Virulence 4:3, 213–222; April 1, 2013; © 2013 Landes Bioscience

Rise of the microbes

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Keywords: emerging pathogens, infectious disease outbreaks, hypervirulence, microbial pathogenesis, vaccines, influenza, meningitis, cholera, Shiga toxin-producing *E. coli*, Salmonella

Infectious diseases continue to plague the modern world. In the evolutionary arms race of pathogen emergence, the rules of engagement appear to have suddenly changed. Human activities have collided with nature to hasten the emergence of more potent pathogens from natural microbial populations. This is evident in recent infectious disease outbreaks, the events that led to their origin, and lessons learned: influenza (2009), meningitis (Africa, 2009), cholera (Haiti, 2010), *E. coli* (Germany, 2011) and Salmonella (USA, 2012). Developing a comprehensive control plan requires an understanding of the genetics, epidemiology, and evolution of emergent pathogens for which humans have little or no pre-existing immunity. As we plot our next move, nature's genetic lottery continues, providing the fuel to transform the most unlikely infectious disease scenarios into reality.

Infectious diseases have caused more suffering and death than any other calamity known to mankind. A comprehensive control plan for emergent pathogens requires knowledge of the selective pressures imposed on natural microbial populations as well as the pathogenic mechanisms leading to the acquisition, expression and transmission of new virulence traits. Where did these pathogens come from? Why did they arise? What can be done to stop them? Answers to some of these questions have come from recent infectious disease outbreaks that have severely impacted public health systems on local, national, and international scales. The lessons learned may shape health care globally for years to come.

Influenza (2009): Dodging a Bullet

The 1918 "Spanish Flu" pandemic had an estimated mortality of 40–100 million deaths worldwide and is among the worst public health disasters of modern history.[1] Pandemic influenza viruses are products of nature's "genetic lottery"—for which humans have little or no pre-existing immunity. There have been three influenza pandemics in the last century: 1918 Spanish flu (H1N1), 1957 Asian flu (H2N2) and 1968 Hong Kong flu (H3N2). On April 15, 2009, humanity was challenged once again with the emergence of a previously uncharacterized influenza virus: type A, subtype H1N1.[2-5] The early pathological reports did not fit the profile of the seasonal flu: 30% of hospitalizations had severe respiratory complications, the most common cause of death was viral pneumonia without a secondary bacterial infection (atypical), and the median age of hospitalized patients was 27 y—within the age group least susceptible to influenza infection.[4-6] On April 26, 11 d after the first case was identified by the Centers for Disease Control and Prevention (CDC), the US declared a National Public Emergency,[7] resulting in school closures, travel advisories and the demand for antivirals and vaccines. On April 27, the CDC selected a seed-stock for a vaccine (A/California/7/2009). However, the development and distribution of a vaccine was still months away as the virus needed to be grown in chicken eggs—a rather slow, cumbersome manufacturing process used to safely and reliably produce flu vaccines for decades. Questions arose: Would it be available for the fall '09 flu season? Would it be safe? Who should be immunized first? By May 19, 9,830 confirmed cases of H1N1 were reported in 40 countries, including 79 deaths.[8] Three conditions must be met for the declaration of a viral pandemic: the virus must (1) be a new subtype for which humans have little or no pre-existing immunity, (2) infect humans and cause illness and (3) confer rapid and sustainable human to human transmission in the general population.[7] One by one, each of these conditions was met. On June 11, the World Health Organization (WHO) raised the pandemic threat status to "Alert Phase 6"—the highest warning available—formally declaring the first influenza pandemic in 40 y.[9] At the onset, the pandemic virus was resistant to one class of antivirals, matrix-channel blockers (adamantine) but was sensitive to another class called neuraminidase inhibitors [e.g., oseltamivir (Tamiflu), zanamivir (Relenza) and peramivir (BioCryst Pharmaceuticals)].[10] The widespread use of antivirals resulted in oseltamivir-resistant mutants in patients, but fortunately they remained sensitive to zanamivir.[11] Then nature fought back. On January 11, 2010, a multidrug-resistant strain emerged from a fatally-infected immunocompromised patient that conferred resistance to all three neuraminidase inhibitors—leaving physicians with few treatment options.[12,13] The CDC estimates that from April 2009 to April 2010, 61 million people were infected, resulting in 274,000 hospitalizations, and 12,470 deaths in the US alone.[14] Now the good news: The pandemic could have been much worse. The H1N1 (2009) virus was mild relative to other pandemic viruses since it lacked a few key virulence functions that compromise innate immune cytokine responses via disruption of antiviral signaling (PB2,[15] PB1-F2[16] and NS1[17])—which would have amplified the cost and casualties considerably.[10,18-23] And remarkably, the H1N1 vaccine was made available for the fall 2009 flu season—in record time. On August 1, 2010 the

