What Is a Pathogen?

Developing a definition of a pathogen requires looking closely at the many complicated relationships that exist among organisms

Stanley Falkow

From a practical perspective, bacterial pathogens are defined as microorganisms capable of causing disease. However, microbiologists recognize that pathogenicity represents a form of versatility and specialization that enables certain microorganisms to replicate within specific animals and damage host cells. Although cellular damage is not clinically apparent in many cases, a significant proportion of infected hosts shows signs of disease or dies. The outcome is as dependent on the host as it is upon the properties of the pathogen. In this article, I seek a definition of a pathogen that better describes the biology of pathogenicity, not just the capacity to cause disease.

Where Did Pathogens Come From?

Infectious diseases have been a major cause of human suffering and death throughout history. The major factors that made infectious diseases a leading cause of morbidity and mortality include:

- Populations large enough to sustain host-to-host transfer of pathogens; the predominant diseases of recent millennia likely did not play a major role among early humans. For one thing, humans have lived in large enough populations to sustain familiar pathogens, such as measles, for only about 5,000 years. However, because of their latency and their tendency to recur, diseases such as treponematosis and infections caused by mycobacteria, some protozoans, worms, and viruses of the herpes group, likely afflicted early humans.
- Poverty, competition for food, and crowding early during history led to social unrest, war, and, undoubtedly, the evolution of parasitic species capable of being maintained and transmitted in human populations.
- The domestication of animals—imagine the impact on human (and animal) health when humans began to husband other animal species in close proximity. Thus, many of our best-known epidemic diseases likely came from microorganisms carried by animal species (Table 1) and adapted to humans only rather recently.

We are in a perpetual evolutionary dynamic with our large and small parasites. In a sense, we currently try to meet the onslaught of microbial infections with genes that are essentially those of hunter-gatherers.

What Are the Attributes of Pathogenicity?

Pathogens fall into two basic types. Primary pathogens regularly cause disease among at least a portion of normal individuals, whereas opportunistic pathogens cause disease only in individuals who are compromised in either their innate or humoral immune defenses.

Nonetheless, an opportunist in one host may be a primary pathogen in another. One helpful distinction between an opportunistic and primary pathogen reflects the essentiality of the host for the long-term survival of a microbe. A primary pathogen's long-term survival absolutely depends upon its ability to replicate and to be transmitted in a particular host, whereas an opportunistic pathogen does not. Whether a primary or opportunistic pathogen, a microbial pathogen must be capable of entering a host, finding a unique niche, somehow avoiding or subverting the host's normal defenses, and multiplying in that setting. Primary pathogens additionally must be transmitted to a new susceptible host or at least establish themselves in the host for extended periods for eventual transmission.

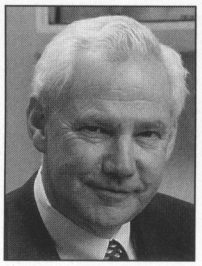

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Table 1. Sources of some human infectious diseases

Disease	Source
Measles	Canine distemper; rinderpest
Rhinovirus	Horse is only natural host
Tuberculosis	From other animals
Leprosy	Water buffalo
Diphtheria	Cow
Syphilis	Monkey
Influenza	Birds

Pathogens first must find a way of entering their hosts. As a species, we embody an evolutionary necessity to resist the constant genetic onslaught of microorganisms surrounding us. Each of our mucous membrane sites that communicates with the outside world is the target of one or more pathogenic species of microorganisms, which are equipped to gain entry to and survive within these sites.

Entry is not simply the result of casual contact between the host and an infectious agent. Upon entering the host, the pathogen's surroundings change profoundly, and it needs genetic machinery enabling it to grow in, or at least tolerate, different environments. Specialized pathogenic traits may not be expressed until the infecting organism encounters a particular environment within its host.

Next, pathogens must find unique niches within hosts. Although we are prey to a small number of frankly pathogenic microorganisms, we are hosts to countless commensal bacteria, fungi, protozoans, and minute insect species. For most commensal bacterial species, their replication on or within us is essential for their survival. Moreover, our own survival is likely dependent on the presence of those microorganisms. Primary pathogens, like commensal species, need to replicate on a particular host. Similarly, both pathogens and commensal species must find, occupy, and be sustained in a unique niche within their host species. In seeking the definition of a pathogen, it is neccessary to define the fine line that separates the pathogen from the commensal.

