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SUPPLEMENT ARTICLE

Interplay Between Antibiotic Resistance and Virulence During Disease Promoted by Multidrug-Resistant Bacteria

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Diseases caused by antibiotic-resistant bacteria in hospitals are the outcome of complex relationships between several dynamic factors, including bacterial pathogenicity, the fitness costs of resistance in the human host, and selective forces resulting from interventions such as antibiotic therapy. The emergence and fate of mutations that drive antibiotic resistance are governed by these interactions. In this review, we will examine how different forms of antibiotic resistance modulate bacterial fitness and virulence potential, thus influencing the ability of pathogens to evolve in the context of nosocomial infections. We will focus on 3 important multidrug-resistant pathogens that are notoriously problematic in hospitals: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. An understanding of how antibiotic resistance mutations shape the pathobiology of multidrug-resistant infections has the potential to drive novel strategies that can control the development and spread of drug resistance.

Keywords. antibiotic resistance; virulence; fitness; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; *Staphylococcus aureus*.

Hospital-borne diseases are the outcome of a complex interplay of several dynamic factors operating at the level of the infecting microorganism, the host patient, and the hospital environment. These include the pathogenicity, drug resistance, and environmental persistence of the microbe; the immune status and microflora composition of the human host; and nosocomial interventions such as antimicrobial therapy. As these factors interact, the bacteria responsible for nosocomial diseases constantly evolve, with the unfortunate outcome being the rapid and widespread rise in intractable, multidrug-resistant (MDR) organisms. There has been much recent interest in how the genetic perturbations responsible for antibiotic resistance modulate bacterial biology and fitness. Studies on the effects of antibiotic resistance on fitness often document fitness costs of varying severity [1]. In many cases, measurements of bacterial growth in animal hosts have revealed fitness costs and virulence attenuations that agree with in vitro tests, leading to the view that pathogens incur fitness trade-offs that compromise their pathogenic potential. Other studies have challenged this view, however, by providing examples in which drug resistance increases pathogen fitness during infection [2–4]. Understanding the consequences of antibiotic resistance mutations on the pathobiology of MDR infections has important implications for controlling the spread of resistance and informing novel treatment strategies.

In this review, we will examine the described effects of antibiotic resistance on bacterial fitness and virulence. Our focus is on 3 key MDR pathogens that are common agents of problematic hospital-acquired infections: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. Experimental measures of fitness performed in bacteriological culture do not always predict fitness at sites of host infection or the clinical outcomes of infection, so we will emphasize studies using animal models to examine fitness effects of antibiotic resistance in vivo and their clinical correlates. In addition, studies examining how resistance-induced reprogramming of bacterial biology may underlie the observed changes in pathogenicity will be discussed. As a point of emphasis, we will orient our view toward the fitness impacts of *acquired* antibiotic resistance, which we define as resistance mechanisms that have arisen by relatively recent genetic changes, including mutation or gene acquisition. This is in contrast to *intrinsic* resistance, which we view as being selected as a consequence of long-term pathogen evolution within hosts, and which thus possess functions that are by necessity intertwined with virulence. Accordingly, examples of intrinsic resistance determinants include outer membrane components that confer low permeability and fine-tuned production of broad-substrate efflux pumps. Examples of acquired resistance mechanisms are mutations to the enzyme targets of antibiotics, resistance gene cassette acquisitions, and altered regulation of intrinsic resistance determinants as a consequence of mutational changes.

P. AERUGINOSA INFECTIONS

P. aeruginosa is responsible for a wide range of nosocomial infections. Although this pathogen encodes a broad swath of potential virulence factors, its ability to cause disease is tied to deficiencies in host defenses, as occurs in patients receiving mechanical ventilation and those with cystic fibrosis (CF) [5]. Rates of multidrug resistance in *P. aeruginosa* continue to rise

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globally [6, 7]. Several studies have analyzed how production of virulence determinants and fitness are altered by antibiotic resistance mutations, particularly those causing misregulated multidrug efflux pump production and mutations leading to β-lactam resistance.

