to N_2O (Ambus *et al*., 2006). The second step is denitrification, which is the utilization of nitrate (NO_3^-) as a terminal electron acceptor during anaerobic respiration of organic compounds (Chapter 15). N_2O is an intermediate in the reduction of NO_3^- to N_2, but can be the end point of nitrate reduction especially in environments where initial nitrate concentrations are low. Thus, N_2O can be produced as an intermediate both in denitrification and in nitrification. Repeated nitrogen fertilization of agricultural soils has been shown to increase N_2O emissions with a stimulation of both bacterial and archaeal ammonia oxidizers (Inselsbacher *et al*., 2011). Interestingly, nitrogen fertilizers also lead to increased CO_2 emissions due to microbial respiration in the rhizosphere (Treseder, 2008).

Why is this a problem? Nitrous oxide gas that is released to the atmosphere from industrial and biogenic sources contributes to global warming as a greenhouse gas. Greenhouse gases are of concern because they absorb long-wave radiation from the sun after it hits the Earth and is reflected back into space. This effectively traps heat in the atmosphere. N_2O is of concern in several respects: it has a long residence time (> 100 years) in the atmosphere; and it is highly efficient in absorbing long-wave radiation. One molecule of N_2O is equivalent in heat-trapping ability to about 200 molecules of CO_2. Therefore, small increases in atmospheric N_2O concentration can have a large impact on warming trends.

An additional concern with N_2O is that in the upper atmosphere, solar radiation can photolytically convert N_2O to nitric oxide (NO), which is a contributor to the depletion of the protective ozone layer (Figure 31.1). The ozone layer acts as a filter to remove biologically harmful ultraviolet (UV) light. Stratospheric ozone depletion occurs through a chemical interaction between sunlight, ozone and certain reactive chemical species, including nitrogen oxides and organohalogens such as chlorofluorocarbons (CFCs).

Depletion of the protective ozone layer can have serious ecological and human health consequences. Increased levels of UV radiation may be inhibitory to certain microorganisms, such as phytoplankton, and may also increase the incidence of skin cancer in humans. Ozone is depleted in the series of reactions shown in Figure 31.1, where light energy begins the reaction by splitting nitrous oxide into N_2 and singlet oxygen, in which one of the electrons is in a high-energy state. This singlet oxygen can react with nitrous oxide to form two molecules of nitric oxide. Nitric oxide in turn reacts with ozone (O_3) to produce nitrogen dioxide and oxygen. The nitrogen dioxide then reacts with singlet oxygen ($O^\bullet$) to produce oxygen and regenerate nitric oxide. The fact that nitric oxide is regenerated in this series of reactions means that for every nitrous oxide molecule released to the atmosphere, a large number of ozone molecules can be destroyed.

Strategies can be implemented to reduce biogenic N_2O emissions from agriculture. A primary factor in N_2O emissions is the low efficiency of utilization of nitrogen fertilizers, which leaves them subject to nitrification/denitrification processes. Several measures can be taken to increase fertilizer utilization efficiency. The most economical approach is simply to manage the amount and time of fertilizer application to a crop. Enough fertilizer must be added to meet crop needs, but overfertilization will result in increased nitrate formation and leaching. A second way to minimize nitrogen losses in irrigated croplands is through control of the timing and amount of irrigation. Remember, plants can take up nitrogen as either ammonia or nitrate, but nitrate is easily removed from the plant root area through leaching processes. Many states in the U.S. have studied and adopted best management practices (BMPs) for fertilizer application and irrigation. These BMPs are region-specific since climate and soil types change dramatically from region to region. Finally, two other approaches to minimizing
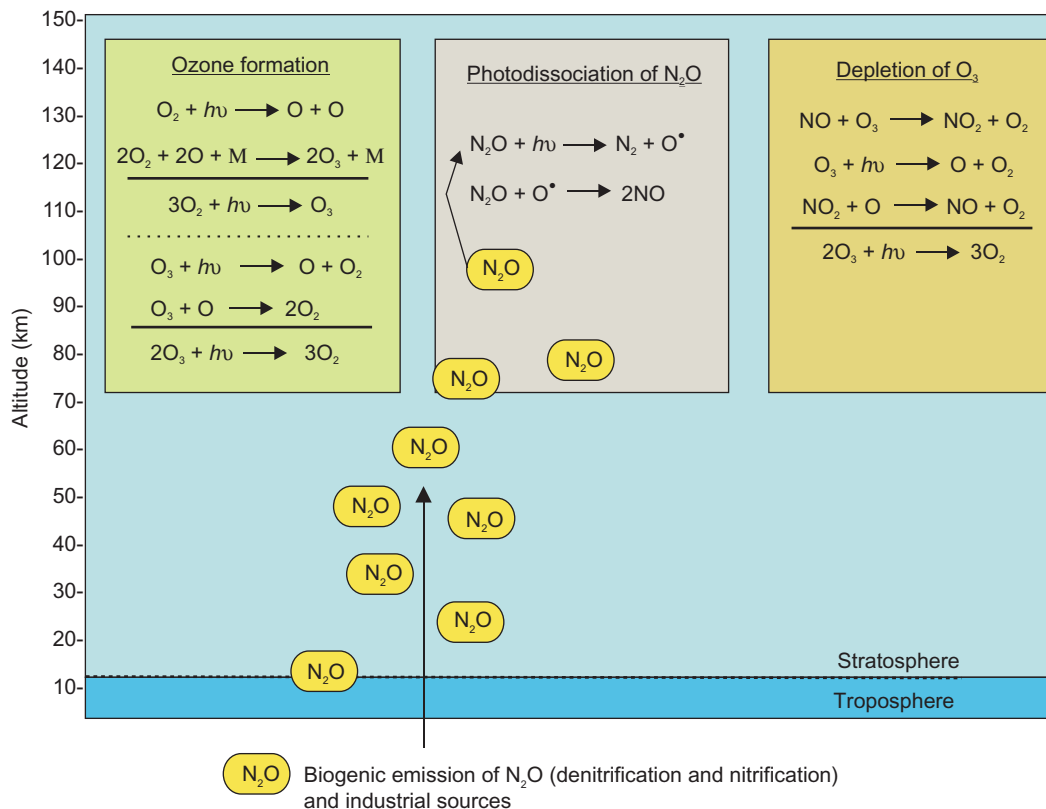
FIGURE 31.1   Equations summarizing ozone formation and the depletion of ozone by reaction with nitric oxide (NO). Solar UV radiation (*h*v) photodissociates molecular oxygen (O_2) into two oxygen atoms (O), which recombine with undissociated O_2 (in the presence of another chemical species, M) to form ozone (O_3). Ozone is then photodissociated back to molecular oxygen (O_2). The constant cycling between ozone and oxygen is important because it consumes harmful UV radiation in the stratosphere. Nitrous oxide (N_2O) emitted to the atmosphere is photodissociated to nitrogen and an oxygen atom in an electronically excited state, $O^{\bullet}$, which reacts with N_2O to produce nitric oxide (NO). Nitric oxide can react with ozone and, in a series of reactions, produce O_2 resulting in a net depletion of ozone.

