Cell mediated immunity follow up in vaccinated rats with multivalent Staphylococcus aureus surface antigens vaccine

By Marwan M. Mohammed

WORD COUNT 4019
TIME SUBMITTED 11-JUN-2024 11:58AM
PAPER ID 109567385

Cell mediated immunity follow up in vaccinated rats with multivalent *Staphylococcus aureus* surface antigens vaccine

Marwan M. Mohammed[1] , Mohammed H. Khudor[2], Hanaa K. Ibraheim[2]

[1] Department of Medical laboratory Technologies, Basrah college of sciences and technology, Iraq

[2] Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq.

mohamed.khudor@uobasrah.edu.iq
https://orcid.org/0000-0002-5699-5241

hanaa.ibraheim@uobasrah.edu.iq

ABSTRACT

Staphylococcus aureus can cause a variety of infectious disease, it can cause serious illnesses in both human and animal. Bacterial inactivated *S. aureus* surface antigen vaccine known as inactivated vaccine prepared in this study by treated the bacterial cells with NaOH to formation an empty envelop with whole bacteria and injected the prepared vaccine in rats to investigate the immunity response.

Material and methods. The prepared vaccine was evaluated by using scanning electron microscope (SEM) and by experimental animals. The experimental animal divided in to four groups (25 animal in each group) which include the non-treated animal (CT) were injected with 300μl PBS which used as negative control, intramuscular immunized group (IM) ,intravenous immunized (IV) and subcutaneous immunized (SC) were injected at zero day with 300 μl of prepared inactivated vaccine and injected with 250 μl bootstring dose at 14 and 28 days, finally 25 rats were used for safety test.

Results and conclusion. The total lymphocytes level was significantly increase in all vaccinated groups compared with non-vaccinated group ($p< 0.05$). Also the CD+4

and CD8+ levels were increased due to immunization with inactivated vaccine in all groups ($p < 0.05$). The results of study showed that prepared vaccine induce immunity and provide a serious protection against challenge test

Key words: *S. aureus*, Surface antigens, Rats, Flow cytometry, CD+4 , CD8+. Immune response

Introduction

One of the most prevalent bacteria that colonizes the nasal cavity and/or the exterior body surfaces of humans and other animals is *Staphylococcus aureus* (*S. aureus*) which it can cause a variety of infectious diseases [1]. In addition to causing poisoning, *Staphylococcus aureus* is an opportunistic human and animal pathogen that could cause serious illnesses like endocarditis, septicemia, osteomyelitis, pneumonia, toxic shock syndrome, and a host of other infections tend to range from skin and soft tissue infections [2]. Because of the misuse of antibiotics (such as utilizing them without a prescription, in high doses, or for no justification), which led to bacterial evolution, *S. aureus* drug resistance has progressively developed in recent years [3]. Methicillin-resistant (commonly known as methicillin-resistant) *Staphylococcus aureus*. Because the activity of penicillin-binding proteins (PBPs) that are suppressed by antibiotics being replaced by PBP (PBP2a) function with minimal affinity for most -lactam antibiotics, *Staphylococcus aureus* (MRSA) is resistant to most -lactam antibiotics [4]. Animal-related methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a major threat to human and animal health. There is a considerable frequency of bovine mastitis, an infected udder illness that spreads to dairy cows' milk glands, in both cattle and udder quarters worldwide. Mastitis in dairy milk prevents frequent milking because it affects the mammary gland cells. In addition, pathogenic bacterial enzymes, including the coagulase enzyme, which causes the conversion of fibrinogen to fibrin, cause clots to develop in the milk of cows with mastitis [5]. Despite the existence of few effective

treatments for multidrug-resistant *S. aureus* strains, immunization is still the most effective strategy to prevent and manage *S. aureus* infections [6].

Developing and implementing innovative targeted delivery methods is gaining traction. Bacterial derivatives, such as extracellular vesicles, bacterial surface antigens and toxins are common biological materials that are future users of vaccines and medications [7]. Inactivated whole bacteria vaccine (whole surface antigens) offer a good practices and potential approach in nonliving vaccine technology.. Transmembrane structures develop on the cell surface antigens, leaving the cell envelopes vacant. In experimental animal models, the resultant stimulate robust immune responses and guard against certain illnesses [8]. As an alternative, a novel method for producing *E. coli* vaccine has been performed by employing the MICs and minimum growth concentrations of sodium hydroxide (NaOH), sodium dodecyl sulfate, and calcium carbonate [9].