The next component of a pathogen's strategy for survival involves avoiding, circumventing, or subverting normal host cell defenses. Once a pathogenic species reaches its unique niche, it may face formidable host defense mechanisms, such as phagocytic cells. A site that is suitable for a pathogen may be devoid of commensal bacteria precisely because it contains host defense measures not found at mucosal surfaces. The ways by which microbes avoid, circumvent, or even subvert such host defense measures typically are different for each species, although certain common tactics are apparent (Table 2).

Moreover, because bacterial pathogenicity is so multifaceted, it can be likened to a symphony in which each part contributes to a common theme. Among enteric pathogens, for example, each species has a unique "style" of survival, even though some of them sometimes deploy homologous instruments that, used separately but in similar fashion, lead to their freedom to multiply effectively in this particular environmental niche.

The obvious goal of any bacterium is to become bacteria. A pathogenic species must multiply sufficiently either to establish its progeny within the host or to move into a new susceptible host. Of course, multiplication rates vary from microbe to microbe. From the medical standpoint, the rate of microbial replication reflects, and often defines, the incubation period of a disease. However, it also defines the stealth phase during which the microorganism avoids destruction by host defenses and, instead, attains sufficient numbers to carry its progeny forward.

We know relatively little about the replication of microorganisms in their native habitats. With new analytic tools, such as nucleic acid amplification, and the availability of very sensitive reporter genes, like the one specifying the green fluorescent protein, we can begin to monitor microbial replication and biosynthetic activities directly in animals, including ourselves.

Less is known about the precise mechanisms involved in the transmission of microorganisms between individual hosts than any other attribute of

Table 2. The pathogenic signature

Avoidance	
	Capsule; sialylation of gonococcal cell envelope; Ig-like proteins of gram+organisms
Subversion	
	Salmonella ruffling; *Yersinia* tyrosine phosphatase; use of host enzymes to activate essential determinants of pathogenicity
Circumvention	
	Coagulase; IgA1 protease; attachment to alternative cell receptors

bacterial pathogenicity. Transmission is not merely a matter of coughing, intimate contact of mucous membranes, or diarrheal expulsion. Instead, many pathogens probably contain finely honed genetic apparatuses to ensure their continued transmission from one host to another. The virulence of a microbe, is determined in large part by its capacity to be transmitted at a high frequency. A low infectious dose along with an efficient transmission strategy is a potent combination strategy for a microorganism to sustain its progeny.

Bacterial Pathogens Are Clonal

The unit of bacterial pathogenicity appears to be a clone or cell line. For instance, disease outbreaks and increases in infection frequency often can be traced to distinct bacterial clones. Such clones typically possess unique combinations of virulence genes. Multilocus electrophoretic studies and DNA sequencing data support the idea that, while the number of allelic variants is large in most bacterial species, recombinational processes make it unlikely that strains with a similar genetic profile can be generated randomly.

Typically, most of the bacteria isolated during a disease outbreak manifest a relatively limited set of genetic profiles, indicating that they probably arise by descent from a single progenitor rather than by convergence from separate pro-genitors through gene flow. Moreover, as illustrated by the molecular population genetics of *Neisseria meningitidis*, temporal variation in disease frequency and severity is often associated with clonal replacement, much like influenza epidemics are driven by antigenic shifts. Such data reinforce the concept that bacterial clones vary dramatically in their virulence potential.

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Certain genotypes of pathogens may arise infrequently or only once. Only rarely, if ever, will they bestow their particular constellation of virulence attributes horizontally to other lineages of the same species or to other microorganisms.

Bacterial pathogens respond to the social and biological factors that affect their hosts. In recent discussions of emerging microbial infections, inadequate emphasis is placed on the impact of changes in the sociology and biology of humans. For example, during the past 50 years humans have come to take more showers and fewer baths. This change has had a profound impact on the aerosolization of microorganisms present in potable water, including *Legionella pneumophila* and *Mycobacterium avium*, both of which have seen sharply increased prevalence in recent years.

The use of antibiotics during this same period also has exerted profound selective effects on global populations of microorganisms. It is not just a matter that the bacteria have become resistant and more difficult to treat. The wide use of antibiotics also encourages more frequent transmission of both general and specific genetic information among microbes throughout the world. Transfers of phages, plasmids, and carrier transposons are not restricted to antibiotic resistance genes.

