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MINIREVIEW

Pathogenic *Acinetobacter*: from the Cell Surface to Infinity and Beyond

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The genus *Acinetobacter* encompasses multiple nosocomial opportunistic pathogens that are of increasing worldwide relevance because of their ability to survive exposure to various antimicrobial and sterilization agents. Among these, *Acinetobacter baumannii*, *Acinetobacter nosocomialis*, and *Acinetobacter pittii* are the most frequently isolated in hospitals around the world. Despite the growing incidence of multidrug-resistant *Acinetobacter* spp., little is known about the factors that contribute to pathogenesis. New strategies for treating and managing infections caused by multidrug-resistant *Acinetobacter* strains are urgently needed, and this requires a detailed understanding of the pathobiology of these organisms. In recent years, some virulence factors important for *Acinetobacter* colonization have started to emerge. In this review, we focus on several recently described virulence factors that act at the bacterial surface level, such as the capsule, *O*-linked protein glycosylation, and adhesins. Furthermore, we describe the current knowledge regarding the type II and type VI secretion systems present in these strains.

Infections caused by pathogenic members of the genus *Acinetobacter* are emerging as a significant threat to human health. These Gram-negative bacteria are responsible for increasing numbers of infections encountered in hospitals, particularly among immunocompromised patients (1, 2), and community-acquired infections are also increasing in prevalence (3). A recently recognized population at particular risk for *Acinetobacter* infections is military service members who have suffered combat-related injuries, who often acquire these infections in field hospitals (4–6). Although *Acinetobacter* spp. primarily cause pneumonia, they are also frequent causes of wound and burn infections, meningitis, urinary tract infections, and sepsis (7). There is a growing trend for these isolates to display high levels of antibiotic resistance, with some being resistant to all clinically available antibiotics (8). Collectively, these attributes have led to a pressing need to elucidate the mechanisms used by pathogenic *Acinetobacter* to cause disease.

The bacterial cell surface plays essential roles in sensing of the environment, interactions with the host, and maintenance of cellular homeostasis (9). The molecular structures present on the cell surface, and those that extend beyond the surface, are of central importance for understanding the pathogenesis of an organism; these are often the key determinants that mediate bacterial virulence and thus represent important targets for novel antimicrobials and vaccines (10). This review highlights the recent research on pathogenic *Acinetobacter* spp., which has led to several key findings on the strategies used by these bacteria to elaborate cell surface and secretion components that are vital for causing disease (Fig. 1).

CELL SURFACE

The cell surface of Gram-negative bacteria plays myriad roles in the physiology of these organisms, including transport of molecules into and out of the cell, interaction with and sensing of the extracellular environment, and protection from external stresses (9). While all bacterial cell surfaces are composed primarily of lipids, carbohydrates, and proteins, the diversity in the molecular composition and arrangement of these structures has vast implications for virulence in pathogenic bacteria. Recent experimental investigation into the arrangement and composition of these structures on *Acinetobacter* cell surfaces has provided important insights into their role in the pathobiology of these important human pathogens.

Lipooligosaccharide. Lipopolysaccharide (LPS), the major component of the outer leaflet of the outer membrane (OM) of many Gram-negative bacteria, is an immunostimulatory molecule that plays an important role in bacterial resistance to external stresses (11). LPS is composed of the endotoxic lipid A, a core oligosaccharide, and a repeating sugar structure called the O antigen (11). During biosynthesis of LPS, the core oligosaccharide is built onto the lipid A moiety in the cytoplasm and flipped into the periplasmic space. The repeat subunit of the O antigen is synthesized separately onto an undecaprenyl phosphate (Und-P) carrier, which is then flipped to the periplasm and ligated to the lipid A core by the WaaL ligase enzyme (12); thus, the WaaL ligase is essential for the production of O antigen. Many pathogens, such as *Escherichia coli* and *Salmonella* spp., produce diverse O-antigen structures that form the basis for serotyping schemes (13–15). However, other important bacterial pathogens, such as *Neisseria* and *Campylobacter*, lack a WaaL ligase ortholog and do not produce O antigen, elaborating only lipooligosaccharide (LOS) (16).