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Submitted: 11/05/12; Revised: 12/19/12; Accepted: 12/20/12
http://dx.doi.org/10.4161/viru.23380

WHO declared "post-pandemic status," officially ending the pandemic threat alert.[24] Humanity dodged a bullet—for now—but nature's genetic lottery continues to lead us back to the past. A novel avian flu (H5N1) strain, for example, is currently circulating in birds that has high pathogenic and pandemic potential if/when a variant arises that confers increased human to human transmission.[25-27] It is only a matter of time.

Influenza A virus is a single-stranded RNA virus comprised of eight gene segments encoding for 12 protein products, including two surface proteins that have reciprocal functions, hemagglutinin (HA) and neuraminidase (NA).[28] HA binds the virus to sialic acid receptors located on respiratory epithelium, whereas NA functions to release progeny virus from infected cells via sialidase activity. Sixteen HA and 9 NA types have been identified and form the basis for the classification of influenza A viruses into subtypes. Further variation among viruses exists and thus isolates are identified by a standard nomenclature specifying: virus type, geographical location based on first isolation, sequential number of isolation, year of isolation and HA and NA subtype, e.g., A/California/7/2009 (H1N1).[7] Influenza mutants are classified into two major groups: those that arise via "antigenic drift," characterized by relatively minor changes in either HA or NA as seen in "seasonal" influenza strains; and those that arise via "antigenic shift," characterized by marked changes in these proteins (and others) that occur when two different viruses recombine or reassort chromosomes with the potential to generate new pandemic influenza strains for which humans have little or no pre-existing immunity. The emergence of H1N1 (2009) was due to antigenic shift, whereby successive rounds of recombination and reassortment of chromosomes from different viruses led to the origin of a new virus subtype not previously detected in humans or animals.[18,29,30]

The identity of "Patient Zero" of the H1N1 pandemic is unknown and will likely remain so. However, the genetic identity of the H1N1 (2009) virus is known to have occurred by means of viral evolution and cross-infection between humans, birds and pigs of viral strains that circulated previously—a quadruple reassortant virus comprised of elements from North American swine (30.6%), Eurasian swine (17.5%), North American avian (34.4%) and North American human (17.5%) influenza viruses.[5,10,18,21,29,30] Each genetic segment of H1N1 genes circulated in swine populations for at least 10 years prior to the genetic reassortment leading to the origin of the virus.[18] Contact between pigs from Eurasia and North America may be responsible for the mixing of viruses necessary to generate the H1N1 virus.[18] And human involvement likely contributed—perhaps in the international hog trade—since there are distinct subtypes among swine influenza A viruses from geographically distant areas due to the lack of contact between these animals[21] and the virus can be transmitted from pigs to humans and from humans to pigs.[28]

Lessons learned: Recommendations have been developed in response to the H1N1 pandemic. (1) Improved surveillance of influenza viruses in pigs is necessary to avoid a pandemic of similar origin.[10,18,28] (2) Obesity, pregnancy and age (> 50 y) have been identified as risk factors and early antiviral therapy is necessary to avoid severe complications.[6,31-33] (3) Infection-control measures for immunocompromised patients need to be strictly enforced to impede the emergence of multidrug-resistant viruses in this patient cohort.[6,12,13]

