Where future emerging pathogens will come from and what approaches can be used to find them, besides VFARs

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ABSTRACT

A consideration of available evidence for some known and well-characterized waterborne pathogens suggests that the diversity of pathogen virulence mechanisms and properties is too great to specifically predict the emergence and future human health impacts of new waterborne pathogens. However, some future emerging pathogens are existing microbes that will be discovered to cause disease. Some will arise from existing ones by either predictable evolutionary and adaptation changes or by unpredictable changes involving a variety of biotic and abiotic mechanisms. Many, and perhaps most, emerging waterborne human pathogens will be zoonotic agents or come from other non-human reservoirs. The emergence of some waterborne pathogens will be related to antibiotic use, resulting in emerging antibiotic-resistant waterborne pathogens. Reliably predicting pathogen emergence and human health effects based on VFARs or other properties of microbes and their hosts is not possible at this time. This is because of (1) the diversity of microbes and their virulence and pathogenicity properties, (2) their ability to change unpredictably, (3) their intimate and diverse interrelationships with a myriad of hosts and dynamic natural and anthropogenic environments and (4) the subtle variations in the immune status of individuals. The best available approach to predicting waterborne pathogen emergence is through vigilant use of microbial, infectious disease and epidemiological surveillance. Understanding the microbial metagenome of the human body can also lead to a better understanding of how we define and characterize pathogens, commensals and opportunists.

Key words | antibiotic resistance, emerging pathogens, microbial ecology, surveillance, virulence, zoonoses

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INTRODUCTION AND BACKGROUND

There are billions to trillions of microorganisms all around us. In contrast, there are only a little over 6 billion humans on this planet. Microorganisms have been on this planet billions of years before humans appeared and it can only be hypothesized that microorganisms are going to persist long after humans have gone extinct. Thanks to recent technological advances, our understanding of the microbial world around us is starting to become much clearer. Recent studies on the microbial metagenome of humans and animals are unraveling a very diverse microbial world within and all around us. Pathogens potentially transmitted by water and other environmental media are diverse, often ubiquitous and usually quite adaptable. They include the range of agents comprising the microbial world: viruses, bacteria, protozoan parasites, mycotic agents, helminths and probably prions. In the future, pathogens will come from the same places from which they have previously come. Specifically, they will come from other people,

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from animals, from other living things and from the surrounding environment. They have diverse properties, infect diverse hosts and occupy diverse environmental niches. Furthermore, they, their hosts and the environments in which they exist are undergoing constant change. Some of this change is predictable and some of it is not. It is these changes in microbes, their hosts and the biotic and abiotic environments in which they exist that contribute to the emergence of new pathogens and the recognition of previously unrecognized pathogens. In some cases, the advent of new technologies can uncover the role of hitherto undiscovered microbial pathogens, while in other cases known microorganisms with unique virulence properties are identified as human pathogens. These new properties may arise due to genetic changes, acquisition of novel genes, regulatory changes in gene expression or changes in the hosts around these microorganisms. In a majority of instances, our improved understanding of the disease process results in the recognition of emerging pathogens.

Because of the diversity and complexity of the microbes, their hosts and the environments in which they exist, knowing when, how and which emerging pathogens arise will continue to be a major challenge. One approach that has been used to identify potential chemical contaminants makes use of the structure–activity relationship (SAR) concept. The use of this concept to predict effects, such as human health effects, may work reasonably well because it pertains to a single, unique chemical structure that may be highly specific and common to a class of chemicals. This structure can inform and perhaps predict how the chemicals interact with epitopes or targets on and in cells, tissues and organs, and how they can be metabolized in living systems. An analogous concept for microorganisms, virulence factor–activity relationships (VFARs), posits that the same principles based on properties of pathogens, namely their virulence properties, can be applied to pathogens, and specifically waterborne pathogens, to identify virulence factor–activity relationships. The central premise of VFARs is to relate the architectural and biochemical components of microorganisms to potential human disease (NRC 2001). Underlying this concept are the assumptions that the number of genetic sequences relevant to virulence is relatively limited and that interactions between proteins encoded by these sequences are predictable. Furthermore, this approach assumes that individual hosts will respond similarly to a given pathogen species.

In the judgment of the authors it is impossible to reliably predict pathogen emergence and human health effects based on VFARs, or other properties of microbes and their hosts because of (1) the incredible diversity of microbes and the factors that control virulence and pathogenicity, (2) their ability to respond unpredictably to changing host and environmental conditions, (3) their intimate and diverse interrelationships with a myriad of hosts and the dynamic natural and anthropogenic environments supporting and influencing them, (4) the inherent and ever-changing variations in susceptibility to infections among humans and (5) our continually changing understanding of what constitutes a "pathogen". The remainder of this paper expands on this position, provides examples of why this is the case at the present time, why our basic definitions of what constitutes pathogens and commensals may undergo fundamental changes, and why various surveillance and vigilance strategies are the appropriate ways to detect, identify and respond to emerging pathogens in an effort to anticipate, manage and minimize their human health effects. It also suggests how, why and where future emerging pathogens will probably arise or become recognized and what approaches are recommended to detect and recognize them.

MICROBIAL DIVERSITY IN RELATION TO VIRULENCE MECHANISMS AND THEIR PROPERTIES

The diversity of microbes and their mechanisms and properties of virulence and pathogenicity are too great to reliably predict emergence and future human health impacts. Microbial pathogens have the ability to infect hosts and cause adverse effects. The range of such microbes, their properties and their effects on hosts are both diverse and often able to change or adapt. There are few, if any, common mechanisms or pathways by which these diverse taxonomic groups express virulence or cause pathophysiological effects. For most of the known pathogens, the microbial properties or constituents that cause or contribute to virulence and pathological effects are either unknown,

or remain poorly understood because of the complexity and diversity of these mechanisms.

There is only a superficial understanding of the properties of most existing pathogens responsible for their virulence and pathogenic effects at the present time. As an example, consider the viruses, which are among the simplest of pathogenic biological agents. At the most fundamental level, a virus can be pathogenic for, and express virulence in, a host only if it can successfully attach to and enter host cells. If the host lacks the appropriate receptor for that virus (or vice versa), no infection occurs and the host is spared. For example, in the case of genogroup I (G1) noroviruses (such as Norwalk Virus), the host must express certain ABH histo-blood group antigens (HBGAs) so that the intestinal cells where infection is initiated can have the appropriate cell surface carbohydrate receptor for attachment, entry and infection to occur (Hutson *et al.* 2004). If the host belongs to an ABH histo-blood blood group lacking or not expressing that carbohydrate, no infection occurs. However, successful norovirus infection and its health effects are not solely dependent on virus binding to a specific carbohydrate receptor on the cell surface. Cells with the correct norovirus receptor that bind the virus do not necessarily support viral replication. Furthermore, the range of different HBGAs responsible for susceptibility to different noroviruses is diverse and their role in the risk of infection and severity of illness varies and remains poorly understood (Huang *et al.* 2005).

