At What Cost Echinocandin Resistance?

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(See the article by Ben-Ami et al, on pages 626–35.)

The treatment and prevention of infectious diseases caused by antimicrobial-resistant pathogens is one of the great challenges confronting modern medicine. Meeting this challenge will require better understanding of the various factors that determine the emergence and spread of resistance. Major determinants of the rate and trajectory of the evolution of resistance include the size of the microbial population exposed to a given agent, mutation rates, and the effect of resistance mechanisms on microbial fitness [1, 2]. Fitness, defined in the Darwinian sense as the ability to survive and reproduce [3], has been extensively studied in bacteria and viruses [2–4]. In many cases, resistance comes at a fitness cost to the organism, which is evident as reduced replication rate, virulence, or transmissibility [2]. Efflux pumps or the synthesis of enzymes that modify antibiotics, for example, may reduce fitness by requiring greater energy and metabolic expenditures [5]. Mutations of genes targeted by antibiotics, on the other hand, may have deleterious effects on the essential cellular processes regulated by the gene products [6]. In some instances, however, resistance confers no change in microbial fitness or may even increase fitness [2], depending on the specific mutation, strain background, and experimental conditions [3, 4]. Moreover, initial decreases in fitness due to resistance may be restored over time by compensatory evolution, which can stabilize the resistant populations and render them as fit as susceptible organisms [2, 6]. Therefore, although the concept of fitness cost is useful, the relationship between resistance and the fitness of microbes is more complex than the term suggests [3].

Antifungal resistance has emerged as a major clinical problem in concert with the increasing populations of immunosuppressed hosts and hospitalized patients at risk for fungal infections. Antifungal resistance mechanisms are best understood for fluconazole among *Candida* species, the most common fungal pathogens. Fluconazole and other azoles are particularly prone to the emergence of resistance, because they are often prescribed for extended periods on a repeated basis, and their fungistatic activity leaves a larger residual population of *Candida* than do fungicidal drugs [1]. In addition, there are multiple mechanisms by which *Candida* strains can become fluconazole resistant, including overexpression or mutation of *ERG11* (the gene encoding the target enzyme lanosterol 14-α demethylase), expression of efflux pumps, gain-of-function mutations in a transcription factor regulating efflux pump expression, loss of mitochondrial function, and changes in chromosome number [1, 7–12]. Given these diverse mechanisms, the effect of fluconazole resistance on fitness is at least as complex as that for bacteria and antibiotic resistance. In fact, studies have reported both increased and reduced candidal fitness, disparities between fitness in vitro and in vivo, and the presence and absence of compensatory evolution [1, 7–12].

In this issue of the *Journal*, Ben-Ami et al report the first study of the effect of echinocandin resistance on fitness of a *Candida* species [13]. The echinocandins have become front-line agents for the treatment of candidiasis because of their broad-spectrum activity, including against fluconazole-resistant *Candida* strains [14]. These agents inhibit the synthesis of 1,3-β-D-glucan synthase, an enzyme complex that encodes a major constituent of the cell wall. To date, echinocandin resistance remains relatively rare among clinical isolates [15], although reports are increasing in number [16]. Compared with azole resistance, the emergence of echinocandin resistance is limited by the candidacidal activity of the class and the fact that the only known mechanism is mutation in the *FKS* genes encoding the glucan synthase complex [17]. As such, the study of fitness costs associated with echinocandin resistance may

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be more straightforward than with azole resistance.