Meningitis (Africa, 2009): Pray for Rain

The Meningitis Belt in sub-Saharan Africa has the highest incidence of bacterial meningitis of any region in the world. Meningococcal disease can develop rapidly, with an incidence approaching 1% of the population and mortality rates exceeding 20%.[34-38] The misery continues long after the epidemic subsides as 10 to 20% of survivors are left with serious debilitating disease—brain damage, deafness and learning disabilities. Epidemics have a predictable episodic cycle: they begin with the dry season—cease with the onset of rain—and resume during the next dry season. This cycle repeats for a duration of 2 to 3 y for a given epidemic.[36] Why the dry season? It signals the arrival of the Harmattan trade winds that churn desert sands with a power so fierce they can block the sun. And with cold nights and seasonal respiratory infections, they combine to damage epithelial tissue of the human upper respiratory tract, which is now ripe for infection with *Neisseria meningitidis*, a natural inhabitant of this site in up to 40% of the human population.[37,39] Also linked to the dry season are events associated with extreme crowding, including pilgrimages and assembly in market places.[38] Collectively, this scenario is a recipe for epidemic disaster. Meningococcal strains are classified into serogroups defined by the composition of a protective polysaccharide capsule that surrounds the microbe. Serogroup A strains are responsible for African epidemics, whereas serogroups B and C are more prevalent in industrialized nations.[34,37,38] Reasons for the geographic distribution of these serogroups remain unclear. The last epidemic (1996) caused by serogroup A was among the largest in African history.[40] The toll: 250,000 cases, 25,000 deaths and thousands with long-term debilitating disease. Epidemics occur every 7 to 14 y and it was time once again.[38] In 2009, two strain variants, serogroups W-135 (ST-11) and X (ST-181), emerged in West Africa that were similar to those seen in earlier cases linked to pilgrimages to Saudi Arabia and localized occurrences in West Africa.[41-43] In a triple-threat attack, the W-135 and X strains combined with serogroup A strains to cause the largest outbreak in the Meningitis Belt since 1996. The toll in 2009 alone: 88,199 cases with 5,352 deaths.[38] With limited access to early diagnosis, antimicrobial therapy and vaccination, residents in the region could do little else but pray for rain.

Epidemic meningitis remains a global health problem with 1.2 million cases worldwide and up to 135,000 deaths annually.[44] Infection with the bacterium can cause a spectrum of illnesses ranging from meningitis and septicemia to pneumonia and arthritis, as well as brain and nerve damage.[45] *N. meningitidis* adheres to nasopharyngeal mucosal tissues via filamentous structures called type IV pili, after which the bacteria proliferate in tight aggregates called microcolonies on the epithelial cell surface.[46] This host cell contact stimulates a microbe-mediated chemical modification of type IV pili that triggers detachment, dissemination, and migration of the bacteria across the epithelial

cell surface. The resultant bacteremia provokes infection of other tissues such as the lung, meninges (lining of the brain) and central nervous system. Initial diagnosis of meningococcal meningitis is made by clinical examination (stiff neck, high fever, headaches and confusion) followed by a lumbar puncture and examination of the spinal fluid.[38,47] Early diagnosis and antibiotic treatment is critical since up to 10% of patients die within 24–48 h after the onset of symptoms. Thus, vaccines have been deemed the most viable approach to minimize human suffering resulting from meningococcal disease. Vaccine efforts have been hampered by the fact that *N. meningitidis* is the "chameleon" of the microbial world. It can rapidly modulate its surface structures (via antigenic variation and phase variation) and switch capsular serogroups with other strains to effectively evade immune clearance mechanisms.[37,47-49] Now the good news: In response to the 1996 epidemic, the Meningitis Vaccine Project (MVP) was established in 2001, spearheaded by the WHO and Program for Appropriate Technology in Health (PATH).[36,40,50] The consortium's ambitious goal: eliminate serogroup A epidemics in Africa. This effort led to the successful development of "MenAfriVac" (Serum Institute of India), a vaccine that was recently shown to be safe and effective against infection with serogroup A strains in African clinical trials.[36,37,40,50] The cost is 40 cents a dose. The "conjugate" vaccine links serogroup A polysaccharides to a protein carrier that stimulates T-cell dependent immunity and an effective memory response for long-term-protection. Immunization reduces the number of asymptomatic carriers and provides protection to nonvaccinated individuals via "herd immunity." This has led to a massive vaccine campaign to immunize 250 million people in 25 African countries between 2010 and 2015.[51]

