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Original Article

A novel peptide cocktail containing B and T cell epitopes confers robust protection against *Staphylococcus aureus* bacteremia in a murine model

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Abstract

Previous *Staphylococcus aureus* vaccines have been unsuccessful, but it seems that including T cell immunity in the vaccine is essential for its success. This study used Metal ABC transporter substrate-binding protein (*MABC*), Nickel ABC transporter (*NABC*), and Phosphatidylinositol phosphodiesterase (*PIc*) B and T cell epitopes as a vaccine in a mouse bacteremia model. Mice immunized with PIc and epitope mixture groups showed robust humoral immunity and high survival rates in the bacteremia model. The highest protection level of mice was shown in the peptide mixture group, which was correlated with robust humoral response, the highest level of interferon-gamma (INF-γ) and the lowest levels of interleukin-2 (IL-2). The peptide mixture group also showed the highest count of CD8 cells. These results demonstrate that including B and T cell epitopes improved both the humoral and cellular immunity and resulted in the best outcome in the *S. aureus* bacteremia model. This finding has important implications for the development of future *S. aureus* vaccines.

Keywords: *Staphylococcus aureus*, Vaccine, Peptide, Bacteremia, B-cell epitopes, T-cell immunity.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a gram-positive opportunistic pathogen responsible for significant morbidity and mortality worldwide (from 20% to 50%, depending on infection severity). *S. aureus* can be a highly lethal pathogen with a mortality rate during bacteremia in developed countries [1]. One of the most challenging factors of *S. aureus* management is the bacterium's capability of the bacterium to expand resistance to many antibiotics [2]. Reports of multidrug-resistant *S. aureus* strains, like methicillin-resistant *S. aureus* (MRSA), represent a global health threat. MRSA infections significantly increase treatment costs and lead to poorer patient outcomes, highlighting the urgent need for a vaccine against *Staphylococcus aureus* [3].

Vaccines target Staphylococcal surface antigens like Clumping factor proteins (*ClfB*)[4], Surface-regulated iron-binding protein B [5], Capsular Polysaccharides 5 and 8 (*CP5 and CP8*) [6], Manganese binding protein C (*Mntc*), Serine-aspartate repeat protein E and D (*SdrE and SdrD*)[7]. These proteins were selected due to their high degree of conservation across *S. aureus* strains [8]. Unfortunately, early vaccine studies in clinical trials, such as StaphVAX [6], in phase III clinical studies, did not effectively protect against *S. aureus* bacteremia infection [9]. In contrast, the following studies targeted a variety of Staphylococcal antigens to improve vaccination effectiveness [8]. Although it was shown that the quadrivalent vaccine "(Pfizer)" could not prevent invasive infections after spinal surgery, clinical development of the vaccine was abandoned in phase IIb, highlighting the challenges associated with Staphylococcal immunization. Furthermore, no antibody-based *S. aureus* vaccines have been successful in clinical trials [5,6]. This failure subsequently led to further consideration of T cells' role in *S. aureus* infection protection, so the design of the *S. aureus* vaccine should consider the inclusion of T cells.

An in-silico reverse vaccinology study was carried out in our lab to identify potential vaccine candidates that were tested in a panel of *S. aureus* clinical isolates [10]. Phosphatidyl inositol phosphodiesterase (*PIc*) gene B cell and T cell epitopes were designed, synthesized, and tested in mice bacteremia models. Results showed promising results in mice survival [10]. Also, the zinc metallopro-

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teinase aureolysin was cloned, expressed, and tested in-vitro against sera collected from humans infected with *S. aureus* and showed high reactivity with the human sera [11]. *S. aureus's* metal transporter protein C mechanisms, particularly those necessary for iron and manganese accumulation, are crucial for its pathogenicity[12]. The current study aimed to test the effectiveness of T cell and B cell epitopes of gene cocktail of (*PIc*), (*NABC* transporter), and (*MABC)* protein against *S. aureus* mice Bacteremia model.

