Chapter 10

Physiology and Pathology of Multidrug-Resistant Bacteria: Antibodies- and Vaccines-Based Pathogen-Specific Targeting

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Additional information is available at the end of the chapter

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Abstract

Multidrug-resistant bacteria (MDR) are increasing rapidly and posing a global threat to mankind. Alternative strategies other than antibiotics have to be explored urgently. In this chapter, we review the current status of nonantibiotics strategies including antibody-based therapy and vaccine development for targeting Gram-positive strains (methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*) and MDR Gram-negative strains (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*). Biologics-based clinical progress against these bacterial infections is updated.

Keywords: multidrug-resistant bacteria, MDR, MRSA, VRE, *A. baumannii*, *P. aeruginosa*, infection, biologics, antibody, vaccine

1. Introduction

Antibiotics treatment for bacterial infections has been extensively used for over half century. This is coupled with increasing reports of bacteria drug resistance to almost all available classes of antibiotics.

The antibiotics multidrug resistance (MDR) situation is particularly severe in clinics and community for the designated ESKAPE notorious bugs (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.) [1, 2].

Given the prevalence of antibiotic resistance to these bacteria-associated infections, alternative strategies are urgently needed. This chapter reviews the current status of nonantibiotics-based

strategies including antibody-based therapy and vaccine development for Gram-positive strains methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) and MDR Gram-negative strains (*A. baumannii* and *P. aeruginosa*). **Figure 1** shows the basic

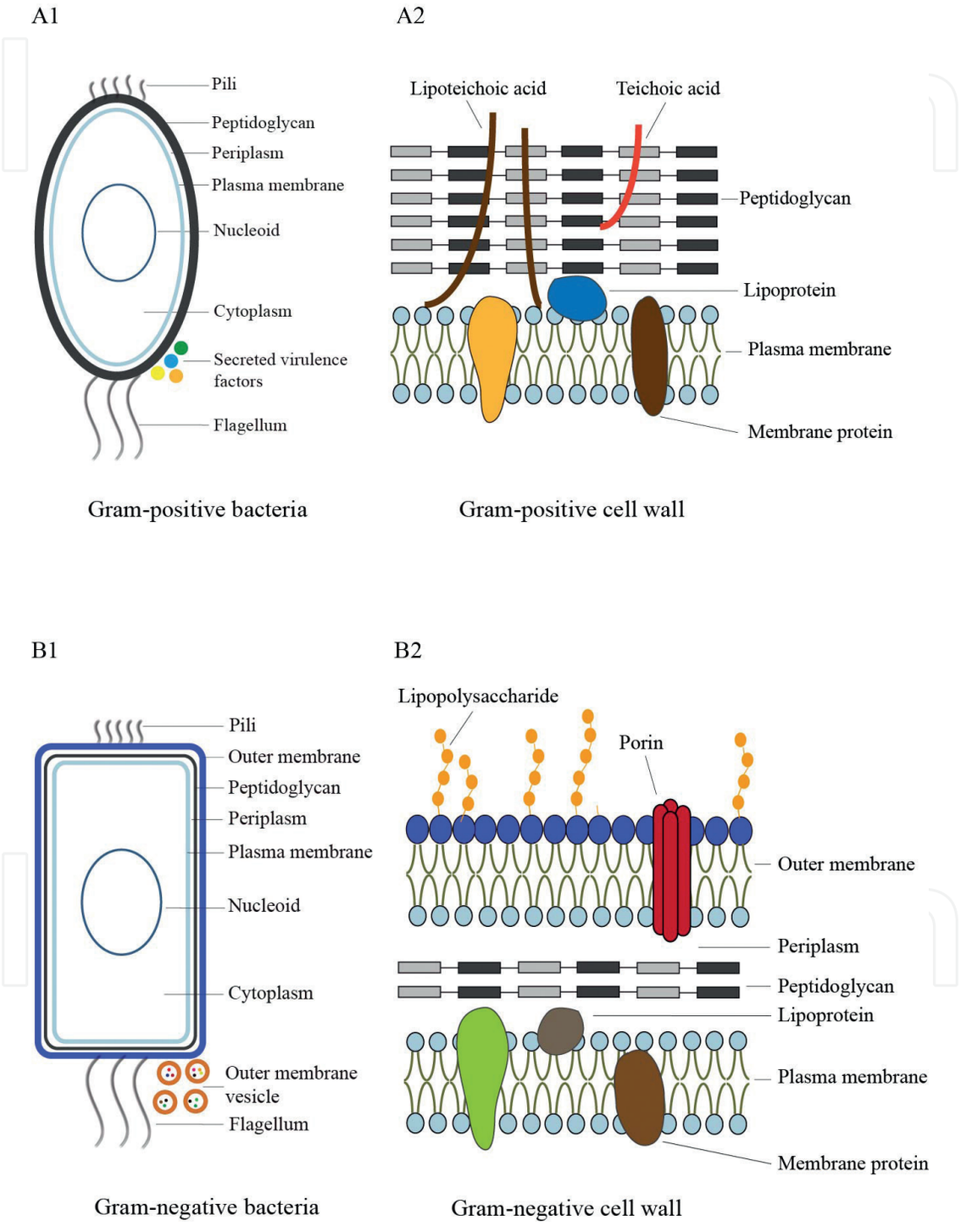

Figure 1. Bacterial cell and detailed cell wall architecture. Gram-positive bacterial cell (A1), the detailed Gram-positive bacterial cell wall (A2), Gram-negative bacterial cell (B1) and the detailed Gram-negative bacterial cell wall (B2) are shown.

