

A Review of Designing New Vaccines to Prevent Hospital-Acquired Antibiotic-Resistant Infections

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Abstract

Hospital-acquired infections are one of the main challenges and concerns of patients and medical staff in hospitals and healthcare centers. Meanwhile, *Clostridium difficile* infection is one of the most important bacterial hospital infections. Prevention is the best and most effective way to deal with these infections. Designing and using vaccines against these infectious microorganisms is the best way of prevention. In this review, we evaluated 80 research articles and guidelines in the field of designing and developing new vaccines against nosocomial infections. The articles were collected from Google Scholar, PubMed, Research Gate, NCBI, CDC, and WHO databases. To date, considering the properties and virulence factors of each microorganism, some vaccines have been designed and made up from cellular complexes or recombinant proteins. The magnitude of vaccine effect varies by bacteria and strain type. Some vaccines have conferred high-levels of protection and immunity. However, there is still no vaccine against some bacteria, which has led to more in-depth research in this area. Although significant successful attempts have been made so far to design vaccines against the most important hospital-acquired infections, there is still an essential need to design and develop new vaccines against these infectious bacteria.

Keywords: Hospital-Acquired Infection, Vaccine, *Clostridium difficile*, Methicillin-Resistant *Staphylococcus aureus*, *Acinetobacter baumannii*

Introduction

Importance of vaccine design for hospital-acquired antibiotic-resistant bacteria strains

Edward Jenner made the first scientific attempt to prevent infectious diseases by vaccination in the 1790s (1). A vaccine is a biological preparation that provides active acquired immunity to a microbial or viral disease (2). A vaccine typically contains a mixture that is in the pathogen structure or is produced by it and is often made from weakened or killed forms of the microorganism, its toxins or one of its surface proteins (2, 3). Vaccination is proved to be the most effective method in preventing infectious diseases (4-7). Hospital-acquired infections (HAIs), also known as nosocomial infections, are infections, which patients acquire while referring to a hospital or any other healthcare facility

to get treatment for their underlying disease. The infectious pathogens may transmit from healthcare personnel and staff, other patients or the hospital environment. The signs and symptoms of nosocomial infections may manifest even after the patient has been discharged from the hospital. Evidence showed that there were an estimated 722,000 HAIs in United States hospitals in 2011; among that the most common infections were pneumonia (21.8%) with 157500 cases and gastrointestinal diseases (17%) with 123100 cases. Of these, about 75,000 patients with HAIs died during their hospitalizations (8). In 2012, according to the Health Protection Agency report, the prevalence of HAIs in England among the hospitalized patients was 6.4%, which is lower in comparison to of 8.2% in 2006; however, this prevalence rate is still considered high (9, 10). Direct and indirect contact with microbial agents, the susceptibility of patients,

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bacterial resistance, and other environmental factors are some of the most influential factors contributing to the development of HAIs. There are many different factors involved that make controlling HAIs a rather difficult endeavor (1). *Clostridium difficile*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), and *Acinetobacter baumannii* are among the most important microbial agents causing hospital infections (1, 4, 9, 11-14). There are multiple ways for the treatment of HAIs including prescribing combined antibiotics; but the treatment procedure is difficult, especially for bacterial strains resistant to antibiotics such as MRSA and *Acinetobacter* strains. Nevertheless, it could be said that preventing these infections, in comparison to treating them, is easier, more effective, less expensive and more accessible to both patients and the health care personnel. Appropriate hand hygiene and glove usage and subsequently disinfection and sterilization of surfaces, application of antibacterial combinations and vaccination of patients and medical health care personnel against infectious bacteria are among the simple methods for preventing HAIs. *C. difficile* is an anaerobic gram-positive bacterium that can create spores and is considered the main cause of diarrhea due to excessive antibiotic consumption and inflammation of the large intestine (colon). *Staphylococci* are gram-positive spherical bacteria, which are frequently found in the oral and respiratory tract and on the skin and are one of the common causes of respiratory infections such as sinusitis, skin infections and food poisoning (15-18). *Pseudomonas aeruginosa* is a common gram-negative, rod-shaped bacterium exhibiting highly increased resistance to antibiotics and can cause hospital-acquired pneumonia and septicemia (19). *A. baumannii* is a gram-negative coccobacillus, which almost exclusively isolated from hospital environments. It is an opportunistic nosocomial pathogen, which causes

a variety of infection including pneumonia and meningitis (20, 21). The prevalence of some pathogens are summarized in Table-1.