Teichoic acid, proteins, and peptidoglycan compensate the *S. aureus* bacterial envelope. In several investigations, *S. aureus* envelope components were thought to be potential vaccine candidates in animal models [10]. Experimental animals that were vaccinated with *S. aureus* peptidoglycan developed protective immunity against a lethal challenge [11]. Recent research revealed that vaccination of experimental animals with a cocktail of four surface antigens (IsdA, IsdB, serine-aspartate repeat A [SdrA], and SdrE) generated in opsonophagocytic antibodies and substantially protected against clinical *S. aureus* isolates. Components of the *S. aureus* cell wall have been reported to both humoral and cell-mediated immunity to be boosted [12].

In the present study, we created the inactivated vaccine and vaccination of rats via intramuscular , subcutaneous, and intravenous methods might elicit the cellular immune responses. Moreover, these immune responses provide defense against a challenge including pathogenic *S. aureus*.

Material and methods

Sample collection.

Five suspected *S. aureus* isolated from cows suspected with mastitis condition where samples obtained and transported by using sterile test tubes [1,14] from Basrah dairy farms. The samples were collected between February 2022 to April 2022, and analysis

was done after collection. The laboratory work was completed in Basrah Veterinary College.

The collected milk where tested for isolation and identification of bacteria was done according to previous study [5,23]. The identification of *S. aureus* performed by using the VITEK 2 system (bioMérieux S.A., France) for each suspected isolate was recognized. The sensitive, intermediately sensitive and resistant results was accompanied with Clinical and Laboratory Standard institute (CLSI) guideline .The positive results of vitek for specific isolates were subjected to molecular analysis.

The genomic DNA of bacterial isolates were extracted by using (Bioneer/Korea) kit genomic DNA from bacterial cells using the Gram-Positive Bacteria methodology. Conventional PCR amplification of essential *nuc* gene was used to amplify by using specific primers. The PCR program (279 bp) was carried out according to [15] as follow; primary denaturation with 95 C for 5min, denaturation with 94C for 1 min, annealing temp 55C, extension temp 72 C for 1 min and extension for 34 cycles then final extension temp was 72C for 10 min (Table 1).

Table (1) *nuc* gene primers sequences with product size

Primer	Sequences of the primer (5 to 3)		Product size
nuc gene	Forward	GCCATTGATGGTGATACGGT	279 bp
	Reverse	AGCCAAGGTTGACGAACTAAAGC	

Preparation of *S aureus* surface antigens vaccine by using NaOH

Determination of NaOH MIC

S. aureus surface antigen vaccine were produced by using the MIC of NaOH method as described previously [15]. In briefly, the biomass of 72-h-old *S. aureus* cells was collected by centrifugation (10,000 g for 10 min at 4°C) and washed three times with phosphate-buffered saline. One milliliter of the MIC of NaOH was added to 2 ml of the bacterial suspension (1 X10^8 CFU/ml) and incubated at 37°C for 75 min. To determine the lysis rate, samples of cells treated with the MIC of NaOH and non-treated control cells were collected at 15-min intervals (15, 30, 45, 60, and 75 min) after treatment and spread onto mannitol salt agar plates. After incubation at 37°C for 24 h, viable colonies were enumerated and results were expressed in numbers of CFU/ml.

Samples of bacterial cells treated with the MIC of NaOH and non-treated control cells were collected. Their genomic DNA was extracted by using isolation kit (persto mini DNA extraction kit), according to the manufacturer's instructions. The extracted genomic DNA was analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide.

Morphological features of Inactivated *S. aureus* vaccine were analyzed by scanning electron microscopy (SEM). According to Vinod study [15] the sample was prepared and examined.

Safety test

Twenty-five animals rat used for safety test by injected these rats subcutaneously with 300 μl of prepared vaccine then followed up for one week in order to determine any undesirable side effect or post vaccination in these animals or in their behavior and the results were recorded.

Experimental animals

One hundred twenty five male Wistar albino rats (8 weeks age and 200-220 grams body weight/animal) were used. 75 of them were used in the vaccination program where as 25 of them were used as a control to determine the protection level of the prepared vaccine in challenge test at the end of the study, finally 25 of them have been used in the safety test to determine safety of each prepared vaccine in this study.

