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MOLECULAR CLONING AND IMMUNOGENICITY EVALUATION OF *SPA* PROTEIN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) VACCINE CANDIDATES FOR MASTITIC COWS

SABRIN IBRAHEEM MOHSIN AND AIDA BARA ALLAWE

Department of Microbiology, College of Veterinary Medicine, University of Baghdad

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ABSTRACT

Mastitis is one of the most common and consequential illnesses globally impacting dairy cows, where the predominant mastitic pathogen is believed to be methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide. Antimicrobial resistance necessitates the development of novel vaccines. This work aimed to produce recombinant *SpA* protein for vaccination against *Staphylococcus aureus* (*S. aureus*)-induced mastitis and to evaluate its effects on IL-12 and IFN-γ production in the spleens of lactating BALB/c mice. Clinical mastitis MRSA strains were cultured in Luria–Bertani broth (LB) at 37°C until ready for real time polymerase chain reaction (PCR) amplification. The recombinant proteins were expressed by cloning in *Escherichia coli (E. coli*) *Rosetta* (DE3) using the expression vector plasmid pET-24a, alongside the expression of the IL-12 and IFN-γ genes in the spleens of mastitic BALB/c mice models. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) bands demonstrate that protein A (35.8 kDa) remained soluble in the lysis solution across all evaluated isopropyl β-d-1-thiogalactopyranoside (IPTG) doses. This was seen at an optical density (OD) of 600 nm of 0.6 after an 8-hour induction at 30°C with 0.1 mM IPTG. 100 mg of *SpA* recombinant protein was acquired. The current study indicated that immunization of mice with *SpA* protein showed significant differences in the increased IL-12 and IFN-γ levels, compared to the control group ($p>0.0001$). Furthermore, the mice that were immunized with the *SpA* protein recombinant protein and Freund's adjuvant exhibited significant increases in IL-12 (macrophage interleukin) and IFN-γ (T-lymphocyte cytokine) levels compared to the control group ($p>0.0001$). The immunization with *SpA* recombinant protein or *SpA* recombinant protein with incomplete Freund's adjuvant exhibited an increase in the expression of IL-12 and IFN-γ, compared to the control group.

***Keywords*:** Mastitis, MRSA, *Staphylococcus aureus*, *SpA* vaccine, IL-12 and IFN-γ genes.

INTRODUCTION

Mastitis is a critical and hazardous concern in dairy cattle management, associated with reduced milk production, cow culling, veterinary expenses, and diminished quality of dairy products (Gharban, 2021 and Sheet, 2022). Despite a notable reduction in the incidence of clinical bovine mastitis attributable to contemporary preventive strategies, subclinical mastitis remains a worldwide threat to the dairy industry (Al-Shuwaili

Corresponding: SABRIN IBRAHEEM MOHSIN
E-mail address: sabrin.i@covm.uobaghdad.edu.iq
Present address: Department of Microbiology, College of Veterinary Medicine, University of Baghdad

and Khudhir, 2022 and Saadoon, 2022). Bovine mastitis is often addressed with antibiotic therapy, yet illness management encounters challenges owing to recurrent infections within herds, the threat of antibiotic resistance, and overall treatment expenses (Al-Rasheed *et al*., 2022). *S. aureus* remains a prominent bacterium associated with the disease, capable of existing at subclinical levels within herds. Treatment for *S. aureus* infections is challenging due to the bacteria's ability to develop mutations that confer antibiotic resistance, and these infections generally exhibit a high mortality rate (Sayhood *et al.,* 2022). Moreover, MRSA presents a significant issue due to its inadequate response to antibiotic therapy; hence, a prophylactic vaccine would be important in mitigating the adverse consequences of mastitis in the dairy industry threatening human health (Mahmood, 2014 and Ning *et al*., 2023).

The subunit vaccines, which include an adjuvant and several *S. aureus* surface proteins, represent the most promising experimental candidates. Protein A (*SpA* protein) is the primary element of *S. aureus* virulence (Ali and Hussein, 2018). A substantial cell-wall component weighing 40–60 kDa is expressed by 99 percent of *S. aureus* strains. *SpA* protein is attached to the bacterial cell wall by a signal sequence and an LPXTG sortase motif (Schneewind and Missiakas, 2019).

