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REVIEW ARTICLE

Vaccine development to control the rising scourge of antibiotic-resistant *Acinetobacter baumannii*: a systematic review

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Abstract

Acinetobacter baumannii has emerged as one of major nosocomial pathogen and global emergence of multidrug-resistant strains has become a challenge for developing effective treatment options. *A. baumannii* has developed resistance to almost all the antibiotics viz. beta-lactams, carbapenems, tigecycline and now colistin, a last resort of antibiotics. The world is on the cusp of post antibiotic era and the evolution of multi-, extreme- and pan–drug-resistant *A. baumannii* strains is its obvious harbinger. Various combinations of antibiotics have been investigated but no successful treatment option is available. All these failed efforts have led researchers to develop and implement prophylactic vaccination for the prevention of infections caused by this pathogen. In this review, the advantages and disadvantages of active and passive immunization, the types of sub-unit and multi-component vaccine candidates investigated against *A. baumannii* viz. whole cell organism, outer membrane vesicles, outer membrane complexes, conjugate vaccines and sub-unit vaccines have been discussed. In addition, the benefits of Reverse vaccinology are emphasized here in which the potential vaccine candidates are predicted using bioinformatic online tools prior to in vivo validations.

Keywords *Acinetobacter baumannii* · Multidrug resistance · Vaccine · Reverse vaccinology · Epitopes

Introduction

Acinetobacter baumannii (*A. baumannii*) is associated with a number of nosocomial infections, such as pneumonia, bloodstream infections, meningitis, skin and urinary tract infections in hospitals and intensive care units(Peleg et al. 2008; Moubareck and Halat 2020). Multidrug resistance (Abdi et al. 2020; López-Durán et al. 2020), biofilm formation ability (Espinal et al. 2012; Yang et al. 2012) and desiccation tolerance (Espinal et al. 2012; Wang et al. 2020) are the foremost characteristics making it a critical priority pathogen. Patients requiring mechanical ventilation in hospitals are major targets of *A. baumannii* causing ventilator-associated pneumonia (VAP) (Brotfain et al. 2017; Čiginskienė et al. 2019). It is a common pathogen found in patients of burn injuries and military personnel injured in war. In these critical conditions, vaccination may emerge as solution and it can prevent the initial bacterial invasion to avoid the massive colonization of bacteria which causes infection. Vaccine development against *A. baumannii* infections is being explored extensively and multiple potential vaccine candidates have been identified (Garg et al. 2016; Singh et al. 2016b, 2017; Fereshteh et al. 2020). Patients who tend to be admitted in hospital intensive care units (ICUs) may be vaccinated with enough time to allow for the generation of an immune response to make them ready for exposure to *A. baumannii*. Method of immunization could be selected by analyzing the potential targets of *A. baumannii*. For example, patients admitted in the hospitals for longer duration can be actively immunized and they will get enough time to develop immunity against *A. baumannii*. On the other hand, passive immunization may serve the patients for whom the risk of infection is not foreseen, such as in traumatic injuries, severe burn injuries or in emergent surgery. Although passive immunization is therapeutic and has the potential to provide instantaneous protective immunity, active immunization is safe and cost effective against bacterial infections.

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Future treatment of *A. baumannii* infections may be through an effective vaccine that augments host responses and results in limiting the bacterial infections. Preparing the host for invading nosocomial pathogens appears to be most promising treatment option as adaptive immune response (Antigen presenting cells, B cells and T cells) leads to protection through cellular or humoral immunity. Vaccination with modified or inactivated pathogens or components derived from these pathogens stimulates adaptive immune response in an antigen-specific manner.

Prevalence of *A. baumannii* in global and Indian context

A. baumannii has established itself as one of the most prominent nosocomial pathogens in hospitals and its antimicrobial-resistant clinical strains have been documented at alarming rate worldwide (Holt et al. 2016; Meumann et al. 2019; Brink 2019; Pormohammad et al. 2020). In certain European, South American and Asian hospitals, *A. baumannii* has shown extensive drug resistance pattern as compared to clinical strains isolated from the patients in ICUs in the Nordic countries, the Netherlands and The USA (Falagas and Karveli 2007). Pormohammad et al. (2020) have reported the global prevalence of colistin resistance at increased rate in South–East Asia and East Mediterranean than any other region in world. The highest and the lowest rates of resistance were observed for cefotaxime (99%) in Africa and colistin (1.1%) in Western Pacific. The rate of colistin resistance was highest in Lebanon (17.5%) followed by China (12%) and was lowest in Germany (0.2%). *A. baumannii* has become highly prominent and has been found all over the regions of India. In 2001, Sengupta et al. reported the incidence of *A. baumannii* in wound infections of burn patients. These infections led to septicemia and in few cases, death. When characterized, these bacteria were found multidrug resistant (Sengupta et al. 2001). The prevalence of metallo-β-lactamases (MBLs) among *A. baumannii* clinical isolates obtained from South Indian tertiary care hospitals has been reported and the strains were characterized for their antimicrobial susceptibility (Karthika et al. 2009). Most of the isolates showed resistance to imipenem (100%), meropenem (89%), amikacin (80%), cefotaxime (89%) and ciprofloxacin (72%). In addition, prevalence of *A. baumannii* in patients is of great concern. Azim et al. reported *A. baumannii* colonization in 37 out of 96 patients included in the study conducted in Lucknow in India. They found simultaneous colonization of *A. baumannii* and *P. aeruginosa* in 12 patients and all were extended-spectrum β-lactamase (ESBL)- and metallo-β-lactamase (MBL)-producing isolates(Azim et al. 2010). Srirangaraj et al (2015) described an antibiotic-resistant *A. baumannii* clinical isolate from a 70-year-old patient who developed urinary tract infection caused due to indwelling urinary catheter, prolonged stay in ICU and exposure to broad spectrum antibiotics. This isolate was found resistant to all the drugs used for urinary tract infections (UTI), such as amikacin, ceftriaxone, co-trimoxazole, nalidixic acid, nitrofurantoin and norfloxacin. Further analysis of this isolate showed resistance to aminoglycosides (gentamycin and tobramycin), fluroquinolones (ciprofloxacin and levofloxacin), piperacillin (tazobactum) and imipenem. This MDR isolate was found sensitive to polymyxin B and colistin only (Srirangaraj et al. 2015). Vijayakumar et al. described the prevalence of carbapenem resistance among the *A. baumannii* isolates from tertiary care hospital in South India. They found *blaOXA*-51 and *blaOXA*-23 genes in all the 103 isolates and 94 were carbapenemase producers. *bla*NDM and *bla*VIM genes were predominant among metallo-β-lactamases and 80% of the isolates had ISAba1 upstream *bla*OXA-23 gene which suggests that this insertion element acts as a promoter and facilitates its increased expression(Vijayakumar et al. 2016).