Drug name	Sponsor (s)	Target	Product class	Indication	Development stage	Ref.
Altastaph	Nabi Biopharmaceuticals	CP5/CP8	*S. aureus* antibody	Treatment of bacteremia and continuing fever	Phase I/II	[48]
Aurexis (Tefibazumab)	Bristol-Myers Squibb	ClfA	*S. aureus* antibody	Treatment of bacteremia	Phase II	[68]
Aurograb	NeuTec Pharma	ABC transporter GrfA	*S. aureus* antibody	Treatment of severe, deep-seated infections	Phase III, failed	[189]
Pagibaximab	Biosynexus Inc./ GlaxoSmithKline	Lipoteichoic acid	*S. aureus* antibody	Prevention of staphylococcal sepsis in very low birth weight infants	Phase II/III, failed	[190]
MEDI4893	MedImmune LLC	α-toxin	*S. aureus* antibody	Prevention of pneumonia	Phase II	[36]
SAR279356 (F598)	Sanofi	PNAG	*S. aureus* antibody	Prevention of pneumonia	Phase II, terminated	[191]
Veronate	Bristol-Myers Squibb	ClfA and SdrG	*S. aureus* antibody	Prevention of infections in neonates	Phase III	[192]
SA3Ag	Pfizer	CP5/CP8/ClfA	*S. aureus* vaccine	Prevention of infections	Phase I/II	[92]
StaphVAX	Nabi Biopharmaceuticals	CP5/CP8	*S. aureus* vaccine	Prevention of infections	Phase III, failed	[50]
STEBVax	National Institute of Allergy and Infectious Diseases	SEB	*S. aureus* vaccine	Treatment for toxic shock syndrome	Phase I	[193]
V710	Merck	IsdB	*S. aureus* vaccine	Prevention of infections	Phase III, failed	[194]
SA4Ag	Pfizer	CP5/CP8/ClfA/ MntC	*S. aureus* vaccine	Prevention of infections	Phase I, II, IIb	[195]
4C–Staph	GSK	HlaH35L/EsxAB/ FhuD2/Csa1A	*S. aureus* vaccine	Prevention of infections	Phase I	[196]
MEDI3902	MedImmune LLC	PcrV/Psl	*P. aeruginosa* antibody	Prevention of pneumonia	Phase II	[197]
KB001-A	KaloBios Pharmaceuticals	PcrV	*P. aeruginosa* antibody	Prevention of infections	Phase II, failed	[198]

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Drug name	Sponsor (s)	Target	Product class	Indication	Development stage	Ref.
PseudIgY	Immunsystem AB	Unknown	*P. aeruginosa* antibody	Prevention of infections	Phase I/II	[199]
KBPA-101	Kenta Biotech Ltd	O-polysaccharide	*P. aeruginosa* antibody	Treatment of infections	Phase I/II	[200]
IC43	Valneva Austria GmbH	OprF/OprI	*P. aeruginosa* vaccine	Prevention of infections	Phase II/III	[201]
Aerugen	Crucell	O-polysaccharide	*P. aeruginosa* vaccine	Prevention of chronic CF infection	Phase III, failed	[140]
Flagella		Subtype-a and subtype-b flagellin	*P. aeruginosa* vaccine	Prevention of chronic CF infection	phase III	[131]
MEP	Univax Biologics	MEP antigen	*P. aeruginosa* vaccine	Prevention of chronic CF infection	Phase I	[142]
Pseudostat	Provalis PLC	Inactivated *P. aeruginosa* strain 385	*P. aeruginosa* vaccine	Prevention of chronic CF infection	Phase I	[145]

CP5/8: serotype 5/8 capsular polysaccharides; ClfA: clumping factor A; PNAG: poly-N-acetyl glucosamine; SdrG: serine-aspartate repeat-containing protein G; SEB: Staphylococcal enterotoxin serotype B; IsdB: iron-regulated surface determinant protein B; MntC: manganese transport protein C; HlaH35L: α-Hemolysin H35L; EsxAB: ess extracellular A/B; FhuD2: ferric hydroxamate-binding lipoprotein; Csa1A: conserved staphylococcal antigen 1A; PcrV: Low calcium response locus protein V; OprF/OprI: Major outer membrane porin F/I; MEP: mucoid exopolysaccharide.

Table 1. Antibodies and vaccines for *S. aureus* and *P. aeruginosa* in clinical development.

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structures of Gram-positive and Gram-negative bacteria that are a key for design and development of antibodies and vaccines to target against these MDR bacterial infections.

Monoclonal antibodies (mAbs) have advantages over traditional chemotherapy in that (1) mAbs can bind target antigen specifically and thus reduce off-target side effects associated with traditional chemotherapy; (2) through Fc neonatal receptor (FcRn) recycling mechanism, mAbs have long serum half-life (ranges in days to weeks) when compared to chemotherapy (ranges in minutes to hours); (3) mAbs can recruit effectors for antibody-dependent cell-mediated phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) through its Fc region, which functions are missing in chemotherapy [3]. By 2015, more than 60 monoclonal antibodies (mAbs) have been approved by the United States Food and Drug Administration to treat cancer, autoimmune disorders, and infections [4].

To conquer the serious antibiotic resistance from bacterial pathogens, passive immunization (mAb treatment against bacterial pathogen) and active immunization (vaccine against bacterial pathogen), as alternative strategies, are being actively explored.

In this chapter, we focus on the current status of antibody and vaccine development against Gram-positive strains (*S. aureus* and *Enterococci*) and Gram-negative strains (*P. aeruginosa* and *A. baumannii*). Antibodies and vaccines under clinical trials are summarized in **Table 1**.

2. Antibody and vaccine development against *S. aureus*

S. aureus establishes infection through a variety of complicated mechanisms. *S. aureus* produces cell envelope-associated proteins, nonprotein glycopolymers, a collection of secreted toxins that mediate host-microbe adhesion, host cell lysis, antibody function interference, complement activation inhibition, and invasion of immune nonprofessional phagocytes [5, 6].

2.1. Antibodies against staphylococcal-secreted virulent factors

2.1.1. Staphylococcal superantigens as antibody targets

S. aureus is a round-shaped, facultative anaerobe, which can produce an array of superantigens (SAgs), including staphylococcal exotoxins, enterotoxins, and toxic shock syndrome toxin 1 (TSST-1). These toxins exert their hyper-stimulatory properties and cause food poisoning, toxic shock syndrome, acute lung diseases, and autoimmune diseases [7–10]. The superantigenicity of SAgs is largely achieved by the activation of APCs and T cells, leading to a massive release of cytokines, including IL-1β, IL-6, and TNFα [11].

Staphylococcal enterotoxin serotype B (SEB) was classified as a category B select agent by the Centers for Disease Control and Prevention (CDC) due to its high toxicity to human and potential use as a biological weapon [12]. Several mAbs targeting on SEB are under investigation. A high-affinity SEB-specific mouse mAb, 20B1, was investigated in mouse models with superficial skin, sepsis, or deep-tissue infections [13]. Treatment of 20B1 significantly increased the

survival in the sepsis model, whereas it reduced bacterial burden and dissemination of bacteria in the superficial skin model. Moreover, 20B1 was shown to decrease pro-inflammatory cytokine levels and T cell proliferation. Remarkably, their following work further showed that isotype switching from original IgG1 to IgG2a, without changing of SEB binding affinity, greatly enhanced the protective ability in *S. aureus* sepsis models [14]. This is consistent with a recent report in which humanized anti-SEB mAbs attenuated virulence of exogenous SEB expressing *S. aureus* in a mouse pneumonia model [15].

