Cards of Virulence and the Global Virulome for Humans

When soil microbes interact with neighboring microorganisms, traits can emerge that may render them pathogenic in animal hosts

Arturo Casadevall

Pathogenic microbes can be put into two general categories: those acquired from the environment and those acquired from other humans and animals. Microbes that are acquired from the environment typically can survive in environmental niches and also in their hosts. In contrast, microbes acquired from other human and animal hosts usually have complex relationships with the originating host, and may utterly depend on their hosts to replicate and survive. Furthermore, diseases caused by environmentally acquired microbes may not be communicable, whereas those caused by host-derived microbes may depend on host-to-host spread for survival of the microbe.

For example, endemic pathogenic fungi such as *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Coccidioides* spp. are found in soils and have no apparent requirement for animal hosts. Yet, these organisms can cause disease in numerous animal hosts and are generally considered nonspecific pathogens. At the other extreme, viruses completely depend on their hosts to replicate and survive, and often are specially adapted to particular hosts. Somewhere between these two extremes one finds microbes such as *Bacillus anthracis*, which lives in soils for prolonged periods but amplifies greatly when it causes anthrax in mammalian herbivores.

The virulence in microbes acquired from other animal and human hosts can be understood as an interaction that damages the new host. For such microbes, infecting new hosts may be essential for their survival, whereas causing damage is not. For example, because all viruses depend on host cell machinery to replicate, they absolutely require a susceptible host to survive. Similarly, many parasites require one or more hosts to complete their respective life cycles, but do not immediately damage their new hosts.

Many free-living bacteria, protozoa, and fungi with pathogenic capacities do not require eukaryotic hosts to survive and face no obvious selection pressures to cause or avoid causing damage to human and animal hosts, with the caveat that killing these hosts will likely return these microbes sooner to their ordinary environmental niches. The potential for soil microbes becoming virulent thus raises some fundamental questions about the origin of virulence. Recent studies of interactions of soil mi-

Summary

- Through interactions with other competing organisms in soil, microbes may acquire traits that lead to virulence when they encounter animal hosts.
- Each soil-dwelling microbe possesses a unique combination of characteristics, including some held widely in common such as the ability to form biofilms, acquire iron, or produce proteolytic enzymes, that can contribute to virulence.
- Not only can soil-dwelling microbial pathogens acquire fitness traits when passing through animal hosts, these sojourns provide opportunities to change rapidly and to move between particular soil environments.
- The virulome for humans consists of an enormous set of traits for configuring both established and emerging pathogenic microbes.

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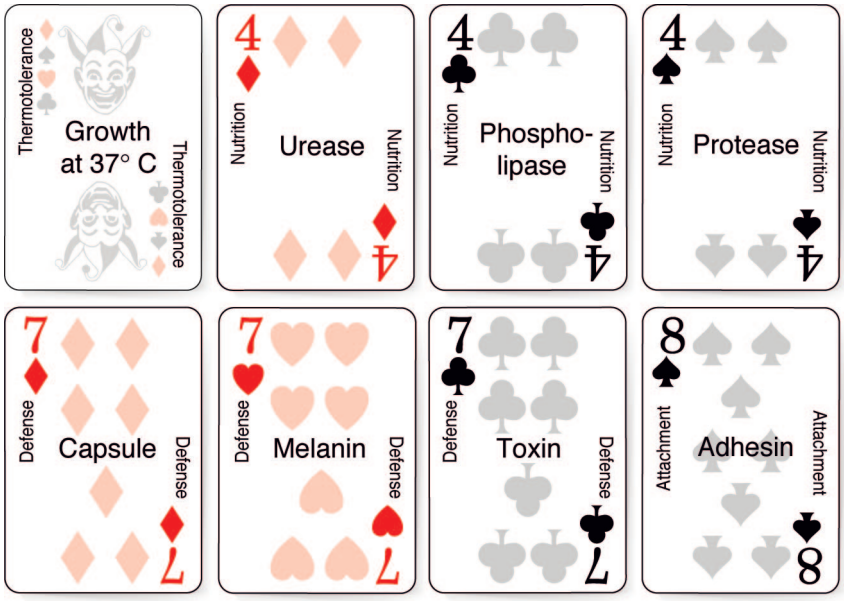
FIGURE 1



The cards of virulence, where each virulence factor is considered a card that contributes to the overall virulence phenotype.

crobes with other microorganisms, such as fungi and amoebae, suggest ways in which virulence can emerge in microbes that otherwise do not require animal hosts.

Microbes Play a Metaphorical Card Game within an Extreme Environment

Soils teem with countless bacteria, fungi, phage, viruses, protists, and small animals—all competing for nutrients in an environment that is subject to extremes of humidity, oxygen tension, temperature, electromagnetic radiation, ionic strength, and pH, because of diurnal cycles, seasons, and climates. For example, 1 g of soil may contain 10^9 microorganisms from more than 4,000 microbial species. Many of them produce powerful enzymes to break down dead organic matter, while some devour other microbes as nutrient sources.