Whether *Acinetobacter* spp. elaborate LPS or LOS on their cell surface has been a topic of considerable debate. Several reports have described the presence of LPS in *Acinetobacter*, many of which have used structural or antibody-based methods to detect O-antigen carbohydrate moieties (17–26). However, most *Acinetobacter* spp. do not show "typical" LPS laddering upon silver

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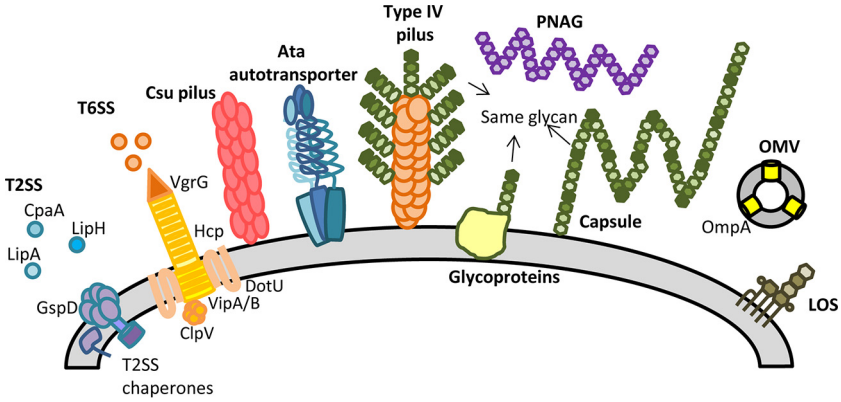

FIG 1 Cell surface components and secretion systems identified in *Acinetobacter* spp.

staining of isolated LPS (27, 28), leading to doubts regarding whether *Acinetobacter* spp. actually produce true O antigen. Intriguingly, *Acinetobacter* spp. possess either one or two genes, depending on the strain, that encode proteins with domains similar to those found in WaaL ligase orthologs (29–32); however, those domains are also found in PglL, the enzyme responsible for *O*-linked protein glycosylation (see below) (29). Bioinformatic analysis alone is not sufficient to distinguish between WaaL and PglL orthologs, and careful experimental analysis is required to determine the true function of the proteins possessing these domains. Recent work has now conclusively demonstrated that, in *Acinetobacter* spp. with a single "*waaL*-like" gene, the gene actually encodes a PglL enzyme with no O-antigen ligase activity (29). In *Acinetobacter* spp. with two *waaL*-like genes, the genes were initially suggested to encode one PglL enzyme and one WaaL enzyme (31), but experimental analysis has identified both enzymes as being exclusively involved in protein *O*-linked glycosylation, with no role in O-antigen biosynthesis (30). In light of these recent data, and in the absence of experimental data showing a protein possessing divergent O-antigen ligase activity, it seems most likely that *Acinetobacter* spp. produce LOS but not LPS. In any case, targeted mutagenesis and random mutagenesis of genes involved in synthesis of the LOS core oligosaccharide have shown that this component is a major contributor to *Acinetobacter* survival and virulence (33–35). The cluster of genes that synthesize the sugar component of LOS are extremely diverse among *Acinetobacter* spp., and a number of different structures have been determined or predicted (36). Modification of the LOS has also been shown to impart resistance to antimicrobials, similar to the findings for other bacterial species. Specifically, these modifications occur on the lipid A structure of *Acinetobacter* spp. and lead to decreased susceptibility to antibiotic and antimicrobial peptides and increased survival during desiccation (37–39). *Acinetobacter* spp. have also been shown to acquire mutations in the lipid A biosynthetic pathway when treated with colistin, resulting in resistance to the antibiotic (40–43). Interestingly, those studies found that mutations in certain lipid A genes resulted in complete loss of LOS.