In addition, Tilahun and colleagues explored the use of combined mAbs targeting on different epitopes of SEB, as well as co-administration of mAb and antibiotic, both of which showed synergistic protection in *S. aureus* infection mouse model [16, 17]. This strategy seems promising as synergistic protection by co-administration of two mAbs recognizing distinct SEB epitopes was also observed independently in another study [18]. To date, there are not any anti-SEB mAbs being tested in clinical trials. Of note, a phase I clinical study of safety of a recombinant SEB vaccine (STEBVax) against toxic shock syndrome has been completed [19].

TSST-1 is a 22 kDa monomeric protein, of which the N-terminal domain binds to the MHC-II on APCs and the C-terminal domain is implicated in β-chain variable region of TCR (TCR-Vβ) interaction [20, 21]. In a recent report, human single chain variable fragments (scFvs) against recombinant TSST-1 were panned out from synthetic human scFv library by phage display technology [22]. The scFvs were demonstrated to be able to inhibit TSST-1–mediated T cell activation and pro-inflammatory cytokine production. Besides, a recombinant TSST-1 vaccine (Biomedizinische Forschungs gmbH) has been completed in phase I clinical study and proved to possess a good safety profile with no observable severe adverse events occurred [23, 24].

2.1.2. α-Hemolysin as antibody target

S. aureus releases a number of cytolytic toxins, among which the pore-forming α-hemolysin (Hla, α-toxin) is the most studied one. Hla is secreted as a 33 kDa monomer consisting almost entirely of β-strands by circular dichroism [25]. It exerts cell lytic activity through a membrane perforating mechanism, which is initiated through binding to membrane lipid or/and its proteinaceous receptor, a disintegrin and metalloprotease 10 (ADAM10) [26]. In detail, Hla monomers assemble into a heptameric structure on susceptible host cell membrane and form a central pore of approximately 1–3 nm in diameter [27, 28]. This allows rapid egress of Ca^{+}, ATP, and low molecular weight molecules through the pore, resulting in alteration of cellular signaling pathways and cell lysis [29–31].

Therapeutic anti-Hla mAbs have been actively developed due to the key role of Hla in Staphylococcal pathogenesis. In a study in which a recombinant Hla, AT62, was used as a vaccine, the study also showed that passive immunization of anti-AT62 IgG reduced wound infection and tissue damage in a mouse model [32]. In a *S. aureus* dermonecrosis model, combined administration of Hla-targeting mAb, MEDI4893*, with frontline antibiotic linezolid or vancomycin, exhibited enhanced protection by reduced lesion size, reduced tissue damage, and accelerated healing in a synergistic manner [33]. Furthermore, MEDI4893 (MedImmune) was generated from MEDI4893* by introducing three amino acids substitution (M252Y/S254 T/T256E) [33]. The YTE mutation

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has been shown to extend half-life by two- to fourfold without affecting distribution properties [34]. MEDI4893 not only abrogated Hla-host cell interaction but also potentially blocked oligomer formation due to steric hindrance [35]. Recently, a phase I clinical trial was completed by evaluating the safety, tolerability, and pharmacokinetics of MEDI4893 in healthy adult subjects [36]. Currently, a phase II study is ongoing to evaluate the safety and efficacy of MEDI4893 in the prevention of *S. aureus* pneumonia [37].

2.2. Antibodies against staphylococcal surface-associated components

2.2.1. Capsular glycopolymer as antibody target

Bacterial capsule is a polysaccharide layer lying outside of the cell wall found in both Gram-positive and Gram-negative bacteria. Capsule produced by pathogens has been involved in promoting adherence, resisting bacterium from host immune attack, and mediating release of virulent factors [38]. Encapsulation of *S. aureus* prevents bacterial phagocytosis by interfering with effective opsonization [39].

Serotype 5 (CP5) and serotype 8 (CP8) capsular polysaccharides predominate among *S. aureus* clinical isolates, representing 75–80% of total isolates [40]. While several CP5 or CP8-specific mAbs were studied [41, 42], serum containing antibodies that recognize the shared epitope of CP5 and CP8 were recently developed [43, 44]. The cross-reactivity was confirmed *in vitro* and the sera were demonstrated to promote opsonophagocytic killing of both CP5 and CP8 *S. aureus* strains. There are no reports on therapeutic antibodies targeting staphylococcal polysaccharide in clinical trials. However, two vaccines, StaphVAX and Altastaph (Nabi Biopharmaceuticals), have been completed for their clinical studies for safety and immunogenicity evaluation [45–48]. Although Altastaph was able to induce significant elevation of anti-CP5 and anti-CP8 antibody levels, unfortunately, it failed to show efficacy in a phase II clinical trial [49]. StaphVAX also showed ineffectiveness in the reduction of *S. aureus* in patients on hemodialysis and thus failed in a phase III trial [50].

Bacterial poly-N-acetyl glucosamine (PNAG) is another major class of surface polysaccharide that has been evaluated as a vaccine. PNAG, which is synthesized by enzymes encoded in intercellular adhesin (ica) locus, contributes to biofilm formation, colonization in host tissue, and immune evasion [51, 52]. Recent work showed that deacetylation of PNAG (dPNAG) by surface protein, IcaB, is a critical step for PNAG association to cell wall and plays key roles in colonization and resistance to host immune defense [53]. Indeed, antibodies specific to dPNAG were better in opsonic killing than that specific to PNAG [54]. In consistence, passive immunization of mice with antisera raised to dPNAG showed efficient clearance of *S. aureus*, compared with that raised to acetylated form [55].

2.2.2. Staphylococcal protein A as antibody target

Staphylococcal protein A (SpA) is anchored to *S. aureus* cell wall by sortase A through amide linking of its C-terminal threonine of LPXTG motif to pentaglycyl crossbridge within peptidoglycan [56]. SpA interferes with immunoglobulin (Ig) function by binding to Fcγ domain

of Ig and prevents the bacterium from opsonophagocytic killing [57]. It also interacts with B cell receptor through binding with VH3-clan of antigen-binding fragment (Fab) region and induces supraclonal B cell responses, resulting in insufficient adaptive responses against infection [58–60].

Based on the mechanistic studies, a mutated form of SpA, SpA(KKAA), was generated to abolish both Fcγ and Fab binding abilities [61]. Vaccination of SpA(KKAA) was able to elicit robust antibody responses against multiple staphylococcal antigens in a MRSA-infection mouse model. In their following studies, passive immunization of antibodies specific for SpA(KKAA) significantly promoted opsonophagocytic clearance, reduced abscess formation, and decreased the mortality [62]. Furthermore, a humanized version successfully conferred protection against *S. aureus* sepsis in neonatal mice [63].