Thus, it is unevenly dispersed at two to four foci, making up around 7% of the staphylococcal surface (Mohammed and Al-Mathkhury, 2023). During the exponential growth phase, *SpA* expression is first triggered and then post-translationally downregulated (Uebele, 2016). The five homologous Ig-binding domains that make *SpA* bind the Fc portion of human immunoglobulin G, which stops *S. aureus* from opsonizing and inhibits Fc receptor-mediated phagocytosis (Boero *et al.*, 2022). The extremely variable area X, which consists of a sequence of appr. In epidemiology, the sequence in which those repeats occur determines the *SpA* type, which is used to identify and categorize strains (Santos-Júnior *et al*., 2016). This work involved the cloning, expression, and purification of the *SpA* protein encoded by *S. aureus*, with the aim of exploring its potential as a candidate for vaccination against *S. aureus*-induced mastitis. Subsequently, the present study evaluated the expression of IFN-γ and IL-12 genes in the spleen of breastfeeding BALB/c mice.

MATERIALS AND METHODS

1. Bacterial strains and *SpA*- protein vector

Luria-Bertani broth (LB) was used to isolate and identify the clinical mastitis bovine MRSA strains at 37°C until they were ready for PCR amplification. *E. coli* Rosetta (DE3) from Novagen, USA, was used in the cloning process to produce recombinant proteins. Its plasmid pET-24a (Novagen, USA) was used as an expression vector.

2. Amplification and Cloning of *SpA gene*

The GenEluteTM plasmid miniprep kit **(**Merk, Argentina**)** was used for DNA extraction. A 750 bp fragment of the *SpA* gene was amplified using NotI-tailed forward (5'-TTG CGG CCG CG AAG CTC AAC AAA ATG CT-3') and XhoI-tailed reverse (5'-GCC GTC TTC TTT ACC AGG TTC TCG AGA A-3') primers. The PCR technique started with an initial denaturation of 5 minutes at 95°C, followed by 33 cycles, including denaturation at 95°C for 30 seconds, annealing at 61°C for 55 seconds, extension at 72°C for 55 seconds, and a final extension at 72 °C for 10 minutes.

PCR reaction, which included 25 µl of 2X PCR master mix, 3 µl of MRSA DNA, 2 µl of each forward and reverse primer, with the total volume adjusted to 50 µl using deionized water. Restriction enzymes were used to cleave the purified *SpA* fragment

prior to its ligation into the pET-24a vector, which contains six residues at the C-terminus to facilitate the purification of the resultant protein. The recombinant vector pET24a-*SpA* was introduced into competent *E. coli* BL21 (DE3) strains using heat shock transformation. Figure (1) demonstrates that colony PCR, restriction endonuclease digestion, and sequencing were used to verify the integrity of the recovered plasmid (Khateb, 2014 and Vahdani *et al*., 2021).

3. Expression of *SpA* protein

Competent BL21 (DE3) was created by transforming the pET24a/SpA construct to overexpress the protein. LB agar supplemented with 50 µg/ml of kanamycin was used to cultivate cultures. The colonies harboring pET24a/SpA were cultivated at 37◦C in 5 ml of LB medium that was treated with kanamycin. The T7 promoter was activated in the vector that was expressing the recombinant protein by injecting IPTG (Sigma, USA) at a final dose of 1 mM. This was done during the early exponential phase of growth, which was measured at an OD of 600 nm of 0.6. After stimulating the expression of the protein for 4 hours with 1.0 Mm IPTG, the cell pellets were subjected to protein expression analysis using 12% SDS-PAGE at 30◦C (Khateb, 2014 and Vahdani *et al*., 2021).

4. Purification of 6xHis-tagged *SpA* protein

The ideal expression parameters (temperature, IPTG concentration, and duration) were determined at the flask level, which included 50 milliliters of bacterial culture. The parameters were then adjusted to reflect one liter of culture. Following the collection of recombinant protein-expressing bacteria, their pellets were resuspended in a lysis solution containing protease inhibitors.