Antibiotic monotherapy has shown limited effect on these bacteria hence antibiotic combinations have been tried against them. Muthusamy et al. demonstrated in vitro activities of rifampicin and polymyxins against carbapenem-resistant *A. baumannii* in a tertiary care hospital from South India. They screened 20,282 clinical isolates obtained from various specimens, such as tracheal aspirate, broncho-alveolar lavage fluid, blood, endotracheal tube tip, sputum, ascitic fluid and wound swabs. All the isolates were found sensitive to polymyxin B and 80% were resistant to rifampicin. Only carbapenem-resistant strains were included in further study and 78% were found sensitive, 12% intermediate sensitive and 10% resistant to colistin. The increasing emergence of MDR *A. baumannii* in all parts of India is alarming and antimicrobial stewardship programs are needed to prevent the emergence and spread of antibiotic resistance (Vijayakumar et al. 2016; Banerjee et al. 2018; Odsbu et al. 2018; Kalal et al. 2020; Nguyen and Joshi 2021).

The need for vaccine against *A. baumannii*

A. baumannii is an important nosocomial pathogen causing various human infections in critically ill patients (Dijkshoorn et al. 2007) and is highly resistant to many antimicrobials (Peleg et al. 2008; Kempf and Rolain 2012; Abdi et al. 2020). Acquired resistance to broad-spectrum β-lactams in *A. baumannii* is mainly due to enzymatic degradation by β-lactamases and resistance to broad-spectrum cephalosporins usually results from overexpression of the chromosomal AmpC-type cephalosporinase (Corvec et al. 2003; Rodríguez-Martínez et al. 2010; Nasr 2020) and from acquisition of extended-spectrum β-lactamases (ESBLs) (Naas et al.

2007). β-Lactamases with carbapenemase activity are most concerning, because the antibiotic resistance determinants may be found on plasmids and/or transposons that could be laterally transferred among bacteria. The rapid worldwide emergence of β-lactams and carbapenem-resistant *A. baumannii* strains shows the adaptation of this bacterium to selective environmental pressure (Peleg et al. 2008; Kempf and Rolain 2012; López-Durán et al. 2020). The emergence of MDR *A. baumannii* has increased the use of antibiotic colistin (Li et al. 2006; Tan et al. 2007; Kempf and Rolain 2012) and has unfortunately led to the discovery of colistin-resistant strains (Kempf and Rolain 2012; Cai et al. 2012; Papathanakos et al. 2020).

In general, this bacterium is considered as a low virulence pathogen, but several virulence factors (Table 1), including adherence and invasion in host cells and host cell death (Choi et al. 2005, 2008), biofilm formation (Eze et al. 2018), capsular polysaccharides (Russo et al. 2013), phospholipase D (Zadeh Hosseingholi et al. 2014), serum resistance (Kim et al. 2009; Bolourchi et al. 2019) and iron acquisition (Zimbler et al. 2009) have been identified which make it a serious pathogen (Fig. 1). These virulence factors along with multidrug resistance trait make this pathogen create havoc, at least in hospitals and it is the emerging cause of nosocomial respiratory and urinary tract infections. Despite studies on several vaccine candidates to confer partial immunoprotection, there is no efficacious vaccine available to prevent *A. baumannii* infections at present.

Development of vaccines against *A. baumannii* is necessary to provide prophylactic protection for susceptible population of immunocompromised and patients in hospitals. As this is a nosocomial pathogen, every individual admitted in hospitals or undergoing antibiotic treatment has the potential risk to acquire infection. The incidence of chronic and persistent infections can be reduced through vaccination. The treatment of *A. baumannii* infections is very difficult due to the inherent resistance of *A. baumannii* to multiple antibiotics as various efflux pumps efficiently remove antibiotics from the bacterial cell that results in multidrug property of the *A. baumannii*. Another reason for antibiotic resistance is biofilm forming ability. In biofilms, the contact between antibiotics and surface of bacteria is hindered resulting in antibiotic failure. In addition, there are changes in the cell envelope reducing the permeability of the cell membrane to the antibiotics. In addition, the range of infections caused by *Acinetobacter* spp. and the potential to develop severe chronic infections in the immunocompromised individuals through the persistence ability make the need for vaccine crucial (Kaur et al. 2018).

Active immunization

Although *A. baumannii* causes nosocomial infections and it may not be suitable to immunize acute patients urgently requiring the protective treatment, but active immunization can evoke immunity and delay the onset of bacteremia to enhance the efficacy of antibiotic treatment in individuals at high risk (Table 2).