Glycoproteins. The posttranslational modification of proteins with glycans, which were once thought to be exclusive to eukaryotes, has been identified in all forms of life. In bacteria, carbohydrates can be attached via the amide of asparagine residues (*N*-linked) or the hydroxyl of serine/threonine residues (*O*-linked). In a series of steps analogous to O-antigen biosynthesis, glycans are assembled onto the Und-P lipid carrier and flipped to the periplasmic face of the inner membrane, where an *O*-oligosaccharyltransferase (*O*-OTase) enzyme catalyzes the transfer of the complete carbohydrate structure to a serine or threonine residue on the cognate acceptor protein (44).

Bioinformatic analysis identified a protein in *Acinetobacter baumannii* that showed homology to the *O*-OTase from *Neisseria meningitidis*, named PglL (29). PglL proteins often contain domains similar to those of WaaL ligases (Wzy_C domains), and bioinformatic identification of a PglL-like protein is not sufficient to differentiate it from WaaL ligases, thus necessitating experimental characterization. When the *pglL*-like gene from *A. baumannii* was deleted, loss of a carbohydrate-specific band was detected after SDS-PAGE analysis, with no change in the LOS profile (29). In-depth characterization of the *A. baumannii pglL* mutant by mass spectrometry determined that the *pglL*-deficient strain lacked a total of seven glycoproteins, which were glycosylated with a pentasaccharide in the wild-type strain. Loss of protein glycosylation in *A. baumannii* resulted in pleiotropic effects on several virulence-associated phenotypes, including biofilm formation and survival in a mouse model of systemic infection (29). Protein glycosylation has been shown to be a conserved modification present throughout the genus *Acinetobacter*, but the composition of the glycan moiety and the number and identity of the modified proteins vary among strains (45). Interestingly, the carbohydrate structure attached to glycoproteins is identical to the repeat units found in capsular polysaccharide (46). Although the phenotypes associated with complete loss of protein glycosylation have been studied, the contributions of individual glycoproteins to these phenotypes remain unknown.

The *pglL* gene in *A. baumannii* is located immediately downstream of the predicted major type IV pilin gene, *pilA*, which encodes a common target for glycosylation in several bacteria. In *A. baumannii* ATCC 17978, this protein was not found to be glycosylated under laboratory conditions but was glycosylated upon overexpression in the presence of PglL (29, 30). Intriguingly, most *Acinetobacter* spp. actually have two proteins with a domain from the Wzy_C superfamily that is common to PglL and WaaL orthologs. It was originally suggested that this second gene could be a WaaL ligase involved in O-antigen biosynthesis (31). Through mutagenesis and functional studies, however, it was determined that both genes actually encode *O*-OTases (30). The *O*-OTase en-

coded by the gene near the pilin gene was found to exclusively glycosylate the cognate pilin protein, similar to the PilO/TfpO protein from *Pseudomonas aeruginosa* (47), while the second gene encoded a general *O*-OTase that was responsible for the glycosylation of multiple proteins, similar to PglL from *Neisseria* (48, 49). Although the functional significance of having two glycosylation systems remains to be determined, this represents the first known case of multiple *O*-OTases present in a single bacterium.