nitrate formation and leaching are the use of slow-release fertilizers, which allow more controlled release of ammonia into the environment, and the application of nitrification inhibitors, e.g., N-serve (2-chloro-6-(trichloromethyl) pyridine), which suppress the formation of nitrate. However, application of slow-release fertilizers or nitrification inhibitors is costly, so neither approach is widely used.

31.1.2 Methane Emissions from Landfills

The formation of another global warming gas, methane (CH_4), is called methanogenesis and is predominantly a microbial process (see Chapter 16). The organisms responsible for this process are a group of obligately anaerobic Archaea called the methanogens. Many of these are commonly found in wetlands and marine sediments where anaerobic conditions prevail. However, methane emissions due to anthropogenic activities include emissions from landfills (Chiemchaisri *et al.*, 2012). Methane

is the third most important greenhouse gas after water vapor and CO_2, and has 25 times the global warming potential of an equal mass of CO_2. It has been estimated that one ton of deposited municipal solid waste results in $160-250$ m^3 of landfill gas, which consists of CH_4 ($50-60\%$ v/v) and CO_2 ($40-45\%$ v/v) (Scheutz *et al.*, 2004). Landfill gas has been shown to increase with increased moisture due to more optimal conditions for growth of anaerobic bacteria. In some modern landfills, anaerobic degradation and methanogenesis are actually encouraged in order to degrade organic materials thus freeing up valuable landfill space while generating CH_4, which can be captured to generate electricity. Examples of landfill methanogens identified through polymerase chain reaction (PCR) detection of the methyl coenzyme-M reductase gene (*mcrA*) are shown in Information Box 31.1. Overall, anthropogenic activities have significantly affected microbial activities, in many cases resulting in enhanced emissions of greenhouse gases and concomitant global warming.

31.2 GLOBAL CHANGE AND MICROBIAL INFECTIOUS DISEASE

In many cases, human activity directly affects the magnitude of exposure to pathogens that can be transmitted via the environment, as in the case of the voluntary risk associated with a decision to drink water that may or may not be fecally contaminated. However, in other situations, human activities are not directly involved such as when whole communities are exposed to pathogens due to accidental contamination of a drinking water supply. Urbanization and deforestation can also cause immense changes in environmental systems that impact exposure to pathogens. It is also increasingly clear that climate change will have an influence on our exposure to pathogens.

Here we discuss the influence of both indigenous environmental and animal-borne (including human) pathogens on human health and infectious disease, as well as the influence of anthropogenic activities on exposure to pathogens. Overall, microbial infectious diseases dwarf all other sources of human deaths annually (Table 31.2). But there is a disparity in where these deaths occur globally. In developing countries, microbial infectious diseases are clearly the leading agent of death (WHO, 2004) (Table 31.3). In developed countries, heart diseases and cancer are more important, although in the United States infectious diseases are still the third leading cause of death.

