Acinetobacter baumannii: An Overview of Virulence Factors, Resistance Mechanisms and Treatment Options of the Emerging Pathogen

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ABSTRACT

In the current healthcare system, Acinetobacter baumannii is unquestionably one of the most aggressive bacteria causing nosocomial infections including pneumonia, bacteremia and wound infections. The frequent occurrence of Acinetobacter baumannii infections and outbreaks highlights the urgent requirement for using efficient antimicrobial agents to treat such cases due to the availability of few effective ones. This prompted the Centers for Disease Control and Prevention (CDC) to consider Acinetobacter baumannii as an urgent threat. Recent research on the virulence factors and resistance mechanisms of Acinetobacter baumannii is necessary to comprehend and combat this threat. Herein, we overviewed different virulence factors and resistance mechanisms involved in the pathogenesis of Acinetobacter baumannii. Virulence factors include outer membrane protein A (porins), biofilm formation, metal acquisition systems and lipopolysaccharide whereas antimicrobials resistance mechanisms involve β-lactamases, aminoglycosides-modifying enzymes, efflux systems, reduced membrane permeability and alteration of the target site of the antibiotics. Finally, a summary of potential and innovative therapies for infections caused by Acinetobacter baumannii was discussed.
**Keywords:** Acinetobacter baumannii, Infection, Virulence factors, Resistance mechanism

1. Introduction

High morbidity and mortality caused by *Acinetobacter baumannii* attracted the attention of the World Health Organization (WHO) to put this pathogen on the top of antimicrobial resistance research priority list (1).

The studies on *Acinetobacter* spp. started when it was first isolated from a soil sample and named *Micrococcus calcoaceticus* in 1911 (2). The genus *Acinetobacter* was officially documented only in 1971, by taxonomists based on common biochemical characters (2, 3). Although these bacteria show a twitching motility and coccobacillary morphology, they were named after the Greek word a-kinetos-bacter which means non-motile rod.

Molecular approaches have permitted identification of many available species within the *Acinetobacter* genus. This genus comprises strictly aerobic, non-fermentative, oxidase-negative, catalase-positive and non-pigmented Gram-negative bacteria (4-6). From the closely related species showing similar phenotypic and biochemical properties that are included in the *Acinetobacter calcoaceticus*–*Acinetobacter baumannii* complex (ACB complex), *A. baumannii* is considered as the commonest clinical species around the world (7). This opportunistic pathogen is one of the life-threatening nosocomial pathogens named ESKAPE, which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. (8). *A. baumannii* causes serious infections, mainly ventilator-associated pneumonia, urinary tract, bloodstream, skin and soft tissue infections, particularly among patients in Intensive Care Units (ICUs) (9).

Unfortunately, a significant increase in the number of multi-drug resistant (MDR) *A. baumannii* isolates has been reported (10). In addition to innate resistance to several antibiotics, *A. baumannii* is flexibly acquiring or upregulating resistance genes, therefore limiting the effective therapeutic choices and rising mortality rates (10). Furthermore, *A. baumannii* has numerous potential virulence factors that permit its persistence in the environment, adherence to surfaces, invasion to host cells and the escape from the host immune system (11-14). *A. baumannii* has been considered as one of the most persistent pathogens causing nosocomial infections (1). As most clinical isolates of this Gram-negative bacteria are found to be multi-drug resistant, suitable alternative therapies are needed to control this infection (15).
2. Clinical Significance

*A. baumannii* is one of the most significant nosocomial pathogens, with known ability to cause infections like urinary tract infections, pneumonia, bacteremia, and wound infections (16). The most common clinical cases with high mortality rates include pneumonia and bacteremia (17). It results in a 26% mortality rate in medical facilities, rising to 43% in ICUs (18). The risk factors for acquiring MDR isolates involve recent exposure to antimicrobials, the application of urinary or venous catheters, severity of disease, duration of hospital accommodation, and recent surgical procedure (19-21). The risk of mortality from invasive *A. baumannii* infection has been also reported to be high, especially for isolates that are resistant to carbapenems (22).

