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REVIEW

Clinical and Pathophysiological Overview of *Acinetobacter* Infections: a Century of Challenges

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SUMMARY *Acinetobacter* is a complex genus, and historically, there has been confusion about the existence of multiple species. The species commonly cause nosocomial infections, predominantly aspiration pneumonia and catheter-associated bacteremia, but can also cause soft tissue and urinary tract infections. Community-acquired infections by *Acinetobacter* spp. are increasingly reported. Transmission of *Acinetobacter* and subsequent disease is facilitated by the organism's environmental tenacity, resistance to desiccation, and evasion of host immunity. The virulence properties demonstrated by *Acinetobacter* spp. primarily stem from evasion of rapid clearance by the innate immune system, effectively enabling high bacterial density that triggers lipopolysaccharide (LPS)–Toll-like receptor 4 (TLR4)-mediated sepsis. Capsular polysaccharide is a critical virulence factor that enables immune evasion, while LPS triggers septic shock. However, the primary driver of clinical outcome is antibiotic resistance. Administration of initially effective therapy is key to improving survival, re-

ducing 30-day mortality threefold. Regrettably, due to the high frequency of this organism having an extreme drug resistance (XDR) phenotype, early initiation of effective therapy is a major clinical challenge. Given its high rate of antibiotic resistance and abysmal outcomes (up to 70% mortality rate from infections caused by XDR strains in some case series), new preventative and therapeutic options for *Acinetobacter* spp. are desperately needed.

KEYWORDS *Acinetobacter*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*

INTRODUCTION

It is uncertain when the first isolation of organisms in the *Acinetobacter* genus occurred (1, 2). Gram-negative coccobacilli that were likely *Acinetobacter* were isolated as early as 1914 and repeatedly through the 1940s but were previously referred to as *Mima polymorphia* (now *Acinetobacter lwoffii*), *Herellea vaginicola* (now *Acinetobacter baumannii* or *A. calcoaceticus*), *Bacterium anitratum*, B5W, and *Moraxella lwoffii* (1, 2). Until quite recently, distinguishing *A. baumannii* from *A. calcoaceticus* was difficult. Thus, literature from decades past likely reflects a mixture of the two species.

The genus *Acinetobacter* is highly diverse, comprised of oxidase-positive and -negative, nonpigmented, Gram-negative coccobacilli. Although there are more than 50 species within the diverse *Acinetobacter* genus (3), the majority are nonpathogenic environmental organisms. The most common species to cause infections is *A. baumannii*, followed by *A. calcoaceticus* and *A. lwoffii* (4). Additional species, including *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. nosocomialis*, *A. pittii*, *A. schindleri*, and *A. ursingii*, have occasionally been reported as pathogens (5–11). *A. seifertii* is an emerging pathogen in Asia; it is genetically closely related to and may be misidentified as *A. baumannii* (12–14). Multivariate analysis of clinical data and studies of animal models (discussed further below) have demonstrated that *A. baumannii* is the most virulent of all the species (15).

In general, *Acinetobacter* spp. are found in wet environments, including moist soil/mud, wetlands, ponds, water treatment plants, fish farms, wastewater, and even seawater (3). These environmental strains often harbor antibiotic resistance mechanisms, including carbapenemases and extended-spectrum β-lactamases (ESBLs) (3), and may thus serve as important environmental reservoirs for resistance elements that transform into clinically relevant strains. Some medically relevant species, such as *A. calcoaceticus*, *A. lwoffii*, *A. nosocomialis*, and *A. pittii*, have been found on vegetables, meat, dairy products, and human skin (16). Such strains have harbored extensive antibiotic resistance repertoires. Furthermore, *A. baumannii* strains harboring extensive antibiotic resistance have contaminated commercial food, including meat, vegetables, and various types of livestock, suggesting multiple environmental routes of transmission into human populations (3, 17–19). However, non-*baumannii* *Acinetobacter* spp. have predominated in surveillance studies of skin colonization, particularly among healthy individuals, whereas *A. baumannii* has rarely been identified as a colonizer of skin among healthy patients (3, 20–23). Strains of *A. baumannii-A. calcoaceticus* complex did colonize 17% of healthy military personnel studied in Texas; however, the colonizing strains were distinct from those found in infected soldiers returning from Iraq and Afghanistan, and hence were unlikely the source of infection (23). Thus, healthy humans seem to rarely harbor more-pathogenic species and strains.

Infections caused by *Acinetobacter* spp. emerged in earnest during the 1960s and 1970s in parallel with increasing utilization of complex intensive care (1, 2). *Acinetobacter* was initially considered a commensal opportunist—a low-virulence pathogen of minimal significance. In subsequent decades, however, the increasing ubiquity and intensity of mechanical ventilation, central venous and urinary catheterization, and antibacterial therapy has caused a surge in the frequency and severity of *Acinetobacter* infections (24–27).

Today, *Acinetobacter* infections have spread rapidly through hospitals across the globe. The highest density of infections occurs in intensive care units (ICUs). U.S.

National Healthcare Safety Network (NHSN) 2009-2010 surveillance data found that *Acinetobacter* spp. caused 1.8% of all health care-associated infections (27). Based on surveillance studies from hospital networks, the frequency is similar in ICUs across Europe and Latin America (28–32). However, in China, Thailand, Taiwan, Vietnam, and some countries in South America, *Acinetobacter* causes a much higher proportion of nosocomial infections and may be the predominant nosocomial pathogen. It is also becoming a predominant nosocomial pathogen in India (33–38). In Asian and certain Latin American countries, *Acinetobacter* is one of the three most common causes of bacteremia and nosocomial pneumonia (39–43). There are an estimated 45,000 (range, 41,400 to 83,000) cases of *Acinetobacter* infections per year in the United States and 1 million (range, 600,000 to 1,400,000) cases globally per year (44).

TRANSMISSION

Acinetobacter spp. are often transmitted to patients via persistence on environmental surfaces and transient colonization of the hands of health care workers (45, 46). However, nosocomial spread by aerosolized bacteria from infected or colonized patients has been reported. For example, in one well-publicized case, a health care worker developed fulminant pneumonia after inhalation of *A. baumannii* aerosolized during endotracheal suctioning of a ventilated patient (47). Another study revealed that nearly a quarter of air samples collected from patient rooms were contaminated with carbapenem-resistant *A. baumannii* (CRAB). These rooms had all housed patients infected with CRAB (46, 48). The air ducts were not colonized, indicating that the patients themselves were the source of the airborne bacteria (46, 48). However, Rock et al. found evidence of air contamination by *A. baumannii* in only one of a dozen patient rooms evaluated, so the frequency of air contamination by *A. baumannii* is variable (49). They surmised that the reduced rate of air contamination might have been due to use of frequent air exchanges combined with the fact that their patients were mechanically ventilated (and hence had closed airway circuits) (49). Nevertheless, the unnerving notion of the spread of organisms by settling on patients from contaminated air suggests that episodic cleaning of environmental surfaces may not be able to prevent dissemination unless there are also efforts to disinfect the air in patients' rooms. This concept of airborne spread represents a particular challenge and may require a shift in approach from an infection control standpoint. Specifically, early control of patient respiratory secretions, patient cohorting, and models aimed at reducing environmental dissemination may be equally important as surface disinfection. Novel technologies to enable air decontamination, such as misting, UV light, or vapor technologies may also have roles to play, although clinical data are lacking thus far.

While it is commonly asserted that *Acinetobacter* spp. cause infections primarily in immunocompromised patients, the predominant predispositions to infection are colonization pressure, selection by exposure to broad-spectrum antibiotics, and disruption of anatomical barriers (e.g., placement of catheters or endotracheal tubes and traumatic or surgical injury to skin and integument). Patients with suppression or depletion of leukocytes constitute the minority of those infected with *A. baumannii* (25, 50–52). Clinically, *Acinetobacter* infections are associated with mechanical ventilation, intravenous and urinary catheterization, surgery, invasive procedures, and prolonged broad-spectrum antimicrobials, especially in patients who suffer from burns, have trauma, or are in ICUs (25, 26, 39, 50, 52, 53). Thus, while *Acinetobacter* is largely an opportunistic pathogen, the "opportunities" that usually result in clinical infection are defects in anatomical host defenses and alteration of normal host flora by exposure to broad-spectrum antibiotics.

Acinetobacter is intrinsically resistant to desiccation, which contributes to its persistence in environments and transmission in health care settings. In addition, community-acquired pneumonia and bacteremia can occur, particularly in hot and humid tropical climates (25, 45). Cases appear to display a seasonal predilection. The National Nosocomial Infections Surveillance (NNIS) System found that between 1987 and 1996 the rate of *Acinetobacter* infections in the United States increased by 54%

between July and October compared to November through June (45). Humidifiers and water baths have often been implicated as environmental reservoirs, and a high level of humidity has been postulated to facilitate growth of the bacteria (45).

PATHOGENESIS

Models of Infection

Various *in vivo* infection models have been used to study the pathogenesis of *A. baumannii*. Healthy mice inoculated in the lung or intravenously are generally resistant to lethal infection caused by many strains of *Acinetobacter* except at very high inocula (i.e., $>10^9$ CFU), suggesting dubious relevance to human pathogenesis (54–58). To circumvent the intrinsic resistance of many mouse strains to *A. baumannii* infection, artificial models have been used, such as infecting mice intraperitoneally (a clinically irrelevant route of entry) or mixing the inoculum with porcine mucin as a foreign body that inhibits the host's immune system from rapidly clearing the organism (59, 60). In addition, mice are often made neutropenic prior to infection even though neutropenia is not a common risk factor for infections caused by *A. baumannii*, and the vast majority of patients infected with *A. baumannii* have neither deficiency in leukocyte numbers nor overt defects in leukocyte function (25, 50–53, 61–68). Results from such models must be interpreted circumspectly given the unclear applicability to clinical disease.

In contrast, examination showed that A/J and C3HeB/FeJ strains of mice are intrinsically susceptible to lethal intravenous and lung infections by some clinical isolates of *A. baumannii* at inocula commensurate to (or lower than) those required for other commonly recognized virulent pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (54, 69–72). A/J mice had delayed neutrophil recruitment to the lungs due to diminished CXC chemokine responses to the bacteria, possibly explaining their susceptibility to pulmonary infection (54). Such an explanation for why C3HeB/FeJ mice are more susceptible than other mouse strains to *A. baumannii* infection has yet to be revealed.

Rats are also susceptible to lethal pneumonia caused by *A. baumannii* without being immunocompromised. Russo et al. found that inoculating *A. baumannii* into the lungs of rats resulted in clinically comparable pneumonia, confirmed by histopathology, inflammatory response, physiological injury, and death (73). They also described a skin and soft tissue infection model in rats in which virulence differences among bacterial strains were detected, including greater virulence for clinical isolates than for environmental isolates (73, 74). Thompson et al. describe a wound infection model of *Acinetobacter* in mice with surgically induced, full-thickness skin incision (68). These investigators used a virulent clinical isolate, *A. baumannii* AB5075, but like other BALB/c models, they had to pretreat mice with cyclophosphamide to create an immunocompromised state the bacteria could exploit to enable a persistent infection. McConnell et al. have summarized models of meningitis, endocarditis, and osteomyelitis caused by *A. baumannii* (60).

The wax moth larva of *Galleria* has also been used as a model of *Acinetobacter* infection. For example, Peleg et al. found that *A. baumannii* was more lethal in the *Galleria* model than non-*baumannii Acinetobacter* species, including *A. baylyi* and *A. lwoffii*, and that antibiotic therapy improved survival of the infected larvae (75). Gebhardt et al. also found that *A. baumannii*, even a strain considered to be avirulent (ATCC 17978), was more virulent than *A. baylyi*, in *Galleria* (76). Wand et al. reported that strain to strain differences in biofilm formation did not correlate with virulence (77). However, if the strains were induced to form biofilms and the biofilm was then disrupted, the sessile bacteria harvested from the biofilms were more virulent in *Galleria* than the same strain taken from planktonic growth (77). Interestingly, the growth phase of the organism at the time of infection may affect a strain's virulence.