Multidrug Resistance Through Efflux Pump Hyperproduction

Drug pumps of the resistance-nodulation-division (RND) class result in clinically significant increases in resistance to a range of antibiotics and other noxious compounds when overproduced in mutant strains of *P. aeruginosa* [8]. The expression levels of these systems are controlled by an array of transcriptional regulators [8]. In many cases antibiotic treatment enriches for mutations within a single regulator causing persistent hyperproduction of a given pump and increased resistance to its substrates. With the MexAB-OprM RND system, which exports a very wide range of structurally diverse antibiotics, detergents, disinfectants, and quorum-sensing (QS) molecules, mutations resulting in hyperproduction are highly common in strains isolated from patients with severe, acute infections and are frequently due to mutations to the *mexR* regulator ("*nalB*"-type mutations) [9–11]. With MexCD-OprJ and MexEF-OprN, overproduction is less prevalent in organisms causing acute disease but has been observed with isolates associated with chronic infections in patients undergoing long-term antibiotic therapy [12–14].

Experimental data generally support the notion that hyperproduction of the clinically important RND pumps confers a fitness cost for the organism growing in tissue sites in the absence of antibiotic pressure. Compared with isogenic wild-type (WT) strains, *mexR* mutants hyperproducing MexAB-OprM showed reduced production of virulence determinants such as phenazines and proteases [15] and lowered virulence in varied animal models [15, 16]. In agreement with these results, a study on *P. aeruginosa* strains isolated during an outbreak in France documented the emergence of isolates with low *mexAB-oprM* levels despite the presence of a *nalB*-type *mexR* allele that should result in overproduction of these proteins [17]. In these isolates second-site mutations were found in the promoter or ribosome-binding sites of the *mexAB-oprM* operon, consistent with the hypothesis that constitutive pump overproduction imposes a fitness burden that can be offset by compensatory mutations.

Fitness costs during growth in animal models are also seen with mutational overproduction of the MexCD-OprJ [15, 18] and MexEF-OprN egress pumps [19, 20], and these costs are often linked to global effects on bacterial physiology. Overproduction of MexCD-OprJ causes pleiotropic changes to metabolism and gene expression [21], including reduced production of the type III secretion system, an important virulence determinant [22]. Overproduction of MexEF-OprN due to *nfxC* mutations was shown to reduce production of virulence factors including pyocyanin, elastase, and rhamnolipids, which are QS regulated [23]. Interestingly, these deficiencies resulted from impaired QS signaling due to excessive removal of QS autoinducer precursors by the overproduced pump [20, 24].

Although the above-mentioned studies demonstrate that fitness costs are associated with acquired resistance through mutational up-regulation of RND pumps, several studies support a role for natively regulated pump production in promoting pathogenesis, illustrating the connection between intrinsic resistance and virulence. Expression of *mexAB-oprM* is induced in animal models [3], and inactivation of the system attenuates fitness or virulence in these same models [3, 25, 26], while reducing invasiveness into cultured epithelial cells [25]. Consistent with these findings, *mexA* and *mexB* mutations resulting in pump deficiencies were observed to accumulate during the course of chronic infection in patients with CF [27, 28], a setting in which selective pressures tend to promote attenuation of virulence through gradual loss of classic virulence factors [29]. Therefore, tight regulation of RND pumps, such as MexAB-OprM, has probably evolved to balance pathogenicity, resistance, and fitness.

Carbapenem Resistance as a Consequence of OprD Deficiency

OprD is a channel for the uptake of basic amino acids and small peptides containing basic residues, as well as for carbapenem antibiotics [30–32]. Carbapenem resistance through null mutations in *oprD* commonly arises in *P. aeruginosa* patient isolates during therapy [33, 34]. Infections with such isolates are associated with worse clinical outcomes [35]. Interestingly, mutations lowering *oprD* expression were found to arise during long-term colonization in patients who have not been treated with carbapenem [36], consistent with the presence of an interplay between the lowered amounts of this porin and adaptation to the human host.

