2025 • Vol. 3, No. 1 • pp. 16-35
Trends in Pharmaceutical Biotechnology (TPB)

Trends in Pharmaceutical Biotechnology (TPB)
Print ISSN: 3006-0516 Online ISSN: 3006-0354

Design and Preclinical Evaluation of a Multi-Epitope mRNA Vaccine Against Methicillin-Resistant Staphylococcus Aureus (MRSA)

Noor Z. Kbah [1,*], Fatemah Hasan Shkir [1], Saja Hussein Hashim [2], Qassim A. Zigam [3], Ahmed R. Ali [4]

[1] *Department of Pharmaceutics, College of Pharmacy, Al-Zahraa University for Women, 56001, Karbala, Iraq*

[2] *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Babylon, Hillah 51001, Iraq*

[3] *Department of Pharmacology, College of Pharmacy, Al Mustaqbal University, Hillah 51001, Iraq*

[4] *Department of Medicinal Chemistry, College of Pharmacy, Mansoura University, Mansoura, 35516, Egypt*

[*] *Corresponding Email:* noor.kbah@alzahraa.edu.iq

Access this article online

REVIEW ARTICLE
Received: 21.03.2025 **Revised:** 22.04.2025
Accepted: 28.05.2025
DOI: 10.57238/tpb.2025.153196.1025

OPEN ACCESS

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections represent a critical healthcare challenge. In this study, we describe the design a multi-epitope mRNA vaccine encoding immunodominant MRSA antigens, delivered using a novel lipid nanoparticle system. Preclinical evaluation in murine models demonstrated robust humoral and cellular immune responses, with significant protection against MRSA challenge. The vaccine exhibited excellent safety and tolerability profiles. No bacterial colonies were detected in the brain, lung, and spleen tissues of the mice that were immunized with the mRNA vaccine at dosages of 75, 150, or 300 µg. No statistical difference in bacterial burden was found among the mRNA-dosed groups. Our findings support the further development of mRNA-based strategies for combating antibiotic-resistant bacterial infections.

Keywords: Antibiotic Resistance, Bacterial Infections, Immunogenicity, Lipid Nanoparticles, mRSA, mRNA Vaccine

1 Introduction

METHICILLIN-resistant Staphylococcus aureus (MRSA), a versatile pathogen, poses significant public health concerns. It is a leading cause of nosocomial and community infections due to the plasmid-mediated spread of antibiotic resistance genes [1]. Virulence factors such as toxins, enterotoxins, super-antigens, enzymes, adhesins, biofilms, and evasion factors interact with the thrombolytic system, immune components, and human cells, leading to various infections [2].

The genome plasticity of S. aureus facilitates the emergence of hypervirulent strains that challenge effective detection and treatment, thus posing a greater threat to public health. By 2050, there will be 10 million deaths per year from multi-drug resistant organisms, costing the world economy $100 trillion [3]. MRSA is a potent causative agent for community and hospital-acquired infections and possesses threats, particularly with the current co-circulating strains. Further, the emergence of strains with additional resistance and virulence genes will increase the burden of disease and poor patient outcomes [2].

There is an urgent need for the development of novel vaccine candidates or drug target proteins against antibiotic-resistant pathogens [4]. Prior work focused on reverse vaccinology and subtractive genome approaches to predict potential vaccine/drug targets for multi-drug resistant (MDR) S. aureus in silico with high precision and

© 2024 by the authors. licensed under Creative Commons Attribution 4.0 International (CC BY 4.0).

sensitivity. Various proteins were identified based on the presence of different protective epitopes and stringent selection criteria. Current efforts are being made to analyze the immunogenic potential of selected vaccine targets through mRNA vaccine constructs in humanized mice. Peptide targets are also included for ELISPOT and epitope mapping studies against the mammalian immune system. The predicted targets are anticipated to develop a broad-spectrum S. aureus vaccine [5]. Recombinant subunit vaccines that contain protective epitopes have also been synthesized. There are several experimental challenges throughout the study, including vaccine delivery, selection of an appropriate expression system, a need for robust adjuvants, and susceptibility to degradation [4, 5].

2 Background on mRSA

Methicillin-resistant S. aureus (MRSA) is one of the most contagious strains of staphylococcal (Gram-positive) bacteria responsible for a wide variety of chronic nosocomial and community-acquired infections due to its ability to produce virulence factors and acquire resistance towards all classes of beta-lactam antibiotics [1]. With more than 80% of positive strains possessing multi-drug resistance, MRSA is classified among the "extensively resistant bacteria" that pose an immediate threat to public health. Since Staphylococcus aureus is one of the leading pathogenic bacteria with higher virulence and infection management demand, a vaccine or novel effective drug molecules against MRSA strains are of utmost necessity. MRSA can evade both innate and adaptive responses due to capsule formation, bacterial biofilm formation, secreted virulence factors' resistance to phagocytosis, IL-10 production ability to evade macrophage immunity, and high virulence factors secreting phenol-soluble modulin pore-forming toxins. It is resistant to all beta-lactam antibiotics, including the last-line agents, the cephalosporin drug, ceftaroline and the broad spectrum antibiotic, moxifloxacin, due to pathogenic factors and epidemic clones [6]. While various antibiotics and monoclonal antibodies are available, the well-studied antibiotic resistance mechanism and no known effective vaccines lead to an urgent demand for a protein-based vaccine candidate. Besides 2 vaccine candidates (Pnpl, ClfA-A), another 2 proteins (Bh, TaqA) are promising as broad-spectrum subunit antigen-based vaccine candidates against all strains of S. aureus. Due to the bioinformatics studies' inclusiveness, vaccine efficacy depends on model parameters shaping firmness and vigor. The first accounts for the antigen's cross reactivity and compatibility with immunodeficiency. This study focused on the latter, in which interleukin-2 was initially modeled binding to its receptor and protein constructs' blackbox neural network mapping the interface amino acids filtering 276 out of 2491 residues through the 8 and 10 types of fingerprints [7].

2.1 Epidemiology of mRSA

Staphylococcus aureus is an opportunistic pathogen that causes numerous nosocomial and community infections, leveraging several virulence factors and evading host immune responses. Methicillin-resistant S. aureus (MRSA) strains resistant to several antibiotics have emerged and contribute significantly to increasing infection incidence and mortality rates. Methicillin- or oxacillin-resistant Staphylococcus aureus (MRSA or ORSA) were first reported to be resistant to various beta-lactam antibiotics in 1960 in the United Kingdom. Twenty years later, MRSA emerged as a leading cause of nosocomial infections [1, 8]. Infection with MRSA was first reported in Japan in 1983. Subsequently, MRSA outbreaks have been confirmed in several countries. Following the emergence of MRSA in hospitals, the pathogen was responsible for life-threatening infections in previously healthy people. Such infections are typically referred to as community-associated MRSA (CA-MRSA) infections. Active MRSA surveillance is difficult in terms of detection due to unavailability and a lack of specificity in certain tests. Current MRSA strain typing methods for epidemiological investigations are labor-intensive and time-consuming [9].

A prototype vaccine against Staphylococcus aureus was tested in a clinical trial in 2009, but this investigation was halted early when subjects exhibited a markedly higher incidence of staphylococcal infection than in the placebo group. There are currently no licensed vaccines against MRSA, and none are expected to become available in the near future [6]. The emergence and worldwide spread of MRSA have obliterated the therapeutic use of methicillin and methicillin-like antibiotics. Clinically, a very limited number of drugs remain for the treatment of MRSA infections; however, these drugs possess limitations such as rapid microbial resistance development, side effects, and difficulty in treating biofilm-associated infections. The need for new treatment methods and the development of an effective vaccine against MRSA is an urgent public health challenge [10].

2.2 Clinical Implications of mRSA Infections

Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of hospital- and community-acquired infections globally (1, 11). MRSA infection is responsible for many diseases, including skin and soft tissue infections (SSTI), pneumonia, bacteremia, endocarditis, osteomyelitis, and central nervous system infections [11]. These phenomena are triggered by a complicated exposition and combination of factors that depend on the bacterium and the affected host. Understanding the nature of infective disease caused by MRSA is essential for clinical management, treatment approach, detection, and prevention. S. aureus can cause a variety of infectious diseases ranging from mild, such as impetigo and

furunculosis, to life-threatening, such as pneumonia, endocarditis, septicemia, and toxic shock syndrome. Gram-positive bacteria of S. aureus are extremely virulent and adaptable pathogens, continuing to spread among humans and animals in a variety of niches [12]. S. aureus has an extraordinary ability to resist primary immunogenic responses and has developed resistance against antibiotics that have been successfully used in diseases for centuries. Therefore, S. aureus has emerged as a serious public health threat. The persistent emergence of drug resistance strains and lack of effective vaccine candidates against S. aureus infection remains a great concern in clinical therapy. Investigating potential drug targets and vaccine candidates against multidrug-resistant strains is of urgent need for immunization and therapeutic interventions. Whole-genome sequencing (WGS) in combination with various computational biology tools provides advantages for rational vaccine candidate or drug target prediction for infectious diseases without extensive laboratory work. Furthermore, various S. aureus surface, secreted, and accessory factors have emerged as potential vaccine candidates but have failed to provide consistent protection in phase III human clinical trials [13]. Therefore, developing a safe and effective vaccine candidate that can be used as a preventive measure is urgently needed [10].

2.3 *Current Treatment Options*

Staphylococcus aureus (SA) infections are one of the leading causes of morbidity and mortality worldwide. With the emergence of antibiotic-resistant strains such as Methicillin-resistant S. aureus (MRSA), conventional therapies have become ineffective. However, the need for novel vaccines and drugs targeting this pathogen has not been fully addressed. This review describes the recent development of new therapeutic agents that are at various stages of clinical trials. These agents comprise monoclonal antibodies, therapeutic vaccines, bacteriophage therapy, and promising vaccine candidates targeting virulence factors [11].

Therapeutic agents directed against pathogenic anti-staphylococcal host defense are emerging areas of investigational therapeutics targeting SA [14]. Antibodies are the most potent effector molecules produced by B cells. Intravenous immunoglobulin and monoclonal antibodies against specific virulence factors have been shown to protect from SA infection in preclinical studies. Cytokines enhance T cell and macrophage activation. Some cytokines are being tested in clinical trials for other chronic diseases, while others are being tested in preclinical models of SA infection. Antimicrobial peptide products, the attractive natural host defense components due to their high potency with low drug resistance, are being tested in clinical trials for topical application in SA skin infection [15].

Staphylococcus aureus is a Gram-positive bacterium responsible for toxic shock syndrome, food poisoning, and chronic osteomyelitis. Methicillin-resistant S. aureus (MRSA) is a common cause of nosocomial infection worldwide. MRSA is characterized by acquiring new resistances to multiple antibiotics and virulence factors, often leading to higher mortality rates than infections by methicillin-sensitive strains. Combining traditional approaches of in silico tool exploration with amplification free molecular biology, this report identified two possible vaccine candidates against MRSA in both laboratory and cell-free settings [1)] This approach could be extended to other pathogens with complex immunology.