Experiment using vaccination and challenge. The animals were separated into four groups and given the names Ct, SC, and IV in order to evaluate the immunogenicity and protective effectiveness of SAGs. Sterilized PBS was administered subcutaneously to group Ct rats (the non-immunized control group). The prepared 300 μl of inactivated vaccine ($1X10^6$cells/ml) with sterile PBS were administered intravenously, subcutaneously, and intramuscular to the rats in group IV, Sc, and IM, respectively. Rats from each of the four groups received three vaccinations at intervals of day 1, day 14 and finally day 28. All rats were administered an intravenously with 300 μl virulent *S. aureus* ($2x\ 10^8$ CFU/ml) of challenge dose with two weeks following the previous inoculation at week 7. Blood samples were obtained from each rat's tail vein to assess the immunological response at several days (day 5, day 19 and day 33) for flow cytometry analysis.

Flow cytometry analysis

Blood samples from vaccinated and non-immunized control rats were collected and testing with same day on the day 5 , day 19 and day 33 following the immunization to assess T-cell markers in order to study the cellular immune response. According to the manufacturer's instructions created (R & D sytem), Fluorescence-activated cell sorter (FACS) analysis was used to obtain the data using a Accuri flow cytometer and Accuri software.

Statistical analysis

SPSS version 24 were used and ANOVA with LSD test used for data analysis. The significant differences between groups measured at P value ≤ 0.05 with mean Standard deviation.

RESULTS

Identification of MRSA

Only five isolates from mastitis cases isolates were identified by Vitek2 system then confirmed by using *nuc* gene (279pb) Figure (1).

By using Vitek 2 compact system for antibiotics sensitivity testing for PCR positive isolates of S. *aureus*. The results for positive showed that *S. aureus* resist to more than 80% (20) antibiotics from different antibiotics family .According to antibiotics sensitivity testing, all isolates was resist to Oxacillin which refer as all isolates were Methicillin resistant *Staphylococcus aureus* (MRSA).

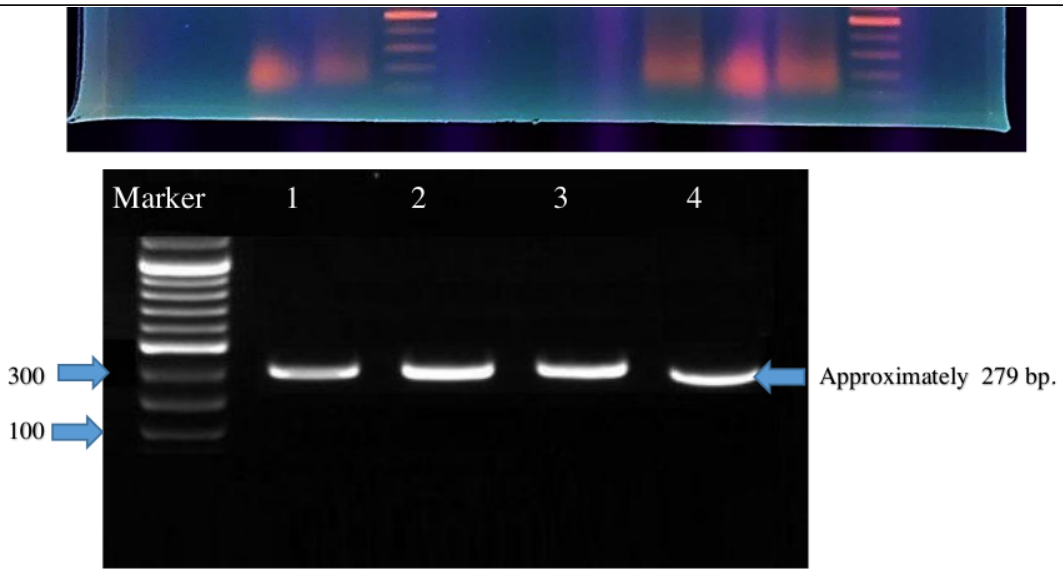

Figure (1) Molecular identification of S .aureus isolates for (lane 1-4) primer with 279 bp ,M: marker. Agarose 1 % concentration.

The results of the Minimum inhibition concentration of NaOH showed that best concentration that appears minor growth was observed with the concentration of 8.0 mg/ml for 75 min that decrease number of CFU on the nutrient plate as in Figure 2.