2.2.3. Clumping factor A as antibody target

Microbial adhesion to host tissue is crucial to infection initiation in most of the bacterial infections. Microbial surface component recognizing adhesive matrix molecules (MSCRAMM), such like clumping factor A (ClfA), plays a vital role in this process [64]. ClfA, a fibrinogen-binding protein, is required for establishing early infection, abscess formation, protection against phagocytosis, as well as bacterial persistence in host [65, 66].

Tefibazumab, a humanized anti-ClfA mAb, was developed and exhibited high affinity and specificity for ClfA [67]. *In vitro* study showed that tefibazumab inhibited fibrinogen-binding ability of ClfA and protected against MRSA infection in murine septicemia and rabbit infective endocarditis models. Safety and pharmacokinetic profile of tefibazumab were evaluated in phase II clinical trial [68]. Unfortunately, it failed to show significant differences between treatment and placebo groups in overall adverse clinical events. A detailed analysis of ClfA-fibrinogen structure observed a modest IC50 value of binding between ClfA and tefibazumab, which might partly explain the unsatisfactory clinical outcome [69].

2.2.4. Autolysin as antibody target

Autolysin (Atl) is a cell wall-associated enzyme with various functions. The major *S. aureus* autolysin (AtlS) contains two distinct domains, amidase and glucosaminidase, which are responsible for enzyme localization to cell wall and peptidoglycan hydrolysis, respectively [70, 71]. Atl participates in biofilm formation, separation of daughter cells after cell division and attachment to host matrix [72]. Moreover, AtlS is highly conserved among strains of *S. aureus* and other *Staphylococci*. These features together make AtlS an attractive target for anti-*S. aureus* mAb and vaccine investigation.

To test it, a mAb, 1C11, was generated to inhibit AtlS glucosaminidase domain and its effect in animal model was assessed [73, 74]. The mAb was shown to impair cell growth and cause cell aggregation and sedimentation in *in vitro* assay. Following this study, administration of 1C11 reduced severity of implant-associated osteomyelitis in a mouse model by decreased abscess numbers and efficient internalization of antibody-opsonized *S. aureus*.

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Immunodominant staphylococcal antigen A (IsaA) is another highly conserved Atl. Similarly, protection was conferred by a mAb specific to IsaA in a mouse model [75]. The mode of action of mAb is mainly through activation of professional phagocytes and induction of oxidative burst activity of neutrophil.

2.3. Antibodies against staphylococcal cell wall components

2.3.1. Lipoteichoic acids as antibody target

Most Gram-positive bacteria produce teichoic acids (TAs) to facilitate their survival under disadvantageous conditions. Teichoic acids covalently link to either peptidoglycan or cytoplasmic membrane, known as wall teichoic acids (WTA) and lipoteichoic acids (LTA), respectively [76]. The roles of TAs in pathogenic bacteria include adherence to host cells [77], activation of complement [78], and cytokine induction [79].

Since structures of LTA are highly conserved across many clinical isolates, including *Enterococci*, *Staphylococci*, and several *Streptococci*, LTA is considered as a promising target for vaccine and therapeutic antibody development [80]. In a recent report, antibodies against *E. faecalis* LTA were used to test cross-activity with other Gram-positive bacteria, including *S. aureus* [80]. The *in vitro* data showed that the antibodies were also able to bind with LTA purified from *S. aureus*. Remarkably, the antibodies exhibited 60–90% opsonophagocytic killing activity across a variety of *S. aureus* strains, and great protection against MRSA infection in a mouse peritonitis model. In accordance with the observation, immunization with a BSA-conjugated LTA fragment, containing a conserved minimal structure in majority of Gram-positive bacteria, was able to induce opsonic killing of *E. faecium* E1162 and *S. aureus* MW2 [81]. Besides, immunization of WTA also elicited an anti-WTA immune response, illustrated by complement-dependent opsonophagocytosis [82, 83].

2.3.2. Peptidoglycan as antibody target

Peptidoglycan (PG) is composed of cross-linked polysaccharide and peptide chains, which forms the backbone of bacterial cell wall. So far, reports on therapeutic antibody or vaccine targeting on PG are scarce. A mAb against deacetylated peptidoglycan, ZBIA5H, was screened with best protective property in mouse models [84]. Surprisingly, ZBIA5H did not show the highest affinity to PG, compared with other mAbs. The superior property of ZBIA5H may be attributed to the unique epitope it recognizes. This study highlights that besides antigen binding affinity, other factors, such as epitope, should also be taken into consideration in therapeutic antibody discovery.

2.4. Antibodies against nutrient transporter proteins

Nutrient acquisition is one of the most basic and essential process virtually in all forms of life. Vertebrate host has evolved powerful strategy, termed nutritional immunity, to limit proliferation of invading pathogens by sequestering essential nutrients [85]. One of the best characterized examples of nutritional immunity is transition-metal-ion sequestration in which

metal ions are predominantly trapped by host metal-binding proteins [86]. To combat with host defensive system, microorganism employs mechanisms to maintain intracellular metal homeostasis. Therefore, these mechanisms could be suitable targets for therapeutic antibody development. For example, an Fab was screened to inhibit acquisition pathway for Mn(II), which is essential for detoxification of reactive oxygen species (ROS) [87, 88]. The mAb is bound to manganese transporter C (MntC) of an ATP-binding cassette (ABC) transporter system and thereby blocks the metal delivery to the channel. *In vitro* assay showed that the Fab increased the sensitivity of *S. aureus* to ROS by over 10-fold.

An earlier report identified ABC transporter as the most commonly associated protein with IgG from the sera of 26 patients suffered with septicemia [89]. ScFvs against the conserved peptides from the ABC transporter were then panned from a phage display library and were shown to reduce the bacterial burden in a mouse model.

2.5. Multicomponent vaccines

So far, neither passive nor active immunization has shown potent efficacy on humans. The failure from basic research to clinical practice could partly be attributed to the limited understanding of the sophisticated events associated with every stage of infection. Prior strategies targeting on single virulent factor showed efficacy only in certain experimental settings. In this regard, novel vaccine formulations targeting on multiple pathogenic components are proposed to offer protection from distinct aspects through a synergistic working mode.

Recently, efficacy of a combination vaccine, 4C–Staph (four-component *S. aureus* vaccine), was evaluated [90]. 4C–Staph is composed of detoxified α-Hemolysin, a fusion of ess extracellular A (EsxA) and ess extracellular B (EsxB), two staphylococcal surface proteins, which are ferric hydroxamate-binding lipoprotein (FhuD2) and conserved staphylococcal antigen 1A (Csa1A). 4C–Staph induced broad and synergistic protection against several *Staphylococcal* clinical isolates in different models. In addition, mechanistic study showed that the protection was mainly antibody dependent.