Capsule. Like many other pathogens, *Acinetobacter* spp. produce an extracellular capsule that provides a layer of protection from external threats such as complement-mediated killing (50). Capsule production and protein glycosylation are exquisitely linked in *Acinetobacter*, as the carbohydrate repeat unit found in the capsule is the same as the single repeat unit attached to proteins (46). The sugar subunits for capsule and protein glycosylation are derived from the same pathway, in which an initiating glycosyltransferase, PglC/ItrA, attaches the first carbohydrate to Und-P, followed by the addition of other sugar monomers by glycosyltransferase enzymes to complete the repeat unit (46). This repeat unit is then flipped to the periplasm, and the capsule and protein glycosylation pathways diverge at this point. In the case of protein glycosylation, this single repeat unit is attached to the target protein by the *O*-OTase (29). For capsule production, individual sugar repeat units are instead polymerized and exported to the cell surface. This bifurcated pathway represents a novel mechanism that illustrates the evolutionary connections between capsule and protein glycosylation, which may allow *Acinetobacter* to adapt rapidly to changing environments. How *A. baumannii* partitions a given carbohydrate repeat unit to the protein glycosylation pathway or capsule production remains to be determined. Although the carbohydrate structures produced by different strains are highly variable, functional studies have shown that capsule production is essential for *Acinetobacter* survival during infection and growth in serum (32, 46, 50, 51). It was recently reported that capsule production could be increased by the presence of subinhibitory levels of antibiotics, which increased resistance to complement-mediated killing and led to a hypervirulent phenotype in a mouse model of systemic infection (52). This capsule hyperproduction phenotype was shown to be controlled by the two-component BfmRS system, which regulates several other important virulence factors in *Acinetobacter* (53). *Acinetobacter* spp. also produce a surface-associated poly-β-1-6-*N*-acetylglucosamine (PNAG) polysaccharide, which is important for virulence and biofilm formation (54).

Pili. Filamentous bacterial surface appendages, termed pili, mediate interactions between the producing organism and their environment. *Acinetobacter* pili have been studied since 1975, when Henrichsen and Blom observed that *Acinetobacter calcoaceticus* strains displaying surface fimbrial structures exhibited twitching motility (55, 56), a form of bacterial locomotion now known to be dependent on functioning type IV pili (57). Furthermore, it has been shown that the nonpathogenic model organism *Acinetobacter baylyi* ADP1 produces both thin and thick pili (58); however, the roles pili play in the biology and pathobiology of pathogenic *Acinetobacter* spp. have been only partially elucidated.

A system of chaperone/usher pili, designated Csu pili, has been identified in all sequenced pathogenic *Acinetobacter* spp.; however, the Csu pili have been primarily studied in *A. baumannii* (59). The *Acinetobacter* Csu pili are required for biofilm formation and maintenance in *A. baumannii* ATCC 19606 but were found not to play a role in adherence to human epithelial cells (60). Another study found that the CsuA/B pilin subunit was the most abundant protein identified within the pellicle matrix of multiple *A. baumannii* strains (61), further strengthening the role of Csu pili in biofilm formation and maintenance. Previous reports also identified a single nucleotide insertion in the *csuB* gene of *A. baumannii* ATCC 17978, suggesting that this system may be nonfunctional in this strain (62, 63); however, recent resequencing of the *A. baumannii* ATCC 17978 genome (GenBank accession no. CP012004) did not find the same insertion event (64). Lastly, other chaperone/usher pilus-like systems have been bioinformatically identified in many *A. baumannii* strains, but none of those systems has been functionally characterized (61, 62). Mass spectrometric characterization of pellicle-associated proteins did find that non-Csu-pilin subunits were present, indicating that these systems may be functional.

Medically relevant *Acinetobacter* spp. have also been shown to produce type IV pili (Tfp), which are dynamic bacterial surface appendages known to mediate twitching motility, horizontal gene transfer, and biofilm formation (65). Although bioinformatic studies have identified genes predicted to encode proteins required for the biogenesis of Tfp in *A. baumannii*, only *Acinetobacter nosocomialis* strain M2 has been shown conclusively to produce functioning Tfp (66, 67), which is glycosylated by a TfpO-like oligosaccharyltransferase (30). Many *A. baumannii* isolates have been found to be naturally transformable and to exhibit twitching motility (63, 68–70), two classic Tfp-associated phenotypes, which strongly indicates their presence. Tfp-like structures were also identified on *A. baumannii* ATCC 17978 (71); furthermore, mutants in predicted Tfp biogenesis components of *A. baumannii* ATCC 17978 exhibited impaired biofilm formation (71) but the major pilin subunit, PilA, has not been shown to be surface exposed and/or associated with the pilin structures observed. Although Tfp, with roles in *Acinetobacter* motility and natural transformation, has emerged as a possible virulence factor, no studies have conclusively linked Tfp to the pathobiology of *Acinetobacter*, as is the case for *Pseudomonas* and *Neisseria*.