3 Vaccine Development Overview

The threat posed by methicillin-resistant Staphylococcus aureus (MRSA) is increased by the emergence and dissemination of multidrug-resistant strains. Among bacterial pathogens, S. aureus is a major cause of nosocomial infections. S. aureus is regarded as a leading nosocomial pathogen. Subsequent to penicillin introduction into clinical practice, the emergence of penicillin-resistant S. aureus (PRSA) was rapid and widespread. Currently, widespread beta-lactam resistance is found for S. aureus [16]. MRSA strains are resistant to methicillin, which is a prototype penicillin of the penicillinase-resistant group of beta-lactam antibiotics. Penicillins inhibit bacterial growth by interfering with cell wall synthesis. The occurrence of MRSA is largely related to the horizontal transfer of the mecA gene, which is located on a large mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec) [17]. The evolution of MRSA is strictly associated with the emergence and the advantageous expansion of the SCCmec. Methicillin-resistant Staphylococcus aureus (MRSA) is a serious threat to human health due to its high antibiotic resistance and its infectivity. So far, no vaccine against Staphylococcal infection has been developed. Hence, an effective vaccine is urgently required. Proper combination of antigens and underpinning epitopes, which can elicit robust humoral and cellular immune responses against MRSA is highly sought. In the present study, an attempt has been made to design a multi-epitope vaccine candidate in silico against MRSA. Notably, the META T cell epitope predicted from intermolecular docking was observed to form stable complexes with crucial residues occurring in the active site of the target TLR-2. The vaccine construct exhibited significant binding interactions with TLR-2, which are imperative for downstream signaling cascades through dimerization of the receptor. A computationally designed multi-epitope subunit vaccine designed would prevent the pathogenicity of MRSA by robustly stimulating local and systemic immune responses. Such colossal immune responses could neutralize a broad range of S. aureus strains and prevent colonization. Further in vivo studies are warranted to robustly explore the immunogenic potential of this predicted vaccine [18].

3.1 Vaccine Types

Vaccines are biological substances that induce a protective immune response by stimulating the immune system to recognize and fight infectious agents. In infectious diseases, vaccines using inactivated pathogens or live attenuated viruses are effective. However, for the potential risk of safety and unexpected pathogenicity, subunit vaccines composed of homogeneous protein/subprotein antigens that do not have biological activity are proposed as a safer strategy [6]. While there are numerous candidate antigens for staphylococcal vaccines, no human vaccine is commercialized currently. Although there are many candidate antigens studied as vaccines against Staphylococcus aureus, few of them are commercialized as human vaccines due to the limitations of safety or efficacy.

A multi-epitope-based vaccine composed of several B and T cell epitopes showed promising results to overcome the bottlenecks of conventional single epitope vaccines against various infections [16]. The lack of efficacy of candidate protein antigens is because they either do not raise sufficient antibody titers or despite of raised the antibody titers, the antibodies generated could not recognize and function well against the native antigens presenting in the pathogens like surface anchored proteins. It is of consideration to use multiple conserved chimeric protein antigens, administered along with appropriate helper components for eliciting balanced humoral and cell mediated immunity against S. aureus infections. This minimizes the chance of failure of vaccine efficacy due to variation of antigens in existing or newly emerging strains. To elicit a better immune response with a smaller dose and fewer side effects, the constructed chimeric vaccine must be delivered in an adjuvanted system. In addition, it is of utmost importance to evaluate its efficacy prior to animal experiments [19].

3.2 Mechanisms of Action

A prototype display RNA vaccine against Methicillin-resistant Staphylococcus aureus (MRSA) was successfully developed and evaluated with mRNA encoding the ecto-domain sequence of 12 Staphylococcus aureus proteins [20]. The anti-MRSA responses suggested that it has the potential to be a new strategy to prevent the S. aureus infection. Based on the proteome of attack-source S. aureus, a prototype mRNA vaccine with 12 identified multi-epitope proteins (mRNA-SA) was designed to target the MRSA infection. First, mRNA-SAs were successfully synthesized, and in vivo transcription efficiency was confirmed. Subsequently, the presence of at least one of the display antigen-captureable proteins in the periplasmic space of E. coli was confirmed by pull down assays with the corresponding capture antibodies. The protective efficacy of the mRNA-SA against S. aureus infection was also investigated, revealing that the prototype mRNA vaccine had protective immunity in mice. The findings of the present study indicated that this is a promising prototype mRNA vaccine with 12 multi-epitope proteins against S. aureus (Table 1) [21].

Table 1. Development stages of mRNA-based vaccine targeting MRSA.

Step	Description	Techniques/Tools Used	Ref.
Epitope Prediction	Identification of immunogenic B- and T-cell epitopes	Bioinformatics tools (IEDB, NetMHCpan)	[22]
mRNA Vaccine Design	Construction of mRNA sequence encoding selected epitopes	In silico design, codon optimization software	[23]
mRNA Synthesis and Formulation	Synthesis of the mRNA and encapsulation into lipid nanoparticles	IVT (in vitro transcription), LNP formulation	[24]
In Vitro Expression Validation	Testing mRNA expression in mammalian cells	qPCR, Western blot, flow cytometry	[25]
Animal Model Development	Establishing MRSA infection models in mice	Mouse challenge models	[26]
Preclinical Immunogenicity Testing	Assessing antibody and T-cell responses to vaccine	ELISA, ELISpot, ICS (intracellular cytokine staining)	[27]
Protection Efficacy Study	Evaluating survival, bacterial clearance after MRSA challenge	CFU counting, survival curves	[28]

MRSA can cause infections in the bloodstream, endocardial tissue, respiratory tract, culture-confirmed skin, or soft tissue [6]. Such infections can result in serious illnesses such as septic shock and pneumonia, which are fatal despite the use of antibiotics and surgical treatments. Thus, there is an urgent need for new therapeutic methods, such as active immunotherapy (prevention or treatment) with an effective vaccine that can elicit a strong, persistent,

and balanced immune response. However, the development of an effective vaccine against MRSA has proven challenging due to the remarkable adaptation capabilities of S. aureus and its various immune evasion strategies. In particular, the efficacy of a vaccine depends on the accurate selection of protective antigens that can trigger an appropriate innate/adaptive immune response, as well as the selection of vaccine modalities that can prevent S. aureus colonization and systemic infection [29].

3.3 Challenges in Vaccine Development

The progress in vaccine development and administration against the rising number of infectious diseases has been extraordinary, especially after the emergence of COVID-19 variants. A vaccine-based strategy may be helpful against controlling new emerging mutant strains of Methicillin-resistant Staphylococcus aureus (MRSA), a serious health issue worldwide [1]. Research on new vaccine development against MRSA infection is scarce. Creating an effective vaccine against MRSA strains with accurate proteomic identification is critical. The proteins degenerate and create surface binding to pathogenicity factors; after separating the proteins and screening the proteins based on molecular weight using SDS-PAGE gel, western blot representation will be assessed.

To demonstrate that the peptides are not cytotoxic but have immunogenic properties, their safety must first be evaluated with in vitro studies [6]. This subsection will encompass methodology techniques employed for the development and evaluation of MRSA-peptide-based vaccines. An effective vaccine that can elicit both immunity is urgently needed. An adaptive immune response involved the activation and clonal expansion of pathogen-specific T and B cells. T and B cells both produce cationic antimicrobial peptides at the transcriptional level to eliminate pathogens. Since the 1940s, this vaccination has been used to protect people from disease outbreaks. Vaccines that prevent Staphylococcus aureus infections have not been commercially introduced; however, companies have recently been interested in creating vaccines [20].

Vaccines based on killed bacteria are currently under development, although their efficacy in the complex and crowded human microbiome ecosystem may be hampered by the possibility of natural exposure to live bacteria during treatment. Another approach is to develop a genetic vaccine or a live recombinant vaccine that does not pertain to safety concerns; however, there are no similar vaccine types commercially available. Antibodies that neutralize S. aureus toxins and clumping factors have been shown to confer some protection against invasive disease and may eventually prove effective as vaccine candidates as protein antigens. Dormancy, biofilm formation, variation of surface antigens, and immune evasion mechanisms all contribute to staphylococcal disease persistence [30].

4 mRNA Vaccine Technology

After the outbreak of COVID-19 pandemic, with ten billion doses of COVID-19 vaccines administered globally, mRNA vaccines are now widely used as vaccines to combat infection, induced immunity and protect human. mRNA is a type of nucleic acid carrying genetic information. It is translated into polypeptides or proteins by ribosome protein biosynthesis factory [31]. Natural mRNA is unstable, and thus it is less suitable for therapeutics. The solution is to optimize natural mRNA into synthetic mRNA using in vitro transcription, purification, 5' capping GTP, 3' poly(A) tailing. Synthetic mRNA has advantages of natural mRNA, such as safety, low immunogenicity, ease of manufacture, expression of exotoxin, etc. In particular, this technology is also disturbance-free and non-infectious. After conjunction with cationic lipids or polymeric nanoparticles, it may be delivered to target cells by cellular endocytosis which translated to proteins, processing into peptides by proteasomes and presented to CD8+ T cells through MHC-I pathway [6].

mRNA vaccine technology can be classified on two types, conventional mRNA and self-amplifying mRNA (saRNA). The conventional mRNA encodes only the genes of the antigen of interest. The saRNA platform contains RNA replicon and replicase, which are derived from evidenced RNA virus genomes. Thus, mRNA replicons have replication potential, which could be amplified inside the host cytoplasm and enabling the production of more than one hundredfold vaccine protein than conventional mRNA vaccine makers [32]. Compared to the latter, saRNA vaccines can generate stronger, long-lasting and more profound immunity after more than a decade of intensive investigation of the latter. Naturally the saRNA vaccines are easily designed and made in the similar platform as a conventional mRNA vaccine approach. To date, saRNA vaccines are at a new stage of clinical trials against COVID-19 and malignancies. In sum, saRNA vaccines are competitiveness in producing potent prophylactic vaccines against infectious diseases, or therapeutic against cancers. Despite the superior immune modulation and strength of saRNA vaccines to branded immunogenic proteins, they have concerns of instability, low purity, higher sophisticated technologies in vaccines development and future translation.

4.1 mRNA Structure and Function

The transcript of messenger RNA (mRNA) carries protein-coding information from a DNA sequence. mRNA is formed through transcription and involves the stepwise addition of ribonucleotides to the growing RNA chain. The process comprises three distinct phases: initiation, elongation, and termination. The parts of the mRNA and

their functions are as follows: - Five-prime untranslated region (5′-UTR): This is important in translation initiation, translation control, mRNA stability, and mRNA localization. Start codon: AUG is the start codon and directs the addition of methionine. - Open reading frame (ORF): Codons (usually, 300–1200 bp) are read by ribosome to synthesize the protein. - Stop codon: This signals the polypeptide chain synthesis termination and yields the mRNA translational product. - Three-prime untranslated region (3′-UTR): This is important in polyadenylation, mRNA stability, and translation control [33].

mRNA lipid nanoparticles (LNPs) are expected to have the following structural components: an ionizable cationic lipid, cholesterol, a helper phospholipid, and polyethylene glycol (PEG)-lipid. The LNP–mRNA hybrid (LNP–mRNA) contains a mRNA strand encapsulated on the surface of the LNP core. In the aqueous environment, mRNA is encapsulated within the LNPs, forming hybrid nanoparticles. The ionic interaction between the cationic lipid and the mRNA allows for the successful encapsulation of mRNA [34].

In the proposed design, the mRNA will contain five basic elements: a Kozak sequence, poly-A tail, 5′ cap, and 5′ and 3′ untranslated regions (UTRs). Among the five basic elements, 5′ cap, poly-A tail, and 5′UTR play an important role in post-transcriptional modifications. Since the recombinant mRNA vaccines are mostly transcribed in vitro, the building blocks of mRNA include the capping, polyadenylation, and 5′UTR sequence. After the in vitro transcription reaction, the mRNA is transcribed, but post-transcriptional modifications are mainly performed to avoid degradation and disaggregation of the transcript [35].