The success of antimicrobial treatments and, indeed, wide use of vaccines could also, in principle, be affecting the evolution of pathogens in ways that we cannot readily detect. Will selection for alternative portals of entry or sites of colonization become more prevalent? Has the recent dramatic eradication of *H. influenzae* type b disease and even its carriage created a "microbial vacuum" that can be capitalized upon by other microorganisms or even non- type b *Hemophilus* strains?

Let me speculate. Consider what happens when disease symptoms betray a particular microorganism and lead public health experts to campaign for its eradication in technologically advanced countries. In so doing, they might inadvertently select for a microorganism that gives rise to fewer or less severe symptoms while retaining its capacity to replicate and be transmitted. Alternatively, such an eradication campaign might select for highly invasive variants that replicate more swiftly, before treatment or host defenses can be mobilized.

Genetic Determinants of Pathogenicity Often Occur Together

Bacterial plasmids and bacteriophages play a key role in the genetically haploid world of microorganisms as carriers of essential components of

pathogenicity. William Hayes, one of the founders of modern-day microbial genetics, noted over 30 years ago that we may too often incriminate the bacterium for the sins of the virus or plasmid. Although a plasmid or bacteriophage may play an essential role in conferring pathogenic traits on a bacterium, the transfer of a single phage or plasmid, even a very large one, would rarely if ever be sufficient to convert a nonpathogenic commensal microbe into a primary pathogen. Many other factors contribute (Table 3).

Recently, investigators realized that many bacterial pathogens carry large inserts of DNA, called pathogenicity islands, that are not found in nonpathogenic members of the comparable genera (Table 4). Thus, for instance, uropathogenic *Escherichia coli* and enteropathogenic *E. coli* possess large fragments of DNA, ranging in size from 35 to 170 kilobases, which include a number of virulence genes that are absent from commensal *E. coli* strains.

Although these inserts differ in size and in the specific genes that they encode, they also have several striking similarities. For instance, both of them contain a common boundary at an uncommon selenocystine tRNA locus. Not all pathogenicity islands in *E. coli* and other enteric bacteria are found within this specific tRNA locus. Other pathogenicity islands are inserted adjacent to other tRNA loci. Circumstantial evidence, including an insertion sequence and both bacteriophage- and plasmid-related sequences within these large DNA inserts, suggest that mobile elements may well have helped in building bacterial pathogens.

Moreover, the DNA composition of these genetic inserts differs markedly from the overall DNA pattern within the genome of these organisms. This finding is also consistent with the pathogenic island DNA being "alien" to the host chromosome, perhaps deriving from horizontal transmission of accessory genetic elements and subsequent recombination. Significantly, homologs of the effector-encoding genes on plasmids in species such as *Yersinia enterocolitica* and *Shigella flexneri* are found in chromosomal blocs in other enteric species such as *E. coli* and *Salmonella typhimurium*. In addition, several clustered chromosomal genes encoding other virulence factors are common among gram-negative pathogens ranging from plant pathogens to *Helicobacter pylori*.

Perhaps the molecular essence of a pathogen resides in the sequences of a relatively small number of effector molecules which are put in place by regulatory genes and effector molecules that conduct many other more general tasks

Pathogens Respond in Complex Ways to Hosts

Bacterial pathogens use complex regulatory pathways to permit them to respond to host environments. During an infection, a pathogen encounters a number of different environments within the host as it traverses extracellular and intracellular compartments. Well-adapted bacterial pathogens express appropriate sets of virulence genes in response to the stimuli they encounter in these varied environments.

In fact, a pathogen probably does not respond any differently than any other microbial species to changes in oxygen, carbon dioxide, nutrient levels, iron, and pH. Thus, many of the regulatory cascades that control bacterial virulence likewise regulate genes that are not directly associated with pathogenicity or at least are not essential for pathogenicity. Yet, pathogenicity is exquisitely regulated, and expression of certain virulence genes at the wrong time during the infection cycle can have devastating consequences for the microorganism.

For example, salmonellae fail to invade host cells unless the organism senses proper levels of oxygen, pH, osmolarity, and an appropriate signal to the PhoP/Q regulon, according to Catherine Lee and her colleagues at Harvard University in Cambridge, Mass. If even one of these conditions is unfavorable, invasion gene expression is repressed and the salmonellae fail to invade the host.