Whole cell vaccines

Whole cell vaccines (first generation), either live attenuated or killed, induce the immune system. Due to their ability to elicit a broad range of immune responses, these vaccines are considered ideal. They have been widely used against dreadful infectious diseases, such as cholera, mumps, measles and tuberculosis (Moyle 2015). Pathogenic strains are weakened by multiple passages in laboratory conditions or immunologically related microorganisms are used that do not use human as the target host. Live attenuated microbes do not cause any pathological or lethal effects in human body but they replicate and are recognized by human immune system

Table 1 Important virulence factors which can be used in vaccine development and their role in the pathogenesis of *A. baumannii*

S. no	Virulence factor	Role	References
1	OmpA	Acts as porin, induces host cell apoptosis, antimicrobial resistance and biofilm formation	Moon et al. 2012; Kwon et al. 2017; Nie et al. 2020a)
2	Biofilm associated protein (BAP)	In biofilm formation and involved in intercellular adhesion within the mature biofilm	(Loehfelm et al. 2008; Brossard and Campagnari 2012)
3	AdeABC	Multidrug efflux complex involved in multidrug resistance and biofilm formation	(Subhadra et al. 2019; Xu et al. 2019)
4	Pili	A type IV pili system required for twitching motility	(Harding et al. 2013; Geisinger et al. 2019)
5	CsuA/BABCDE	Usher-chaperone fimbriae required for pili biogenesis and biofilm formation	(Tomaras et al. 2003; Longo et al. 2014)
6	PNAG	A surface polysaccharide involved in biofilm formation,	(Bentancor et al. 2012a; Geisinger et al. 2019)
7	Capsule	Provides protection desiccation and disinfection regimes as well as host immune responses	(Russo et al. 2010; Geisinger et al. 2019; Hu et al. 2020)

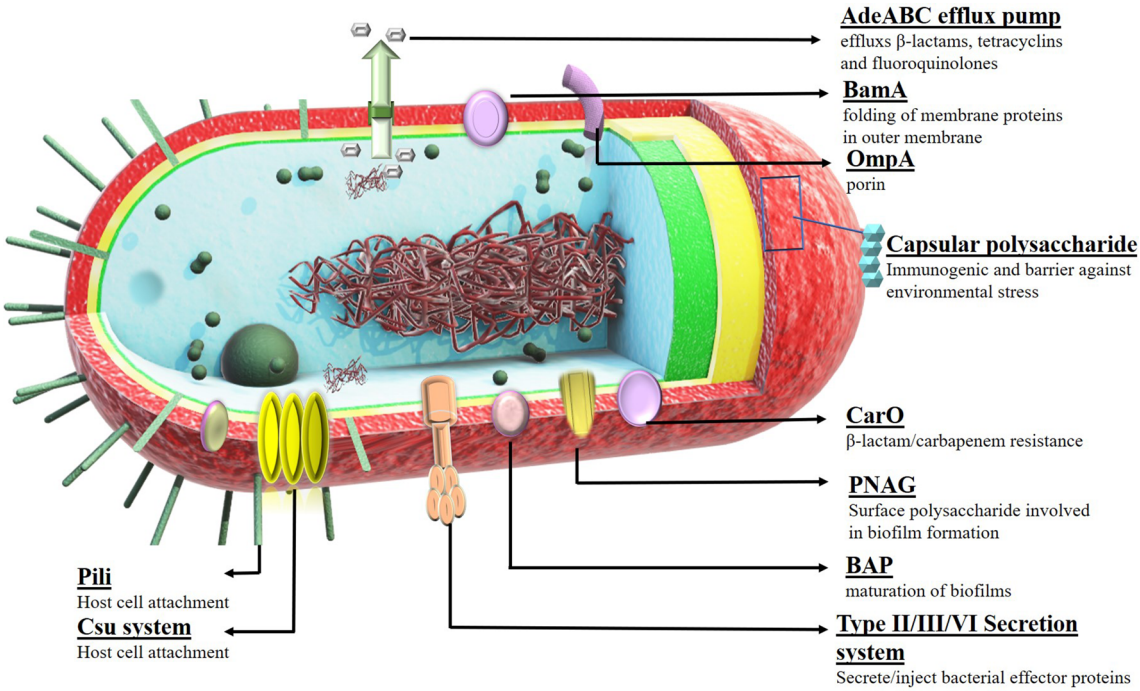

Fig. 1 A schematic illustration of virulence factors of *A. baumannii* with their roles. *AdeABC* RND efflux superfamily, *BamA* outer membrane protein assembly factor, *OmpA* outer membrane protein A, *CarO* carbapenem associated resistance protein, *PNAG* polysaccharide poly-N-acetylglucosamine, *BAP* biofilm associated protein, *Csu* Csu operon

as foreign invaders. By knocking out the virulence genes essential for pathogenesis, well defined weakened or attenuated live vaccines can be developed (Robbins and Robbins 1986; Nascimento and Leite 2012; Lin et al. 2015; Morais et al. 2019).

Killed whole cell vaccines consist of non-living pathogens that are unable to replicate and yet remain immunogenic in host which makes them extremely safe. This type of vaccine has been successfully used against anthrax, Q fever and whooping cough (Ada 2005). These vaccines elicit humoral immune response resulting in high concentration of neutralizing antibodies. Although killed whole cell vaccines are known to not stimulating the strong cell mediated immunity, resulting in multiple doses of vaccination for long lasting protection but they induce a broad immune response against multiple surface antigens. In case of *A. baumannii*, McConnell and Pachon reported high immunogenicity of whole cell vaccine inactivated by formalin as it produced significant antibody titer against multiple outer membrane proteins. Although this vaccine had contamination of high levels of LPS but it generated strong immune response resulting in reduction of bacterial loads in organs and reduced serum pro-inflammatory cytokine levels compared to unimmunized mice and protected mice from *A. baumannii* ATTC 19,606 and two clinical strains (McConnell and Pachón 2010). KuoLee et al. used inactivated whole cell vaccination approach against *A. baumannii*. In this study, a clinical isolate of *A. baumannii*, LAC-4, causing 100% mortality in mice by acute pneumonia and bacteremia, was used for bacterial challenge. Intranasal immunization with formalin-killed *A. baumannii* LAC-4 cells resulted in high levels of IgG1 and IgG2 responses and ultimately clearing bacteria from lungs and serum (KuoLee et al. 2015). An inactivated whole-cell vaccine derived from antibiotic-exposed MDR *A. baumannii* (I-M28-47–114) (Shu et al. 2016) and radiation inactivated cells were found to be protective(Dollery et al. 2021).