For genogroup II noroviruses, other cell surface receptors besides HBGAs can serve as host susceptibility factors. HBGA-negative individuals have antibodies against human noroviruses and develop clinical illness after GII norovirus challenge (Lindesmith *et al.* 2005, 2008). Recent experimental studies suggest that carbohydrate ligand binding patterns of norovirus GII.4 virus-like particles (used as norovirus surrogates) have changed over time, suggesting norovirus strain variability and evolution influencing human susceptibility. Indeed, for Genogroup II noroviruses, the current globally predominant genogroup, there is no association between histo-blood group antigens and susceptibility to clinical infections (Halperin *et al.* 2008). In an investigation of two outbreaks of acute gastroenteritis in military units in Israel, GII viruses belonging to the Lordsdale cluster were capable of infecting all secretors of HBGA, regardless of their ABO type. Hence, we continue to lack sufficient understanding of virus–host interactions leading to infection and illness to predict the emergence of new epidemic strains of waterborne virus pathogens like noroviruses.

Other factors besides host cell surface receptors contribute to virus infectivity and virulence in hosts. In the case of some enteroviruses such as the polioviruses, it is known that the ability to cause paralytic poliomyelitis or other neurological disease is dependent on the ability of the virus to attach to and infect neural cells. If the virus lacks the surface epitope that is needed to attach to a specific receptor of host neural cells, no infection of neural cells and tissues takes place and hence no paralysis will occur. However, despite knowing the complete nucleotide sequence of the polioviruses and several other enteroviruses and knowing some of the receptors to which the viruses must attach to to successfully infect neural cells, the mechanisms of their virulence and pathogenicity remain poorly understood (Racaniello 2006; Nathanson 2008). Likewise, our understanding of the pathogenicity and virulence properties of other viruses, bacteria and protozoan parasites is both limited and lacking in predictive ability relevant to identifying and prioritizing emerging pathogens.

Our understanding of the cues for bacterial virulence in humans is also in its infancy. Cell–cell signaling molecules such as AI-2 and host–cell signaling molecules such as AI-3 have been reported to be responsible for enteric bacterial infections in humans (Bansal *et al.* 2008; Soni *et al.* 2008*a*). Recent reports suggest that a host signal (epinephrine) is critical for *E. coli* O157:H7 to invade the host cells (Walters & Sperandio 2006). Similarly, hitherto unknown cell signaling factors are found to be operating within *Campylobacter*, *Pseudomonas*, *Salmonella* and other key human pathogens (Reading & Sperandio 2005). Studies show how the function of these cell–cell signaling molecules can be influenced by the components within the food matrix on which these organisms may be present (Widmer *et al.* 2007; Girennavar *et al.* 2008; Soni *et al.* 2008*b*,*c*). Given that many of the pathogens enter humans via the foods we eat and the water we drink, our understanding of the virulence triggers in these environments is virtually unexplored.

SOME EMERGING PATHOGENS ALREADY EXIST BUT ARE UNRECOGNIZED

Some future emerging pathogens are already existing organisms that will be discovered to cause disease and become better recognized and appreciated for their health effects and disease burden. Future emerging pathogens will come from the recognition of their existence and from increased awareness of their previously unrecognized or under-appreciated roles in human disease. There are many recent examples of human pathogens whose ability to cause disease was either unrecognized or underestimated. For example, the bacterium *Kingella kingae*, a part of the pharyngeal flora of young children, has emerged as an important cause of invasive infectious diseases, including septic arthritis, osteomyelitis, spondylodiscitis, bacteremia, endocarditis, lower respiratory tract infections and meningitis (Yagupsky 2004, 2006). Discovered in 1960, *Kingella kingae* is probably not a truly "new" pathogen. Instead, it is likely that the organism is now being recognized more frequently as a result of improved culture techniques and growing familiarity with its identification by clinical microbiology laboratories.

Another example is parvovirus B19. This virus, which was first discovered in 1975, was first associated with disease in 1981, when it was linked to aplastic crisis in a patient with sickle-cell disease (Corcoran & Doyle 2004). Parvovirus B19 is now recognized as a significant human pathogen causing erythema infectiosum (fifth disease) of children, arthritis and arthralgias (most commonly in adults but also in children), fetal loss and severe disease in immunocompromised patients. It has been identified as an important source of blood-borne infection and illnesses. Similarly Crohn's disease in humans is now being attributed to *Mycobacterium avium* subsp. Parapseudotuberculosis exposure through milk (Naser *et al.* 2000; Khare *et al.* 2004).

Among the protozoan parasites, *Cyclospora cayetanensis*, which was discovered in the intestines of moles in the 1870s, was not recognized as a human pathogen until the late 1970s (Eberhard & Arrowood 2002; Shields & Olson 2003; Karanja *et al.* 2007). It did not get attention as an enteric pathogen until the mid-1990s, when it was clearly identified as a coccidian parasite and linked to a series of outbreaks of gastroenteritis from the consumption of fecally contaminated fresh produce.

A fourth agent that has been in our midst for a long time but was not recognized or appreciated for its human health risks until recently is the prion responsible for the neurodegenerative disease of human spongiform encephalopathy, the Creuzfeldt–Jacob Disease variant (vCJD) of Bovine Spongiform Encephalopathy (BSE) and related agents. BSE was discovered in cattle in the United Kingdom and rapidly spread to trading partners in Europe via contaminated cattle feed supplements (McKintosh *et al.* 2003; Ricketts 2004; Smith *et al.* 2004). However, it was not until 1996 that vCJD was recognized as a human disease caused by consumption of the BSE agent from beef, which led to international efforts to control this foodborne disease threat (Doherr 2003; McKintosh *et al.* 2003; Smith *et al.* 2004). Creuzfeldt–Jacob Disease was recognized as a neurodegenerative disease in the early 1920s, but it was not until the 1950s and 1960s that this disease and similar diseases in humans (kuru) and animals (scrapie in sheep) were recognized as caused by transmissible, infectious agents. It was not clearly documented and accepted that these agents were infectious proteins (prions) until the 1980s (Prusiner 1991; Yokoyama & Mohri 2008). Chronic wasting disease (CWD) of deer and elk, which is also caused by a prion, is endemic in parts of the western USA, which raises as yet unproven concerns that it may also be transmissible to humans (Belay *et al.* 2004).

Traditionally accepted "end-points" of microbial infection and disease are also undergoing radical re-thinking. Human personality disorders are now being linked to a microbial etiology. Neuroticism and concealed aggression are now being shown to be markers of functional gastrointestinal disorders (Lars & Ulrik-Fredrik 2001). Schizophrenia and rheumatoid arthritis are both chronic relapsing diseases of unknown etiology (Torrey & Yolken 2001). Lyme borreliosis is a tick-borne infection in humans which is associated with atypical presentations of psychiatric syndromes with relapsing and remitting progressive deterioration (Fallon *et al.* 1993).

