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Antibiotic efficacy in the complex infection environment

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Abstract

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.

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 in 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.

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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 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 phagocytic cells following phagocytosis, 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 fail to penetrate 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 and host death. 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.

These 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 prerequisite 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 and acyl-homoserine lactone mediate multidrug-tolerant persister cell formation within populations of *Streptococcus mutans* and *P. aeruginosa*, respectively (24, 25). In an interesting example of interspecies crosstalk, indole production by the native commensal *E. coli* was demonstrated to induce antibiotic tolerance in pathogenic *Salmonella enterica* Typhimurium (26). Similarly, interception of *Haemophilus influenzae* autoinducer-2 (AI-2) by *Moraxella catarrhalis* significantly increases *M. catarrhalis* tolerance to multiple antibiotics through the induction of *M. catarrhalis* biofilm formation (27). Indeed, biofilm-associated infections have long been associated with antibiotic treatment failure, and these infections are often polymicrobial in nature (28). Biofilm matrix production by one microbial species may induce antibiotic tolerance in another. In a recent example, it was demonstrated that *C. albicans* extracellular matrix production during dual-species biofilm formation protects *S. aureus* from antibiotic killing *in vivo* (29).

In all, studying bacterial pathogenesis outside of artificial monoculture is not only more representative of the conditions encountered during infection, but also reveals instances where factors produced by one species can inadvertently influence the susceptibility of another to antimicrobial activities. Interspecies microbial interactions can induce antibiotic resistance or tolerance, which may mitigate antibiotic efficacy. It is also likely that synergistic interactions occur that increase antibiotic efficacy, although this remains to be seen. Identifying the determinants of antibiotic susceptibility in complex communities rather than relying on potentially misleading information garnered from monoculture susceptibility assays is essential for improving our ability to efficiently treat polymicrobial infection.

Metabolic determinants of antibiotic susceptibility

Currently, antibiotic susceptibility is measured in nutrient rich media, under aerobic conditions, free of most stressors typically encountered during infection. However, the complex “macro-ecosystem” of a host consists of a variety of physiologically distinct microenvironments subject to bacterial colonization. Nutrient availability and overall physiological states within these distinct niches can vary drastically, and promote stark differences in bacterial metabolism. Even within the same spatial niche there often exists a significant degree of environmental heterogeneity, with aerobic, microaerophilic and anaerobic microniches in close proximity. Such is the case in late stage CF patients, where decreased mucociliary clearance promotes the formation of mucus plugs within the alveoli of the lungs, creating anoxic microenvironments within the aerobic lung (30). Oxygen penetration is also often severely hampered in wound infections and abscesses (31, 32).

Indeed, obligate anaerobes are frequently isolated from the CF lung as well as from polymicrobial wound infections, implying that anoxic microenvironments exist within these infection sites (30, 33). Within the heterogeneous infection environment, facultative anaerobes such as *S. aureus*, *E. coli* or *Streptococcus pneumoniae* can colonize both aerobic and anaerobic niches to cause disease, and life within these niches requires specific metabolic adaptation.

Metabolic heterogeneity in the infection environment may play a significant role in dictating antibiotic susceptibility. Indeed, certain antibiotic classes are active only against either aerobically or anaerobically growing bacteria. Metronidazole, for instance, must be reduced by nitroreductases in order to exhibit bactericidal activity, and reduction only occurs in anaerobically growing bacteria (34). Conversely, PMF-dependent uptake of aminoglycosides generally restricts their activity to aerobically respiring bacteria (35, 36). Active cellular respiration has also been linked to the lethality of other bactericidal antibiotics (37). We recently demonstrated that intracellular ATP levels are predictive of antibiotic susceptibility in *E. coli* and *S. aureus*, and that entrance into a low-ATP state is associated with increased tolerance to the bactericidal effects of antibiotics (38, 39). Respiration is a more efficient ATP generating process than fermentation, thus, actively respiring cells under oxygen rich conditions are expected to be higher in energy and more susceptible to antibiotic killing than cells in anoxic environments undergoing fermentation. In support of this, frequently acquired mutations that result in defective electron transport in *S. aureus* are commonly associated with persistent infection, as the small colony variants (SCVs) that result are highly resistant to antibiotic killing activity (40). SCVs are selected for by oxidative stress and low pH as well as interaction with *P. aeruginosa*, further exemplifying how host and interspecies interaction can alter antibiotic susceptibility (41, 42).

