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Different Paths to Pathogenesis

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Abstract

In a recent issue of *Cell Host & Microbe*, Elsen and colleagues identify a novel hemolysin in a highly virulent *Pseudomonas aeruginosa* strain that lacks a type 3 secretion system. Their analysis provides another example of how individual strains of *P. aeruginosa* utilize different virulence mechanisms to cause severe infections.

Pseudomonas aeruginosa is an important opportunistic pathogen and a leading cause of severe healthcare-associated infections. As with many pathogens, there is great concern over an alarming increase in antibiotic resistant strains, which present a significant clinical challenge for the treatment of *P. aeruginosa* infections. The success of *P. aeruginosa* as a pathogen relates to its ability to colonize the host, scavenge nutrients, break down host defenses, and circumvent host immune responses (1). To accomplish these pathogenic steps, *P. aeruginosa* employs a considerable armamentarium of virulence factors encoded by a large and complex genome. The *P. aeruginosa* genome is commonly viewed in two parts. The core genome represents those regions that are common to all isolates, while the accessory genome is composed of elements found in some strains within the species but not others (2). The core genome encompasses approximately 90% of the chromosome and contains an abundance of genes associated with basic housekeeping, metabolic, and pathogenic functions characteristic of nearly all strains of *P. aeruginosa*. For example, the genes for type IV pili, Psl polysaccharide, Rhl quorum sensing, and type II secretion are part of the core genome. These and other core virulence genes presumably allow all *P. aeruginosa* strains to cause infections (Figure 1) (3). In contrast, the accessory genome houses genes that confer strain-specific traits. Such genes are commonly parts of larger mobile genetic elements that are acquired through horizontal gene transfer. Incorporation of acquired elements occurs at specific regions of genomic plasticity found throughout the *P. aeruginosa* genome (4). There are several examples of how accessory genomic elements enhance the virulence of individual *P. aeruginosa* strains. Transposable elements containing antibiotic-resistance cassettes may become widely disseminated among bacterial

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populations. Prophage elements have been implicated in the increased competitiveness of epidemic strains in cystic fibrosis patients (5). Integrative and conjugative elements (ICEs), including *Pseudomonas aeruginosa* Pathogenicity Island (PAPI)-1 and *Pseudomonas aeruginosa* Genomic Island (PAGI)-5, contain several regions that contribute to virulence in animal infection models (6, 7). However, in contrast to the core genome, the accessory genome of *P. aeruginosa* has attracted much less attention and has not been systematically explored for the presence of virulence genes.

Two well characterized virulence factors encoded by the accessory genome are the type 3 secretion system (T3SS) effector proteins ExoU and ExoS. ExoU is a phospholipase A2 cytotoxin, whereas ExoS exhibits both GTPase activating protein (GAP) activity and ADP-ribosyltransferase activity. Both of these effector proteins have been implicated in resistance to killing by phagocytes, disruption of epithelial barriers to facilitate dissemination, and a marked enhancement of infection severity. The gene encoding ExoU has been found within the genomic island PAPI2 and the ExoU islands A, B, and C. The *exoS* gene is also a variable trait and appears to be inserted by itself into the core genome (3). Interestingly, most strains of *P. aeruginosa* contain either the *exoU* or the *exoS* gene but not both. This observation led to the hypothesis that ExoU and ExoS use different strategies to accomplish the same goal: to facilitate severe infections (Figure 1).

Recently, a *P. aeruginosa* strain named PA7 was shown to lack T3SS genes (8). Elsen and colleagues identified another member of the PA7 family, CLJ1, that was cultured from a patient with severe hemorrhagic pneumonia (9). This prompted them to ask the question of how this strain could be so virulent in the absence of type 3 secretion. In their recent publication, they describe a novel and very interesting cytotoxin produced by CLJ1 (9). This toxin shares homology with hemolysins found in several Gram-negative pathogens and was termed Exolysin. Exolysin (ExlA) was determined to have potent cytolytic activity responsible for rapid damage to cellular membranes. This resulted in the breakdown of pulmonary barriers, severe alveolar hemorrhage, and subsequent dissemination to systemic sites in a murine pneumonia model. As with its homologues, ExlA is exported by a second protein (in this case ExlB) through a two-partner secretion mechanism, but how ExlA intoxicates its target cells is currently unknown. ExlA homologues have features of a broad class of recently described polymorphic toxins (10). The central portions of polymorphic toxins contain several conserved repeat domains, such as Rhs/YD repeats or the filamentous hemagglutinin repeats within ExlA. These domains have been hypothesized to form a filamentous structure that facilitates the delivery of a toxic C-terminal domain into host cells (10).