31.2.1 Global Warming and Microbial Infectious Disease

It is now well documented that global warming is occurring and that anthropogenic activities are at least partially to blame. Currently, human activities add about $7-9$ gt (gigatons) of carbon to the atmosphere as carbon dioxide as well as other greenhouse gases that contribute to this process (see Chapter 16). Global warming is resulting in changing weather patterns and the increased incidence of extreme weather events (Patz *et al.*, 2000). Severe weather events including prolonged droughts in arid areas, and excessive precipitation that results in flooding

TABLE 31.2 Leading Causes of Human Deaths Worldwide

Cause	Percent of deaths	Deaths per 100,000 per year		
		All	Male	Female
All causes	100.00	916.1	954.7	877.1
All microbial infectious disease	50.4	461.8	484.0	439.2
Cardiovascular diseases	29.34	268.8	259.3	278.4
Ischemic heart disease	12.64	115.8	121.4	110.1
Malignant neoplasms (cancers)	12.49	114.4	126.9	101.7
Cerebrovascular disease (stroke)	9.66	88.5	81.4	95.6
Respiratory diseases	6.49	59.5	61.1	57.9
Unintentional injuries	6.23	57.0	73.7	40.2
Chronic obstructive pulmonary disease	4.82	44.1	45.1	43.1
Perinatal conditions	4.32	39.6	43.7	35.4
Intentional injuries (suicide, murder, war, etc.)	2.84	26.0	37.0	14.9
Lung cancers	2.18	20.0	28.4	11.4
Road traffic accidents	2.09	19.1	27.8	10.4
Childhood diseases	1.97	18.1	18.0	18.2
Neuropsychiatric disorders	1.95	17.9	18.4	17.3
Diabetes mellitus	1.73	15.9	14.1	17.7
Hypertensive heart diseases	1.60	14.6	13.4	15.9
Suicide	1.53	14.0	17.4	10.6
Stomach cancer	1.49	13.6	14.1	13.1
Diseases of the genitourinary system	1.49	13.6	14.1	13.1
Cirrhosis of the liver	1.38	12.6	16.1	9.1
Nephritis/nephropathy	1.19	10.9	11.0	10.7
Colorectal cancer	1.09	10.0	10.3	9.7
Liver cancer	1.08	9.9	13.6	6.2

Adapted from WHO (2004).

TABLE 31.3 Leading Causes of Human Deaths: Developed vs. Developing Economics

Causes of death in developing countries	Number of deaths (annually)	Causes of death in developed countries	Number of deaths (annually)
HIV/AIDS	2,678,000	Ischemic heart disease	3,512,000
Lower respiratory infections	2,643,000	Stroke	3,346,000
Ischemic heart disease	2,484,000	Chronic obstructive pulmonary disease	1,829,000
Diarrhea	1,793,000	Lower respiratory infections	1,180,000
Cerebrovascular disease	1,381,000	Lung cancer	938,000
Childhood diseases	1,217,000	Car accident	669,000
Malaria	1,103,000	Stomach cancer	657,000
Tuberculosis	1,021,000	High blood pressure	635,000
Chronic obstructive pulmonary disease	748,000	Tuberculosis	571,000
Measles	674,000	Suicide	499,000

Adapted from WHO (2004).

Information Box 31.2   Influence of Global Warming on Microbial Infectious Disease

Mechanism	Impact	Example
Severe weather events (floods, droughts, heat waves)	Vector- and waterborne diseases increase	Most waterborne outbreaks occur after above normal rainfall events
Enhanced sea temperatures (El Niño)	Vector- and waterborne diseases increase	Enhanced growth of *Vibrio cholerae*, and increased malaria
Enhanced freshwater temperatures	Water-based pathogen numbers increase	Enhanced growth of the protozoan *Naegleria fowleri*
Decreased potable water sources	Waterborne disease increases	Use of source water of a lower quality

Information Box 31.3   Mosquito-borne Microbial Diseases

Disease	Type of Microbe	Genus
Malaria	Protozoan parasite	*Plasmodium*
Dengue fever	Virus	*Flavivirus*
Rift Valley fever	Virus	*Phlebovirus*
Encephalitis	Virus	West Nile virus

in other areas, can also affect the incidence of microbial infectious disease (Information Box 31.2).

Direct mechanisms for enhanced infectious disease due to global warming include enhanced incidence of vector-borne diseases, water-related pathogens and water-based pathogens. One example is mosquito-borne microbial diseases, which are particularly prevalent worldwide (Information Box 31.3). Malaria is an infectious disease carried by mosquitoes that occurs in tropical and subtropical regions including the Americas, Asia and Africa. The cause of the disease is a protozoan parasite in the genus *Plasmodium* (Snow *et al.*, 2005). Global warming is now believed to be increasing the

incidence of malaria in areas such as the East African highlands of Kenya, where previously high elevation and cooler temperatures limited the disease (Martens, 1999). Other vector-borne diseases including dengue fever and rift valley fever are also thought to be on the increase due to global warming and associated El Niño/Southern Oscillation (ENSO)-related climate anomalies. Rift valley fever is a viral zoonosis that primarily affects domestic livestock, but can be passed to humans resulting in a fever.