Meanwhile, phagocytic unicellular organisms such as amoebae, slime molds, and protists such as paramecia and hydra also prey on bacteria and fungi for nutrition. Small worms and snails provide yet another layer to the food chain feeding on the microbial flora. Many soil organisms are subject to infections and selective pressures from bacteriophages and viruses. Hence, soils are "extreme" environments.

Nonphagocytic microbes in soils depend on hydrolytic enzymes, including proteases, phospholipases, lipases, and DNAases, to digest nearby organic matter. To ward off phagocytic predators, soil microbes depend on various chemical defenses, including capsules and toxins. Furthermore, each microbial species in soil has its own optimal growth range regarding pH, temperature, and other chemical and physical factors. Other elements of diversity include different means for acquiring iron, quorum sensing, forming biofilms, and two-component signaling.

With such extensive genetic diversity, each soil microbe possesses a unique combination of characteristics and attributes that is analogous to a set of cards in a game of survival. Moreover, in this complex card game, each soil microorganism plays uniquely different hands, albeit with some players holding some cards in common (Fig. 1). Among the commonly held cards are biofilm formation, iron acquisition, and enzymes such as proteases to acquire foods.

Soil Microbes Encountering Hosts

For soil microbes, a host is any living entity that can encompass, constrain, or contain them, including unicellular microbes such as amoebas or small invertebrates such as the worm *Caenorhabditis elegans*. Encounters between soil microbes and hosts can lead to one of three outcomes: death of the microbe, death of the host, or persistence of the microbe in the host.

Some soil host-microbe interactions result from unicellular eukaryotic hosts feeding on bacteria and fungi. For example, amoebae and amoeboid slime mold cells ingest and digest bacteria. Because soil hosts are numerous and highly diverse, this environment proves unforgiving for many microbes, suggesting that only the availability of special characteristics allows survival after encounters with soil hosts. Microbes that survive in soils encounter many predatory hosts with different types of receptors and antimicrobial defenses. By contrast, non-

soil-dwelling microbes are more likely to display survival strategies that reflect their coping with only one or a few host species.

Hence, microbes that live in soils are likely to carry a more diverse array of characteristics to defend against predatory hosts than do those microbes that cope with only one host. In terms of the playing card analogy, soil microbes carry a more diverse set of cards than do host-associated microbes.

Soil Microbes Hold Various Cards of Virulence

Pathogenic microbes have virulence factors—or "cards"—that singly or in combination allow them to damage animal hosts. In this scheme, each soil microbe has a different "hand," making the likelihood of any particular animal-microbe encounter resulting in disease a stochastic process. Hence, only those microbes with the required virulence cards could colonize a particular animal host.

Consider the thermotolerance card that enables a microbe to persist at higher-than-ambient temperatures. Such a card could be essential for this microbe to cause disease in a mammal but not in insects or invertebrates. Other traits that would contribute to virulence in mammalian hosts include proteolytic enzymes, phospholipases, and enzymes for producing melanin.

Let's compare the human pathogenic fungus *Cryptococcus neoformans* to 21 other species of heterobasidiomycetous yeasts. Among them, only *C. neoformans* has a capsule gene associated with virulence, the ability to survive at mammalian body temperatures, and the ability to make melanin, a pigment associated with virulence. We do not know how *C. neoformans* acquired these traits. Because of the close concordance between cryptococcal factors that lead to its virulence for both mammals and amoebae, these traits may reflect adaptations that enhance the survival of *C. neoformans* when faced with amoeboid predators (see table).

Histoplasma capsulatum, a facultative intracellular fungal pathogen of mammalian macrophages, is commonly found in the Ohio and Mississippi river valley soils. This fungus adheres to and is ingested by mammalian macrophages where it survives in phagosome by inhibiting acidification. Although *H. capsulatum* causes disease in many animal species, contact with macrophages must be an exceedingly rare event for the overwhelming majority of *H. capsulatum* organisms that currently inhabit the planet. What selective pressures led to its exquisite strategy for intracellular pathogenesis within macrophages? The answer perhaps arises from the observation that *H. capsulatum* replicates inside amoeba. Hence, like *C. neoformans*, pressure from phagocytic predators in the soil could have selected for an intracellular survival strategy that is readily applicable to survival in macrophages.