The lysis solution had a pH of 8.0 and included 8 M urea, 0.1 m NaH2PO4, and 0.01 M Tris. The cell suspension was centrifuged for 20 minutes at 10,000 revolutions/ min and underwent sonication. Ni-nitrilotriacetic acid (NiNTA) affinity chromatography was used to purify recombinant proteins (about 35.8 kDa) from the supernatant under denaturing conditions, using His-tag interactions post-centrifugation. G Bioscience, according to the manufacturer's guidelines. An elution buffer with a pH of 4.5 was used to elute fractions of pure *SpA* protein after washing steps using a wash buffer composed of 100 mM NaH2PO4, 10 mM Tris-HCl, and 8 M urea at a pH of 6.4. The Bradford protein assay and Nanodrop spectrophotometer measured the protein concentration in the output fractions, which were then analyzed using SDS-PAGE. After dialysis with 0.1 m phosphate-buffered saline (PBS, pH = 7.4) for 72 hours to eliminate urea, the *SpA* solutions were filtered (0.22 µm) and preserved at −70 ◦C (Khateb, 2014 and Vahdani *et al*., 2021).

5. Animals and immunization

The current study has been done in Animal House, College of Veterinary Medicine, University of Baghdad. BALB/c female lactating mice (n=48) weighing 20–25 g were kept in ordinary cages with unrestricted access to food and drink for a week. Mice were divided into four groups (number of each=12). G1 and G2 were injected intramammary with phosphate buffer saline and MRSA broth only, respectively. G3 and G4 were injected with *SpA* protein and *SpA* protein 20µg with an equal volume of incomplete Freund's adjuvant, respectively (Mandelli *et al*., 2024). A booster dose of vaccine post 14 days was administrated. At 21 days, all tested mice were challenged with 5×10^8 MRSA CFU/ml, where their spleen tissue samples were collected on 7,9 &11 days of challenge, granted with liquid nitrogen by mortar, and then centrifuged at 10,000×g for 15 min to extract (Dai *et al*., 2015).

6. Real time PCR

6.1. RNA extraction

RNA was extracted using TransZol Up Plus RNA Kit (Transgenbiotech, China) according to the manufacturer's recommendations.

6.2. Primers

PCR was performed in this study as follows:

IL-12, F (5'- GGGACCAAACCAGCACATTGA -3').
R (5'- TACCAAGGCACAGGGTCATCA -3').
IFN-γ, F (5'- CAGCAACAGCAAGGCGAAAAA-3').
R (5'- AATCTCTTCCCCACCCCGAAT -3').

Furthermore, the GAPDH was used as a housekeeping gene:

F (5'- CGGGTTCCTATAAATACGGACTG -3').
R (5'- CCAATACGGCCAAATCCGTTC -3').

6.3. Real time PCR amplification

The conversion of RNA to cDNA according to EasyScript® First-Strand cDNA Synthesis SuperMix.

7. Statistical analysis

The statistical analysis was performed with version 22 of the SPSS software (ANOVA).

RESULTS

1. Amplification and cloning of SpA gene

As shown in Figure (1), the amplified PCR products of the Staphylococcal protein A (*SpA*gene) were separated by 3% agarose gel electrophoresis.

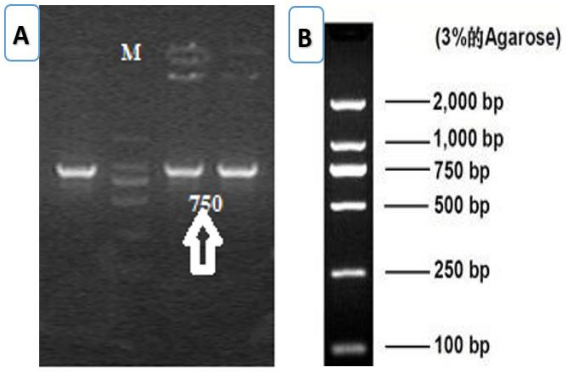

Figure 1 A: PCR product electrophoresis of *SpA* gene, B: DNA maker.

This study involved the cloning of *S. aureus* into pET28a, a recognized *E. coli* expression vector. The *SpA* gene was initially cloned using the XhoI site within the multiple cloning site of the pET28a plasmid, leading to its insertion (Figure 2).

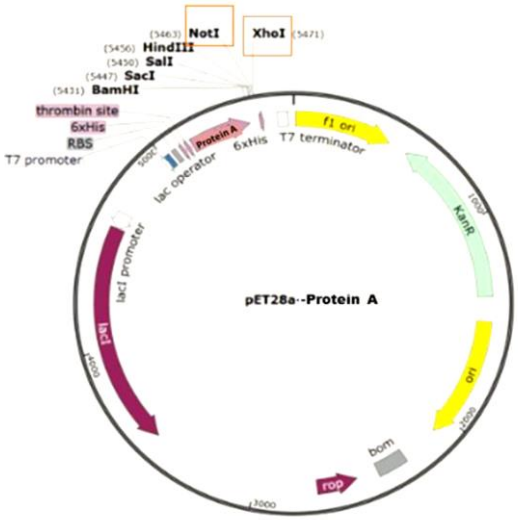

Figure 2: Showing Map design of Pet28a plasmid carrying Protein A of *SPA* gene of MRSA and showin g restriction enzyme of Xho1 and Not1 sites that are used for cloning.