Lessons learned: The success of the Meningitis Vaccine Project has given hope to ongoing efforts for the eradication of meningococcal disease.[36,37,47,49] Efforts include: (1) Development of multivalent vaccines that confer protection against other serogroups (W-135 and X). (2) Implementation of improved surveillance along with genotypic strain typing to detect fluctuations in incidence and shifts in serogroups and genotypes. (3) Development of novel vaccination methods for serogroup B strains, which are necessary since B-capsule similarity to neuronal cell adhesion molecules renders it poorly immunogenic due to immune tolerance.

Cholera (Haiti, 2010): The Perfect Storm

Cholera epidemics have plagued mankind for centuries but had spared the island nation of Haiti. That was about to change. On January 12, 2010, an earthquake decimated the country—leaving a quarter of a million people dead and 2 million homeless.[52] Nine months later, on October 21, a cholera outbreak was confirmed in Haiti.[53] It spread like wild fire—fueled by inadequate sanitation, clean water and health care infrastructure, as well as Hurricane Thomas, which struck the island on November 5–6.[52,54,55] By December 17, a total of 121,518 cases of cholera, resulting in 63,711 hospitalizations and 2,591 deaths, were reported.[56] The outbreak strain was identified as *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor.[53] DNA sequencing indicated that the outbreak strain was remarkably similar to those endemic to South Asia and not those of neighboring Latin American regions.[57] Epidemiological and molecular analysis confirmed that the outbreak strain was inadvertently introduced into Haiti by United Nations security forces from Nepal, wherein cholera outbreaks had occurred shortly before troop deployment.[58-61] Insufficient treatment of sewage from the Haitian encampment led to contaminated river water that was the likely route of spread. These findings led to considerable political unrest in a country that was ravaged by nature and a disease that was imported during relief efforts—a perfect storm. The cholera outbreak caused nearly 300,000 illnesses and more than 4,500 deaths, and continues to sicken people and claim lives in Haiti.[61] These events exemplify the explosive and lethal nature of cholera outbreaks, and have forever changed the global response to natural disasters.

Cholera is one of the most rapidly fatal diseases known, capable of killing within 12–24 h of the onset of diarrhea (several liters/day).[62] The WHO estimates 3 to 5 million cholera illnesses and up to 130,000 deaths occur globally each year.[63] Mankind is suffering the seventh cholera pandemic since 1817.[63,64] The first six pandemics were caused by the "classical" biotype that has been replaced with the "El Tor" biotype (origin: Indonesia, 1961)—the causative agent of the most extensive cholera pandemic in modern history. The El Tor biotype confers increased environmental persistence and asymptomatic carriage in humans and, thus, may be more likely to become endemic upon introduction to a naïve region.[62] The El Tor biotype has undergone two major changes in the last two decades.[62,64] First, it acquired a new lipopolysaccharide (LPS) structure—from O1 to O139—that resulted in a marked capacity to cause disease in previously immune populations.[65,66] Second, the O139 El Tor strain was subsequently replaced with an O1 El Tor "hybrid" variant that had acquired the more potent cholera toxin from the classical biotype as well as other factors that are associated with increased human disease, dissemination/transmission and environmental persistence.[57,67-69] Accordingly, the O1 El Tor hybrid variant has the capacity to fuel explosive and lethal disease outbreaks on a global scale. It has now become the predominant strain in many regions of Asia[67-69] and may replace the pandemic strains in Latin America following its introduction to the region during the Haitian outbreak.[57]