The El Niño/Southern Oscillation (ENSO) is a global-coupled ocean—atmospheric phenomenon. Specifically, ENSO is a prominent outcome of climate change that occurs due to enhanced temperature changes in surface waters of tropical areas of the Pacific Ocean. Currently, it is believed that global warming is enhancing El Niño effects, causing global climate anomalies and weather patterns including periods of excessive rainfall in some areas and enhanced drought in others. El Niño events are thought to be responsible for the enhanced incidence of several infectious diseases (Kovats *et al.*, 2003). El Niño rains resulted in enhanced cholera outbreaks in Uganda in

2002−2003 (Alajo *et al.*, 2006). Outbreaks of cholera in Peru were significantly correlated with elevated sea temperatures due to a 1997−1998 El Niño. Interestingly, the onset of cholera epidemics was linked not only to warmer sea temperatures, but also to plankton populations. When sea temperatures increase, phytoplankton populations first become abundant utilizing sunlight for energy. Subsequently, zooplankton populations increase with a concomitant increase in the cholera-causing bacterium *Vibrio cholerae* (Gil *et al.*, 2004). El Niño has also been linked to malarial epidemics in South America (Gagnon *et al.*, 2002). In the southwestern United States, the incidence of disease caused by hantavirus and West Nile virus is also thought to be related to El Niño events (Information Box 31.4).

In addition to clearly documented effects of El Niño on microbial infectious disease, other effects of global warming are now being noticed that have not yet been well studied. These include anecdotal reports of increased *Naegleria fowleri* infections in the United States, and increased Legionnaires' disease in the United Kingdom. In both instances, the water-based microorganisms involved in the infectious disease prefer warm, fresh water habitats for growth and reproduction. Whether or not these outbreaks are linked to global warming remains to be seen, but clearly from a broad perspective, global warming is now a major factor influencing the incidence of microbial infectious disease.

There are other ways in which humans impact the environment and influence infectious disease. For example, climate change-induced drought combined with increasing human populations and demand for potable water has strained the availability of potable water supplies. This has led to an increase in the consumption of fecally-contaminated water, particularly in developing countries. Floods can also enhance microbial infectious disease. In developed countries, excessive precipitation can result in storm water loads that overwhelm wastewater treatment and drinking water treatment plants, and wash sewage, animal manures and other

sources of pathogens into surface waters. Such was the case in the infamous Milwaukee, Wisconsin, U.S. *Cryptosporidium* outbreak in the early 1990s.

31.2.2 Urbanization and Deforestation

Urbanization can affect the incidence of microbial disease both positively and negatively. In developing countries, increased population growth within urban centers can lead to densely packed housing and inadequate water supplies. Such conditions can frequently lead to enhanced waterborne disease due to poor sanitation. Interestingly, the incidence of vector-borne disease has been shown to decrease during urbanization due to less available breeding sites for mosquitoes (Hay *et al.*, 2005). However, urbanization can also result in the creation of new pools of water such as dams, rice paddies or artificial wetlands, which serve as habitats that can enhance vector-borne disease including malaria.

Concomitant with urbanization are associated land cover changes that affect the incidence of infectious disease. Such anthropogenic land surface changes include: deforestation; road construction; agricultural encroachment; urban sprawl; and extractive industries such as mining, quarrying and oil drilling (NRC, 2007). Recently, deforestation, urban spawn and biodiversity loss were linked to an increase in the incidence of Lyme disease (Schmidt and Ostfeld, 2001). Lyme disease is now endemic in at least 19 states in the U.S. (Nadelman and Wormser, 2005). The disease is due to the spirochete bacterium *Borrelia burgdorferi*, which is transmitted to humans by ticks. Similarly, expansion and changes in agriculture were shown to be intimately associated with the emergence of Nipah virus in Malaysia (Lam and Chua, 2002). Thus, in many instances urbanization and deforestation can result in increased contact between humans and an infectious agent. Also of concern are the zoonotic pathogens of nonhuman origin with the potential for adaptation to cause human infections. It is thought that microbial agents causing infectious disease in humans often originate from disturbances of the natural environment. Emerging microbial diseases can be caused by infectious agents of wildlife that either recently adapted to infect humans, or were pre-adapted, and come into opportunistic contact with humans (Taylor *et al.*, 2001). Examples of such emerging pathogens include the HIV/AIDS virus, Ebola and West Nile virus.

In summary, anthropogenic activities that result in global climate change and changes in land use are influencing the incidence of known microbial infectious disease, and in some cases allowing for the discovery of new infections of microbial origin. As the human population continues to grow, these phenomena are unlikely to disappear.

Information Box 31.4   El Niño Enhanced Microbial Infectious Disease

Country/Region	Disease
Peru, Uganda	Cholera
Ecuador, Peru, Bangladesh, India	Malaria
Thailand, Brazil	Dengue fever
Southwestern United States	Hantavirus, West Nile virus

Adapted from Gagnon *et al.* (2002); Alajo *et al.* (2006).