The history of our experiences with, and awareness of, diverse infectious agents as human pathogens clearly document the likelihood that at least some future emerging pathogens will be "discovered", recognized as etiological

agents of disease and become appreciated for their disease risks and burdens from among the microbes that already exist, some of whom we already know. With regards to VFARs, predictive determination of these pathogens would not be possible, given that the genetic and biochemical makeup of these organisms would be that of a known microbe previously considered non-pathogenic.

PREDICTABLE AND UNPREDICTABLE PATHOGEN EMERGENCE

Future emerging pathogens will arise from the existing ones by predictable changes of evolution and adaptation and largely unpredictable changes that occur by a variety of biotic and abiotic mechanisms. Pathogens will emerge from predictable and unpredictable changes in existing microorganisms and as a consequence of changing interactions with their hosts and the environment. The large numbers of pathogens that are already known or suspected are genetically diverse, adaptable and capable of both acquiring and shedding genetic information that influences their virulence and human pathogenicity. However, even the ability to predict or anticipate the changes of already well-known pathogens has been of little help in predicting the next changes to occur or their future impacts on human health. Examples of well-known pathogens for which there is exquisite genetic and physiological knowledge but as yet little ability to fully understand or predict their health effects or other consequences include viruses, such as polioviruses and influenza viruses, and bacteria, such as *Vibrio cholera* and non-typhoid multiple antibiotic-resistant *Salmonella spp*.

The prediction of emergent pathogenic enteric viruses based on "virulence" properties is unlikely to be successful. This is because: (1) the genetic determinants responsible for or contributing to the virulence of enteric viruses do not fit a distinct or common genetic pattern (such as a common toxin gene), (2) they can be spread over different parts of the virus genome (not in pathogenicity islands), (3) they can change due to serial infections of hosts or by genetic exchange (with other viruses or with host genes) and (4) they depend on host genetic and phenotypic factors, such as cell surface receptor genes and their expression.

Polioviruses and poliomyelitis

Polioviruses are single-stranded RNA viruses belonging to the *Picornaviridae* family and the enterovirus genus and consist of three genetically distinct types. These viruses initially infect the gastrointestinal tract and can spread via the bloodstream and lymphatic system to the central nervous system, where they infect cells and cause paralysis in their human hosts. The virus-specific factors responsible for poliovirus neurovirulence remain inadequately understood at the genetic, protein or virion level. For instance, neurovirulence is mediated by the ability of the virus to successfully infect neurons and cause high levels of virus production and death of these cells. Because of the human health risks of paralytic disease caused by wild-type, neurovirulent polioviruses, avirulent or attenuated polioviruses were selected as vaccine strains in the mid-20th century. This was done by serially passing wild-type, neurovirulent viruses in atypical hosts (primates and cell cultures) at different cell culture incubation temperatures. Using molecular techniques such as RT-PCR and sequencing, it is now known that these live oral poliovirus vaccine strains differ from the wild-type viruses by having several different point mutations that are associated with the ability to infect neural cells (Minor 1999). These nucleotide changes are located in different regions of the viral genome, including regions encoding specific amino acids in virus capsid proteins VP1 and VP3, the 3D polymerase and both the 3′ and 5′ untranslated regions (Minor 1999; Racaniello 2006; De Jesus 2007). Supposedly critical to attenuation is a nucleotide substitution in the 5′ untranslated region corresponding to position 480 in type 1, 481 in type 2 and 472 in type 3 polioviruses and probably several other mutations in the 3′ untranslated region of the viral genome (Evans *et al.* 1985; Racaniello 2006; De Jesus 2007). The 5′ untranslated region containing the point mutation is believed to be an internal ribosome entry site in which the mutation alters RNA secondary structure and decreases replication in neural cells (Racaniello 2006; De Jesus 2007).

Neurovirulence depends not only on a range of virus-specific factors but also on host factors and environmental conditions. The unpredictable adaptability of polioviruses as modulated by host and environmental factors has been dramatically demonstrated by back mutations of the

attenuated live oral vaccine strains that cause reversion to wild-type viruses and paralytic poliomyelitis in vaccine recipients. Mutation rates of polioviruses, like all single-stranded RNA viruses, are high, resulting in rapid reversion of vaccine polioviruses to genotypes with neurovirulent properties among the excreted viruses of vaccine recipients (Minor 1999; Racaniello 2006; De Jesus 2007; Nathanson 2008). Serial transmission of vaccine poliovirus strains among susceptible human hosts has resulted in the accumulation of mutations, which eventually led to the selection, and further serial transmission of neurovirulent vaccine-derived paralytic polioviruses (VDPVs) (Kew *et al.* 2002, 2004; Shimizu *et al.* 2004). Poliomyelitis outbreaks associated with circulating VDPVs have occurred in Hispaniola (2000–1), the Philippines (2001), Madagascar (2001–2) and other geographic areas (Kew *et al.* 2002, 2005; Rousset *et al.* 2003; Kew & Miyamura 2004; Shimizu *et al.* 2004). Based on the extent of genetic change (about 3%), the VDPVs of some of these outbreaks had apparently been spreading from person to person over a period of about two years or longer. Furthermore, among the VDPVs from Hispaniola were isolates that contained non-capsid genomic sequences that were derived from other enteroviruses. At least four different enteroviruses recombined with the poliovirus type 1 VDPV during its circulation in Hispaniola (Kew *et al.* 2002). The extent to which these recombinations with non-polio enteroviruses contributed to the virulence and pathogenicity of these VDPVs is uncertain but a cause for concern.

The continuing lack of understanding of the role of point mutations in poliovirus neurovirulence and attenuation is highlighted by a recent study that found no reverse mutation in position 480 in type 1 VDPVs of high neurovirulence (Zhang *et al.* 2007). The authors concluded that the effect of the 480 point mutation in determining neurovirulence has been overestimated, other mutations also may play an important role, and more information is needed to elucidate the mechanism of poliovirus attenuation and virulence. Therefore, despite knowledge of the complete nucleotide sequence of polioviruses for more than two decades, the cloning and expression of the cell surface receptor of the virus, the development and use of a transgenic mouse model for neurovirulence, and considerable efforts to identify the neurovirulence mechanisms in cell culture and animal systems, the mechanisms have not been fully elucidated (Kew *et al.* 2005; Racaniello 2006; De Jesus 2007; Nathanson 2008).