Bacteriophages play a key role in the evolution and emergence of more potent *V. cholerae* strains via horizontal gene transfer and bacteriocidal selection[64]—most notably with the acquisition of cholera toxin (CT) that is encoded on a lysogenic bacteriophage (CTXϕ).[70] Further, genomic analysis of pandemic strains has indicated that other phages have supplied genes involved in increased virulence in humans and/or increased environmental fitness.[71] Bacteriophages are also involved in the natural control of cholera epidemics via "phage predation"—a bacteriocidal mechanism whereby phage-sensitive strains are selectively killed in favor of those that are phage-resistant.[72-74] Collectively, these phage-mediated processes likely contributed to the elimination of the classical biotype and the origin/maintenance of the El Tor biotype—responsible for the most extensive cholera pandemic in duration and geographic spread in the modern world.[64]

Lessons learned: Recommendations have been developed in response to the Haitian cholera outbreak.[52,54,62,75] (1) Provisions for adequate sanitation, clean water and minimal health care infrastructure are necessary to meet acute patient needs and for education of both residents and response personnel. (2) Antibiotic administration at the onset may cull transmission and prevent lethal outbreaks as *V. cholerae* is hyperinfectious after human passage;[76] more potent, single use drugs are now available. (3) Prophylactic vaccination, reactive vaccination (during an outbreak) and vaccine stockpiling comprise a comprehensive approach toward the prevention and containment of cholera outbreaks.

E. coli (Germany, 2011): A Royal Flush

The foodborne outbreak of hemolytic uremic syndrome (HUS) in northern Germany (2011) embodies the fact that massive multi-national foodborne outbreaks are now a reality—irrespective of food safety standards. In May through June of 2011, two separate outbreaks of bloody diarrhea and hemolytic uremic syndrome occurred in Europe. One was centered in Germany and comprised 3,816 cases of bloody diarrhea, 845 cases of HUS and 54 deaths; whereas the other was centered in France and comprised 15 cases of bloody diarrhea, nine of which progressed to HUS.[77-83] Both outbreaks were caused by a strain of Shiga toxin-producing *Escherichia coli* (STEC) of serotype O104:H4, representing the highest frequency of HUS and death recorded from a STEC strain. Epidemiological investigation determined that contaminated sprouts were the source of the outbreak: a consequence of tainted fenugreek seeds from an exporter in Egypt that were obtained by a German seed distributor supplying a German sprout farm.[84] A portion of the original seed shipment was also sent to an English seed distributor, which repackaged the seeds, and supplied them to French garden stores, leading to the outbreak in France.

Clues to the origin of the outbreak strain have come from genome analysis revealing similarity to an enteroaggregative *E. coli* (EAEC) strain isolated from an HIV patient in the Central African Republic with persistent diarrhea.[85,86] However, the O104:H4 outbreak strain harbors a prophage encoding Shiga toxin 2 (Stx2), a potent eukaryotic protein synthesis inhibitor,[77,86,87] Stx2 causes damage to the colon and the microvasculature in humans, characterized by hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome (hemolytic anemia, thrombocytopenia and acute renal failure).[88,89] The outbreak strain also possesses an unusual assortment of EAEC virulence factors that may promote colonization, biofilm formation and mucosal damage—which could also facilitate the production/dissemination of Stx2 into circulation. The O104:H4 outbreak strain, for example, harbors the EAEC virulence plasmid that mediates colonization/biofilm formation (type I aggregative adherence fimbriae[88,90]) and bacterial movement along the intestinal mucosa (dispersin[91]). It also contains EAEC chromosomal virulence factors—Shigella enterotoxin 1 (ShET1)[92] and an unusual assortment of serine proteases [serine protease autotransporters of Enterobacteriaceae (SPATEs)]—thought to play a role in mucosal damage and colonization.[93,94] Lastly, the O104:H4 outbreak strain possesses a multidrug-resistance plasmid encoding extended-spectrum β-lactamase (ESBL) that render the bacterium resistant to several antibiotics.[77,86] The selective pressure for maintenance of ESBL is likely due to environmental exposure as patients with STEC infections are not usually treated with antibiotics since these drugs may promote toxin production.[59,95] This scenario issues a clear caution to physicians treating patients infected with newly-emergent pathogens.[86] Collectively, these data support the view that the O104:H4 outbreak strain harbors an unusual assortment of virulence functions that provide a means for increased pathogenicity, heightened disease manifestations and multidrug resistance—a veritable royal flush.

