

# Toward a more systematic understanding of bacterial virulence factors and establishing Koch postulates in silico

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Human health is threatened by various infectious bacterial pathogens but is also supported by many commensal bacteria particularly in the intestinal tract.<sup>1–3</sup> However, many of the bacterial pathogens and commensal bacteria are taxonomically indistinguishable, with pathogenic and commensal bacteria existing in the same genus and even species. A typical example is *Escherichia coli*,<sup>4</sup> which is commonly found in the lower intestine of humans. But some serotypes can cause severe diseases.

Virulence factors are assumed to explain the differences between pathogenic and commensal bacteria.<sup>5–8</sup> When a bacterium harbored a specific virulence factor, e.g., *Escherichia coli* secreting Shiga toxin, it could turn commensal to pathogenic. However, with more factors revealed to be involved in the bacterial virulence, the boundary between pathogenic and commensal bacteria defined by virulence factors becomes obscure. Some virulence factors playing vital roles in bacterial infection are also found to be encoded in genomes of commensal bacteria.<sup>5,6</sup> The conflicts between the definitions of infection experiments and the genomic distributions of virulence factors raise critical questions on the understanding of bacterial pathogenicity and virulence factors.

In this issue of *Virulence*, Niu et al. recognized the contradiction and classified bacterial virulence factors into two groups.<sup>9</sup> One group is formed by those virulence factors only found in pathogenic bacteria, called pathogen-specific

virulence factors. The other group consists of virulence factors that are also found in non-pathogenic and even commensal bacteria, called common virulence factors. They conducted a systematic analysis of the differences of these two groups with respect to their genomic and functional distributions.

Significantly, they observed that the common virulence factors are more likely to be involved in the general pathogen–host interaction processes, e.g., modulating the host environments for tight adhesion and nutrients, while the pathogen-specific virulence factors are more likely to be directly linked to virulence. Significantly, they also observed that pathogen-specific virulence factors are more likely to be encoded in the genomic islands that are often acquired and evolved through horizontal gene transfer while common virulence factors are inclined to evolve vertically. Therefore, their observations retain the potential to define or predict the pathogenicity of a bacterium through the common/specific classification of virulence factors.<sup>10</sup>

However, the bacterial pathogenicity seems not easy to be predicted by singular virulence factors. Because successful bacterial infections to human cells always involve multiple processes such as adhesion, invasion, modulation, colonization, transmission and immune escape,<sup>7</sup> failure in any step may prevent a bacterium from becoming pathogenic. With the development of the understanding of virulence factors, especially the insights obtained by

systematical studies, it is time to revisit the relationship of pathogenicity and virulence factors from a “set theory” view. The discrimination of common virulence factors from pathogen-specific virulence factors by Niu et al. just provides such an example,<sup>9</sup> which implies that the formation of complete pathogenicity for a bacterium may require both of the common and pathogen-specific virulence factors.

The type III secretion systems (T3SSs) in gram-negative bacteria provide a typical molecular example to explain the set formation of bacterial pathogenicity. A complete and functional T3SS should include structural proteins that build the functional needle structure, effector proteins that are injected into the host cells to modulate the host environment, and chaperons that protect those effectors in bacterial cytoplasm and direct them toward the needle structure for injection.<sup>11</sup> Niu et al. demonstrated that the structural proteins and chaperons of T3SSs are generally common virulence factors whereas the effector proteins are generally pathogen-specific virulence factors.<sup>9</sup> Those structural proteins and chaperons may constitute a highly efficient injector but those specific effector proteins serve as “drugs”. When the drugs are beneficial to the host, the bacteria–host relationship becomes commensal and even symbiotic. When the drugs are toxic to the host, the relationship will become infectious. The effector proteins cannot exert their functions without the structural proteins and chaperons of T3SSs which are common

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in both pathogenic and nonpathogenic bacteria.

Set view of the relationship between pathogenicity and virulence factors not only contributes to a systematic understanding of host–bacteria interactions, but also provides more avenues to interfere with bacterial infection. In the virulence set, some virulence factors are functionally dependent on others. Thus, some virulence factors may have higher centrality. Drugs targeted to the critical virulence factors may be able to functionally inhibit other virulence factors through inhibition of the function of the virulence set besides a specific factor. As demonstrated in T3SSs by Niu et al., those common virulence factors, i.e., the structural proteins and chaperons, are generally more conserved than and are likely to be dependent on those pathogen-specific effector factors.<sup>9</sup> These common virulence factors are critical to the virulence set and are promising drug targets to inhibit infection.

Niu et al. conducted a systematic but summary analysis of the laws of bacterial virulence factors based on the currently available bacterial genomic data. In this genomic era, more and more bacterial genome sequences will be available in the databases. In particular, with the development of next-generation sequencing technologies, the sequence of a bacterial genome can be obtained within several days. A promising trend for the future

may be to predict the pathogenicity of a sequenced bacterium solely based on its genome sequence, i.e., establishing “Koch postulates” in silico.<sup>8,10</sup> Koch postulates in silico would allow high-throughput and rapid screen of pathogenic bacteria by computational methods, which can greatly shorten the response time of humans to those bacteria identified in foods and other specimens and can reduce the infection experiments on model organisms. Set view of the composition of bacterial pathogenicity may provide a promising approach for the establishment of Koch postulates in silico by adopting the state-of-the-art machine learning and data mining techniques. The work of Niu et al.<sup>9</sup> should make the first step toward this goal and much work is needed in the future.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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