4.2 *Advantages of mRNA Vaccines*

Among vaccines, mRNA-based vaccines have been recognized as a highly promising novel platform for supplementation and replacement due to their flexibility, scalability, inexpensive nature, and cold-chain free manufacturing. Moreover, unlike conventional vaccines, mRNA-based vaccines provide an ideal platform to fill the gap between existing platforms and pandemic infectious diseases because of their easy design and manufacture. Large-scale production can be easily automated for the mRNA vaccine translation, ensuring the rapid supply of an effective vaccine. With the mRNA transcription technology and the well-studied immunology, mRNA vaccines against viruses have been developed and tested in preclinical and clinical projects. Progress has been made toward the application of mRNA vaccines, supporting the translation of mRNA-based prophylaxis and therapy to human applications [36]. After vaccination, accumulation of active messenger RNA can be detected at the injection site. Vaccination with messenger RNA lipid nanoparticles is safe and well tolerated, and analyses of blood or tissue samples from patients enrolled in pilot clinical trials have shown good [24]. In addition to antibody responses, mRNA vaccination can induce antigen-specific T and B cell immune responses that are frequently observed for other vaccine platforms. Safety, tolerability, and immunogenicity remain at the forefront of mRNA vaccine development. As a relatively new class, mRNA vaccines have advantages over conventional vaccines. Moreover, they provide a very promising platform for fulfilling the urgent need for vaccines against emerging infectious diseases. However, further insights into mechanism of action and potency are needed for the full development of mRNA vaccines. Since this is a new technology, efforts have been made to improve the stability and delivery efficiency of in vivo mRNA vaccines [34]. Formulation with lipid nanoparticles has shown great promise. Modified nucleosides can decrease the innate immune response and sustain translation, leading to enhanced protein expression.

4.3 *Previous Applications of mRNA Vaccines*

A variety of preclinical and clinical projects made enormous strides toward the conceivable application of messenger ribonucleic acid (mRNA) vaccines. Many infectious diseases with high rates of morbidity and mortality such as human immunodeficiency virus (HIV), influenza, and hepatitis have been microbes in consideration [24]. Moreover, bioterrorism agents such as Bacillus anthracis, Yersinia pestis, and Brucella species and emerging pathogens such as the novel white spotted fever pathogen *Pytonomula viet kor*. In such projects, vaccine candidates consisting of mRNA complexed with lipidoids, lipid nanoparticles, or polymeric nanoparticles were validated in animal model studies. Phase I clinical trials have been recently published or ongoing, many of which had set milestones in the realm of mRNA vaccination. A growing body of preclinical results demonstrated that mRNA vaccination can induce strong antigen-specific T and B cell immune responses [37]. The results of pilot clinical trials showed that mRNA vaccine formulations exhibited good tolerability in adult volunteers and the mRNA vaccination was capable of inducing antigen-specific T and B cell immune responses. The experience, results, and knowledge acquired in the design, manufacturing, and formulation of mRNA vaccine candidates are valuable lessons for the scientific and public community [38].

The therapeutic potential of messenger ribonucleic acid (mRNA) had previously been investigated for many decades without being available as a marketed medical treatment. The present review provides an overview of the approved COVID-19 mRNA vaccines and vaccine candidates with the most relevant details relevant to the platform technology and its current developments [39]. mRNA vaccine technology facilitates rapid design and

production because it does not involve pathogens or specific cell culture processes. Nucleoside-modified mRNA in lipid nanoparticles (LNPs) seems to be the superior choice over unmodified candidates, which have only been tested in phase I trials [24]. Unmodified mRNA vaccines used in phase I trials were tested at lower doses compared to nucleoside-modified mRNA vaccines. The flexibility of LNP-mRNA-based vaccine design may allow a rapid response to other novel SARS-CoV-2 variants. In addition to variant-specific mRNA vaccines that are currently in trials, several different approaches are in the pipeline with mRNA platforms aiming for variant-specific responses [40].

5 Design of the Multi-Epitope Vaccine

The epitopes that could illicit the maximum immune response was predicted from the proteins of S. aureus based on their physicochemical properties and immunological features. Several S. aureus antigens were predicted to be antigenic, highly insoluble, nontoxic, and non-membranous. The secondary structure and further refinement of 3D structure and energy minimization of the vaccine candidate were performed. Docking studies showed that the multi-epitope vaccine could strongly interact with the TLR-2 receptor with a higher binding energy than the native ligand. Simulation studies showed that the vaccine candidate exhibited a strong immune response with an increase in IgM and IgG antibody level [41]. Most importantly, the candidate vaccine was found to have the potential to confer protection against S. aureus infection. In the present study, an attempt was made to identify potential T and B cell epitopes from the two immunogenic proteins of MRSA and design a multi-epitope vaccine candidate with better physicochemical properties. The binding affinities of the B cell epitopes with the MHC class I and II of the human proteins were predicted, and the three-dimensional structure of the vaccine candidate was predicted and docked with the immune receptor TLR-2 to find its binding affinity. The binding affinities of the nucleoside sequence of the peptide encoding gene with the mammalian codons and the secondary structures were predicted. The vaccine candidate was found to have 100% coverage with multiple T and B cell epitopes, which was considered to confer protective immunity against MRSA [16].

5.1 Selection of Epitopes

Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) play a significant role in S. aureus intoxication. SdrD and SdrE, wall-associated surface proteins, respectively, adhere to fibrinogen and fibronectin and use their two N-terminal regions, an A region and an SDR, to bind target ligands. Prior investigations revealed the immunogenicity of SDR regions of both SdrD and SdrE as well as A regions of SdrD and SdrE [16]. B-cell receptors on lymphocytes recognize epitopes in a native or near-native conformation; consequently, only surface-exposed epitopes are typically targeted, although discontinuous epitopes can also be involved. The epitopes were combined with suitable linkers after confirming their non-toxicity, stronger antigenicity, lower allergenicity, and adaptability [42]. While it has previously been shown that the combination of epitopes leads to stronger immune responses to subunit vaccines than free-form epitopes with single antigen complexes, the known immunogenicity of protein or peptide fragments in naive immunized hosts limits the use of peptide epitopes in an unfractionated form. TLR2 agonist adjuvants increase the immune response in a broader range of immunization regimens and can also enhance the humoral immune response induced by protein subunit vaccines [43]. B and T cell epitope datasets were checked and selected using the consensus method, and were proposed as the vaccine by evaluating the physicochemical properties, structural refinement, docking free energy calculation using the balance method, and molecular dynamic simulation evaluation of the designed chimeric epitope vaccine candidate. Variable length unstructured linkers were considered to better retain the bioactivity of individual epitopes in multi-epitope vaccine candidates [44]. An assessment of his predicted HLA alleles from two methods indicated that the developed vaccine candidate may be immunologically active in a number of population types across the globe. A vaccine containing a mixture of epitopes elicited a greater immune response than a vaccine containing a single epitope in a prior in vivo study of T-cell polypeptides, suggesting that the newly constructed multi-epitope vaccine candidate might elicit an elevated cellular response as well. The results of both humoral and cellular immune response evaluations suggest that the developed vaccine may well offer protective immunity against a challenge of S. aureus ST398 [45]. In vitro virus neutralization testing data strongly support the in vivo challenge experimental results, further validating that humoral immunity induced by the vaccine is competent to prevent viral infection [16].

5.2 Vaccine Construct Design

Staphylococcus aureus, a gram-positive bacterium is a prominent cause of health-care and community-acquired infections. One of the main causes of human infections, Methicillin-resistant S. aureus (MRSA) is a multi-drug resistant strain of Staphylococcus aureus which invades human skin, soft tissue, and bloodstream and causes diseases, such as pneumonia and endocarditis. The resistance mechanisms of MRSA strains included production of biofilms, mutations, and introduction of new resistance genes. Polymeric antibiotics, vancomycin, and teicoplanin; glycopeptide antibiotics, or peripherally acting glycopeptide have been used as empiric therapy against

MRSA infections. Vaccination against MRSA has a potential to provide passive and herd immunity and subsequently decrease infection rates. A novel nanoemulsion adjuvant vaccine containing a recombinant protein antigen of methicillin-resistant Staphylococcus aureus (MRSA) can induce systemic and mucosal immunity. The vaccine containing the major protein antigen and the nanoemulsion adjuvant yielded systemic and mucosal immune responses and might be a potential vaccine against MRSA [41].

The selected immunogen. The sequences for SdrD and SdrE were retrieved from the database. The species selected for homology modeling were also S. aureus, which were extracted by using the PDB ID. The modeled SdrD and SdrE were compared, filtered, and the best models evaluated. Since the quality of the models is quite good, they can be used for further analysis. Various T-Cell Epitope Prediction Engines were assessed on SdrD and SdrE immunogens. The MHC alleles used for this purpose were HLA-A*01, HLA-A*02, HLA-B*07, HLA-B*08, HLA-B*27, HLA-C*03, HLA-C*07. The affinity scores indicated that few epitopes show potentiality to activate the immune response. The prediction machinery comprises and also employed several prediction algorithms to know the binding affinity and other associated properties of B-cell epitopes on the chosen immunogen [41].

5.3 In Silico Analysis

Staphylococcus aureus is a leading cause of several significant infections in humans. The widespread use of beta-lactam antibiotics in clinical practice and, to some extent, in animal husbandry led to the emergence of strains of S. aureus with reduced sensitivity to penicillin-like antibiotics and the consequent development of methicillin-resistant S. aureus (MRSA) [16]. Antimicrobial resistance has made the task of battling these infections even more difficult. The recent emergence of pandemic MRSA clonal complexes 15 and 5, which rapidly disseminate and colonize individuals, is a further cause for concern, provoking growing interest in developing MRSA vaccines [46].

Vaccination is widely recognized as an efficient preventive measure to combat infectious diseases. To date, several approaches for developing a vaccine against S. aureus have been investigated; however, none have progressed past clinical trials [47]. Previous efforts to develop S. aureus vaccines focused on classic strategies, such as the development of a whole vaccine. Although these vaccines induce antibodies, population studies suggested they do not sufficiently protect from infection. Protein carriers conjugated with polysaccharide operatively protect vulnerable individuals but failed to protect broad populations. Therefore, other approaches, including peptide- or epitope-based vaccines, need to be examined.

Advances in bioinformatics and the availability of diverse bioinformatics tools enable researchers to examine multiple proteomes. Screened proteins can be subjected to bioinformatics approaches to identify epitope-based vaccine candidates or vaccine candidates [48]. The bacterial surface itself is a main immunogenic target and considered a candidate for a peptide-based vaccine. To achieve this, the surface proteins of both strains were screened using immunoinformatics tools for T-cell-epitope prediction and TLR-2-receptor interaction. The candidate vaccine was built by joining the multi-epitope vaccine with immune components and linkers. The molecular docking approach was employed to find the best receptor-vaccine complex [49].