2.1. Hospital-Acquired Pneumonia

One of the most common clinical cases associated with *A. baumannii* is hospital-acquired pneumonia (HAP), especially for patients on mechanical ventilator assistance (23). HAP is an infection of the pulmonary parenchyma in patients at least 48 h after admission to the hospital, or in 14 days after leaving the hospital. The clinical case of HAP principally involves the presence of “new lung infiltrate of an infectious source, leukocytosis, new-onset fever, purulent sputum, as well as deteriorated oxygenation” (24). On the other hand, ventilator-associated pneumonia (VAP) is an infection of pulmonary parenchyma appearing at least 48 h after endotracheal intubation, including also the same clinical situation of HAP. Despite the notable improvements in the understanding of the influencing causes and prevention, HAP and VAP remain to be frequent complications of hospitalized patients (24). Zhang and co-workers also reported in meta-analysis performed in China that the incidence of HAP was 12.8–20.4%, and that of VAP was 31.4–36.1% (25). The mortality rate of HAP has been reported to be 21–37.4% according to Behnia and co-workers (26), while the occurrence of HAP has been reported to extend hospital accommodation by 18–30 days (25). In large surveillance studies from the United States, 5 to 10% of cases of ICU-acquired pneumonia were caused by *A. baumannii* (27). The proportion of MDR *A. baumannii* among causative agents of HAP and VAP has been reported to be approximately 30–34% (28). The mortality rate of *A. baumannii* pneumonia was 37.2–48.1% (29). Upon screening of *A. baumannii* isolates obtained from patients with late-onset VAP hospitalized in some Egyptian
University hospitals, MDR-carbapenem resistant *A. baumannii* were frequently found with about 84% (30).

### 2.2. Bloodstream Infection

Bacteremia is a principal and widespread cause of the total mortality in patients with *A. baumannii* infection that ranges broadly from 29% to 63% (31-33). Together with the increasing exposure to antibiotics, multidrug resistance and carbapenem resistance, rates of bacteremia have been obviously escalating over years. Many previous studies have explored predictors of mortality in patients with *A. baumannii* bacteremia. Risk factors independently linked with mortality include selecting the suitable antimicrobial therapy, drug resistance, acuteness of illness, malignancy, and other comorbidities such as immunosuppression (34). In a study on bloodstream infections in patients with febrile neutropenic cancer in Egypt, MDR *A. baumannii* was responsible for about 13% of MDR Gram-negative bacteria causing bloodstream infections (35).

### 2.3. Wound Infection

*A. baumannii* wound infections have become an issue of interest with statements of rising incidences of outbreaks among victims of battle injuries and natural disasters (6). *A. baumannii* is commonly isolated from wounds of combat casualties from Iraq or Afghanistan (36). Highly resistant strains of *A. baumannii* were reported to be among of the most common pathogens causing severe and sometimes fatal wound infections (37). Moreover, *A. baumannii* may occasionally cause skin infections outside of the military population. The pathogen caused 15% of hospital-acquired skin infections in a previous study (38). It is a well-recognized pathogen in burn units and may be difficult to eradicate from such patients (39). While wound infection is regarded polymicrobial, the contribution of *A. baumannii* is progressively being linked to bad outcomes and may require specific treatment (37).

### 3. Transmission of Infection

*A. baumannii* can be transmitted through the closeness of patients or colonizers such as hospital surfaces, and even medical tools. Contamination of respiratory support equipment, suction appliances, and that used for intravascular access are actually potential modes of transmission of infection (40).
4. Virulence Factors

Numerous virulence factors help in the interaction of *A. baumannii* infection with the host effectively (13). The virulence potential of *A. baumannii* and the host reactions to infection generated by this bacterium are poorly understood (41). Virulence factors of *A. baumannii* were reported to include outer membrane proteins (porins), cell envelope factors, enzymes, biofilm production, motility, micronutrient acquisition systems and protein secretion systems (13). The discussed virulence factors are summarized in Table 1.