31.3 MICROBIAL REMEDIATION OF MARINE OIL SPILLS

The effects of human activities on microorganisms in the age of the Anthropocene is explicitly illustrated by oil spill disasters such as the *Exxon Valdez* spill in 1989, and the BP *Deepwater Horizon* spill in 2010. Petroleum hydrocarbons occur naturally in all marine environments, with the result that numerous diverse microorganisms have evolved the capability to degrade similar hydrocarbons following an oil spill, as they utilize them as a source of carbon and energy for growth. Overall, there are hundreds of species of Bacteria, Archaea and Fungi that can degrade petroleum (Atlas and Hazen, 2011). Factors affecting the microbial degradation of petroleum hydrocarbons are shown in Information Box 31.5. Overall, oil-degrading indigenous microorganisms can play a significant role in the cleanup of marine oil spills. The *Exxon Valdez* oil spill in 1989 was notorious because of its impact on Alaskan wildlife and the pristine environments of Prince William Sound. The BP *Deepwater Horizon* spill became imprinted in the minds of the U.S. public due to the incessant nightly television images of oil gushing out of an oil wellhead far beneath the ocean surface for 87 days. The notoriety of both spills resulted in frequent comparisons, even though the two events were in fact quite different. However, in both cases biodegradation of oil by indigenous microorganisms significantly reduced the environmental impact of each disaster (Case Study 31.1).

Information Box 31.5   Factors Affecting Microbial Biodegradation Rates of Petroleum Hydrocarbon

Factor	Influence on Biodegradation
Type of oil	Lighter crude oils degrade more readily than heavier crudes or polycyclic aromatic hydrocarbons (PAHs)
Number and activity of oil-degrading microorganisms	Acclimation needed to enhance pre-spill degrader numbers
Essential nutrients	Lack of nitrates, phosphates and iron can limit rates of degradation
Environmental redox conditions	Aerobic conditions enhance degradation rates relative to anaerobic conditions
Oil surface area: volume ratio	Can limit degradation rates
Dispersants	Increase oil surface area and degradation rates

31.4 ANTIBIOTIC-RESISTANT BACTERIA

Bacteria are prokaryotic organisms with the ability to metabolize and replicate very quickly. They are also very adaptable genetically. When confronted with an antibiotic, there need only be one bacterial cell with a genetic or mutational change that confers resistance to that antibiotic to subsequently allow for the proliferation of antibiotic-resistant bacteria. Thus, the more antibiotics are used, the greater the likelihood of antibiotic-resistant strains developing. The greatest concern with antibiotic resistance is the potential for human pathogenic strains to become resistant to popular antibiotics, which subsequently cannot contain the infectious agent. Commonly used antibiotics and their mode of action are shown in Information Box 31.6. The widespread sometimes indiscriminant use of antibiotics has raised the question "Can antibiotic resistant genes be transferred from nonpathogenic bacteria to human pathogenic strains?" Here we address this issue by examining the incidence of antibiotic-resistant bacteria, and the potential for horizontal gene transfer between different groups of bacteria. In particular, we examine which anthropogenic practices are most likely to induce antibiotic-resistant bacteria.

31.4.1 Development of Bacterial Antibiotic Resistance

Antibiotic drug resistance is the acquired ability of an organism to resist the effects of a chemotherapeutic agent to which it is normally susceptible. In general, there are two ways in which a microbe can become resistant:

1. Mutational change (spontaneous): One way microbes can become more tolerant of an antibiotic is to alter the target of an agent within the cell. For example, spontaneous mutations in the genes encoding ribosomal RNAs can prevent antibiotics such as tetracycline from binding and blocking gene translation.
2. Introduction of foreign DNA: In this case, genes are obtained from other host microorganisms that offer protection against antibiotics. Well-studied examples here include efflux of antibiotics such as tetracycline, or inactivation of penicillins by β-lactamases. The genes encoding these resistances can be localized on a plasmid or on the chromosome. A gene responsible for tetracycline resistance encodes a membrane transporter responsible for pumping tetracycline out of the cell. The β-lactamases cleave the β-lactam ring of most penicillins thereby rendering them ineffective.

Because antibiotic-resistant genes can be transferred within and between bacteria, the issue of horizontal gene transfer becomes relevant to the discussion. Horizontal gene transfer (HGT) is the transfer of genetic material horizontally between bacteria of the same species, or other species or genera. The genetic material can consist of discrete pieces of DNA, whole genes or multiple genes contained within plasmids or transposons. The mechanisms

Case Study 31.1   The *Exxon Valdez* and BP *Deepwater Horizon* Oil Spills

In 1989 the *Exxon Valdez* oil tanker ran aground in Prince William Sound, Alaska, resulting in the second largest oil spill in U.S. history. Due to the nature of the heavy crude oil and concern about the use of dispersants, the decision was made that oil dispersion would not be attempted.