The relationship between mutational inactivation of *oprD* and pathogenicity was examined in a series of studies by Skurnik, Roux and colleagues [3, 4], who provided evidence that loss of *oprD* increases bacterial fitness and virulence in mouse infection models. In a large-scale screen of transposon inactivation mutants for altered relative fitness during mouse gastrointestinal infection, *oprD* mutations yielded the paradoxical result of enhanced colonization and increased systemic dissemination [4]. This effect was shown with isogenic laboratory strains and with related clinical isolates varying in *oprD* expression. The loss of *oprD* function also increased resistance to killing by human serum and low pH, and increased bacterial-driven killing of murine macrophages. In a subsequent study, the authors showed that *oprD* mutants were also more virulent than their *oprD*$^+$ counterparts in a mouse pneumonia model [3]. Genome-wide transcriptional profiling by RNA-seq of WT and *oprD*$^-$ strains uncovered a large number of genes whose relative expression levels were a function of the *oprD*

allele, but well-established virulence genes such as the type III secretion system and exopolysaccharides were unaffected by the absence of this gene [4]. This suggests that altered fitness during disease may be directly a consequence of OprD function, or may result from large-scale expression changes that affect processes other than the direct interface of this protein with host-derived molecules.

We note that the model positing that lowered *oprD* expression increases virulence is in contrast to a prior study using a different *P. aeruginosa* laboratory strain background [16]. In that study pulmonary infection with an *oprD* deletion strain led to reduced mouse mortality when compared with a parental WT strain, although the reversibility of the virulence phenotype was not interrogated with a genetic complementation test.

β-Lactam Resistance via *ampC* Overexpression

Resistance to noncarbapenem β-lactams is highly prevalent in *P. aeruginosa* and is commonly due to mutational derepression of the *ampC* β-lactamase [7]. In WT strains, a regulatory system maintains *ampC* expression at low basal levels in the absence of antibiotics and allows highly induced expression with β-lactam treatment. Mutations to this induction circuit result in constitutively increased *ampC* expression and β-lactam resistance [7]. Mutations are diverse and often found in the *ampD* amidase, *ampR* regulator [37, 38], or the nonessential penicillin-binding protein PBP4 (*dacB*) [39].

Regarding the amidase *ampD*, *P. aeruginosa* encodes 3 homologues [7]. In laboratory strains, mutation of 1 of the amidase genes results in partial *ampC* derepression, while combined inactivation of the 3 amidase genes results in constitutive high-level *ampC* derepression [7]. In clinical isolates, single *ampD* mutants seem most prevalent [40]. This finding may reflect a compromise between pathogenicity and resistance, because single mutations eliminating only 1 of these amidases show no fitness or virulence trade-off in a mouse systemic infection model, whereas double or triple *ampD* mutants show significant fitness costs and attenuated virulence [40]. Combinatorial *ampD* mutants fully-derepressed for *ampC* production probably mimic the situation with *Salmonella typhimurium*, in which deletion of its single *ampD* gene was shown to reduce in vivo fitness owing to the build-up of muropeptides within the bacterial cytoplasm [41].

Several studies have described potential infection-promoting roles for components of the *ampC* induction circuit in *P. aeruginosa*. For example, *ampR* was shown to regulate diverse genes beyond *ampC,* such as the virulence determinants pyocyanin and elastases [42]. Roux et al [3] showed that inactivation of *ampC* reduces fitness during mouse gastrointestinal infection in the presence of penicillin and that the gene is induced transcriptionally under these conditions.

Hypermutators

Hypermutator variants arise during chronic infections with *P. aeruginosa,* as occurs in patients with CF [43], and this state has been shown to promote the development of antibiotic resistance as well as compensatory adaptation to the fitness costs of resistance [44, 45]. Hypermutator mutations themselves decreased overall population fitness in vivo in a mouse chronic lung infection model [46]. Interestingly, antibiotic pressure can reverse the fitness disadvantage associated with Δ*mutS* hypermutators in animal infection models [47].

A. BAUMANNII INFECTIONS

Infections with the opportunistic pathogen *A. baumannii* have emerged around the globe as increasingly problematic for clinicians, especially in intensive care unit settings. Rates of multidrug resistance have risen rapidly in recent years, with increasingly common resistance to last-line antibiotics, such as colistin. Although infections with *A. baumannii* are often highly difficult to treat, the organism has generally been considered to have relatively low virulence potential in immunocompetent hosts. Strains with increased pathogenicity in animal models, however, have been recently isolated [48, 49]. Here we discuss the relationship between the in vivo fitness of this microorganism and pathways leading to colistin resistance and pump overproduction, and highlight recent work on genetic and phenotypic adaptations that can reversibly modulate its resistance and virulence potential.