The emergence of VDPVs has raised serious concerns about how to achieve global polio eradication and the "end-game" in which eventually all polio vaccination would cease. Can we stop polio immunization completely, and if we do, will the live attenuated polioviruses still circulating re-emerge as paralytic mutants to occupy the niche previously occupied by the wild-type paralytic viruses? Even if polioviruses are successfully eradicated globally, there is concern that the human niche occupied by paralytic polioviruses will become occupied by other viruses, such as other highly adaptable and neurovirulent enteroviruses closely related to poliovirus (Rieder *et al.* 2001). There is growing evidence that polioviruses and some type A coxsackieviruses (CAVs) have a common ancestry and very similar genetic organization and composition (Mueller *et al.* 2005; Jiang *et al.* 2007). A main difference between PVs and CAVs is the use of different surface cell receptors to initiate infection. The possible emergence of polioviruses (PVs) from C-cluster coxsackie A viruses (CAVs) has important implications for global poliovirus eradication and the potential re-emergence of similar neurovirulent enteroviruses. If the CAVs mutated under selective pressure or recombined with other viruses that have the needed virus receptor to infect the same cell surface receptor as used by polioviruses, they could potentially infect neuronal cells and tissues now infected by polioviruses and essentially replace them as neurovirulent viruses, causing paralysis and other neurological health effects.

Why a particular virus or virus group has adopted a certain surface molecule to enter a host cell is currently unknown. A wide variety of cell surface molecules with diverse functions can serve as virus receptors and there is evidence of both adaptive and evolutionary changes in virus and host cell receptors (Lentz 1990; Baranowski *et al.* 2001). There are no discernable and consistent patterns or rules that govern the virus receptor preferences, sometimes even within the same virus genus. In some cases very similar viruses within the same genus use quite chemically, structurally and functionally different cell surface receptors (Lentz 1990; Baranowski *et al.* 2001; Rossmann *et al.* 2002). These observations indicate that viruses have remarkable

adaptability to evolve different receptor specificity. Such lack of consistency and predictability of virus binding sites for host cells is further evidence that relying on the structure and function of macromolecules in or on a microbe or microbe group cannot provide a systematic and useful basis to predict virulence and pathogenicity for the purpose of identifying and prioritizing future emerging virus pathogens, at least not now nor in the near future.

Influenza and influenza viruses (Orthomyxoviruses)

Influenza viruses, the cause of "the flu", are single-stranded RNA viruses having eight distinct genomic segments. This segmented genome increases the potential for recombinants to form by interchange of gene segments (reassortment) if two different viruses infect the same cell. Influenza viruses constantly evolve and this results in recurrent annual epidemics of disease (Alexander & Brown 2000; Hilleman 2002; Alexander 2006; Solorzano *et al.* 2007). Two types of genetic changes occur: progressive *antigenic drift* of influenza A and B viruses due to the mutability of the RNA genome and periodic *antigenic shift* caused by reassortment that leads to the emergence of novel influenza A subtypes to which the population has little immunity and thereby causes severe pandemics (Webster *et al.* 1992; Hampson & Mackenzie 2006; Landolt & Olsen 2007). Antigenic shift occurs primarily in strains of influenza A, in which there is complete replacement of either or both of the two main surface glycoproteins (antigens) on the virus envelope, the neuraminidase (N) and hemagglutinin (H). These surface antigens are responsible for host cell attachment and entry and for eliciting an immune response in infected hosts.

The appearance of new pandemic strains of influenza viruses by reassortment has been well documented to occur periodically, with identifiable subtypes going back to the late 1800s. In many cases the new H and N antigens come from animal sources, such as birds, swine and horses. The new, recombinant viruses are designated by a numbering system for the H and N antigens, such as H1N1 or H1N5. Human pandemic influenza strains apparently arise from influenza viruses circulating in a natural animal reservoir, and the presence of intermediate hosts is sometimes involved in this process. Pigs and birds play a major role in the ecology of influenza viruses by providing an environment in which co-infection by two influenza viruses can change their phenotype by reassortment that results in antigenic shift with the potential for new viruses with expanded host range, zoonotic transmission to humans and efficient human-to-human transmission (Webster *et al.* 1992). Birds are considered the main reservoir and in these hosts the viruses often cause inapparent infection. The emergence of new strains of influenza virus often occurs in regions of south and southeast Asia, where humans live in intimate contact with swine, poultry and other animals under poor sanitation conditions. These conditions create opportunities for human infection by avian subtypes or co-infection of swine, leading to the emergence of new reassortant viruses. Transmission of avian H5N1 and H9N2 viruses directly to humans during the late 1990s shows that land-based poultry can serve as a link between aquatic, migratory birds and humans as intermediate hosts of influenza viruses (Morens & Fauci 2007; Webster *et al.* 2007). So far these avian influenza virus strains have not been highly transmissible in humans but the risks of this occurring have raised concerns about the emergence of a human pandemic influenza subtype of avian origin (Nguyen-Van-Tam & Sellwood 2007; Peiris *et al.* 2007).

Despite exquisite knowledge of the ecology, biology and molecular biology of influenza viruses, it is not possible to predict the properties of the newly emerging pandemic strains, exactly where and when they will emerge and what effects they will have on human health. As with other pathogens, the specific properties of the virus and its location and time of appearance as a new pandemic influenza virus subtype cannot be deduced or predicted by analysis of existing subtypes or strains. Such predictive ability is also poor even for the emergence and identification of influenza A and B subtypes and strains that will circulate annually. The properties of the viruses responsible for emergence, transmission and human health impact in future years remain unclear partly because of the role of the host in this process. While the ability of the influenza virus to infect and efficiently replicate in a susceptible human host and cause disease is important, this is not considered to be the primary factor determining virus emergence as a new pandemic influenza subtype in humans. A key feature of a potentially pandemic influenza virus probably is its

transmissibility, which is the ability to spread efficiently from infected to susceptible (uninfected) hosts. However, the molecular basis of influenza virus transmissibility and other aspects of pathophysiology have not been adequately elucidated (Korteweg & Gu 2008). Although new pandemic subtypes of influenza probably will emerge as they have done for their recorded history, it is unlikely that VFAR or any other approach based primarily on knowledge of the structural and functional properties of the viruses alone will have the ability to identify and predict their appearance or properties as human pathogens. The random or stochastic nature of the selection process, the diversity of the pool of influenza viruses circulating in human and animal hosts, and our continued lack of understanding of the factors governing transmissibility, pathogenicity and virulence prevent prediction of forthcoming emergent pandemic strains or subtypes of influenza.

Cholera and *Vibrio cholerae*

The disease cholera is caused by the bacterium *Vibrio cholerae*. Cholera can be acquired through ingestion of contaminated food or water or contact with feces or vomitus of infected persons. While *V. cholerae* is an important human enteric pathogen transmitted by the fecal–oral route, its ecology or natural history is intimately associated with natural aquatic environments and the organism is characterized by a wide range of hosts, reservoirs and ecological niches. *Vibrio cholerae* are associated with fresh, brackish and marine warm waters, phytoplankton, copepods, bivalve mollusks (oysters) and aquatic vegetation and they can live and proliferate in these habitats and hosts, often in biofilms (Colwell 1996, 2004).