SA3Ag (Pfizer), a tri-component vaccine, consists of CP5 and CP8 individually linked with a nontoxic form of diphtheria toxin, and a recombinant mutant form of clumping factor A (rClfAm) [91]. A phase I clinical trial was completed to evaluate safety, tolerability, and effect of SA3Ag [92]. This vaccine showed a relatively safe profile among older and young adults.

In order to further enhance protection against *S. aureus*, another component, MntC, was added to SA3Ag to form a four-component vaccine SA4Ag (Pfizer) [93]. In phase 1/2 clinical trials, single-dose administration of SA4Ag was well-tolerated among young and older adults, shown by mild or moderate local reactions and comparable systemic events with placebo control [94, 95]. More excitingly, SA4Ag induced a rapid, robust, and sustained functional antibody response.

2.6. Antibody-antibiotic conjugate

While *S. aureus* has classically been considered as an extracellular pathogen, a growing body of evidence reveals that it is capable to survive and persist within host cells, including phagocytic cells, which are responsible for bacterial clearance [96, 97]. Although phagocytic cells,

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particularly neutrophils and macrophages, can efficiently kill majority of invading bacteria, a small population of persisters can however turn the circulating phagocytes to "Trojan horses" to facilitate bacterial dissemination via bloodstream [98]. Meanwhile, intracellular persistence allows bacteria to escape from antibiotic and immune attack. Indeed, most of the current antibiotics are less efficient in intracellular *S. aureus* killing, which may partly explain the poor response to treatment and the high frequency of recurrence in clinical practice [99, 100].

Based on these findings, therapies specifically targeting on intracellular pathogen may promote clinical outcome. Similar to antibody-drug conjugate (ADC), which has been successfully applied for cancer therapy, antibody-antibiotic conjugate (AAC) was first proposed and evaluated by Lehar and his colleagues in 2015 [101]. The AAC is composed of three building blocks: an antibody to target on bacteria, a highly bactericidal antibiotic payload, and a linker to attach antibiotic payload to the antibody. The AAC was designed with no antibacterial activity as antibiotic serves as a prodrug when covalently linked. However, when planktonic AAC-tagged bacteria are internalized by host cells, the antibiotics can be efficiently released in their active form by cleavage from host protease. Thus, the AACs take bacteria as "Trojan horses" to deliver potent antibiotics to cytoplasmic compartment and resulting in intracellular antibacterial effect. To their anticipation, the AAC was shown to efficiently restrict intracellular *S. aureus* growth when treatment was initiated several hours after intravenous infection. In contrast, poor efficacy was observed by delayed treatment of vancomycin. This result is particularly interesting as majority of bacteria were found to associate with neutrophils within 10–15 minutes [97]. Moreover, the AAC was able to limit metastasis of *S. aureus* to brain in an intravenous infection model.

3. Antibody and vaccine development against *E. faecium*

Different from *S. aureus,* which produces an array of virulent factors, pathogenesis of *Enterococci* is largely determined by their adherence to host tissue mediated by surface adhesion components. Several most-studied components include aggregation substance proteins, collagen adhesins, enterococcal leucine-rich repeat-containing proteins, pili, polysaccharides, and glycolipid [102], which are potential targets for antibody and vaccine development.

3.1. Enterococcal pili as antibody target

Enterococcal surface pili are filamentous proteins with Ig-like folds and LPXTG motifs, which have been implicated in biofilm formation, endocarditis, and catheter-associated urinary tract infections (CAUTIs) [103, 104]. Endocarditis and biofilm-associated pilus A (EbpA), one of the most-studied pili in *Enterococci*, is widely present among *Enterococcal* species and highly conserved in N-terminal domains [105]. In detail, N-terminal domain of EbpA (EbpANTD) binds to host fibrinogen deposited on urinary catheter to facilitate Enterococcal colonization [106]. Sera against EbpANTD was recently shown to provide universal protection in a murine model by reducing bacterial titers of a broad spectrum of Enterococcal isolates, including *E. faecalis*, *E. faecium,* and VRE [105]. Consistently, vaccination of EbpA or EbpANTD, but not its carboxyl-terminal domain, diminished biofilm formation and prevented CAUTIs in *E. faecalis* infection model [106].

3.2. Polysaccharide antigens as antibody targets

Based on a previous serotyping analysis, about 60% of *E. faecalis* isolates fall into four serotypes from CPS-A to CPS-D [107]. CPS-C and CPS-D can express capsular polysaccharide, whereas CPS-A and CPS-B are nonencapsulated due to deficiency of essential gene locus [108]. In an early study, antibodies raised against LTA from CPS-A strain only opsonized acapsular CPS-A and CPS-B strains, but not encapsulated ones [109, 110]. To develop antibodies against capsule-bearing CPS-C and CPS-D strains, a novel diheteroglycan was identified from capsular polysaccharide [110]. As a result, passive immunization of anti-diheteroglycan antibodies successfully protected CPS-C and CPS-D *E. faecalis* bacteremia mouse model. However, it was observed that considerably lower susceptibility of CPS-C and CPS-D strains to opsonic killing by naturally acquired antibodies was present in healthy human sera as compared with CPS-A and CPS-B [111]. Therefore, capsule may be a natural barrier to access therapeutic antibody by masking antigens underneath.

3.3. Lipoproteins as antibody targets

A transcriptomic analysis from an *E. faecalis* infection mouse model identified two ABC transporter substrate-binding lipoproteins upregulated upon infection: PsaAfm for manganese transport and AdcAfm for zinc transport [112]. Treatment of antibodies raised from recombinant proteins showed increased opsonic killing *in vitro* and reduced colony counts in a mouse bacteremia model. Protective role was also seen in treatment with antibodies against distinct ABC transporter proteins [113], suggesting the potential of ABC transporter as a therapy target in enterococcal infection.

4. Antibodies and vaccines against *P. aeruginosa*

Effective control of *P. aeruginosa* infections remains a challenging problem due to its remarkable ability to evolve resistance to many antibiotics. Antibodies and vaccines are considered to be a promising and alternative strategy to treat or prevent *P. aeruginosa* infections in susceptible populations. The identified *P. aeruginosa* antibody and vaccine targets include the lipopolysaccharide (LPS) O-antigens, pilus, flagella, alginate, outer membrane proteins (OMPs), mucoid exopolysaccharide (MEP), and antigens from the type III secretion system (T3SS) [114].

