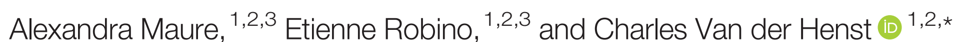
CellPress
OPEN ACCESS

Trends in
Microbiology

30th anniversary Special issue: The emerging pathogen defense arsenal

Review

The intracellular life of *Acinetobacter baumannii*



Alexandra Maure,[1,2,3] Etienne Robino,[1,2,3] and Charles Van der Henst[1,2,*]

Acinetobacter baumannii **is a Gram-negative opportunistic bacterium responsible for nosocomial and community-acquired infections. This pathogen is globally disseminated and associated with high levels of antibiotic resistance, which makes it an important threat to human health. Recently, new evidence showed that several *A. baumannii* isolates can survive and proliferate within eukaryotic professional and/or nonprofessional phagocytic cells, with *in vivo* consequences. This review provides updated information and describes the tools that *A. baumannii* possesses to adhere, colonize, and replicate in host cells. Additionally, we emphasize the high genetic and phenotypic heterogeneity detected amongst *A. baumannii* isolates and its impact on the bacterial intracellular features. We also discuss the need for standardized methods to characterize this pathogen robustly and consequently consider some strains as facultative intracellular bacteria.**

Highlights

Acinetobacter baumannii is a problematic pathogen resistant to (i) antibiotics, (ii) environmental stresses such as desiccation, (iii) hospital disinfectants, and (iv) first-line immune-system components such as human serum and phagocytic cells.

Several *A. baumannii* isolates can invade, persist, and even use different eukaryotic (professional and nonprofessional phagocytic) host cells as proliferation niches, pointing towards facultative intracellular abilities.

Critical steps of intracellular trafficking and several virulence factors are identified, but more efforts to better characterize this host–pathogen interaction are still required.

The intracellular behavior of *A. baumannii* isolates can impact environmental persistence, antibiotic treatments, and/or human infections.

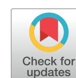
A. baumannii

A. baumannii is a nonflagellated, coccobacillus-shaped, and aerobic Gram-negative bacterium with extensive genomic and phenotypic heterogeneity amongst isolates [1–3]. It belongs to the **ESKAPE** group (see Glossary), which includes the six most problematic nosocomial multidrug-resistant (MDR) pathogens [4]. The World Health Organization (WHO) ranks *A. baumannii* on the priority pathogens list for new antibiotics (www.who.int/). At the same time, the Centers for Disease Control and Prevention classified it as an urgent threat to public health (www.cdc.gov/). *A. baumannii* is associated with life-threatening hospital-acquired infections [5], taking advantage of wounds and invasive treatments (such as catheters and ventilation systems) to cause bacteremia, pneumonia, and urinary-tract or skin and soft-tissue infections [6]. As a nosocomial pathogen, the risk factors associated with *A. baumannii* infections are prolonged hospital stay, heavy trauma, open wounds or burns, and impaired immune system [2]. Finally, comorbidities, including diabetes, pulmonary or renal diseases, high consumption of alcohol, and smoking favor infections, leading to significant morbidity and mortality rates [7,8]. *A. baumannii* exhibits multi-, extensively- and pan-drug resistances towards antibiotics [9–11]. In addition, this bacterium resists desiccation, disinfectants, oxidative stress, human serum, and phagocytosis, allowing its environmental persistence and transmission within hospital settings [12–15]. *A. baumannii* displays extensive genomic heterogeneity with a core genome identity of 14.5 to 16.5% and an open-pan genome with continuously acquired new genes [3,16]. This heterogeneity explains the genetic and phenotypic divergence between the new isolates and the historical ATCC17978 and ATCC19606 non-MDR strains. Recently, *A. baumannii*'s interaction with eukaryotic host cells highlighted the intracellular abilities of this pathogen. *A. baumannii* can survive inside eukaryotic cells *in vitro*, such as within endothelial and epithelial cells, macrophages, neutrophils, and amoebae [17–20], with some bacterial strains able to replicate intracellularly [21–23]. *In vivo*, intracellular *A. baumannii* can persist in bladder epithelial cells after infection resolution, leading to resurgent infection [24]. These recent insights highlight the importance of identifying the mechanisms or virulence factors involved in *A. baumannii* intracellular trafficking to enable the discovery of new therapeutic targets development of new diagnostics against this pathogen.

[1]Microbial Resistance and Drug Discovery, VIB-VUB Center for Structural Biology, VIB, Flanders Institute for Biotechnology, Brussels, Belgium
[2]Structural Biology Brussels, Vrije Universiteit Brussel (VUB), Brussels, Belgium
[3]These authors contributed equally to the work.

*Correspondence:
charles.van.der.henst@vub.be
(C. Van der Henst).

© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).



Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

CellPress
OPEN ACCESS

This review examines the processes enabling *A. baumannii* to establish an intracellular niche in eukaryotic cells, including host cell adhesion, internalization, survival, replication, and host cell death. Moreover, we discuss the heterogeneity amongst strains regarding intracellular features and the need for a methodological consensus to characterize them. Finally, based on the information provided by this review, we suggest considering some specific *A. baumannii* strains as facultative intracellular pathogens.

Adhesion

Adhesion to host cells is the first critical step for a successful infection, and **pili** are key factors for bacterial attachment to abiotic and biotic surfaces [25]. ATCC17978 strain lacking the Hfq protein expresses fewer fimbriae than the wild type (WT) and therefore adheres less to A549 and H_{292} lung epithelial cells and HEK 293 kidney epithelial cells [26,27]. As Hfq is a transcriptional regulator interacting with small noncoding RNA (sRNAs) in other bacteria, key findings could be obtained by searching for sRNAs regulating fimbriae expression and eukaryotic cell adhesion [28]. The clinical *A. baumannii* UPAB1 strain isolated from a urinary-tract infection (UTI) binds fibrinogen of host cells via the Abp1D and Abp2D tip-adhesive proteins of its Abp1 and Abp2 **chaperone-usher pathway pili (CUP pili)** [29,30]. Accordingly, a Δ*abp1*Δ*abp2* UPAB1 strain exhibits an attenuated catheter-associated UTI (CAUTI). Although the data suggest that CUP pili are essential for *A. baumannii* UPAB1 to colonize catheters, their role in adherence to urothelial cells remains to be demonstrated [29,30]. Additionally, *A. baumannii* has a chaperone-usher pilus system, commonly named Csu pili, which may mediate the adherence of *A. baumannii* to human lung epithelial cells, depending on the studies [31–33]. The different results could be due to technical variations in the adherence assays and the strains used in the studies. In the latest study, Ahmad *et al.* suggested an interplay between Csu and type IV pili of *A. baumannii* AB5075 [31]. This type IV pilus and its PilA pilin subunit have been studied for adherence to epithelial cells, motility, and biofilm formation in *Acinetobacter nosocomialis* M2 [34,35]. *A. nosocomialis* M2 Δ*pilA* exhibits reduced adhesion to A549 and nasopharyngeal Detroit 562 epithelial cells compared to the WT strain [34]. Complementation of *A. nosocomialis* M2 Δ*pilA* with PilA of *A. baumannii* AB5075, ACICU, or BIDMC57 restores the mutant adhesion [35]. However, *A. baumannii* AB5075-Δ*pilA::Tn* mutant shows the same adherence ability on A549 cells as the WT strain [31]. Given the diversity of the results regarding the pili, further studies are still required to determine their precise role in the adhesion of different *A. baumannii* strains to host cells.