Single sub-unit vaccines

Sub-unit vaccines contains minimal microbial components necessary to stimulate long lasting specific protective and/or therapeutic immune responses against the pathogen (Moyle and Toth 2013). However, preparation of subunit components as vaccines requires extremely stringent safety protocols as their preparation involves cultivation of live pathogenic bacteria in large scale culture.

Protein candidates

A. baumannii immunogenic proteins used are mostly extracellular or surface exposed proteins, such as outer membrane proteins (OMPs), porins, receptors, channels and other functional and structural components on the bacterial surface.

Table 2 Characteristics of *Acinetobacter baumannii* vaccine candidates

Vaccine type	Vaccine formulation	Examples	References
Whole cell (killed or attenuated)	–Formalin inactivated cells with aluminium-based adjuvant, Alhydrogel 2% (w/v) –Formalin killed cells with saline –Formalin killed cells with Freund's adjubant –100µL of 1.0×10^7 irradiated bacterial cells in PBS	1. Inactivated LPS-deficient whole cells 2. Inactivated *A. baumannii* Lac4 strain 3. An inactivated whole-cell vaccine derived from antibiotic-exposed MDR *A. baumannii* (I-M28-47–114) 4. Gamma radiation inactivated whole cells	(García-Quintanilla et al. 2014) (KuoLee et al. 2015) (Shu et al. 2016) (Dollery et al. 2021)
Outer membrane vesicles and complex	–Outer membrane vesicles (OMVs) in PBS with aluminium phosphate adjuvant –Outer membrane complexes (OMCs) in PBS with aluminium phosphate adjuvant	1. OMVs 2. OMCs	Huang et al. 2014c; Badmasti et al. 2015a; Pulido et al. 2020) (Huang et al. 2014b) (Pulido et al. 2018)
Protein only	–500 µl of 1:50 diluted heat-inactivated sera injected 24 h before the challenge –3 µg of rOmpA in 0.1% $Al(OH)_3$ –50 µg of fusion protein thioredoxin–OmpW adjuvanted by 1 mg of Alum –5, 10, 20 or 50 µg of fusion protein thioredoxin–Omp22 adjuvanted by 1 mg of Alum –20 µg FilF in Freund's adjuvant –5–50 µg of nuclease in Freund's adjuvant –20 µg BamA in 2% $Al(OH)_3$	1. BAP 2. OmpA 3. OmpW 4. Omp22 5. FilF 6. Nuclease 7. BamA	(Fattahian et al. 2011)(Darzi Eslam et al. 2020) (Luo et al. 2012) (Huang et al. 2015) (Huang et al. 2016) (Singh et al. 2016a, b) (Garg et al. 2016) (Singh et al. 2017)
Conjugate candidate	–25, 50 and 25 µg of $OMV_{(PagL)}$, $AbOmpA_{(8-346aa)}$ and $Bap_{(1-487aa)}$ in 2% $Al(OH)_3$	1. Conjugate of OMV (PagL), Bap (1–487aa) and AbOmpA (8–346aa), and Bap (1–487aa)	(Badmasti et al. 2015)
Epitope candidate	–30 µg of multi-epitope assembly in Freund's adjuvant –100 µg of rOmp22 in PBS	1. B-cell and T-cell epitopes from FilF and NucAb, peptide of Ata linked by peptide GGGGSGGGG 2. B-cell and T-cell epitopes of Omp22 conjugated by 6-amino-caproic acid, encapsulated by chitosan (CS) and poly (lactic–co-glycolic) acid (PLGA)	(Ren et al. 2019) (Du et al. 2021)

OmpA (Luo et al. 2012), Ata (Bentancor et al. 2012b), BamA (Singh et al. 2017), NucAb (Garg et al. 2016), FilF (Singh et al. 2016b), Bap (Fattahian et al. 2011), OmpW (Huang et al. 2015) and Omp22 (Huang et al. 2016) are the major outer membrane proteins that are antigenically conserved and surface exposed in sequenced strains and clinical isolates and immunization with these Omps as immunogens elicited opsonizing, cross reactive and protective antibodies in mouse pneumonia or sepsis model. Moreover, *A. baumannii* outer membrane proteins are highly conserved as compared to high heterogeneity of outer membrane vesicles or outer membrane complexes.