This kind of regulation appears to be a common theme among bacterial pathogens. Thus, although blocs of virulence genes may have derived from a foreign source, many of the regulatory links controlling those genes appar-

Table 3. Extrachromosomal determinants of pathogenicity

Plasmid-mediated determinants
Shigella invasion
Tetanus neurotoxin
Enterotoxigenic *E. coli* toxin and adhesins
B. anthracis toxins
Bacteriophage-encoded determinants
C. diphtheriae toxin
S. pyogenes erythrogenic toxin
C. botulinum neurotoxin
Enterohemorrhagic *E. coli* Shiga-like toxin

ently are native to the specific microorganisms. And, why not? All prokaryotes respond to environmental conditions, such as temperature, carbon dioxide, oxygen, iron, pH, stationary phase, and specific nutrients, that reflect their particular niche in nature. Regulating expression of pathogenic determinants is thus a common theme, consistent with other examples of bacterial specialization.

Furthermore, blocs of genes, even large ones, are responsible for delivering a relatively few functional effectors to the bacterial cell surface and assembling them there for the specific tasks they perform. Perhaps the molecular essence of a pathogen resides in the sequences of a relatively small number of effector molecules which are put in place by regulatory genes and effector molecules that conduct many other more general tasks for the bacterium. Understanding the interplay between such factors undoubtedly will become increasingly important for the design of living vaccine strains and will provide new targets for antimicrobial therapy.

Personal Thoughts about Redefining Pathogens

As a graduate student, I learned that bacterial pathogens arose as commensal species lost key functions and came to depend on the host to provide them with the essentials needed for growth and survival. Thus, as commensal organisms became increasingly host dependent, they eventually crossed a line and began to replicate at the expense of the host, causing disease in some individuals.

However, the more I studied pathogens, the less this concept seemed to ring true. For instance, the fact that a bacteriophage mediates diphtheria toxin production and that Ent and K88 plasmids specify pathogenic traits in pathogenic *E. coli* are examples in which nonpathogens gain rather than lose genetically specified functions to become pathogenic.

Table 4. Molecular and phenotypic properties of selected pathogenicity islands

Pathogen	Chromosomal location	Molecular features	Virulence phenotypes
Uropathogenic *E. coli*			
Pai I	(I) 82 min	Both unstable flanked	Hemolysis
70 kb	*selC*	by nucleotide repeats	Serum resistance
Pai II	(II) 87 min		Mannose-resistance hemagglutination
190 kb	*leuX*		Uroepithelial cell binding
EPEC			
LEE	82 min	Stable	Enterocyte effacement
35 kb	*selC*	No direct repeats	
Y. pestis			Hemin binding and storage
102 kb		Unstable	Pesticin sensitivity
		flanking IS100s	Iron-reg. OMP expression
			Growth in iron limited conditions
S. typhimurium			
40 kb	60 min, *spiI*	Not stable in all clones	Invasion of non-phagocytic cells, apoptosis
	28 min, *spiI*	Stable (?)	Persistence in infected animals

Even fastidious pathogens, such as the gonococci, do not fit the model of gaining host dependency by losing genes as they move from innocuous to pathogenic. For example, IgA protease gene sequences are absent from commensal *Neisseria* species but are present exclusively in the pathogenic species, according to Michael Koomey, who cloned and studied that gene while working in my laboratory. No slow, adaptive evolution of preexisting genes is going on here!

I began thinking that pathogenicity might change by quantum jumps rather than by slow adaptive steps. However, the large DNA inserts now called pathogenicity islands had not been recognized. A decade ago, plasmids, lysogenic phages, and modest-sized transposons seemed the more likely agents of such changes. In any event, pathogenicity, or at least increased virulence, apparently can be gained when an organism acquires whole blocs of genetic information devoted to one or more steps. The microbial groups in which this process has been the means to becoming pathogenic are not fully known.

A key distinction is that a pathogen has an inherent capacity to breach host cell barriers, whereas a commensal species and opportunistic pathogens do not

Pathogens Are Equipped To Breach Defenses, Damage Cells

These and other observations lead to further questions. What are the distinguishing features of commensal species and of pathogens? Also, what enables a commensal species to act as an opportunistic pathogen? And if a pathogen causes widespread infection but little overt disease, are the disease and cellular damage merely an accidental outcome of the host-parasite relationship?

Perhaps the cardinal difference between these two categories is that pathogens can gain access to privileged niches that normally are not available to commensal species. Pathogens typically reach these sites by damaging host cells either directly or indirectly. Well-tempered pathogens generally interact with, or stay close to, a specific type of host cell. In this context, commensals may cause opportunistic infections when they are introduced into such privileged host sites or if some other ordinarily insurmountable host defense is breached. The key distinction then is that a pathogen has an inherent capacity to breach host cell barriers, whereas a commensal species and opportunistic pathogens do not.