2. Materials and methods

2.1. Experimental Animals and Ethics Statement

The animal house of the Holding Company for the Production of Vaccines, Sera, and Drugs (VACSERA -Egy-Vac) provided the six-week-old female BALB/c mice with an average body weight of 20 g (n=20/group). After a week of acclimation, the animals were separated into six groups of 20 mice/group. They were kept in plastic cages in a room with conventional lighting and a 12-hour cycle of light and dark at a temperature of 25±1°C. The animals were housed under standard conditions with ad libitum access to food and water. All animal experiments complied with the Egyptian regulations for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) / Research Ethics Committee of the Faculty of Pharmacy, Suez Canal University (Approval Reference: PM-NR3-PHD-17).

2.2. Bacterial strains

S. aureus bacteremia induced by standard *S. aureus* strain ATCC 25923 1×107cfu/mL [13], which was purchased from the Microbiological Resources Center (Cairo MIRCEN). Bacterial strains were cultured in Trypticase soy agar (Trypticase soy broth 30 gm, agar 15 g, distilled water 1 liter), autoclaved for 15 minutes at 121 ˚C, and aerobically at 37 °C overnight.

2.3. Data Retrieval

For *S. aureus* full genomes downloaded from GenBank, Standalone Blast-p [14] was used. The National Center for Biotechnology Information against *S. aureus* subsp *aureus* NCTC 8325 (Reference genome provided by University of Oklahoma Health Sciences Center) (ftp:/ftp.ncbi.nlm.nih.gov/genomes/archive/old GenBank/Bacteria) [15]. According to [16], Blast-P was used and approved proteins having alignment results of at least 80% coverage, 40% identity, and cutoff e-value of 10-12. The predicted proteins have been listed as prospective vaccination candidates because they fulfilled the requirements specified by the Vaxign server. Homologous proteins to humans, pigs, or mice were excluded. The VaxiJen v2.0 server was used to predict the antigenicity of the complete protein sequence [17], which may be accessed at www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html, with a threshold of 0.45. Proteins with antigenic scores of less than 0.45 were chosen. Initially, selection criteria included 10 genes included conservation of the chosen antigens across *S. aureus* genomes. Two Genes were selected based on our previous study results [10,11], *PIc* (Accession No. YP498656) and Nickel ABC transporter (Accession No. YP501226). The third gene is (Gene name SAUSA300_0618), Metal ABC transporter substrate-binding protein, selection of Metal ABC transporter substrate-binding protein (*MABC*) from previous study [18], the same peptides were used in our recent research in a skin infection model [19].

2.4. Target Peptide

The first gene was (*MABC*) B cell epitopes; (113–136 amino acid); (209–232 amino acid) and (263–286

amino acid) as previously reported [18]. The second gene (*NABC)* was nominated as a potential gene candidate in our previously published study [10]. B-cell epitope prediction was made using DiscoTope 2.0 Server (www.cbs.dtu.dk/services/Disco Tope). T cell prediction was made using. The SignalP 5.0 server (www.cbs.dtu.dk/services/SignalP). Their sequence is shown in Table 1. The third gene peptide was the previously published peptide sequence of (*PIc*) (B cell epitope172-185 amino acid, T cell epitope 187-201, and 48-96 amino acids) [10]. Peptide synthesis was done by GenScript, USA. Each peptide was prepared by reconstitution with sterile water to a concentration of 1mg/ml.

2.5. Murine Bacteremia Model

Mice groups: 1-4 were intraperitoneally immunized with *MABC, NABC, PIc*, and Mixed peptides in equal concentration and adjuvanted with Alum. For the mixture group, an equal concentration of each peptide was used. Booster doses were applied two weeks post the primary injection using the half concentration of the first dose of each peptide in each group, as shown in Table 2. Mice were challenged intravenously with 0.5 ml inoculum containing 10^7 CFU *S. aureus* ATCC 25923 on day 30. Two control

Table 1. Chemically synthesized peptides and their characteristics.