**Table 1. Potential Virulence factors of *A. baumannii*.**

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Role in interaction with host</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Outer membrane protein A</td>
<td>Attachment process and host cell invasion</td>
<td>(42)</td>
</tr>
<tr>
<td>Biofilm formation</td>
<td>Attachment to epithelial cells and survival in hospitals</td>
<td>(43)</td>
</tr>
<tr>
<td>Metal acquisition systems</td>
<td>Acquiring patient nutrients leading to survival</td>
<td>(9, 44, 45)</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>Resistance to human serum, survival in vivo and adhesion to host cells</td>
<td>(46-48)</td>
</tr>
</tbody>
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According to reports, *A. baumannii* outer membrane protein A have a considerable impact on signal processing and pathogenesis (49). It is a significant surface-bound protein that aids in the attachment process, stimulating apoptosis at the beginning of infection, in addition to its role in epithelial cell invasion (42).

In order to survive in unfavourable environmental conditions and during host infection, bacteria often form complex structures known as biofilms that are attached to biotic or abiotic surfaces and implanted in extracellular polymeric materials in the form of matrix (50). Numerous host variables, including growth conditions, light, cell density and quorum sensing affect the formation of biofilms (51). The ability of *A. baumannii* to quickly attach to epithelial cells and a variety of medical devices, is essential for the pathogen's invasion of vulnerable hosts and survival in hospitals (43). Clinical strains of *A. baumannii* have the ability to create biofilm which is
especially prevalent on abiotic surfaces, in patients with sepsis, urinary tract catheters, or even shunt-associated meningitis (52).

The ability of *A. baumannii* to acquire patient nutrients, such as iron, manganese, and zinc, and adapt to the patient's metal-limited atmosphere is a key component in its endurance as a nosocomial pathogen (45). *A. baumannii* primarily uses five clusters of siderophores, which are iron-chelating molecules with high ability to capture iron. Additionally, the pathogen has direct iron receptors and transporters like FecA and FecI that enable the use of heme (53). Iron transporter alteration have been reported to lower virulence by reducing biofilm development and oxidative stress resistance (44).

Lipopolysaccharide (LPS), one of the virulence factors in Gram-negative pathogens, is involved in several stages of the illness process. The LPS from *A. baumannii* is crucial for resistance to typical human serum and provides a survival advantage in vivo. As an initial step in colonization, the antigenic O-polysaccharide of the LPS in conjunction with pili may encourage adhesion to host cells (46). *A. baumannii* pathogenicity is mostly determined by the existence of a capsule surrounding the bacterial surface, in addition to LPS. The capsule's repeating, tightly packed sugars operate as a barrier against a variety of environmental factors, including dryness, phagocytosis by host’s immune system, and certain antimicrobials (48). Despite variations in *A. baumannii* capsular sugars, the pathogen can always survive throughout infections and flourish in serum when enclosed in the capsule (54).

### 5. Resistance

Bacterial resistance is a normal outcome of how bacteria interact with their niche in the environment. Bacteria have accumulated a variety of defense mechanisms over time to secure their survival in a harsh environment. Consequently, it is thought that bacterial strains that are resistant to one or more antimicrobial substances have an intrinsic resistance, which is mediated by the resistance determinants (55). The development of the enzymes that can destroy the antimicrobial agent or stop its intracellular interaction to the target site is the general base of intrinsic resistance. This property of bacteria is demonstrated by their ability to continue exhibiting some level of antimicrobial compound resistance even in the absence of prior encounter (56). Bacteria have two genetic defense mechanisms against antibiotics: mutations, which typically alter the way the drug
works, and horizontal gene transfer acquisition of external genetic material (57). In terms of acquiring external material, mobile genetic elements allow bacteria to take up and transfer genes that are crucial to the spread of antibiotic resistance. Enzymatic (production of β-lactamases) and non-enzymatic processes including alteration of membrane permeability, activation of efflux pumps and alteration of the target site are the major mechanisms of the acquired antibiotic resistance. The wide cluster of antimicrobial resistance systems that have been depicted for A. baumannii is great and opponents those of other Gram-negative microbes (58, 59). The fast development of multi- and pandrug-resistant strains of Acinetobacter features its capacity to rapidly adapt to environmental pressing factors. The upregulation of innate resistance mechanisms combined with procurement of foreign determinants are special abilities that have brought A. baumannii incredible regard as a MDR pathogen (60). The discussed antimicrobial resistance mechanisms are presented in Figure 1.