Caenorhabditis elegans has also been used as an occasional invertebrate model of *Acinetobacter* infection (78, 79). In at least one study, however, virulence outcomes in *C. elegans* did not correlate with outcomes in mice, so caution is warranted in interpreting translatability of results in this model (80). Finally, a recent study described a

model of *Acinetobacter* virulence in zebrafish larvae (81). In this model, mutant strains that had been shown to have attenuated virulence in mice also had attenuated virulence, and host defenses depended on neutrophil and macrophage uptake, suggesting commonalities between this invertebrate model and mice. Again, caution may be warranted in that the *A. baumannii* strain background studied was ATCC 17978, a lab-adapted strain which was highly virulent in zebrafish but is essentially avirulent in healthy mice with a normal immune system, suggesting differences between the zebrafish and murine models. The authors also found that bacterial phenylalanine production was critical to triggering neutrophil chemoattraction to the site of infection (81). The phenylalanine catabolic pathway degrades phenylalanine, and a key step in that pathway is the *paaA* gene. Wild-type strains with intact phenylalanine catabolism produced less phenylalanine, resulting in less neutrophil attraction to the site of infection, whereas a strain with a disrupted *paaA* gene produced more phenylalanine, resulting in more neutrophil chemoattraction. Thus, phenylalanine degradation may be an important virulence factor of the bacteria, which ameliorates neutrophil chemoattraction and therefore protects the bacteria against early innate immune clearance, at least in zebrafish.

In the aggregate, animal models have been important to identify potential virulence factors driving the outcome of the interactions between the host and *Acinetobacter* spp. Indeed, *in vitro* assays, including adherence to human cells (e.g., epithelial cells and/or pneumocytes), cell invasion, and biofilm formation have often lacked correlation with *in vivo* virulence of *Acinetobacter* when studied head to head (77, 80, 82, 83). Similarly, a recent survey of *A. baumannii* and *A. pittii* clinical isolates found no evidence that the strains mediated adherence, invasion, or damage to lung epithelial cells in *in vitro* assays despite the fact that they had caused infection and disease in patients (84). These results underscore two points. (i) Discrepancy exists between clinical phenotype and *in vitro* assays. (ii) Caution must be taken in using such *in vitro* assays to describe or define virulence factors.

Thus, confirmation of virulence traits *in vivo*, and in particular in models relevant to human infection, is of great importance to advancing the field. Furthermore, confirmation of physiological injury is important in *in vivo* models when defining virulence, as 10- to 100-fold differences of bacterial density in the first 24 to 48 h of infection have not translated into survival differences in mice (85).

Acinetobacter Virulence Factors

Multiple studies indicate that *A. baumannii* intrinsically has more human virulence potential than other *Acinetobacter* spp., including *A. calcoaceticus*, *A. lwoffii*, *A. junii*, *A. baylyi*, and *A. haemolyticus*. For example, *A. baumannii* grew better at 37°C and was better able to resist macrophage uptake than these other species in one study (86). As mentioned, *A. baumannii* strains were also more lethal to the wax moth larva of *Galleria* than strains of *A. baylyi* and *A. lwoffii* (75, 76). In another study, a strain of *A. junii* was nonlethal in neutropenic mice, whereas several *A. baumannii* strains were lethal (85). Chusri et al. compared clinical outcomes in patients infected with *A. nosocomialis*, *A. pittii*, and *A. baumannii* and then compared the clinical isolates in an animal model (15). By multivariate analysis, infection with a non-*baumannii Acinetobacter* species resulted in a nearly a 9-fold reduction in mortality compared to *A. baumannii*. Furthermore, the clinical strains of non-*baumannii Acinetobacter* species were substantially less lethal during infection in wax moth larvae of *Galleria*. Similarly, in a case-control study, patients infected with *A. ursingii* had much lower 28-day mortality than those infected with *A. baumannii* (6% versus 37%) even though multidrug resistance and inadequate initial therapy were as likely to occur in patients infected with either species (10).

New advances in genetics and molecular biology have facilitated our understanding of basic *Acinetobacter* physiology and virulence factors (87, 88). Many have made transposon mutant libraries in an effort to better understand *Acinetobacter baumannii* virulence phenotypes. These libraries have utilized transposon insertion sequencing (TnSeq) in an attempt to identify potential virulence determinants (76, 89–91). When

combined, the current mutant collections and whole-genome sequencing provide an invaluable resource for both virulence and antibiotic susceptibility screenings (57, 74, 89, 92).

Paradoxically, despite the etymology of *Acinetobacter*—from *a-kineto*, Greek for "nonmotile"—bacteria of this genus are decidedly motile; in fact, motility is one of the genus' putative virulence factors (60, 93). Furthermore, as mentioned, *Acinetobacter* is resistant to disinfection and desiccation. Ethanol enhanced the growth of *A. baumannii* in culture media and its salt tolerance, allowing it to grow despite salt concentrations that were inhibitory without alcohol (94). Ethanol exposure also led to marked changes in the organism's proteome and to enhanced virulence in *Galleria* (95). The bacterial enzyme RecA mediates bacterial DNA repair and resistance to desiccation and prevented *A. baumannii* killing inside macrophages and contributed to lethality in mice (96).

Under dry conditions, *A. baumannii* undergoes morphological changes, including thicker cell walls (97, 98), likely contributing to its impressive persistence on environmental surfaces. In outbreak investigations, *A. baumannii* remained viable in hospital units after months—even years—on a solid surface, underscoring the challenge to eliminating environmental transmission of the organism once it has colonized nosocomial surfaces (97, 98). Subsequent experimental models also highlight a propensity for epidemic isolates to persist in dry conditions (99).

Numerous other potential virulence factors have been suggested, including formation of biofilm, adherence mechanisms, iron acquisition characteristics, activities of polysaccharide membrane and outer membrane protein phospholipases, alteration in penicillin-binding proteins, and outer membrane vesicles (OMVs) (Table 1). Of particular mention is outer membrane protein A (OmpA) which has been suggested to have a variety of functions, including adhesion to host epithelial cells, biofilm function, and complement resistance (100). In a recent lethal model of *A. baumannii* pneumonia, transposon-mediated disruption of OmpA reduced mortality in small numbers of mice, suggesting a virulence function of the OmpA (100). Additionally, overexpression of chromosomal efflux systems has received considerable attention. The overproduction of these systems confers increased multidrug resistance to antimicrobial agents (101–103).

However, much of the *in vivo* pathogenesis work published thus far has used avirulent, lab-adapted strains (chiefly ATCC 17978) and/or nonlethal murine models to measure modest changes in bacterial density over time. Table 1 summarizes results of a systematic PubMed search of *Acinetobacter* virulence research, using title-word "Acinetobacter" and title-word/abstract "virulence." We have separated studies by whether the assays were strictly *in vitro*, included invertebrate animal outcomes, included nonlethal vertebrate animal outcomes, or included lethal vertebrate animal outcomes, either conducted or not with artificial introduction of porcine mucin to prevent innate immune clearance of the bacteria (Table 1). It is imperative to consider the results of these pathogenesis studies in the context of the model systems and endpoints used, given their potential limitations.

It is unclear how to interpret virulence factors characterized solely *in vitro* or in *in vivo* models in which the animals do not die or suffer physiological injury or in models using routes of infection that rarely occur clinically. Furthermore, as mentioned, in lethal *in vivo* models, porcine mucin is often used as a foreign body to protect the bacteria from rapid innate immune clearance (which is not physiologically relevant) or render mice immunocompromised by making them neutropenic to achieve the same effect (Table 1). Such models likely mask host-microbe interactions that are of primary importance in determining host outcome. Still, they may unmask secondary virulence functions that become relevant in the context of patients whose immune systems are highly dysfunctional.

In the aggregate, repeated themes become discernible when evaluating virulence studies of *A. baumannii* (Table 1). Capsule and its negative surface charge may well be the primary virulence function of the pathogen, as it is the primary defense the bacteria

TABLE 1 Summary of putative virulence factors for *Acinetobacter* spp.

Model type and virulence factor(s) or gene(s), process, or organelle[a]	Model	Outcome(s)	Reference
In vitro only			
OmpA	Cell cytotoxicity	OmpA was administered to eukaryotic cells and induced cell death (note that endotoxin levels on the protein not reported)	321
OmpA	Complement lysis of OmpA mutant of *A. baumannii* 19606 vs wild type	OmpA mutant resisted alternative pathway complement lysis *in vitro*	322
OmpA	Knockout of OmpA in *A. nosocomialis* ATCC 17903	The knockout had reduced biofilm formation and adherence to lung epithelial cells, with no difference in cytotoxicity	323
CpaA	Blood coagulation	Purified CpaA protease reduced coagulation of human plasma	324
BfmS	Various assays comparing BfmS mutant on ATCC 17978 background to the wild type	BfmS mutant had diminished biofilm formation, reduced adherence to cells, and sensitization to serum killing	325
Porins (CarO and OprD-like)	Growth rate, cytotoxicity of a clinical isolate vs ATCC 19606 strain (nonisogenic pair)	A clinical pan-drug-resistant isolate with reduced CarO and OprD-like expression grew more slowly and was less cytotoxic in a cellular assay	326
CFTR inhibitory factor (CiF)	Gene expression and function	Gene homologous to CiF from *Pseudomonas aeuruginosa* is found in *A. nosocomialis* and *A. baumannii*	327
Biofilm gene (*LH92_11085*)	Characterization of gene expression and biofilm formation in *A. baumannii* MAR002	MAR002 overexpresses biofilm and has 25-fold increased expression of *LH92_11085*	328
Oxidative resistance (KatG and KatE)	Mutants of *A. baumannii* and *A. nosocomialis* tested *in vitro*	Mutants had increased susceptibility to oxidative killing and neutrophil killing	329
Adherence, invasion, and cytotoxicity	5 clinical isolates of *A. baumannii* and 6 clinical isolates of *A. pittii* tested in adherence, invasion, and cytotoxicity of lung epithelial cells	Adherence, invasion, and cytotoxicity not detected despite testing strains that had caused clinical disease	84
In vivo–invertebrate models			
NfuA (iron acquisition scaffold protein)	NfuA knockout in ATCC 19606 strain vs wild-type strain	Knockout strain more sensitive to oxidative stress and modestly less lethal in *Galleria*	312
EntA (enterobactin precursor synthetic gene)	EntA knockout in ATCC 19606 vs wild-type strain	Knockout strain modestly less lethal in *Galleria*	311
Superoxide dismutase (SOD)	SOD knockout in ATCC 17978 vs wild-type strain	Knockout strain more sensitive to oxidative stress and less lethal in *Galleria*	330
TonB (energetics of nutrient uptake)	TonB mutant of ATCC 19606 vs wild-type strain	Variable impact on lethality in *Galleria* but impacted adherence to epithelial cells	82
OXA-40 gene (carbapenemase)	Clinical isolates with or without the OXA-40 gene	OXA-40-containing isolates appeared to kill *Galleria* more slowly	331
AbuO (outer membrane protein)	AbuO knockout of *A. baumannii* AYE (origin unclear) with infection in *C. elegans*	Knockout displayed increased susceptibility to antibiotics and disinfectant and modestly reduced lethality in *C. elegans*	332
SecA (iron acquisition)	Transposon mutant disruption of SecA in *A. baumannii* 19606	Mutant displayed modest reduction in lethality in *Galleria*	315
pmrB (colistin resistance due to altered LPS charge)	Clinical isolate with spontaneous *pmrB* mutation	Mutant displayed no reduction in strain fitness, growth, or lethality in *Galleria*	333
lpxACD, *pmrB* (colistin resistance due to loss of LPS synthesis genes [*lpx*] or altered LPS charge [*pmr*])	Clinical strains serially passaged on colistin	*lpxACD* mutants had growth defects and loss of virulence, whereas *pmrB* mutants had no change in growth or virulence in *Galleria*	334
Phospholipase D	Disruption of 3 phospholipase D genes in ATCC 19606	Reduced virulence in *Galleria*	335
Type VI secretion system (T6SS)	T6SS was compared in ATCC 17978, a nonclinical isolate (DSM30011), and 3 clinical isolates	Only the nonclinical isolate expressed a highly functional T6SS, which played a role in colonization in *Galleria*	336
lpxD (colistin resistance due to loss of LPS synthesis)	Clinical isolate of *A. noscomialis* serially passaged in subtherapeutic colistin	*lpxD* mutant had modestly attenuated virulence in *C. elegans*	337

(Continued on following page)

TABLE 1 (Continued)