Shedding from asymptomatic animal reservoirs likely applied to the chain of events leading to the *E. coli* O104:H4 strain outbreak, as animal reservoirs provide a continued source of contamination of food and water supplies leading to human disease.[96] Healthy cattle, swine and deer are asymptomatic carriers since they lack the Stx2 glycolipid receptor, globotriaosylceramide (Gb3),[97] which promotes attachment, translocation and resultant intoxication in humans via Stx-mediated inhibition of protein synthesis.[88,89,96] Tainted water may have led to contamination of sprout seeds that were exported from Africa and distributed to farms in Europe, wherein sprouts were consumed with resultant disease in local residents and those abroad due to travel to and from the source area. Notably, an emergency treatment protocol was approved to reduce organ damage and mortality in severely ill O104:H4 patients. It involved the administration of a humanized monoclonal antibody against complement component C5 (eculizumab, Alexion Pharmaceuticals) to reduce complement activation, and the protocol had promising patient outcomes.[98] However, the long and short-term effects of this and other promising therapies—as well as the extremely high costs of these innovative procedures—have yet to be fully vetted.

Lessons learned: A multi-national investigation of the *E. coli* O104:H4 outbreak has led to the following recommendations.[99] (1) Develop diagnostic methods for emergent STEC and integrate molecular typing into routine surveillance. (2) Utilize product tracing as an epidemiological tool. (3) Research the pathogenesis, clinical course and new treatment options (antimicrobials and antitoxin). (4) Study pathogen evolution among the human host, the environment and in animal reservoirs.

Salmonella (2012): The Trojan Horse

Salmonella is the greatest foodborne disease burden in the US—representing the leading cause of infections, hospitalizations and deaths—1.03 million illnesses with the medical costs alone reaching $11.4 billion per year and billions more being incurred by the food industry (recalls, litigation and reduced consumer confidence) and by state, local and federal public health agencies acting in response to Salmonella outbreaks.[100-102] The disease burden is mind-boggling when translated on a global scale, with an estimated 93.8 million cases of salmonellosis and 155,000 deaths each year,[103] and Salmonella has emerged as the leading cause of bacteremia in sub-Saharan Africa with a case fatality rate up to

25%.[104,105] Moreover, the problem has been exacerbated due to the prolonged administration of antibiotics to livestock that has resulted in the emergence of multidrug-resistant strains that have disseminated globally. The pandemic spread of *S.* Typhimurium DT104, for example, has caused a high number of foodborne disease outbreaks over the last two decades and is resistant to four of the five most commonly used antibiotics in veterinary medicine (tetracycline, β-lactams, aminoglycosides and sulfonamides).[106,107] These multidrug-resistant strains are more virulent and cause increased hospitalizations and bacteremia.[108,109] Now the bad news: the Salmonella disease burden is poised to worsen with the potential emergence of more potent strains that, when combined with multidrug resistance, pose a significant health risk due to the lack of therapeutics available to fight these infections when they occur.[110-113] These dire predictions may have been realized as "hypervirulent" Salmonella were isolated from natural microbial populations (2012) that are among the most virulent microbes encountered of this species.[114] These strains are 100 times more virulent then other clinical isolates, more capable of killing vaccinated animals and not detectable under standard laboratory test conditions as the hypervirulent state is only expressed during the infective process. The key to their identification was to assess virulence immediately after infection before their rapid transition to a less-virulent state outside of the animal. These hypervirulent strains utilize a "Trojan Horse" strategy whereby virulence functions are only revealed during the infective process (**Fig. 1**). Entry into an animal host induces distinct transcriptional responses in hypervirulent strains that were not altered, or altered to the same extent, in conventionally virulent strains. Such altered gene expression is characterized by elevated production of an actin cytotoxin and immunomodulatory molecules that, in combination, confer profound effects on microbial virulence and the capacity of the host to mount an effective immune response. Reciprocally, exposure to ex vivo conditions signals a rapid reversion to a less-virulent state characterized by more competitive growth in the environment. This rapid and reversible switching between virulence states provides a means to quickly adapt to disparate hosts/environments without undergoing irreversible changes in the genome, and may contribute to the maintenance of hyperinfectious strains in nature. These events have sounded the alarm to the medical community, as hypervirulent strains of other pathogens may be present among natural microbial populations—posing a previously unrecognized risk to human health.