Colistin Resistance

Resistance to colistin, a drug increasingly used to combat MDR *A. baumannii*, is relatively rare but has been observed to arise during therapy [50]. Mutations usually occur in the *pmrAB* 2-component system, leading to increased modification of the lipid A target of the drug [51–53]. Such mutations have been shown in numerous studies to confer some degree of fitness cost in vitro and in various in vivo models [54–56], although isolates that have developed high-level colistin resistance without loss of virulence have been described using the *Galleria mellonella* infection model [57, 58]. Colistin-resistant isolates resulting from lesions in *pmrB* also showed cross-resistance to host antimicrobial peptides [59], providing a potential mechanism that counteracts inherent fitness costs at sites of host infection. An alternate mechanism of colistin resistance observed in some isolates is complete loss of lipopolysaccharide due to mutations in genes responsible for its biosynthesis. These mutations are more frequently associated with laboratory selection on bacteriological medium, however [60, 61] and, predictably, they show severe in vivo fitness trade-offs [58, 60].

Snitkin and colleagues [53] captured the evolution of colistin resistance in *A. baumannii* strains isolated longitudinally during and after withdrawal of colistin therapy. Resistance due to multiple types of *pmr* mutations occurred independently during therapy. In all cases susceptible isolates reappeared quickly on therapy withdrawal, supporting the idea that *pmr* mutations leading to colistin resistance are costly for fitness and infectivity.

Genomic analysis revealed a number of additional findings of interest. First, in several resistant isolates, mutations predicted to affect translation arose, pointing to a possible compensatory mechanism offsetting the in vivo fitness costs of colistin resistance. Second, in 1 isolate that regained sensitivity after colistin treatment, the original *pmrB* resistance allele was retained, while a mutation arose in *pmrA* predicted to attenuate hyperactivation of the system. Notably, this compensatory mutation was shown to constrain the ability to re-evolve colistin resistance. Third, a low-cost *pmr* mutation resulting in intermediate resistance not detectable by standard clinical microbiological testing was identified in a subset of isolates [53]. This study illustrates the power of genome sequencing to uncover multiple mutational pathways to drug resistance and their fate after antibiotic withdrawal.

Multidrug Efflux Pumps

As with *P. aeruginosa,* overproduction of efflux pumps is a widespread mechanism that contributes to MDR phenotypes in *A. baumannii*. Three egress systems of the RND class, known as AdeABC, AdeIJK, and AdeFGH, confer increased resistance to a broad range of antibiotics in many clinical isolates [62]. Hyperproduction of either AdeABC or AdeIJK is most frequently observed and usually results from mutations within the transcriptional regulator genes that control their expression [62, 63]. In the absence of regulator mutations, AdeABC production is tightly controlled at a very low basal level, whereas the AdeIJK operon is expressed at relatively high basal levels, accounting for its role in supporting intrinsic resistance in WT strains [62]. Compared with isogenic WT, overproduction of AdeABC or AdeIJK resulted in minimal fitness costs during the course of systemic infection in mice, and no significant fitness cost during lung infection in a mouse pneumonia model [64], consistent with the high prevalence of clinical strains overproducing these pumps [63]. Although histopathology revealed similar lesions in lungs, AdeABC-overproducing bacteria caused changes to inflammatory markers within bronchoalveolar lavage fluid that were consistent with increased neutrophil activation compared with WT bacteria, suggestive of an altered host inflammatory response [64]. Further work is needed to determine the molecular basis of such changes and their impact on pathogenesis.

Overproduction of AdeABC and AdeIJK results in additional phenotypes that may have consequences for host-pathogen interactions, including altered membrane protein levels and reduced biofilm growth [62]. Pump-overproducing mutants may show altered physiology or host interactions through multiple possible means including the direct effects of increased pump activity or through global transcriptional changes brought about by the mutated regulator gene. Recent RNA-seq analysis comparing a clinical strain overproducing AdeABC with isogenic strains deleted for either the pump or its regulator system indicates that gene expression changes are driven by both of the above mechanisms [65]. Deletion of the cognate regulator (*adeRS*) or the pump itself resulted in changes to the transcription levels of hundreds of genes, including those encoding pili and a siderophore transport system with potential roles in virulence [65]. Additional analysis is required to define the direct gene targets of *adeRS* and the connections between AdeABC overproduction and global alterations in gene expression.