A. baumannii possesses adhesins recognizing different components of the host-cell surface (Figure 1). The biofilm-associated protein (Bap) of *A. baumannii* is known to be involved in developing mature biofilm on abiotic surfaces [12,36,37]. It can also act as an adhesin for the adhesion of *A. baumannii* strain 307-0294 to normal human bronchial epithelial (NHBE) and human neonatal keratinocyte (NHNK) cells *in vitro* [38]. Additionally, *A. baumannii* strain AYE has two Bap-like proteins (Blps), which display a role in adherence to A549 cells, with AYE-Δ*blp1* and AYE-Δ*blp2* mutant strains showing 2.9- and 1.7-fold reduction in adherence compared to the WT, respectively [39]. Accordingly, *blp1* deletion in *A. baumannii* strains belonging to clonal lineage 1 (IC1) reduced adhesion by threefold to LL/2 lung carcinoma cells [40]. However, *blp1* is not required for the IC2 strain's adhesion to LL/2 cells, suggesting functional redundancy of adhesive components in these strains [40]. The invasin-like adhesin InvL, secreted by the type II **secretion system** (T2SS), also acts as an adhesin, which binds α5β1 integrin, collagen V, and fibrinogen of host cells. A Δ*invL* mutant exhibits a significantly decreased adhesion to kidney (MDCK) and bladder (5637) epithelial cells, causing attenuated CAUTI pathogenesis [41]. In this study, the *invL* gene is conserved in 74% of 2728 *A. baumannii* isolates [41]. Thus, the remaining isolates may encode other unknown invasins or adhesins. *A. baumannii* also harbors the adhesin *Acinetobacter* trimeric autotransporter (Ata), a type V autotransporter secretion system

Glossary

Apoptosis: a programmed and structured cell death initiated by the cell to maintain homeostasis.
Autophagy: a mechanism of intracellular degradation by which cytoplasmic materials or organelles are delivered to the lysosomes for degradation.
Chaperone-usher pathway pili (CUP pili): pili composed of an outer-membrane pore-forming usher protein, a periplasmic chaperone protein, pilus subunits, and in most cases, a tip adhesin protein.
ESKAPE: group composed of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp. which represent the most problematic nosocomial pathogens for human health.
Outer-membrane protein (OMP): protein located at the outer membrane of Gram-negative bacteria that has essential roles in cell survival and virulence.
Outer-membrane vesicles (OMVs): vesicles, derived from the outer membrane of Gram-negative bacteria, which can transport numerous cargoes, including virulence factors.
Pili: long, extracellular polymers that mediate diverse functions, such as bacterial adherence, mobility, and substrate transport.
Pyroptosis: cell death which limits the replication of intracellular pathogens by eliminating infected immune cells, exposing released bacteria to other phagocytic cells and releasing inflammatory content.
Secretion systems: systems used to transport small molecules, proteins, and DNA into the extracellular space or target cells; they are varied in size, composition, and architecture.
TIMER fluorescent protein: a fluorescent protein that shifts color over time, depending on the expression system.

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

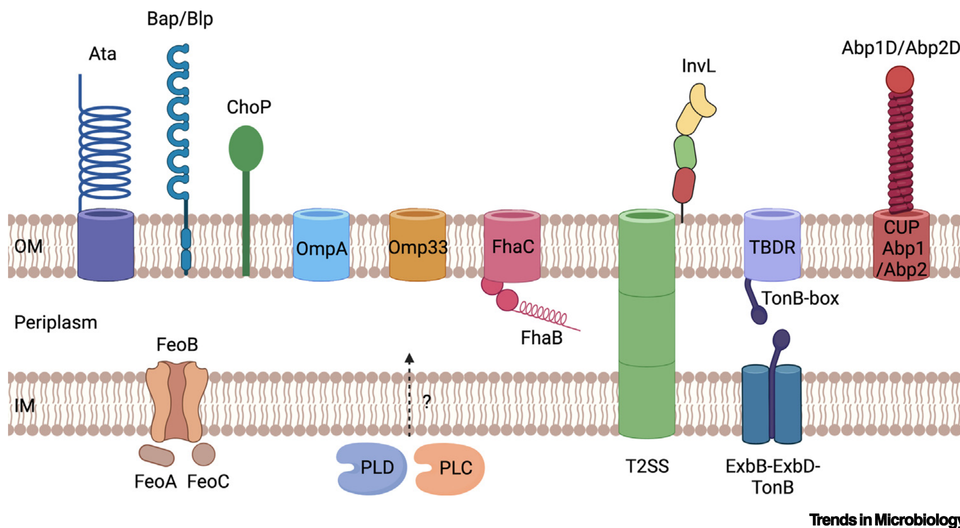


Figure 1. Virulence factors of *Acinetobacter baumannii* involved in host–pathogen interaction. *A. baumannii* uses different virulence factors to adhere to eukaryotic cells such as pili (CUP), type V autotransporter secretion systems (Ata and FhaB/C), outer-membrane proteins (OmpA), invasin-like adhesin (InvL) secreted by T2SS, adhesins (Bap/Blp), the TonB system (of ExbB, ExbD, TonB and TBDR) and the ferrous iron transport system (FeoABC). For uptake into host cells, *A. baumannii* uses PLD, and ChoP. *A. baumannii* induces host cell toxicity via Omp33 and PLC. Abbreviations: Ata, *acinetobacter* trimeric autotransporter; Bap, biofilm-associated protein; Blp, Bap-like protein; ChoP, phosphorylcholine; CUP, chaperone-usher pathway; Feo, ferrous iron transporter; Fha, filamentous hemagglutinin; IM, inner membrane; InvL, invasin-like protein; OM, outer membrane; Omp, outer membrane protein; PLC, phospholipase C; PLD, phospholipase D; T2SS, type II secretion system; TBDR, TonB-dependent receptor. Figure created with BioRender.com.

composed of a membrane-associated transporter domain and a large repetitive passenger domain [12]. Ata is located at the bacterial surface and acts as a lectin that binds multiple extracellular matrix components of the host, including type IV collagen and fibronectin, via glycan recognition [42,43]. *A. baumannii* ATCC19606 adheres to primary human umbilical cord vein (HUVEC) cells, HMEC-1 endothelial cells, and epithelial cells in an Ata-dependent manner [44,45]. *A. baumannii* ATCC19606 Δ*ata* mutant loses its ability to bind A549, HeLa, and HepG2 epithelial cells [44,45]. This mutant also shows a sixfold reduction in HUVECs binding, while overexpression of *ata* in a complemented strain increases *A. baumannii's* adhesion rate on HUVECs by more than 20-fold, compared to WT [45]. Genomic screening discovered another type V secretion system, the two-partner secretion (TPS) system, in *A. baumannii* AbH12O-A2 [46]. Deletion of the filamentous hemagglutinin C (FhaC) from this TPS system (AbFhaB/FhaC) reduces the adhesion of *A. baumannii* AbH12O-A2 to fibronectin by 2.37-fold compared to the WT strain. Subsequently, it decreases fibronectin-mediated adherence to A549 cells [46]. Accordingly, it would be interesting to know if *A. baumannii* type V autotransporter **secretion systems**, like T2SS, secrete other virulence factors that could act as an adhesin. So far, several adhesins involved in eukaryotic cell binding have been identified in *A. baumannii* (Figure 1). Considering the heterogeneity amongst the bacterial isolates, other adhesins can be discovered depending on the origin of the strain (respiratory, urinary tract, or wound infections) and the host cell type used as the infection model.

Among the virulence factors used by *A. baumannii* to adhere to host cells, **outer-membrane proteins (OMPs)**, especially OmpA, are extensively studied. OmpA mediates the adherence of *A. baumannii* ATCC19606 and ATCC17978 to epithelial cells, as inactivation of OmpA (either by gene mutation or compound inhibition with cyclic hexapeptide AOA-2) prevents adhesion to host cells [18,47,48]. OmpA binds fibronectin [49] and host integrins, cytoskeleton, and junctional adherence proteins of host cells based on chemical cross-linking coupled to mass spectrometry

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

CellPress
OPEN ACCESS

experiments [50]. Specifically, OmpA from the AB5075 strain crosslinks desmosomes and hemidesmosomes that mediate cell–cell contact in host epithelia, destabilizing cell–cell interactions and allowing *A. baumannii* intrusion through the epithelium [50]. In the same study, the TonB system, composed of ExbB, ExbD, TonB, and a TonB-dependent outer membrane receptor (TBDR), such as BauA, interacts with host cells [50] and, more specifically, with fibronectin [49]. Playing a role in biofilm formation, TonB is also involved in the adherence of *A. baumannii* ATCC19606 and ATCC17978 to host cells as a TonB-dependent receptor-deficient mutant shows a decreased adherence to A549 cell monolayers [51,52]. Sato *et al.* suggested that the number of *A. baumannii* clinical isolates adhering to A549 cells could depend on the OmpA mRNA level [53]. Hence, overexpression of OMPs could be a virulence mechanism used by *A. baumannii* to adhere to eukaryotic cells. A global transcriptomic analysis performed on *A. baumannii* ATCC17978 during murine pneumonia infection revealed that FeoA is a valuable virulence factor for *A. baumannii* infection [52]. FeoA is a part of the ferrous iron transport cluster (*feoABC*) used for iron uptake and is upregulated during infection *in vivo* in strains ATCC17978 and AbH12O-A2 [52]. Accordingly, the ATCC17978 *feoA* mutant has limited fitness and virulence, with a significantly reduced ability to adhere to A549 cells *in vitro* [52,54]. Further investigations are needed to decipher the virulence factors involved in host–*A. baumannii* interaction, such as type IV pili, adhesins, or secretion systems [55]. Deciphering the mechanisms of *A. baumannii* adhesion is also important to understand the next stage of the infection process: internalization.