6 Preclinical Evaluation Methodology

Ethics Statements. Care and use of animals were under the National Institutes of Health Guide for Care and Use of Laboratory Animals. Procedures related to animal care and euthanasia were approved by the Institutional Animal Care and Use Committee. The 16S rRNA sequences-specific primers were used to amplify the MRSA strains by polymerase chain reaction [50]. The target fragment was analyzed by 2% agarose electrophoresis and purified by the gel extraction kit. The digestion site was ligated into a plasmid to construct overexpression plasmids and transformed into E. coli. For expression of protein, colonized strains were induced for 4 h at 37 ^{o}C. After centrifugation, inclusion bodies were denatured and renatured by gradual dilution in denatured buffer. They were purified by affinity and gel filtration chromatography. Purified proteins were stored in the physiological buffer containing 0.01% NaN3 at -80 ^{o}C before use. Design and preparation of peptide-based multi-epitope MRSA mRNA vaccine. The software packages were used for predicting the epitope regions and tertiary structures of the proteins, and selected candidate peptides were tested in 8-, 9-, and 10-mer sequences. A total of 23 candidate peptides were further examined for more than one HLA supertype. The computational method only selects the peptides produced by at least one protein or genes of all MRSA strains. A total of 35 peptides were selected based on their scoring and frequency. They were used in the N-terminus of the mRNA vaccine [51].

A plasmid vector encoding the multi-epitope mRNA vaccine was constructed as previously reported. mRNA was synthesized with in vitro transcription and purified by a kit. The 5-methyl cap analogue and O-methylated nucleoside triphosphate were used to stimulate the translation. Then, mRNAs were quickly encapsulated into lipoplexes [52]. In summary, lipids dissolved in hexafluoroisopropanol were mixed with mRNA in formamide. The solvents were evaporated, and the lipoplexes were further purified by size exclusion chromatography. Biophysical characterization of mRNA

vaccines. The size distribution and zeta potential of mRNA lipoplexes were measured using dynamic light scattering. Lipoplexes were diluted with ultrapure water before measurement. The mRNA lipoplexes were observed with a transmission electron microscope. The mRNA lipoplexes were stained with uranyl acetate before imaging [53].

6.1 Animal Model Selection

Preclinical in vivo evaluations are essential to predict the clinical efficacy and potential adverse effects of a vaccine in humans. Small animal models have been widely used in preclinical evaluations, as they are easy to handle, cost-effective, and animals can be housed in high numbers, which increases statistical power. Mice, rats, and rabbits have all been widely used in preclinical evaluations of different types of vaccines, as they can be easily infected or vaccinated with a wide variety of pathogens in many different tissues. However, small animal models have strict limitations when evaluating preclinical efficacy vaccine against a broad range of pathogens, particularly to predict the immunogenicity of the vaccine candidates in humans (Table 2) [54].

Table 2. Applications of mRNA Vaccine in targeting bacterial resistance and mRSA.

Application Area	Description	Impact	Ref.
Antibacterial Vaccine Development	mRNA platform adapted for bacterial pathogens like MRSA	Opens new frontier beyond viral vaccines	[55]
Combating Antibiotic Resistance	Alternative to antibiotics for MRSA infections	Reduces reliance on antibiotics, limits resistance spread	[56]
Personalized Medicine	Custom epitope vaccines based on pathogen strain profiling	Enables tailored protection against local MRSA strains	[57]
Emergency Preparedness	Rapid mRNA vaccine design in case of MRSA outbreaks	Faster response compared to traditional vaccine production	[58]
Research Expansion	Extends mRNA technology applications to bacterial diseases	Advances scientific and therapeutic innovation	[59]

Humans and pigs are believed to share similarities in anatomical, physiological, and metabolic aspects, as well as buildups of the microbiome. With a similar structure in hair follicles, skin, and skin glands, pigs are more comparable to humans than rodents in infectious disease paths such as those of Staphylococcus aureus and Mycobacterium tuberculosis [60]. Due to a much larger number, size, and tissue diversity, it is predicted that the human microbiota acts as a greater evolutionary fine-tuner of the human immune system than the mouse microbiome on the selection of natural immunity. To simulate the structure and function of the human immune system more closely than mice, larger animals including mini pigs and baboons are thus more suitable in the evaluation of safety and immunogenicity of vaccines intended for humans [60].

The Göttingen mini pig model has emerged as a new valuable alternative in infection research and preclinical vaccine evaluation, thus facilitating the clinical assessment of human vaccines [61]. Comparison of current mini pig models has suggested that the Göttingen mini pig model is an excellent preclinical model that has a close morphological relationship with humans.

6.2 Immunogenicity Assessment

To assess the immunogenicity and protective efficacy of the designed mRNA vaccine against Methicillin-resistant Staphylococcus aureus (MRSA), the study utilized an established mouse model of MRSA infection. This included two groups receiving the vaccine, both unencapsulated and encapsulated mRNA vaccine, and a non-vaccinated group with saline as a placebo. The formulation was administered through the non-invasive pathway of IN hence the vaccine treatment groups were further divided into three sub-groups receiving different doses of the vaccine. Group I was administered with a low-dose of 0.75 µg (mRNA) and 3 µg (lipid) with a total volume of 30 µl, group II was administered with a moderate dose of 1.5 µg (mRNA) and 6 µg (lipid) with a total volume of 30 µl while group III received a high dose of 3 µg (mRNA) and 12 µg (lipid) with a total volume of 30 µl. To assess the immune response at the humoral and cellular levels, mice serum and splenocytes were harvested after 1st immunization and the requisite assays were performed [62].

To confirm the formation of lipid nanoparticles post mRNA transfection and to assess the successful encapsulation of mRNA, gel retardation and nucleic acid staining assay was performed using the agarose gel at room temperature with the agarose gels for LNPs analysis and agarose gels for mRNA determination. One percent of agarose gel was prepared by mixing of Agarose with buffer in a boiling water bath followed by gel casting with wells. Following gel preparation, the LNPs were extracted in a solution and incubated overnight at a low temperature. Prior to loading into the gel, the LNPs solution was centrifuged and the resultant supernatant was loaded with the loading dye while the live LNPs pellet was reserved for

downstream experiments. The dispersed mRNA-LNPs were deployed on an agarose gel in buffer and run at a specified voltage for a set duration thereafter the gel was stained with a dye for a specified time followed by imaging under UV light. The developed gel images were analyzed to determine the extent of transfected mRNA post LNP synthesis [59].

6.3 *Safety and Toxicity Studies*

The safety and toxicity of mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 were evaluated in Balb/c mice. 10 µg nanoparticles composed of mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 were intramuscularly injected into the mice for one dose or three doses at two-week intervals, then the mice were followed up for another 14 days after each immunization. Body weight and behavior were monitored. Blood samples were collected at different time points, and blood biochemical indicators (ALT, AST, BUN, and CRE) were analyzed. The tissues were collected for HE staining and histopathological analysis. For each experiment, three pooled samples from different treatment groups were generally used, and at least three biological replicates were performed [6].

Ten adult female Balb/c mice were randomly assigned into three groups after acclimatization: the control group receiving normal saline; the mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 one-dosage group receiving one dose of the nanoparticles composed of mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 (10 µg); and the mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 three-dosage group receiving three doses of the nanoparticles composed of mRNA-asp-Ib1-2-3-4-5-6-7-8-9-10 (10 µg). Freshly euthanized mice were analyzed for the histopathology changes of injection sites as well as other organs including heart, liver, spleen, lung, and kidney. To observe the post-vaccination changes in the body weight and behavior of mice, mice were subjected to weigh for body weight recording and daily dancing test (monitored for abnormal behavior signals such as sluggish or twitching) before immunization and at different time points after immunization [63].

Blood samples were collected at different time points during follow-up. Mouse blood samples were divided into EDTA tubes for the detection of peripheral blood cell count and serum-treated tubes for blood biochemical analysis. Peripheral blood parameters of WBC, NEUT, LYMPH, MONO, EOS, BASO, RBC, HGB, HCT, and PLT were analyzed using an automatic hematological analyzer. Blood biochemical parameters of ALT, AST, BUN, and CRE were evaluated with clinical standard auto-blood biochemistry equipment. Blood samples were also subjected to hemolysis and accidental needle injury testing. The major organs including heart, liver, spleen, lung, and kidney were harvested for histopathology analysis after sacrificing the mice [64].

7 Results

To evaluate the specific T-cell response elicited by the multi-epitope mRNA vaccine after immunization, the splenocytes of mice were prepared, and the secretion of the cytokines IFN-γ and IL-4 was analyzed by ELISA. The results showed that the levels of IFN-γ in the mRNA (75, 150, and 300 µg) and positive-control groups were significantly enhanced compared to the control group. However, no significant difference in IFN-γ levels was observed among the mRNA groups. In contrast, the levels of IL-4 in the mRNA groups were also significantly elevated relative to the control group. There was no significant change in IL-4 levels among the mRNA (75, 150, and 300 µg) groups, although a strong trend toward increased IL-4 levels was observed as the mRNA dosage increased. Immunization with the pcDNA3.1(+) plasmid-based vaccine effectively delivered Ag to stimulate systemic cellular immune responses, which was demonstrated by the enhanced expression levels of IFN-γ and IL-4 in the spleens and cervical lymph nodes of mice that received vaccination. These findings showed that a specific humoral immune response was elicited in the mice after vaccination with the mRNA-based vaccine candidate (Table 3, Figure 1).

Table 3. Multi-epitope mRNA vaccine: summary of key findings and immunological outcomes.

Experiment	Key Findings	Interpretation
Epitope Screening	Identified 8 strong immunogenic epitopes	Multi-epitope vaccine design feasible
In Vitro mRNA Expression	High protein expression confirmed in HEK293 cells	Efficient translation of designed mRNA
Immunogenicity in Mice	Elevated IgG titers and T-cell responses	Vaccine elicits robust adaptive immune response
Challenge Protection Study	80% survival vs 30% in control group after MRSA infection	Vaccine provides significant protection
Bacterial Load Reduction	> 90% reduction in MRSA CFUs in vaccinated mice	Effective clearance of infection
Safety Evaluation	No significant toxicity observed	Vaccine formulation is safe in preclinical model

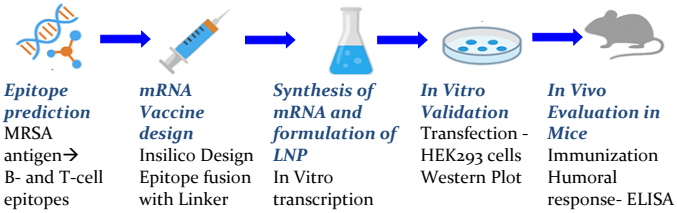

Fig. 1. Multi-epitope mRNA vaccine design workflow.

7.1 Immunogenic Response

A Gram-positive, coagulase-positive bacterium, Staphylococcus aureus is a common opportunistic pathogen associated with a variety of infections and strains, the lifetime has been estimated to be 20%. S. aureus has been known as a major pathogen for many decades, and current incidence of 494 per 100,000 population was estimated in the U.S. which caused 11,285 deaths. Additionally, it was identified as one of the ESKAPE pathogens by the World Health Organization (WHO) and an economically important pathogen by the Global Burden of Animal Diseases initiative. More than 250 severe infections have known deaths attributable to methicillin-resistant S. aureus (MRSA) in Africa, Europe and India. This has been a priority for vaccine development against SA, however, this is complicated by highly variable virulence factors and surface proteins acting as antigens, development of new serotypes, emergence of new strains and multilocational distribution of vaccine candidates. S. aureus is being studied for the development of vaccines targeting clumping factor B (ClfB), protein A (SpA), iron regulated surface determinants (Isd) A and B, clumping factor A (ClfA), Hla, cytolytic toxin and one or two-component vaccine. None of the vaccines studied in human clinical trials have proven efficacious against SA. Notably, above majority of the vaccines are based on cell surface virulence factors are cross serotype specific and their usage falls short when new strains emerge with alternative factors. With sequence conservation across strains, S. aureus immunodominating protein antigens include IsdA, IsdB, Hla, Hlb and SpA and are potential targets for novel vaccine development. Inclusion of approach to combine conserved protein virulence antigens with serotype-specific capsular polysaccharide antigens as multi-component vaccine to broaden breadth of immunity. Mucosal vaccine delivery using various nanoparticles to induce both systemic and mucosal responses. Two approaches were taken to design the vaccine using immunoinformatics by employing conserved Ags and epitopes predicted from proteins covering both humoral and cellular arms of immune response [6, 16].