PROTEIN SECRETION

Extracellular export of proteins is a fundamental process of all forms of life. Protein secretion systems of Gram-negative bacteria are extremely diverse in function and composition and often are important mediators of virulence. Recent research has elucidated several of the mechanisms *Acinetobacter* uses to secrete proteins and the role they play in the biology of *Acinetobacter*. Here we describe the recent insights into macromolecular protein secretion systems present in *Acinetobacter*, with a focus on the systems that export proteins out of the cell and that have been characterized experimentally.

Type II secretion. The most recently described secretion system is a functional type II secretion system (T2SS) identified in both *A. nosocomialis* strain M2 (119) and *A. baumannii* ATCC 17978 (72); moreover, it was shown that clinical isolates of *Acinetobacter pittii*, *A. baumannii*, *A. calcoaceticus*, and *Acinetobacter junii* all were able to secrete type II substrates, indicating that functioning type II secretion systems seem to be the rule and not the exception. With regard to the T2SS of *A. nosocomialis* strain M2, a two-dimensional differential gel electrophoresis approach identified multiple putative type II substrates; the LipA and LipH lipases and the CpaA metallopeptidase were vali-

dated as *bona fide* type II secretion substrates. Interestingly, both LipA and CpaA required specific membrane-associated chaperones for secretion, which indicates that T2SS chaperones are more widespread than previously recognized. Importantly, it was shown that an *A. nosocomialis* strain M2 *gspD* mutant lacking the outer membrane secretin of the T2SS was severely attenuated in both the *Galleria mellonella* and murine pulmonary infection models. Specifically, mice intranasally infected with the *gspD* mutant strain had ~2-log lower bacterial burdens in both the lungs and the spleen after 36 h, compared to both the parental strain and the complemented mutant. In *A. baumannii* ATCC 17978, Johnson et al. identified a lipase, LipA, secreted in a T2SS-dependent manner that was required for growth on medium containing lipids as a sole carbon source (72). Mutants with mutations in *lipA* or the T2SS structural gene *gspD* were less competitive than the wild-type strain in a mixed-infection murine model of bacteremia. Collectively, these findings indicate that the *Acinetobacter* T2SS is a previously unrecognized virulence factor mediating pathogenesis in a relevant mammalian model. Interestingly, a recent study by Wang et al. utilized an *A. baumannii* ATCC 17978 *gspN* mutant for validation of their insertion sequencing murine pulmonary infection studies, and they subsequently found that the *gspN* mutant did not display any virulence defect in survival or competition models, compared to the parent strain (51). Although these data are in contrast to the newly defined role of type II secretion in *Acinetobacter*, it was demonstrated previously that *gspN* homologs were not required for a functioning T2SS in *Klebsiella oxytoca* (73); furthermore, *gspN* homologs are absent from numerous known T2SSs in other Gram-negative bacteria (74), indicating the dispensable nature of GspN in functioning T2SSs.

Autotransporters. A type V autotransporter has been characterized in *A. baumannii*. The *Acinetobacter* trimeric autotransporter (Ata) was found to be crucial for the ability of certain *A. baumannii* strains to adhere to extracellular matrix components, including collagen I, III, IV, and V (75). Ata is also an important mediator of *A. baumannii* biofilm formation and maintenance, as an *A. baumannii* ATCC 17978 *ata* mutant had significantly diminished biofilm production and was less virulent in a murine intraperitoneal infection model, compared to the parental and complemented strains (75).