Internalization

Following adhesion, pathogen uptake into host cells occurs by different processes, such as (i) active invasion by the bacterium itself, resulting in internalization by nonphagocytic epithelial cells, and (ii) phagocytosis by professional immune cells (Figure 2).

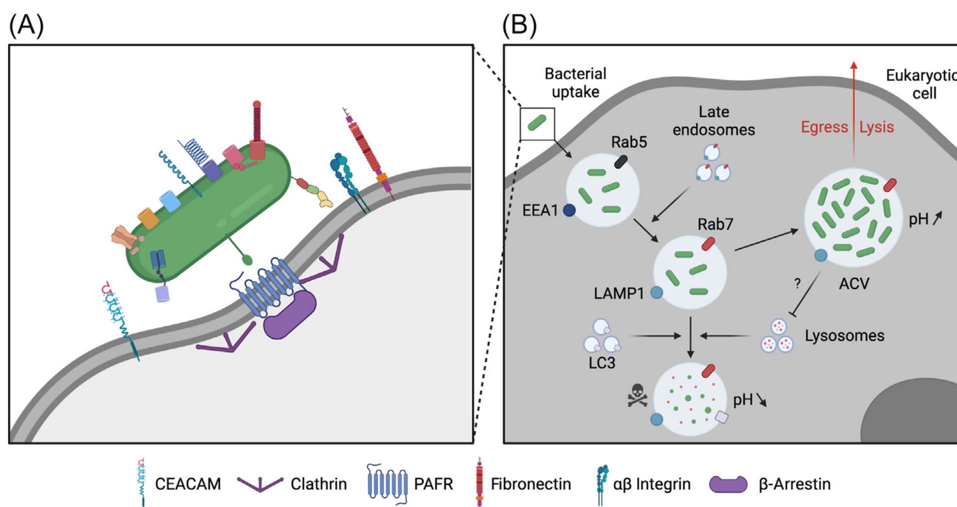


Figure 2. Current model summarizing the interactions described between *Acinetobacter baumannii* and the eukaryotic host cell. (A) *A. baumannii* interacts with the eukaryotic cell via its various virulence factors before being taken up by the host cell. (B) Upon internalization, *A. baumannii* resides in a vacuole decorated with early endosomal markers: EEA1 and Rab5. After maturation, the vacuole acquires the late endosomal markers: LAMP1 and Rab7. Vacuole containing nonreplicative *A. baumannii* follows the phagosomal pathway, is decorated with LC3 markers, and then fused with lysosomes, resulting in the killing of the bacterium. ACVs containing replicative *A. baumannii* do not interact with lysosomes. The ACV pH increases due to ammonia production, allowing bacterial replication and egress from the eukaryotic cell. Abbreviations: ACV, *Acinetobacter*-containing vacuole; CEACAM, carcinoembryonic antigen-related cell adhesion; EEA1, early endosomal antigen 1; LAMP1, lysosomal associated membrane protein 1; LC3, protein light chain 3; PAFR, platelet-activating factor receptor. Figure created with BioRender.com

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

The phospholipase D (PLD) from *A. baumannii* strain 98-37-09 is essential for serum resistance and epithelial cell invasion [56]. Indeed, a Δ*pld* mutant presents a decreased capacity to invade HeLa and BEAS-2B epithelial cells [56]. This agrees with a study showing that the strain ATCC19606 has three PLDs and that double or triple *pld* knock-out mutants present impaired A549 cell invasion [57]. However, how *A. baumannii* PLD modulates host cell signaling to trigger internalization into nonphagocytic cells successfully is unknown. *A. baumannii* AB5075 interacts with carcinoembryonic antigen-related cell adhesion (CEACAM) -1, -5, and -6 on the surface of A549 cells, triggering bacterial internalization in a membrane-bound vacuole [58]. This recent article corroborates one of the first studies on *A. baumannii* internalization, which shows that the pathogen is internalized by a zipper-like mechanism in a membrane-bound vacuole [21]. Furthermore, the phosphorylcholine (ChoP)-containing OMP binds platelet-activating factor receptor (PAFR) located on A549 cells, which then activates pathways composed of G protein-coupled phospholipase C, clathrin, and β-arrestin, and mediates the internalization of *A. baumannii* clinical isolate 77 into host cells [59]. It is important to note that the invading potential of *A. baumannii* is dependent on the method, the cells, and the strain used. For example, *A. baumannii* strain NCTC 7844 can colonize only 0.3% of HeLa cells after 2 h of infection [60].

Like nonprofessional phagocytic cells, increasing evidence suggests that neutrophils and macrophages can phagocytose some *A. baumannii* strains (Box 1), as shown for the 0057 and ATCC19606 strains by human peripheral blood neutrophils [61,62]. Moreover, the antimicrobial peptide CRAMP from murine macrophages seems to promote phagocytosis by neutrophils [63], whereas interleukin-10 deficiency leads to impairment of phagocytosis against *A. baumannii* ATCC15150 [64]. *A. baumannii's* phagocytosis requires functional microfilament and microtubulin systems, as shown for the ATCC19606 strain and J774A.1 macrophages [65]. This agrees with another study in which cytochalasin D pretreatment of *Drosophila* S2 macrophage-like cells limits the internalization and replication of the pathogen [20]. Interestingly, inhibition of the phagosome and autophagosome biogenesis by wortmannin (a class III phosphatidylinositol 3-kinase inhibitor), SP600125 (mitogen-activated protein kinase (MAPK)/c-Jun NH2-terminal kinase (JNK) inhibitor), or Rab5 and Rab7 by interferent RNA, reduces *A. baumannii* ATCC17978 internalization (Boxes 1 and 2) [20].

The *A. baumannii* capsule could also play a role in promoting phagocytosis. Indeed, *A. baumannii* HUMC1, which naturally presents a disruption of the *gtr6* gene in the capsular locus, is intrinsically resistant to phagocytosis by neutrophils and macrophages [66]. The authors suggest that *gtr6*-disrupted strains produce a capsule structure unrecognizable by phagocytic receptors [66]. *A. baumannii* capsule structure and production varies between strains, subsequently inducing

Box 1. Overview of the phagocytosis process

Pathogen recognition by macrophage triggers several signaling pathways that result in the uptake of the pathogen. The phagocytosis forms an intracellular vacuole, named the phagosome, delimited by a membrane [102]. This latter undergoes a maturation process mostly mediated by the Rab family of GTPases. The new phagosome merges directly with early endosomes mainly via the small GTPase Rab5. Rab5 recruits the early endosome antigen 1 (EEA1) and class III phosphoinositide 3-kinase (PI3K) human vacuolar protein-sorting 34 (VPS34). VPS34 produces phosphatidylinositol 3-phosphate (PI3P) which retains EEA1 at the cytosolic leaflet of the phagosome and promotes the recruitment of proteins involved in phagosomal maturation [102]. As the phagosome matures, Rab5 is replaced by Rab7 [102]. This Rab GTPase regulates the fusion of the early phagosome with late endocytic compartments. Fusion with late endosomes and elements of the Golgi complex allows the incorporation of lysosomal-associated membrane proteins and luminal proteases (cathepsins, proteases, and hydrolases) within the phagosome [103]. Also, the lumen of the phagosome becomes more acidic (pH 5.5–6.0) via a gradual accumulation of V-ATPases on the membrane and the translocation of protons (H^+) [103]. The last stage of the phagosome maturation involves fusion with lysosomes to become the phagolysosome [103]. The phagolysosome has a very acidic lumen (pH around 5.0–5.5). It contains hydrolytic enzymes including cathepsins, proteases, lysozymes, and lipases which have their optimal efficacy at this pH, allowing bacterial clearance [103].