Antibiotic tolerance increases significantly during periods of nutrient limitation or diauxic carbon-source transition, and starving bacteria of specific nutrients during *in vitro* growth markedly increases antibiotic tolerance (43–45). Similarly, it has been proposed that persister formation in *E. coli* can be mediated by the stringent response, which is activated upon amino acid starvation (46). As bacteria compete both with other microorganisms, and the host for nutrient acquisition during pathogenesis, nutrient availability undoubtedly plays a role in determining antibiotic susceptibility *in vivo* as well.

Biofilm-associated growth represents a major source of metabolic heterogeneity during infection. Nutrient and oxygen consumption by cells at the periphery of the biofilm coupled with limited nutrient diffusion can result in a starvation-induced state of dormancy for cells at the center of the biofilm that is associated with increased tolerance to antibiotic killing (47). Supplying biofilms with limiting nutrients can restore bacterial susceptibility to antibiotic killing, suggesting that starvation induced antibiotic tolerance may be responsible for the recalcitrance of biofilm infections to antimicrobial treatment (43, 48, 49).

Further investigation into the influence of bacterial metabolism on antibiotic susceptibility and of the metabolic state of pathogens at various infection sites is of major importance. In order to eradicate a bacterial infection, we must adjust our strategy to also target populations that have adapted to nutrient and oxygen depleted conditions. In support of this, the

antibiotic ADEP4, which kills without any requirement for metabolic activity or ATP, is capable of killing persisters *in vitro* and *in vivo* resulting in eradication of *S. aureus* populations (50).

Closing Remarks

Though antibiotic susceptibility is traditionally examined in simple homogenous conditions *in vitro*, more and more studies are revealing the dynamic and complex nature of antibiotic efficacy in the infection environment (Figure 1). The administration of antibiotics without consideration of these environmental factors may result in treatment failure, exacerbated disease progression, and the rise of resistant microorganisms. Moreover, pathogen sensitivity to antibiotic killing is contingent not only on genotype, but also the pathogen's metabolic state, as well as interactions with the host and co-infecting microorganisms. Further elucidation of key determinants of efficacy *in vivo* may lead to a more sophisticated and personalized approach to antibiotic therapy in order to eradicate infection in a given patient as efficiently as possible, thereby reducing the likelihood of treatment failure and the incidence and spread of antibiotic resistance.

Acknowledgments

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Highlights

- The infection environment influences antibiotic susceptibility
- Clinical susceptibility assays frequently fail to predict drug efficacy in patients
- Host interactions and microbial interactions alter antibiotic susceptibility
- The metabolic activity of a pathogen also determines antibiotic susceptibility
- Environmental factors may potentiate or antagonize antibiotic activities
- Consideration of these factors may improve antibiotic treatment of patients

Figure 1. Overview of extrinsic factors influencing antibiotic susceptibility within the host
 Environmental factors can antagonize or potentiate antibiotic efficacy killing of a pathogen. Antimicrobial peptides (AMPs) can synergize with antibiotics to increase killing of pathogens. Conversely, pathogen engulfment by phagocytic cells can inhibit antibiotic killing by preventing drug access to the pathogen or by directly influencing pathogen metabolism and physiology through production of reactive oxygen or nitrogen species (ROS/RNS), vacuole acidification or nutrient sequestration. Inter- and intraspecies interactions can positively and negatively impact a pathogen's susceptibility to antibiotic killing either through signaling processes or via direct interaction, such is the case in polymicrobial biofilms. Finally, heterogeneity in oxygen or nutrient concentration within the infectious environment can influence bacterial metabolism with significant consequences for antibiotic susceptibility.