Determination of the DNA concentration of prepared vaccine by electrophoresis

After extraction of treated and control isolates DNA by electrophoresis in 1% agarose gel stained with ethidium bromide, the results showed clear bands of total extracted DNA of control while

there was no clear bands for prepared vaccine treated isolates with NaOH .

Scanning electron microscope (SEM) examination

After examination of treated cells by using scanning electron microscope (SEM) the images showed the formation of transmembrane lysis tunnel structures in treated cells the procedure of treatment with MIC of NaOH not changed in cell morphology or cause damage to cells which that consider as a main feature of *S. aureus* cell surface antigens vaccine.

Safety test

After injection the ten rats that isolated for safety test and followed up their vital signs for 7 days for detection of any side effects that would appeared with demonstration of prepared vaccine. the rats showed no change in their vital signs and behavior and no side effects appeared after injection with prepared vaccine.

Flow cytometry analysis

Lymphocytes

The results showed an increase in the numbers of lymphocytes in all vaccinated groups in comparison with the Ct group. In the IV groups, the total number of lymphocytes was higher than Sc and IM groups on day 5. On day 19 the IV number.

The results showed an increase in the numbers of lymphocytes in all vaccinated groups in comparison with the Ct group. In the IV groups, the total number of lymphocytes was higher than Sc and IM groups on day 5. On day 19 the IV number was highest than the other IM and SC group. Finally, IM group's total lymphocytes were elevated than Sc and Iv group on day 33 (Figure 3).

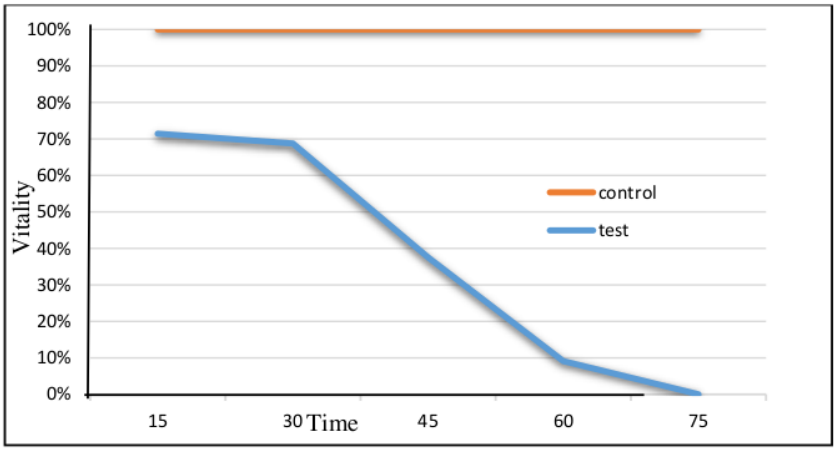

Figure (2) The Curve of MRSA in CFU/ml different time point after treated with NaOH

was highest than the other IM and SC group. Finally, IM group's total lymphocytes were elevated than Sc and Iv group on day 33 (Figure 3).

CD+4 percentage analysis

The flow cytometry analysis for CD+4 cells percentage was increased two fold in all vaccinated groups (SC, IM, and IV) compared with the Ct group .On Day 5 the IV group CD4 percentage results were higher than the IM group and SC group. A great matching was revealed in CD4 + percentage between all groups (SC, IM and IV) on day 19. Finally, a higher percentage of CD+4 was shown in the IM group than in the SC group and IV group on day 33 (Figure 4).