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

CellPress
OPEN ACCESS

Box 2. Overview of the autophagy process

Autophagy is a mechanism of intracellular degradation by which cytoplasmic materials are delivered to the lysosome for degradation [104]. Autophagy is essential for immunity by triggering intracellular pathogen degradation, which is a type of autophagy called xenophagy. Autophagy is a process involving the formation of a double-membrane structure named the autophagosome [104]. This formation is a four-step process: (i) autophagosome initiation, (ii) autophagosome elongation, (iii) autophagosome closure, and (iv) autophagosome fusion with lysosomes where autophagosomal contents are degraded [104]. Initiation of the autophagosome formation is highly regulated by signaling pathways involving autophagy-related proteins (ATGs) [104]. The first step occurs mostly via activation of the mammalian target of rapamycin (mTOR) that binds to the serine/threonine kinase ULK1 complex. This complex then activates the PI3K complex (composed notably of VPS34, Beclin-1 and ATG14L) that generates PI3P, thus marking the autophagosome formation [105]. Autophagy may also be activated by the mitogen-activated protein kinase (MAPK)/c-Jun NH2-terminal kinase (JNK). JNK activation results *in fine* in the liberation of Beclin-1 which stimulates autophagy [106]. Elongation of the autophagosome is directed by covalent linkage between ATG12 with ATG5/ATG16L1 and microtubule-associated light chain 3 (LC3), also known as ATG8. LC3-I, generated by the proteolytic cleavage of pro-LC3 by ATG4, is conjugated to phosphatidylethanolamine, becoming LC3-II, by a series of reactions that involve the ATG5/ATG12/ATG16 complex, ATG7 and ATG3 [104]. After closure, the mature autophagosome fuses with lysosomes to form an autolysosome where content degradation occurs. This step is mediated by a fusion machinery including the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family like syntaxin, such as STX17 [105].

various phenotypes and virulence levels *in vitro* and *in vivo*. Complete *in vivo* virulence requires the presence of a capsule, and a noncapsulated and avirulent AB5075 strain can revert to a capsulated and virulent strain upon scarless excision of an IS*Aba13* insertion sequence [3,67]. Due to the high degree of heterogeneity in capsule production and type amongst clinical isolates [3], *A. baumannii* may be able to regulate its capsule production, thickness, or density depending on the stage of the infection process (adhesion, internalization, replication, or host cell death) and the level of virulence required. Therefore, studies should further investigate the consequences of the presence or absence of bacterial capsules, the specific role(s) of the capsule types, and their regulation in *A. baumannii*'s virulence.

Intracellular survival and proliferation

It is well established that different *A. baumannii* strains adhere to, and invade, (non-) phagocytic cells, and more and more findings highlight the bacterial fate once inside cells. Several studies point out the location of some *A. baumannii* strains within vacuoles, related or not to **autophagy** and the autophagosomal pathway (Box 2 and Figure 2).

ATCC17978 activates transcription factor EB (TFEB) once inside A549 cells, inducing upregulation of the genes related to the autophagic pathway, including the gene coding for the protein light chain 3 (LC3) [68]. Following *A. baumannii* 98-37-09 internalization, the beclin-1-dependent autophagy pathway is induced in HeLa, MH-S, and THP-1 cells via the AMPK/ERK/mammalian target of rapamycin [69]. Inside RAW 264.7, ATCC17978 resides within a double-membrane structure, with bacterial Omp33-36 promoting the accumulation of p62 and LC3b autophagy proteins [70]. In TC-1 cells, the ATCC17978-containing autophagic vacuoles are decorated with the early endosomal antigen 1 (EEA1) at an earlier time of infection and with the late endosomal marker lysosomal associated membrane protein 1 (LAMP1) at later time points [20]. After cell invasion, *A. baumannii* AB5075-UW is located inside membrane-bound vacuoles decorated with Rab5 (on early endocytic compartments), Rab7 (on late endocytic compartments), and LC3 [58]. Following bacterial internalization, strain ATCC19606 is rapidly killed by J774A.1 macrophages and human neutrophils [23,62,65]. *A. baumannii* AB5075-UW transiently survives intracellularly but is eventually killed after vacuole acidification [58]. Activation of autophagy following *A. baumannii* 98-37-09 uptake induces bacterial clearance in MH-S and THP-1 cells [69]. *In vitro,* experiments reveal that IL-10 enhances phagocytosis and bacterial killing of *A. baumannii* ATCC15150 by bone marrow-derived macrophages by upregulating the macrophage receptor with collagenous structure (MARCO) [64]. However, discordant results obtained

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

by An *et al.* show that purified OmpA from ATCC19606 strain triggers incomplete autophagy in HeLa and RAW264.7 cells through the MAPK/JNK signaling pathway when coincubated for 24 h [71]. Moreover, a 24 h coculture with strain ATCC19606 and HeLa cells may lead to the inhibition of synthaxin 17 (STX17) in a growth arrest-specific 5 (GAS5)-dependent manner, resulting in an impairment of the autophagosomes and lysosomes fusion [72]. The fate of intracellular *A. baumannii* bacteria differs depending on the studies and the strains used. Technical variations such as incubation times post contact and multiplicity of infection highlight the need for a methodological consensus to conclude about intracellular *A. baumannii*-related pathways (see section 'Heterogeneity of the strains and methods').

Recent studies demonstrate that isolated *A. baumannii* multidrug-resistant clinical strains survive and replicate within spacious vacuoles named *A. baumannii*-containing vacuoles (ACVs) inside a wide range of host cells such as macrophages, A549 epithelial cells, EA.hy 926 endothelial cells, and primary NHNK cells (Figure 2) [21–23]. Depending on the cell type and the bacterial strain, the vacuoles have either a single [22] or a double membrane [21]. Although ACV biogenesis following bacterial infection remains unclear, ACV formation in J774A.1 mouse macrophage with *A. baumannii* UPAB1 appears to depend on the type I secretion system and the pAB5 plasmid [21]. ACVs of recent multidrug-resistant clinical isolates UPAB1, 398, C4, and ABC141 strains are LC3-negative; ACVs from C4, ABC141, and 398 strains are decorated with LAMP1 [21–23]. *A. baumannii*'s survival is associated with lower acidification of these vacuoles. Before bacterial death, strain AB5075 causes a delay of the detrimental vacuolar acidification at an early stage of infection [58]. Only a small percentage of ACVs were lysotracker-positive at the late stages of infection with *A. baumannii*. Distel *et al*. discovered that, compared to ATCC19606, *A. baumannii* 398 increases the ACV's luminal pH via ammonia production [23]. As pointed out by Rubio *et al.,* the ability of recent *A. baumannii* clinical isolates to survive without multiplication inside A549 cells, despite their clonal lineage, is frequent, showing a major evolution on *A. baumannii* intracellular features [22]. Of note, on 46 isolates tested, only five *A. baumannii* isolates (C4, ABC141, BMBF_193, ABC020, and R10) can replicate intracellularly in LAMP1-positive vacuoles [22]. Interestingly, a subpopulation of *A. baumannii* ATCC19606 can establish a replicative niche in vacuoles decorated with the endoplasmic reticulum marker calreticulin in *Drosophila* S2 cells, thereby avoiding interactions with degradative lysosomal compartments [20]. These various intracellular behaviors result from the high heterogeneity of *A. baumannii* strains. High-throughput techniques, as developed by Rubio *et al.,* could enable a large classification of clinical isolates regarding their intracellular abilities [22]. It could also help to determine if different bacterial growth phases and/or coculture conditions with host eukaryotic cells can influence the intracellular features of *A. baumannii*. Comparing these newly isolated clinical *A. baumannii* genomes with selected reference genomes could help to identify key genes or new virulence factors involved in *A. baumannii* intracellular trafficking. In this context, using Acinetobase, a genetic and phenotypic database on *A. baumannii,* appears helpful in selecting appropriate strains related to a specific biological question [73]. Dual-RNA sequencing could also be an alternative approach to characterize the interplay between the host and different replicative strains during infection, possibly highlighting genes or virulence factors essential for replication [74].

ACVs are not restricted to mammalian cells. Indeed, *A. baumannii* Ab1 and Ab2 environmental strains are also located in vacuoles in the cytoplasm of the amoeba *Acanthamoeba castellanii,* in trophozoïte form after 1 and 3 days, but also between the double walls of cysts after several weeks [17]. This survival in *Acanthamoeba* sp. makes amoebae a potential major environmental reservoir that can facilitate the growth and dissemination of resistant strains, even in hospital settings.