Materials and Methods

In this systematic overview, the data were collected and summarized from relevant databases including PubMed, Research Gate, NCBI, CDC, and WHO. The criteria for including studies and review articles were the focus on disease prevention methods especially through designing vaccines against microorganisms accounting for the majority of hospital-acquired infections. Other forms of immunizing, e.g. passive immunization using transferring active antibodies were excluded. Keywords of “*Clostridium difficile*,” “*Staphylococcus aureus*,” MRSA, “*Acinetobacter baumannii*,” “Vaccine,” “Nosocomial infection,” “Vaccine research” and “Nosocomial infectious disease” were used.

Result

Vaccine design for prevention of hospital-acquired C. difficile strain

The pathophysiology of infections caused by *Clostridium* is multifactorial and complex (13-18). Typically, the natural flora of human intestinal tract prevents *C. difficile* colonization and consequently, if a person is exposed to the bacteria in the active form or spore, it rarely causes infections. If the normal flora of the intestinal tract is disrupted (for example, from the use of broad-spectrum antibiotics), the remaining flora provides an opportunity for *C. difficile* spores to become active and colonize in the intestinal tract leading to the development of signs of infection caused by *Clostridium* (19-28). The return of gastrointestinal flora to its natural condition prevents

Table 1. Prevalence of pathogens causing nosocomial infections (8)

Pathogens	Gastrointestinal tract infections (%)	Surgical infection (%)	Pneumonia(%)	HAIs(%)
<i>Clostridium difficile</i>	70.9	0	0	12.1
<i>Staphylococcus aureus</i>	1.2	15.5	16.4	10.7
<i>Klebsiella pneumoniae</i> or <i>K. oxytoca</i>	1.2	13.6	11.8	9.9
<i>Escherichia coli</i>	1.2	12.7	2.7	9.3
<i>Enterococcus</i> species	5.8	14.5	1.8	8.7
<i>Pseudomonas aeruginosa</i>	1.2	6.4	12.7	7.1
<i>Candida</i> species	3.5	2.7	3.6	6.3
<i>Streptococcus</i> species.	2.3	7.3	6.4	5.0
Coagulase-negative <i>staphylococcus</i> species	0	6.4	0	4.8
<i>Enterobacter</i> species	0	4.5	2.7	3.2

the recurrence of *C. difficile* infection (CDI). The host immune system also plays a significant role in CDI (29). For example, if the host immune system was unable to produce anti-toxin antibodies for *C. difficile* or unable to develop an adequate humoral immunity and protection against *C. difficile* toxins, this can contribute to the intensification of CDI (29). The design of recombinant antibiotics, biological products, and immunological treatments and vaccinations are among the new and effective ways of preventing and treating CDI. In recent years, numerous studies have been carried out on immunological treatments and vaccinations. Level of IgG serum of antibodies against *C. difficile* A and B toxins in patients with *C. difficile* colonization without apparent symptoms are less severe than in normal human who is likely to develop diarrhea for *C. difficile* (29). These data reveal that humoral immunity against *C. difficile* toxins prevents CDI development. Similarly, in patients with CDI, an increase in the IgM and IgG concentrations against *C. difficile* toxin A inhibits the recurrence of infection (30). The protective effect of antibodies against toxin A is interesting and important because the experiments conducted on the hamster model of CDI and genetic deactivation models on toxin genes suggest that toxin B is also essential for pathogenicity (31). Thus, the results of the host immune response to *C. difficile* indicate that passive and active immunization can play a crucial role in both the prevention and treatment of *C. difficile* infection (32). Among CDI prevention measurements, vaccination is one of the most effective ways for long-term protection against CDI. There are three candidate vaccines currently undergoing phase 2 and 3 clinical evaluation for CDI prevention (Table-2) (33). *C. difficile* toxoid vaccine candidate developed by Sanofi Pasteur has undergone several phases 1 tests, and CDI recurrence has been reported in three patients in phase 2 clinical trials (34, 35). According to the latest results from clinical phase 1 tests, 3 concentrations of conjugated (2, 10 and 50 micrograms) toxoid vaccine were given by intramuscular injection to healthy volunteers