4.1. Antibody and vaccine development against T3SS translocation protein PcrV

Type III secretion system (T3SS), as a key virulence determinant in *P. aeruginosa*, is encoded by at least 42 genes and assembled as a needle-like apparatus that can directly inject bacterial effector proteins into host cell to elicit pathological response [115]. PcrV is located at the tip of needle-like apparatus and closely involved in translocation of effector proteins from *P. aeruginosa* to host cell [115].

Fab 1A8, a human Fab antibody fragment, can specifically target against *P. aeruginosa* PcrV antigen and elicit protective effects for mice with lethal pulmonary *P. aeruginosa* challenge

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[116]. KB001, a PEGylated anti-PcrV Fab fragment in clinical phase-2a trial for ventilator associated and *P. aeruginosa* colonized but not for infected patients in intensive care units (ICUs), showed good safety, tolerability, and pharmacokinetic profile. Although statistical significance was not observed for patients with KB001 treatment and placebo treatment, incidence of *P. aeruginosa* pneumonia was decreased in KB001 treatment group (31%) as compared to that of placebo treatment group (60%) [117]. Identification of anti-PcrV IgG from human sera confirms that PcrV is a vaccine target [118]. Moreover, human high titer anti-PcrV sera clearly have prophylactic effect for mice with lung *P. aeruginosa* infection [118].

4.2. Antibody and vaccine development against PsI

By construction and phenotypic screening of human scFv phage display libraries from peripheral blood B cells of healthy individuals and patients recovered from recent *P. aeruginosa* infections, mAbs against one epitope of Psl, the exopolysaccharide important for *P. aeruginosa* attachment to host cell and biofilm maintenance, was identified to show potent protection in several animal *P. aeruginosa* infection models [119]. Also, this finding suggests that PsI can be used as a vaccine target. However, most patients suffered from *P. aeruginosa* bloodstream infection (BSI) had low anti-PsI titer that showed nonprotective to *P. aeruginosa* BSI infection [120]. MEDI3902, the combination of anti-PsI and anti-PcrV in a bispecific format, showed synergistic protection against *P. aeruginosa* murine pneumonia models as compared with each parental mAb [121]. Moreover, MEDI3902 can synergize several classes of antibiotics for the treatment of clinical antibiotics resistant isolates [121].

4.3. Antibody and vaccine development against outer membrane proteins (OMPs)

OMPs form porins and other structural and functional components on the bacterial cell surface. CFC-101, a mixture of OMPs from *P. aeruginosa*, was used to immunize healthy human volunteers in a phase I/IIa clinical trial [122]. CFC-101 was safe and immunogenic in eliciting human mAbs after immunization that can passively protect mice from lethal *P. aeruginosa* challenge [122].

OprF and OprI are the major OMPs that are surface-exposed and conserved in wild-type strains of *P. aeruginosa* [123]. In phase I human trials, OprF-OprI vaccine (IC43) conjugating with aluminum hydroxide was safe and induced specific antibodies in healthy volunteers and burn patients by intramuscular administration [124, 125]. Intranasal immunization of OprF-OprI vaccine followed by systemic boost elicited a long-lasting systemic and local lung mucosal antibody response in patients with chronic pulmonary diseases [126]. Recently, phase II study on ICU *P. aeruginosa* infection showed that IC43 also produced a significant immunogenic effect without mortality or safety concerns [127].

4.4. Antibody and vaccine development against flagellins and pilins

Flagella are essential for motility, chemotaxis, invasiveness, and adhesion of *P. aeruginosa* to activate host inflammatory responses [128]. Flagellin is the primary protein component of flagella and consists of subtype a and subtype b [129].

A monovalent *P. aeruginosa* flagella vaccine was safe and immunogenic in healthy human adults by intramuscular immunization and showed high and long-lasting serum antibody (IgG and IgA) titers against flagella positive *P. aeruginosa* [130].

Then, a bivalent flagella vaccine, containing some of the flagella subtype antigens (a0a1a2 and b), was evaluated over a 2-year period on cystic fibrosis (CF) patients not colonized with *P. aeruginosa* in phase III trial. The vaccine lowered the risk of patients for initial infection as compared with that from the placebo group, though not statistically significant. Therefore, multivalent vaccine against *P. aeruginosa* flagella subtypes a and b is needed to improve overall efficacy of vaccine to more flagella subtypes [131]. A multivalent protein fusion vaccine consisting of flagellin subtype a and b, Oprl and OprF epitope 8, was used to immunize mice that induced specific IgGs against each individual antigen [132]. Although these IgGs elicited potent ADCC and increased clearance of nonmucoid *P. aeruginosa*, which reflect the initial colonization of *P. aeruginosa*, they were less effective for mucoid *P. aeruginosa*, which represent the colonized and chronic *P. aeruginosa* biofilm formation [132]. Conjugation vaccine of flagellin subtype a (FLA) with polymannuronic acid (PMA) built from mannuronic acid, the major component of alginate and biofilm, induced protection against mucoid *P. aeruginosa* in mice and rabbits [133].

Pili, as one key virulent factor, are filaments of pilin polymers located at the pole of *P. aeruginosa* and are responsible for adhesion of *P. aeruginosa* to host epithelial surfaces and twitching motility [134, 135]. A disulfide loop (DSL) at the C-terminal of pilin is the major epitope in bridging adherence of *P. aeruginosa* to host cell [134, 135]. Single copy of DSL was not an effective immunogen in mice, whereas multi-copy of DSL peptides increased IgG response 1000 times [136]. Immunization of mice with full length pilin of *P. aeruginosa* induced mAbs that inhibited pili-mediated epithelial cell adhesion [137].

4.5. Antibody and vaccine development against LPS

LPS is the major component of the outer membrane of *P. aeruginosa*. LPS has two types, smooth or S-type and rough or R-type. S-type LPS consists of O-polysaccharide (O-antigen) repeats linked with a core-conserved oligosaccharide and a lipid A moiety, while R-type LPS lacks O-antigen and only contains the core oligosaccharide [138]. The S-type LPS is involved in nonmucoid and in early stage of *P. aeruginosa* infection in CF patients, whereas the R-type LPS is associated with mucoid and late stage of *P. aeruginosa* infection in CF patients [139]. The O-antigen is immunogenic in the host for the induction of protective antibodies, whereas lipid A is the core endotoxic component for induction of inflammatory responses [138]. More than 20 serotypes of O-antigens have been identified [138].