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

During infection, *A. baumannii* faces stresses imposed by the host immune system, including essential nutrient metal sequestration, such as iron (Fe) and zinc (Zn), and the production of reactive oxygen species (ROS) by phagocytic cells. To overcome the lack of metals, *A. baumannii* has different metal homeostasis systems, such as siderophores, that bind and scavenge Fe and Zn from host cells [75,76]. For example, *A. baumannii* ATCC19606 produces an acinetobactin transport system in response to Fe-limiting conditions imposed by the A549 cell's intracellular environment, which is needed to persist within these cells [77]. Intracellular *A. baumannii* also faces ROS production by neutrophils and macrophages, such as superoxide, H_2O_2, hypochlorous acid, and peroxynitrate [78–80]. In response to these ROS, *A. baumannii* induces catalase, peroxidase, and superoxide dismutase to detoxify and prevent oxidative damage. MDR *A. baumannii* clinical isolates exhibit upregulated catalase activity, which primarily depends on the expression level of the *katE/G* genes [80]. These systems are regulated by OxyR, which binds and represses the *kat* promoter [81]. In addition, an excess of Fe increases the level of ROS via the Fenton reaction [82]. Therefore, Fur tightly regulates iron acquisition, and its deletion reduces the ability of *A. baumannii* to detoxify ROS [82]. A study of proteomic changes in THP-1 macrophages infected by *A. baumannii* AB5075 shows the upregulation of several cold-shock proteins, namely Csp2, Csp1, and the oxidative stress resistance-associated thioredoxin TrxA, glutaredoxins GrxC, and GrxD, peptide-methionine (S)-S-oxide reductase MsrA [83]. Authors suggest that *A. baumannii* may utilize cold-shock proteins and proteins of the thioredoxin superfamily to facilitate bacterial resistance against redox stress and H_2O_2 encountered in macrophages and to promote its survival [83,84]. Further similar proteomic analyses using intracellular replicative *A. baumannii* strains at different critical steps of the infection could enable the discovery of other stress tolerance strategies.

A. baumannii has numerous systems which confer the ability to survive under acidic and limiting nutrient conditions and oxidative stress within eukaryotic host cells. Nevertheless, further studies are needed to comprehensively overview other mechanisms that this pathogen uses to survive and replicate.

Host cell death

The completion of the infection cycle by intracellular *A. baumannii* can be harmful to the eukaryotic host cell. UPAB1 escapes from macrophages by a lytic process [21]. ATCC17978 and ATCC19606 strains induce alveolar MH-S macrophage death at 48 h post-infection but not the death of TC-1, MLE-12 epithelial cells [20]. *A. baumannii* harboring phospholipase C (PLC) has an enhanced ability to lyse A549 cells [85,86], and PLC inhibition by the phosphatidylcholine analog miltefosine significantly reduced this cytolytic effect [87]. *A. baumannii* OMPs also play a role in host cells cytotoxicity. *A. baumannii* ATCC17978 expressing Omp33-36 induces **apoptosis** through caspase activation in RAW264.7 [70]. AB5075 WT strain exhibits enhanced cytotoxicity on H_{292} epithelial cells compared to Δ*ompA* AB5075 [50]. Additionally, *A. baumannii* Ab_{169} with **outer-membrane vesicles (OMVs)** deficient in OmpA shows a reduced ability to induce J774A.1 macrophages and A549 cell death [88]. OmpA induces mitochondrial fragmentation and subsequently increases cellular ROS levels, consistent with another study showing that OmpA-containing OMV interacts with the protein G elongation factor mitochondrial 1 (GFM1), affecting ROS generation [50,89]. *A. baumannii* also activates the NLRP3 inflammasome via OmpA, Omp34, or an inflammatory bioactive factor which results in **pyroptotic** cell death [88,90–92] (Box 3). Time-resolved transcriptional profiling in HeLa cells after *A. baumannii* NCTC 7844 infection shows that host cells upregulate the TGFB1-induced antiapoptotic factor 1 (TIAF1), as well as the DNA damage-inducible transcript 4 protein (DDIT4), which induces apoptosis and inhibits cell growth, respectively [60]. Fourteen clinical isolates belonging to the clonal lineage IC2 with a carbapenemase resistance gene OXA-23 can induce apoptosis in infected

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

Box 3. Overview of the inflammasome process

Inside eukaryotic cells, recognition of pathogen-associated molecular patterns by membrane pattern recognition receptors (PRRs) – for example, Toll-like receptors (TLRs) – or cytosolic PRRs – for example, nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, or NLRs) – is the core of the immune responses. Among these responses, activation of NLRs triggers the assembly of protein complexes called inflammasomes [107]. NOD-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome activates caspase 1 in response to various stimuli, notably upon bacterial infection. NLRP3 inflammasome activation requires a priming signal with the upregulation of its expression (linked to TLR or NLR stimulation of the cell) followed by a stimulus like ionophores (K^+, Ca^{2+}, Na^+, and Cl^-), mitochondrial ROS, or lysosomal content to be active [107]. This binding leads to a conformational change, allowing the recruitment of the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) protein and pro-caspase 1 [107]. Indeed, inflammasomes mainly activate caspase 1, resulting in the production of proinflammatory IL-1β and IL-18 molecules. IL-1β and IL-18 are present in the cell as inactive precursors (pro-IL-1β, not constitutively expressed, and pro-IL-18, constitutively expressed) which are then cleaved into mature cytokines [108]. IL-1β binds to its receptor, IL-1R, which stimulates the release of proinflammatory cytokines [108]. Similarly, IL-18 binds to IL-18R. The presence of IL-1R or IL-18R results in the activation of a signaling cascade and activation of transcription factors, all regulating the inflammation process [108]. Inflammasomes also induce specific death of infected cells, named pyroptosis, via the mediator gasdermin D (GSDMD) [109]. Pyroptosis is a cell death which limits the replication of intracellular pathogens by eliminating infected immune cells, exposing released bacteria to other phagocytic cells, and releasing inflammatory content such as IL-1β and IL-18 [109].

HUVECs from 4.59% to 22.58% of the total cell population [93]. Through the complexome profiling of mitochondrial proteins, this apoptosis is linked to the perturbation of oxidative phosphorylation leading to the loss of mitochondrial membrane potential and interruption of ATP production, a drop in mitoribosome complexes, and interferences with mitochondrial metabolic pathways [93]. Surprisingly, *A. baumannii* ABC141 and C4 strains invade A549 cells and form intracellular bacterial clusters without causing cell lysis, suggesting unknown mechanisms used by *A. baumannii* to escape cells without causing cell death [22]. Identifying virulence factors that enable *A. baumannii* to kill host cells would be a significant breakthrough at a clinical level. Targeting these factors could lead to discovery of new therapeutics, thereby changing the outcome of potentially lethal *A. baumannii* infections. Identification of strains that can kill eukaryotic cells would also be helpful for the prediction of the infection outcome.

A. baumannii isolates also show different killing abilities against amoebae. A recent paper highlights the internalization and intracellular degradation of the AB5075 strain by environmental and nondomesticated amoebae belonging to the genus *Tetramitus* [94]. In addition, nondomesticated amoebae belonging to the genera *Acanthamoeba*, *Vahlkampfia*, *Stemonitis*, and *Tetramitus*, and the species *Vermamoeba vermiformis*, have bactericidal activity in cocultures with the isolate *A. baumannii* 40288, the strain AB5075, and a constitutively mucoid mutant of strain AB5075 [94]. On the contrary, the 1656-2 clinical isolate and strain ATCC19606 can kill *A. castellanii* after 24 h of coincubation [95]. Environmental isolates Ab1 and Ab2 show amoebicidal activity against *A. culbertsoni* [17]. Overall, the fact that some *A. baumannii* strains grow in the presence of amoebae, and even kill them, suggests that *A. baumannii* can be considered an amoebae-resistant bacterium. Studying these host–pathogen interactions further is required to determine if amoebae can act as an environmental and/or clinical cellular reservoir for *A. baumannii*.

Heterogeneity of the strains and methods

As stated previously, *A. baumannii* displays extensive genomic heterogeneity between strains. Related to this heterogeneity, it is essential to emphasize that the strategies used by *A. baumannii* to adhere, invade, survive, and replicate within eukaryotic host cells are not shared by all strains, with some having specific virulence factors. For instance, *A. baumannii* NFAb-1 and NFAb-2 share 846 genes absent in the ATCC19606 or ATCC17978 genomes and display a different capacity to adhere to A549 cells [96]. Ata is found in 78% (1899/2434) of *A. baumannii*

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

CellPress
OPEN ACCESS

analyzed genomes in one study and 58.6% (44/75) of *A. baumannii* in another [43,44]. Out of 541 strains of *A. baumannii*, 20% lack the *bap* gene in their genome [39]. In addition to this heterogeneity, spontaneous genetic mutations occur in established culture collections, as shown in a study indicating that the chromosome sequences of ATCC19606 differ between isolates from different laboratories [97]. Recently, Vesel *et al.* demonstrated the importance of epigenetic DNA modification in horizontal gene transfer among *A. baumannii* strains [98]. This newly described system could be one of the mechanisms explaining heterogeneity. *A. baumannii* isolates also describe phase variation, resulting in changes in phenotypes associated with different virulence levels and resistance to immune antimicrobials or antibiotics [15,99]. In this light, we advise carefully using historically established ATCC19606 and ATCC17978 strains, as they might not reflect the clinical relevance of recent intracellularly adapted isolates.