2. Expression and purification of 6xHis-tagged *SpA* protein

The SDS-PAGE bands indicated that Protein A (35.8 kDa) was soluble in the lysis buffer for all tested IPTG concentrations (Figure 2). This was observed at OD 600 nm of 0.6 following an 8-hour induction at 30°C with 0.1 mM IPTG (Figure 3).

The *SpA* gene was purified as 35.8KDa (Figure 3). In a system like Rosetta™(DE3) pLysS competent cells, the *SpA* gene was used to synthesize the corresponding protein. The 323 amino acids represent the part length of *SpA* protein, where each triplet of nucleotides in the coding sequence translates into a specific amino acid (Figure 4). The analysis sequence of cloned Protein A, sequence and calculations of amino acids using Mega 6 alignment program (Table 1).

3. Real-time PCR amplification

Table (2) and Figure (5) illustrate substantial differences (P<0.0001) in IL-12 and IFN-γ between all vaccinated groups and the control group. The mice inoculated with vaccine protein A and adjuvant exhibited a substantial elevation of IL-12 and IFN-γ compared to the control group (P=0.0001).

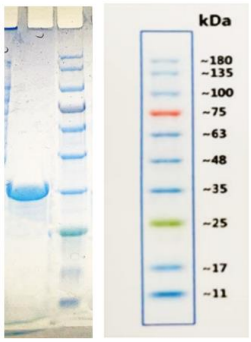

Figure 3: SDS-PAGE was used to separate the soluble and insoluble fractions of recombinant protein in 20 mM Tris/1 mM EDTA/0.1% Triton X-100 (pH8). The IPTG-induced *E. coli* (DE3) samples were normalized by absorbance at 600 nm (IPTG 0.1 mM).

Table 1: Amino acids compositions of purified protein (*SpA* gene) 323KDa, molecular weight and molarity of each amino acids.

Amino acid Composition	Number	Mol%
A	30	9.29
C	0	0.00
D	24	7.43
E	30	9.29
F	14	4.33
G	15	4.64
H	2	0.62
I	11	3.41
K	34	10.53
L	28	8.67
M	3	0.93
N	45	13.93
P	19	5.88
Q	32	9.91
R	5	1.55
S	19	5.88
T	2	0.62
V	5	1.55
W	0	0.00
Y	5	1.55
Total: 323	Molecular weight: 36170.57	100.00%

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  1 - GAAGCTCAACAAAATGCTTTTTATCAAGTGTTAAATATGCCTAACTTAAACGCTGATCAA - 60
  1 - E  A  Q  Q  N  A  F  Y  Q  V  L  N  M  P  N  L  N  A  D  Q  - 20

 61 - CGTAATGGTTTTATCCAAAGCCTTAAAGATGATCCAAGCCAAAGTGCTAACGTTTTAGGT - 120
 21 - R  N  G  F  I  Q  S  L  K  D  D  P  S  Q  S  A  N  V  L  G  - 40

121 - GAAGCTCAAAAACTTAATGACTCTCAAGCTCCAAAAGCTGATGCGCAACAAAATAACTTC - 180
 41 - E  A  Q  K  L  N  D  S  Q  A  P  K  A  D  A  Q  Q  N  N  F  - 60

181 - AACAAAGATCAACAAAGCGCCTTCTATGAAATCTTGAACATGCCTAACTTAAACGAAGCG - 240
 61 - N  K  D  Q  Q  S  A  F  Y  E  I  L  N  M  P  N  L  N  E  A  - 80

241 - CAACGCAATGGTTTCATTCAAAGTCTTAAAGACGATCCAAGCCAAAGCACTAACGTTTTA - 300
 81 - Q  R  N  G  F  I  Q  S  L  K  D  D  P  S  Q  S  T  N  V  L  - 100