Model type and virulence factor(s) or gene(s), process, or organelle[a]	Model	Outcome(s)	Reference
SurA1 (surface antigen protein)	Knockout of SurA1 from *A. baumannii* CCGGD201101 (an isolate from diseased chicks)	Knockout had decreased growth rate, increased killing in serum, and decreased virulence in *Galleria*	338
AdeRS (*Acinetobacter* drug efflux pump regulator)	Deletion of AdeRS from *A. baumannii* AYE or S1	Knockouts had decreased biofilm formation; S1 but not AYE knockout had decreased virulence in *Galleria*	103
gacA and *gacS* (regulator genes), *abaI* (quorum sensing), *paaA* (phenylalanine catabolism)	ATCC 17978 and knockouts infected via blood in zebrafish embryos	Knockout strains had attenuated virulence in the zebrafish model, and the *paaA* knockout produced more phenylalanine, which triggered more neutrophil attraction to the site of infection	81
Multiple genes regarding stress response, osmotic stress, capsule, and LPS genes	Comparison of *Acinetobacter* strains in *Galleria*, including transposon disruptants	*Galleria* distinguished known avirulent (ATCC 17978) and virulent (5075) strains of *A. baumannii*, with the former causing some lethality and the latter 100% fatal. *A. baylii* ADP1 was less virulent than *A. baumannii* ATCC 17978. A variety of genes disrupted by transposon insertion in *A. baumannii* 5075 modulated mortality in *Galleria*.	76
In vivo–nonlethal vertebrate models			
Serum/complement resistance	Clinical isolates in Long-Evans rat soft tissue infection (subcutaneous)	Sensitivity to complement correlated with rapidity of soft tissue clearance *in vivo*	73
Phospholipase D	C57BL/6 intranasal lung infection ($>3 \times 10^8$ inoculum) with transposon mutant clinical CSF isolate 98-37-09 vs wild type	Disruption resulted in serum sensitivity and no difference in lung bacterial density, but the mutant strain had lower bacterial blood density following pneumonia	57
Heme consumption	Nonlethal intranasal infection with *A. baumannii* LAC-4 clinical isolate, treatment with an inhibitor of heme acquisition vs placebo	Mice infected with LAC-4 and treated with heme acquisition inhibitor had modestly reduced lung bacterial density and bacteremia	313
PTK and EpsA (capsular polysaccharide regulators)	Long-Evans rat soft tissue infection with knockouts on *A. baumannii* 307-0294 clinical isolate background vs wild type	Disruption resulted in diminished growth in human ascitic fluid, human serum, and rat soft tissue	74
OmpA, LpsB, GacA	Transposon mutant library of *A. baumannii* ATCC 17978 infected intranasally into C57BL/6 mice	CFU differences at 24 h detected for strains with mutations of various genes, including *lpsB* (LPS biosynthesis), *ompA*, and *gacA*	89
Outer membrane vesicles (OMVs)	Outer membrane vesicles purified from *A. nosocomialis* ATCC 17903 administered to cells *in vitro* and BALB/c mice intratracheally	OMVs were toxic to eukaryotic cells and triggered inflammatory cytokine production in mouse lungs	339
LipA (lipase)	Tail vein infection of DBA mice made neutropenic with cyclophosphamide and infected with LipA knockout in *A. baumannii* ATCC 17978 vs wild type	LipA knockout demonstrated reduced competition fitness during nonlethal infection in mice	340
gspD (type 2 secretion system [T2SS])	Intranasal inoculation of C57BL/6 mice with *A. nosocomialis* M2 with knockout of *gspD* vs wild type	Modest differences in survival in *Galleria* and modest differences in bacterial densities during nonlethal infection in mice	341
AdeABC and AdeIJK (efflux pump regulators)	Intranasal and intraperitoneal infection of C57BL/6 mice with clinical isolate *A. baumannii* BM4587 or its isogenic Ade mutants	Increase in bacterial burden during intraperitoneal infection with the *adeABC* mutant, decreased with *adeIJK* mutant, no change after lung infection (intranasal)	101
Zur (zinc uptake regulator)	Intranasal infection of C57BL/6 mice with *A. baumannii* ATCC 17978 or a Zur knockout strain	No difference in lung bacterial burden, but liver burden lower for the Zur knockout strain	319

(Continued on following page)

TABLE 1 (Continued)

Model type and virulence factor(s) or gene(s), process, or organelle[a]	Model	Outcome(s)	Reference
ZigA (zinc chaperone)	Intranasal infection of C57BL/6 mice with *A. baumannii* ATCC 17978 or a ZigA knockout strain	No difference in lung bacterial burden, but liver burden lower for the ZigA knockout strain	320
FeoB (ferrous iron transport), DDC (cell wall cross-linking), PntB (pyridine metabolism), FepA (enterobactin receptor)	Intravenous infection in CBA/J mice with a transposon mutant library of *A. baumannii* ATCC 17978 treated with cyclophosphamide to make them neutropenic, using competitive growth by spleen bacterial density, or in human serum, as read-outs	Defects in these genes altered competitive growth/relative bacterial density in the spleens or serum	90
In vivo–lethal vertebrate models			
Inoculum mixed with porcine mucin			
pmrB	Intraperitoneal infection in C57BL/6 mice with $\geq 10^8$ organisms of *pmrB* mutant in *A. baumannii* ATCC 19606 vs wild type	*pmrB* mutant had lower bacterial density and less mortality	59
pmrB	Intraperitoneal infection in C57BL/6 mice with $\geq 10^{7.9}$ organisms of a clinical spontaneous *pmrB* mutant of *A. baumannii* CR17 (cerebrospinal fluid strain) vs its pretreatment parent strain	*pmrB* mutant had reduced *in vivo* fitness in competition with wild type and lower mortality at low, but not high (i.e., $>10^5$) inocula	342
pmrA (altered LPS charge)	Intratracheal lung infection in Sprague-Dawley rats with a spontaneous mutant of *A. baumannii* respiratory clinical isolate vs its pretreatment isogenic strain	*pmrA* mutant had reduced lethality	343
Ciprofloxacin resistance (mutation not described)	Intraperitoneal infection in C57BL/6 mice with 10^6, 10^7, or 10^8 *A. baumannii* clinical strain serially passaged in subtherapeutic ciprofloxacin vs its parent	Ciprofloxacin-resistant strain induced lower mortality	344
Acinetobactin (iron siderophore)	*Galleria* as well as intraperitoneal infection in C57BL/6 mice with 10^6 or 10^5 *A. baumannii* acinetobactin knockouts in ATCC 19606 vs wild type	Knockout strain induced lower mortality in *Galleria* and in mice	316
$PglL_{Ab}$ (O glycosylation)	*Galleria* and intraperitoneal infection with porcine mucin in BALB/c mice, using a PglL knockout in ATCC 17978 vs wild type	Mutant less lethal in *Galleria*, and less competitive than wild type during growth in mice	345
pglC (capsule)	Intraperitoneal infection in BALB/c mice infected with *pglC* knockout in ATCC 17978 vs wild type	Capsule-deficient mutant strain was avirulent compared to wild type	105
Omp33	Intraperitoneal infection in C57BL/6 mice with 10^6, 10^7, or 10^8 Omp33 knockout in *A. baumannii* 17978 vs wild type	Knockout strain displayed growth defect *in vitro*, and reduced lethality in mice	171
MapA (Omp33-36)	Intraperitoneal infection in C57BL/6 mice with Omp33-36 knockout in *A. baumannii* 17978 vs wild type	Knockout displayed a 12-h delay in death (but all mice died)	346
gacS (sensor kinase) and *paaE* (phenylacetic acid [PAA] catabolic pathway)	Intraperitoneal infection in BALB/c mice infected with knockouts in ATCC 17978 vs wild type	*gacS* and *paaE* mutant strains had attenuated mortality in mice	347
Infections in wild-type mice without porcine mucin			
RecA (DNA damage repair)	Intraperitoneal infection in CD1 mice with 2×10^8 RecA knockout of *A. baumannii* ATCC 17978 vs wild type	RecA mutant was more sensitive to oxidative damage, macrophage killing, and heat exposure *in vitro* and caused mildly reduced lethality compared to wild-type strain (7% vs 20%)	96

(Continued on following page)

TABLE 1 (Continued)

Model type and virulence factor(s) or gene(s), process, or organelle[a]	Model	Outcome(s)	Reference
pmrB, *lpxA*, *lpxA*, *lpxC*, *lpxD* (LPS genes)	Intraperitoneal infection in BALB/c mice with knockouts in *A. baumannii* 19606 vs wild type	*lpx* mutants had reduced *in vitro* growth while *pmrB* mutant did not; *lpx* mutants had attenuated virulence in both *C. elegans* and in mice, but *pmrB* mutant had attenuated virulence only in *C. elegans* and not in mice	80
OmpA	*In vitro* studies followed by tracheal aspiration pneumonia using Ab5075 strain in wild-type C57BL/6 mice	Transposon-disrupted OmpA strain was nonlethal in 5 mice, whereas 3 of 4 mice infected with wild-type died (note the small numbers of mice)	100
Capsule	Intraperitoneal infection of C57BL/6 mice with *A. baumannii* ATCC 17978 strains which were induced to overproduce capsule or strains with mutations in capsule production	Strains expressing enhanced capsule were resistant to serum/complement, and more lethal in mice	107
UspA (universal stress protein A)	Intranasal and intraperitoneal infection of C57BL/6 mice with UspA knockout in *A. baumannii* ATCC 17978 vs wild type	Modest difference in lung CFU during nonlethal infection and no significant difference in survival during intraperitoneal lethal infection	348

[a]CFTR, cystic fibrosis transmembrane conductance regulator; PTK, protein tyrosine kinase; DDC, D-Ala-D-Ala-carboxypeptidase.

have against complement-mediated destruction and opsonization, as well as phagocytic uptake (74, 104–107). Hypervirulent strains have been reported that resist innate immune uptake, which may have unusual or mutated capsular phenotypes (70, 105, 108–110). In one compelling study, the investigators used the lack of virulence of the lab-adapted *A. baumannii* strain ATCC 17978 to demonstrate a remarkable gain of function of virulence by treating the strain with small molecules that triggered overexpression of capsule (107). Recent elucidation of the O-glycation systems in *A. baumannii* has begun to clarify capsular chemical structures that protect against innate defenses; *A. baumannii* glycation favors short-chain, branched, negatively charged amino-containing surface sugars which both protect against host immunity and may serve as a target for future immune interventions to help clear the pathogen (111–116).

Multiple studies have also shown that lipopolysaccharide (LPS) (endotoxin) has a major impact on the organisms' virulence (discussed more below). However, it is not clear that the *pmrB* mutation, which disrupts phosphoethanolamine addition to the LPS core and thereby affects the negative charge of LPS, actually disrupts virulence. The study results are mixed, likely resulting from yet uncharacterized virulence factor differences underlying the diverse *pmrB* mutant strains (Table 1). In contrast, defects in the *lpxABCD* cluster (responsible for LPS biosynthesis) clearly affect the ability of the organism to grow, thereby diminishing virulence (Table 1).

Also of importance to *A. baumannii* virulence is iron acquisition by multiple routes (Table 1). This is intuitive: without iron, the organism cannot grow. Phospholipases have been suggested in several studies to be important for virulence functions, but the *in vivo* data are not definitive in vertebrate models.

Immunological Defense Mechanisms

The immune defenses operative against *Acinetobacter* infection are slowly being elucidated (Table 2). In one of the earliest investigations, van Faassen et al. reported that intranasal pneumonia in mice resulted in rapid neutrophil recruitment to the lung, and as the infection was cleared by the innate immune system, neutrophil recruitment ceased and inflammation rapidly resolved (117). Antibody-mediated depletion of neutrophils increased lung bacterial burden and allowed for extrapulmonary dissemination (117). Nevertheless, the infection remained nonlethal; although inflammation resulted, it remained relatively mild. The nonlethal nature of the model may be the result of using a lab-adapted *A. baumannii* strain, ATCC 17961, in intrinsically resistant C57BL/6 and BALB/c mice.