![Figure 1. Acinetobacter baumannii antimicrobial resistance mechanisms](image)

### 5.1. Enzymatic Mechanisms

The existence of transferable plasmids expressing a wide variety of enzymes implicated in the propagation of β-lactam resistance has promoted the prevalence of β-lactamase production in Gram-negative bacteria (55). Based on molecular and functional structure analysis, β-lactamases
were categorized (61). Using the amino acid sequences, Ambler divided the β-lactamases into four classes (A, B, C, and D). Serine is present in the active enzyme site for classes A, C, and D, whereas Zn-dependent metalloenzymes are present in class B (62). *A. baumannii* strains include all of the β-lactamases classified under the Ambler system.

The most common cause of β-lactam resistance is class A lactamases. These enzymes can hydrolyze penicillins and cephalosporins more effectively than carbapenems and are blocked by clavulanate (63). Over 2000 class A β-lactamases have been identified by phenotypic and biochemical investigations, with the majority of them being found in Gram-negative bacilli (64). Different molecular variants of the functional kinds of class A β-lactamases exhibit their capacity to hydrolyze cephalosporins and carbapenems (65). Numerous class A β-lactamases, including TEM, GES, CTX-M, SHV, SCO, PER, CARB, VEB, or KPC, were discovered in *A. baumannii*. Most of these (SHV-5, PER-1, PER-2, PER-7, TEM-92, CTX-M-15, VEB-1, GES-14, CARB -10, CTX-M-2) are Extended-spectrum β-lactamases (ESBL), while some (TEM-1, SCO-1) are narrow-spectrum β-lactamases.

Class B β-lactamases, often known as Metallo-β-lactamases (MBLs), have Zn or another heavy metal in the catalytic site as compared to class A enzymes, which have serine in the enzyme active site (66). Chelating substances like ethylenediaminetetraacetic acid (EDTA) can reduce the enzymatic activity of these forms of β-lactamases due to the chelation of the metal from the active enzyme site. *A. baumannii* has a variety of MBLs that have been identified (67). The *bla-ampC* gene that belongs to Class C β-lactamases was found in 65 of the 105 MDR *A. baumannii* strains in China (68). All 23 Taiwanese strains of the bacteria *A. baumannii* tested positive for *ampC*-type β-lactamases, according to the investigation (69).

In healthcare settings, carbapenem-resistant *A. baumannii* (CRAB) has emerged as a significant issue due to high prevalence of drug resistance. The most common mechanisms of resistance in CRAB are carbapenem hydrolyzing class D β-lactamases (CHDL), followed by class B MBLs (70). CHDL, also known as oxacillinases (OXA) for their capacity to hydrolyze oxacillin, contain serine in the active catalytic site. More than 400 OXA enzymes have been identified, the majority of which can hydrolyze carbapenems (61). One of the key mechanisms of resistance in *A. baumannii* is the existence of OXA-type β-lactamases, which hydrolyze carbapenems (71, 72). In *A. baumannii* strains, OXA enzymes such OXA-23, OXA-24/40, OXA-58, OXA-143, and OXA-235 are among the most common. OXA-23 was discovered in Scotland, spread over the
world, and is now present in large numbers in A. baumannii isolates (73, 74). OXA-type β-lactamase genes have been found on chromosomes or plasmids in A. baumannii strains (75). New Delhi Metallo β-lactamase-1 (NDM-1), a B β-lactamase pattern with a high occurrence, is receiving attention on a global scale. NDM-1 were classified as superbugs that were reportedly known to be impossible to treat. Using one of the detection techniques in a previous study performed in Egypt, the percentage of presence of NDM-1 gene was 44.44% of the MBLs producers (76).