OmpA is most studied *A. baumannii* outer membrane protein and potential therapeutic target for *A. baumannii* infections(Choi et al. 2008; Gaddy et al. 2009; Park et al. 2012; Moon et al. 2012; Confer and Ayalew 2013; Samsudin et al. 2016; Jahangiri et al. 2017; Nie et al. 2020). Amount of vaccine candidate administered affects its immunology in the host. OmpA is well explored protein in *A. baumannii* and variations in its immunization doses was found to affect the antibody titer and immune responses. Larger vaccine doses (30–100 µg) as compared to lower (3 µg) was found to induce higher IgG and IgG subtypes, epitope restriction for IFN gamma producing lymphocytes, a polarized IL-4/type 2 response, while lower doses induced lower antibody response and a balanced IFN-γ-IL-4 immune response (Lin et al. 2013). *ompA* gene was sequenced in six clinical strains of *A. baumannii* and found 99% identical. Immunization with recombinant outer membrane protein A resulted in high antibody titer, protected mice from infection and reduced bacterial load in various organs (Luo et al. 2012). *Acinetobacter* trimeric autotransporter or Ata protein plays a role in *A. baumannii* infections by acting as adhesion immobilizing collagen type IV and promotes biofilm formation. Immunization with Ata significantly reduced bacterial load in different organs of mice 24 h postinfection (Bentancor et al. 2012b). Chiang et al. analyzed whole proteome of *A. baumannii,* cloned 3 candidate proteins viz. OmpK, Ompp1 and FKIB in *E. coli.* Mice were immunized with these recombinant proteins along with complete/incomplete Freund's adjuvant and significant antibody titer was observed resulting in partial mice survival (60%) after lethal bacterial challenge (Chiang et al. 2015). Similarly, OmpW protected mice from lethal bacterial challenge with 100% survival rates (Huang et al. 2015). A 22-kDa outer membrane protein of *A. baumannii* was found as a novel and safe antigen for developing antisera or effective vaccine to control *A. baumannii* infections (Huang et al. 2016). Omp22 was found more than 95% conserved in 851 reported *A. baumannii* strains. Recombinant Omp22 elicited significant titers of specific IgG in mice. Both active and passive immunizations with Omp22 suppressed the bacterial burdens in the organs, increased the survival rates of mice (100%) and reduced the levels of serum cytokines and chemokines. In addition, Omp22 antiserum had efficient bactericidal activities against clinical *A. baumannii* isolates. In addition, high dose of purified protein (500 µg) did not cause pathological changes in mice. BamA showed its immunoprotective potential against lethal doses of MDR *A. baumannii* and promising candidate for active and passive immunization (Singh et al. 2017). Recently, Omp87 showed significant immunoprotective potential against *A. baumannii* in sepsis model (Rasooli et al. 2020). The development of protein-based vaccine shows promising results as it relies on the identification of safe and effective vaccine candidates for the prevention of *A. baumannii* infections.

Non-protein candidates

Capsular polysaccharide is a well explored virulence factor, major component of the outer complex of *A. baumannii* and due to its surface accessibility, it has remained the widely characterized and tested vaccine candidate(Geisinger and Isberg 2015; Singh et al. 2019; Geisinger et al. 2019; Moubareck and Halat 2020). Russo et al. studied the effect of monoclonal antibodies against K1 capsular polysaccharide of *A. baumannii* in rat model. These monoclonal antibodies promoted in vitro neutrophil-mediated bactericidal activity and reduced post infection bacterial loads in various organs of rat. In addition, these monoclonal antibodies reacted to 13 out of 100 *A. baumannii* strains isolated from body sites and different geographic locations (Russo et al. 2013). However, capsule may interfere with the antiOMP antibodies (Wang-Lin et al. 2017) but CPS-induced antibodies have been found to provide 55% protection against *A. baumannii* challenge in mouse (Yang et al. 2017).

Outer membrane vesicles

A. baumannii secrete outer membrane vesicles (OMVs) during growth (Jin et al. 2011). They are composed of phospholipids, proteins and lipopolysaccharides (LPS) forming spherical vesicles with diameter ranging from 20 to 300 nm (Ellis and Kuehn 2010). OMVs play role in the delivery of virulence factors to the host cells thus initiating the infection (Alaniz et al. 2007). The production of OMVs increases under stress and harsh conditions, such as infection (McBroom and Kuehn 2007). Exact mechanism of OMV formation is unknown but Deatherage et al. proposed and explained a model of OMV formation in *Salmonella* species (Deatherage et al. 2009). They described that OMVs are produced in cell envelope regions, where the density of outer membrane–peptidoglycan associations is temporarily decreased. They also found that outer membrane proteins present in OMVs have specific domains that could interact with peptidoglycan and could modulate OMV production. In

addition, *Salmonella* mutants lacking few outer membrane proteins, such as OmpA, LppAb, and Pal, were able to produce more OMVs than wild type. In case of *A. baumannii*, OmpA was found to play important role in biogenesis of OMVs and protein composition (Moon et al. 2012).

OMVs have been tried as vaccine candidates against infections caused by *A. baumannii*. Jun et al. proposed OMVs as potent stimulators of innate immune response with membrane proteins in OMVs playing critical role in it. Immunization with OMVs successfully stimulated the pro-inflammatory response, recruitment of neutrophils and exudates in the lungs of neutropenic mice (Jun et al. 2013). Huang et al. studied immunoprotective efficacy of OMVs of a clinical isolate against MDR *A. baumannii* in both sepsis and pneumonia mice model. Immunization with these OMVs resulted in significant IgG antibody (64,000 titer, 21 days after 2nd booster) response in female ICR mice. These OMVs were found effective in providing 100% mice survival due to decreased bacterial burden in organs, decreased pro-inflammatory cytokines and less damage to the organs after bacterial challenge (Huang et al. 2014). McConnell et al. have shown that OMV immunization reduced post-infection organ burden loads and increased survival after lethal challenge (McConnell et al. 2011b). Badmasti et al. isolated OMVs from *A. baumannii* expressing lipid A deacylase PagL and combined them with two other *A. baumannii* proteins viz. Bap and AbOmpA. These formulations induced robustly antibodies, Th1 and Th2 responses and protected mice from bacterial challenge (Badmasti et al. 2015). Recently, OMVs isolated from an Acinetobacter strain deficient in lipopolysaccharide (LPS) due to mutation in *lpxD* elicited immunity against infection and elicited antibody titers (IgG, IgG1, IgG2c and IgM) similar to the wild type (Pulido et al. 2018, 2020). These OMVs were highly immunogenic and their immunization resulted in significantly reduced post-infection spleen bacterial loads, serum IL-1beta and IL-6 levels and provided 75% mice protection from bacterial challenge.