Type VI secretion. Bacteria interact with each other in a multitude of ways; these interactions are often competitive in nature and play important roles in niche establishment (76). The bacterial type VI secretion system was first formally described for *Vibrio cholerae* and *P. aeruginosa* and was suggested to play a role against eukaryotic hosts (77, 78). While several T6SSs have been determined to secrete antieukaryotic toxins, it has recently been appreciated that many bacteria use their T6SSs to secrete antibacterial toxins to kill competing bacteria (79, 80). The T6SS is composed of approximately 15 conserved structural proteins and a variable number of accessory factors, which work in concert to secrete proteins extracellularly (81). Important components include Hcp, which forms a polymerized tubular structure that is secreted out of the cell and is essential for protein secretion, and VgrGs, which are present at the tip of this structure and can have effector activity or facilitate effector secretion (82). The T6SS bears striking similarity to bacteriophage, both structurally and functionally (83).

The presence of a T6SS was initially predicted bioinformatically for *A. baylyi*, and Hcp was subsequently detected in supernatants of *A. baumannii* ATCC 19606 (84, 85). The genetic organization and sequences of T6SS genes are remarkably well conserved across *Acinetobacter* spp. Based on homology with T6SS genes in other bacteria, the single *Acinetobacter* T6SS locus includes most of the genes required for apparatus assembly and function (86, 87). Notably, the main T6SS cluster does not contain *vgrG* genes, which are instead scattered throughout the genome. The VgrG proteins of *Acinetobacter*, which differ in number from strain to strain, do not seem to include effector domains (86, 88). Instead, bioinformatic analyses suggest that the proteins are most likely to mediate the secretion of adjacently encoded toxic effectors, with cognate immunity proteins being encoded nearby (88). However, no *bona fide* T6SS-dependent effectors have been experimentally characterized in *Acinetobacter*. In *A. baylyi*, mutation of three PAAR proteins, which interact with VgrG proteins, results in loss of Hcp secretion, and one of those PAAR proteins has been experimentally determined to be secreted, although it is not clear whether the proteins have any effector functions themselves (89). The primary function of the T6SS in *Acinetobacter* seems to be to kill competing bacteria, and *Acinetobacter* spp. with active T6SSs are able to kill a wide variety of other bacteria, including other strains of the same species (64, 87–89).

T6SS expression often is tightly controlled and is activated only under certain conditions; the molecular mechanisms used to achieve this regulation are extremely diverse and complex and differ from organism to organism and even between strains of a given species (82). Although little is known about T6SS regulation in *Acinetobacter*, recent studies have provided insight into some of the regulatory mechanisms used by these organisms. T6SS activities vary widely in different strains and species of *Acinetobacter*, with some strains showing robust T6SSs and bacterial killing and others seeming to have inactive systems under laboratory conditions (64, 86–88). However, the available data suggest that strains with T6SSs invariably express the main protein Hcp to at least some level, with variations in whether the protein is secreted (thus determining whether the system is active) (64, 86, 88). In *A. baumannii* ATCC 19606, Hcp is constitutively secreted in wild-type cells but is lost in mutants lacking lipid A, potentially due to membrane disruptions (85). In *A. baumannii* ATCC 17978, T6SS activity is controlled by a plasmid (see below), and a chromosomally encoded histone-like nucleoid-structuring (H-NS) protein may also regulate the T6SS (90). Several *A. baumannii* isolates harbor a resistance plasmid that encodes repressors of the T6SS (64). Upon spontaneous loss of this plasmid and subsequent loss of the repressors, the T6SS is activated and the resistance genes are lost. The functional significance of this remains to be elucidated but, considering the tremendous amount of energy required for T6SS activity (91) and the fitness defects often caused by harboring multiple antibiotic resistance genes (92), this may represent a mechanism to maintain both systems while avoiding potentially deleterious effects of having them be active at the same time. In the absence of antibiotic pressure, *Acinetobacter* strains do not require resistance genes but are more likely to encounter competitors, thus losing the resistance plasmid and activating the T6SS, which could provide a competitive advantage. Because the cells that lose the plasmid will lose resistance to antibiotics, this strategy could constitute an altruistic mechanism to ensure *Acinetobacter* population survival. It should be noted that several recent multidrug-resistant (MDR) *A. baumannii* strains seem to have permanently inactivated their T6SSs through chromosomal gene loss; it has

been suggested that this may be a result of the antibiotic pressure being great enough to make it evolutionarily advantageous to completely lose the T6SS, rather than maintaining it in an inactive state (93). Although there are limited data, there appears to be a link between antibiotic resistance and T6SS status in *Acinetobacter*; strains that are multidrug resistant express but do not secrete Hcp, while those that are not multidrug resistant are more likely to have an active T6SS (64, 86, 88).