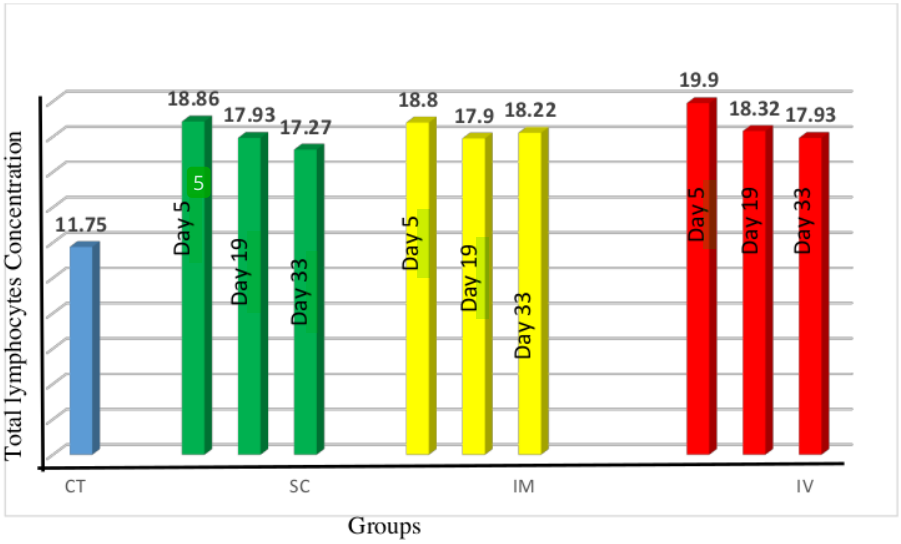

Figure (3) Total lymphocytes numbers changes in SC, IM and IV groups comparison with Ct group

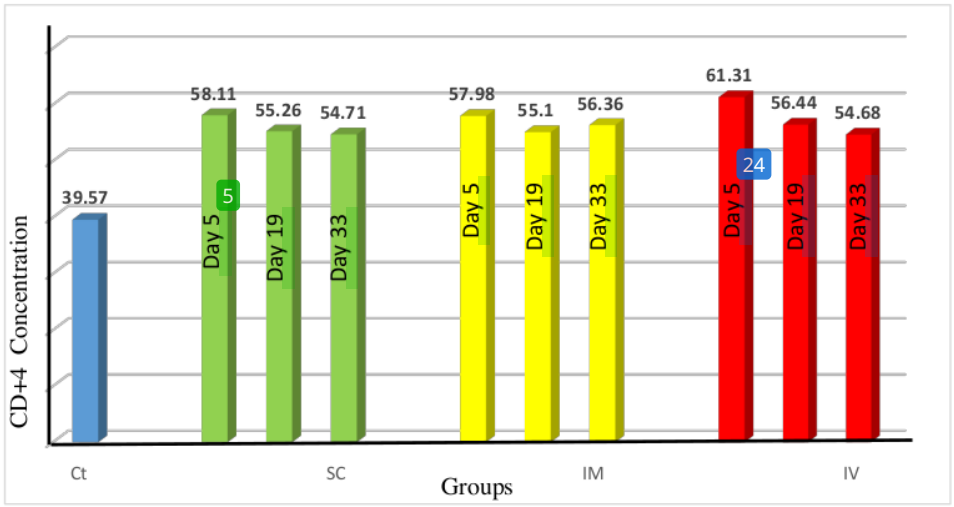

Figure (4) The CD+4 Percentages fold changes in SC, IM and IV groups comparison with Ct group

CD+8 percentage analysis

In comparison between all vaccinated groups with the control non-vaccinated group, the results of CD+8 percentage showed a significant increase in CD+8 percentages. On day 5 a higher percentage was noticed in the IV group compared with IM and Sc groups. There were no significant changes recorded on day 19 in all vaccinated group results of CD+8 percentages but still approximately two-fold higher than the Ct non-vaccinated group. On day 33, the IM

results were higher than other SC and IV results of CD+8 percentages while the percentage of CD+8 that lower than on day 5 and day 19 for both SC and IV groups (Figure 5).