TABLE 2 Summary of host defense elements determining outcomes of *Acinetobacter* infection

Model type and host defense factor(s) or process(es)	Model	Outcome(s)	Reference
In vitro models			
Cytokines and pattern recognition receptors	LPS from *A. baumannii* clinical isolates exposed to human macrophages	High levels of IL-8 and TNF produced but only if TLR4 is functional, not through TLR2—however, whole killed *A. baumannii* induces cytokines via both TLR4 and TLR2	123
Cytokines	LPS from *A. baumannii* clinical isolates exposed to mouse splenocytes	High levels of TNF released, mitogen stimulation occurred	122
In vivo–nonlethal models			
Neutrophils	Intranasal infection with *A. baumannii* ATCC 17961 in C57BL/6 or BALB/c mice with or without neutrophil depletion	Depletion of neutrophils substantially increased bacterial burden in the short term and enabled extrapulmonary dissemination, but inflammation remained mild, and the infection was cleared within several days with no deaths in any group	117
Neutrophils and macrophages	Intranasal infection with *A. baumannii* ATCC 17978 in Fus1 knockout or wild-type mice	The knockout mice displayed enhanced NF-κB activation, higher IL-17A production, lower IL-10 production, more rapid neutrophil and macrophage chemotaxis to the lung, and lower bacterial burden	121
Macrophages	Intranasal infection with *A. baumannii* ATCC 17961 of C57BL/6 mice treated with liposomal clodronate to deplete macrophages or with placebo	In healthy mice, macrophages are the predominant cells present in the lung through the first 4 h and rapidly take up bacteria. Depletion of macrophages results in substantial increase in bacterial density, but not mortality, as inflammatory cytokine levels are lower (despite higher bacterial density) in depleted mice.	120
Zinc and manganese sequestration	Mice with calprotectin disrupted or wild-type mice infected intranasally with ATCC 17978	Mice with calprotectin disrupted had higher bacterial burden in the lung	317
Pattern recognition receptors and host defense	Intranasal infection with a clinical isolate of *A. baumannii* RUH 2037 in C57BL/6 mice or congenic CD14, TLR4, or TLR2 knockouts	CD14 and TLR4 knockout mice had higher bacterial density at 4 h and had lower neutrophil influx. At 24 h only, the TLR4 knockout mice had higher bacterial density, but they had cytokine levels similar to those of wild-type mice, and they did not die of infection. TLR2 knockout mice had earlier cellular influx, but their cytokine levels and bacterial density were similar to those of the wild-type mice.	124
Pattern recognition receptors and host defense	Intranasal infection with *A. baumannii* ATCC 17978 in C57BL/6 mice or isogenic TLR9 knockout mice	TLR9 knockout mice had higher bacterial burden, attenuated cytokine production, and more severe lung pathology, with no mouse deaths	129
In vivo–lethal models			
Iron sequestration	Treatment with transferrin or placebo in C3H/FeJ mice lethally infected intravenously with hypervirulent *A. baumannii* HUMC1	Mice treated with transferrin had lower blood and tissue bacterial burden and marked improvement in survival	72
Neutrophils	Intraperitoneal infection with clinical isolates of *A. baumannii* in C57BL/6 and C3HeB/Fe mice	Antibody depletion of neutrophils but not abrogation of IL-17A or KC (keratinocyte-derived chemokine) increased lethality and bacterial burden of infection	118

(Continued on following page)

TABLE 2 (Continued)

Model type and host defense factor(s) or process(es)	Model	Outcome(s)	Reference
Neutrophils	Intranasal infection with *A. baumannii* ATCC 17961 in A/J or C57BL/6 mice	A/J mice experienced delayed neutrophil influx into the lungs, resulting in early rapid microbial replication, and later severe inflammation and death, whereas C57BL/6 mice cleared the infection	54
Superoxide production	Intranasal infection of C57BL/6 mice or congenic gp91$^{phox-/-}$ mice or nitric oxide synthase-deficient mice with *A. baumannii* ATCC 17961	Gp91$^{phox-/-}$ mice had normal neutrophil and macrophage recruitment to the lung by 4 h, but 1,000-fold-higher bacterial density, which resulted in marked increase in inflammatory cell influx into the lung by 24 h and death by 48 h. The nitric oxide-deficient mice had relatively normal phenotype and did not die.	119
Avoidance of innate effector uptake	C3HeB/Fe mice were infected i.v. via the tail vein with >40 clinical isolates of *A. baumannii*, and then the experiments were repeated with selected strains depleted of combinations of complement, neutrophils, or macrophages in a fatal model of infection	Clinical strains clustered into one of 3 groups: hypervirulent (HUMC1 and LAC-4), virulent (almost all others, including 5075), and avirulent (ATCC 17978 and R2). The differences in virulence (and hence fate of the animal) were definable within 1 h postinfection depending on ability to persist in the blood. Depletion of any of the three components increased bacterial density of the avirulent strain but nonlethally. Double depletion increased bacterial density further but nonlethally, and triple depletion converted the avirulent strain into a hypervirulent strain inducing rapid lethality, while depletion of any individual component had marginal impact on the hypervirulent strain.	70
LPS-TLR4 governance of outcome	C3HeB/FeJ and C3H/HeJ (TLR4 mutant) mice treated with LpxC inhibitor (blocks LPS production) and C57BL/6 or congenic TLR4 knockout mice, infected i.v. via the tail vein with hypervirulent *A. baumannii* HUMC1 or avirulent ATCC 17978	TLR4 mutant and knockout mice had dramatically lower inflammatory cytokine levels, sepsis biomarkers, and 100% survival compared to 100% fatality in the wild-type mice, despite having similar bacterial densities (dissociation of bacterial density from outcome). LpxC inhibition also blocked cytokine levels and protected mice from lethal infection and modestly lowered CFU likely by enhancing macrophage uptake of the bacteria.	69
Morphine	C57BL/6 and C3HeB/Fe mice with intraperitoneal infection of *A. baumannii* clinical isolates, treated with morphine or not treated with morphine (control)	Morphine treatment resulted in fatal subcutaneous infection, whereas no control mice died and naltrexone (opiate antagonist) reversed the effect. Morphine did not affect bacterial growth *in vitro* but increased bacterial density and inflammatory cytokine output *in vivo* and suppressed phagocyte recruitment to the site of infection.	130

Similarly, in the intraperitoneal infection model (absent porcine mucin), pretreatment of mice with antineutrophil antibodies converted the same nonlethal inoculum of clinical *A. baumannii* isolates into rapidly lethal infections (118). Interestingly, abrogation of interleukin 17A (IL-17A) or keratinocyte-derived chemokine (KC), both predom-

inant governors of neutrophil chemotaxis, did not alter the outcome. Thus, neutrophils, but not necessarily traditional chemokines that summon neutrophils, are important in early host defense against *A. baumannii*. Since peritoneal infection by *Acinetobacter* is highly artificial and rarely encountered in human patients, caution is warranted in interpreting these results. Furthermore, findings in the bloodstream model of infection in another study did not indicate that specific elimination of neutrophils converted nonlethal infection to lethal infection (below).

The role of oxidative killing of *A. baumannii* in early, innate host defense was delineated by Qiu et al. (119). They reported that *gp91*$^{phox-/-}$ mice (superoxide-deficient model for chronic granulomatous disease) were hypersusceptible to *A. baumannii* lung infection compared to congenic wild-type control mice. By 4 h postinfection, the knockout mice had normal levels of neutrophil or macrophage influx, but they were not able to clear the bacteria. The knockout mice developed 1,000-fold-higher bacterial burden such that by 24 h postinfection, they had also developed markedly worse inflammatory influx in the lungs compared to wild-type mice. The knockout mice died by 48 h, while the congenic C57BL/6 wild-type mice, or mice with disrupted nitric oxide synthase, cleared the infection. These results indicated that superoxide production is important in clearance of *A. baumannii*; this study constitutes one of the earliest to suggest that the inflammatory response to the organism is one of the primary mechanisms by which host death occurs.

Qiu et al. also reported that wild-type A/J mice were more susceptible to pneumonia caused by *A. baumannii* than C57BL/6 mice were (54). The cause of the increased susceptibility was delayed recruitment of neutrophils which allowed early and rapid microbial replication in the lung, resulting in severe inflammation at later time points. Beyond neutrophils, macrophages are also critical at early stages. The same investigators found that macrophages predominated in bronchoalveolar lavage samples at the time of infection and 2 h postinfection in mice (120). By 4 h postinfection, macrophages were still the predominant cell type present (75%, with neutrophils comprising 25% of cells) and had taken up substantial numbers of *A. baumannii* in the lung. When mice were depleted of macrophages with liposomal clodronate, bacterial densities rose sharply. However, the infection remained nonlethal and inflammatory cytokine levels were actually lower in the mice with depleted macrophages. These results once again underscore that bacterial density is only one component of determining whether the host survives and demonstrate that the host inflammatory response to infection seems critical to determining outcomes of *A. baumannii* infection. Even if bacterial density is higher, when inflammatory cytokine output is lower, the host may survive with minimal damage.

The role of early neutrophil and macrophage recruitment in protecting against lung infection caused by *A. baumannii* is given further credence by a study of mice deficient in the mitochondrial protein Fus1. Fus1-disrupted mice had enhanced neutrophil and macrophage recruitment to the lungs at early time points, likely due to more rapid activation of nuclear factor kappa beta (NF-κB) and resulting enhanced IL-17A and decreased IL-10 production (121). Due to enhanced phagocytic recruitment, the knockout mice had lower bacterial burden in the lungs.

LPS from *A. baumannii* induces inflammatory cytokines, such as IL-8 and tumor necrosis factor (TNF), from mouse splenocytes and human macrophages at levels equivalent to those stimulated by LPS from *Escherichia coli* (122, 123). The LPS activates cytokine production in a manner requiring TLR4, whereas whole *A. baumannii* is able to activate cytokines via both TLR4 and, for unclear reasons, TLR2 (123).

In vivo, TLR4-deficient mice exhibited minimal inflammation in response to LPS harvested from *A. baumannii* that was intranasally administered (124). Furthermore, TLR4-deficient mice had substantially lower and delayed cytokine and chemokine induction in lungs after intranasal infection with *A. baumannii*, resulting in slower neutrophil recruitment and slower bacterial clearance. Despite having higher bacterial density at 24 h postinfection, the mice had lower inflammatory cytokine levels, and they ultimately cleared the infection. In contrast, TLR2-deficient mice had accelerated

inflammation and bacterial lung clearance. A follow-up study used an alternative method (distant turpentine injection) to suppress lung cytokine and chemokine responses during intranasally inoculated pneumonia in mice (125). Turpentine suppressed early cytokine expression and neutrophil recruitment, resulting in higher bacterial density. Yet the treated mice had significantly less alveolar filling consistent with pneumonia and hence less host damage. Once again these data underscore that host outcome is driven by cytokine levels and inflammation as much as or more than bacterial density, and that dampening the inflammatory cytokine response can blunt host damage despite higher bacterial burden.

Nevertheless, since mice with disrupted TLR4 had higher bacterial density, it has been assumed that activation of TLR4 by *A. baumannii* LPS was critical for host defense. We reiterate that these *in vivo* models were nonlethal and the outcome measured was slower clearance of bacteria. Thus, the role of TLR4 in host defense against lethal infection was not fully elucidated by these studies. However, these results informed more recent efforts to better delineate the balance between bacterial density and inflammation as well as the factors governing that balance.

More-Recent *In Vivo* Virulence Assessments

In neutropenic mice with pneumonia, differences in *A. baumannii* strain virulence were noted using a mortality endpoint (85, 126). To begin describing virulence factors that drive the host's physiological response in noncompromised mice, more than 40 unique clinical isolates of *A. baumannii* were evaluated in the intravenous infection model of immunocompetent C3HeB/FeJ mice (70). *A. baumannii* strains were readily separated into virulent and less virulent phenotypes based on survival and sepsis outcomes in the mice (70). *In vitro* growth variance did not correlate with *in vivo* virulence differences. However, bacterial blood densities of each strain at 1 h postinfection predicted lethality and correlated with virulence (70). Thus, clinical outcome could be predicted within 1 h after infection, indicated by the bacterial density achieved in blood, even though severe sepsis had not yet developed.

Hypervirulent *A. baumannii* strains maintained high blood bacterial densities ($>10^7$ CFU/ml) at 1 h postinfection, which persisted for 24 h (70). In contrast, the virulent strains achieved 1,000-fold-lower bacterial densities at 1 h postinfection but underwent minimal clearance over the subsequent 23 h (70). The avirulent strain achieved a bacterial density at 1 h postinfection similar to the virulent strains, but underwent an additional 100-fold decrease by 24 h postinfection (70).