aged 18 to 55 years and adults aged 65 years and older at 0, 28 and 56 days; these results were published in 2012 (36). During this research, the seroconversion rate for toxin A was quicker and more pronounced than for toxin B. Antibody responses to vaccination among the younger age volunteers (18 to 55 years) were faster than in older ones. The additional data were collected during phase 2 double-blind multi-stage randomized controlled trial. In the first step, the suitable vaccine formulations were determined by comparing five dose groups: low dose, low dose with aluminum salts, high dose, high doses with adjuvant and placebo (37). The safety of high dose plus adjuvant was greater than other formulations. In the second step, a range of doses for injection schedule of high dose with adjunct was assessed. The three injection schedules on days 0, 7, and 30; 0, 7 and 180; and 0, 30 and 180 were compared (38), with the vaccine injection schedule on days 0, 7 and 30 soliciting the desired immune response. Since the administration of high doses of vaccine with adjuvant did not cause any particular problems for volunteers, research on vaccine formulation, injection scheduling and dosing in phase 3 clinical trials are under investigation (39). The vaccine under development by Pfizer was initially produced with a mutation in the second *C. difficile* enzymatic cytotoxin (40). The small amounts of toxicity remaining from this procedure can be deactivated by adding formaldehyde. Likewise, Valneva has developed fusion protein containing the receptor binding domains of *C. difficile* toxin A and toxin B, which elicited protection in hamster models. Phase 1 clinical testing in humans have been performed using a dosing schedule with 4 doses at days 0, 7, 28, and 56, with or without aluminum adjuvant in 2012 (41). The design and development of oral-mucosal vaccines seem to be a very promising idea, although this idea has not yet reached the clinical trial phase. There are several carriers used for this vaccine, among which *Bacillus subtilis* spore is designed in such a way that it can provide the repeat binding sites for *C. difficile* toxins A and B

Table 2. Vaccines under development against CDI

Vaccine	Antigen	Formulation and scheduling	Clinical phase	Reference
Sanofi Pasteur <i>C.difficile</i> toxoid vaccine	Formalin-inactivated toxins A and B from VPI 10463	Intramuscular injection at days 0, 7 and 30	Phase 3	(37,38)
Pizfar 3-dose <i>C. difficile</i> vaccine	Chemically treated recombinant vaccine and with genetic modifications	- / + Adjuvant, intramuscular injection at days 1, 8 and 30	Phase 2	(40)
vaccine Valneva Austria GmbH VLA84 <i>C. difficile</i>	The recombinant fusion protein of toxin a and b binding sites	- / + Aluminum adjuvant, intramuscular injection at days 0, 7 and 30	Phase 1	(41)

in conjunction with the outer spore coat proteins such as CotB and CotC (42). Interestingly, when the binding site of *C. difficile* toxins A was used alone, it had the greatest impact on the elevated levels of secretory IgA in response to both toxin A and B. Oral vaccine administration to hamsters at days 0, 14, 35 and 57, resulted in 75% of the hamsters surviving exposure to *C. difficile* strain 630, suggesting that the oral vaccination could be an effective candidate for experimental models (43). There are several questions regarding the impact of the *C. difficile* vaccine. Through targeting toxins, A and B, vaccine candidates can prevent clinical illness; however, it seems that they are unable to disrupt bacterial colonization of the gastrointestinal tract. Given the importance of preventing *C. difficile* colonization, there was a lot of focus on targeting non-toxic bacterial surface antigens to prevent colonization and thus avoiding person-to-person transmission. Currently, relative immunity has been achieved by vaccines targeting surface proteins. Some studies have identified virulence factors that can play a decisive role in the binding of microorganisms to *C. difficile* bacteria and, ultimately, preventing gastrointestinal colonization. These include capsules, proteolytic enzymes such as Cwp84, and adhesions that contribute to the mucus and cells adhesion (44-47).