Standardizing the methods used to characterize intracellular *A. baumannii* represents a milestone for future research in this field. The invasive and replicative strains UPAB1, ABC141, LAC-4, C4, and 398 represent useful strains to characterize intracellular pathways within different eukaryotic cell types [21–23]. Researchers should also consider stationary or exponential bacterial growth phases. Rubio *et al*. showed that the growth stage is critical to confer a hyperinvasive and replicative phenotype to ABC141 [22]. At another level, organoids could represent an advanced model used to deepen our knowledge of host–*A. baumannii* interactions [100,101]. Using organoids allows us to go beyond monolayers containing a single cell type or immortalized cell lines, with different tissues studied, such as the lung, bladder, and brain tissues, all representing anatomical sites of infection for *A. baumannii* [100,101]. Although the gentamicin protection assay remains robust for studying intracellular bacteria such as *Mycobacterium tuberculosis*, *Brucella abortus,* or *Salmonella* spp., gentamicin-resistant *A. baumannii* isolates represent a challenge. In this context, other antibiotics, like colistin, which does not effectively cross the host cell membrane, or a combination, represent effective alternatives [21–23]. Any observation should be confirmed using complementary techniques such as microscopy, and cell viability assessment after infection should be done to strengthen any conclusions. In recent studies, successful application of the **TIMER fluorescent protein,** coupled with flow cytometry or microscopy, transmission electron microscopy, confocal microscopy, and immunolabeling methods, described intracellular *A. baumannii*, paving the way for well-designed methods describing viability, and the metabolic and replicative states of intracellular *A. baumannii* [21–23,60].

Concluding remarks and perspectives

A. baumannii is a top-priority pathogen with increased antibiotic resistance, including pan-resistant strains spreading worldwide. Resistance to desiccation, disinfectants, and the immune system also represents a significant part of this pathogen's protective arsenal. In this review, we highlighted the different molecular mechanisms used by *A. baumannii* to successfully invade and establish a replicative niche within different eukaryotic cells, thus expanding the environmental and mammalian reservoirs available for this pathogen. Consequently, asymptomatic carriage of intracellular *A. baumannii* may explain some hospital-acquired infections. It will be relevant in some cases, such as after the resolution of an *A. baumannii* UTI, to search for intracellular bacteria in patients as a new strategy to prevent a resurgent infection. Intracellular *A. baumannii* within eukaryotic cells may prevent antibiotics from reaching the pathogen, leading to treatment failure. Understanding the *A. baumannii* intracellular route and its associated virulence factors could enable the design of new therapeutics to treat emerging intracellular MDR clinical isolates. Overall, there are significant biological questions regarding the survival mechanisms and proliferation within host cells, and several virulence factors remain to be identified (see Outstanding questions). Due to high heterogeneity, we cannot define general intracellular features for all *A. baumannii* isolates. Nevertheless, it is now possible to consider some specific strains of *A. baumannii* as facultative intracellular pathogens.

Outstanding questions

What are the different steps characterizing the intracellular trafficking of *A. baumannii*?

What are the molecular mechanisms (virulence factors) involved in the intracellular resistance of *A. baumannii*?

As *A. baumannii* is a highly heterogeneous bacterium, what proportion of isolates falls in the facultative intracellular category?

Are there other eukaryotic cell types used as intracellular niches in addition to the ones described in this review?

Can we observe *A. baumannii* within eukaryotic cells during human infection?

What is the long-term fate of host cells with internalized *A. baumannii* during human infection?

What are the implications of the intracellular abilities of *A. baumannii* during human infection, animal infections, and environmental resistance?

What are the consequences of this intracellular behavior on antibiotic resistance and susceptibility?

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

Acknowledgments

The authors acknowledge Jesus S. Distel, Charline Debruyne, Suzana P. Salcedo, and Mario F. Feldman for discussion and helpful suggestions. We thank Juliette Van Buylaere for reading this manuscript and providing feedback.

Declaration of interests

No interests are declared.

References

1. Howard, A. *et al.* (2012) Acinetobacter baumannii. *Virulence* 3, 243–250
2. Whiteway, C. *et al.* (2022) Acinetobacter baumannii. *Trends Microbiol.* 30, 199–200
3. Valcek, A. *et al.* (2023) Phenotypic characterization and heterogeneity among modern clinical isolates of *Acinetobacter baumannii*. *Microbiol. Spectr.* 11, e0306122
4. Pendleton, J.N. *et al.* (2013) Clinical relevance of the ESKAPE pathogens. *Expert Rev. Anti-Infect. Ther.* 11, 297–308
5. Ayoub Moubareck, C. and Hammoudi Halat, D. (2020) Insights into *Acinetobacter baumannii*: a review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. *Antibiotics* 9, 119
6. Wong, D. *et al.* (2017) Clinical and Pathophysiological Overview of *Acinetobacter* infections: a century of challenges. *Clin. Microbiol. Rev.* 30, 409
7. Ballouz, T. *et al.* (2017) Risk factors, clinical presentation, and outcome of *Acinetobacter baumannii* bacteremia. *Front. Cell. Infect. Microbiol.* 7, 156
8. Dexter, C. *et al.* (2015) Community-acquired *Acinetobacter baumannii*: clinical characteristics, epidemiology and pathogenesis. *Expert Rev. Anti-Infect. Ther.* 13, 567–573
9. Shi, J. *et al.* (2020) Multidrug resistant and extensively drug resistant *Acinetobacter baumannii* hospital infection associated with high mortality: a retrospective study in the pediatric intensive care unit. *BMC Infect. Dis.* 20, 597
10. Gottesman, T. *et al.* (2021) An outbreak of carbapenem-resistant *Acinetobacter baumannii* in a COVID-19 dedicated hospital. *Infect. Prev. Pract.* 3, 100113
11. Karakonstantis, S. *et al.* (2020) Excess mortality due to pandrug-resistant *Acinetobacter baumannii* infections in hospitalized patients. *J. Hosp. Infect.* 106, 447–453
12. Harding, C.M. *et al.* (2018) Uncovering the mechanisms of *Acinetobacter baumannii* virulence. *Nat. Rev. Microbiol.* 16, 91–102
13. Tacconelli, E. *et al.* (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* 18, 318–327
14. Farrow, J.M. *et al.* (2018) Desiccation tolerance in *Acinetobacter baumannii* is mediated by the two-component response regulator BfmR. *PLoS One* 13, e0205638
15. Tipton, K.A. *et al.* (2015) Phase-variable control of multiple phenotypes in *Acinetobacter baumannii* strain AB5075. *J. Bacteriol.* 197, 2593
16. Imperi, F. *et al.* (2011) The genomics of *Acinetobacter baumannii*: Insights into genome plasticity, antimicrobial resistance and pathogenicity. *IUBMB Life* 63, 1068–1074
17. Cateau, E. *et al.* (2011) *Acanthamoeba* sp. promotes the survival and growth of *Acinetobacter baumannii*. *FEMS Microbiol. Lett.* 319, 19–25
18. Choi, C.H. *et al.* (2008) *Acinetobacter baumannii* invades epithelial cells and outer membrane protein A mediates interactions with epithelial cells. *BMC Microbiol.* 8, 216
19. Kamoshida, G. *et al.* (2016) A novel bacterial transport mechanism of *Acinetobacter baumannii* via activated human neutrophils through interleukin-8. *J. Leukoc. Biol.* 100, 1405–1412
20. Qin, Q.-M. *et al.* (2020) A tractable *Drosophila* cell system enables rapid identification of *Acinetobacter baumannii* host factors. *Front. Cell. Infect. Microbiol.* 10, 240
21. Sycz, G. *et al.* (2021) Modern *Acinetobacter baumannii* clinical isolates replicate inside spacious vacuoles and egress from macrophages. *PLoS Pathog.* 17, e1009802
22. Rubio, T. *et al.* (2022) Incidence of an intracellular multiplication niche among *Acinetobacter baumannii* clinical isolates. *mSystems* 7, e0048821
23. Distel, J.S. *et al.* (2023) Replicative *Acinetobacter baumannii* strains interfere with phagosomal maturation by modulating the vacuolar pH. *PLoS Pathog.* 19, e1011173
24. Hazen, J.E. *et al.* (2023) Catheterization of mice triggers resurgent urinary tract infection seeded by a bladder reservoir of *Acinetobacter baumannii*. *Sci. Transl. Med.* 15, eabn8134
25. Hospenthal, M.K. *et al.* (2017) A comprehensive guide to pilus biogenesis in Gram-negative bacteria. *Nat. Rev. Microbiol.* 15, 365–379
26. Kuo, H.-Y. *et al.* (2017) Functional characterization of *Acinetobacter baumannii* lacking the RNA chaperone Hfq. *Front. Microbiol.* 8, 2068
27. Sharma, A. *et al.* (2018) The unusual glycine-rich C terminus of the *Acinetobacter baumannii* RNA chaperone Hfq plays an important role in bacterial physiology. *J. Biol. Chem.* 293, 13377
28. Sarshar, M. *et al.* (2022) The *Acinetobacter baumannii* model can explain the role of small non-coding RNAs as potential mediators of host-pathogen interactions. *Front. Mol. Biosci.* 9, 1378
29. Tamadonfar, K.O. *et al.* (2023) Structure–function correlates of fibrinogen binding by *Acinetobacter* adhesins critical in catheter-associated urinary tract infections. *Proc. Natl. Acad. Sci.* 120, e2212694120
30. Di Venanzio, G. *et al.* (2019) Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic *Acinetobacter baumannii* chromosomal genes. *Nat. Commun.* 10, 2763
31. Ahmad, I. *et al.* (2023) Csu pili dependent biofilm formation and virulence of *Acinetobacter baumannii*. https://www.researchsquare.com/article/rs-2812735/v1 https://doi.org/10.21203/RS.3.RS-2812735/V1
32. de Breij, A. *et al.* (2009) CsuA/BABCDE-dependent pili are not involved in the adherence of *Acinetobacter baumannii* ATCC19606T to human airway epithelial cells and their inflammatory response. *Res. Microbiol.* 160, 213–218
33. Chen, C.-L. *et al.* (2022) d-mannose-sensitive pilus of *Acinetobacter baumannii* is linked to biofilm formation and adherence onto respiratory tract epithelial cells. *J. Microbiol. Immunol. Infect.* 55, 69–79
34. Ronish, L.A. *et al.* (2019) The structure of PilA from *Acinetobacter baumannii* AB5075 suggests a mechanism for functional specialization in *Acinetobacter* type IV pili. *J. Biol. Chem.* 294, 218–230
35. Piepenbrink, K.H. *et al.* (2016) Structural diversity in the type IV pili of multidrug-resistant *Acinetobacter*. *J. Biol. Chem.* 291, 22924–22935
36. Loehfelm, T.W. *et al.* (2008) Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. *J. Bacteriol.* 190, 1036–1044
37. Harding, C.M. *et al.* (2017) Pathogenic *Acinetobacter* species have a functional type I secretion system and contact-dependent inhibition systems. *J. Biol. Chem.* 292, 9075–9087
38. Brossard, K.A. and Campagnari, A.A. (2012) The *Acinetobacter baumannii* biofilm-associated protein plays a role in adherence to human epithelial cells. *Infect. Immun.* 80, 228–233
39. De Gregorio, E. *et al.* (2015) Biofilm-associated proteins: news from *Acinetobacter*. *BMC Genomics* 16, 1–14
40. Skerniškytė, J. *et al.* (2019) Blp1 protein shows virulence-associated features and elicits protective immunity to *Acinetobacter baumannii* infection. *BMC Microbiol.* 19, 259