Dual-use virulence factors of *Cryptococcus neoformans*

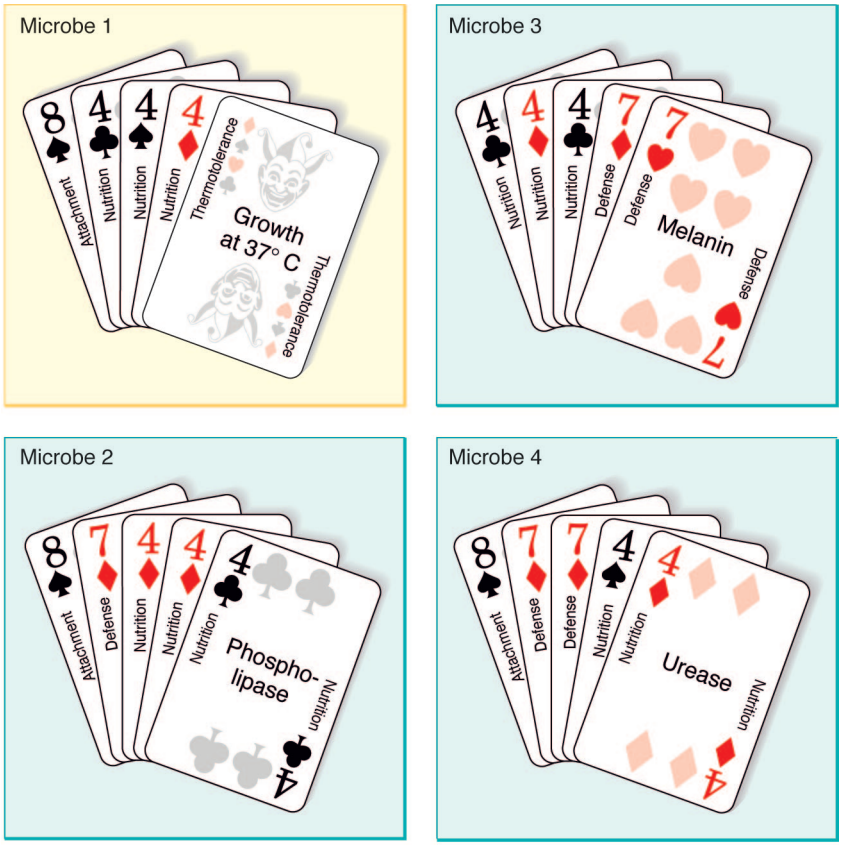
Virulence Factor	Function in mammalian virulence	Function in environment
Capsule	Antiphagocytic Immunomodulator Intracellular replication	Antiphagocytic Delays desiccation Radioprotector
Melanin	Free radical sink Immunomodulator Fe acquisition?	Enhances survival in amoebae Protects against heavy metals Protects against temperature extremes Resistance against enzymatic degradation Fe acquisition? Protects against ultraviolet light
Phospholipase	Intracellular replication Eicosanoid production	Nutritional role Survival in amoebae
Proteases	Host tissue damage	Nutritional role
Laccase	Melanin synthesis Interference with oxidative burst	Melanin synthesis Wood degradation
Urease	Microvascular sequestration	Nutrition
Mannitol	Antioxidant	Osmotic stress Thermotolerance

Traits Conferring Virulence Play Other Roles That May Depend on Context

Medical mycologists share an intense interest in fungal dimorphism, in which pathogenic fungi undergo a morphological transition between yeast and hyphal forms. For example, *H. capsulatum* and *Blastomyces dermatitidis* exist as yeast cells at temperatures that prevail within mammalian hosts but as hyphal cells at lower temperatures that are more likely encountered in soils.

Yeast and hyphal cells express different types of molecules and surface antigens. Because tissue forms of these fungi are usually yeast cells, dimorphism is considered central to virulence in dimorphic pathogenic fungi. Although switch-

FIGURE 2



Microbial card sets for four hypothetical microbes. Each microbe has a set of cards that includes potential virulence factors. The ability of a microbe to cause disease in a particular host species is a function of the virulence traits that are available to that microbe. Microbe 1 has the potential for being virulent in a mammalian host because it is the only one with the ability to grow at 37 °C.

ing between ambient and mammalian temperatures can induce morphological transitions between yeast and hyphal forms, a switch from yeast to hyphal form also is triggered when *H. capsulatum* is exposed to amoebae.

Shifting to the hyphal form may enable *H. capsulatum* to escape phagocytic predators. However, the yeast form of *C. neoformans* proves more vulnerable to amoebae than does its pseudohyphal cell form. Thus, the relative resistance of these two cellular morphotypes to phagocytic predators appears to vary with each microbial species, and perhaps for each phagocytic predator.

When considered in the environmental context, factors that render soil microbes virulent for animals sometimes have other ways of enabling them to survive in soil. Even those virulence factors that appear highly specialized may play equally critical roles in soil environments that are totally different from their role in pathogenesis in animal hosts. However, other virulence factors, such as proteases and phospholipases, may play similar roles in both animal hosts and the environment such as helping to acquire nutrients while, in the case of pathogens, also destroying host tissues.