For example, pathogenic *Escherichia coli* do not usually cause disease in the colon, where facultative commensal strains ordinarily reside. However, enteropathogenic species and uropathic species target sites outside the colon along relatively unoccupied (by enteric species) mucosal surfaces of the small bowel and urethra or, in rarer cases, within the colon at a specific cellular niche. Indeed, for those microbial genera with both pathogenic and nonpathogenic representatives, the pathogens typically occupy niches that are not ordinarily available to commensal members of the genus.

The way in which the *E. coli* and *S. typhimurium* chromosomes are organized also is consistent with this view. For example, many of the *Salmonella* genes that encode products involved in penetrating host epithelial and macrophage cells occur within a 40-kilobase segment of DNA, known as SpiI, which is located between minutes 59–60 on the *Salmonella* chromosome. Although no such segment is found on the *E. coli* chromosome, similar DNA sequences adjacent to the SpiI segment are found on both the *Salmonella* and *E. coli* chromosomes.

By using signature-tagged mutagenesis to search for novel genes, David Holden and his colleagues at Hammersmith Hospital in London, England, recently identified a second *Salmonella* pathogenicity island, designated SpiII, near minute 31 on the chromosome. Genes along this segment help the pathogen to survive during the period after it gains entry to its host.

These distinct pathogenicity islands hold clues to how pathogens exploit host cell biology and cellular immunology during infections. For example, the SpiI segment of *S. typhimurium*, which contains this pathogen's "invasion" genes, apparently is no longer expressed after the bacteria move from the ileum into Peyer's patches. However, continuing expression of the genes of the second segment, SpiII, enables the bacteria to gain access to the spleen and to survive within phagocytic cells. Meanwhile, the virulence plas-

mid of *Salmonella* carries yet other genes that are important for the bacteria to survive during systemic infection, including within the spleen.

Is this merely genetic redundancy or a reflection of the need to replicate within the reticuloendothelial system and another vital component of adaptation? By obtaining mutations in the genes that are part of these three genetic inserts, we may learn what roles the products they encode play during pathogenesis.

One can imagine that each of these large DNA insertions was acquired by an ancestral microbe that gave the prototype *Salmonella* strain the capacity to translocate the mucosal barrier and subsequently survive within particular target organs of the host. Thus, certain clones of commensal bacteria apparently expanded into relatively privileged sites at a time when there was little competition from other microorganisms for those sites. In a sense, these pathogens were the early microbial pioneers searching the mammalian body for new territories that were less crowded, where nutrients were relatively abundant, but where immune defense mechanisms posed greater risks to survival. As with many human pioneers, such microbial explorations sometimes gave way to exploitation, and even anarchy or imperialism.

If asked to define a pathogen, my answer is more complicated than that purely pragmatic definition. Instead, I define a pathogen as being any microorganism whose survival is dependent upon its capacity to replicate and persist on or within another species by actively breaching or destroying a cellular or humoral host barrier that ordinarily restricts or inhibits other microorganisms. This capacity to reach a unique host niche free from microbial competition and possibly safe from host defense mechanisms sets the foundation for the expression of specific determinants that permit such microbes to establish themselves within a host and to be transmitted to new susceptible hosts.

ACKNOWLEDGMENTS

I began to think seriously about the biological basis of pathogenicity when I was a member of the first NIH recombinant DNA advisory committee. We were asked to define a pathogen. Over the years, my mentors and my students (who are also my mentors) always found time to discuss this fundamental issue (sometimes with considerable passion). However, I first outlined the basic features presented in this paper when I made a serious attempt to define the genetic and molecular basis of pathogenicity as a divisional lecturer at the ASM annual meeting held in St. Louis in 1984. I am grateful to my wife Lucy Tompkins for encouraging me (then and now) to try to develop these ideas in a coherent way and for being my sounding board for ideas. In the last few years, the current members of my laboratory have borne the brunt of my whining about this topic. They have showed considerable patience, if not tolerance. Finally, I note that many of the ideas presented in this article came about from my presentation entitled "What Is a Pathogen?" at the 1994 ASM General Meeting and from my preparation of a chapter on the evolution of pathogenicity in the enteric bacteria, which appeared recently in the 2nd edition of the tome *Escherichia coli* and *Salmonella: Cellular and Molecular Biology*, from ASM Press.

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