Pseudogen, a heptavalent O-antigen vaccine, showed efficacy in nonrandomized trials among adult cancer and burn patients in preventing fatal *P. aeruginosa* infections but no benefit in leukemia and CF patients [139]. Furthermore, Aerugen, an octavalent vaccine, was developed by conjugating purified O-antigens from eight *P. aeruginosa* strains with exotoxin A. This vaccine induced high levels of specific opsonizing antibodies in CF patients and significantly reduced the frequency of chronic infection for 10 years without apparent adverse effects in a nonblind trial. However, a subsequent double blind, randomized, placebo-controlled phase III trial failed to confirm the initial positive results and the further development of this vaccine was suspended [140].

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4.6. Antibody and vaccine development against alginate

Alginate or mucoid exopolysaccharide (MEP), a linear polymer of partially acetylated D-mannuronic acid and L-guluronic acid, is the major component of the *P. aeruginosa* biofilm matrix and thus critical in persistence of the bacteria in the CF lung [141]. MEP is relatively conserved between strains, which makes it an attractive vaccine antigen for CF patients. A high molecular weight MEP vaccine elicited long-lived opsonic antibodies in 80–90% of the volunteers in phase I trial [142]. MEPs conjugated to various carrier proteins successfully enhanced the MEP-specific immune responses and elicited opsonizing antibodies against heterologous MEPs in mice and rabbits [143]. However, a successful clinical product has not yet been developed, indicating that vaccine of MEP alone may not be sufficient for potent immunization in human and conjugation with other vaccine targets may be considered.

4.7. Inactivated whole-cell vaccine and antibody development against *P. aeruginosa*

Whole cell-inactivated vaccines contain multiple bacterial antigenic components and thus can potentially induce diverse immunologic responses against various targets of *P. aeruginosa*. Oral immunization of bronchiectasis patients with an enteric-coated whole-cell killed vaccine resulted in significant reduction of *P. aeruginosa* in the sputum by specific lymphocyte responses [144]. Oral immunization of healthy volunteers with killed *Pseudomonas* vaccine was safe and increased *Pseudomonas*-specific serum antibodies, most notably IgA, and promoted phagocytosis elimination of *P. aeruginosa* [145]. Whole cell inactivation by X-ray irradiation kept antigen expression functional but inhibited replication in *P. aeruginosa* [146]. Mice immunized with this vaccine showed statistically significant protection against *P. aeruginosa* challenge in acute pneumonia model via opsonic killing, recruitment of CD4+ T lymphocytes and neutrophil cells [146].

4.8. Antibody and vaccine development against exotoxin

Exotoxin A is a key virulence factor secreted by around 90% *P. aeruginosa* clinical isolates and around 10,000 times more lethal than LPS [147, 148]. Exotoxin A is an ADP-ribosyltransferase and can kill macrophages, polymorphonuclear leukocytes, and other immune-related cells by receptor-mediated endocytosis and inhibition of protein synthesis elongation factor 2 [148].

mAbs against two epitopes of exotoxin A after immunization of rabbits showed potent inhibition of exotoxin A-induced cytotoxic activity *in vitro* [149]. Furthermore, these mAbs showed protective effects against *P. aeruginosa* infection for mice after immunization and enhanced the survival rate of mice model when antibiotic amikacin was combined [150]. Similarly, immunization of mice with exotoxin A showed 93.8% protection efficacy against mice burn and *P. aeruginosa*-challenged models when compared with unimmunized mice group that all died within the 70-day period [151].

Chimeric vaccine composed of a nontoxic (active-site deletion) exotoxin A and a key pilin fragment sequence was used to immune rabbits subcutaneously [152]. The produced antibodies could target against both pilin to weaken *P. aeruginosa* adherence and exotoxin A to neutralize its cytotoxic activity *in vitro* [152]. Intranasal immunization of chimeric vaccine (pilin and exotoxin A) in

mice elicited serum and saliva immune responses [153]. Moreover, saliva samples contain antibodies that can inhibit pilin-dependent *P. aeruginosa* adherence and neutralize exotoxin A [153]. This approach of immunization may be useful to provide protection against *P. aeruginosa* early-stage adhesion and infection via oropharyngeal airway [153].

5. Antibody development against *A. baumannii*

5.1. Iron-regulated outer membrane proteins (IROMP) as antibody and vaccine target

Iron is essential for bacteria to survive within host. Bacteria have evolved several ways to compete with host for iron uptake. Expression of iron-regulated outer membrane proteins (IROMPs) in bacteria is one such way. IROMPs, with molecular weight ranging from 77 to 88 kDa, are a class of specific cell surface receptors that can bind iron chelator siderophore with high affinity and subsequently lead to the internalization of iron-loaded siderophore and iron assimilation in *A. baumannii* [154, 155]. Goel et al. [155] used IROMPs from *A. baumannii* to immunize BALB/c mice and identified several mAbs of IgM isotype that can block interaction of siderophore with IROMPs and induce bactericidal and opsonizing activity *in vitro*.

5.2. Inactivated whole cell, outer membrane complexes (OMCs), and outer membrane vesicles (OMVs) as vaccine and antibody target

Immunization of mice with inactivated whole *A. baumannii*, prepared from formalin-treatment, elicited protective antibody response against *A. baumannii* post-infection challenge in mice sepsis model [156]. Subsequently, these antibodies separated from immunized mice sera also showed passive protection against mice with *A. baumannii* infection [156]. As inactivated whole *A. baumannii* vaccine contains LPS (endotoxin) that may complicate immune responses after immunization, LPS-deficient and inactivated whole *A. baumannii* cell was used to immune mice [157]. Similar humoral and cellular immune responses was observed as compared with wild-type inactivated whole *A. baumannii* vaccine in protection against different mouse models with disseminated *A. baumannii* infections of various strains [157].

Vaccine made of outer membrane complexes (OMCs) from *A. baumannii* induced protective humoral and cellular immune responses against murine sepsis model [158]. Similarly, passive transfer of antiserum from immunized murine to naive mice rescued these mice from *A. baumannii* infection [158].

Outer membrane vesicles (OMVs), released from Gram-negative outer cell wall surface, have a diameter within the range of 50–250 nm and contain all constituents as Gram-negative outer cell wall, such as proteins, LPS, phospholipids, DNAs, and RNAs [159–161]. OMVs play important pathological roles by delivering virulence factors into host cell and coordinate group communications known as quorum sensing [160, 161]. High-dose challenging of mice with OMVs (200 μg) triggered a strong pro-inflammatory cytokine release that may be pathological to host [162].