	Name	Peptide	Epitope type	Antigenicity	Conservancy (%)	Amino acid position	Purity (%)
	EPIc-1(A)	KKVIAVSKDVKPIYLNGEEGNKDK	B-cell (*MABC*)	-	100%	113–136	95.2%
MABC	EPIc-2(B)	FKYFSKQYGITPGYIWEINTEKQG	B-cell (*MABC*)	-	100%	209–232	96.1%
	EPIc-3(C)	MESLSEETKKDIFGEVYTDSIGKE	B-cell (MABC)	-	100%	263–286	97.1%
	EPIc-1(D)	KDSDVRQKDGKN	B-cell (*NABC*)	2.97	100%	336-347	98.8%
NABC	EPIc-2(E)	DMNPHVYGGSMSAES	T-cell (*NABC*)	1.32	100%	21-35	99.7%
	EPIc-3(F)	YLQAEFKKMGIKLNI	T-cell (*NABC)*	1.37	100%	367-381	99.1%
	EPIc-1(M)	TGSNANPTLKETKG	B-cell(*PIc)*	2.59	100%	172-185	92.5%
PIc	EPIc-2(N)	IVLFNRMGGTYIKSG	T-cell(*PIc)*	0.63	100%	187-201	91%
	EPIc-3(O)	KSGVRFFDIRGRASA	T-cell(*PIc*)	1.89	100%	84-96	94.6%

*Epitopes A, B, C belong to MABC, Epitopes D, E, F belong to NABC transporter and Epitopes M, N, O belong to Pic.

Table 2. Mice immunization groups and peptide concentrations in bacteremia model.

	Group 1 (MABC)	Group 2 (NABC)	Group 3 (PIc)	Group 4 (Mixture)	Group 5 (Negative control 2)	Group 6 (Negative control 1)
First dose (Day 0)	50µg(A)+50µg(B) + 50µg(C) +40 µg Alum	50µg(D)+50µg(E)+ 50µg(F) + 40 µg Alum	50µg(M) +50µg(N) + 50µg(O) + 40µg Alum	16.67µg(A) +16.67µg(B) +16.67µg(C) +16.67µg(D) +16.67µg(E) +16.67µg(F) +16.67µg(M) +16.67µg(N) +16.67µg(O) +40µg Alum	Negative control2, 40µg Alum	Plain/ Negative control 1
Booster dose 1 (Day 14)	25µg(A)+25µg(B) + 25µg(C) +40µg Alum	25µg(D)+25µg+ 25µg(F)+40µg Alum	25µg(M) + 25µg(N) + 25µg(O) + 40 µg Alum	8.33µg(A)+ 8.33µg(B)+ 8.33µg(C) + 8.33µg(D) + 8.33µg (E) +8.33µg(F) +8.33µg(M) +8.33µg(N) +8.33µg(O)+40 Alum	Negative Control2, 40µg Alum	Plain/ Negative control 1
Booster dose 2 (Day 28)	12.5µg(A) +12.5µg(B) +12.5µg(C) + 40µg Alum	12.5µg(D)+12.5µg(E) + 12.5µg(F)+ 40 µg Alum	12.5µg(M) +12.5µg (N) +12.5µg (O) +40 µgAlum	4.16µg(A)+ 4.16µg(B)+ 4.16µg(c) +4.16µg(D) + 4.16µg(E) + 4.16µg(F) +4.16µg(M) +4.16µg(N) +4.16µg(O) +40µgAlum	Negative control2, 40 µg Alum	Plain/ Negative control 1

groups were used; the negative control 2 group was challenged with 0.5 ml inoculum containing 10^7 CFU *S. aureus* ATCC 25923 on day 30, and the negative control group 1 (plain/normal mice). The survival rate was monitored after the challenge [13]. In addition, the sera of each animal group were collected from the tail vein for analysis of IgG using ELISA [20] at intervals (0 days, 14 days, 30 days, 37 days). The sera of each animal group were collected from the tail vein for analysis of Interleukin 10, Interleukin 2, and Interferon-gamma (IFN- γ) at intervals (30 days, 37 days, 58 days, and 86 days).

2.6. Enzyme-linked immunosorbent assay (ELISA)

High protein-binding 96-well microtiter plates (Costar - USA) were coated with 5 µg of each peptide in 50 µl sodium bicarbonate buffer (PH 9.6) as the coating buffer for the ELISA test. A 1% addition of bovine serum albumin was made for non-specific binding. Following washing, serum samples were diluted (1:50) and treated for 1 hour at 37 °C with coated antigen. HRP goat anti-human IgG (Sigma-Aldrich, USA) was used as the secondary antibody at dilution of 1:2000. ELISA was detected using HRP substrate solution for 15 minutes at 30 °C. With the addition of 4M sulfuric acid, the reaction was halted after 15 minutes, and a microplate reader (Bio-Rad) was used to measure the absorbance at 450 nm [21]. Positive wells read three times higher than the mean of wells treated with antigen alone without serum[22].