Outer membrane vesicles. A special case of protein secretion is the production of outer membrane vesicles (OMVs), which are blebs of outer membrane (OM) released from the bacterial cell surface (94). There is significant debate regarding whether OMVs are produced by a directed process or simply represent cellular debris. Proteomic comparisons between the OM and OMVs of some bacteria have shown that the protein profiles differ between these two fractions, indicating that some OM proteins are excluded from OMV recruitment and suggesting that OMV formation is a directed process (94). However, many studies also detected cytoplasmic proteins in OMV preparations, indicating that cell lysis could also be a major contributor to OMV formation (94). OMVs have been implicated in numerous biological functions, with particular attention being devoted to their role in virulence (95). Several studies on OMVs in *Acinetobacter* have suggested that they have many functions, including roles in horizontal gene transfer, antibiotic resistance, and virulence. A wide variety of cargo types have been identified in OMVs from different *Acinetobacter* strains, including virulence proteins, antibiotic resistance determinants, and DNA (96–102). An important virulence factor of *A. baumannii*, OmpA, has also been found to be associated with OMVs, and OMVs have been suggested to act as a mechanism for delivery for this protein to host cells (103). Furthermore, OmpA has been suggested to be involved in the biogenesis of OMVs (104). OMVs from *Acinetobacter* may have an important role in the development of novel therapeutics, as they can stimulate a strong immune response and are protective when administered as a vaccine (105–107).

FUTURE DIRECTIONS

The surge of infections caused by *Acinetobacter* spp. has led to increased interest from the scientific and medical communities and attempts to understand the disease-causing mechanisms of these organisms. Many recent studies have greatly increased our understanding of *Acinetobacter* infection mechanisms but have also illuminated what a formidable pathogen the health care community is facing. There is intense interest in discovering novel strategies to fight this pathogen, which is quickly becoming untreatable with our current antibiotic repertoire. Although the vast majority of patients who become infected with *Acinetobacter* spp. are immunocompromised, which complicates intervention strategies, vaccines have been proposed as an alternative method to fight MDR *Acinetobacter* (108, 109), and several promising candidates have been described (107, 110–114). Individuals for whom immunocompromise could potentially be predicted *a priori*, such as patients undergoing cancer treatments or surgery and military personnel entering conflicts, may benefit from a prophylactic vaccination strategy (115). Carbohydrate structures present in *Acinetobacter*, such as the capsule and glycoproteins, represent attractive antigenic targets for vaccine development; since the carbohydrate moieties are the same in both, targeting these structures may provide broad protection (46). Indeed, capsule-based vaccines have shown efficacy in soft tissue, pneumonia, and bacteremia rodent models (116, 117). A drawback to this approach, however, is that the strain-to-strain variations in carbohydrate structures are so great that a multivalent vaccine to target all pathogenic *Acinetobacter* strains is unrealistic. Extensive epidemiological data on the capsular/glycoprotein serotypes most prevalent in a given health care institution may allow for a more directed approach to vaccine design. Indeed, PCR-based schemes have been proposed to accomplish this, and advances in the speed and cost of genome sequencing may make this a feasible approach (118). Because the production of capsular polysaccharides and glycoproteins is essential for virulence, targeting common steps in the biosynthetic pathway of these structures may be more feasible. Given the genomic plasticity of *Acinetobacter*, it is likely that a "one size fits all" solution to the problem is not possible, and multiple strategies should be investigated in order to determine the most beneficial approach for a given health care setting.

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