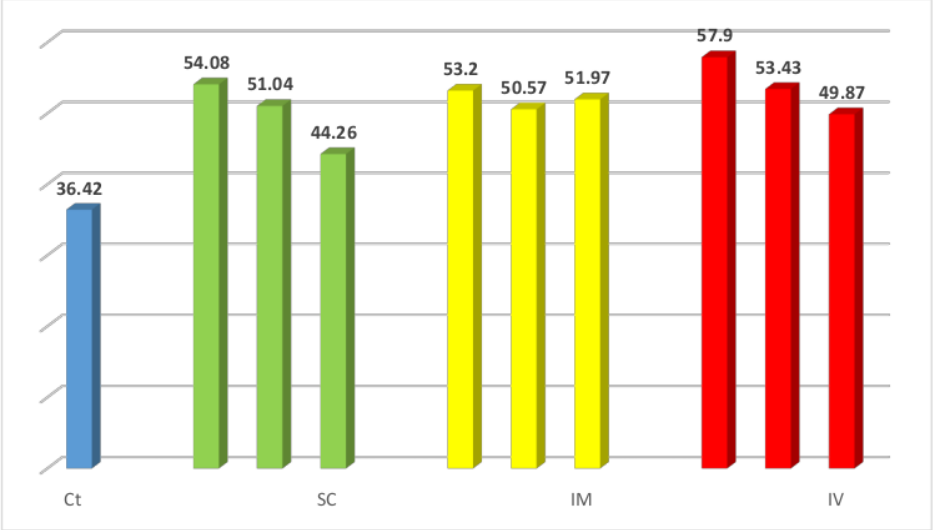

Figure (5) The CD+8 Percentages changes in SC, IM and IV groups comparison with Ct group

Challenge test

The challenge test was conducted on day 42 of the study. The test revealed the vaccinated animals survival rates were 100% in all groups while 70% of death were occurred in the unvaccinated group of displaying the virulent bacterium with a dosage of $5{,}526 \times 10^{10}$ CFU/ml, which represented LD50 (Table 2).

Table (2): Percentage and number of death and a live animal after challenge test

Group Name	Number of animal	Live	Death	Survival Percentage
Ct	10	3	7	**30%**
SC	10	10	0	**100 %**
IM	10	10	0	**100 %**
IV	10	10	0	**100 %**

Discussion

Several studies conducted in Iraq revealed that *Staphylococcus aureus* was the main bacterium causing clinical cattle mastitis [5,13,14,16]. In this study, we noticed that all suspected isolates that had a clear growth on mannitol salt agar had positive results as an *S . aureus* by using Vitek system with a higher confidence rate (99%). Also, all these positive isolates appeared clear bands of *nuc* gene when we performed conventional PCR analysis. The matching between PCR and vitek system results was agree with many studies such as [17] and [18] which these studies evaluated the performance of Vitek system with other methods. The vitek system is regarded as one of the more effective and affordable choices for the regular detection of microorganisms.

The use of cefoxitin as a surrogate marker for detection of *mecA* gene-mediated methicillin resistance is due to the fact that certain isolates were resistant to cefoxitin, which refers to all isolates being Methicillin Resistant *Staphylococcus aureus* (MRSA). The results agreement with [19,20] and Which they recorded as a higher percentage of MRSA with *S. aureus* isolates. The frequent use of antibiotics and unclean farming techniques by farmers may be to blame for the prevalence of multidrug-resistant MRSA in milk samples in this study. This is a potential consequence to the owners' public health.

The *S. aureus* infection management and prevention urgently require the development of a safe inactivated vaccine against *S. aureus*. Compared to alternative methods like the E-mediated lysis technique, the use of NaOH was quicker and less expensive [21]. In this study, we established that the development of a new *S. aureus* surface antigens vaccine may be achieved by rapidly and effectively lysing *S. aureus* cells using NaOH. The *S. aureus* cells were treated with 8.0 mg/ml of MIC NaOH, which showed a high lysis ratio after 60 minutes. These similar to the [22] study conducted a research in which *S. aureus* cells were treated with 7.5 mg/ml of NaOH, and the effectiveness of the lysis appeared after 60 minutes, indicating that the chemical approach of NaOH MIC was the most efficient and swift.

We utilized SEM to examine the generation of chemically inactivated vaccine in treated cells treated with MIC NaOH versus untreated cells. the images demonstrated presence of transmembrane lysis tunnel after completed the lysis period of NaOH treatment the images showed same results of another study [22] which observed the

similar morphological alterations in the treated cells due to transmembrane tunnel development, which caused the cells to be evacuated and become empty cells.

After exposing the cells with MIC of NaOH for 60 min, agarose gel electrophoresis revealed no DNA bands in contrast to control cells, which revealed a distinct band of genomic material. It was taken into consideration as a significant indicator that the generated inactivated vaccine was missing both DNA and cells' genetic contents. These results matching with study [21]. This study back up the theory that the NaOH approach may successfully induce DNA free of prepared inactivated vaccines.

By testing the blood samples from a variety of groups, we evaluated at changes in the CD4+ and CD8+ populations. During the study period, the IV and SC groups had greater CD4+ and CD8+ percentages, whereas the IM group had lower CD4+ and CD8+ ratios which these results agree with [22] that both studies confer the prepared inactivated vaccine that used had elicit a higher percentage of both CD4+ and CD8+ in vaccinated group compared with control group. The potential of inactivated vaccine to activate CD4 and CD8 and both types of lymphocytes capable of providing protection against *S. aureus* was discovered in this investigation.