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

CellPress
OPEN ACCESS

41. Jackson-Litteken, C.D. *et al.* (2022) InvL, an invasin-like adhesin, is a type II secretion system substrate required for *Acinetobacter baumannii* uropathogenesis. *mBio* 13, e0025822
42. Tram, G. *et al.* (2021) The *Acinetobacter baumannii* autotransporter adhesin Ata recognizes host glycans as high-affinity receptors. *ACS Infect. Dis.* 7, 2352–2361
43. Bentancor, L.V. *et al.* (2012) Identification of Ata, a multifunctional trimeric autotransporter of *Acinetobacter baumannii*. *J. Bacteriol.* 194, 3950–3960
44. Weidensdorfer, M. *et al.* (2015) Analysis of endothelial adherence of *Bartonella henselae* and *Acinetobacter baumannii* using a dynamic human *ex vivo* infection model. *Infect. Immun.* 84, 711–722
45. Weidensdorfer, M. *et al.* (2019) The *Acinetobacter* trimeric autotransporter adhesin Ata controls key virulence traits of *Acinetobacter baumannii*. *Virulence* 10, 68–81
46. Pérez, A. *et al.* (2017) The FhaB/FhaC two-partner secretion system is involved in adhesion of *Acinetobacter baumannii* AbH12O-A2 strain. *Virulence* 8, 959–974
47. Gaddy, J.A. *et al.* (2009) The *Acinetobacter baumannii* 19606 OmpA protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. *Infect. Immun.* 77, 3150–3160
48. Vila-Farrés, X. *et al.* (2017) Combating virulence of Gram-negative bacilli by OmpA inhibition. *Sci. Rep.* 7, 14683
49. Smani, Y. *et al.* (2012) Role of fibronectin in the adhesion of *Acinetobacter baumannii* to host cells. *PLoS One* 7, e33073
50. Schweppe, D.K. *et al.* (2015) Host–microbe protein interactions during bacterial infection. *Chem. Biol.* 22, 1521–1530
51. Abdollahi, S. *et al.* (2018) The role of TonB-dependent copper receptor in virulence of *Acinetobacter baumannii*. *Infect. Genet. Evol.* 60, 181–190
52. Martínez-Guitián, M. *et al.* (2021) Global transcriptomic analysis during murine pneumonia infection reveals new virulence factors in *Acinetobacter baumannii*. *J. Infect. Dis.* 223, 1356–1366
53. Sato, Y. *et al.* (2017) Virulence characteristics of *Acinetobacter baumannii* clinical isolates vary with the expression levels of omps. *J. Med. Microbiol.* 66, 203–212
54. Álvarez-Fraga, L. *et al.* (2018) Pneumonia infection in mice reveals the involvement of the feoA gene in the pathogenesis of *Acinetobacter baumannii*. *Virulence* 9, 496–509
55. Cecil, J.D. *et al.* (2019) Outer membrane vesicle–host cell interactions. *Microbiol. Spectr.* 7. https://doi.org/10.1128/microbiolspec.PSIB-0001-2018
56. Jacobs, A.C. *et al.* (2010) Inactivation of phospholipase D diminishes *Acinetobacter baumannii* pathogenesis. *Infect. Immun.* 78, 1952–1962
57. Stahl, J. *et al.* (2015) *Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases D. *PLoS One* 10, e0138360
58. Ambrosi, C. *et al.* (2020) *Acinetobacter baumannii* targets human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) for invasion of pneumocytes. *mSystems* 5, e00604-20
59. Smani, Y. *et al.* (2012) Platelet-activating factor receptor initiates contact of *Acinetobacter baumannii* expressing phosphorylcholine with host cells. *J. Biol. Chem.* 287, 26901–26910
60. Crua Asensio, N. *et al.* (2021) Time-resolved transcriptional profiling of epithelial cells infected by intracellular *Acinetobacter baumannii*. *Microorganisms* 9, 354
61. Gandhi, J.A. *et al.* (2014) Alcohol enhances *Acinetobacter baumannii*-associated pneumonia and systemic dissemination by impairing neutrophil antimicrobial activity in a murine model of infection. *PLoS One* 9, e95707
62. Lázaro-Díez, M. *et al.* (2017) Human neutrophils phagocytose and kill *Acinetobacter baumannii* and *A. pittii*. *Sci. Rep.* 7, 4571
63. Kang, M.-J. *et al.* (2020) Cathelicidin-related antimicrobial peptide contributes to host immune responses against pulmonary infection with *Acinetobacter baumannii* in mice. *Immune Netw.* 20, 1–13
64. Kang, M.-J. *et al.* (2020) IL-10 protects mice from the lung infection of *Acinetobacter baumannii* and contributes to bacterial clearance by regulating STAT3-mediated MARCO expression in macrophages. *Front. Immunol.* 11, 270
65. Qiu, H. *et al.* (2012) Role of macrophages in early host resistance to respiratory *Acinetobacter baumannii* infection. *PLoS One* 7, e40019
66. Talyansky, Y. *et al.* (2021) Capsule carbohydrate structure determines virulence in *Acinetobacter baumannii*. *PLoS Pathog.* 17, e1009291
67. Whiteway, C. *et al.* (2021) Scarless excision of an insertion sequence restores capsule production and virulence in *Acinetobacter baumannii*. *ISME J.* 16, 1473–1477
68. Parra-Millán, R. *et al.* (2018) Intracellular trafficking and persistence of *Acinetobacter baumannii* requires transcription factor EB. *mSphere* 3, e00106-18
69. Wang, Y. *et al.* (2016) Critical role of bacterial isochorismatase in the autophagic process induced by *Acinetobacter baumannii* in mammalian cells. *FASEB J.* 30, 3563–3577
70. Rumbo, C. *et al.* (2014) The *Acinetobacter baumannii* Omp33-36 porin is a virulence factor that induces apoptosis and modulates autophagy in human cells. *Infect. Immun.* 82, 4666–4680
71. An, Z. *et al.* (2019) *Acinetobacter baumannii* outer membrane protein A induces HeLa cell autophagy via MAPK/JNK signaling pathway. *Int. J. Med. Microbiol.* 309, 97–107
72. An, Z. and Ding, W. (2021) *Acinetobacter baumannii* upregulates LncRNA-GAS5 and promotes the degradation of STX17 by blocking the activation of YY1. *Virulence* 12, 1965–1979
73. Valcek, A. *et al.* (2022) Acinetobase: the comprehensive database and repository of *Acinetobacter* strains. *Database* 2022, baac099
74. Westermann, A.J. *et al.* (2017) Resolving host–pathogen interactions by dual RNA-seq. *PLoS Pathog.* 13, e1006033
75. Murdoch, C.C. and Skaar, E.P. (2022) Nutritional immunity: the battle for nutrient metals at the host–pathogen interface. *Nat. Rev. Microbiol.* 20, 657–670
76. Mortensen, B.L. and Skaar, E.P. (2013) The contribution of nutrient metal acquisition and metabolism to *Acinetobacter baumannii* survival within the host. *Front. Cell. Infect. Microbiol.* 3, 95
77. Gaddy, J.A. *et al.* (2012) Role of acinetobactin-mediated iron acquisition functions in the interaction of *Acinetobacter baumannii* strain ATCC 19606T with human lung epithelial cells, *Galleria mellonella* caterpillars, and mice. *Infect. Immun.* 80, 1015–1024
78. Liu, Z. and Xu, W. (2022) Neutrophil and macrophage response in *Acinetobacter baumannii* infection and their relationship to lung injury. *Front. Cell. Infect. Microbiol.* 12, 890511
79. Chen, W. (2020) Host innate immune responses to *Acinetobacter baumannii* infection. *Front. Cell. Infect. Microbiol.* 10, 486
80. Sato, Y. *et al.* (2019) Multidrug-resistant *Acinetobacter baumannii* resists reactive oxygen species and survives in macrophages. *Sci. Rep.* 9, 17462
81. Juttukonda, L.J. *et al.* (2019) *Acinetobacter baumannii* OxyR regulates the transcriptional response to hydrogen peroxide. *Infect. Immun.* 87, e00413-18
82. Ajiboye, T.O. *et al.* (2018) Contributions of ferric uptake regulator Fur to the sensitivity and oxidative response of *Acinetobacter baumannii* to antibiotics. *Microb. Pathog.* 119, 35–41
83. Kho, Z.Y. *et al.* (2022) Correlative proteomics identify the key roles of stress tolerance strategies in *Acinetobacter baumannii* in response to polymyxin and human macrophages. *PLoS Pathog.* 18, e1010308
84. May, H.C. *et al.* (2019) Thioredoxin-A is a virulence factor and mediator of the type IV pilus system in *Acinetobacter baumannii*. *PLoS One* 14, e0218505
85. Kareem, S.M. *et al.* (2017) *Acinetobacter baumannii* virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB). *Microb. Pathog.* 110, 568–572
86. Fiester, S.E. *et al.* (2016) Iron-regulated phospholipase C activity contributes to the cytolytic activity and virulence of *Acinetobacter baumannii*. *PLoS One* 11, e0167068
87. Fiester, S.E. *et al.* (2019) Miltefosine reduces the cytolytic activity and virulence of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 63, e01409-18