For some bacterial pathogens such as *V. cholerae*, it has been possible to identify key properties and changes occurring in them that are responsible for or contribute to virulence (Faruque & Mekalanos 2003). The mechanisms of pathogenicity have been elucidated in terms of physiological effects linked to clinical illness. Molecular, biochemical and physiological methods have been used to explore the acquisition and evolution of these virulence properties in relation to the microbial ecology of this bacterial pathogen. Current understanding of the ecology and epidemiology of cholera requires analysis of the complex interplay of global and seasonal weather patterns, aquatic reservoirs, bacteriophages harboring numerous cholera toxin genes, zooplankton and shellfish, the collective behavior of surface-attached cells (quorum-sensing phenomena), deep sea, estuarine and freshwater environments that influence properties of the bacterium with an adaptable genome and human host responses and behaviors, including their travel, food preferences and handling practices, as well as their water management and use. Despite extensive study and understanding of the ecology, physiology, pathogenicity, molecular biology and evolution of *V. cholerae*, our knowledge and understanding of this pathogen have been of limited value in predicting the emergence, outcomes and human health effects of pandemic *V. cholerae* strains and their human disease burdens (Colwell 2004; Fabiano *et al.* 2004; Faruque *et al.* 2004, 2006; Emch *et al.* 2008).

Recorded evidence of cholera dates back to the 1500s in India. However, cholera disease in the modern era has been marked by periodic pandemics, which have been recorded since 1817 (Pollitzer 1959; Faruque *et al.* 1998; Kilbourne 2006). The seventh pandemic (1961–present) is noteworthy because it has the most extensive geographical spread compared to the previous pandemics. It was initiated by a new *V. cholerae* biotype (El Tor), and it began on the island of Sulawesi, Indonesia, in contrast to previous pandemics that emerged from the Indian subcontinent. An eighth pandemic, which began in late 1992 in India and Bangladesh, is also unique because it is caused by a new strain, *Vibrio cholera* serotype 0139.

The species *V. cholerae* is well defined based on biochemical tests, DNA homology and recently a complete genetic map based on nucleotide sequencing of one *V. cholerae* strain and considerable genetic analysis of strains with varying pathogenicity ((Heidelberg *et al.* 2000; Fabiano *et al.* 2004). Not all groups, serotypes or strains of *V. cholera* are capable of causing disease in humans (Tantillo *et al.* 2004). The ability of pathogenic *V. cholerae* to cause disease appears to mostly depend on the expression of two virulence factors: cholera toxin (CT), a potent enterotoxin, and a pilus colonization factor known as toxin coregulated pilus (TCP). Both of these pathogenicity traits are encoded by genes that are part of larger genetic elements. The genes encoding CT are part of a gene cluster called CTX that is the genome of the CTX bacteriophage. The genes for

TCP are part of a Vibrio pathogenicity island (VPI). These virulence factors are under the control of ToxT, an AraC/XylS family protein that activates transcription of the genes encoding TCP and CT (Childers & Klose 2007). ToxT is under the control of a virulence regulatory cascade known as the ToxR regulon, which responds to environmental stimuli to ensure maximal virulence-factor induction within the human intestine. Until recently, only *Vibrio cholerae* of the 01 serogroup, which produces CT, have been historically associated with epidemic and pandemic cholera. However, the new human pathogenic variant of *Vibrio cholerae* (serogroup 0139) that emerged in eastern India and Bangladesh produces the cholera toxin and appears to be genetically related to both the 01 and non-01 serogroups.

International travel and commerce contributes to the spread of cholera as documented in pandemics. In the first pandemic, cholera was spread by ship from Ceylon to Mauritius, which began the dissemination of cholera out of India (Guerrant 1994; Guerrant *et al.* 2003). Cholera was spread to the new world (Quebec, Canada) via ships from Ireland carrying immigrants, marking the beginning of the second pandemic. During the third pandemic, cholera was rampant throughout the US and was carried west by wagon trains of the pioneers (Kaper *et al.* 1995). The introduction of cholera to Guinea in 1970 was probably caused by a returning traveler and was subsequently spread along the coast and into the interior via rivers during the seventh pandemic (Kaper *et al.* 1995).

Cholera has not been endemic in the United States for decades and the infrequent cases that occur are linked to foreign travel or related importation. For example, in 1991 26 cholera cases were reported, of which 18 were associated with travel to Latin America and 11 were related to crabs brought back in suitcases (MMWR 1991*a*, 1992; Finelli *et al.* 1992). In 1991 and 1992 toxigenic *Vibrio cholera* was recovered from ballast, bilge and sewage water from five cargo ships docked in ports in the US Gulf of Mexico. Four of these ships had taken on ballast water in cholera-infected countries (McCarthy & Khambaty 1994). Thus, discharging ballast waters in ports may spread cholera to areas that are cholera-free. This is a proposed mechanism by which cholera was reintroduced into Latin America in 1991.

Human demographic and behavior factors, including population increases, urban sprawl, overcrowded living conditions and large population movements in Africa and other regions of the developing world have resulted in millions of people who are subjected to cholera risks associated with inadequate sanitation, lack of water and poor food supplies. Outbreaks of cholera associated with behaviors involving the consumption of contaminated food and water include drinking unboiled municipal water stored in open containers into which hands were introduced (Ries *et al.* 1992; Swerdlow *et al.* 1992), consumption of popular raw seafood dishes such as oysters (Blake 1983), consumption of food and beverages purchased from street vendors, using harbor water contaminated with sewage to rinse, unload and process shellfish, and consumption of rice-based meals prepared by persons who had previously prepared the bodies of cholera victims for burial (St Louis *et al.* 1990). Funerals and other large gatherings have also played a role in the spread of cholera in Africa during the seventh pandemic. Inadequate technology in the form of fecally contaminated water supplies and lack of or inferior sanitation infrastructure continues to provide the means for efficient dissemination of cholera and other infectious diseases throughout the developing world.

The food and beverage industry also impacts the spread of cholera in both the developed and developing world. Spring water contaminated with cholera and used by a bottling plant was distributed throughout Portugal in 1974, causing over 2000 cases of cholera from its consumption (Blake *et al.* 1977). This is a cause for concern considering the current trend of drinking bottled water for health, aesthetic and convenience reasons. The ability to process food in order to preserve it for storage and transport also contributes to the occurrence of cholera and other food-borne diseases. For example, cases of cholera in Maryland in 1991 were attributed to the consumption of frozen coconut milk imported from Thailand. Freezing was insufficient to kill the cholera organisms and the bacteria proliferated after prolonged storage at room temperature (MMWR 1991*b*).