301 - GGTGAAGCTAAAAAATTAAATGAATCTCAAGCACCGAAAGCTGACAACAATTTCAACAAA - 360
101 - G  E  A  K  K  L  N  E  S  Q  A  P  K  A  D  N  N  F  N  K  - 120

361 - GAACAACAAAATGCTTTCTATGAAATCTTGAACATGCCTAACTTGAACGAAGAACAACGC - 420
121 - E  Q  Q  N  A  F  Y  E  I  L  N  M  P  N  L  N  E  E  Q  R  - 140

421 - AATGGTTTCATCCAAAGCTTAAAAGATGACCCAAGTCAAAGTGCTAACCTTTTAGCAGAA - 480
141 - N  G  F  I  Q  S  L  K  D  D  P  S  Q  S  A  N  L  L  A  E  - 160

481 - GCTAAAAAGTTAAATGAATCTCAAGCACCGAAAGCTGACAACAATTTCAACAAAGAACAA - 540
161 - A  K  K  L  N  E  S  Q  A  P  K  A  D  N  N  F  N  K  E  Q  - 180

541 - CAAAATGCTTTCTATGAAATCTTACATTTACCTAACTTAAACGAAGAACAACGCAATGGT - 600
181 - Q  N  A  F  Y  E  I  L  H  L  P  N  L  N  E  E  Q  R  N  G  - 200

601 - TTCATCCAAAGCTTAAAAGATGACCCTTCAGTGAGCAAAGAAATTTTAGCAGAAGCTAAA - 660
201 - F  I  Q  S  L  K  D  D  P  S  V  S  K  E  I  L  A  E  A  K  - 220

661 - AAGCTAAATGATGCACAAGCACCAAAAGCTGACAACAAATTCAACAAAGAACAACAAAAT - 720
221 - K  L  N  D  A  Q  A  P  K  A  D  N  K  F  N  K  E  Q  Q  N  - 240

721 - GCTTTCTATGAAATTTTACATTTACCTAACTTAACTGAAGAACAACGTAACGGCTTCATC - 780
241 - A  F  Y  E  I  L  H  L  P  N  L  T  E  E  Q  R  N  G  F  I  - 260

781 - CAAAGCCTTAAAGACGATCCTTCAGTGAGCAAAGAAATTTTAGCAGAAGCTAAAAAGCTA - 840
261 - Q  S  L  K  D  D  P  S  V  S  K  E  I  L  A  E  A  K  K  L  - 280

841 - AACGATGCTCAAGCACCAAAAGAGGAAGACAATAACAAGCCTGGCAAAGAAGACGGCAAC - 900
281 - N  D  A  Q  A  P  K  E  E  D  N  N  K  P  G  K  E  D  G  N  - 300

901 - AAGCCTGGTAAAGAAGACGGCAACAAACCTGGCAAAGAAGATGGCAACAAACCTGGTAAA - 960
301 - K  P  G  K  E  D  G  N  K  P  G  K  E  D  G  N  K  P  G  K  - 320

961 - GAAGACGGC - 969
321 - E  D  G  X                                   - 340
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Figure 4: The map of *SpA* gene of MRSA that showing translated Protein a 323 amino acid.

Table 2: Fold change expressions by using real time PCR

$2^{-\Delta\Delta CT}$(Fold change) *IL-12* Mean ±SE									
Control negative	**C1+**	**C+2**	**C+3**	**V1**	**V2**	**V3**	**VA1**	**VA2**	**VA3**
1.0866± 0.375	٠,٩٥٢± ٢,٧٧٤	**2.482 ±0.٧٥٣**	± ٠,٣٨١ ١,٥٣٧	**4.442 ±0.866**	٠,٥٦٨± ٣,٣٠٥	**2.575 ±0.٥٥٠**	٢,٠٤١± ٧,٤٣٤	**4.233 ±١.٤٨٥**	١,٤٢٢± ٣,٢٤٥
P-value = 0.0001									
$2^{-\Delta\Delta CT}$(Fold change) IFN-γ Mean ±SE									
Control	**C1+**	**C+2**	**C+3**	**V+1**	**V+2**	**V+3**	**VA1**	**VA2**	**VA3**
0.411± ١,٠٧٥	١,٧٨٣± ٣,٩٦٥	**٣.٢٧٨ ±0.٤٤٢**	± ١,١٦٣ ٢,٦٦٣	**٤.٥٢١± 0.221**	٠,٥١١ ٤,١٠١±	٣,١١٥± ٠,٤٤٢	٠,٨٩٤± ٥,٤٤٠	٤,٣٦٦± **0.٨٢٥**	١,٤٢٩± ٣,٧٥٣
P-value = 0.0001									