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.

88. Skerniškytė, J. *et al.* (2021) OmpA protein-deficient *Acinetobacter baumannii* outer membrane vesicles trigger reduced inflammatory response. *Pathogens* 10, 407
89. Tiku, V. *et al.* (2021) Outer membrane vesicles containing OmpA induce mitochondrial fragmentation to promote pathogenesis of *Acinetobacter baumannii*. *Sci. Rep.* 11, 618
90. Dikshit, N. *et al.* (2018) NLRP3 inflammasome pathway has a critical role in the host immunity against clinically relevant *Acinetobacter baumannii* pulmonary infection. *Mucosal Immunol.* 11, 257–272
91. Tiku, V. *et al.* (2022) *Acinetobacter baumannii* secretes a bioactive lipid that triggers inflammatory signaling and cell death. *Front. Microbiol.* 13, 870101
92. Kang, M.-J. *et al.* (2017) NLRP3 inflammasome mediates interleukin-1 β production in immune cells in response to *Acinetobacter baumannii* and contributes to pulmonary inflammation in mice. *Immunology* 150, 495–505
93. Leukert, L. *et al.* (2023) Infection of endothelial cells with reveals remodelling of *Acinetobacter baumannii* mitochondrial protein complexes. *Microbiol. Spectr.* 11, e0517422
94. Bornier, F. *et al.* (2021) Environmental free-living amoebae can predate on diverse antibiotic-resistant human pathogens. *Appl. Environ. Microbiol.* 87, e0074721
95. Tamang, M.D. *et al.* (2011) Interaction of *Acinetobacter baumannii* 19606 and 1656-2 with *Acanthamoeba castellanii*. *J. Microbiol.* 49, 841–846
96. Grier, J.T. *et al.* (2021) Two *Acinetobacter baumannii* isolates obtained from a fatal necrotizing fasciitis infection display distinct genomic and phenotypic characteristics in comparison to type strains. *Front. Cell. Infect. Microbiol.* 11, 1
97. Artuso, I. *et al.* (2022) Genome diversity of domesticated *Acinetobacter baumannii* ATCC 19606T strains. *Microb. Genom.* 8, 749
98. Vesel, N. *et al.* (2023) DNA modifications impact natural transformation of *Acinetobacter baumannii*. *Nucleic Acids Res.* 1, 13–14
99. Chin, C.Y. *et al.* (2018) A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. *Nat. Microbiol.* 3, 563–569
100. Aguilar, C. *et al.* (2021) Organoids as host models for infection biology – a review of methods. *Exp. Mol. Med.* 53, 1471–1482
101. Ramírez-Flores, C.J. and Knoll, L.J. (2021) Breakthroughs in microbiology made possible with organoids. *PLoS Pathog.* 17, e1010080
102. Rosales, C. and Uribe-Querol, E. (2017) Phagocytosis: a fundamental process in immunity. *Biomed. Res. Int.* 2017, 1–18
103. Nguyen, J.A. and Yates, R.M. (2021) Better together: current insights into phagosome–lysosome fusion. *Front. Immunol.* 12, 636078
104. Wu, M.-Y. and Lu, J.-H. (2019) Autophagy and macrophage functions: inflammatory response and phagocytosis. *Cells* 9, 70
105. Ktistakis, N.T. and Tooze, S.A. (2016) Digesting the expanding mechanisms of autophagy. *Trends Cell Biol.* 26, 624–635
106. Zhou, Y.-Y. *et al.* (2015) MAPK/JNK signalling: a potential autophagy regulation pathway. *Biosci. Rep.* 35, 1–10
107. Kelley, N. *et al.* (2019) The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int. J. Mol. Sci.* 20, 3328
108. Schroder, K. and Tschopp, J. (2010) The inflammasomes. *Cell* 140, 821–832
109. Swanson, K.V. *et al.* (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19, 477–489

Descargado para Anonymous User (n/a) en National Library of Health and Social Security de ClinicalKey.es por Elsevier en diciembre 15, 2023. Para uso personal exclusivamente. No se permiten otros usos sin autorización. Copyright ©2023. Elsevier Inc. Todos los derechos reservados.