Of fundamental importance are the *de novo* creation, selection and subsequent emergence of new pathogenic serotypes and strains of *V. cholerae* that become the cause of pandemics. This process appears to occur largely independent of human activity in the natural aquatic environments in which *V. cholerae* and other bacteria

thrive. New pathogenic strains of cholera appear to emerge by horizontal transfer of CT genes via CTX phage (Faruque *et al.* 1998). There also may be phage-mediated transfer of the TCP–ACF element that is also associated with virulence and pathogenicity (Davis & Waldor 2003). In an effort to understand the evolutionary aspects and possible selection mechanisms involved in the emergence of pathogenic *Vibrio cholerae*, Faruque *et al.* (2004) analyzed and compared diverse strains isolated from among the many strains present in environmental waters of Bangladesh by direct enrichment in the intestines of adult rabbits and by conventional laboratory culture. Most (99.25%) of strains isolated by conventional culture were negative for the major virulence gene clusters encoding toxin-coregulated pilus (TCP) and cholera toxin (CT) and were non-pathogenic in animal models. However, all strains isolated in rabbits were able to colonize infant mice and most (56.8%) carried genes encoding TCP alone or both TCP and CT. By ribotyping, toxigenic O1 and O139 strains from the environment were similar to pandemic strains, while non-O1 and non-O139 strains and TCP^- nontoxigenic O1 strains were widely divergent from the seventh pandemic O1 and the O139 strains. Distinct groups of environmental strains of *V. cholera* carrying various combinations of virulence genes were observed, which is consistent with the assumption that aquatic strains acquire different virulence gene clusters in distinct steps. However, the ribotypes of most of the environmental strains were widely different from those of pandemic strains, suggesting that the acquisition of virulence-associated genes such as TCP and CT genes alone and the corresponding ability to be human pathogenic do not fully create emergent pandemic strains.

The results of these and other recent studies suggest that (i) environmental *V. cholerae* populations in a cholera-endemic area are highly heterogeneous, (ii) selection in mammalian intestines enriches environmental strains having virulence potential, (iii) *V. cholerae* pathogenicity involves more virulence genes than currently appreciated and (iv) most environmental *V. cholerae* strains are unlikely to become of pandemic potential by acquisition of TCP and CT genes alone. Because most recorded cholera pandemics originated in the Ganges Delta region, this ecological setting is probably favorable for the extensive genetic exchange among *V. cholerae* strains that promotes the rare, multiple-gene transfer events critical to combining the genes required for pandemic spread (Faruque *et al.* 2004, 2006; Alam *et al.* 2007).

Despite the critical role of the environment in the creation of new *V. cholera* strains. Faruque, Mekalanos and their colleagues state that "The evolutionary success of the seventh pandemic clone of *V. cholerae* as an endemic and pandemic pathogen may be more related to its improved interaction with the human host than to its improved fitness within environmental reservoirs". In another paper they also say that "besides virulence genes and genetic elements mediating their transfer, the single most important contributor to the evolution of pathogenic *V. cholerae* is the human host itself, which supports the selective enrichment of pathogenic strains from an immensely diverse mixture of environmental Vibrio strains". Their findings and these statements further support the authors' contention that the roles of the host and the environment are critical elements in the emergence of bacterial pathogens like *V. cholerae* and certainly are no less important in this process than the specific virulence traits or related pathogenic properties of the microbes. Continued and largely unpredictable changes in microbes creates opportunities for the random emergence of new pathogenic strains, but studying the properties of the pathogens alone, genetically or otherwise, is not sufficient to elucidate or predict emerging pathogens and their resulting impacts on human health.

In summary, a lot is understood about the ecology and natural history of the disease cholera and of the bacterium *V. cholerae*, both pathogenic and non-pathogenic serotypes and strains. The emergence and spread of new pandemic strains of pathogenic *V. cholera* is a phenomenon that has been elucidated in terms of contributing factors and mechanisms, but still remains poorly understood and characterized in terms of predictive ability. Why and how new pandemic strains of *V. cholerae* arise and what factors cause their emergence has been carefully investigated, at least in terms of contributing factors and their mechanisms. It is now well documented that factors contributing to emergence of pathogenic pandemic *V. cholerae* include international travel and commerce, inadequate or absence of water, sanitation and hygiene measures, changes in human demographics and behavior, including urbanization and migration, changes in land use and economic development,

seasonal factors related to temperature that influences aquatic biota, and microbial change by adaptation and evolution. Despite this rich knowledge, it remains impossible to predict exactly when and where future pandemic *V. cholerae* strains will emerge, what their properties will be, how and where they will spread and how we can identify and find them in advance of their adverse impacts on human health in the form of pandemic cholera.

EMERGING ZOONOTIC PATHOGENS

Many, and perhaps most, waterborne emerging human pathogens will likely come from animals and other non-human reservoirs; however, which ones, their impacts and importance cannot be predicted. A variety of different kinds of bacterial, viral and parasite pathogens that infect animals are of concern for human exposure from water (Slifko *et al.* 2000; Enriquez *et al.* 2001; Schlundt *et al.* 2004; World Health Organization 2004; Skovgaard 2007). These pathogens are shed in feces, urine, respiratory secretions and from the skin, fur or feathers. Many of these pathogens, especially those of enteric origin, are likely to be present in animal manures and other wastes. Some of the important pathogens potentially present in animal manures are not present in the United States. However, there are growing concerns that non-endemic pathogens may be introduced either accidentally or deliberately into United States animal populations or spread to humans from animals in other countries and enter the United States with travelers. New zoonotic pathogens continue to be discovered in wild and agricultural animal populations and, while the host ranges of these pathogens are uncertain, it may include humans. A recent example of an emerging virus pathogen that came to the United States in this way is the coronavirus responsible for Severe Acute Respiratory Syndrome or SARS (Cheng *et al.* 2007).

The role of fecally contaminated water has been suggested for SARS coronavirus (SARS CoV). There is circumstantial epidemiological evidence that a SARS coronavirus outbreak may have been caused by airborne spread of the virus via airborne fecal droplets emanating from faulty toilet plumbing and spread by the apartment exhaust fans in high-rise buildings of a large apartment complex (McKinney *et al.* 2006). There are concerns that other recently discovered animal enteric and respiratory pathogens may be able to infect humans or that they have the potential to recombine with human pathogens and produce new strains capable of infecting humans via contaminated water and other transmission routes. Particular pathogens of concern in this regard are the hepatitis E virus and orthomyxoviruses (influenza viruses).

The fate of pathogens in manure management systems of agricultural animals such as swine and poultry is a public health concern (Cole *et al.* 1999; Gerba & Smith 2005). This is especially so for large scale, multi-stage systems involving animal waste collection, treatment or storage followed by land application at production facilities with large numbers of animals and minimum acreage (confined animal feeding operations). Because of the magnitude of the quantities of animal wastes generated by these facilities and the potentially high pathogen loadings that can result if the treated manure residuals still contain high pathogen concentrations, waterborne and airborne transmission is a human health concern. Investigation of the fate of pathogens in these systems and their surrounding environments documents microbial release into the aquatic environment (Anderson & Sobsey 2006).