* **C1+** control positive at 7 days after challenge. * **C2+** control positive at 9days after challenge.
* **C3+**control positive at 11days after challenge. ***V1** *SpA* protein only at7days after challenge.
***V2** *SpA* protein only 9 days after challenge. ***V3** *SpA* protein only 11 days after challenge
***VA1** *SpA* protein plus incomplete Freund's adjuvant at7days after challenge
***VA2** *SpA* protein plus incomplete Freund's adjuvant at 9 days after challenge
***VA3** *SpA* protein plus incomplete Freund's adjuvant a t 11days after challenge

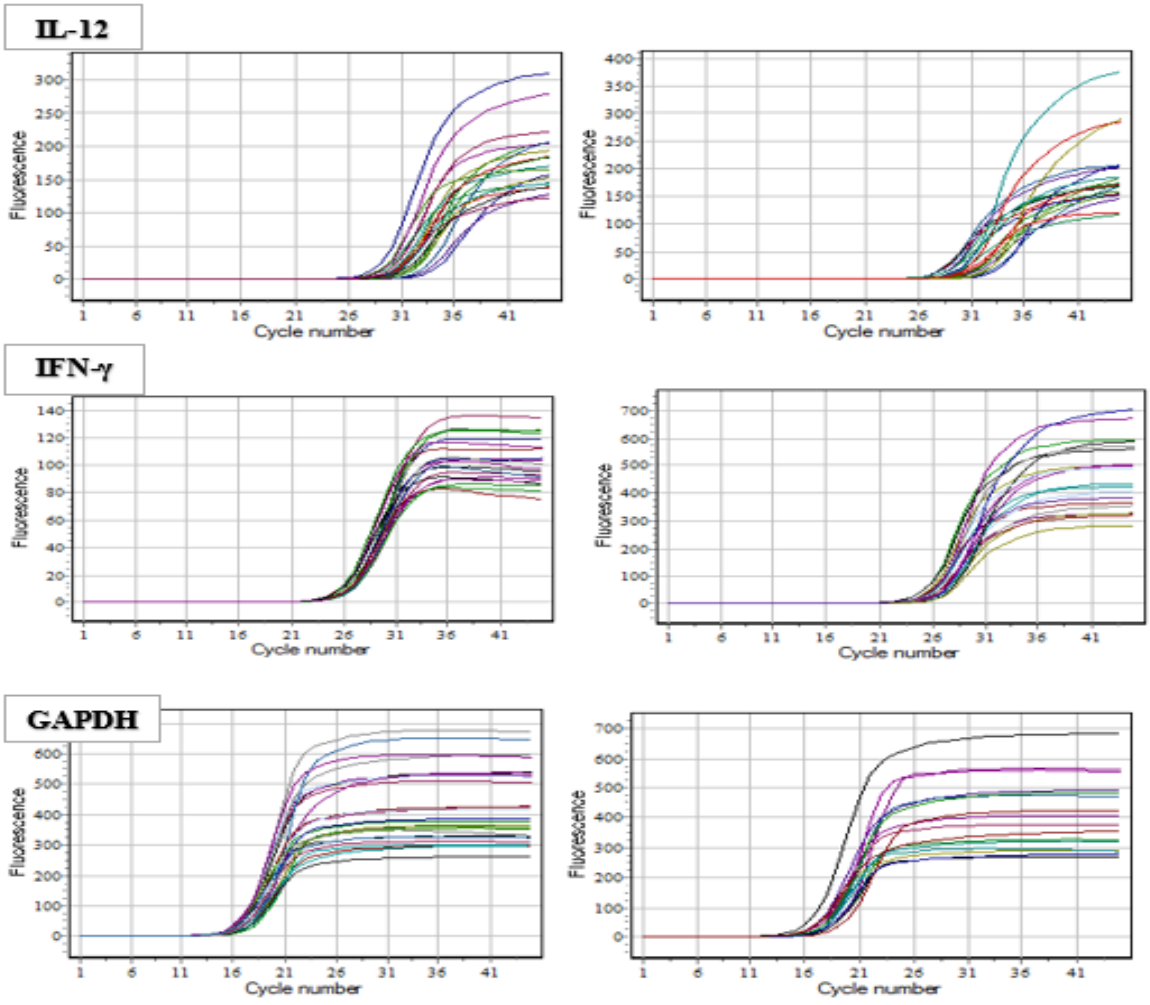

Figure 5: Amplification of IL-12, and IFN-γ cDNA product and housekeeping GAPDH genes were submitted to the real time PCR.