The avirulent strain was very susceptible to complement-mediated killing, whereas virulent and hypervirulent strains were considerably less susceptible (70). Macrophage uptake and killing of *A. baumannii* correlated inversely with strain virulence (70). Furthermore, mice depleted of either macrophages, complement, or neutrophils had modest but significant (10- to-100-fold) increases in blood bacterial density when infected with the avirulent strain (ATCC 17978) (70). Double depletion of any two effectors compounded the increase in blood bacterial density, but the remaining effector was able to maintain a bacterial blood density below the lethal threshold (70). Triple depletion of all three effectors, however, synergistically increased blood bacterial density to levels equivalent to those achieved by the hypervirulent strain in healthy mice; the result was 100% mortality within 24 h postinfection with the avirulent strain (70). These results indicated that lethal infection was not triggered by a specific exotoxin or virulence factor elaborated by *A. baumannii* ATCC 17978; rather, ATCC 17978 was cleared effectively by all three arms of the innate immune effectors, and all three had to be depleted to enable the organism to achieve sufficient bacterial density in blood to trigger the lethal sepsis response with its LPS.

One of the hypervirulent strains was also tested; *A. baumannii* HUMC1 is a lung and blood clinical isolate (simultaneously isolated from a patient with bacteremic ventilator-associated pneumonia) that is extremely drug resistant (XDR)—clinically resistant to all antibiotics except colistin (127). In contrast to ATCC 17978, HUMC1 was already relatively resistant to innate effector clearance at baseline, so disruption of individual

effectors only marginally impacted its bacterial density (70). Triple depletion, however, further exacerbated bacterial density and severity of infection, indicating that the murine host was simultaneously relying on marginally effective efforts from complement, neutrophils, and macrophages to attempt to control the organism's replication in blood. Only by working in concert were all three arms able to mediate bacterial clearance and growth inhibition *in vivo*.

Similarly, in studies of a different hypervirulent strain, *A. baumannii* LAC-4, Harris et al. established lung infection by intranasal inoculation of healthy, wild-type C57BL/6 or BALB/c mice (128). They achieved rapidly lethal infection from severe alveolar pneumonia with secondary bacteremic spread and very potent inflammatory responses in the lungs. Other results with LAC-4 compared to other strains found that LAC-4 and HUMC1 were similarly virulent; both were far more virulent than any other strain they tested (70). Both strains are capable of substantial evasion of initial innate immune clearance by complement and phagocytosis—more so than other strains tested.

Histopathological studies of mice were of further interest. When healthy mice were lethally infected intravenously with *A. baumannii* HUMC1, no tissue abscesses were revealed (69). Rather, the coccobacilli displayed a surprising inability to extravasate into the parenchyma, instead remaining trapped in the vasculature (69). Thus, *Acinetobacter* adherence to or penetration through tissues does not appear to occur to a significant degree during systemic infection.

In contrast, interactions between bacterial LPS and host TLR4 clearly drove survival in the model. Contrary to results with nonlethal infection models, TLR4 was antiprotective during lethal infection and mice were protected via disruption of LPS in the bacteria or TLR4 in the host (69). Surprisingly, bacterial burden did not differ between wild-type and TLR4-disrupted mice infected with *A. baumannii* HUMC1 despite marked, statistically significant differences in sepsis biomarkers and survival (69). Thus, outcomes of infection were driven at least as much by the host response to infection as bacterial density.

Inhibition of LPS production in the *A. baumannii* HUMC1 bacteria by growth in the presence of an LpxC inhibitor did not slow growth of the bacteria but did abrogate its virulence in mice (69). Treatment of infected mice with the LpxC inhibitor markedly decreased inflammatory cytokines, sepsis response, and death from HUMC1 infection. Bacterial density was also modestly reduced, likely because bacteria exposed to the LpxC inhibitor were easier to phagocytose and clear from blood (69).

TLR9, which recognizes CpG DNA motifs found in bacterial and not eukaryotic cells, also may play a role in host defense against *A. baumannii*. In nonlethal models of pneumonia or peritoneally administered disseminated infection, TLR9-disrupted mice had increased bacterial burden and attenuated inflammatory responses (129). However, the model was nonlethal, likely due to the use of the lab-adapted *A. baumannii* ATCC 17978 strain. This study once again demonstrated a dissociation between higher bacterial burden and inflammatory cytokines, where no deaths occurred despite higher bacterial burden likely because inflammation was attenuated.

Interestingly, the critical importance of TLR4 in governing host outcome from *A. baumannii* may in part account for the proclivity of soldiers injured in battle in Iraq and Afghanistan, who may be treated with morphine, to suffer from *A. baumannii* infections or at least more severe disease manifestations of infection. Breslow et al. found that morphine treatment resulted in a marked enhancement of death in mice from intraperitoneal infection by clinical isolates of *A. baumannii* (130). Naltrexone, an opiate antagonist, reversed the effect, demonstrating the specificity of the opiate effect. Morphine-treated mice had markedly increased inflammatory cytokines and bacterial density but suppressed phagocytic recruitment. Morphine has been demonstrated to have the potential to blunt adaptive tolerance to endotoxin, resulting in more persistent septic shock in morphine-treated mice in response to LPS than in control-treated mice (131). Thus, morphine may exacerbate the underlying pathogenesis of *A. baumannii*, and possibly other Gram-negative, infections. This further highlights the finding that while environmental factors may represent an opportunity for initial exposure, the

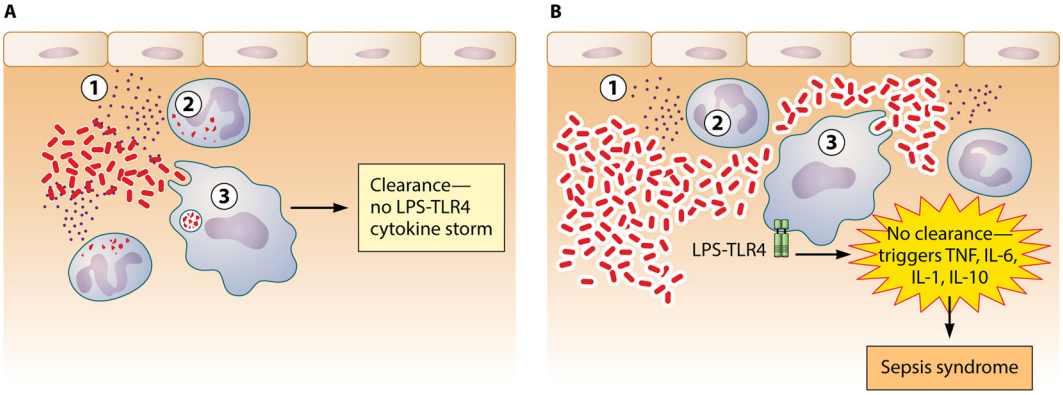

FIG 1 Host fate during *Acinetobacter* infection is determined in two stages. (A) Early clearance of the microbe by the three primary innate effectors, complement (circled 1), neutrophils (circled 2), and macrophages (circled 3), results in prevention of a sustained LPS-TLR4 activation and subsequent cytokine storm. (B) If the organism can resist initial innate effector clearance and replicate, it triggers sustained LPS activation of TLR4, resulting in cytokine storm and sepsis syndrome. One mechanism by which the organism may be able to evade clearance is by expression of an altered capsule that resists complement and phagocytic uptake (denoted by thicker shell around the bacteria).

innate immune system and a variety of host factors play a crucial role in determination of the extent and manifestations of pathogenicity.

Integrated Summary of Current Understanding of *A. baumannii* Pathogenesis

From these studies, an integrative overview of *Acinetobacter* species virulence is beginning to coalesce (Fig. 1). *A. baumannii* virulence appears to be driven initially by its ability to evade complement and phagocytosis, likely through its capsular composition and abundance. A large infectious inoculum and depletion or reduction of host innate effectors are also ways that the balance can be tipped in favor of microbial escape. If the organisms are able to evade innate immune clearance, the second virulence phase is initiated by LPS triggering of TLR4-mediated sepsis.

This integrated overview also underscores why clinical outcomes of *Acinetobacter* infections are much worse when ineffective therapy is administered initially. Early effective therapy helps the host rapidly clear the bacteria, avoiding subsequent host damage from the sepsis response, whereas early administration of ineffective therapy enables the bacteria to persist at higher blood or tissue bacterial densities, triggering host damage.

Antibiotic Resistance and Virulence

As discussed in depth below, the most important determinant in clinical outcome of *Acinetobacter* infections is antibiotic resistance. While antibiotic resistance may not be a traditional virulence factor, it is by far the biggest driver of clinical outcome by precluding the clinician's ability to kill the infecting strain. Traditional thinking has been that antibiotic resistance causes a metabolic cost to the bacterium and hence is an "anti-virulence" factor. Indeed, in a recent study, high-level antibiotic resistance was shown to exert a decrease in virulence for one *A. baumannii* strain in mice (132). However, the attenuated virulence was modest. Mutations leading to resistance delayed lethal infection in mice by several days, yet almost all the mice infected with the resistant strain still died in the end. In another recent study, introducing a multidrug-resistant phenotype by altering the *Acinetobacter* drug efflux (Ade) systems either had minimal impact on virulence or, in one strain, enhanced virulence as assessed by competitive fitness and bacterial burden in nonlethal mouse models (101). Thus, the role of resistance in affecting intrinsic virulence is complex.

Perhaps more clinically important, the therapeutic power of antibiotics is by far the most influential variable in outcome of infections. Resistance to our therapeutic armamentarium does not intrinsically exacerbate the virulence of the infecting strain. However, by eliminating the efficacy of antibiotics, antibiotic resistance precludes our

ability to speed clearance of the organism and, in accordance with the model of pathogenesis described above, thereby affects clinical outcomes in patients. Therefore, a limitation of our current models of virulence and infection are that they are not performed in the presence of antibiotics and do not account for antibiotic treatment when assessing an organism's virulence potential. To the extent that studies of virulence expect to correlate with clinical outcomes, new models of understanding virulence and its interactions with antibiotic resistance may be warranted.

ANTIBIOTIC RESISTANCE DRIVES OUTCOMES

The primary challenge of treating *Acinetobacter* infections centers upon overcoming antibiotic resistance. As early as 1977, a case series found that ventilator-associated pneumonia caused by *Acinetobacter* initially treated with effective antibiotics had a 14% mortality rate compared to an 82% mortality rate among patients infected with strains resistant to standard β-lactam therapy who received ineffective initial therapy (1). Unfortunately, *Acinetobacter* is one of the most resistant organisms encountered in clinical medicine, making initiation of effective empirical therapy challenging.

The general resistance of *Acinetobacter* to antibiotics stems in part from the very small number and size of porins in its outer membrane. The reduced outer membrane porin content confers upon *Acinetobacter* a low permeability to antibiotics, indeed far lower than for other Gram-negative organisms (133). As a matter of scale, the coefficient of permeability (rate of diffusion from outside to inside the bacteria) for cephalosporins is two- to sevenfold larger in *P. aeruginosa* than in *Acinetobacter* (133). Furthermore, the rate of carbapenem diffusion into liposomes containing purified outer membrane derived from *A. calcoaceticus* was 1 to 3% compared to *Escherichia coli* outer membrane (134). In addition, *Acinetobacter* possesses constitutive low-level expression of one or more active efflux systems (e.g., AdeABC and AdeIJK) (133). This interplay between low permeability to antimicrobials and constitutive efflux allows for an inherent resistance to a broad array of antibiotics resulting in limited therapeutic options.

In addition, *Acinetobacter* possesses a massive resistance island within its genome, which is comprised of 45 resistance genes (135, 136). Moreover, it has the capacity to rapidly acquire additional genetic entities for resistance from other bacterial species (135, 136) and the ability to develop resistance to antibiotics in the middle of a course of therapy (137).

A critical problem is the rise of carbapenem resistance among *Acinetobacter*. *Acinetobacter* isolates have shown a complex interaction of multiple mechanisms of resistance to carbapenems, with the production of naturally occurring oxacillinases (OXA) and the absence of PBP2 being most commonly observed; for some isolates, an additional downregulation of porin expression and subsequent reduction in carbapenem entry has been observed (138). The predominant oxacillinases (OXA-23, OXA-24 or -40, OXA-51, OXA-58, and OXA-143) are responsible for the majority of phenotypic resistance to carbapenems detected in the United States, Latin America, Europe, Asia, and in many parts of the world (31, 139–150). OXA-23 is a plasmid- or transposon-encoded β-lactamase, while OXA-51 is a chromosome-based enzyme and is intrinsic to *Acinetobacter*. OXA-24/40 can be chromosomal or plasmid based, and OXA-58 is plasmid encoded. These class D β-lactamases are not very robust carbapenemases, but the presence of an insertion sequence (IS) element, such as IS*Abal* and IS*Aba9*, increases expression of the carbapenemase significantly, resulting in clinical carbapenem resistance (140, 148, 151–155).