ANTIBIOTIC RESISTANCE AND EMERGING WATERBORNE PATHOGENS

Antibiotic-resistant bacterial pathogens are well documented, they continue to emerge and yet their risks to human health from waterborne exposures remain unknown. Antibiotic-resistant enteric bacteria in water as well as food are an emerging public risk associated with widespread antibiotic use in human and veterinary medicine, agriculture and aquaculture (Cabello 2006; Kim & Aga 2007; Mathew *et al.* 2007). The development of resistance to antimicrobial agents has been repeatedly observed since the development and use of antibiotics (Aarestrup *et al.* 1998; Hawkey 2008). Every time a new class of antibiotics is developed, microbial resistance inevitably develops, compelling the pharmaceutical industry to look for new cellular targets and new antimicrobial chemicals. Antimicrobial chemicals have different cellular targets and resistance to

these different chemicals can be caused by different mechanisms. A variety of different antimicrobial resistance phenotypes result from the acquisition of external genes that can provide resistance to an entire class of antimicrobials. These genes are frequently associated with large transferable extrachromosomal DNA elements, plasmids, on which there may be other mobile DNA elements such as transposons and integrons.

Bacteria can possess, develop (by mutation) or acquire numerous genetic traits for antimicrobial resistance properties. For some antimicrobial agents, such as the Beta-lactams that target sites on the bacterial cell surface, the cell can contain an enzyme (Beta-lactamase) that inactivates the antimicrobial agent (Cha *et al.* 2008). Likewise, for aminoglycoside antibiotics, which work by binding to ribosomes and thereby interfere with protein synthesis, resistance can also be due to aminoglycoside-modifying enzymes, of which there are more than 50 different ones (Shakya & Wright 2007). Some antibiotics target a cell enzyme for inhibition, such as the fluoroquinolones that attack bacterial topoisomerase enzymes (namely, DNA gyrase or bacterial topoisomerase II and topoisomerase IV). These bacterial topoisomerases are essential bacterial enzymes that alter the topology of double-stranded DNA (dsDNA) within the cell, and interference with them results in rapid cell death. Resistance to fluroquinilones can be due to alterations (by mutation at critical sites) in the target enzymes within the cells or by alterations in the outer cell layer that limit the permeability of the drug to the target (Piddock 1999). The target enzymes are most commonly altered in domains near the enzyme active sites, resulting in reduced drug binding affinity.

For macrolide, lincosamide, and streptogramin (MLS) antibiotics, which are chemically distinct inhibitors of bacterial protein synthesis, several mechanisms of resistance have been documented (Roberts 2004, 2008). One mechanism is posttranscriptional modifications of the 23S rRNA by adenine-*N*6-methyltransferase, altering a site common to the binding of MLSB antibiotics. Another resistance mechanism is due to mutations in genes that code for efflux proteins. These mutations make it possible for efflux proteins to pump the antibiotic out of the cell and thereby keep intracellular concentrations of the antibiotic low and away from ribosomes. A third mechanism of fluoroquinolone resistance is due to enzymes. The bacteria can acquire genes encoding enzymes which hydrolyze streptogramins or modify the antibiotic by adding an acetyl group (acetyltransferases).

A diverse range of mechanisms are responsible for the action of antibiotics and for the corresponding development of antibiotic resistance (Tenover 2006*a*,*b*). For the glycopeptide antibiotics, vancomycin and teicoplanin, antimicrobial activity is due to the presence of a gene that encodes for an enzyme that alters the peptidoglycan target within the cell, causing reduced binding of the antimicrobial molecule to specific sites on the peptidoglycan. For tetracyclines, which act by binding to the 30S ribosomal subunit and thereby inhibit protein synthesis, the two main mechanisms of cell resistance are due to genes that code for efflux activities (mediated by energy-dependent efflux pumps) or ribosomal protection (due to an elongation factor G-like protein). Trimethoprim is an analog of dihydrofolic acid (an essential component in the synthesis of amino acids and nucleotides) that competitively inhibits the enzyme dihydrofolate reductase (DHFR). Resistance to this antimicrobial is caused by overproduction of the host DHFR, mutations in the structural gene for DHFR and the acquisition of a gene encoding a resistant DHFR enzyme.

Another important aspect of antimicrobial resistance is the development of multiple resistances, often on the same genetic element, and the movement of these grouped or linked multiple resistance traits among bacteria by various mechanisms of gene transfer. The mobility of antimicrobial resistance traits, especially the multiple resistance traits, as a single transmissible genetic element further complicates efforts to control antimicrobial resistance or predict the effects of resistance. If many different antimicrobial resistance genes accumulate on a single mobile element, multiple antibiotic resistances can be acquired as a consequence of a single genetic event. Because bacterial populations have repeatedly demonstrated the ability to adapt to the presence of antimicrobials and changing environmental conditions, and because of their facility in exchanging DNA, antibiotic resistance and multiple antibiotic resistance is probably an inevitable biological phenomenon that will likely continue despite vigorous efforts to prevent it. An example of an emerging enteric bacterial pathogens with multiple antibiotic resistances is *Salmonella typhimurium* DT104

(Threlfall 2000, 2002). The international spread of this multidrug-resistant pathogen and its role in foodborne disease outbreaks associated with agricultural animals and pets raises concerns about its ability to be spread by contaminated water and other environmental routes.

Another challenge to understanding and predicting the consequences of antimicrobial resistance is the growing evidence that some antimicrobial resistance traits are also co-selected with resistance to microbiocides, such as triclosan and quaternary compounds (Sidhu *et al.* 2001). Recently, Braoudaki & Hilton (2004) reported that exposure of *Salmonella enterica* and *Escherichia coli* O157 to sublethal concentrations of antibacterial agents contributed to their development of adaptive resistance to both biocides and antibiotics. Benzalkonium chloride-resistant *Salmonella enterica* serovar Virchow showed elevated resistance to chlorhexidine. *E. coli* O157 acquired high levels of resistance to triclosan after only two sublethal exposures and, when adapted, repeatedly demonstrated decreased susceptibilities to various antimicrobial agents, including chloramphenicol, erythromycin, imipenem, tetracycline and trimethoprim.

Biocides tend to act concurrently on multiple sites within the microorganism and resistance to them is often mediated by non-specific mechanisms that may be associated with properties such as cell wall composition and function and efflux pumps. Co-resistance to antibiotics and biocides has been associated with both efflux pumps and cell walls. Efflux pumps have the potential to act on a range of chemically dissimilar compounds and have been implicated in both biocide- and antibiotic-resistant bacteria. Cell wall changes may also play a role in the observed cross-resistance between biocides and antibiotics, probably by reducing permeability. In *Salmonella enterica* adapted to erythromycin, benzalkonium chloride and triclosan, cell surface hydrophobicity and the presence of active efflux were suggested as contributors to such resistance (Braoudaki & Hilton 2005). However, the specific mechanisms of such co-resistance remain poorly understood.

