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Antibiotic efficacy in the complex infection environment

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Accurate prediction of antimicrobial efficacy is essential for successful treatment of bacterial infection. Beyond genetically encoded mechanisms of antibiotic resistance, the determinants of antibiotic susceptibility during infection remain poorly understood, and treatment failure is common. Traditional antibiotic susceptibility testing fails to account for extrinsic determinants of antibiotic susceptibility present in the complex infection environment and is therefore a poor predictor of antibiotic treatment outcome. Here we discuss how host–pathogen interaction, microbial interspecies interaction, and metabolic heterogeneity contribute to the success or failure of antibiotic therapy. Consideration of these factors during the treatment of disease will improve our ability to successfully resolve recalcitrant bacterial infection and improve patient health.

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Since the discovery of penicillin in 1928, antibiotics have become an essential component of modern healthcare. They have made once life-threatening infections readily treatable, greatly prolonged the lives of immunocompromised individuals, and made possible the routine undertaking of invasive surgical procedures. Currently, however, we are facing a growing crisis as resistance to antibiotics continues to spread, while the discovery of new antibiotics has stagnated [1,2]. Thus, it is more important than ever to use currently available antibiotics as effectively and appropriately as possible. As part of this effort, it is essential that we develop a more sophisticated understanding of antibiotic efficacy in the infection environment. Clinical antibiotic susceptibility testing consists primarily of *in vitro* minimum inhibitory concentration (MIC) assays that measure the ability of an antibiotic to inhibit growth of a pure culture grown under artificial conditions. Consequently, these assays do not assess the ability of a drug to eradicate an existing bacterial population, and fail to account for extrinsic determinants of antibiotic susceptibility present in the complex infection milieu. Indeed, several studies have demonstrated poor correlation between MIC testing and subsequent treatment outcome [3,4]. This poor correlation is particularly problematic in the case of deep-seated, chronic infections that fail to respond to prolonged antibiotic therapy despite apparent drug susceptibility.

In this review, we discuss how host–pathogen interactions, interspecies microbial interactions and metabolic heterogeneity in the infection environment can contribute to the success or failure of antibiotic therapy in patients. Identification and consideration of all the factors in the infection environment that impact the ability of an antibiotic to inhibit bacterial growth and/or kill bacterial cells will improve our ability to predict efficacy in patients, reduce the duration of antibiotic therapy, and decrease the risk of treatment failure, thereby minimizing the development and spread of antibiotic resistance.

Host interaction and antibiotic susceptibility

Antibiotics can be divided into two broad categories, bacteriostatic and bactericidal, based on their ability to inhibit growth or kill bacteria. Inhibition of bacterial growth by bacteriostatic antibiotics gives the host immune system a chance to contain and eliminate an infectious bacterial population. While bactericidal antibiotics lead to bacterial cell death, even powerful bactericidal agents fail to eradicate bacterial populations, as antibiotic tolerant persister cells can survive in the presence of the antibiotic for long periods of time [5,6•]. Hence, both bacteriostatic and bactericidal antibiotics rely on co-operation with the immune system to fully eradicate an infection. In some cases, this co-operation may simply be additive, wherein an antibiotic inhibits growth or kills a portion of the population, and the immune system then eliminates the survivors. On the other hand, specific host-bacterial interactions may specifically inhibit or potentiate antibiotic efficacy. Such antagonistic or synergistic interactions are only recently coming to light, and their impact on *in vivo* efficacy is yet to be fully appreciated.

By comparing antibiotic efficacy in the presence or absence of host factors, Sakoulas *et al.* observed that β-lactam antibiotics can synergize with the host immune system to potentiate bactericidal activity. Specifically, they found that ampicillin treatment can kill 'ampicillin

resistant' populations of *Enterococcus faecium* by facilitating alteration of surface charge, leading to increased sensitivity to the action of host antimicrobial peptides (AMPs) [7•]. Similarly, *Staphylococcus aureus* populations, considered β-lactam resistant by MIC testing, were sensitized to killing by various host factors following β-lactam exposure [8]. Furthermore, in a murine model of intratracheal infection, it was found that the macrolide antibiotic, azithromycin, synergizes with the host cathelicidin antimicrobial peptide, LL-37, resulting in bactericidal activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and more recently *Stenotrophomonas maltophilia*, despite an apparent lack of susceptibility to azithromycin by MIC testing [9••,10•]. It is likely that other as yet unidentified interactions with host factors synergize with commonly used antibiotics to promote efficacy within a patient.