Outer membrane complexes

McConnell et al. analyzed the proteome of outer membrane complex of *A. baumannii* ATCC 19,606 and found 41 proteins associated with the cell surface. They used antibodies generated against multiple proteins in OMCs to recognize the surface proteins from clinical isolates (McConnell et al. 2011a). Immunization with OMCs reduced the bacterial load in organs and reduced the pro-inflammatory cytokines viz. IL-1β, IL-6 and TNF-α. Immunized mice showed increased survival rate after infection with ATCC 19,606 as well as with a pan–drug-resistant strain. Passive immunization with antisera raised against OMCs rescued the infected mice indicating the potential of OMC vaccines. However, preparation of vaccine using OMCs is tricky due to difficulties in standardization of levels of the multiple components of OMCs.

Conjugate vaccines

There are few reports of conjugate vaccines against *A. baumannii*. Gening et al. targeted Poly-*N*-Acetyl-β-(1–6)-Glucosamine (PNAG), which is produced by several pathogens including *A. baumannii*, and prepared a conjugate of oligoglucosamines containing either 5- or 9-mer fully acetylated monosaccharides (5GlcNAc or 9GlcNAc) or 5- or 9-mer fully non acetylated monosaccharides ($5GlcNH_2$ or 9 $GlcNH_2$) and tetanus toxoid. This conjugate produced significant antibody titer which protected mice from *S. aureus* in skin abscesses murine model and from *E. coli* in lethal peritonitis model (Gening et al. 2010). Bentacor et al. demonstrated the efficacy of a conjugate of synthetic oligosaccharide and tetanus toxoid against a high PNAG producing *A. baumannii* strain. Antisera raised in rabbit against this conjugate successfully reduced the bacterial load in mice lungs resulting in high survival rates after lethal bacterial challenge. In addition, antiserum to conjugate showed significant opsonophagocytic activity against various *A. baumannii* clinical isolates (Bentancor et al. 2012a).

Passive immunization

Passive immunization has the potential to provide effective and prompt protection. Either in combination with antibiotic therapy or alone, antibacterial antibodies could treat the *A. baumannii* infections. Antibody preparations have been widely used to delay bacteremia onset by reducing the bacterial count in bronchoalveolar lavage fluid (BALF) or blood.

Several recombinant proteins of *A. baumannii* have been used to produce antisera that were assayed for the opsonophagocytic killing. Antisera generated by immunization with OMCs of *A. baumannii* have been shown to effectively rescue the infected mice (McConnell et al. 2011b). Inoculation with antisera 1 h after the bacterial infection resulted in survival of infected mice. Garg et al. have shown improved survival by passive immunization over active immunization using antisera raised against an outer membrane nuclease, NucAb. Active immunization with recombinant NucAb resulted in significant IgG titer of $1–5 \times 10^5$ resulting in 20% survival which increased to 40% after passive immunization (Garg et al. 2016).

Antisera against an outer membrane protein Omp22 exerted specific bactericidal activity at different dilutions – 39.8% at dilution of 10, 24% at 100 and 11.8% at 1000-fold dilutions(Huang et al. 2016). The bactericidal activity was complement dependent as complement-inactivated antisera showed 22.2% killing by 10X dilutions. Removal of phagocytes from serum led to loss of killing

activity which proved that opsonophagocytic killing. BamA showed 40% mice protection from bacterial lethal challenge on passive immunization, mainly by the opsonophagocytic activity of serum (Singh et al. 2017).

Reverse vaccinology for treating *A. baumannii* infections

Reverse vaccinology is the use of genomic and proteomic information available in databases to predict potential vaccine candidate proteins against pathogens. In conventional vaccinology, pathogen is first cultured then its immunogenic components are identified, isolated and purified by laboratory protocols followed by their validation in animal models. Due to inability to work upon uncultivable pathogens, time consumption in experiments and serious biohazards associated with highly infectious pathogens, reverse vaccinology was discovered as a new approach to identify potential vaccine candidates. This technique requires complete proteomes of sequenced pathogens and online tools which predict the vaccine candidates and screen large number of isolates for homology (Rappuoli 2000, 2001; Sette and Rappuoli 2010; Singh et al. 2016a; Solanki and Tiwari 2018).

Reverse vaccinology represents a complete and successful example of computer-aided biotechnology providing vaccine candidates for most complex and difficult to treat infections (Vivona et al. 2008). Whole genome and proteome of pathogens are available and plethora of computations tools help in identification of potential vaccine candidate proteins out of them. NERVE (Vivona et al. 2006), Vaxign (He 2010), VaxiJen (Doytchinova and Flower 2007), Jenner-Predict (Jaiswal et al. 2013), VacSol (Rizwan et al. 2017) and Bowman-Heinson (Bowman et al. 2011; Heinson et al. 2017) and Vaxgin2 ((Ong et al. 2021)) are the major RV tools to predict potential vaccine candidates. Due to major limitations in conventional vaccinology, RV has become an initial and standard approach to search ideal vaccine candidates possessing certain characteristics (Rappuoli 2000; Singh et al. 2016a). The underlying principles in this approach are selection of proteins localized on cell surface as surface exposure of antigens is the most important requirement for the host–pathogen interaction. High level of antigen conservation is another fundamental requirement in this approach, as to provide broad spectrum immunity covering all the strains of pathogen is necessary. Other than these, vaccine candidate should contain minimum number of trans-membrane helices (less than 2), high adhesion probability, ability to bind to the MHC molecules, high conservation among the clinical strains and most important, its dissimilarity with human and mouse proteome to avoid the generation of autoimmune response by host. These characters can be easily predicted by online tools and servers. This approach has successfully led to identify antigens capable of eliciting protective immunity against *N. meningitidis* group B (Pizza et al. 2000; Kelly and Rappuoli 2005; Masignani et al. 2019).