Vaccine design for prevention of hospital-acquired MRSA

Iron acquisition factors such as IsdB, manganese uptake receptors such as MntABC, fibronectin binding proteins (ClfA, ClfB), polysaccharide capsule molecules (CP5 and CP8) and toxin are among known virulence factors of *S. aureus* (48-51). To date, vaccine candidates have targeted individual cell surface components, such as the polysaccharide capsule, extracellular polysaccharides or cell wall-associated proteins that aid attachment, invasion or act as a receptor (e.g., hemoglobin for iron utilization). Although multiple vaccine candidates, including StaphVAX, have shown promise through preclinical development in a range of animal models, those that have reached late-stage clinical testing have failed to demonstrate efficacy in human trials (52, 53). Currently, much of the attention has been focused on designing vaccines targeting a combination of multiple surface antigens. Clinical tests and the results related to the design of the *S. aureus* vaccine are shown in Table-3. A great deal of research is being done to develop MRSA vaccine; however, there remain numerous obstacles to successful vaccine development. Some of these challenges are the high number of virulence factors, which allows the bacteria to demonstrate high resistance to host immune response. Also, *S. aureus* strains

Table 3. Vaccine candidates against MRSA infections (54)

Drug	Company	Mechanism	Target	Status
StaphVAX	NABI	Vaccine	CP5/CP8	failed phase 3
Altastaph	NABI	Antibody	CP5/CP8	ended
Pentastaph	NABI/GSK	Vaccine	CP5/CP8	failed phase 3
Aurograb	NOVARTIS	Antibody	lipoprotein	failed phase 3
Veronate	INHIBITEX	Antibody	ClfA	failed phase 3
Tefibazumab	INHIBITEX	Antibody	ClfA	ended
Pagibaximab	BIOSYNEXUS	Antibody	LTA	failed phase 3
V710	MERCK	Vaccine	IsdB	failed phase 3
SAR279356	SANOFI	Antibody	PNAG	ended
NVD3	NOVADIGM	Vaccine	Als3	phase 1/2
STEBVax	IBT	Vaccine	Seb	phase 1
SA3Ag	PFIZER	Vaccine	CP5+8/ClfA	phase 2b
PF-06290510	PFIZER	Vaccine	CP5+8/ClfA/MntC	phase 2b
MEDI4893	MEDIMMUNE	Antibody	Hla	phase 2b

are geographically diverse and very versatile in their antigenic repertoire. Current animal models for staphylococcal disease do not have good predictive value. Assays that can reflect physiological endpoints are needed to evaluate the host's potential to identify and eliminate *S. aureus*. The specific types of antigens which could be used to induce protective immunity have not yet been identified. We currently do not know whether a vaccine that protects against *S. aureus* soft-tissue infection can also protect against other forms of *S. aureus* infection (i.e., bacteremia, pneumonia, and osteomyelitis) (55-57).

Vaccine design for prevention of hospital-acquired A. baumannii

Diverse antibiotic resistance mechanisms and high tolerance have made *A. baumannii* one of the major causes of nosocomial infections in recent years (58). *A. baumannii* infections mostly occur in intensive care units, considerably increasing death rates and hospitalization time (59, 60). The ability to develop biofilm, produce siderophores and hydrolytic enzymes and also quorum sensing are some of the most important virulence factors of *A. baumannii* (61, 62). In general, the research on finding an appropriate vaccine candidate for *A. baumannii* is focused on two methods: design of whole-cell vaccines and pure protein-based vaccines. In studies with mice injected by formalin Inactivated Whole *A. baumannii* Cells (IWC), formulated with aluminum phosphate as the adjuvant, the intramuscular injection of the IWC preparation resulted in a rapid robust of antibody titers. The immunized mice had less bacterial burdens in the infected tissues, and a reduced production of pro-inflammatory cytokine serum levels of IL-1b, TNF-a, and IL-6 that are normally associated with sepsis (63). This kind of vaccine candidate is easy to prepare, inexpensive, and does not require expensive denaturation of antigens, that may induce conformational changes of the epitopes; Also, according to the nature of the vaccine, the immunity against several antigens leads to protection against several *A. baumannii* strains. However, the safety of using this vaccine is controversial; considering the incomplete inactivation of the bacterial cell, they might initiate infection, or the possibility of being contaminated with pyrogenic endotoxin during injection can also be problematic (63). Researchers overcame this obstacle by basing the vaccine development on an *A. baumannii* strain, which cannot produce lipopolysaccharide (64). In another study, the outer membrane complex (OMC) was applied with adjuvant and used as a vaccine. The OMC vaccine was confirmed to be highly reproducible and can limit post infection pro-inflammatory cytokines associated with septic shock. It was able to increase IFN-c production to be highly potent and heterologous, and reduce the bacterial burdens in tissues by 105-fold. The humoral response was able to provide a protective immunity with