DISCUSSION

Vaccination was exclusively responsible for stimulating the production of antibodies against the transmembrane protein phosphoglycerate kinase (FGQ) in cured animals (Cunha *et al*., 2020).

The initiation of staphylococcal intramammary infection necessitates the synthesis of many virulence factors that facilitate adhesion, colonization, and invasion of bovine mammary cells by MRSA, as well as evasion of the immune defence mechanisms and survival within the host environment (Campos *et al*., 2022) with high transmissibility and infections rates due to multidrug resistance (Lawrence *et al*., 2012).

Surface proteins of MRSA are extremely important in the interaction between host cells and pathogens, as well as in the pathogenesis of the infection and the development of the immune response of the host (Yang *et al*., 2020). Protein A, often known as *SpA*, is the most major virulence component that MRSA possesses. Many virulence factors such as matrix adhesion molecules, as one of the most common categories of surface proteins, mediate bacterial binding to host tissue and the extracellular matrix, such as collagen, fibronectin, and fibrinogen (Latifpour *et al*., 2022).

Until now, there has been no complete success in staphylococcal vaccination to be capable of preventing invasive pathogen colonization. Vaccines were initially developed as a result of the realization that preexposure to certain diseases or survival may possibly offer protection that would last a lifetime (Clegg *et al*., 2021).

Few studies upon the uses of *SpA* recombinant protein in the immunization of mastitic animals, where it has a great polymorphism in the x-region (Bhati *et al*., 2016). Moreover, few studies on the uses of incomplete Freund's adjuvant accompanied by *SpA* recombinant protein. In the present study, administration of nontoxigenic protein A, infected mastitic mice were able to induce antibody responses that are protective against MRSA infection with a highly significant increase ($P<0.0001$) (Table 2) stimulating adaptive immune responses (Kim *et al*., 2015).

The properties make *SpA* a valuable protein for commercial companies, allowing them to produce and sell it at a high price; consequently, developing an accessible recombinant version of *SpA* in research labs could help researchers reduce costs and ensure that the protein is readily available for future applications (Lee *et al*., 2021).

The designed map of the *SpA* gene was established with 35.8 amino acids. The reason why a partial *SpA* gene was used is that the full gene is toxic to Rosetta (DE3) competent cells. This study agreed with studies conducted by Abouelkhair *et al*. (2018) and Mandelli *et al*. (2024). These are specially engineered cells often used in the expression of difficult proteins. They contain a plasmid that allows the production of protein efficiently, along with plasmids that provide extra tRNAs for rare codons, improving protein yield and solubility (Deshmukh *et al*., 2022).

It can be inferred that *SpA* may also bind to immunoglobulins from other animal species (Lee *et al*., 2021). Protein A can be changed to create a different protein with different properties. There are various immunological mechanisms involved in vaccination response, especially in the context of the adaptive immune system, which could explain the upregulation of IL-12 and IFN-γ in the present study among all groups vaccinated (with both protein A and adjuvant, as well as in all protein A-vaccinated – Table 2 and Figure 5) as Sareneva *et al*. (2000); Fernandes *et al*. (2014); Ma *et al*. (2015) and Roy *et al*.

(2019) had concluded. An effective anti *S. aureus* vaccine remains elusive as the correlates of protection are ill-defined. Targeting specific T cell populations, IFN-γ is essential for stimulating macrophages and improving their capacity to eliminate infections; this is essential for fighting illnesses that cause the immune system to become very active (Salim *et al*., 2016).

Also, in the present study, the adjuvant-vaccinated groups had been more positive immune response than protein A-only groups (Table 2 and Figure 5). It is reported that the Th1 response is aided by the adjuvant and protein A since the substances known as adjuvants strengthen the immune system's reaction to an antigen (Wen *et al*., 2011 and Orosco and Espiritu, 2024). Adjuvants have the potential to improve the vaccine protein (protein A), which in turn can boost T cell activation and cytokine production, which raises IL-12 and IFN-γ levels (Cao *et al*., 2024).