Interactions with the host may also be inhibitory to certain antibiotic activities. The clearest example of this antagonistic relationship is the ability of numerous pathogens to survive within host cells, where the adoption of an intracellular lifestyle often correlates with decreased antibiotic sensitivity [11–13]. Within this niche, bacteria are not only physically protected from certain antibiotics, such as aminoglycosides, that penetrate poorly into host cells, but the intracellular environment itself can induce tolerance to numerous antibiotic classes. For instance, innate defenses within macrophages can induce phenotypic resistance to the last-line antibiotic colistin in *Enterobacter cloacae* via activation of the histidine kinase PhoQ [14••]. Importantly, in this study Band *et al.* explicitly demonstrate that an *E. cloacae* isolate described as colistin-susceptible via common clinical susceptibility testing can proliferate in the presence of colistin *in vivo*, leading to treatment failure. Phagosome acidification and nutrient sequestration in macrophages has also been shown to induce the formation of antibiotic tolerant persister cells in *Salmonella*, resulting in a more difficult to treat infection [15•]. Likewise, activated macrophages produce nitric oxide (NO) and reactive oxygen species (ROS), with unintended, deleterious consequences in regards to pathogen antibiotic susceptibility. For instance, host NO production can inhibit proton motive force (PMF)-dependent uptake of aminoglycoside antibiotics by inhibiting bacterial respiration and thus PMF generation [16], and DNA damage from exposure to ROS can induce persister cell formation in *Escherichia coli* via the upregulation of toxin and drug efflux pump expression [17•]. Though the influence of ROS on *E. coli* persister cell formation has largely been studied *in vitro*, host ROS generation may illicit this same response *in vivo*. The importance of the induction of antibiotic resistant sub-populations or tolerant persister populations by the immune system remains to be fully elucidated. It has been proposed that persister populations act as reservoirs for the infection, leading to relapse once antibiotic therapy is ceased [18,19]. Further examination of persister formation *in vivo* and development of therapies to eliminate persisters may have a major impact on the treatment of chronic and relapsing infection.

The above examples likely represent a microcosm of the many host–microbe interactions that influence antibiotic efficacy during infection. Further elucidation of host mediators of antibiotic activity will improve our ability to predict antibiotic efficacy *in vivo*. Furthermore, the consideration of key host factors during antibiotic discovery and development may lead to novel antibiotics with increased activity within the host.

Antibiotic susceptibility and microbial interactions

Rather than existing in isolation, invading microorganisms frequently encounter a complex polymicrobial community within the host, where interactions with the resident microbiota or co-infecting pathogens can directly influence the overall structure and dynamics of the community. Antibiotic susceptibility of an organism within this complex environment may vary dramatically from that of the same organism grown in pure culture. An excellent example of community based antibiotic resistance can be seen in the deactivation of an antibiotic by a single bacterial species, extracellularly or intracellularly, leading to *de facto* antibiotic resistance of the entire community [20•,21]. In this case, antibiotic sensitive pathogens may elude antibiotic killing due to the activities of a co-existing organism. For instance, it was recently shown that deactivation of chloramphenicol by a resistant population of *Streptococcus pneumoniae* facilitated the growth of a 'freeloader' chloramphenicol sensitive *S. pneumoniae* population in a mouse undergoing chloramphenicol therapy [20•].

In addition to antibiotic deactivation, interspecies interactions can alter microbial metabolism and physiology to induce transient resistance or tolerance to antibiotics. For instance, production of the respiratory toxin 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) by *P. aeruginosa* elicits aminoglycoside resistance in *S. aureus* by inhibiting the electron transport chain and depleting *S. aureus* cellular PMF, a necessary pre-requisite for aminoglycoside uptake [22••]. *P. aeruginosa*-produced HQNO has also been shown to induce vancomycin tolerance in *S. aureus* by shifting *S. aureus* into a fermentative lifestyle [23]. As these pathogens frequently co-exist within the cystic fibrosis lung and in chronic wound infections, these interactions may be an important determinant of antibiotic treatment outcome.

Intraspecies quorum sensing (QS) has also been associated with changes in the susceptibility of a population to antibiotic killing. Production of the QS molecules CSP