just a single vaccine dose in as few as 6 days post immunization and was maintained for 21 days. This aspect can be life-saving in the case of outbreaks or critical conditions. However, safety concerns still arise about possible endotoxin contamination (65). Outer membrane vesicles (OMV) are secreted by *A. baumannii* during growth. These spherical, non-viable, and non-cellular nanovesicles contribute to bacteria colonization during infection (66). OMV has a vital role in spreading antibiotic resistance genes and quorum sensing ability (67). A hundred and thirteen different proteins were identified and found to be packed within OMV, majorly consisting of OmpA; other outer membrane proteins are including CarO protein, tissue degrading enzymes, as well as, lipopolysaccharide and nucleic acids (68). Mice were vaccinated with OMV formulated with Adjuvant, then boosted after 3 weeks by the same preparation. It was found that the IgG increased by 60-fold, bacterial tissue burdens were reduced by 106-fold, and the pro-inflammatory cytokines IL-6 and IL-1b decreased post-infection (69). OMV vaccine provided full heterologous protection to immunized mice against several *A. baumannii* strains including pan-resistant isolates in both pneumonia and sepsis models. Since OMV is non-cellular, it is much safer than inactivated whole cell vaccines and also produces less adverse toxic events although endotoxin is still present and might trigger safety issues (70). According to studies regarding vaccines designed based on pure protein, in 2011, a mixture of purified proteins, which were majorly OMPs and fimbriae proteins, was patented as an effective vaccine against *A. baumannii* (71). Later on, in silico mapping, the potential of OmpA type 1 was confirmed in particular (72). The preparation of recombinant OmpA (rOmpA) with aluminum hydroxide as the adjuvant in a dose of 3l g/kg was used to immunize mice. Immunization marked an increase in mice survival rates by decreasing bacterial tissue burdens and induced high anti-OmpA IgG antibodies titers that soared by increasing the dose up to 30-fold. OmpA vaccines are very promising as they are highly reproducible, easy to be manufactured commercially, and also safer than the whole complex preparations (73). However, when it is in a purer OmpA form, it becomes insoluble thus making its delivery method a concern (74). Further research on the OmPs such as Omp22 (75), OmpW (76), as well as the epitope mapped outer membrane nuclease (Nuc) Ab (77), and the selected OmPs proved their potential as vaccine candidates. The candidate Bap protein has a high molecular weight of 854 kDa and is one of the most acidic proteins found on the surface of *A. baumannii*. It was identified as an important key regulator of biofilm maturation and also as a key factor in the maintenance of the biofilm structure, thickness, and volume. The Bap's presence increases the bacterial cell hydrophobicity which enhances its adherence to the host cells and helps the bacteria to be safe against the effect of phagocytes (78).

Active immunization with Bap targets the most virulent character of *A. baumannii* and deprives it of biofilm formation. The subunit of recombinant Bap was prepared, emulsified with complete Freund's adjuvant, and used to vaccinate mice. Immunized mice exhibited high IgG antibody titers, with a complete bacterial clearance from the infected tissues, which in turn increased mice survival rates. Bap subunit vaccine has around 20 antigenic determinants and 55 separate B-cell epitopes, hence and therefore it demonstrated immune-dominancy (79). Further research on the combination of Bap with OMV or OmpA of *Acinetobacter* proved an augmented potency in comparison with the individual components (80).

Conclusion

Given the emergence of resistant strains to common antibiotics among hospital-acquired infectious bacteria and the difficulties involved in the treatment of infections caused by these bacteria, it seems that there is an urgent need to design effective vaccines against these bacteria and prevent these infections and diseases. So far, considerable progress has been made in regards to designing vaccines. The evolution of the vaccine design from the entire body and subsequently, recombination and the use of nanoparticles as an auxiliary compound to increase efficacy have successfully produced vaccines against these resistant bacteria. Therefore, it seems that the use of vaccines, along with other strategies for the prevention of hospital-acquired infections, can be very effective.

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Conflict of interest

There are no conflicts of interest.

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