Acinetobacter also possesses class B β-lactamases (metallo-β-lactamases [MBLs]) (156). The increase in the number of MBLs in *A. baumannii* is an ominous development in the global emergence of resistance in this pathogen. The MBLs described—IMP, VIM, SIM, and NDM—are all found in *Acinetobacter* (156, 157). The presence of NDM, a widespread MBL, in *Acinetobacter* deserves special mention. *Acinetobacter* may play a very important role in spreading bla_{NDM} genes from its natural reservoir to *Enterobacteriaceae*. In *Acinetobacter*, the bla_{NDM}-type genes are found to be located on either the

plasmid or chromosome. Among NDM-producing *A. baumannii*, the bla_{NDM} gene is usually reported to be found between two copies of the IS*Aba125* element, forming a composite transposon named Tn*125* (158–160). Identification of a truncated form of this composite transposon in *Enterobacteriaceae*, while reported in its entire form in *A. baumannii*, strongly suggests that *Acinetobacter* spp. were the source of those bla_{NDM} genes before their transmission to *Enterobacteriaceae* (158, 160). *Klebsiella pneumoniae* carbapenemases (KPCs) and Guiana extended-spectrum (GES) β-lactamases are rarely reported in *Acinetobacter* (161–165).

In addition to the aforementioned enzymes, porins contribute to carbapenem resistance, the major ones being CarO and OprD (166–171). These porins constitute channels for influx of carbapenems and can contribute to resistance whereby a reduction in *carO* transcription results in downregulation of the CarO porin system and thus a decrease in carbapenem entry.

One of the remarkable features of antibiotic resistance in *Acinetobacter* is the flexibility and ease of its transmission. Transposon-mediated passage of resistance mechanisms are well described, including those for AmpC cephalosporinases, OXA carbapenemases, KPC serine carbapenemases, and NDM or VIM metallo-carbapenemases, and aminoglycosides (101, 135, 136, 147, 148, 150, 155, 158, 159, 172–178). Indeed, transposon-mediated resistance to classically described antibiotic classes, including β-lactams, tetracyclines, aminoglycosides, and sulfonamides, had occurred in a global lineage of *A. baumannii* by the late 1970s; new resistance was subsequently acquired in transposon lineages in the 1980s as new antibiotics became available (136). Transposons can transmit chromosomal resistance mechanisms to plasmids and vice versa and can readily insert in preexisting resistance elements, increasing their expression and hence resulting phenotypic resistance. Furthermore, transposon transfer of resistance can lead to accumulations of large copy numbers of resistance genes or transposons. For example, a patient treated with tobramycin experienced a remarkable rise in resistance concurrent with the isolated strain (172). The MIC increased from 0.5 to 16 μg/ml in only 4 days of therapy and was due to amplification of copy numbers of the Tn*6020* transposon containing the *aphA1* gene mediating resistance to tobramycin. In only 4 days, the number of the transposons in the strain increased to 65 copies, leading to high-level antibiotic resistance. These data underscore the fluidity and flexibility of *A. baumannii* to become antibiotic resistant in the middle of a course of antibiotic therapy and the important role that transposons play in this emergence of resistance.

Without a doubt, a key determinant in patient survivability from *Acinetobacter* infections is the early initiation of effective antimicrobial therapy. Unfortunately, initiation of effective therapy is a particular problem for *Acinetobacter* infections given the frequency of resistance. Consequently, ineffective antimicrobial treatment is more common for *Acinetobacter* than most other pathogens, resulting in a dramatic increase in mortality (24, 26, 179, 180).

Acinetobacter is one of several Gram-negative species that routinely demonstrates an XDR phenotype, defined as resistance to all available systemic antibiotics except for those that are known to be less effective or more toxic compared to first-line agents used to treat susceptible pathogens (127). The hallmark of the XDR phenotype is carbapenem resistance. CRAB strains tend to be XDR—that is, resistant to all other antibiotics, with the exception of polymyxins, tigecycline, and sometimes aminoglycosides (50, 52, 53). NHSN and Eurofins surveillance data highlight that carbapenem resistance occurs in more than 50% of *A. baumannii* ICU isolates in the United States, by far the highest rate of resistance for any pathogen surveyed (27, 181, 182). Resistance rates are even higher in eastern and southern Europe, Latin America, and many Asian countries. Overall, the proportion of global XDR strains of *A. baumannii* has increased from $<$4% in 2000 to $>$60%, while the proportion of XDR strains of *A. baumannii* in some regional nosocomial settings has more recently approached 90% (27, 31, 35, 42, 43, 183–190).

XDR *A. baumannii* infections are generally only treatable with relatively ineffective second-line agents, such as tigecycline or polymyxins (discussed further below). Such

infections cause longer hospitalization, increased costs, and greater mortality than infections caused by carbapenem-susceptible strains (44, 183). Indeed, due to their resistance to first-line agents, XDR *A. baumannii* bloodstream infections result in >50 to 60% mortality (44, 191). Furthermore, by multivariate analysis, *A. baumannii* was one of only two organisms strongly linked to increased mortality, out of 19 microorganisms evaluated (46). The odds ratio for death caused by *A. baumannii* was 1.53—the highest of all Gram-negative species (192). Similarly, a study of multiple ICUs in a Brazilian hospital found that infection caused by *Acinetobacter* was more commonly treated with initially ineffective therapy (88% versus 51% of the time) and nearly doubled the mortality rate of infection compared to infection caused by other pathogens encountered (hazard ratio of death of 1.9 by multivariate analysis) (193).

The excess mortality caused by XDR *Acinetobacter* is underscored by a study in Taiwan, where the most common cause of ICU bloodstream infections is *A. baumannii* (153). The overall mortality of 33%, while alarming, was even more pronounced when examining strains that were carbapenem resistant or XDR. Nearly a third of patients were infected with such strains. Despite having similar comorbidities to other patients, those infected with XDR strains had a significantly elevated mortality rate of 70% versus the mortality rate of 25% caused by susceptible strains (153). Administering initially effective therapy was the key to improving outcomes for infections caused by all strains (both XDR and non-XDR); 30-day mortality rates were 60% without initially effective therapy compared to 20% with effective therapy (153). For infections caused by XDR/carbapenem-resistant strains, treatment with tigecycline or colistin within 48 h still markedly reduced the mortality rates from >88% to <38% (153). Thus, ineffective initial therapy was likely the primary driver of outcome differences, rather than any other virulence differences between carbapenem-susceptible and -resistant strains. Similarly, in a recent study of patients infected with *A. baumannii* in U.S. ICUs, receipt of initially ineffective therapy doubled mortality (180).

CLINICAL MANIFESTATIONS

The two most common clinical manifestations of *A. baumannii* are nosocomial pneumonia and bacteremia. Nosocomial pneumonia occurs as a result of aspiration. In particular, the presence of an endotracheal tube creates an ideal nidus for the environmental transmission of *Acinetobacter*, which avidly adheres to plastic and can establish biofilms on the tube (194, 195). Aspiration of droplets of *Acinetobacter* directly into the alveoli of mechanically ventilated patients circumvent natural host barriers, allowing for establishment of infection in tissue. Similarly, *A. baumannii* bloodstream infections typically occur in the presence of a central venous catheter or secondarily due to extensive pneumonia, facilitating dissemination. Other well-described manifestations of *A. baumannii* include urinary tract infections (typically associated with urinary catheters or percutaneous nephrostomy tubes), wound infections or osteomyelitis (typically postsurgical or trauma related), endocarditis, and meningitis (typically postsurgical or in the presence of a ventriculostomy) (26, 27, 196–198).

Community-acquired infections predominantly occur in warm, humid, tropical environments, especially in parts of Australia, Oceania, and Asia, including China, Taiwan, and Thailand (199, 200). A prevailing finding has been the presence of comorbidities in such patients, including diabetes, kidney disease, cancer, or chronic obstructive pulmonary disease, and particularly with regard to pneumonia, heavy smoking, and excessive alcohol consumption (26, 199). These community onset infections present with acute pneumonia and, in rare occurrences, with meningitis, cellulitis, or primary bacteremia (26). As with nosocomial cases, inappropriate initial antimicrobials were strongly associated with increased mortality for community onset infections (201).

Another scenario in which *Acinetobacter* has been a major cause of infection is among wound, soft tissue, and invasive (blood and bone) infections in soldiers in Afghanistan and Iraq, particularly after traumatic injury (197, 202–208). Similar infections have occurred in trauma victims after natural disasters, such as floods and earthquakes, or bystanders in areas of active military conflicts (209–215).

Recent reports of necrotizing fasciitis caused by *A. baumannii* or *A. calcoaceticus* are especially alarming reflecting a deadly convergence of virulent infections and antibiotic resistance. These cases have typically been reported for immunocompromised patients, mostly in patients with HIV, hepatic cirrhosis, solid-organ transplant, or diabetes mellitus (216–221). They have been either community- or hospital-onset cases and have not necessarily been the result of recognized, antecedent trauma. *Acinetobacter* has even caused septic shock due to necrotizing fasciitis in a house cat (222).

The latter case emphasizes that household pets can carry and become infected by *Acinetobacter*. Two recent studies from Réunion Island found that 5 to 10% of household pets (dogs and cats) seen in veterinary clinics on the island carried *A. baumannii* (223, 224). Most of the pets were seen for routine outpatient clinic visits and were not hospitalized. Although *Acinetobacter* is an established cause of infection in veterinary hospitals (225), carriage among healthy pets has not been well described outside this unique island setting and merits further investigation in the future.

CURRENT TREATMENT OPTIONS

Because of its propensity to develop resistance to antibiotics, current treatment strategies for *Acinetobacter* remain extremely limited. β-Lactam antibiotics are the preferred antibacterial choices for susceptible *A. baumannii* infections, and susceptible pathogens respond briskly to therapy. Due to rising resistance, carbapenems have become an increasingly critical therapeutic option for these infections. For carbapenems (as all β-lactams), the best predictor of efficacy is the time serum carbapenem concentrations remain above the MIC. Extended infusion of carbapenems can maximize time above MIC, thereby optimizing outcomes, particularly for resistant pathogens (226–228). As such, leveraging this property can result in a significant therapeutic effect in strains with low- to intermediate-range MICs.

Risk analysis by Monte Carlo simulation for *Acinetobacter* spp. predicted that a dose of 1 g meropenem administered every 8 h (administered as a 3-h infusion) would provide optimal bactericidal rates (227). Similarly, a meta-analysis of retrospective clinical studies (not specific for *Acinetobacter* infections) found that extended carbapenem infusion times resulted in lower mortality rates than rapid infusion, without any evidence of increased rates of resistance emergence (229). However, the pharmacokinetic cost of prolonging the infusion is a decline in the peak level of the drug. At carbapenem MICs of >16 μg/ml, it is likely that peak levels will never exceed the MIC, and modeling indicates lower ability to achieve the time above MIC necessary to optimize bacterial killing with extended infusion (227). Hence, extended infusion should be strongly considered for isolates with MICs of 4 to 16 μg/ml, but intermittent dosing to achieve peak levels above the MIC may be preferred for isolates with higher MICs.

Unfortunately, as mentioned, carbapenem resistance rates for *A. baumannii* have been rising dramatically both in the United States and globally. For carbapenem-resistant strains, the antibiotic armamentarium is quite limited. There is no consensus on optimal antimicrobial treatments for such strains.

One option to treat XDR infections is tigecycline. While tigecycline often has a low MIC (<2 μg/ml) for *A. baumannii* strains, the drug's serum concentrations are also low, and in clinical trials, outcomes in patients with ventilator-associated pneumonia and bacteremia have been clearly inferior to alternative agents (230–232). In particular, retrospective clinical data validate the susceptibility breakpoint of tigecycline at 2 μg/ml. Specifically, infections caused by *Acinetobacter* strains with tigecycline MICs of ≥2 μg/ml result in significantly increased mortality when treated with tigecycline (137, 233–235). Even if the MIC is ≤2 μg/ml, *Acinetobacter* bacteremia may have inferior outcomes when treated with tigecycline, including worse survival, failure to clear bacteremia, and development of breakthrough bacteremia (236). A recent systemic review and meta-analysis (not specific to *Acinetobacter* infections) found that tigecycline therapy resulted in higher in-hospital mortality, inferior microbial eradication, and a trend toward longer hospital stays (237).