Tidal currents and winds then resulted in a significant portion of the oil floating ashore. Overall, approximately 1300 miles ($\cong$2100 km) of coastline in Prince William Sound and
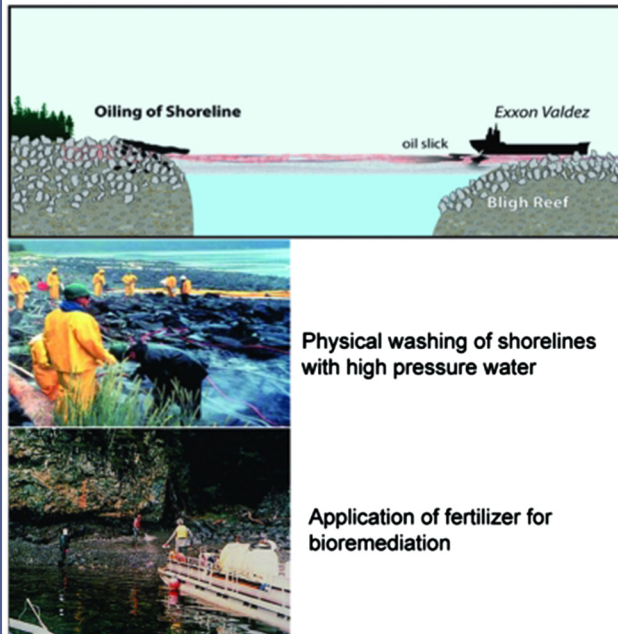
the Gulf of Alaska were contaminated with oil to some degree (Atlas and Hazen, 2011). Initially, physical washing and collection of the oil was the first cleanup strategy employed, but quickly it was realized that *in situ* bioremediation would likely be more effective. A graphic depiction of the *Exxon Valdez* spill and cleanup is shown in Figure 31.2.

In 2010, the *Deepwater Horizon* oil rig exploded and sank, killing 11 people. Subsequent to this, a ruptured wellhead released oil into the Gulf of Mexico for almost 3 months 1500 m below the ocean surface. Unlike the *Exxon Valdez* spill, attempts were made to disperse the oil, since this was a light crude. The dispersant COREXIT 9500 was injected directly into the wellhead, resulting in a deepwater "cloud" of dispersed oil droplets, which remained beneath the ocean surface, and moved away from the wellhead. Overall, this resulted in fewer oil slicks forming at the surface of the ocean above the wellhead. The dispersion of the oil allowed for enhanced rates of oil biodegradation. A graphic depiction of the spill is shown in Figure 31.3. A comparison of the *Exxon Valdez* and BP *Deepwater Horizon* oil spills is shown in Table 31.4.

Bioremediation Aspects of the Two Spills

In the *Exxon Valdez* oil spill, enhanced *in situ* bioremediation was used to degrade oil on the contaminated shores. Rates of biodegradation were increased through the application of slow-release nitrogen fertilizers that also contained phosphate. Overall 107,000 lb (48,000 kg) of nitrogen were applied. Evidence of significant biodegradation of soil was shown by rapid increases in the numbers of naturally occurring oil-degrading bacteria from 103 CFU/ml of seawater ($\cong$1−10% of total heterotrophs) to 105 CFU/ml by late 1989 (up to 40% of total heterotrophs). The success of the bioremediation efforts was documented by National Oceanic and Atmospheric
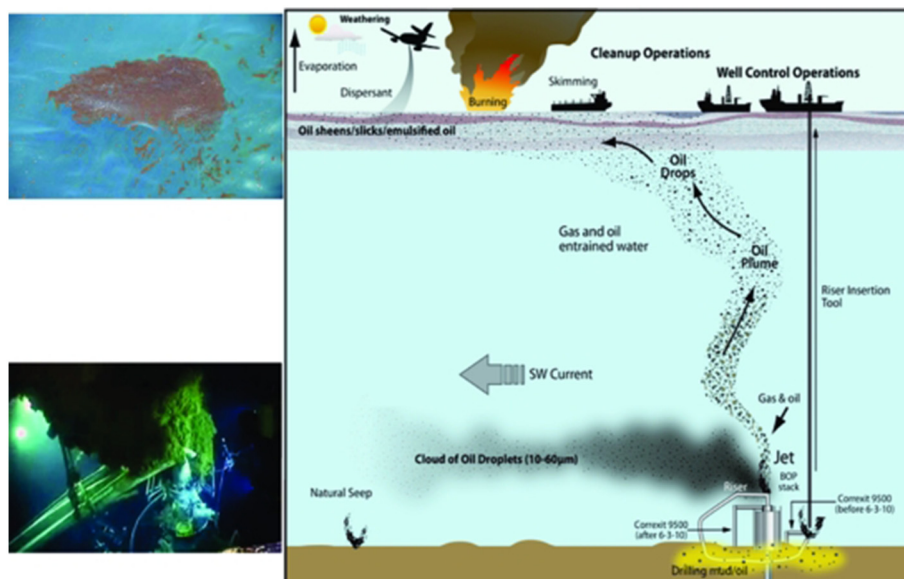

FIGURE 31.2   The *Exxon Valdez* oil spill. *Source:* Atlas and Hazen (2011).

FIGURE 31.3   The BP *Deepwater Horizon* oil spill. *Source:* Atlas and Hazen (2011).