The growing evidence that both antimicrobial resistance and biocide resistance are often genetically linked traits on plasmids and other transmissible elements indicates that microbes are highly adaptable in ways that are not only difficult to predict but also difficult to respond to in terms of plans, initiatives, policies and regulations. In trying to understand the human or animal health and other impacts of antimicrobial resistance traits it is important to recognize that possession of a gene for an antimicrobial resistance trait does not necessarily mean that resistance will be expressed by the bacterium. Furthermore, a gene that is not expressed *in vitro* may be expressed *in vivo* and vice versa. Therefore, relying on genomic analysis of antimicrobial resistance traits alone, such as by PCR or the application of microarrays, cannot predict the extent of gene expression *in vivo*.

Microbes are clearly highly adept at developing resistance to the wide variety of antimicrobial agents that have been developed. These experiences indicate that prior knowledge of the type of cellular target, the mode of action of the antimicrobial agent and molecular basis of the target (the gene and its expression) have been of little value in achieving success in predicting the response of the microbe or microbial impacts on human health and the environment. Previous experiences in the continued development of antimicrobial agents and the emergence of antimicrobial resistance in enteric bacteria found in water and food demonstrates the inability to control microbial impacts on human health, to predict their human health effects or anticipate the consequences of attempts to predict or control microbes and their responses (Ahl & Buntain 1997). The unintended consequences of developing and introducing antibiotics and the resulting development of antimicrobial resistance in waterborne pathogens is another metaphor for the anticipated unlikelihood of success in using an approach such as VFAR to predict or prioritize future emerging waterborne pathogens. The adaptability of microbes remains far more diverse and complicated than our ability to predict their properties, their emergence or their effects.

SUMMARY AND CONCLUSIONS

Microorganisms have existed on Earth billions of years before humans appeared. Long before oxygen materialized on this planet, microorganisms warmed the atmosphere by methane. Cyanobacteria and algae modulated and continue

to modulate oxygen concentrations and nitrogen fluxes in the oceans. Whether we like it or not, microorganisms also appear to have controlled, and continue to control, every aspect of human, plant and animal life on this planet. Microorganisms influence our soils, the crops we grow, our health and the foods we eat. Yet, our understanding of microorganisms, in terms of their structure, function and activity, is still in its infancy.

Emerging pathogens, including those that will be waterborne, will arise in a variety of ways from a variety of sources, many of which will be difficult, if not possible, to predict. In all likelihood, the emergence of many new waterborne pathogens will be stochastic. Hence, the emergence, properties and health effects will not likely be predictable by conventional deterministic approaches that are based on identifying the molecular, physiological and phenotypic properties of microbes at a given place and time and tracking the somewhat predictable changes undergone by some microbes and their hosts. However, based on the current knowledge and previous experience regarding where and how pathogens have emerged, we can expect that pathogen emergence, spread and health effects will continue to be largely unpredictable.

The factors that will contribute to the creation and/or emergence of pathogens include: zoonotic reservoirs from which microbes will "jump" to human hosts, changes in environmental conditions that cause pathogens to enter new geographic areas and encounter new hosts, such as global warming, changes in host susceptibility to microbes such as immunodeficiency and nutritional deficiencies, migration or movement of humans to new areas where they encounter pathogens with which they previously had no contact, viruses, plasmids and other mobile genetic elements that carry virulence genes and other traits of pathogenicity and virulence to new microbial hosts, the creation of new or altered environments that have new or different opportunities for microbes to encounter new hosts, iatrogenic transmission of new or altered pathogens by injection, transplantation, and other biomedical or substance abuse routes, continued anthropogenic activities that alter microbes, such as the creation and use of antibiotics that lead to antimicrobial resistance, genetically altered microbes introduced into the environment or into hosts (vaccines), the eradication or attempted eradication of pathogens leading to the occupancy of an ecological and host niche by a different pathogen or the re-introduction of the same pathogen, and the continued complex and diverse interactions of microbes and their human, animal and other hosts that lead to small and large changes in short or long periods of time.

Because these factors and the interactions they create are stochastic, much like a complex kaleidoscope of processes and conditions, the emergence of specific pathogens and their properties will continue to be unpredictable and unexpected for many of them. For this reason, the approaches that need to be used to predict, identify and detect these emerging pathogens go beyond what can now be achieved with currently available scientific disciplines and tools. Molecular genetic analytical methods (genomics), proteomics, bioinformatics and other advanced tools and analytical methods will continue to identify and characterize virulence genes and improve our understanding of virulence and the role of virulence factors in human disease. Likewise studies of the biological and pathophysiological processes of infection and disease, efforts to understand the roles of genetic and other factors of susceptibility, and elucidating the role of the immune response in infection and disease and their roles in protection from the same will also continue to improve our understanding of virulence and pathogenicity of microbes.

However, greater efforts will be needed to understand and characterize the role of the environment, the effects of environmental change, the impacts of environmental manipulation by human activity and the continual environmental pressure humans have put on themselves, the biosphere and the entire planet in the emergence of microbes responsible for human infectious disease. The role of microbial hosts, microbe interactions with other biotic entities and the effect of the environment on microbe–host interactions need to be better understood and characterized. Also needed are greater efforts to understand the many diverse and complex aspects of microbe–host interactions that contribute to the emergence or re-emergence of pathogens by evolutionary changes, adaptations and other processes. The complexity of microbes, their hosts and the other biological and abiotic agents they encounter in diverse and ever-changing environmental setting makes it difficult, if not impossible,

to predict what the next emerging pathogens will be and what properties and effects they will have.

The best that can be done to detect and identify emerging pathogens is to use a range of tools for active surveillance, including microbial analyses, molecular biology, epidemiology, environmental health sciences, behavioral and other social sciences, syndromic surveillance and rapid response systems in ways that establish or increase vigilance for the appearance of new microbial threats to human health from water and other exposure routes. These surveillance activities can take a variety of forms. They include molecular biological and microbial analyses to look for and track pathogens of concern in various media and hosts. They also include syndromic and other infectious disease surveillance for the appearance of characteristic disease syndromes that are the hallmark of the appearance or emergence of a pathogen in new places or hosts.

Active surveillance for diseases and pathogens and contingency plans for rapid and effective response in the form of prevention and control measures are what are needed to minimize the risks from emerging pathogens and achieving containment and possibly temporary, if not permanent, eradication or containment. The approaches listed here are among the most important ones now being implemented as surveillance measures against bioterrorism threats and in the creation and facilitation of greater biosecurity against natural and anthropogenic microbial threats. Extending these surveillance systems to encompass the appearance or introduction of emerging pathogens from water, other environmental media and other hosts and reservoirs seems both a logical and scientifically sound approach that can be devised and implemented quickly by building on the newly created and expanded capacities that address threats to biosecurity.

As a tool to identify and prioritize pathogens, waterborne or otherwise, the VFAR concept is too simplistic because of its deterministic context, by its focus on microbial genetic and other constitutive properties of microbes, by its lack of consideration of the fundamental roles of humans and other hosts, and the randomness of the wide range of host–microbe interactions in diverse environmental settings. For these reasons it will not be a useful predictive tool for emerging pathogens in water or in general any time soon, if ever.

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