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EDITORIAL

Burkholderia cenocepacia virulence microevolution in the CF lung: Variations on a theme

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Bacteria of the *Burkholderia cepacia* complex (Bcc) are commonly isolated from soil and aquatic environments, but have also found notoriety as opportunistic intrinsically-multidrug resistant pathogens of patients with cystic fibrosis (CF). Twenty Bcc species have been identified to date, although just 2 species, *Burkholderia cenocepacia* and *Burkholderia multivorans*, are the most clinically significant members of the Bcc.[1] Specifically, *B. cenocepacia* causes lung infections that result in significantly decreased survival rates in CF patients. Whereas both *B. cenocepacia* and the more prevalent CF bacterial pathogen *Pseudomonas aeruginosa* can chronically persist in the lungs of CF patients for years, in some patients, *B. cenocepacia* infection can progress to a rapid deterioration in lung function associated with necrotizing pneumonia, bacteremia and sepsis that results in death.[2] To illustrate the complexity of CF infections, for *P. aeruginosa*, it was determined that gene products associated with virulence in one strain were different from those creating virulence in closely-related strains,[3] suggesting that bacterial virulence elaboration during infection is dependent upon specific bacterial genetic backgrounds, as well as the polymicrobial nature of CF infections, the underlying patient-to-patient immune dissimilarities, and the state of disease progression. Previous work by several investigators[4,5] has shown that upon acquisition, initially highly-virulent strains of *P. aeruginosa* adapt to the CF lung environment by becoming less motile and less virulent, thus establishing chronic infections that can persist in opposition to the host immune system for many years, if not decades. This adaptation by *P. aeruginosa* to the CF lung environment has been termed "microevolution," and is characterized by the phenotypic acquisition of mucoidy, and the genotypic acquisition of numerous point mutations, indels, and epigenetic alterations in gene expression.[6]

To determine whether similar microevolutionary processes are involved in the chronic carriage of *B. cenocepacia* in the lungs of CF patients, in this article,[7] Moreira et al. sought to compare the virulence potential of sequential isolates from 3 different patients infected with *B. cenocepacia* utilizing the alternative infection models *Caenorhabditis elegans* and *Galleria mellonella*. Like *P. aeruginosa*, pathogenicity caused by *B. cenocepacia* is multifactorial; many different gene products contributing to the overall virulence capacity of the bacterium, and a principal subset of different virulence factors is expressed in different mammalian and non-mammalian hosts.[3,8] For *B. cenocepacia*, these virulence factors include extracellular enzymes, toxins, adhesins, secretion systems, mechanisms of nutrient acquisition, and regulatory and cell–cell communication systems, which work together to produce motility, biofilm formation, adhesion, intracellular invasion, and immune evasion.[9] As tested in animal infection models, the combined expression of these virulence factors results in pathogenicity as measured by organismal morbidity or mortality.[10,11]

It is acknowledged (though controversial, 12) that the virulence potential of *P. aeruginosa* declines over time throughout chronic infection, based on the comparative assessment of late isolate virulence versus early isolate virulence in animal infection models.[4,5] Given that *B. cenocepacia* in CF patient lungs is not consistently a chronic pathogen, it is understandable that the results presented by Moreira et al.[7] support that the idea that *B. cenocepacia* virulence potential of isolates over time is highly variable. This is likely due to a number of factors. First, because of the presence of a large population of

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mobile genetic elements, *B. cenocepacia*'s genome is likely more plastic than that of *P. aeruginosa*. Second, strain-to-strain differences in the genome content of the Bcc can vary by up to 10%, even for closely related strains. This is at least partially the result of the Bcc having a very large ~8 Mb genome, but also because the Bcc do not have the protection provided by a CRISPR-Cas system. Third, as in *P. aeruginosa*, even for isolates with exactly identical genomes, differences in virulence factor production can result because of epigenetic influences greatly altering the virulence capacity of clones.[13] Finally, whereas *P. aeruginosa* adaptation toward avirulence over time in the CF lung is a survival advantage, no such advantage is apparent for *B. cenocepacia*. This is evident from the phenotypic tests of the 39 isolates, as presented in Figure 5 [7]; there is no clear virulence trend over time within a single strain or between the 3 strains, with respect to important virulence-related traits. Two of the 3 strains examined did have significantly less swarming motility over time, and one of the 3 strains did have significantly less swimming motility over time, but for iron uptake, protease production, and quorum sensing, no trend was clearly evident. Moreover, for the animal infection models, pathogenicity varied randomly over time.[7] Although the results from the 2 infection models do not correlate well with each other, a point that has been observed previously,[10] even within each infection model, related *B. cenocepacia* cells isolated from one timepoint to the next can exhibit maximal or minimal virulence. This divergent microevolution scenario thus allows the observation of 2 isolates from the same timepoint in the infection that vary oppositely, one isolate being extremely virulent, the other exhibiting avirulence.[7] This suggests, that for *B. cenocepacia* in the CF patient's lungs, there is a mixed microevolved population of clonal cells expressing different levels of different virulence factor genes, with all cells similarly capable of surviving the CF lung environment.

The presented results mirror analogous findings for *B. cenocepacia* previously observed by other researchers. O'Grady and Sokol[11] showed that virulence factor genes identified through in vitro analysis are not necessarily the same genes expressed during in vivo infection.[11] However, their analysis does similarly show that *B. cenocepacia* flagella and pili are down-regulated during rat lung infections. Conversely, Zlosnik et al.[14] observed that loss of motility was uncommon in 551 isolates obtained during chronic *B. cenocepacia* infection, and also could not link motility to clinical outcome. For *B. cenocepacia*, motility was occasionally linked to conversion to a shiny phenotype, sometimes referred to as mucoidy, although mucoidy and shininess may be distinct cell surface modifications. Accordingly, Moreira et al.'s results[7] indicate that *B. cenocepacia* exopolysaccharide (EPS) production was found throughout the infection of patient J, whereas no EPS production was identified in late *B. cenocepacia* isolates from patients AB or patient AN. These results are comparable to those observed by Zlosnik et al.,[15] where 45% (23 of 51) *B. cenocepacia* CF lung infections were initiated by mucoid isolates, and during the course of infection, 3 converted from mucoid to nonmucoid, and one converted from nonmucoid to mucoid. In light of the results presented herein by Moreira et al.,[7] it is apparent that selection does not occur over time for *B. cenocepacia* toward avirulence and chronic carriage in the CF lung, that *B. cenocepacia* phenotypic and virulence factor variation does not mimic that of *P. aeruginosa*, and that clinical isolate virulence potential variability is the norm for *B. cenocepacia* across the duration of the infection in CF patients.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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