TABLE 31.4 Characteristics of the Two Major Oil Spills

Exxon Valdez	BP *Deepwater Horizon*
• 41.6 million liters (11 m gallons)	• 779 million liters estimated by the National Incident Command's Flow Rate Technical Group (FRTG) ($\cong$ 200 m gallons)
• North Slope Heavy Oil (API 29) tanker spill with known volume discharged	• Light Louisiana oil (API 35.2)
• Surface spill impacted cobble/rocky shorelines of nearby islands	• Large amounts of methane also released
• Bioremediation used extensively	• Discharged at wellhead at a depth of 1500 m
• Fate of oil remnants still studied more than 21 years after spill	• Spill occurred 77 km offshore
• Scientific and operating experience gained is applicable to other spills	• Impact by deep-sea cloud of fine droplets of low concentration oil, marshs affecting and sandy beach shorelines
• No dispersants utilized	• Took 84 days to seal off leak
	• Aerial and subsurface dispersants used extensively
	• Largest remediation and emergency response to an oil spill ever worldwide

Adapted from Atlas and Hazen (2011).

Administration (NOAA) surveys between 2001 and 2003, wherein 97.8% of all samples assayed had no oil residues (Short *et al.*, 2004). However, in localized areas some residual oil still persists, sequestered within fine-grained sediments, and even in 2013, the debate continues as to whether further bioremediation efforts should be conducted to remove all traces of the oil contaminants.

In the BP *Deepwater Horizon* oil spill, bioremediation efforts were also successful, although the situation was very different from the Prince William Sound spill. Rapid attenuation of oil has been observed in the aftermath of the BP spill, in part due to large populations of marine microorganisms capable of degrading oil. These populations are most likely due to adaptation following natural seeps of oil in the Gulf contributing more than 400,000 barrels of oil a year. Bioremediation was particularly significant in the "cloud" of dispersed oil, where bacterial density within the cloud (10^5 CFU/ml) was two orders of magnitude higher than outside of the cloud. Using a 16S rRNA microarray, it was shown that 16 classes of oil-degrading γ-*Proteobacteria* were significantly enriched within the cloud, with three families in the class *Oceanospirillales*

dominating (Hazen *et al.*, 2010). Subsequent studies utilizing metagenomic and metatranscriptomic sequencing revealed that genes for mobility, chemotaxis and aliphatic hydrocarbon degradation were significantly enriched and expressed in hydrocarbon plume water samples as compared to uncontaminated seawater. Sequencing of two *Oceanospirillales* single cells showed that both cells possessed genes coding for n-alkane and cycloalkane degradation (Mason *et al.*, 2012). Finally, note that significant populations of methanotrophs arose in contaminated seawater, presumably in response to the methane that was also released during the spill.

Multiple remedial actions were utilized following the BP spill including skimming, burning and *in situ* bioremediation. In addition, significant evaporation of alkanes with chain lengths up to C-20 occurred. Overall, it is estimated that 26% of the oil released still remains, much of it within shoreline and marsh sediments, where anaerobic conditions result in reduced rates of biodegradation (Atlas and Hazen, 2011). It is not known what the long-term environmental impacts of these oil remnants will be, and it will likely be decades before all effects are fully understood.

for gene transfer are shown in Information Box 31.7. Overall, acquisition of resistance via mutations has been shown to be less effective than complete gene acquisitions (Tomasz, 2006). Horizontal gene transfer in soils has been well documented. However, rates of gene transfer in soil are relatively low due to spatial separation of donors and recipients, and relatively low numbers of donors compared to nondonor organisms (Rensing *et al.*, 2002). Thus, rates of transfer of resistance genes to human pathogens in soil are likely to be low. However, public concern has still focused on the potential for increasing the number of antibiotic-resistant bacteria in the environment, which would increase the potential for transfer of resistance to human pathogenic bacteria. Despite this, a recent study has shown that antibiotic-resistant strains of bacteria are already indigenous and highly prevalent in soils (D'Costa

et al., 2006). In this study, *Streptomyces* isolates from diverse soil locations were tested for antibiotic resistance. All 480 isolates were resistant to at least six antibiotics, and some to as many as 20. The authors of this study therefore titled the article "Sampling the Antibiotic Resistance." Clearly, then, soils are a source not only of antibiotics but also of antibiotic resistance.