Cytokine Assays: Interleukin 10 (IL-10), Interleukin 2 (IL-2), and Interferon-gamma (IFN- γ) assessment was conducted using RayBio®Mouse IL-10 (Cat. N.: ELM-IL10), IL-2 (Cat. N: ELM- IL 2), and IFN-γ (Cat. N.: ELM-IFN γ). Biotinylated anti-mouse for IL-10, IL-2, and IFN-γ were used to coat 96-well plates. Controls and samples were pipetted into the target wells, and sera samples were bound to the immobilized antibody. The plates were incubated and washed, and biotinylated antibodies were added. HRP-conjugated streptavidin was pipetted as 100 µL/ well, TMB substrate solution was added as 100 µL/ well post-decanting of streptavidin, and the developed color was in proportion to the number of cytokines bound. The Stop Solution turned the color from blue to yellow, and the ELISA reader (ELX-800 Biotek, USA) was used to assess the color's intensity at 450 nm.

2.7. Histological study

At the end of the experiment (86 days), liver and spleen samples were collected from animals. Samples were removed after mice scarification. All samples were processed [22], fixed in 10% formalin, processed in ascending grade alcohol series (70-90-100%), cleared in xylene and embedded in paraffin, and cut 4-5 µm thickness.

The sections were loaded on glass slides, stained with hematoxylin & eosin (H&E), and microscopically examined at 200X and 400X magnification for light microscopic (Hund Germany) observations.

2.8. Flow cytometry

T-cell differentiation was carried out using a flow cytometer (FACScan; Becton Dickinson, Mountain View, CA, USA) assay. The spleens were taken out and weighed eighty-six days after the inoculation. Single spleen suspensions were created using a syringe plunger and 10 ml of phosphate-buffered saline (PBS) solution on a Petriplate. The mixture was filtered to create single-cell suspensions by using a cell strainer to obtain single-cell suspensions, which were then centrifuged at 300×g to collect cell pellets. The pellets were 70% methanol fixed and re-suspended in PBS with 2% benzene sulfonic acid. Two samples were made of re-suspended cells from each mouse's spleen and separately incubated for 30 min with an excess of fluorescein isothiocyanate-conjugated mouse rat anti-CD4 monoclonal antibody and PE-rat anti-CD8a (BioLegend, San Diego, CA, USA).

2.9. Statistical analysis

Using SPSS, the statistical analysis was completed (Social Science version 28.00). The independent T-test was the best tool for comparing the means of two sets of quantitative variables in a parametric analysis. Two-way ANOVA using Fisher's at a 95% confidence level. The

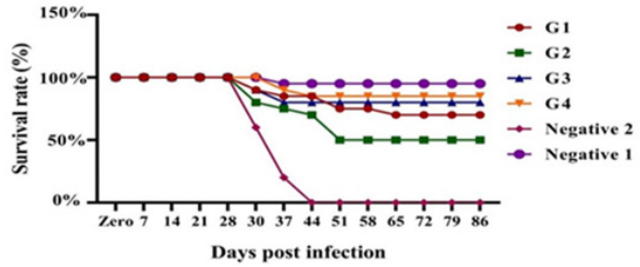


Fig. 1. The survival rates of Balb /c mice (n=20) injected with vaccine candidates MABC (G1), NABC (G2), Pic (G3), Peptide mixture group (G4). Negative control 1 mice were a plain group. Negative control 2 is mice injected with alum only, then challenged by S. aureus strain ATCC 25923 (1 × 10^ CFU/ml).

quantitative nature of the analytical data led to the parametric distribution of the Levane test being conducted. Charts produced with GraphPad Prism8.

3. Results

3.1. Three peptide vaccines provided high protection against *S aureus* in Bacteremia model

The protective efficacy of the peptide vaccine in the bacteremia model was assessed by monitoring mouse survival. In female BALB/c mice, the highest survival rate (85%) was observed in the group immunized with a mixture of B- and T-cell epitopes from *PIc, MABC,* and *NABC.* The *PIc* epitope group showed 80% survival, followed by the *MABC* group (70%) and the *NABC* group (50%), as shown in Figure 1.