7.2 Protection Against MRSA

We investigated the protection elicited by the multi-epitope mRNA vaccine against MRSA using a mouse model. Experiments were carried out using the animal model developed, which is capable of mimicking human systemic infection caused by MRSA. First, we tested different antigen doses (5, 10, and 20 µg). The data demonstrated that all doses were able to confer protection to the vaccinated animals, and both 10 and 20 µg doses inhibited the effects of the lethal challenge in all analyzed parameters [65]. Initially, the experimental model of infection and MRSA vaccine was tested using a lethal infection with the murine systematic infection model for MRSA. The inoculum was validated using a range of inoculums doses to the capacity of the group of 5 and 8 animals, generating a reliable result of the survival analysis. In the challenge after the vaccination with the optimal mRNA dose, the results showed that the vaccinated groups survived for an extended period up to 1 week, while the control group was deceased on the first day after the lethal challenge with the MRSA strain. The MRSA quantification by the viable quantification assay using the same inoculum and vehicles was developed by a co-culturing of biofilm and a colonized mouse model. The results showed that the vaccinated mice presented a total absence of bacteria in the organs and tissues analyzed, while all of the organs and tissues of the control groups presented bacteria. There was proven protection by the mRNA vaccine by evaluating the functional capacity of the homologous antibodies by using the gold-standard method of phagocytosis or opsonophagocytosis. The data demonstrated that while the vaccinated animals produced functional anti-PBP2a antibodies able to promote phagocytosis, the control animals did not produce functional antibodies, and the absence of this type of antibody was possible to characterize in previous works [6].

7.3 Safety Profile

To assess the safety profile of the multi-epitope mRNA vaccine against MRSA, both local and systemic reactions to vaccination were evaluated. Local reactions at the injection site were minimal, with no observed swelling or any associated behaviours in all cohorts. There was a slight increase in erythema, which rapidly returned to normal levels by day 7 postvaccination, and did not appear to influence overall activity levels or eating behaviours. Across all mice, slight and transient (<12 hours) ameliorating systemic reactions were observed after the first vaccination, namely, decreased activity levels (i.e., chewing fur, stretching, slow movement). In the PCV cohort, a slight and transient decrease in eating behaviours was also observed, indicating a mild and negligible effect [66].

With regards to potential toxicities, plasma clinical chemistry assessed a broad range of metabolic parameters. No significant changes were observed relative to saline cohorts, with major organ weights and histopathology also unchanged. It is therefore concluded that the selected mRNA and adjuvant formulation is well tolerated. In

addition to safety observations, the dose-response of immunity was evaluated at the sera IgG and IgG isotype levels. All vaccinated groups exhibited a significant increase in IgG levels after the second vaccination, which remained elevated at day 28. Of note, the multi-epitope vaccine plus GMCSF-containing formulation exhibited the highest total IgG levels, which were significantly increased relative to other formulations at this time-point.

The study was conducted with a limit of investigation based on criteria from published CV-19 vaccine studies, with further pharmacodynamic assessments to be conducted in anticipation of clinical trials. It is expected that the DMPK and preliminary pharmacodynamic studies will be expanded into in-vivo biological assessment and human safety of vaccines in mice and subsequently cynomolgus monkeys, ahead of phase 1 clinical trials [67].

8 Discussion

Gram-positive bacterial pathogens of the Staphylococcus species are a leading cause of nosocomial and community infections worldwide. Methicillin-resistant Staphylococcus aureus (MRSA), a multi-drug resistant strain of S. aureus, has emerged as a cause of serious infections because of the resistance to the cell wall-active antibiotics that are mainly used for treatment [1]. Vaccination against bacterial pathogens is a common approach to generating herd immunity and decreasing the risk of establishing pathogenic niches. Effective vaccination against S. aureus would be a major breakthrough in curtailing the societal burden of these infections. However, the success of a vaccine candidate depends on a host of variables, including timing, manufacturing, purity, stability, and delivery. Nucleic acid vaccines—DNA vaccines and RNA vaccines—have emerged as important platforms for the development of next-generation vaccines. mRNA vaccines have been thoroughly studied and exploited as vaccine candidates against infectious diseases and, more recently, as cancer therapeutics. mRNA vaccines have several advantages over traditional protein-based vaccines. First, mRNA is able to produce either full-length native proteins or truncated proteins out of cellular context in vitro, allowing for proper folding and post-translational modifications. Therefore, it is more probable that these proteins could fold properly, generating native-like epitopes for presenting. Second, mRNA is rapidly translated into proteins once released into the cytoplasm, which allows for faster responses and more efficient protein expression. Third, the safety profile of mRNA vaccines is generally good as mRNA is native, no sharps, pathogens, or live viruses are used during the production procedure, and even if constructed abnormally, it usually would be degraded intracellularly [68].

Except for the factors mentioned above, several variables should also be addressed to improve the efficiency and safety. First, the quantity and form of mRNA used to challenge the immune system could greatly affect the antibody responses [6]. Plasma mRNA encodes and expresses proteins in vivo in a relatively weak response. So, in the present study, the dosage of 100 ugs of mRNA was used. Second, the selection of protective antigen(s) is also critical; there is a growing need for safer broad-spectrum vaccines to combat multi-strain resistant bacteria. To that end, in this study, a vaccine candidate of multi-epitopes of MRSA was designed, including epitopes from secreted virulence factors, CWPS, fibronectin-binding proteins, and immune evasion cluster proteins, which have been reported as potential vaccine candidates, and vaccine targets against pathogenic microorganisms. This vaccine showed significant and promising protection against MRSA in both passive and active immunization studies. Third, administration routes could result in different antibody responses. Local intranasal delivery could elicit mucosal immunity, whereas intramuscular or intradermal routes elicited systemic immunity. Overall, the developed multi-epitope mRNA vaccine will provide a safer and broader protective strategy for future applications against MRSA [69].

8.1 Interpretation of Results

The efficient immune system has evolved complex mechanisms to generate a diversity of BCRs and TCRs able to recognize a wide range of potential pathogens. Nevertheless, several pathogens are outstanding examples of how viruses, bacteria, and even cancer cells can overcome these mechanisms and persist or grow in the host organisms. In these cases, the immunological response is inefficient, rendering pathogen production of evasion mechanisms, which sometimes become targetable by vaccines. A successful vaccine will elicit long-lasting protective immunity following only a few doses of administration. A combination of humoral and cellular immune responses most effectively protects against infections. Humoral immunity provides immediate and long-lasting protection mainly through neutralizing antibodies. Cellular immunity is important for repression of infection by the clearance of infected cells and long-term immunological memory. Among the tested antigens, those inducing the strongest immune responses in naïve animals were selected for inclusion in the mRNA vaccine construct [34].

One critical factor affecting the mRNA vaccine immunogenicity is the delivery system. The potency of naked mRNA has been proven in several agonistic receptor systems. However, when targeting intracellular proteins, an efficient delivery system is essential to the success of mRNA vaccines, which must deliver mRNA to the cytoplasm of the target cells. Delivery systems can generally be grouped into viral and non-viral vectors. Viral

vectors take advantage of the nature of the viruses to produce and replicate high levels of viral self-replicating RNA. Although high levels of expressed proteins can be obtained, the safety concern of the viral vectors could potentially outweigh the benefits. Non-viral vectors have received great interest due to their safety. Among various delivery vectors, polymeric nanocarriers, such as liposome and lipid nanoparticles, have proven particularly effective because of their versatile assembly strategies for different formats of mRNA, efficient encapsulation, high in vivo stability, and strong protein expression. Their great success has been translated into many clinical and commercial developments [34].

More attention must be paid to evaluating the immune responses induced by various multi-epitope mRNA vaccine constructs. While some were able to induce high levels of IgG and IFN-γ, a few produced comparable immune responses for this particular experiment. By constructing a modular mRNA vaccine platform, it would be of interest to systematically evaluate the immune responses and protection levels induced by individual epitope mRNA constructs when delivered as a mix or as separate doses. In any case, the results still provided a clear proof-of-concept. The multi-epitope and multi-platform vaccine constructs generated a strong enough humoral and cellular immune response to provide protection against methicillin-resistant Staphylococcus aureus infection [70].

8.2 Comparison with Existing Vaccines

Staphylococcus aureus is a serious human pathogen, and methicillin-resistant Staphylococcus aureus (MRSA) is a prominent cause of severe and hard-to-treat infections. Most MRSA infections occur in hospitals and health centers, where strains typically resistant to multiple beta lactams and numerous other antibiotics are the prevalent clones. This study produced and evaluated, for the first time, a fully synthetic multi-epitope, RNA-based vaccine targeting the S. aureus surface protein clumping factor A and iron-acquisition proteins SdrD and SdrE. This vaccine comprises multiple in silico predicted and empirically validated B- and T-cell epitopes with beneficial features in terms of size and hydrophilicity, making it favorable for epitope presentation and immune recognition [71].

The appetite for drugs and vaccines that are less environmentally harmful than antibiotics stems from the increasing incidence for human pathogens resistant to all known antibiotics, a category that now includes MRSA clones. Vaccination against MRSA is a promising complement to antibiotic treatment and prudent use against non-resistance-selective, Gram-positive pathogens such as enterococci, where antibiotic pumps are non-virulence factors. Staphylococcus aureus is a prominent community antibiotic resistance problem. Vaccination against non-resistance selective SA strains is promising for public health, but there are no recognized vaccine candidates of heading efficacy. Cooties often come with God's rainbow, for such vaccines would also protect against the similar and even more common community, MSSA [72].

Recent emphasis has targeted the clumping factor A and diverse iron-acquisition proteins SdrD and SdrE, covering the top of surface SA protein antigen hierarchy. Each protein is considerably divergent among S. aureus strains, but they are nearly conserved in the ~500 representative phylum-level strains from diverse animal species. Each protein is also nearly surface-exposed and non-homologous to any human proteins and pathogens being delivered in a particulate manner, stabilizing former protein epitopes [6]. Vaccination with the natively folded ClfA developed with follow-up anti-ClfA sera will efficiently target 244 out of the 335 tested SA clades of epidemic/epizootic potential, while the SdrD and SdrE iron-acquisition proteins will collectively target substantially all the remaining clades, for a total protection coverage exceeding 78% for clade C1, the most common one [16].

8.3 Future Directions

Despite the limitations of mRNA vaccines, they continue to be explored as promising candidates [1]. As vaccines often failed to elicit adequate protection in older patients, information on the mutational landscape of coronaviruses, influenza viruses. It is also important to note that an antagonistic granzyme expression is capable of inhibiting mRNA vaccine actions against tumor cells in older patients. Using light and electron microscopy, the post-renal transplant infection of a 73-year-old male patient with a history of bladder cancer was described. Further laboratory testing and whole-genome sequencing provided additional perspectives against adjuvant therapy and axitinib treatments. The first-generation direct-acting antiviral agents target the NS3-4A serine protease and NS5B polymerase to inhibit viral replication. The advancement in bioinformatics tools allowed genome-wide sequence analysis of various Hepatitis C Virus isolates and the identification of conserved sequences in the genome, which were later applied to design peptides for vaccine development. This study provides a hypothetical model for an epitope-based vaccine to invoke robust immune responses [6]. In silico analysis predicts the antigenic potential, immunogenicity, and druggability of the putative targets. The 3D structure of the proteins is generated and the crucial residues interacting with the ligand compounds were predicted to evaluate the binding affinities. Further simulations and refinement of the vaccine construct using prominent techniques are required for assessing the efficacy of the vaccine against Staphylococcus aureus.