In contrast, other virulence factors appear to play radically distinct roles in the host and the environment. For example, the major adhesin of *B. dermatitidis* is a surface protein known as BAD-1 that is also a powerful immune modulator. Because BAD-1 is involved in acquiring calcium, it likely also plays a functional role in the environment. Similarly, HSP90, a major adhesin of *H. capsulatum,* enables it to bind macrophages, but presumably acts as a surface heat-shock protein when it grows in soils. When either *B. dermatitidis* or *H. capsulatum* grows in soils, it seems unlikely that either BAD-1 or HSP90 is expressed in these fungi, respectively, for pathogenic purposes. Instead, these properties might be examples of molecular mimicry, endowing each of these organisms with the ability to bind critical animal cell structures.

Pathogenic Traits Might Provide Advantages in Soil Environments

Soil-dwelling microbial pathogens might acquire traits that improve fitness in the environment when passing through their animal hosts. In a sense, animal hosts offer extreme environments for such pathogens, bombarding them with antimicrobial molecules, restricting iron and other nutrients, and unleashing phagocytic cells to engulf and digest them.

Because the number of microbial encounters with animal hosts is enormous, some microbial variants that return to the soil when the host dies and decomposes likely arrive there endowed with traits arising from immune selection that could affect fitness in that environment. For instance, a specific microbe that is ordinarily

under selection by soil phagocytic predators may return to the soil from a mammalian host with characteristics that affect its susceptibility to those soil-dwelling hosts. Stochastically, passaging repeatedly through animal hosts and the extremes of the soil environment could provide the microbe with new dual-use cards for surviving in soil and animal environments.

Animal passage also provides opportunities to travel between specific soil environments. Soil microbes ordinarily are limited in their ability to disperse to different ecologic niches. However, animal passage can be an effective mechanism for transporting and dispersing them. For example, comparing *Coccidioides* spp. isolates from the North American Southwest and South America led investigators to propose that the latter reached their current range because of human migrations. Apparently, *Coccidioides* spp. that were acquired by humans or domestic animals in North America were later transported to South America, and presumably became established in this nonnative soil after their migratory hosts died.

Passage through an animal host may also provide soil microbes with opportunities for rapid change. For example, the immune system may select for an antigenic variant that was acquired from the environment. Because antigenic differences reflect structural changes on surface components, host-selected antigenic changes may endow the microbe with changes that enhance its survival in soil. Hence, the capacity for virulence, even if accidental, could provide a fast means for acquiring critical changes. Meanwhile, perhaps the broadest defense barrier against environmental microbes becoming pathogens for mammals and birds is endothermy, which restricts growth to those microbes capable of withstanding elevated temperatures.

The Global Virulome for Humans

The concept that soil pressures can select for traits that also confer virulence leads to the sobering thought that soil or other environments could be a regular source of new pathogens. Let's designate all traits that could contribute to virulence for a given microbial species its "virulome." Meanwhile, for humans the virulome would consist of all microbial genes that could contribute to virulence in that host, but of course would differ from one host to another. The virulome would not be limited to genes in known human pathogenic microbes but would also include genes found in nonpathogenic organisms that could damage a human host.

The global virulome for a particular species is undoubtedly much larger than the sum of virulence genes in its known pathogenic microbes. For example, we know that capsules are important virulence factors for many bacteria and fungi, yet the capsules of known pathogens are only a minute proportion of all capsule-like structures in the microbiota. Similarly, iron limitation is an important mammalian defense mechanism, yet many microbes that are not pathogens have means for acquiring iron that readily might serve roles in virulence if some of those microbes developed other pathogenic attributes. The global virulome of humans includes all virulence factors from known human pathogens as well as those with the potential to contribute to human disease. Even if we restrict this scope to soil microbes, their enormous diversity includes a staggering number of genes.

Because virulence is a microbial attribute that is expressed only in the context of a susceptible host, virulence factors cannot be identified without some relevant measure of virulence. Nonetheless, it may be possible to identify many new components of the virulome by examining how microbes there interact with hosts such as amoebae, slime molds, and worms, even though this approach could not identify those virulence traits that elicit harmful immune responses in mammals.

The global virulome for humans consists of an enormous set of traits and their combinations for configuring both established and emerging pathogenic microbes. Recombination, mutation, selection, and gene exchange combined with climate changes as well as host and microbial extinctions ensure that global virulomes change with time. Of concern is that the human virulome is a vast resource pool for the construction of biological weapons. Defining the size and composition of the global virulome for humans is probably not possible with our existing technology and knowledge, and may remain beyond the reach of prediction since virulence factors cannot be defined independently of a host. Nevertheless, current efforts to identify virulence genes in many microbial species could help in defining the boundaries of the virulome for humans.

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