3.2. B-cell adaptive immunity against *S aureus* in Bacteremia

Immunization with the peptide vaccine induced a strong antibody response. The highest IgG titer was observed in the *PIc* group, measuring 54.26 µg at day 30 post-first vaccination and remaining elevated at day 37. The peptide cocktail group generated a titer of 40.52 µg, followed by the *MABC* group (29.38 µg). The *NABC* group showed the lowest IgG response after the vaccination protocol (Figure 2).

3.3. Differential cytokine levels in mice Bacteremia model (IL-2/IL-10/ INF- γ)

From monitoring IL-2 in a mixture of tripeptides, the best level was shown after 37 days (10.05 pg/ml), then it reached at the end of the experiment (9.1 pg/ml), *PIc* peptides groups showed low levels of IL-2 (7.584 pg/ml) after 30 days and relatively the same level at the end of the experiment (7.308 pg/ml) as shown in Figure 3(A). IL-10 in the mixture of tripeptides group was 490.4 pg/ml after 30 days of vaccination, after challenge, it became 537.6 pg/ml, and after 86 days, it remained high (559.8 pg/ml). *PIc* peptides group was 510.4 pg/ml after 30 days and 466.7 pg/ml after 37 days, and at the end, it became 552.5 pg/ml as shown in Figure 3B. INF- γ in the mixture of tripeptides group was 1.541 pg/ml after 30 days of vaccination and *PIc* peptides group was 1.491 pg/ml. After the challenge (37 days), the mixture group recorded 2.155 pg/ml, while the PIc group recorded 2.799 pg/ml. At the end of the experiment, the mixture group level became 2.03 pg/ml, and the *PIc* group level was 2.262 pg/ml, as shown in Figure 3C.

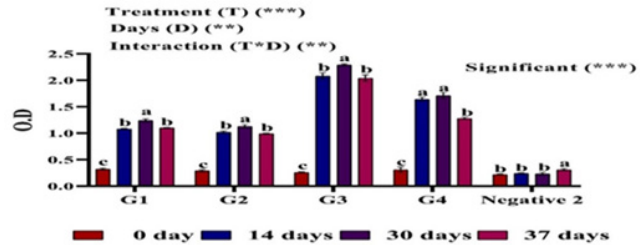


Fig. 2. Level of IgG of four mouse groups and negative control at different intervals. The data are shown as means SE (n=20), with a lowercase letter after each bar. Each group's mean values are denoted with a different letter to signify a significant difference (a-b). Significant and highly significant differences are shown in the two-way ANOVA with Fisher's post-hoc test) and the level of significance ($p<0.05$, $p<0.01$, $p<0.001$).

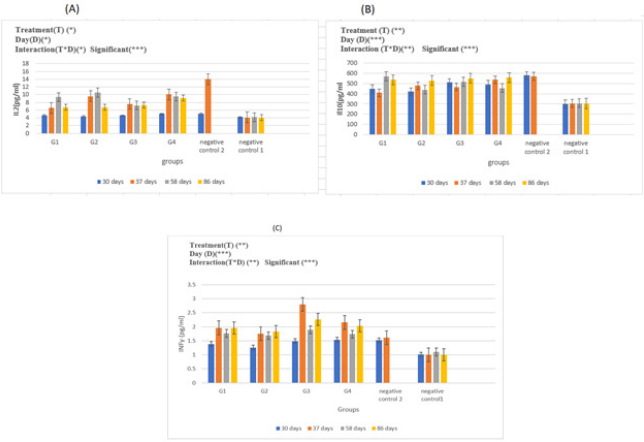


Fig. 3. Interleukin 2 (IL-2), B Interleukin 10 (IL-10) and C Interferon-gamma (IFN γ). Significant differences are shown in the two-way analysis of variance (ANOVA) by the signs *, **, and ***, respectively. G1 (MABC); G2 (NABC); G3 (PIc); G4 (peptide mixture); negative control1; negative control 2, which are mice injected with alum only, then challenged by S. aureus strain ATCC 25923.