Despite an overreaction, it may cause unfavourable side effects or exacerbate inflammatory disorders (Souto *et al.,* 2024). Finally, overexpression of these cytokines usually indicated a protective positive immunological response. The adjuvant and protein A proved synergistic effects on the immune system, which stimulated Th1 and upregulation of IL-12 and IFN-γ (Aricò and Belardelli, 2012). This demonstrates a successful immune response to the vaccination technique and strengthens the body's ability to establish an effective defense against infections (Zheng *et al*., 2022).

CONCLUSION

S. aureus is a primary infectious pathogen causing mastitis in cattle. *Protein A* is a critical virulence component of *S. aureus* that enables the germs to circumvent immune responses by obstructing phagocytosis. The current findings indicated that immunization with *SpA* protein showed significant differences compared to the control group (*p>0.0001*). Furthermore, the mice that were immunized with the *SpA* protein and with incomplete Freund's adjuvant exhibited significant increases in IL-12 and IFN-γ levels compared to the control group (*p>0.0001*). The vaccination with nontoxic protein A, which doesn't attach to immunoglobulin, triggers the production of neutralizing antibodies that help animals with mastitis build both innate and adaptive immune responses.

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ETHICS STATEMENT

The authors affirm that this study was conducted in accordance with the journal's ethical norms, as shown on the author guidelines page.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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التقييم المناعي لبروتين *SpA* المحضر بالاستنساخ الجزيئي كلقاح لبكتريا المكورات العنقودية الذهبية المقاومة للميثيسيلين للأبقار المصابة بألتهاب الضرع

صابرين ابراهيم محسن ، عايدة برع علاوي
قسم الميكروبيولوجي، كلية الطب البيطري، جامعة بغداد

Email: sabrin.i@covm.uobaghdad.edu.iq, Assiut University web-site: www.aun.edu.eg

يعد التهاب الضرع أحد أكثر الأمراض شيوعًا وتأثيرًا التي تؤثر على أبقار الحلوب على مستوى العالم. يُعتقد أن البكتيريا السائدة المرتبطة بالتهاب الضرع هي المكورات العنقودية الذهبية. هناك حاجة عالمية لتطوير لقاحات جديدة لأن طرق العلاج الحالية لمعالجة المكورات العنقودية الذهبية ليست ناجحة باستمرار. يهدف هذا العمل إلى إنتاج بروتين *SpA* معاد التركيب للتطعيم ضد التهاب الضرع الناجم عن المكورات العنقودية الذهبية وتقييم آثاره على إنتاج IL12 وIFN-γ في الطحال لدى لفئران BALB/c المرضعة. تم زراعة المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA) على مرق لوريا بيرتاني (LB) عند درجة حرارة ٣٧ درجة مئوية حتى تصبح جاهزة لتفاعل البلمرة المتسلسل ثم التضخيم والتعبير عن البروتينات معاد التركيب من خلال الاستنساخ باستخدام بكتيريا *Escherichia coli* Rosetta (DE3) وناقل التعبير (البلازميد pET-24a) بالإضافة إلى التعبير عن جين IL12 وIFN-γ في الطحال لفئران BALB/c المرضعة. تشير نطاقات SDS-PAGE إلى أن البروتين A (٣٥،٨ كيلو دالتون) كان قابلاً للذوبان في محلول التحلل لجميع تركيزات IPTG التي تم اختبارها. وقد لوحظ هذا عند OD600 من ٠،٦ بعد الاستقراء لمدة ٨ ساعات عند ٣٠ درجة مئوية مع ٠،١ مللي مول IPTG. تم الحصول على ١٠٠ ملغرام من البروتين *SpA* معاد التركيب. أشارت النتائج إلى أن التحصين ببروتين *SpA* أظهر اختلافات معنوية كبيرة في زيادة مستويات IL-12 وIFN-γ مقارنة بالمجموعة الضابطة (P>٠،٠٠٠١). علاوة على ذلك، أظهرت الفئران المحصنة ببروتين *SpA* مع المادة المساعدة فرويند زيادة كبيرة في مستويات IL-12 وIFN-γ مقارنة بالمجموعة الضابطة (P>٠،٠٠٠١). أظهر التحصين باستخدام بروتين *SpA* المعاد تركيبه، وبروتين *SpA* المعاد تركيبه مع المادة المساعدة فرويند غير المكتمل، زيادة في التعبير عن IL12 وIFN-γ مقارنة بالمجموعة الضابطة.