As an alternative to tigecycline, interest has emerged in the use of minocycline to treat *Acinetobacter* infections (238–240). Minocycline may retain antimicrobial activity even against strains resistant to other tetracyclines (including tigecycline)—although cross-resistance has been seen. A recent, large, international survey of more than 1,000 global *Acinetobacter* strains (including XDR strains) found that minocycline was the most active tetracycline *in vitro*, with more than 70% of strains susceptible per the CLSI breakpoint of MIC of ≤4 μg/ml (241). It should be emphasized that the breakpoint is not well validated based on clinical outcome data and that its value is the same as the mean, peak blood level of minocycline when administered as a 200-mg intravenous (i.v.) dose (239). Thus, caution may be warranted when using minocycline to treat a strain with an MIC of 4 μg/ml, particularly as monotherapy and in the setting of bacteremia. Only limited clinical data are available describing outcomes of *Acinetobacter* infections treated with minocycline. Goff and colleagues described perhaps the largest case series, in which 55 patients were treated with minocycline, but only 3 received monotherapy (240). More than 70% of patients had a favorable clinical response; 25% died. It is difficult to interpret this efficacy rate absent a control group.

As an alternative to tetracyclines, a unique feature of *Acinetobacter* infections is the potential therapeutic role of sulbactam. Sulbactam is used to inhibit β-lactamase enzymes for most pathogens, but it has direct antimicrobial activity against *Acinetobacter*. Sulbactam is a class A β-lactamase inhibitor similar in structure to β-lactams, but it has an intrinsic affinity for the *A. baumannii* penicillin-binding proteins (242, 243). A major disadvantage of sulbactam is that it is only available in combination with ampicillin within the United States. A more concerning feature is that there have been increasing rates of sulbactam resistance encountered (243). Two major studies (one in Spain and another in Taiwan) identified the rate of sensitivity of *Acinetobacter* to sulbactam at only 47% and 30%, respectively (243). However, high-level sulbactam resistance may, at least in animal models, convey a significant fitness cost to the organism (242).

Additional potential options for definitive therapy if β-lactams cannot be used include fluoroquinolones and aminoglycosides, although neither should be considered preferred options for empirical therapy because rates of resistance to both classes are high. Resistance to fluoroquinolones occurs via chromosomal mutations of the *parC* and *gyrA* genes encoding bacterial topoisomerase IV subunits (151, 155, 244, 245). Reduced permeability of the bacterial outer membrane to quinolones and active efflux mechanisms also contribute to the organism's resistance (244). Among the aminoglycosides, amikacin and tobramycin offer the greatest reliability in susceptibility of *Acinetobacter* isolates (246). Aminoglycoside-modifying enzymes are widespread and are particularly prevalent in integrons within multidrug-resistant *Acinetobacter* strains (247). Surveillance data from 2009 found a 59% rate of sensitivity to tobramycin; it is unsurprising that the rate has decreased further since then (248). Prolonged use of aminoglycosides is often avoided due to concerns of drug toxicity. However, a retrospective cohort study found comparable toxicity and microbiologic clearance with tobramycin compared to colistin (246). Therefore, when susceptibility permits, aminoglycosides can be a potential treatment option.

Given the limitations of most of the non-β-lactam antimicrobial options, presently, polymyxins are often the last treatment option for XDR *Acinetobacter*. Unfortunately, polymyxins suffer from high rates of nephrotoxicity and neurotoxicity. They possess no therapeutic window: doses that are effective are also toxic. Although it remains uncommon, resistance to polymyxins has been reported and is increasing (182, 249).

Clinical data are now emerging that evaluate MIC breakpoints for colistin resistance. In one recent series, patients treated with colistin whose infecting isolate of *Acinetobacter* had a colistin MIC of 1 to 2 μg/ml had twice the risk of 14-day mortality as patients infected with isolates having lower MICs (250).

A potential limitation to polymyxin therapy is its relatively poor epithelial lining fluid penetration in the lung (251). Imberti et al. found that in critically ill patients, colistin

was not detected in bronchoalveolar lavage specimens taken 2 h after intravenous infusion of the drug (251). In contrast, nebulizing polymyxins has the potential to achieve very high concentrations in the lungs while minimizing systemic exposure and toxicity. Numerous investigators have described favorable outcomes of patients with nonbacteremic *Acinetobacter* pneumonia who were treated with nebulized polymyxins (252–261). In one case-control study, use of inhaled colistin increased microbial eradication rate from the airways in ventilated patients compared to systemic therapy (257). In another study, patients with ventilator-associated pneumonia had superior cure rates, with similar mortality, when inhaled colistin was added to intravenous therapy (258). In contrast, Demirdal et al. found no difference in microbiological eradication, clinical outcome, recurrence, or mortality among 80 patients with *Acinetobacter* pneumonia who received systemic colistin alone versus 43 patients who received systemic colistin combined with inhaled therapy (262). Fortunately, addition of inhaled colistin did not increase the rate of acute renal injury. Thus, at this point, the data are mixed regarding whether adding inhaled colistin to systemic therapy improves clinical cure. Importantly, several studies have reported favorable outcomes of patients with ventilator-associated pneumonia caused by highly resistant Gram-negative bacteria, including XDR *A. baumannii*, treated with monotherapy inhalational colistin without adjunctive systemic therapy (255, 259, 261, 263). However, these results should be viewed cautiously, as cases were nonrandomized leading to a potential selection bias.

While it may be true that inhalational therapy minimizes systemic toxicity, colistin can be toxic to lung tissue and induce bronchospasm, and nephrotoxicity can occur with accumulated dosing. Furthermore, resistance has emerged during therapy, although with variable frequency depending on the case series (255, 261, 263).

Indeed, rising rates of resistance to the last-line agents, tigecycline and polymyxins, among *A. baumannii* are of substantial concern. These strains tend to be pan-resistant, with no available antibiotic therapies left. Tigecycline resistance now exceeds 50% in some regions, and polymyxin resistance rates as high as 20% have been seen in Greece, and are increasingly reported in other regions as well (189, 249, 264–266). These pan-resistant isolates underscore the critical need to identify alternative therapeutic strategies.

COMBINATION ANTIMICROBIAL THERAPY

Given the limitations of monotherapy for XDR strains and the emergence of pan-resistant strains, combination therapy has been advanced as a potential option to improve treatment outcomes and possibly to prevent new emergence of resistance. Experts have long debated whether the use of combination regimens of antibiotics could prevent the emergence of resistance among typical bacterial pathogens, as is clearly the case for *Mycobacterium tuberculosis* (tuberculosis [TB]) (267–269). However, there are few data to suggest that combination regimens can reduce the emergence of resistance *in vivo*, particularly for Gram-negative bacterial infections (268, 270, 271). For *Acinetobacter* specifically, systematic reviews have not found that combination therapy is more effective at preventing emergence of resistance and could not draw general conclusions for therapeutic outcome based on heterogeneity of the data (272, 273). A recent case series from Greece of XDR *A. baumannii* infections also found no clinical advantage of colistin-based combination regimens compared to colistin alone (189).

With respect to emergence of resistance, it is important not to draw conceptual parallels regarding the use of combination therapy to treat *Acinetobacter* or TB. *M. tuberculosis* has no environmental reservoir, spends the majority of its life cycle during infection in a nonreplicating persister state, and is generally treated with drugs with minimal antimicrobial activity against typical bacterial pathogens (e.g., isoniazid, ethambutol, and pyrazinamide; rifampin being the exception). Thus, use of multiple drugs to treat TB in patients results in minimal selection of resistance to other pathogens among the patient's normal flora, and there are no TB bacilli in the environment to have resistance selection occur. For *Acinetobacter*, the situation is very different. The antimicrobial agents used to treat *Acinetobacter* do select for resistance,

often on mobile genetic elements, among normal flora and among environmental reservoirs of organisms. Even if administration of more than one antibiotic to a patient were to slow the emergence of resistance at the site of infection in that patient, selection of resistance to each drug administered would occur in the patient's normal flora and in the environment. Conceptually, in the long run, this creates the risk of accelerated emergence of resistance, i.e., to each of the multiple drugs used in a combination regimen rather than a single drug used as monotherapy. At the current time, it is not known if there are advantages to combination regimens to prevent the emergence of resistance among *Acinetobacter* (272, 273) or how rapidly combination regimens would result in selection of resistance to each drug separately among normal flora and in the environment.

However, aside from preventing resistance, combination regimens may be useful to improve clinical outcomes or microbial eradication in patients infected with XDR strains. *In vitro* models suggest that rifampin is bactericidal for *Acinetobacter* isolates (59). Despite promising small studies, in a larger randomized, multicenter trial, the mortality was unchanged between patients with XDR *A. baumannii* infections treated with colistin and rifampin versus colistin alone (274). However, there was superior microbial eradication by culture confirmation in patients randomized to the combination arm. Thus, combination therapy with rifampin may be of benefit in situations where there is an infected foreign body or where the infection is in a sequestered site (e.g., central nervous system [CNS], bone) into which most antibiotics penetrate poorly and would have difficulty sterilizing tissue.

Other combination regimens have been examined and although randomized human studies have not been conducted, *in vitro* and animal models suggest increased efficacy of sulbactam in combination with cefepime, meropenem, imipenem, amikacin, or rifampin (243). Alternative combinations include colistin-tigecycline and colistin-carbapenem therapy, each without clear evidence of benefit (233). One point is clear: tigecycline combination therapy should not be used to treat isolates with a tigecycline MIC of $\geq$2 μg/ml. Patients infected with such strains displayed excess mortality when tigecycline was used, even as part of a combination regimen (137, 233).

Among all the combination regimens, the one that may be most rational is colistin-carbapenem, which has been found to be synergistic in multiple *in vitro* studies (275–279). This synergy may be observed in particular for isolates with intermediate resistance to the carbapenems (e.g., if the carbapenem MIC is 4 to 16 μg/ml). Peak levels of carbapenems exceed these MICs at standard dosing, and *in vitro* synergy has been described between colistin and carbapenems in this range (276).

Given the complexity and limitations of the data, there remains considerable debate regarding the utility of combination treatment for XDR infections. A retrospective review evaluating monotherapy for *Acinetobacter* found comparably low efficacy with either tigecycline or colistin, achieving unfavorable clinical success rates of 47% and 48%, respectively (61). However, combination therapy, chiefly with carbapenem or sulbactam, showed a trend toward lower mortality and improved clinical and microbiological success rates (61). Prospective, randomized studies of combination therapy are critically needed to determine whether combination regimens are superior to monotherapy and, if so, what is the preferred combination of agents.

While there are intriguing suggestions that combining colistin with glycopeptides may result in superior outcomes (280), a recent comparative clinical study found no difference in survival or clinical cure, despite a marked decrease in nephrotoxicity among patients treated with the combination versus colistin alone (55% versus 28% [$P = 0.04$]) (281). Conversely, a retrospective review of 68 patients treated for more than 5 days with colistin plus glycopeptide therapy found no difference in nephrotoxicity and a higher survival rate than colistin monotherapy (282). Given the mixed results—particularly the negative finding in the prospective study—these data await confirmation in a randomized controlled trial.

In summary, absent clear evidence of benefit with combination therapy, monotherapy is preferred for β-lactam-susceptible strains. For XDR strains, however, one

rational approach is to consider combining colistin and carbapenem therapy, particularly if the carbapenem MICs are 4 to 16 μg/ml and preferably utilizing extended infusion administration if the MICs are 4 to 8 μg/ml. This approach attempts to supplement the therapeutic effect of the last-line polymyxin therapy with a pharmacokinetically optimized carbapenem. Rifampin may be useful to add for infections of the CNS, bone, or prosthetic materials.

Finally, in cases of nonbacteremic XDR *Acinetobacter* pneumonia, it is reasonable to consider addition of inhaled colistin, minimizing toxicity and maximizing levels delivered to the lung, as an adjunct to systemic therapy with carbapenem or other agents. The use of nebulized colistin as monotherapy may be a reasonable alternative in nonbacteremic patients with pneumonia to avoid systemic nephrotoxicity. However, the potential for nephrotoxicity is not completely eliminated by this approach, bronchospasm can occur, and the potential for resistance selection has not been fully elucidated. Further study is needed to determine whether other combination regimens are useful, preferably in the form of randomized controlled trials.