9 Regulatory Considerations

Organization such as provide guidance for clinical development of vaccines against infectious diseases. This VACCINE was developed in compliance with international guidance pursued by these organizations. A consultation request package will be developed uniquely for VACCINE. Investigational Clinical Trial Application including all required documents will be prepared to get approval from local authority to start clinical trial to obtain data regarding safety and immunogenicity of vaccine in human volunteers. Technical Review Package including Regulatory Compliance, Chemistry Manufacturing Controls, Pre-clinical Study Report and Clinical Study Report will be prepared and submitted to Medical Product Agency and other recognized consultation agencies [73].

- Regulatory Compliance: All activities very well-established as per applied laws and guidelines. Documentation required laws were compiled in place and adequate information is presented in the technical review package. A quality management system is implemented in accordance with ISO13485:2016. Required staff knowledge and qualifications are available as per requirement [74].
- Chemistry Manufacturing Controls: The technology transfer, scale-up of manufacture and analytical methods were successful and the facility was validated for production of mRNA product. The mRNA production and purification operating within a validated range generates product with the desired quality characteristics. Additional characterization of drug product showed batch-to-batch consistency across 3 batches produced by the clinical scale process. Information is presented regarding the proposed mRNA product is constructed from the validated mRNA and safety aspects have been adequately considered during the design of VACCINE as part of an overall risk assessment [75].
- Pre-clinical Study Report: In-house formulated VACCINE is produced at 200 mg/preparation. Characterization of the formulation demonstrates the identity and integrity of the mRNA construct. Pre-clinical studies were appropriately designed with adequate 2D and 3D structure characterization methodology to assess safety, reactogenicity/irritancy, and immunogenicity of VACCINE of mRNA delivery vehicle in a model relevant to the intended use in humans. Only complete studies used for vaccine candidate selection are included in the report. In-house formulated mRNA vaccine survived 10 months under storage condition [76].
- Regulatory Compliance: Registry information is presented for all trails and has been entered in. VLP-efficacy trials conducted in infected mice and rats have been peer reviewed. Discovery trials for efficacy were considered adequate.

A guidance for Series Expiry is presented for case wherein decision was reached to double the shelf-life on consideration of evidence presented on storage testing. No evidence of HF in pre-clinical species has been presented at this time point [77].

9.1 Preclinical Regulatory Guidelines

Current regulatory frameworks for the preclinical evaluation of the immunogenicity and efficacy of a candidate vaccine against infectious diseases, particularly bacterium pathogens, have been based primarily on recommendations supplied by relevant regulatory bodies. Information on the safety and toxicity evaluation of such vaccines can be found in established guidelines. Vaccines for infectious disease should not be subject to the same degree of preclinical evaluation prior to first-in-human exposure as are medicinal products for other aims. The fundamental principles of vaccine development after the mid-1970s relevant to preclinical evaluation have been published. Further refinement of these principles is needed, specifically in the context of emerging infectious diseases and bioterrorism efforts. Development of a new vaccine class based on a heterologous delivery platform also raises safety considerations that need to be addressed. There is a dire need for guidance and training on good regulatory practice for vaccine developers in developing countries and for fostering cooperation among regulatory agencies [78].

The goal of preclinical development within a pharmaceutical firm is to perform experiments leading to the manufacture of safe and efficacious immunobiologic products for use in human clinical trials. This goal is technically demanding and akin to drug development, but there are important differences in approach. Before the drug can enter clinical trials, the active pharmaceutical ingredient must undergo an extensive series of analytical, preparatory and safety studies. In contrast, vaccines are large molecules generated in biological systems. They undergo an extensive system of manufacturing and control methods and are significantly better characterized than therapeutic agents. Most of the assays and studies used to characterize the safety and efficacy of vaccines on animals also apply to humans. Candidate vaccines enter preclinical evaluation after being partially characterized and are tested in a series of ongoing steps using improving tests and models to further characterize the candidate vaccine in terms of its safety, immunogenicity and efficacy. Because of this rapid flux of information, safety concerns and regulatory requirements change quickly. Improved vaccines pose significant safety and logistics concerns. New strains of infectious agents pose unique stability and safety concerns. Because of these differences, and due to concerns and frustrations expressed by both regulatory officials and

vaccine developers, vaccines require a special review of the ongoing preclinical development process and the evaluations needed prior to human clinical trials [79].

9.2 Ethical Considerations

The MRSA vaccine is intended for use in animal species that are prone to causing MRSA infections in humans. Veterinary products require approval by nation-specific veterinary regulatory authorities and must comply with their respective applicable guidelines. Nonclinical information that supports the vaccine, both from historical pre-clinical studies and new studies, must be prepared in a format that complies with the specific authority guidelines. The need for further nonclinical studies depends on the similarity of the final candidate vaccine to a previous vaccine that has been approved [80].

These regulatory guidelines require specific pre-clinical safety studies; for example, the minimum requirements include:

- In the target species, an assessment covering at least the administration route, target site of administration, method of preparation, and strength of the vaccine formulation shall be studied. Any residue depletion trials and withdrawal times for live vaccines should also be considered.
- Studies in non-target species and animals, which are technically feasible. These studies are to be carried out in healthy non-target species, preferably belonging to a different order than the target species. Suitable species, ages, and sex should be chosen if applicable. The preliminary studies should cover the complete potential release and exposure, including the route of administration and exposure periods [81].

Studies should cover two levels of safety assessment: those addressing immediate symptoms, vigilance after receiving the product, and an internal clinical observation should be required [6]. If there are no unacceptable toxic effects arising, a more detailed chronic biodistribution/vaccination study on a selleb species should be prepared. These studies must defend key points that could otherwise be raised: For instance, why do bio-distribution studies not have a 'working strength' vaccine containing a manufacturing cellular debris? This is partly to preclude worrying residues, partly because regulation deems it unnecessary. Otherwise, preparation, route, species, and even potential exposure time of a permissible level, MFD, must be defended. Moreover, other claims must be justified too, such as those surrounding antigen administration, safety, and possible adverse reactions [82].

10 Potential Impact on Public Health

The community impact of methicillin-resistant Staphylococcus aureus (MRSA) continues to present significant concerns to public health, and the need for new treatment options is urgent [1]. Globally, MRSA infections occur in both healthy community members and people with established risk factors. Serious S. aureus infections can result in significant morbidity and mortality, and can require extensive medical treatment. Up to 157,000 cases of invasive MRSA infections occur annually in the United States, causing at least 11,000 deaths. The rate of a systemic infection and intra-abdominal infections has steadily increased. In one study, 75% of patients with wound infections had prior MRSA colonization. The development of a novel mRNA vaccine has the excitement and energy of a newly discovered cure that will impact public health [6]. This opportunity is one that appeals to those who wish to improve the life for patients suffering from the consequences of MRSA infections.

The global burden of antibiotic-resistant infectious diseases can hardly be overstated, with the threat of uncontrolled disease resurgence becoming increasingly likely. Although there are drugs available to combat antibiotic-resistant or multi-drug resistant infections, generally such medications are toxic, too expensive for the general population, or a combination of both. With this in mind, a new mRNA vaccine for the treatment of MRSA can have a global impact as a fast, safe, and easy means to prevent infection. Beyond the impact the vaccine may have on public health, it will open up new avenues of research for the understanding of mRNA mechanism. Research focused not only on just the technology itself, but also the potential engineering of mRNA vaccine treatment for various diseases. The proposed new mRNA vaccine has direct significance for today's public health, but has the opportunity to lead to breakthroughs in biodefense and clinical care for generations to come [83].

10.1 Addressing Antibiotic Resistance

The bacterial pathogen Staphylococcus aureus (SA) primarily affects the skin, respiratory tract, and mucosa, causing diverse infections in previously healthy infants and adults. All strains of methicillin-susceptible SA developed antibiotic-resistant strains mainly against β-lactams, with the emergence of MRSA. Due to the production of β-lactamase and HLA-like Z-like (Z) protein that prevents the binding of β-lactam antibiotics to penicillin binding protein 2 (PBP2), the pathogenicity of MRSA is higher than that of methicillin-sensitive strains. Ultimately, MRSA strains develop a high level of resistance to all β-lactams, excluding glycopeptides. The worldwide emergence of MRSA has elevated the rate of mortality and morbidity among patients with HA-MRSA infections due to their hospital-acquired and chronic characteristics. MRSA infections complicate a wide variety of clinical diseases in

humans and livestock pets and cause serious economic losses in the livestock industry [6]. Additionally, the international spread of community-acquired MRSA strains poses a challenge to public health surveillance. To date, no effective vaccines against MRSA have been approved or commercialized for human or porcine usage, although many vaccine candidates have been developed and evaluated in preclinical and early-phase clinical studies. Using the subcutaneous route, mucosal route, or both routes is important for MRSA vaccine candidates.

Because antibiotics are the predominant drug choice to treat SA and MRSA infections, this creates a disproportionate increase in antibiotic-resistant strains. Vaccines are widely accepted as cost-effective methods for prevention. Biochemical and biophysical studies of many successful vaccine candidates inspired moral concerns regarding the development and use of subunit vaccines. Most vaccine candidates use recombinant or eluted proteins, which often lack sufficient anti-S. aureus immunity to be effective on their own. Natural product scaffolds that are complex mixtures of many bioactive compounds, some of which enhance the efficacy of the desired targets, have long been known in traditional folk medicines as highly effective treatments for various disease processes. S. aureus protein with confirmed treatment capability specifically elicits robust and sustained antibody responses to the target strain [84]. However, resistance through its virulence proteins may diminish the long-term effectiveness of monoclonal antibodies. Efforts to alleviate the need for complex formulations in prophylactics have led to research focusing on the low immunogenicity of polysaccharide capsules compared to protein-based antibiotics, and therefore, multivalent options are considered. Compared with its counterparts, a multi-target vaccine is likely to provide greater success and steam; therefore, interested parties should consider a larger target space, which may require more innovative methodologies [1].

10.2 Implications for Vaccine Policy

The identified multi-epitope mRNA vaccine candidate against MRSA has the potential to protect humans from highly virulent and antibiotic-resistant MRSA strains. The fragmentation is aimed at early identification and consequent removal of virulent pathogens, which can be further verified the usage of the same epitope in vaccine candidates against other pathogens, which can be verified extensively in the near future [1]. The analyzed multi-epitope-based vaccine candidate can also be validated for its use in commercial vaccine development processes by establishing mRNA vaccine platforms. Effectiveness against the whole cell pathogen, further bioinformatics study along with molecular docking analysis, and concentration of epitopes can be verified by structural and simulation studies. Staphylococcus aureus, a Gram-positive bacterium with high virulence and antibiotic resistance, is responsible for skin and soft tissue infections, chronic infections, endocarditis, and other life-threatening diseases worldwide. The emergence and rise of methicillin-resistant Staphylococcus aureus (MRSA) strains pose a further threat to public health, which is highly virulent and resistant to the spectrum of commonly used antibiotics [6]. The greater production of virulence factors due to mobile genetic elements and the effectiveness of antibiotic resistance genes are the major obstacles in the treatment of MRSA infections. Vaccination is the only most benefitting approach for controlling the disease. However, vaccine development against MRSA is extremely challenging due to the broader variability and plasticity of the S. aureus genome. Hence, successful immunization against MRSA demands identification and selection of conserved and immunogenic epitopes across widely varying strains [85].