Salmonella is acquired via the fecal-oral route and is comprised of more than 2,500 serovars (serological variants) based on carbohydrate, lipopolysaccharide and flagellar composition;[112,115,116] and can result in any of four distinct syndromes: enterocolitis/diarrhea, bacteremia, enteric (typhoid) fever and chronic asymptomatic carriage.[117,118] Salmonella puts everyone at risk irrespective of dietary preferences—poisoning meats, poultry, livestock-derived food products, fruits, nuts and vegetables. In the US (2011–2012), the CDC has reported several Salmonella outbreaks due to the consumption of contaminated ground beef, turkey burgers, chicken livers, fish, cantaloupe, mangoes, papayas, pine nuts, alfalfa sprouts, dry dog food, peanut butter or

Figure 1. Hypervirulent Salmonella utilize a "Trojan Horse" strategy—exposing their virulence functions only during the infective process—but appearing much like other less-virulent strains in the environment. Entry into an animal host signals a dramatic shift in gene expression that is characterized by elevated toxin production coupled with the disruption of the host innate immune cytokine response necessary to execute antimicrobial activities. Exposure to ex vivo conditions signals a rapid transition to a less-virulent state characterized by more competitive growth in the environment. This rapid and reversible switching allows the bacterium to rapidly adapt to disparate hosts/environments without undergoing irreversible changes in the genome—providing a means for increased immune evasion/disruption, heightened disease and increased maintenance in nature. Hypervirulent Salmonella are among the most virulent of this species and are difficult to detect by current diagnostics. Credit: Peter Allen, UC Santa Barbara.

via exposure to any of a wide range of live "backyard" animals (poultry, turtles, frogs and hedgehogs).[119] The number of illnesses reported during each of these outbreaks represents only the "tip of the iceberg" as it is estimated that for every confirmed case, there are as many as 30 that go unreported.[100] Therefore, an outbreak of 350 reported cases may affect more than 10,000 people. Moreover, false implication of food products can be devastating to a particular industry; e.g., tomatoes were falsely implicated in the Salmonella "jalapeno and serrano pepper" outbreak (2008), resulting in losses exceeding $200 million as tomato consumption plummeted.[120] Salmonella control efforts are problematic due to the widespread distribution and diversity of pathogenic strains in animal reservoirs and water supplies.[121-123] The standard approach for many years has been the widespread use of antibiotics in livestock, but this option has now become limited due to the emergence of multidrug-resistant strains that are a bona fide risk to human health.[110-113] To improve food safety, the FDA and USDA have thus made the development of improved methods to

reduce Salmonella contamination from farm-to-fork a top priority, with particular emphasis on reduced contamination of food and water supplies at the outset of the livestock production process.[120,124,125] Early monitoring, intervention and prevention methods, for example, would reduce pathogen exposure, transmission, animal disease and the direct contamination of livestock-derived food products as well as the indirect contamination of fruit and vegetable food products via contaminated water.

Lessons learned: Recommendations have been developed to limit the size, frequency, and severity of foodborne outbreaks, the emergence of multidrug-resistant strains, and the false implication of other food products.[120,124,125] (1) Elimination of growth-promoting antibiotics in animal feeds. (2) Development of cross-protective vaccines. (3) Irradiation of post-harvest foods. (4) Improved monitoring by the FDA and USDA. (5) Improved surveillance/traceability for source-product identification.