Reverse vaccinology is highly successful in case of *Acinetobacter baumannii* (Table 3) (Chiang et al. 2015; Singh et al. 2016b, 2017; Hassan et al. 2016; Fereshteh et al. 2020). Using this approach, Chiang et al. analyzed the whole proteome of *A. baumannii* that provided several potential vaccine candidates. Out of these proteins, three were cloned and expressed in *E. coli* and immunization with these individually purified recombinant proteins conferred partial survival in *A. baumannii* mouse pneumonia model (Chiang et al. 2015). In silico analysis of several *A. baumannii* outer membrane proteins has highlighted their vaccine potential. Biofilm associated proteins present on the bacterial surface show high molecular weight, contain a core domain of tandem repeats and play critical role in bacterial infection processes. Rahber et al. analyzed these proteins using online tools and reported the four functional and conserved regions which could be effective antigens. Out of these regions, a construct serving as a potential agent for diagnostic test based on antigen–antibody interaction was described (Rahbar et al. 2010, 2012). BamA (beta barrel assembly machine protein) is also a potential vaccine candidate having several conserved B cell and T cell epitopes. BamA is an outer membrane protein belonging to Omp85 family conserved in all the Gram-negative bacteria. The B cell, MHC class I and MHC class II epitopes were predicted and docked with the HLA molecule to find their affinity towards HLA molecules prevalent in north Indian population (Singh et al. 2014).

An outer membrane putative pilus assembly protein, FilF, was identified using reverse vaccinology approach and FilF immunization significantly decreased bacterial load in lungs in *A. baumannii* pneumonia murine model resulting in decrease in pro-inflammatory cytokines, reduced infiltration of neutrophils in lung alveoli hence, improved survival (50%) as compared to unimmunized infected mice (Singh et al. 2016b). Similarly, an *A. baumannii* outer membrane protein nuclease, NucAb, was found to possess all the attributes of a promising vaccine candidate, such as outer membrane localization, one transmembrane helix only, high adhesin probability (0.53), non-homology to human proteins, totally conserved among all the sequenced *A. baumannii* strains and presence of B-cell and T-cell epitopes binding with high affinity (percentile rank ≤ 1) to HLA alleles prevalent in North Indian populations. Recombinant NucAb (25 µg) immunization elicited high antibody titer ($1–5 \times 10^5$) and reduced the bacterial load by 5 log cycles in lungs, reduced pro-inflammatory cytokines (TNF-α and IL-6), whereas anti-inflammatory (IL-10) cytokine increased in serum and lungs. Active immunization resulted in 20% survival rate in mice which was improved to 40% with passive immunization (Garg et al. 2016). BamA (Singh et al.

Table 3 Antigens identified as potential vaccine candidates by Reverse vaccinology approach in *Acinetobacter baumannii*

S. no	Procedure	Major proteins identified	Tools used	References
1	Investigation of immunoprotective efficacy of recombinant OMPs in pneumonia mouse model	BamA, FilF, Nuclease	–Vaxign, IEDB, ClusPro	(Singh et al., 2017) (Singh et al. 2016b) (Garg et al. 2016)
2	Analysis of *A. baumannii* OMV secretome, and investigated their immunoprotective potential in mouse model	OmpK, Ompp1and FKIB	Microbial Genome Database, PSORTb, MEGA6	(Chiang et al. 2015a)
3	Comprehensive analysis of all completely sequenced *A. baumannii* strains to predict antigenic proteins	AdeK, PonA, FhuE receptor, OmpA/MotB, peptidoglycan associated lipoprotein and peptidyl-prolyl cis–trans isomerase	RNAmmer, HMMTOP, Proped, PropedI, NetSurfP, MHCpred and Virulentpred	(Hassan et al. 2016)
4	Subtractive proteomics to identify novel drug targets	promiscuous antigenic membrane proteins	RNAmmer, PSORTb, VaxiJen, STRING, NetCTL server, IEDB, ToxinPred	(Solanki and Tiwari 2018)
5	Analysis of 33 *A. baumannii* genomes	NlpD, FimA, PapC, and PapC, FhuA, BfnH, FatA-like protein, and IutA	Vaxign, IEDB, Phyre2, LOMETS, ModRefiner, ElliPro and Consurf web tool	(Fereshteh et al. 2020)
6	Designing of multi-antigen vaccine	Study of experimental behavior of the 25 potential vaccine candidate proteins	SignalP 5.0, HMMER 3.0, Soluprot 1.0, TMHMM 2.0, AllerTop 2.0, ToxinPred, IEDB, NetMHCII-pan 3.2	(McConnell and Martín-Galiano 2021)
7	Designing of chimeric vaccine	vaccine constructs having B cell derived T cell epitopes	PSORTb, ABCPred, ProPred and ProPred1, HADDOCK server, C-ImmSim	(Shahid et al. 2021)

2017) and Omp87(Rasooli et al. 2020) are most promising vaccine candidates identified by Reverse Vaccinology as they elicited high antibody titer in mice, reduced cytokine levels and bacterial burden in organs and protected mice from lethal bacterial challenge by active and passive immunization. Recently, Fereshteh et al. analyzed *A. baumannii* proteomes using Reverse Vaccinology and predicted putative vaccine candidates mostly involved in cell division, pili or fimbria assembly and iron acquisition processes (Fereshteh et al. 2020).