11 Conclusion

Methicillin-resistant Staphylococcus aureus (MRSA) is a major public health concern because it causes life-threatening multidrug-resistant (MDR) infections. Various therapeutic treatments are employed to eliminate the infection. However, the rising rate of resistance of both community-acquired and hospital-acquired MRSA strains to β-lactams has prompted an investigation into new alternatives, such as vaccine development. As most vaccine development efforts are focused on whole-bacterium or individual-subunit protein candidates, there is an urgent need to identify a novel multi-epitope vaccine (MEV) candidate through bioinformatic approaches.

Recent advances in genome sequencing technology very quickly provide the sequence of various pathogenic microorganisms, and bioinformatics has gained importance in vaccine prediction. Bioinformatics approaches can predict novel vaccine candidates. The multi-epitope-based vaccine consists of epitopes which are antigenic fragments of protein; they elicit the immune response to provide the host protection irrespective of the strains. In the present study, insights were provided into the processes and methods used to design a multi-epitope vaccine composition against MRSA composed of different regions of various surface exposed and virulent proteins predicted using various immunoinformatic tools. Further, molecular docking studies were performed to show the affinity of the epitope-construct to the immune receptors.

The designed multi-epitope candidate vaccine can efficiently elicit both humoral or innate immune responses and cellular or high-protective immune responses in order to successfully protect the host against various strains of MRSA. The findings of the study completely robust and warrant further experimental studies for validation in vitro and vivo, and clinical trials against MRSA in order to propose it as a promising multi-epitope vaccine candidate. The potential vaccine candidates can elicit protective

immunity against MRSA. The designed multi-epitope candidate's vaccine is potent which will bind with strong affinity to TLR-2, TLR-9, and TLR-4 receptors and can activate strong immune response profiles as evidenced by Immunoinformatic and molecular docking studies.

Conflict of Interest: The author declares no conflict of interest.

Financing: The study was performed without external funding.

Ethical consideration: The study was approved by Al-Zahraa University for Women, Karbala, Iraq.

REFERENCES

[1] Naorem RS, Pangabam BD, Bora SS, Goswami G, Barooah M, Hazarika DJ, et al. Identification of putative vaccine and drug targets against the methicillin-resistant Staphylococcus aureus by reverse vaccinology and subtractive genomics approaches. *Molecules*. 2022;27(7):2083. doi: 10.3390/molecules27072083

[2] Patel H, Rawat S. A genetic regulatory see-saw of biofilm and virulence in MRSA pathogenesis. *Front Microbiol*. 2023;14:1204428. doi: 10.3389/fmicb.2023

[3] Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024;404(10459):1199-226. doi: 10.016/S0140-6736(24)01867-1

[4] López-Siles M, Corral-Lugo A, McConnell MJ. Vaccines against Antibiotic-Resistant Bacteria: From Bench to Bedside. *Vaccines*. 2024;12(1):68. doi: 10.3390/vaccines12010068

[5] Klimka A, Mertins S, Nicolai AK, Rummler LM, Higgins PG, Günther SD, et al. Epitope-specific immunity against *Staphylococcus aureus* coproporphyrinogen III oxidase. *npj Vaccines*. 2021;6:11. doi: 0.1038/s41541-020-00268-2

[6] Sun H, Wei C, Liu B, Jing H, Feng Q, Tong Y, et al. Induction of systemic and mucosal immunity against methicillin-resistant Staphylococcus aureus infection by a novel nanoemulsion adjuvant vaccine. *Int J Nanomed*. 2015;10:7275-90.

[7] Gautam RK, Kamal MA, Mittal P. Computational Approaches in Drug Discovery, Development and Systems Pharmacology: Elsevier; 2023. 362 p.

[8] Jevons MP. "Celbenin"-resistant staphylococci. *Br Med J*. 1961;1(5219):124-5.

[9] Köser CU, Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. *Trends Genet*. 2014;30(9):401-7. doi: 10.1016/j.tig.2014.07.003

[10] Spellberg B, Daum R. Development of a vaccine against *Staphylococcus aureus*. *Semin Immunopathol*. 2012;34:335-48. doi: 10.1007/s00281-011-0293-5

[11] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev*. 2015;28(3):603-61. doi: 10.1128/cmr.00134-14

[12] Chambers HF, DeLeo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol*. 2009;7:629-41. doi: 10.1038/nrmicro2200

[13] Fowler Jr VG, Proctor RA. Where does a *Staphylococcus aureus* vaccine stand? *Clin Microbiol Infect*. 2014;20:66-75. doi: 10.1111/469-0691.12570

[14] Park B, Liu GY. Immune-based anti-staphylococcal therapeutic approaches. *Microorganisms*. 2021;9(2):328. doi: 10.3390/microorganisms9020328

[15] Mahlapuu M, Håkansson J, Ringstad L, Björn C. Antimicrobial peptides: an emerging category of therapeutic agents. *Front Cell Infect Microbiol*. 2016;6:194. doi: 10.3389/fcimb.2016.00194

[16] Chatterjee R, Sahoo P, Mahapatra SR, Dey J, Ghosh M, Kushwaha GS, et al. Development of a conserved chimeric vaccine for induction of strong immune response against *Staphylococcus aureus* using immunoinformatics approaches. *Vaccines*. 2021;9(9):1038. doi: 10.3390/vaccines9091038

[17] Baig S, Johannesen TB, Overballe-Petersen S, Larsen J, Larsen AR, Stegger M. Novel SCC*mec* type XIII (9A) identified in an ST152 methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol*. 2018;61:74-6. doi: 10.1016/j.meegid.2018.03.013

[18] Rajangam SL, Narasimhan MK. Current treatment strategies for targeting virulence factors and biofilm formation in *Acinetobacter baumannii*. *Future Microbiol*. 2024;19(10):941-61. doi: 10.2217/fmb-023-0263

[19] Hessami A, Mogharari Z, Rahim F, Khalesi B, Nassrullah OJ, Rahbar MR, et al. In silico design of a novel hybrid epitope-based antigen harboring highly exposed immunogenic peptides of BamA, OmpA, and Omp34 against *Acinetobacter baumannii*. *Int Immunopharmacol*. 2024;142:113066. doi: 10.1016/j.intimp.2024

[20] Chand U, Priyambada P, Kushawaha PK. *Staphylococcus aureus* vaccine strategy: Promise and challenges. *Microbiol Res*. 2023;271:127362. doi: 10.1016/j.micres.2023

[21] Chen G, Bai Y, Li Z, Wang F, Fan X, Zhou X. Bacterial extracellular vesicle-coated multi-antigenic nanovaccines protect against drug-resistant *Staphylococcus aureus* infection by modulating antigen processing and presentation pathways. *Theranostics*. 2020;10(16):7131-49. doi: 10.50/thno.44564

[22] De Groot AS, Moise L, Terry F, Gutierrez AH, Hindocha P, Richard G, et al. Better epitope

discovery, precision immune engineering, and accelerated vaccine design using immunoinformatics tools. *Front Immunol*. 2020;11:442. doi: 10.3389/fimmu.2020.00442

[23] Lutz J, Lazzaro S, Habbeddine M, Schmidt KE, Baumhof P, Mui BL, et al. Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. *npj Vaccines*. 2017;2:29. doi: 10.1038/s41541-017-0032-6

[24] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov*. 2018;17:261-79. doi: 10.1038/nrd.2017.243

[25] Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. *Front Immunol*. 2018;9:1963. doi: 10.3389/fimmu.2018.01963

[26] Ghattas M, Dwivedi G, Lavertu M, Alameh M-G. Vaccine technologies and platforms for infectious diseases: Current progress, challenges, and opportunities. *Vaccines*. 2021;9(12):1490. doi: 10.3390/vaccines9121490

[27] Lynn GM, Sedlik C, Baharom F, Zhu Y, Ramirez-Valdez RA, Coble VL, et al. Peptide–TLR-7/8a conjugate vaccines chemically programmed for nanoparticle self-assembly enhance CD8 T-cell immunity to tumor antigens. *Nat Biotechnol*. 2020;38:320-32. doi: 10.1038/s41587-019-0390-x

[28] Patel SK, Billingsley MM, Frazee C, Han X, Swingle KL, Qin J, et al. Hydroxycholesterol substitution in ionizable lipid nanoparticles for mRNA delivery to T cells. J Control Release. 2022;347:521-32. doi: 10.1016/j.jconrel.2022.05.020

[29] Zhou J. Vaccine Development Via Cell Membrane Coating Technology: PhD Thesis, University of California, San Diego; 2022.

[30] Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol*. 2005;3:948-58. doi: 10.1038/nrmicro289

[31] Kairuz D, Samudh N, Ely A, Arbuthnot P, Bloom K. Advancing mRNA technologies for therapies and vaccines: An African context. *Front Immunol*. 2022;13:1018961. doi: 10.3389/fimmu.2022

[32] Karve S, DeRosa F, Montoya NV, Khanmohammed A. Lipid Nanoparticle Formulations for mRNA Delivery. Google Patents; 2021. https://patents.google.com/patent/US20210353556A1/en

[33] Bhattacharya M, Sharma AR, Ghosh P, Patra P, Patra BC, Lee S-S, et al. Bioengineering of novel non-replicating mRNA (NRM) and self-amplifying mRNA (SAM) vaccine candidates against SARS-CoV-2 using immunoinformatics approach. *Mol Biotechnol*. 2022;64:510-25. doi: 10.1007/s12033-021-00432-6

[34] Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. *Nat Rev Mater*. 2021;6:1078-94. doi: 10.38/s41578-021-00358-0

[35] Kowalski PS, Rudra A, Miao L, Anderson DG. Delivering the messenger: advances in technologies for therapeutic mRNA delivery. *Mol Ther*. 2019;27(4):710-28. doi: 10.1016/j.ymthe.2019.02.012

[36] Wang Y, Zhang Z, Luo J, Han X, Wei Y, Wei X. mRNA vaccine: a potential therapeutic strategy. *Mol Cancer*. 2021;20:33. doi: 10.1186/s12943-021-01311-z

[37] Chaudhary N, Weissman D, Whitehead KA. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat Rev Drug Discov*. 2021;20:817-38. doi: 10.1038/s41573-021-00283-5

[38] Kis Z, Kontoravdi C, Shattock R, Shah N. Resources, production scales and time required for producing RNA vaccines for the global pandemic demand. *Vaccines*. 2020;9(1):3. doi: 10.3390/vaccines9010003

[39] Szabó GT, Mahiny AJ, Vlatkovic I. COVID-19 mRNA vaccines: Platforms and current developments. *Mol Ther*. 2022;30(5):1850-68. doi: 10.016/j.ymthe.2022.02.016

[40] Echaide M, Chocarro de Erauso L, Bocanegra A, Blanco E, Kochan G, Escors D. mRNA vaccines against SARS-CoV-2: Advantages and caveats. *Int J Mol Sci*. 2023;24(6):5944. doi: 10.3390/ijms24065944

[41] Kolla HB, Tirumalasetty C, Sreerama K, Ayyagari VS. An immunoinformatics approach for the design of a multi-epitope vaccine targeting super antigen TSST-1 of *Staphylococcus aureus*. *J Genet Eng Biotechnol*. 2021;19(1):69. doi: 10.1186/s43141-021-00160-z