One of the most crucial antibiotic groups for treating nosocomial infections brought on by A. baumannii strains is the aminoglycoside family (77). The primary mode of resistance in A. baumannii is the enzymatic alteration of aminoglycosides through the development of aminoglycoside-modifying enzymes that are divided into acetyltransferases, phosphotransferases, and nucleotidyl transferases depending on how they function (78). The primary aminoglycoside-modifying genes implicated in aminoglycoside resistance in A. baumannii are aac (3′)-I, aph (3′)-I, aac (6′)-Ib, ant (2″)-Ia, ant (3′)-I, aac(3)-Ia [aacC1], aac(3)-IIa [aacC2], aac(6′)-Ib [aacA4] (79).

5.2. Non-Enzymatic Mechanisms

When overexpressed, chromosomal genes encoding A. baumannii efflux systems can confer resistance to a number of antibiotics (80). Chromosome-encoded MDR efflux systems often contribute to intrinsic resistance by constitutive expression or acquired resistance through expression following mutation (81). Resistance Nodulation Division superfamily (RND), Multidrug and Toxic compound Extrusion family (MATE), Major Facilitator Superfamily (MFS), and Small Multidrug Resistance transporters (SMR) are four kinds of efflux pumps found in A. baumannii (71). The RND system, which includes the AdeABC pump and is the most prevalent one among the four efflux systems in A. baumannii, plays a crucial part in the organism's ability to withstand antibiotics, particularly aminoglycosides. The fusion protein AdeB, the outer membrane factor (AdeC), and the inner membrane of the pump (AdeA) make up the AdeABC pump (82). The adeRS operon, which encodes AdeABC, is expressed when the efflux pump is exposed to an excessive amount of hazardous substances or antibiotics, resulting in an MDR phenotype. This operon also contributes to the development of biofilm in A. baumannii (83). MATE is a different class of efflux pumps found in A. baumannii. AbeM, that belongs to MATE family was reported with other efflux pumps to play a role in the resistance to imipenem,
gentamicin, doxorubicin, norfloxacin, and ciprofloxacin (84). The MFS superfamily is crucial in the resistance of A. baumannii to many antibiotics including doxycycline, minocycline, chloramphenicol, tetracycline and fosfomycin (85-87). SMR, another efflux pump class reported in A. baumannii, which involves AbeS, with indicated participation in resistance to various antibiotics (88).

The Antimicrobial resistance might rise if the membrane permeability is reduced. By facilitating the transfer of molecules, the pores of the outer membrane play a crucial role in the virulence and resistance of different strains of A. baumannii (89). Reduced membrane porin density in A. baumannii is linked to increased carbapenem resistance (90).

One key mechanism of bacterial resistance is alteration of the target site of the antibiotics. This mechanism typically relies on point mutations that barely affect the homeostasis of bacterial cells. Mutation-mediated resistance mechanisms of A. baumannii to common antibiotics were reported. Spontaneous mutations in the gyrase and topoisomerase IV-encoding genes parC, gyrA, and gyrB occurred as resistance mechanisms to fluoroquinolones (91, 92). There are two known pathways for colistin resistance: First, mutations in the PmrAB two-component system modify the lipid A from LPS, and second, mutations in the lpxA, lpxC, and lpxD genes reduce the ability to produce LPS (93).

6. Treatment Options

Sensitive isolates of A. baumannii can be treated with conventional antibacterial agents, including the third and fourth generations of cephalosporin, carbapenems or fluoroquinolones (94). It was known that carbapenems were particularly effective at treating A. baumannii infections in vitro. Thus, carbapenems are one of the most important lines of defense for treating infections brought on by MDR A. baumannii. However, clinical strains of A. baumannii have become more resistant to carbapenems, especially in Europe, Latin America, Asia, and Australia (95).

Combined therapy is indicated once all conventional antibacterial agents become unsuccessful against A. baumannii (96). Combined therapy has shown exceptionally bactericidal activity against the MDR clinical isolates of A. baumannii (97, 98). Such synergic combinations include blends of two or three classes of the following antibacterials: sulbactam, tigecycline, aminoglycosides, polymyxins or β-lactams, such as broad-spectrum cephalosporins or carbapenems (99, 100).
Sulbactam, a common β-lactamase inhibitor, possesses an innate mechanism that makes it effective against *A. baumannii* isolates (101). Promising results of sulbactam against *A. baumannii* infections have been monitored both in vivo and in vitro (98). However, resistance to sulbactam has recently increased significantly so it is not recommended to use it empirically (102, 103).