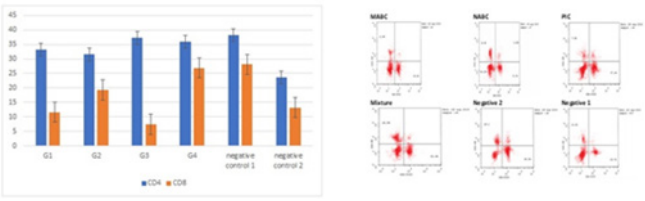


Fig. 4. A) Effect of four groups on the levels of CD4 and CD8. The values are defined as means ± SE (n=20) (***; highly significant at p-value<0.001 G1(MABC); G2(NABC); G3(PIc); G4(mixture); negative control 2 and, negative control 1 (plain group). B) Flow-cytometry picture in the 6 mice groups; G1(MABC), G2 (NABC), G3(PIc), G4(mixture). Negative control 2 mice injected with alum and challenged by S. aureus strain ATCC 25923 (1×107cfu/mL), negative control 1 (plain group).

3.4. Histopathology of Liver and Spleen

Histological evaluation showed that the best vaccination candidates were Gp 3 (*PIc* peptides), Gp4 (peptide mixture), *MABC* peptides, and *NABC* peptides. Similar findings in the histology of the liver between Gp3 and Gp4 include modestly dilated portal veins and portal tracts, notable central vein enlargement with a moderate peri-venular inflammatory infiltrate, minor intra-lobular inflammation, and scattered apoptosis. The level of inflammation is higher in Gp2 than in Gp 1(Fig 5.A).

Histological evaluation of spleen sections was performed to assess the immune response (Fig. 5B). In Group 3 (*PIc*-vaccinated), the best-performing group, spleens exhibited mildly expanded red pulp due to small lymphocytes and few scattered giant cells. Group 4 (antigen mixture-vaccinated) showed average-sized lymphoid follicles with tangible-body macrophages and a mildly expanded red pulp containing excess macrophages and few giant cells. In contrast, the positive control spleens had relatively small lymphoid follicles with central arterioles and a markedly expanded red pulp infiltrated by numerous macrophages and many scattered giant cells.

4. Discussion

Designing a single and universal vaccine for *S. aureus* is a highly complicated process, partly due to *S. aureus* genetic heterogeneity and the wide spectrum of virulent factors and multiple virulence pathways[23]. Despite our best efforts, we could not significantly reduce circulatory system contamination, regardless of the antigen combinations or adjuvants used in the study. Alum is utilized as an adjuvant; While Alum significantly improves humoral immunity, it has little impact on cellular immunity[24]. The method of delivery and adjuvant choice significantly impact the type of immune response a vaccination induces [24]. Our study demonstrated the immune protective efficacy of *PIc* peptides in mice bacteremia, as shown previously in our pilot study [10]. Nickel ABC-transporters are involved in nickel transport in *S. aureus*, which is essential for urease activity and implicated in *S. aureus* UTI pathogenesis [25]. Metal ABC-type permease systems typically feature two membrane-associated nucleotide-binding domains that link energy to metal import, two integral membrane domains. Provide a transport channel and a metal-binding protein that scavenges the metal with high affinity[26]. Studies using mutagenesis that specifically target ATP-binding cassette (ABC) transporter components have shown that a subset of these transporters is crucial for the survival and growth of infections. Our recent study results demonstrated efficacy of the the used peptide cocktail in mice skin infection model [19].

The constant rise in IgG levels throughout the *PIc* peptide immunized group and then the peptide mixture group after challenge, which demonstrated the effectiveness of the proposed peptides, indicates the robust humoral immune response after vaccination. Antibodies against *PIc* neutralize this enzyme, prevent hydrolysis, and may facilitate opsonophagocytic. The cytokine network is essential in controlling the inflammatory response to bacterial infections. In order to ensure that the host produces the proper response to combat the invasive pathogen while also limiting needless pathology from excessive inflammation, the balance between proinflammatory cytokine (IL-2, IFN- γ) and anti-inflammatory (IL-10) cytokines should be kept. Our findings showed that IL-10 plays an important role in survival during systemic acute *S. aureus* infection [27]. The role of IL-10 is determined by the location of infection. IL-10 protects the host during systemic *S. aureus* acute peritoneal infection by limiting proinflammatory responses (IL-2), particularly T-cell responses, reducing excessive inflammation and accompanying host damage, as indicated by histopathology findings [28]. IL-2 cytokine encourages both T effector and T regulatory cells to survive and grow. Group 4 showed the best levels of IL-2 stimulation of CD4 and CD8 and Gp3(*PIc*), respectively [29].