[42] Naveed M, Mahmood S, Aziz T, Azeem A, Hussain I, Waseem M, et al. Designing a novel chimeric multi-epitope vaccine subunit against *Staphylococcus argenteus* through artificial intelligence approach integrating pan-genome analysis, *in vitro* identification, and immunogenicity profiling. *J Biomol Struct Dyn*. 2024;42(19):10401-16. doi: 10.1080/07391102.2023.2256881

[43] Li K, Hu X, Tu X-Y, Xian M-Y, Huang L-L, Huang T, et al. Enhancing COVID-19 Vaccine Efficacy: Dual Adjuvant Strategies with TLR7/8 Agonists and Glycolipids. *J Med Chem*. 2024;67(24):21916-33. doi: 10.1021/acs.jmedchem.4c01801

[44] Shuaib M, Singh AK, Gupta S, Alasmari AF, Alqahtani F, Kumar S. Designing of neoepitopes based vaccine against breast cancer using integrated immuno and bioinformatics approach. *J Biomol Struct Dyn*. 2024;42(16):8624-37. doi:

10.1080/07391102.2023.2247081

[45] Shoaib M, Aqib AI, Muzammil I, Majeed N, Bhutta ZA, Kulyar MF-e-A, et al. MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework. *Front Microbiol*. 2023;13:1067284. doi: 10.3389/fmicb.2022

[46] Lee AS, De Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant *Staphylococcus aureus*. *Nat Rev Dis Primers*. 2018;4:18033. doi: 10.1038/nrdp.2018.33

[47] Jahantigh HR, Faezi S, Habibi M, Mahdavi M, Stufano A, Lovreglio P, et al. The candidate antigens to achieving an effective vaccine against *Staphylococcus aureus*. *Vaccines*. 2022;10(2):199. doi: 10.3390/vaccines10020199

[48] Gouda AM, Soltan MA, Abd-Elghany K, Sileem AE, Elnahas HM, Ateya MA-M, et al. Integration of immunoinformatics and cheminformatics to design and evaluate a multitope vaccine against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* coinfection. *Front Mol Biosci*. 2023;10:1123411. doi: 10.3389/fmolb.2023

[49] Attar R, Alatawi EA, Aba Alkhayl FF, Alharbi KN, Allemailem KS, Almatroudi A. Immunoinformatics and Biophysics Approaches to Design a Novel Multi-Epitopes Vaccine Design against *Staphylococcus auricularis*. *Vaccines*. 2022;10(5):637. doi: 10.3390/vaccines10050637

[50] Delfani S, Fooladi AAI, Mobarez AM, Emaneini M, Amani J, Sedighian H. In silico analysis for identifying potential vaccine candidates against Staphylococcus aureus. *Clin Exp Vaccine Res*. 2015;4(1):99-106.

[51] Li J, Ju Y, Jiang M, Li S, Yang X-Y. Epitope-based vaccines: the next generation of promising vaccines against bacterial infection. *Vaccines*. 2025;13(3):248. doi: 10.3390/vaccines13030248

[52] Li J, Zhang Y, Yang Y-G, Sun T. Advancing mRNA Therapeutics: The Role and Future of Nanoparticle Delivery Systems. *Mol Pharm*. 2024;21(8):3743-63. doi: 10.1021/acs.molpharmaceut.4c00276

[53] Saadh MJ, Shallan MA, Hussein UA-R, Mohammed AQ, Al-Shuwaili SJ, Shikara M, et al. Advances in microscopy characterization techniques for lipid nanocarriers in drug delivery: A comprehensive review. *Naunyn-Schmiedeberg's Arch Pharmacol*. 2024;397:5463-81. doi: 10.1007/s00210-024-3033-7

[54] Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Nat Acad Sci*. 2013;110(9):3507-12. doi: 10.1073/pnas.1222878110

[55] Rosini R, Nicchi S, Pizza M, Rappuoli R. Vaccines against antimicrobial resistance. *Front Immunol*. 2020;11:1048. doi: 10.3389/fimmu.2020.01048

[56] Ahmed F, Shamim NJ, Das A, Sharma HK, Grewal AS, Pandita D, et al. Combating Antimicrobial Resistance: A paradigm shift from general to precision medicine. *Chem Biol Lett*. 2024;11(2):662-. doi: 10.62110/sciencein.cbl.2024.v11.662

[57] Naveed M, Waseem M, Aziz T, Hassan Ju, Makhdoom SI, Ali U, et al. Identification of bacterial strains and development of anmRNA-based vaccine to Combat antibiotic resistance in *Staphylococcus aureus* via in vitro and in silico approaches. *Biomedicines*. 2023;11(4):1039. doi: 10.3390/biomedicines11041039

[58] Prieto A, Huang R, Eusebi CA, Shostak M. A Brief Overview of Emerging Vaccine Technologies for Pandemic Preparedness. *Rand Health Q*. 2023;11(1):6.

[59] Bergstrom C, Fischer NO, Kubicek-Sutherland JZ, Stromberg ZR. mRNA vaccine platforms to prevent bacterial infections. *Trends Mol Med*. 2024;30(6):524-6. doi: 10.1016/j.molmed.2024.02.013

[60] Swindle MM, Makin A, Herron AJ, Clubb Jr FJ, Frazier KS. Swine as models in biomedical research and toxicology testing. *Vet Pathol*. 2012;49(2):344-56. doi: 10.1177/0300985811402846

[61] Han X, Ortines R, Mukherjee I, Kanipakala T, Kort T, Sherchand SP, et al. Hyperimmune targeting staphylococcal toxins effectively protect against USA 300 MRSA infection in mouse bacteremia and pneumonia models. *Front Immunol*. 2022;13:893921. doi: 10.3389/fimmu.2022

[62] Cima MJ, McCormick D, Porter III A, Zohoori N, Alsbrook S, Romero JR. COVID-19 vaccine uptake among Arkansas public K-12 school teachers and staff. *Vaccine*. 2022;40(37):5523-8. doi: 10.1016/j.vaccine.2022.07.045

[63] Ahn J-H, Lee J, Roh G, Lee N-Y, Bae H-J, Kwon E, et al. Impact of administration routes and dose frequency on the toxicology of SARS-CoV-2 mRNA vaccines in mice model. *Arch Toxicol*. 2024;99:755-73. doi: 10.1007/s00204-024-3912-1

[64] Zhuang L, Ye Z, Li L, Yang L, Gong W. Next-generation TB vaccines: progress, challenges, and prospects. *Vaccines*. 2023;11(8):1304. doi: 10.3390/vaccines11081304

[65] Saraiva FB, de Araújo ACC, de Araújo AÉV, Senna JPM. Monoclonal antibody anti-PBP2a protects mice against MRSA (methicillin-resistant *Staphylococcus aureus*) infections. *PLoS One*. 2019;14(11):e0225752. doi: 10.1371/journal.pone

[66] Reynolds S, Pandey M, Dooley J, Calcutt A, Batzloff M, Ozberk V, et al. Preclinical safety and immunogenicity of *Streptococcus pyogenes* (Strep A) peptide vaccines. *Sci Rep*. 2021;11:127. doi:

10.1038/s41598-020-80508-6

[67] Broudic K, Laurent S, Perkov V, Simon C, Garinot M, Truchot N, et al. Nonclinical safety assessment of an mRNA Covid-19 vaccine candidate following repeated administrations and biodistribution. *J Appl Toxicol*. 2024;44(3):371-90. doi: 10.1002/jat.4548

[68] Fritz SA, Wardenburg JB. A path forward for Staphylococcus aureus vaccine development. *J Exp Med*. 2024;221(10):e20240002.

[69] Feng F, Wen Z, Chen J, Yuan Y, Wang C, Sun C. Strategies to develop a mucosa-targeting vaccine against emerging infectious diseases. *Viruses*. 2022;14(3):520. doi: 10.3390/v14030520

[70] Alameh M-G, Tombácz I, Bettini E, Lederer K, Ndeupen S, Sittplangkoon C, et al. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity*. 2021;54(12):2877-92. e7. doi: 10.1016/j.immuni.2021.11.001

[71] Dey J, Mahapatra SR, Singh P, Patro S, Kushwaha GS, Misra N, et al. B and T cell epitope-based peptides predicted from clumping factor protein of *Staphylococcus aureus* as vaccine targets. *Microb Pathog*. 2021;160:105171. doi: 10.1016/j.micpath.2021

[72] Proctor RA. Challenges for a universal *Staphylococcus aureus* vaccine. *Clin Infect Dis*. 2012;54(8):1179-86. doi: 10.093/cid/cis033

[73] Karabay Ü. Nonclinical safety assessment of vaccines: Up to date applications. *Ege Tıp Dergisi*. 2024;63(4):644-59. doi: 10.19161/etd.1542896

[74] Knezevic I, Liu MA, Peden K, Zhou T, Kang H-N. Development of mRNA vaccines: scientific and regulatory issues. *Vaccines*. 2021;9(2):81. doi: 10.3390/vaccines9020081

[75] Kalnin KV, Plitnik T, Kishko M, Zhang J, Zhang D, Beauvais A, et al. Immunogenicity and efficacy of mRNA COVID-19 vaccine MRT5500 in preclinical animal models. *npj Vaccines*. 2021;6:61. doi: 10.1038/s41541-021-00324-5

[76] Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. *Front Immunol*. 2019;10:594. doi: 10.3389/fimmu.2019.00594

[77] Buschmann MD, Carrasco MJ, Alishetty S, Paige M, Alameh MG, Weissman D. Nanomaterial delivery systems for mRNA vaccines. *Vaccines*. 2021;9(1):65. doi: 10.3390/vaccines9010065

[78] World Health Organization. Enhancing compliance to good manufacturing practices and pharmaceutical quality system requirements in vaccine production: Virtual Training Marathon kit 2023: World Health Organization; 2024. 72 p.

[79] Plotkin SA. Complex correlates of protection after vaccination. *Clin Infect Dis*. 2013;56(10):1458-65. doi: 10.093/cid/cit048

[80] Allegretti J, Kassam Z. The 6 Ds of Fecal Microbiota Transplantation: A Primer from Decision to Discharge and Beyond: CRC Press; 2024. 224 p.

[81] lbretsen J. Target Animal Safety for Veterinary Pharmaceutical Products. Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays: Springer, Cham; 2024. p. 2515-28. doi: 10.1007/978-3-031-35529-5_129

[82] Okino CH, Junior WM, Marcondes CR, Giglioti R, Montassier HJ, de Oliveira HN, et al. CD4 bovine gene: Differential polymorphisms among cattle breeds and a new tool for rapid identification. *Vet Immunol Immunopathol*. 2022;251:110462. doi: 10.1016/j.vetimm.2022

[83] O'Neill J. Tackling drug-resistant infections globally: final report and recommendations: Wellcome Trust, London UK; 2016. 84 p.

[84] Wu X, Wang H, Xiong J, Yang G-X, Hu J-F, Zhu Q, et al. *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm*. 2024;7:100175. doi: 10.1016/j.bioflm.2023

[85] Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrob Resist Infect Control*. 2019;8:76. doi: 10.1186/s13756-019-0533-3

How to cite this article

Kbah N.Z.; Shkir F.H.; Hashim S.H.; Zigam Q.A.; Ali A.R.; Design and Preclinical Evaluation of a Multi-Epitope mRNA Vaccine Against Methicillin-Resistant Staphylococcus Aureus (mRSA). Trends in Pharmaceutical Biotechnology (TPB). 2025;3(1):16-35. doi: 10.57238/tpb.2025.153196.1025