As mentioned, *P. aeruginosa* CLJ1 is a member of the PA7-like taxonomical group, whose members lack T3SS genes. Analysis of eight PA7-like strains isolated from various clinical sources revealed that all examined members of this lineage carry the Exolysin genes (9). Moreover, these genes were absent from other type III secretion-positive strains, indicating that the *exlA* gene is part of the accessory genome. Thus, the Exolysin toxin represents a novel virulence strategy used by some *P. aeruginosa* strains as an alternative to the T3SS. Recent studies suggest the accessory genome is a rich reservoir of virulence genes, so Exolysin, ExoU, and ExoS may be just the “tip of the iceberg” with regard to virulence

factors employed by some strains of *P. aeruginosa* but not others. These genes may allow some *P. aeruginosa* strains to cause especially severe infections or infections with particular characteristics, such as hemorrhagic features (Figure 1). Modern sequencing technologies will continue to facilitate the mining of the accessory genomes for these new factors within the global *P. aeruginosa* virulome. Identification and characterization of these novel virulence factors will provide new insights into the spectrum of pathogenic strategies used by *P. aeruginosa*. It will be interesting to see whether these factors can serve as biomarkers for highly virulent strains that allow clinicians to identify patients in need of particularly aggressive interventions. In any case, the study by Elsen and colleagues elegantly demonstrates that *P. aeruginosa* uses more than one path to pathogenesis.

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References

1. Casadevall A. Cards of virulence and the global virulome for humans. *Microbe*. 2006; 1:359–364.
2. Kung VL, et al. The accessory genome of *Pseudomonas aeruginosa*. *Microbiol Mol Biol Rev*. 2010; 74:621–641. [PubMed: 21119020]
3. Wolfgang MC, et al. Conservation of genome content and virulence determinants among clinical and environmental isolates of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A*. 2003; 100:8484–8489. [PubMed: 12815109]
4. Mathee K, et al. Dynamics of *Pseudomonas aeruginosa* genome evolution. *Proc Natl Acad Sci U S A*. 2008; 105:3100–3105. [PubMed: 18287045]
5. Winstanley C, et al. Newly introduced genomic prophage islands are critical determinants of in vivo competitiveness in the Liverpool Epidemic Strain of *Pseudomonas aeruginosa*. *Gen Res*. 2009; 19:12–23.
6. Battle SE, et al. Hybrid pathogenicity island PAGI-5 contributes to the highly virulent phenotype of a *Pseudomonas aeruginosa* isolate in mammals. *J Bacteriol*. 2008; 190:7130–7140. [PubMed: 18757543]
7. He J, et al. The broad host range pathogen *Pseudomonas aeruginosa* strain PA14 carries two pathogenicity islands harboring plant and animal virulence genes. *Proc Natl Acad Sci U S A*. 2004; 101:2530–2535. [PubMed: 14983043]
8. Roy PH, et al. Complete genome sequence of the multiresistant taxonomic outlier *Pseudomonas aeruginosa* PA7. *PloS One*. 2010; 5:e8842. [PubMed: 20107499]
9. Elsen, S., et al. A Type III Secretion Negative Clinical Strain of *Pseudomonas aeruginosa* Employs a Two-Partner Secreted Exolysin to Induce Hemorrhagic Pneumonia. *Cell Host Microbe*. 2014. <http://dx.doi.org/10.1016/j.chom.2014.01.003>
10. Zhang D, et al. Polymorphic toxin systems: Comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. *Biology Direct*. 2012; 7:18. [PubMed: 22731697]

Figure 1.

Pseudomonas aeruginosa strains utilize different pathogenic mechanisms to facilitate severe infections. Virulence factors that account for the basic pathogenic functions of all *P. aeruginosa* strains are presumably found within the core genome. Severe *P. aeruginosa* infections may require additional virulence factors encoded within the accessory genome that differ in their delivery mechanisms and functions. Abbreviations: T3SS, type 3 secretion system; TPSS, two-partner secretion system.