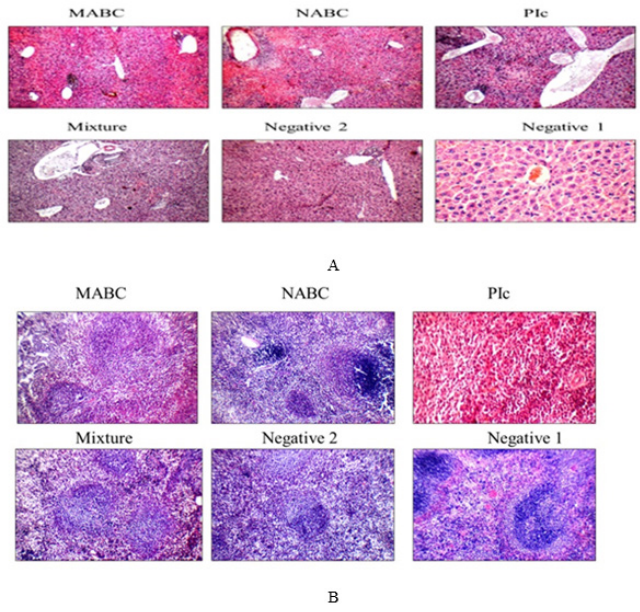


Fig. 5. A) Histopathology of liver in the 6 mouse groups: G1(MABC), G2 (NABC), G3(PIc), G4(mixture group). B) Histopathology of Spleen in the 6 mouse groups: G1(MABC), G2 (NABC), G3(PIc), G4(mixture group). Negative control 2 mice were injected with alum and challenged by S. aureus strain ATCC 25923 (1×107 CFU/ml), negative control 1 mice were a plain group.

The ability of Th1 and Th17 cells to influence susceptibility is crucial for developing resistance to *S. aureus* infection [30,31]. When administered intravenously, a vaccine targeting *S. aureus*- induced pneumonia significantly decreased bacterial load; this effect was countered by IFN- γ and IL-17 generated by CD4 cells"In our experiment PIc follow by G4 showed the optimal level of IFN γ [13].".T helper cells were crucial in orchestrating the phagocytic effector cells' response to eradicate the illness, but they did not actively attack bacteria or infected cells. INF-γ activated macrophages and cytotoxic T cells, whereas IL-17 recruited neutrophils to disease sites [32]. Another T cell-resistant structure, known as CD8 cells, also released INF- γ and IL-17 and improved resistance to *S. aureus* infection [33]. In terms of resistance to *S.aureus* infection, components of CD4 and CD8 cells, as well as INF- γ and IL-17 released by these cells, have received widespread consideration. By adjusting the recruitment of neutrophils, dendritic cells, and macrophages, these cells and the cytokines they release are thought to have strong promise as vaccine targets, *S. aureus* contamination, and in controlling the onset of resistance responses to lung and skin diseases[34,35]. At the end of the experiment, the count of CD4/ CD8 was elevated. Bacteremia mice showed a significant difference between the mice vaccinated with the mixture and negative control groups for CD4 and CD8 counts. CD4 count in the four groups was significantly higher from negative mice, which indicates the antigenicity of the peptides for both B cells and T cells.

5. Conclusion

Reverse vaccinology is a sophisticated technique that may be implemented to identify new vaccine candidates against a number of microorganisms, and due to the exces-

sive quotes of antibiotic resistance without successful vaccines in the marketplace for *S. aureus*. Our study results demonstrated that including multiple genes, B cell and T cell epitopes improved both the humoral and cellular immunity and resulted in the best outcome in the bacteremia mice model. A more expanded in-vivo study is recommended for testing (*MAB*C, *NABC* transporter, and *PIc)* as B cells and T cells peptides cocktail as a promising *S. aureus* vaccine.

Conflict of Interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
This research was approved by the ethics committee of the faculty of pharmacy, Suez Canal
University in Egypt (Reference numberPM-NR3-PHD-17).

Informed Consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
All authors contributed to the study's conception and design. SK developed, optimized protocol and applied experiments. SS wrote this manuscript. SK, SS, and AH reviewed this draft and contributed and approved the final manuscript.

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Use of Artificial Intelligence Tools
Some sentences in this manuscript were revised with the assistance of artificial intelligence–based language models to improve clarity and readability. All intellectual content, interpretations, and conclusions are solely the responsibility of the authors.

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