In their study, Ben-Ami and colleagues compared resistant *Candida albicans* strains in which homozygous *fks1*-S645F or *fks1*-S645P mutations arose in response to echinocandin exposure in clinical or laboratory settings with their respective wild-type *FKS1* parents. To date, these are the most commonly reported *FKS* mutations. Grown in the absence of an echinocandin, the *fks1* mutants exhibited reduced maximum catalytic velocity (V_{max}) of glucan synthase, thickened cell walls due to increased chitin content, decreased growth rates and filamentation in vitro, and significantly attenuated virulence in *Drosophila melanogaster* flies and mice. Strikingly, the aberrant phenotypes correlated directly with chitin content. Increased chitin also was associated with a blunted Dectin-1–mediated inflammatory response from murine macrophage cells during coincubation experiments in vitro, suggesting that the cell wall salvage responses triggered by the *FKS1* mutation may act to minimize the tissue damage that contributes to mortality during disseminated candidiasis. Most importantly, for 1 mutant-parent pair, the investigators confirmed the reduced fitness using highly sensitive competitive growth assays in vitro and within mouse kidneys following hematogenous dissemination.

Indeed, the study is notable for its logical design and scientific rigor, which strengthen the authors' conclusion that *C. albicans FKS* mutations that confer resistance to echinocandins come at costs in fitness and virulence. There are several caveats to this conclusion that do not diminish the value of the paper. First, the results are valid for the specific mutations studied, in these specific *C. albicans* strains, under these specific experimental conditions. As prior experience with various antibiotic-resistant bacteria and fluconazole-resistant *Candida* strains has taught, results may differ for other mutations, *C. albicans* strains, *Candida* species, or fitness and virulence assays. Second, the authors' suggestion that the attenuated fitness of *fks* mutant *C. albicans* strains may place them at an evolutionary disadvantage in the absence of echinocandin exposure and, thereby, limit the potential for spread within the population is tempered by the fact that clinical studies testing this hypothesis for drug-resistant *Mycobacterium tuberculosis* and other bacteria have been inconclusive [2, 3]. In fact, most data suggest that antimicrobial resistance, once introduced into a population at the individual or community level, is not easily reversed by removal of antimicrobial selection pressure [2]. Finally, the authors acknowledge that their study does not assess the effect of *FKS1* mutations on genome-wide expression patterns, chromosomal stability, or other adaptive responses. As they note, the attenuated fitness and virulence of the clinical *fks1* mutants suggest that the phenotypes that they describe remain stable within the host environment. Nevertheless, it is not clear whether this stability would remain evident during long-term persistence within the host or whether changes due to compensatory evolution would eventually emerge. These considerations are particularly important for *C. albicans* and most other *Candida* species, given their normal life cycles as human commensals.

This study appears at a time when numerous investigations into the clinical relevance of echinocandin resistance are being undertaken. Although the echinocandins are an important advance in the treatment of candidiasis, therapeutic failures and breakthrough infections are well recognized, and mortality rates during candidemia remain high. The relative contribution of resistance to failed echinocandin therapy and poor outcomes is unclear, and at present, clinicians do not have a validated laboratory test to identify patients who are unlikely to respond to these agents. Clearly, the presence of *FKS* mutations is associated with elevated minimum inhibitory concentrations (MICs) and reports of failed therapy [16], but the role, if any, of genotypic testing in improving outcomes is undefined. Breakpoint MICs for resistance have recently been revised to define more clinical isolates as echinocandin resistant [18], but their effect is as yet unknown. In fact, data from clinical trials of caspofungin suggested that treatment responses were better, rather than worse, among patients infected with *Candida* isolates having elevated caspofungin MICs [19]. Ben-Ami and colleagues speculate that their results may provide a biological explanation for these seemingly paradoxical clinical findings. Their hypothesis will come under intense scrutiny in the upcoming years, and if validated, it would have profound clinical implications. Rather than relying solely on conventional susceptibility methods such as echinocandin MIC measurements or *FKS* genotyping, for example, it may be that clinicians will need to incorporate fitness assays into their decision making at the bedside. In this regard, the work of Ben-Ami and colleagues is important because it highlights the fact that our ability to improve the treatment and prevention of infectious diseases such as candidiasis will depend on sound research that considers the interactions between infecting organisms, the host, and therapeutic interventions.

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