31.4.2 Anthropogenic Activities that Enhance the Incidence of Antibiotic-resistant Bacteria

Human activities such as land application of municipal biosolids or animal manures have been implicated as potentially increasing the concentrations of antibiotic-resistant bacteria within soil. However, in a

Information Box 31.6   Commonly Used Antibiotics and Their Mode of Action

Name	Spectrum	Mode of Action
Chloramphenicol	Broad spectrum	Inhibits protein synthesis by binding to 50S ribosomal subunit
Erythromycin	Mostly Gram-positive	Inhibits protein synthesis by binding to 50S ribosomal subunit
Tetracycline	Broad spectrum	Inhibits protein synthesis by binding to 30S ribosomal subunit
Streptomycin	Broad spectrum	Inhibits protein synthesis by binding to 30S ribosomal subunit
Polymyxin	Gram-negative bacteria, especially *Pseudomonas*	Disrupts cell membrane
Nalidixic acid	Gram-negative bacteria	Inhibits DNA synthesis
Novobiocin	Gram-negative bacteria	Inhibits DNA synthesis
Trimethoprim	Broad spectrum	Inhibits purine synthesis
Rifampicin	Gram-positive bacteria	Inhibits RNA synthesis
Penicillin	Mostly Gram-positive bacteria	Inhibits cell wall peptidoglycan synthesis

Information Box 31.7   Gene Transfer Mechanisms

- Conjugation: Transfer of genes from one prokaryotic organism to another by a mechanism involving cell-to-cell contact and a plasmid
- Transformation: Transfer of nucleic acid via uptake of free DNA. It does not require cell-to-cell contact
- Transduction: Transfer of nucleic acid mediated by a virus

recent study, concentrations of antibiotic-resistant bacteria did not increase even after 20 continuous years of land application of biosolids, when compared to neighboring soil that had not received biosolids (Brooks *et al.*, 2007). In contrast, soils receiving dairy manures have been shown to have enhanced concentrations (Esiobu *et al.*, 2002). Most concern over soil-borne antibiotic-resistant bacteria relates to the introduction of animal manures from swine, poultry or cattle fed antibiotics as part of their diet. For example, prophylactic dosing of antibiotics in confined animal feeding operations results in relatively high concentrations of antibiotic-resistant bacteria in animal manures compared to biosolids (Chee-Stanford *et al.*, 2009). Antibiotics are used as growth promoters to increase feed efficacy and subsequent daily growth of animals. Since many of these drugs are closely related to antibiotics used for treating human diseases, the transfer of these strains and the resistances they carry from animals to humans is of concern.

One area of concern is chickens which are fed Ciprofloxacin. Chickens are also frequently infected with *Salmonella* and/or *Campylobacter*. Therefore, there is the potential for resistance to Ciprofloxacin to increase, since microorganisms develop resistance to this antibiotic fairly easily. If a family consumes chicken that is not cooked properly, they may become infected with a Ciprofloxacin-resistant *Campylobacter*. Of additional interest is the fact that Ciprofloxacin is one of the primary drugs for the treatment of anthrax.

As another example, cattle are frequently fed a 90 to 100% grain diet prior to slaughter to enhance fat marbling in the meat. However, this diet is unnatural for cattle that are used to feeding on low-nutrition grasses, since they are ruminants. High grain diets can result in acid production, which causes ulcers in the cattle's stomachs. Bacteria can then migrate from the ulcers to the liver, where additional abscesses form. Because of this cattle are fed antibiotics to suppress the bacteria. Antibiotics also increase feed conversion efficiency.

However, new studies dispute the ease with which antibiotic resistance develops. A recent study examined 448 *Campylobacter* isolates from U.S. feedlot cattle for resistance to 12 antimicrobials (Englen *et al.*, 2005). These included: tetracycline; nalidixic acid; trimethoprim/sulfamethoxazone; and Ciprofloxacin. The study demonstrated only low levels of resistance to a broad range of commonly used antibiotics, relative to other recent studies. Therefore, the complete story on this emerging issue has yet to be written.

The major contributing factor for the selection and propagation of antibiotic-resistant strains is the use of antibiotics. In developed countries, many of the problems of antibiotic resistance are generated within hospitals because of the intensive use of antibiotics (Levy and Marshall, 2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) is probably the most important antibiotic-resistant bacterium associated with hospital-acquired infections (HAI), since staphylococci are the most common pathogens causing bacteremia (blood infection). Bacteremia caused by MRSA are much more difficult to treat, and causes thousands of deaths every year in the United States.

Measures to manage and prevent the spread of drug resistance in hospitals include:

- isolation of infected patients that are potentially hazardous

- early identification and prompt implementation of control measures
- effective hand washing measures
- new therapeutic approaches

Overall, antibiotic-resistant bacteria appear to be widely dispersed throughout the environment, and yet it is only in specialized environments that their presence appears to adversely affect human health and welfare. In hospitals, antibiotic-resistant bacteria are selected due to high usage of antibiotics, and the fact that sterilized surfaces reduce biotic competition. Therefore, the potential for antibiotic-resistant pathogens is high as is the potential for nosocomial infections (hospital-acquired infections). The potential adverse effects from livestock feeding of antibiotics also warrants caution. In contrast, in environments such as soils or biosolids, the potential for gene transfer to human pathogenic bacteria would appear to be lower. Antimicrobial resistance has become one of the most important public health issues faced by the industrialized world. It is estimated that the annual cost of infections caused by antibiotic-resistant bacteria in the United States is U.S.$4−5 billion.

QUESTIONS AND PROBLEMS

1. In your opinion, what is the greatest threat posed by global emerging microbial issues in the Anthropocene era?
2. Do soil microorganisms enhance or diminish global warming? Discuss.
3. Will we run out of new effective antibiotics? Discuss.

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