Epitope based broad spectrum vaccine

Epitopes are the smallest molecular entities recognized by the host immune system. Developing epitope-based vaccine is challenging due to eminently polymorphic nature of MHC molecules and distinct frequencies of human leukocyte antigens (HLAs) (Sette and Fikes 2003; Parvizpour et al. 2020). A plethora of online tools are available now to predict T-cell epitopes and compare their population coverage in different geographical locations. Importantly, epitope-based vaccines can be designed to maximize the population coverage and minimizing the complexity or variability in human population. There is huge genomic and proteomic information available because of next generation sequencing that can be explored to identify the epitopes which are conserved among the strains of *A. baumannii* or Genus *Acinetobacter* and can be used to develop as broad-spectrum vaccine. Immune-dominant B and T-cell epitopes can generate robust immune response in host.

Whole proteomes of pathogens are screened for highly conserved surface exposed proteins and then, B cell and T cells are predicted using online epitope prediction tools. BamA (Singh et al.), Phospholipase D(Zadeh Hosseingholi et al. 2014), NucAb (Garg et al. 2016), FilF (Singh et al. 2016b), TolB (Song et al. 2018), Omp34 (Jahangiri et al. 2018), Ton-B dependent copper receptor (Abdollahi et al. 2018), Polysaccharide export outer membrane protein (Ahmad and Azam 2018), Chaperone-usher pathway protein B, CsuB (Ahmad and Azam 2018), Iron regulated proteins(Bazmara et al. 2019), Rhs(Ahmad et al. 2019) and fimbrial biogenesis outer membrane usher protein, FimD (Ahmad et al. 2019) are the *A. baumannii* proteins used for epitope prediction. However, epitope vaccine candidates have been designed as chimeric construct containing more than one epitope or combination of B or T cell epitopes from different proteins. Ahmad et al. investigated a tigecycline-resistant *A. baumannii* strain and predicted BamA, FimD and Rhs from core proteome as surface proteins, essential, localized at the pathogen surface, non-homologous to humans and mice. Epitopes FPLNDKPGD (BamA), FVHAEEAAA (FimD) and YVVAGTAAA (Rhs) were predicted which had high affinity for the prevalent alleles in human populations.

These epitopes were linked, attached to an adjuvant to enhance its antigenicity and docked with TLR4 receptor showing high affinity (Ahmad et al. 2019). Omp34 is present in > 1600*A. baumannii* strains with > 98% identity and its antigen construct was designed as antigen with high epitope density in which copies of antigenic peptides were increased by replacing non-antigen sequences(Jahangiri et al. 2018). Ren et al, 2019 constructed multi-epitope assembly peptide by linking two B-cell epitopes of Ata, one CD4 + T-cell epitope from FilF and two B-cell epitopes and one CD + epitope from NucAb and its immunoprotective efficacy was investigated in mice and found as promising vaccine candidate (Ren et al. 2019). Vaccine candidate proteins obtained by in silico analysis of *A. baumannii* proteomes are subjected to conservation analysis and sequential epitope mapping (Moriel et al. 2013; Chiang et al. 2015; Garg et al. 2016; Singh et al. 2016b, 2017; Hassan et al. 2016; Ren et al. 2019; Fereshteh et al. 2020; Du et al. 2021; Shahid et al. 2021).

Conclusion

Vaccine development against fierce infectious microorganisms has remained one of the great human achievements. It has primarily focused on the pathogenesis of pathogen based on its virulence factors causing the infection. In addition, constant evolution of multidrug-resistant strains is challenging the antibiotic regime and indicating the vaccine development as an appropriate and effective treatment option. Current recombinant immunization strategies target a single or multiple outer membrane proteins which are easy to prepare, safe as there is no risk of pathogen reverting back to its virulent form and very few adverse effects as compared to other forms of vaccination. Recombinant vaccines are designed for broad range of protection by selecting only those proteins which are conserved throughout the strains of a specific species or species of a particular genus. Conservation analysis of these proteins are performed either by in silico analysis using genomic and proteomic information or by checking them in clinical isolates using polymerase chain reaction (PCR). Completely and partially sequenced strains of *A. baumannii* can be screened for proteins conserved in maximum of strains and these candidates can be evaluated for their vaccine potential. Moreover, identifying specific host immune pathways induced by *A. baumannii* infection or immunization will facilitate the discovery of new and potential immunoprotective candidates to help eliminate this emerging public health crisis. Multidrug-resistant *A. baumannii* is a major source of concern and constitutes a serious therapeutic problem as use of newer generation antibiotics is expensive. Better hygienic conditions and sanitation, routine microbiological surveillance and proper in vitro testing prior to the use of antibiotic may help in control, prevention and treatment of these infections. In addition, there is a dire need for accurate identification of these pathogens to reduce outbreaks.

Future perspective

Vaccine development against multidrug-resistant pathogens have shown significant potential to reduce the clinical burden, mortality and morbidity caused by them. Preparing the host for incoming nosocomial pathogens may be the most promising treatment option as adaptive immune response (APCs, B cells and T cells) leads to protection through cellular or humoral immunity. Genomic and proteomic data available are being explored to find out broad spectrum vaccine candidates by analyzing multiple strains at once. The dearth of effective therapeutics to treat multidrug *A. baumannii* has energized the researchers to seek novel approaches and ingenious strategies. Reverse vaccinology is such a time saving approach to predict potential vaccine candidates exploiting huge bioinformatic data. Moreover, several vaccine development attempts have provided important details about *A. baumannii*'s antimicrobial resistance patterns, diversity, active/passive vaccination and animal infection model development that can assist in more potent and efficacious vaccine development against *A. baumannii.*

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Declarations

Conflict of interest The authors report no conflict of interest.

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