Tigecycline, a synthetic derivative of minocycline, that acquires a particular mechanism of action with reported bacteriostatic activity against CRAB isolates (104). The treatment of *A. baumannii* infections, such as VAP, bacteremia, and skin infections using combination therapies including tigecycline, was previously reported (105, 106).

Clinicians have reconsidered the use of polymyxins, colistin in particular, for MDR *A. baumannii* infections as a result of the lack of adequate therapeutic choices (107). The effect of the bactericidal agent, colistin, depends mainly on the used concentration against *A. baumannii* (108). Colistin has demonstrated intravenous efficacy in the therapeutic outcomes of patients with meningitis or VAP, either in mono- or combination therapy (109, 110). However, the frequent use of colistin for the treatment of *A. baumannii* infections in patients with critical illness may result in rising resistance (111).

To develop better curative options for topical application, many institutions are working on small-molecule antibiotics that have a more narrow-spectrum with direct activity on *Acinetobacter* species (6).

### 7. Innovative Approaches for Treatment of *A. baumannii* Infections

Knowing that different *A. baumannii* strains has become resistant to many of the available antibiotics, the research has started to focus on the "post-antibiotic era," with special emphasis on the creation of innovative methods to stop the spread of MDR *A. baumannii*. The latest prevention approaches that include phage therapy, antimicrobial peptides, and the CRISPR Cas system will then be discussed.

Bacteriophages are viral parasites, that when genetically modified, can be used to make resistant strains more susceptible to antibiotics. Phage therapy has been revived as a result of the worrying rise in resistance rates to boost bacterial susceptibility by eradicating resistance and virulence markers (112). Additionally, in vitro and in vivo studies have demonstrated that phage
therapy has a promising likelihood of serving as an efficient and secure treatment for MDR A. baumannii strains (113-115).

Antimicrobial peptides (AMPs) might serve as a better option than antibiotics for preventing the spread of MDR A. baumannii strains. AMPs can be regarded as principal defense against infectious organisms. The capacity of AMPs to destroy cell membranes and cell walls, the inhibition of protein synthesis, nucleic acids, and the promotion of apoptosis and necrosis are the basic mechanisms of their antimicrobial effect. Due to these characteristics, AMPs have been proposed for preventing the spread of nosocomial infections (116). Several recent studies have reported the effect of AMPs on A. baumannii (117-120). Currently, the main goal of scientific research is to create technologies that will increase the effectiveness and specificity of AMPs in vivo, along with improving their safety profile and production costs (118, 121, 122).

Prokaryotes use the CRISPR-Cas system as an immune mechanism to battle off the invasion of foreign genetic material. A CRISPR array, a leader sequence, and Cas-related proteins are the typical components (123). Understanding how to disarm pathogens, develop effective therapeutics, and stop the horizontal gene transfer of antimicrobial resistance genes requires fundamental research into pathogen defense mechanisms and immunity, such as CRISPR-Cas systems. Recent studies involved understanding the CRISPR-Cas systems in A. baumannii (124, 125).

8. Conclusion

Because of its growing clinical value, there are plenty of studies about A. baumannii. It has a well-known capacity to endure in various healthcare settings and to develop antimicrobial resistance. The most problematic aspect of A. baumannii is its multidrug resistance, which renders new therapeutics useless against it. Antibiotic resistance mechanisms of A. baumannii include β-lactamases, enzymatic modification of aminoglycosides, overexpression of efflux pumps, reduced membrane porins, and alterations of target sites. To tackle the A. baumannii infection widespread in the meantime, we must understand how to maximize the efficacy of our present antimicrobial agents, possibly with combinational regimens concurrently with studying the different weapons of the threatening pathogen.

- **Conflict of Interest**

The authors declare no conflict of interest.
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