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Pseudomonas aeruginosa virulence and antimicrobial resistance: Two sides of the same coin?

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Successful nosocomial pathogens must overcome two major obstacles to cause infections: the host immune defense system and the antibiotics that are ubiquitous in the hospital setting. The bacterium *Pseudomonas aeruginosa* is adept at scaling both of these hurdles. It produces a plethora of pathogenic factors that target different aspects of host defenses, and it ranks high on the list of antibiotic resistant bacteria.

P. aeruginosa uses an arsenal of toxins to target cells of the innate immune system. Of these, ExoU appears to be particularly critical. This protein is injected directly into host cells through a needle-like apparatus called the type III secretion system [1]. Once in the cytosol, it is targeted to the host cell plasma membrane and activated by host cell factors to become a potent phospholipase. The net result is cleavage of phospholipids in the host cell plasma membrane, which causes lysis and death of the host cell. Recent studies have demonstrated that neutrophils are a prime target for ExoU injection during the early stages of *P. aeruginosa* pneumonia [2]. The subsequent death of these neutrophils results in a localized "neutropenia" in the lungs and creates an environment in which *P. aeruginosa* thrives [3]. Although only one-fourth of *P. aeruginosa* clinical isolates harbor the gene encoding ExoU [4], these isolates appear to be particularly virulent and have been linked to especially severe infections [5, 6].

P. aeruginosa's credentials in the forum of antibiotic resistance are no less impressive. It has been designated an ESKAPE organism, one of six bacteria for which novel antimicrobial agents are most needed [7]. Loss of susceptibility to the widely used fluoroquinolones is especially problematic, with resistance rates now over 30% in U.S. hospitals [8]. This resistance has been linked to worse clinical outcomes [9]. Since fluoroquinolone resistance often results from over-expression of efflux pumps, it is frequently linked to multidrug resistance. Thus the association between fluoroquinolone resistance and poor outcomes is thought to occur because of an increased likelihood of inadequate empiric therapy prior to the availability of antimicrobial susceptibility results.

Interestingly, previous reports indicated that the *exoU* gene and resistance to fluoroquinolones (as well as other antibiotics) cluster together in the same strains of *P. aeruginosa* [10–12]. These strains therefore have the capacity to both thwart the innate immune response and resist the sterilizing action of one of the most frequently used antibiotics. In this month's issue of Critical Care Medicine, Sullivan and colleagues present findings suggesting that such strains more frequently lead to pneumonia [13]. In a retrospective analysis of 218 consecutive patients with respiratory cultures that grew *P. aeruginosa*, the single most significant predictor of pneumonia (as opposed to bronchitis or colonization) in a multivariate regression model was the combined traits of fluoroquinolone resistance and the *exoU* gene. These findings suggest that strains with both the propensity to produce ExoU and the ability to resist the killing effects of fluoroquinolones are more likely cause progression to pneumonia rather than merely colonize the respiratory tract or cause bronchitis. The authors go on to suggest that diagnostic tests capable of rapidly identifying such strains could prove clinically useful in allowing clinicians to rapidly intervene to prevent pneumonia or to treat it early in its course.

One of the more interesting questions generated by this study is how the linkage between fluoroquinolone resistance and the *exoU* gene developed. At first glance, one might assume that fluoroquinolone resistance occurred in an ancestral *P. aeruginosa* strain that harbored the *exoU* gene, and that this resistance phenotype was subsequently disseminated in a clonal manner. However, both strain genotyping and fluoroquinolone resistance allele sequencing studies indicate that these strains are non-clonal [13, 14]. Perhaps the most likely explanation, as suggested by the authors, lies in the fact that antimicrobial agents work best in the presence of a robust immune response. Experience with neutropenic patients confirms the importance of neutrophils in facilitating bacterial eradication by antibiotics. By targeting neutrophils, ExoU allows persistence of *P. aeruginosa* in the host for longer times [15] and therefore more prolonged exposure of viable bacteria to fluoroquinolones in patients treated with these agents. This prolonged exposure enhances the chance of fluoroquinolone resistance mutations arising. Additional studies designed to follow the emergence of fluoroquinolone resistance in *exoU+* strains as patients progress from colonization to pneumonia would be informative in this regard.

The study by Sullivan and colleagues has several limitations, which are acknowledged by the authors. Most importantly, it is retrospective, and respiratory cultures were presumably obtained at the discretion of the treating physicians. Also, the use of sputum and endotracheal cultures could have misidentified the true etiology of pneumonia, especially when these cultures grew multiple organisms.

What are the implications of this study? First, while the poor outcomes observed with fluoroquinolone-resistant strains of *P. aeruginosa* have been ascribed to inappropriate empiric therapy, it is also possible that the co-existing *exoU* gene and presumably secretion of the ExoU toxin is at least partially responsible for these worse outcomes [10]. Second, additional studies are needed to determine whether other bacterial virulence determinants are also associated with increased levels of antimicrobial resistance. If the phenomenon reported by Sullivan and colleagues is indeed more general, our conventional view that antibiotic resistance and pathogenic factors both independently lead to worse clinical outcomes may be too simple. These two characteristics may instead be interdependent—two sides of the same coin.

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