The expression of efflux pumps at basal levels in nonoverproducing WT strains of *A. baumannii* has also been shown to contribute to fitness in animal models. In a large transposon inactivation screen, disruption of the *adeIJK* genes in a nonoverproducing isolate diminished fitness in a *G. mellonella* infection model [66]. In their study exploring the relationships between resistance and bacterial fitness in vivo, Roux and colleagues [3] analyzed 2 *A. baumannii* genes annotated as RND pumps. They showed that inactivation of the genes (A1S_1649 and A1S_1801) resulted in both reduced sensitivity to the aminoglycoside tobramycin and delayed killing of mice after intraperitoneal injection.

Our own informatics examination sheds additional light on the putative connections of these genes with resistance and virulence. Querying the 2 gene products with the National Center for Biotechnology Information Conserved Domain Database revealed a close relationship to the major facilitator superfamily, not the RND, family of pumps. The first product (A1S_1649) is encoded in an operon containing Fur boxes and siderophore biosynthesis machinery homologues and was highly up-regulated by iron limitation [67]. Interactions between iron and antibiotic resistance [68–70] and the well-known roles of iron acquisition in pathogenesis [71] add complexity to the hypothesis that the efflux activity of this pump determines resistance or modulates virulence. Complementation tests would have provided important supportive evidence that each gene was involved in virulence and aminoglycoside resistance, particularly because the phenomenon of phase variation in *A. baumannii* is known to affect these phenotypes [72]. Nevertheless, these studies serve to highlight the deep-rooted connections between intrinsic resistance and virulence.

Multidrug Resistance Plasmid Acquisition

Many *A. baumannii* strains carry a large conjugative plasmid containing a variable array of resistance determinants [73]. The plasmid also encodes regulators that repress expression of a type VI secretion system (T6SS) capable of intoxicating bacterial competitors [73]. Spontaneous loss of the resistance plasmid results in de-repression of the T6SS, enabling the antibiotic-sensitive *A. baumannii* bacteria to attack and kill "prey" microorganisms such as *E. coli* during coculture. Therefore, presence of the plasmid endows multidrug resistance on *A. baumannii* at the expense of (1) a strategy to outcompete neighboring bacteria

and (2) a hypothesized fitness cost associated with maintenance of the large plasmid [1]. Repression of the T6SS may serve to lessen fitness trade-offs associated with unnecessary production of the system, such as during antibiotic therapy, which in the host would reduce commensal competitors. The hypothesized cost of an active T6SS in the presence of antibiotic pressures remains to be determined, as do fitness costs of maintaining the resistance plasmid and the benefits of having an active T6SS during growth during the disease process in the absence of antibiotics.

Phenotypic Adaptations That Modulate Virulence and Resistance

Owing to their broad resistance, infections with MDR pathogens such as *A. baumannii* are often initially treated with inappropriate empiric antibiotics, with negative consequences for the patient [74]. Several studies have examined how antibiotic exposures at levels under the minimum inhibitory concentration modulate the virulence properties of resistant pathogens (for review, see [75]). We found that *A. baumannii* responds to such antibiotic levels by augmenting its virulence, converting a normally low virulence organism into one that causes lethal disease [76]. This result was connected to the ability of the antibiotics chloramphenicol and erythromycin, which target the 50S ribosome, to reversibly augment the production of capsular exopolysaccharide via induction of a transcriptional response in the cell. Profiling of genome-wide transcriptional changes and responses to varied antibiotic classes in future studies may reveal additional determinants of virulence amplification in this opportunistic pathogen.

A. baumannii also adapts to conditions characteristic of the host niche with increased phenotypic resistance. Hood and colleagues [77] demonstrated that physiological concentrations of sodium chloride induce increased resistance to aminoglycosides, carbapenems, quinolones, and colistin. This phenotype was associated with transcriptional changes in antibiotic resistance determinants, including up-regulation of efflux pumps and down-regulation of porins, pointing to a multifactorial basis for this response.