FUTURE TREATMENT OPTIONS

New Antibiotics

Several companies are working at the preclinical stage on small-molecule antibiotics that have specific activity focused on *Acinetobacter*. Entasis Therapeutics has a novel β-lactamase inhibitor combined with sulbactam (283). Spero Therapeutics has a polymyxin-based potentiating peptide that is combined with other antibiotics for Gram-negative bacteria, including *Acinetobacter* (284). Melinta is developing a novel, ribosomal protein synthesis inhibitor with broad Gram-negative activity, including against *A. baumannii* (285). Tetraphase Pharmaceuticals is developing eravacycline, a novel fluorotetracycline which has expanded activity against *Acinetobacter*. Eravacycline has completed phase III clinical trials for complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI); more trials will likely be required based on unexpectedly low cure rates for cUTI (286).

Antimicrobial Peptides

Novel therapies remain under investigation and offer exciting potential for bypassing the permeability, efflux, and enzymatic resistance mechanisms that make *Acinetobacter* a tremendously adaptable pathogen. For example, antimicrobial peptides are a common host defense mechanism, and most commonly, they function to disrupt bacterial membranes. The failures of antimicrobial peptides in clinical development are countless, and some key opinion leaders have begun to doubt whether antimicrobial peptides will be viable therapeutic options for systemic infections (in contrast to topical applications) without substantial further scientific advancements (287). Nevertheless, such peptides can have broad-spectrum activity against highly resistant Gram-negative bacteria, including *Acinetobacter*. For example, cecropin A-melittin hybrid proteins have shown *in vitro* bactericidal activity against resistant *A. baumannii*, but a short half-life has limited their utility thus far (288). Peptides derived from frog and toad skin have been shown to have strong bactericidal activity. Highlighting this potential, in preclinical models of blood and wound infections treatment with A3-APO (a proline-rich antibacterial peptide) improved survival and decreased bacterial density more than either imipenem or colistin (288). Before peptide therapies can be reliably used systemically, they must overcome problematic pharmacology, toxicity, and inactivation in the context of biological matrices such as serum, surfactant, and other biological fluids and tissues.

Phototherapy

Phototherapy utilizes the combination of oxygen, infrared light, and a photosensitizer (a nontoxic, photoreactive dye) to generate reactive oxygen species that can damage DNA and disrupt cellular membranes. This modality is limited to topical use and carries the potential for local tissue injury from reactive oxygen species. Although

few studies have attempted to examine the roles of photosensitizer agents in *Acinetobacter* infection, tetrapyrrole porphyrins and phenothiazinium salts have the most support. Studies suggest that phototherapy can exert a bactericidal effect via disruption of lipopolysaccharide with tetrapyrrole choline applied topically, effectively reducing bacterial density 1,000-fold in a mouse model (288).

Bacteriophages

At long last, given the marked rise in resistance to antibiotics and failure of the antibiotic pipeline, there has been renewed interest over the last 2 decades in bacteriophages capable of lysing bacteria. While interest in bacteriophages predates the antibiotic era, by the late 1920s to early 1930s, there were already concerns about the quality of data supporting efficacy in patients and concerns about the potential for biological matrices to neutralize bacteriophage effects (289). While it remains unclear if phages will demonstrate efficacy for systemic infections (as opposed to topical or gastrointestinal), recent years have witnessed a substantial renewed interest in their potential (290).

With respect to *Acinetobacter*, AB1 and AB2 were identified in 2010 as the first two phages specific for *A. baumannii*; since that time, numerous other phages have been identified with lytic activity of *Acinetobacter* (288, 291). Several rodent studies have suggested that strain-specific phages can improve outcomes from intranasally inoculated lung infection, intraperitoneal sepsis, or wound infections (288, 292–294). Lysin peptides from phages have also been studied as effective treatments for *A. baumannii* infections in mice (295, 296).

The limitations of phage therapy remain substantial. For example, phage therapy requires very precise matching of phage to bacterial strain type; *Acinetobacter* is an exceedingly diverse genus. In one study, phage AB1 induced lysis in only one of five multidrug-resistant isolates; likewise, phage AB2 was capable of infecting only 25 of 125 (20%) of clinical isolates examined (288). Subsequent studies of phage Abp53 and phage ZZI were able to lyse only 27% and 13% of multidrug-resistant isolates, respectively (288). More recently identified phage strains have shown greater efficacy, but even phage AP22 had activity in only 89 of 130 isolates (68%) and remains one of the most broadly acting phages identified thus far (288).

A cocktail of multiple phages may be able to overcome the limitations of each individual phage. Limited studies of a multiphage cocktail resulted in growth inhibition, but again, with an effect highly dependent on the *Acinetobacter* strain (297). Regeimbal et al. describe the efficacy of a five-member phage cocktail in reducing the bioburden in a neutropenic murine full-thickness wound (298). Interestingly, this cocktail included four phages that did not directly kill bacteria but rather acted in a combinational manner to delay bacterial growth and targeted encapsulated strains, shifting the population to a predominantly unencapsulated state. The caveat remains that mice were neutropenic, blunting a potential concurrent host immune response that could neutralize the phages, the phage cocktail was individualized to the inoculated *Acinetobacter* strain, and the cocktail was administered by both intraperitoneal and topical administration. This raises questions about the large-scale applicability of producing personalized phage cocktails and their potential efficacy in a systemic infection model. However, it does raise unique options for phage therapeutics as a means to target specific virulence factors, in this case bacterial capsule, to increase sensitization for eradication by antimicrobial, host immune, or other combinational modalities. Beyond bactericidal activity, phages also hold the potential for decolonization and for disruption of biofilm formation; phage AB7-IBBI and AB7-ABB2 were capable of disrupting 75% of preformed biofilms in one study (288).

As promising as studies have been, phage therapy is far from clinical use. Additional concerns with phage therapy include the potential for secondary effects on human flora, emergence of phage resistance akin to antibiotic resistance, and the potential for production of host inflammatory responses following phage administration. Likewise, the long-term and reproducible viability of phage therapies may be a concern due to

rapid clearance by human macrophages and the induction of antiphage antibodies. Clarification of the potential for phage therapy to treat *Acinetobacter* infections awaits additional study.

Active and Passive Vaccination

Another interest is the development of vaccines targeting *Acinetobacter* for preventative or therapeutic purposes (299). A major challenge of developing a vaccine targeting *Acinetobacter* is the diversity of the genus overall and of the *A. baumannii* species in particular. Nearly 40 serotypes have been identified in *A. baumannii*, and the prevalence of each serotype is largely unknown. A monoclonal antibody targeting the K1 capsular polysaccharide recognized only 13% of *Acinetobacter* strains (113). Capsule is an attractive target for an antibody-based vaccine, but such a vaccine would have to be multivalent given the number of different capsular types that might need to be included.

With respect to protein targets, the one that has garnered the most attention is outer membrane protein A (OmpA). OmpA is highly conserved among clinical *A. baumannii* isolates and has several extracellular loops that might be amenable to vaccination (55). Vaccination with recombinant OmpA did indeed engender protection against bacteremic sepsis in a manner that appeared to be dependent on antibody (55). Higher doses of vaccine were found to result in superior humoral immune responses due to type 2 cytokine polarization (300). Another promising target that has been reported to have protective activity as a vaccine in mice is highly conserved nuclease, NucAb (301). The vaccine markedly reduced bacterial burden, although its improvement of survival was less impressive. Other outer membrane proteins, such as OmpW, have also been reported to be effective vaccines (302, 303). However, a challenge of any active vaccine for *Acinetobacter* is to define a patient population that is of sufficiently high short-term risk of infection to make efficacy definable in a reasonably sized clinical trial, while still allowing sufficient time for the at-risk patient to generate protective immunity to an active vaccine before infection begins.

In contrast, in passive immunization, antibodies could be administered and result in immediate protection, without having to wait for a lymphocyte response to a vaccine. Indeed, passive transfer of polyclonal anti-OmpA, anti-OmpW, or anti-NucAb immune serum from vaccinated mice transferred protection to recipient mice, validating the approach (300, 301, 303). In other experiments, antibodies against the outer membrane transporters and polysaccharides were able to facilitate opsonization and subsequent phagocytosis of A. *baumannii* leading to decreased tissue loads (304). The challenge for passive immunization will be to identify a monoclonal antibody or mixture of several monoclonal antibodies that are capable of covering 90 to 95% of clinical isolates of this highly diverse genus or even the *A. baumannii* species.

Trace Metal Sequestration

Sequestration of host iron and other trace metals is another novel strategy that has potential to ameliorate the severity of *Acinetobacter* infections (305–308). Multiple studies conducted by prominent laboratories have demonstrated that iron or zinc limitation can suppress *Acinetobacter* growth and that the organisms have multiple iron and zinc acquisition mechanisms, including catechol production, production of very high-affinity siderophores (e.g., acinetobactin) and companion siderophore uptake receptor and energetic mechanisms, and heme utilization (82, 121, 308–320). Small-molecule iron chelators have *in vitro* activity at killing *Acinetobacter* during log growth (318). Thus, combatting iron or zinc uptake in the bacteria is a novel, potentially impactful therapeutic approach.

Transferrin is a predominant iron-sequestering agent in the blood of mammals (306). Despite the much higher affinity for iron of bacterial siderophores compared to transferrin (e.g., affinities of $\geq 10^{-30}$ M versus 10^{-24} M), sufficient quantities of transferrin can overwhelm and outcompete the siderophores produced by bacteria; the bacteria are thus unable to acquire sufficient iron to maintain viability. Specifically, exposure of *Acinetobacter* (and other pathogens) to transferrin *in vitro* resulted in depletion of intracellular iron and zinc levels in the bacteria, with resulting cell

membrane depolarization due to inability to maintain cellular energetics absent sufficient iron, and static growth inhibition by time-kill curves (72). Furthermore, i.v. transferrin therapy of mice infected intravenously with XDR, hypervirulent *A. baumannii* HUMC1 resulted in marked improvement in survival (72).

Another metal-sequestering agent demonstrated to have activity against *Acinetobacter* is calprotectin (317). Calprotectin is a neutrophil-derived host defense molecule that sequesters zinc and manganese and inhibited *A. baumannii* growth *in vitro* (317). Mice with disrupted calprotectin, which diminishes their ability to mediate zinc and manganese sequestration, had higher bacterial burden during *A. baumannii* pneumonia (317). These data underscore the potential for novel iron-sequestering approaches to serve as viable therapeutic, or perhaps even prophylactic, approaches in patients in the future.

Cytokine and Pattern Recognition Receptor Modulation

Finally, there is great potential for modulation of host inflammation based on LPS-TLR4 interactions to improve outcomes from *Acinetobacter* infections. We found that disruption of TLR4 resulted in profound resistance of mice to otherwise lethal infection caused by hypervirulent *A. baumannii* HUMC1 (69). Furthermore, as mentioned previously, small-molecule inhibition of LpxC in *A. baumannii*, while not altering growth *in vitro*, completely abrogated its virulence in mice (69). Thus, developing small-molecule or biological approaches that abrogate the LPS-TLR4 interaction during *Acinetobacter* infection should result in great therapeutic benefit.

Given the relatively barren antimicrobial pipeline for new agents targeting *Acinetobacter* infections, combined with the dramatic rise in XDR and pan-resistant strains, new therapeutic agents are critically needed for these infections. Novel biological and pathogen-specific approaches are promising to improve outcomes, slow the spread of resistance, and ameliorate the pressure of continuously developing new antibiotics to treat *Acinetobacter* infections. Given the diversity of approaches under investigation and the proof of concept for many of them at the preclinical stage, it is likely that one or more such approaches will be available clinically in the coming 10 to 15 years.

CONCLUSIONS

After a century of studying this tenacious pathogen, *Acinetobacter* remains an ever elusive foe, and a tremendous challenge for physicians. The prompt administration of effective therapy remains critically important yet is very difficult to achieve with rising rates of resistance and a dry pipeline targeting this organism. When this pathogen is susceptible, β-lactam antibiotics are the preferred treatment. Whether combination regimens are beneficial is not yet known; however, for XDR strains, and particularly those with carbapenem MICs of 4 to 16 μg/ml, combination carbapenem-polymyxin therapy is a rational approach. For isolated pulmonary disease caused by XDR strains in the absence of bacteremia, inhaled colistin is a reasonable choice to deliver high levels of drug while minimizing systemic exposure.

Ultimately, given our limited therapeutic options, addressing *Acinetobacter* infections must combine a multidisciplinary approach, including vigilant infection control practices, antimicrobial stewardship, and the combined efforts of multiple health care providers. Additional research on new therapeutics holds promise to improve outcomes in the future. In the meantime, we must learn how to optimize the efficacy of our current antimicrobials, potentially with combinational regimens and extended infusion, to combat the crisis of *Acinetobacter* infection.

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