Conclusions: Dead Smart

Infectious disease outbreaks manifest the impact of emergent pathogens on human health, animal welfare and modern agriculture—a clear indication of how fragile public health has become. Pathogen emergence is fueled by the acquisition of genes encoding novel virulence functions and/or the altered expression of pre-existing functions. The resultant shifts in serogroups and genotypes lead to an unusual assortment of virulence capabilities for which humans have little or no pre-existing immunity. In some cases, genetic alterations in one species results in the acquisition of variations that allow them to overcome barriers and infect new hosts—in these cases, devastating outbreaks can occur. A paradigm for this process is cross-species virus transmission and emergence of new epidemic diseases: HIV, Marburg and Ebola.[126-128] Human activities have hastened these natural processes of microbial evolution. This is evident in the aftermath of recent infectious disease outbreaks, highlighting the importance of adequate sanitation, clean water, health care infrastructure and the implementation of improved surveillance/genotyping that monitor pathogen emergence. It is also evident in our public health care systems wherein hospitalizations for septicemia or sepsis have more than doubled from 2000 to 2008 at an annual cost of $14.6 billion in the US alone.[129] Numerous factors are thought to have contributed to the rise in sepsis patients including an aging population with chronic conditions, increased use of invasive procedures, immunosuppressive drugs, chemotherapy and increased use of antibiotics—creating a fertile breeding ground for multidrug-resistant superbugs.[130,131]

Physicians must carefully evaluate patient treatment practices from hospital admittance to discharge and beyond to avoid exacerbating the current vicious (and deadly) cycle: more potent pathogens—more acute illnesses—more aggressive treatments—and the emergence of even more potent pathogens. Totally drug-resistant (TDR) *Mycobacterium tuberculosis* has emerged with treatment options limited to removal of portions of the infected lung (Iran, 2009; India, 2012).[132,133] Extensively drug-resistant *Streptococcus pneumoniae* (South Korea, 2012)[134] has recently forced treatment of pneumococcal infections with vancomycin—usually reserved for patients with MRSA (methicillin-resistant *Staphylococcus aureus*).[135] Vancomycin-resistant *S. aureus* strains now abound in the United States and elsewhere.[136] Notably, multidrug-resistant strains are often more virulent—causing more illnesses, hospitalizations and deaths[108,109]—and their enrichment and maintenance in nature can occur at extremely low antibiotic concentrations that are commonly found in the environment (e.g., ground water).[137] Another example of human activities that have hastened microbial evolution is pneumococcal vaccination. Although highly successful in preventing pneumococcal disease, vaccination has led to changes in capsular serogroup prevalence in human populations.[138] Since each pneumococcal serotype varies in its ability to cause invasive disease,[139-141] some vaccination benefits may be offset by altered serotype prevalence and, in combination with treatment of pneumococcal patients with front-line antibiotics, may lead to the emergence of highly invasive multidrug-resistant strains that escape vaccine-conferred immunity. Lastly, careful consideration must be given to emergent pathogens that may already be lurking within natural microbial populations waiting for the appropriate signal(s) to launch a covert attack on human populations—with potentially devastating outcomes.[114,142]

However, there is new hope in the ongoing battle against infectious diseases. Genomic sequencing methods are now available to rapidly determine the presence of antibiotic resistance genes as well as specific virulence or antigenic determinants in newly emerging pathogens prior to making decisions about what antibiotics or vaccines to administer.[57,86] In addition to new therapeutics, there are a number of novel vaccine efforts that are either ongoing or in the pipeline: universal influenza vaccines against pandemic and seasonal viruses;[143] epidemic meningitis vaccines,[37,47] prophylactic/reactive cholera vaccines,[62,75] STEC vaccines[144] and cross-protective salmonellae vaccines.[125] Meanwhile, as we exhaust all available resources to combat emergent pathogens, nature's genetic lottery is continuous and unrelenting—fueling the rise of the microbes for the next generation. Some things never change.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We would like to thank Bob Sinsheimer for critically reading the manuscript. This work was supported by G. Harold and Leila Y. Mathers Foundation, US Army (W911NF-09-D-0001), Cottage Hospital Research Program (2012-265), USDA Agriculture and Food Research Initiative (2008-01452) (to M.J.M.) and National Science Foundation Graduate Research Fellowship DGE-1144085 (to J.Z.K.).

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