S. AUREUS INFECTIONS

S. aureus is an important source of resistant infections in the hospital and in the community. Diseases caused by this pathogen result from a variety of virulence determinants controlled by multiple regulatory systems. One key system is the accessory gene regulator (*agr*), which coordinates the expression of pathogen genes based on bacterial population density [78]. Here we discuss how the major mechanisms behind antibiotic resistance in acute and chronic infections with *S. aureus* interact with its formidable virulence capacities.

Methicillin Resistance

Increased resistance to β-lactam antibiotics in methicillin-resistant *S. aureus* (MRSA) strains is determined by the acquisition of a gene set known as the staphylococcal cassette chromosome (SCC*mec*), which encodes an altered penicillin-binding protein with decreased affinity for β-lactams. Strains of MRSA that are able to spread successfully in the community, termed community-associated (CA) MRSA [79], have been shown to display high virulence in animal models and carry expanded sets of virulence determinants that are produced at higher levels than sensitive strains [80]. CA-MRSA strains carry distinct, smaller forms of the SCC*mec* cassette (SCC*mec* types IV or V) compared with the larger forms most commonly present in hospital-acquired (HA) MRSA strains [80]. Deletion of the CA-MRSA–type cassette did not affect fitness in culture [81, 82] or attenuate fitness or virulence in animal models [83, 84]; by contrast, presence of the larger HA-MRSA–type SCC*mec* incurred a fitness cost in bacteriological medium [81, 82], lowered cytotoxicity with cultured immune cells [81, 85], and reduced virulence in mice [85].

Interestingly, HA-MRSA–type SCC*mec* elements contain a gene, *psm-mec,* that is absent from CA-MRSA strains and that modulates virulence in a complex manner [86, 87]. This gene encodes a regulatory RNA that directly represses *agr* expression, lowering toxin production and virulence in certain HA-MRSA strains [86], and it also encodes a cytolytic toxin that was shown to promote virulence in a different isolate of HA-MRSA [87]. The effects of *psm-mec* on pathogenicity probably depend on the variable regulatory network and toxin repertoire present in different strain backgrounds. Overall, these studies provide a mechanistic basis consistent with the different disease-causing capacities of the MRSA subtypes, although many additional factors are clearly at play [80].

In addition to genetic factors that modulate HA-MRSA virulence, treatment with β-lactam antibiotics, to which these organisms are highly resistant, can shape pathogenicity. MRSA strains exposed to β-lactams were shown by Muller and colleagues [88] to produce a structurally altered, hyperinflammatory cell wall that caused increased stimulation of macrophages and more severe immunopathology in a mouse skin infection model. This study provides a potential connection between inappropriate empiric antibiotic treatment of nosocomial MRSA infections and worsened clinical outcomes [89].

Vancomycin Resistance

Reduced susceptibility to vancomycin typically arises in MRSA infections in the presence of prolonged vancomycin therapy [90]. Strains with intermediate level resistance (vancomycin-intermediate *S. aureus* [VISA]) represent the most prevalent form and are typically characterized by a thickened cell wall hypothesized to sequester the drug from critical sites of growth inhibition [90]. Several clinical studies indicate that VISA strains display lowered virulence [80], which is supported by experimental data showing that mutations that increase vancomycin resistance result in attenuated virulence in *G. mellonella*

[91], a rat model of endocarditis [92], and systemic infection of mice [93]. The altered virulence of these strains may relate to a number of factors. Dysfunction of the key global virulence regulatory system, *agr*, is frequently observed [80]. In addition, increased capsular polysaccharide, decreased surface protein A, and reduced innate immune activation were observed in VISA isolates emerging during bacteremic infection, compared with susceptible counterparts [94]. The precise molecular determinants that reduce virulence as a consequence of the VISA state remain to be fully elucidated.

The genetic pathways leading to increased vancomycin resistance are heterogeneous and not yet completely understood [90]. Genomic approaches have led to the discovery of novel mutations that drive the altered resistance and virulence phenotypes in VISA isolates [95, 96]. One such mutation is in the *stp1* gene encoding a eukaryotic-like phosphatase. Engineered mutations that inactivate *stp1* recapitulate the VISA phenotype and result in decreased hemolysin production and attenuated virulence in mouse systemic infection models [80, 97, 98]. Interestingly, the substrates of Stp1 are phosphorylated cysteines on proteins including members of the SarA family of global transcriptional regulators [98]. Many virulence genes are targets of this family [99], suggesting an additional mechanistic pathway linking altered vancomycin resistance and virulence.