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Interestingly, immunization of mice with low dose OMVs (10 μg) from one clinical MDR *A. baumannii* isolate induced clear protection against mice pneumonia and sepsis models after *A. baumannii* challenge [163]. The protective mechanism is in part from specific anti-OMV antibody induced opsonophagocytic activity and suppressed pro-inflammatory cytokine release [163].

Recently, OMVs were engineered as a delivery vehicle to package and display Omp22 at the OMV surface [164]. The displayed Omp22-OMV can induce high-titer anti-Omp22 specific antibodies and protect mice from sepsis after lethal *A. baumannii* challenge [164].

5.3. Targeting outer membrane protein A (OmpA)

Outer membrane protein A (OmpA), previously known as Omp38, is a lethal and most abundantly expressed surface virulence factor in *A. baumannii* [165, 166]. OmpA belongs to the porin family with low permeability that may be a key factor contributing to its multidrug resistance [167]. OmpA can bind with host cell directly, internalize within mitochondria and nuclei compartments of host cell, and induce host cell death [165, 166]. Moreover, OmpA is highly conserved within six clinical isolates (99% protein sequence identity) and 14 other NCBI GenBank deposited sequences from different isolates of *A. baumannii* (89% protein sequence identity), while OmpA shows no homology to human proteins [168].

Thus, OmpA from *A. baumannii* is a potentially ideal vaccine and antibody target.

In agreement with the sequence identity analysis, immunization of diabetic mice subcutaneously with recombinant OmpA induced markedly protective effect upon lethal, extreme drug resistant-*A. baumannii* challenge; use of antibodies against OmpA also elicited similar protective effect on diabetic mice with lethal *A. baumannii* infection [168]. Interestingly, dosage of *A. baumannii* rOmpA vaccine correlates with various B cell epitopes and immunodominant T cell epitopes, emphasizing dosage needs to be taken into account for vaccine development [169]. Recently, intranasal immunization of mice with OmpA can trigger both mucosal and systemic protective antibodies against MDR *A. baumannii* infection [170].

Omp22 is an outer membrane protein with molecular weight of 22-kDa. Omp22 is more than 95% conserved within 851 reported *A. baumannii* strains [171]. In contrast, there is no homology with human proteins. This unique and conserved sequence makes Omp22 an ideal vaccine candidate. Immunization of mice with recombinant Omp22 induced clear protection from MDR *A. baumannii* infections, showing a potential vaccine candidate [171].

FilF is a highly conserved outer membrane protein predicted to be involved in pilus assembly in *A. baumannii* [172]. Immunization of mouse pneumonia model induced high titer of antibody, decreased the bacteria lung burden, and rescued around 50% of mice from lethal *A. baumannii* infection [172]. These promising results may suggest that FilF is a promising vaccine candidate for further evaluation [172].

5.4. Biofilm related proteins as vaccine and antibody target

Biofilms are bacterial communities connected by a surface of extracellular matrix with complicated compositions that may vary based on different bacteria and different living

environments [173]. Identified biofilm components contain polysaccharides, proteins, and extracellular DNAs and play essential pathological roles in bacterial adhesion to host cell and shielding bacteria from nearby pressures such as antibiotics [173, 174].

Surface polysaccharide poly-beta-(1-6)-N-acetylglucosamine (PNAG), as a major component of biofilm, is a key virulence factor in *A. baumannii* [175]. Immunization of rabbit with conjugation of a synthetic oligosaccharide, mimicking PNAG, with tetanus toxoid induced antibodies that can opsonize clinical isolates of *A. baumannii* with surface expression of PNAG *in vitro* and protect *A. baumannii* challenged mice [176].

Biofilm-associated protein (Bap) in *A. baumannii*, 8620 amino acids in length, is one of the largest proteins identified within bacterial proteins and plays a vital role in biofilm formation [177]. Bap, containing seven tandem repeats of modules, is 41–66% conserved among clinical isolates and its expression is induced by low iron concentration [177, 178]. Immunization of mice with one region of Bap from *A. baumannii* elicited protective immunity against *A. baumannii* of different strains, suggesting that Bap is conserved and can be used as a potential vaccine candidate [179].

Ata, a trimeric transporter and a key virulence factor in *A. baumannii*, is essential in biofilm formation [180]. Rabbit sera from Ata vaccination can opsonize *A. baumannii* isolates effectively in complement and polymorphonuclear cells dependent manners [181]. Moreover, the rabbit sera can significantly lower the burden of mice lung infection from MDR *A. baumannii* strains, showing that Ata is one more potential vaccine target [181].

5.5. Targeting K1 capsular polysaccharide

K1 capsular polysaccharides are an important virulence factor that helps *A. baumannii* to establish infections within host [182]. Immunization of mice with sub-lethal and K1 capsular polysaccharide positive *A. baumannii* induced generation of specific anti-K1 capsular polysaccharide IgM monoclonal antibody (13D6) [183]. Moreover, 13D6 can induce efficient neutrophil-mediated in vitro opsonization and in vivo passive protectivity in rat soft tissue infection model [183]. However, only 13% of 100 collected *A. baumannii* strains were positive against 13D6, suggesting other capsular polysaccharide serotypes that may be unexplored. Additionally, lack of immunoglobulin class switch from IgM to IgG may not effectively trigger adaptive long-term immune memory response. Failure of class switching may be the inherent property of most capsular polysaccharides that only elicits a T cell independent immune response after immunization [184]. Thus, to target more *A. baumannii* strains effectively, identification of more capsular polysaccharide serotypes and conjugation of capsular polysaccharide with carrier proteins may be needed. As a matter of fact, this strategy has been successfully applied in clinics for the prevention of *Streptococcus pneumoniae* infection by the introduction of 23-valent nonconjugated and 13-valent conjugated capsular polysaccharide vaccines [185].

6. Concluding remarks

Antibody and vaccine are important treatment options in the mobilization of human immune system passively or actively to recognize, kill bacteria enemies, and moreover memorize these

enemies for the long-term protection. Antigen selection is the key for antibody and vaccine development, which needs to be immunogenic and conserved. Initially, antibody and vaccine development mainly focused on individual antigen. It is now clear that multivalent antigens should be more potent in eliciting immune responses against bacteria. Combination of pan-genomics, proteomics, and reverse vaccinology analysis of bacteria revealed a list of conserved antigens as potential vaccine or antibody targets and some of these antigens are already known as virulence factors of related bacteria [186, 187]. These bioinformatics-based "omics" analysis will undoubtedly facilitate effective vaccine and antibody target identification and development.

Other alternatives to antibiotics, including short antimicrobial peptides, antibiofilm peptides, and host defense peptides, are not covered in this chapter; readers can refer to a recent excellent review and references therein for further information [188].

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