Small Colony Variants

Small colony variants (SCVs) of *S. aureus* and other pathogens are associated with relapsing infections that persist despite antibiotic therapy. The mechanisms underlying the SCV phenotype have been linked to mutations in electron transport or thymidine biosynthesis, resulting in altered metabolism, slowed growth, and resistance to antibiotics, particularly the aminoglycosides [100]. Additional pathways have been shown to drive the SCV phenotype in the clinic, such as activation of the stringent response due to point mutations in *relA* [101]. In agreement with clinical observations on the chronic, recalcitrant nature of infections with SCVs [100, 102], isolated SCVs in pure culture display decreased virulence in animal models compared with their WT counterparts, while generally retaining an ability to cause persistent infection with substantial bacterial burden [100, 101, 103].

The switch to a persistent lifestyle seems to be determined by diverse mechanisms. For some SCV isolates, reduced *agr* expression is observed, resulting in decreased production of toxins and increased production of surface attachment factors. Such reprogramming is thought to promote a persistence niche when bacteria are in close association with host cells [104]. In 1 study, SCV strains containing mutations in *relA* and *rpoB* showed enhanced expression of *agr*; however, these mutations also triggered up-regulation of capsule biosynthesis genes and increased resistance to killing by serum and antimicrobial peptides, properties that may explain enhanced persistence in vivo [101].

SCV strains are often isolated as subpopulations from mixed bacterial communities that comprise the infection site [105, 106]. A study by Hammer et al [107] tested the hypothesis that interbacterial interactions within such communities at sites of infection can permit bypass of the virulence deficiency seen with individual SCV isolates in monoculture. The authors cocultured 2 strains with SCV phenotypes due to distinct mutations affecting electron transport—one affecting heme biosynthesis and the other menaquinone biosynthesis. Interestingly, coculture led to enhanced growth of the overall population in bacteriological medium and increased fitness and virulence in a murine model of osteomyelitis, an infection commonly associated with the development of SCV isolates in humans. Similar growth enhancements were found in interactions between defined *S. aureus* SCVs and slow-growing strains of other bacterial species frequently coisolated with *S. aureus* in patients. The data support a model in which interstrain exchanges of metabolites promoting respiration underlie the mutualistic interactions [107], although the precise mechanisms of such exchanges remain to be elucidated.

CONCLUSIONS AND FUTURE DIRECTIONS

The continual evolution of nosocomial pathogens under selective pressures from antibiotics, the host, and the environment presents both challenges and opportunities. Mutations increasing antibiotic resistance have a range of effects on bacterial fitness during infection manifesting as decreased or increased pathogenic potential. Future studies should further elucidate the determinants of altered virulence potential in resistant pathogens and illuminate the mechanisms by which resistance traits modulate the outcome of disease. It is clear that the disease process provides selective pressures to constrain the spectrum of resistance. The selective forces present in the hospitalized human host are complex, and animal experiments that take a reductionist approach to disentangle these complex environments will play a major role in explaining how individual pressures shape the fate of resistant pathogens during infection. Some of the specific selective forces highlighted in this review include prior treatment with sublethal antibiotics and defined polymicrobial interactions.

Several gaps in our knowledge of how the disease process shapes resistance development remain, however. A key gap is how the host immune status—that is, the presence or absence of specific effectors of the immune system—modulates the evolution of resistance traits in pathogens. One possible approach to bridge this gap is parallel testing of bacterial fitness in animal hosts with or without deficiencies in defined innate immune functions. Using such animals as hosts for experimental microbial evolution [108] in the presence of antibiotic treatment would represent an additional strategy to shed light on the roles of host pressures in influencing the emergence and fate of resistance mutations. Another important gap is the interplay

of resistance with additional stages of the disease process, such as transmission from environmental reservoirs and epithelial colonization. These stages can shape the acquisition of antibiotic resistance, and resistance mutations likely affect bacterial fitness at these sites in ways different from those seen at sites of outright infection. Understanding these phenomena has the potential to inform the development of improved strategies to control the spread of antibiotic resistance in clinical settings.

Notes

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