Finally, challenge test results of the vaccinated group and non-vaccinated group after being injected with virulent isolate demonstrated the 100% protection of the vaccinated group compared to non-vaccinated group. In contrast to the non-vaccinated group, which had a death rate of 70%, the vaccinated group had a mortality risk of zero. The explanation, that the ready inactivated vaccine was given a complete immunity and protection against pathogenic virulence *S. aureus* strains, which can give animals protection against homologous challenge.

Conclusion

Staphylococcus aureus can cause a variety of infectious disease, it can cause serious illnesses in both human and animal. Because of the misuse of antibiotics, which led to bacterial evolution, *S. aureus* drug resistance has progressively developed in recent years. The ready inactivated vaccine was given a complete immunity and protection against pathogenic virulence *S. aureus* strains, which can give animals protection against homologous challenge. The challenge test results demonstrated the 100% protection of the vaccinated group compared to non-vaccinated group. In contrast to the

non-vaccinated group, which had a death rate of 70%, the vaccinated group had no mortality risk .

References

1. Abbas,B.A., Khudor,M.H., Idbeis,H.I(2013). Investigation of the activity and pathogenecity of *Staphylococcus aureus* enterotoxin c by ligated ileal loop assay inrabbits. *Basrah Journal of Veterinary Research*, *12*(2).

2. Gao, C., Zhu, L., Jin, C. C., Tong, Y. X., Xiao, A. T., & Zhang, S. (2020). Proinflammatory cytokines are associated with prolonged viral RNA shedding in COVID-19 patients. *Clinical Immunology*, *221*.

3. Santos, G. A. C., Dropa, M., Rocha, S. M., Peternella, F. A. S., & Razzolini, M. T. P. (2020). *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in drinking water fountains in urban parks. *Journal of Water and Health*, *18*(5).

4. Srednik, M. E., Crespi, E., Testorelli, M. F., Puigdevall, T., Pereyra, A. M. D., Rumi, M. V., Caggiano, N., Gulone, L., Mollerach, M., & Gentilini, E. R. (2019). First isolation of a methicillin-resistant *Staphylococcus* aureus from bovine mastitis in Argentina. *Veterinary and Animal Science*, *7*.

5.. Idbeis, H. I..& Khudor, M. H (2019). Detection Of Intracellular Adhesion Gene (Icaa and Icad) and Biofilm Formation *Staphylococcus aureus* Isolates From Mastitis Milk of Sheep and Goat. *Bas.J. Vet.Res. Vol.18,No.2*.

6. Ebensen, T., Paukner, S., Link, C., Kudela, P., de Domenico, C., Lubitz, W., & Guzmán, C. A. (2004). Bacterial Ghosts are an Efficient Delivery System for DNA Vaccines. *The Journal of Immunology*, *172*(11).

7. Langemann, T., Koller, V. J., Muhammad, A., Kudela, P., Mayr, U. B., & Lubitz, W. (2010). The bacterial ghost platform system. *Bioengineered Bugs*, *1*(5).

8. Hajam, I. A., Dar, P. A., Won, G., & Lee, J. H. (2017). Bacterial ghosts as adjuvants: Mechanisms and potential. In Veterinary Research (Vol. 48, Issue 1).

9. Hoseini Shahidi, R., Hashemi Tabar, G., Bassami, M. R., Jamshidi, A., & Dehghani, H. (2019). The design and application of a bacterial ghost vaccine to evaluate immune response and defense against avian pathogenic *Escherichia coli* O2:K1 serotype. *Research in Veterinary Science*, *125*.

10. Fowler, V. G., & Proctor, R. A. (2014). Where does a *Staphylococcus aureus* vaccine

stand? In Clinical Microbiology and Infection (Vol. 20, Issue S5).

11. Chen, H., Ji, H., Kong, X., Lei, P., Yang, Q., Wu, W., Jin, L., & Sun, D. (2021). Bacterial ghosts-based vaccine and drug delivery systems. In Pharmaceutics (Vol. 13, Issue 11).

12. Stewart-Tull, D. E. S. (1980). The Immunological Activities of Bacterial Peptidoglycans. *Annual Review of Microbiology*, *34*(1), 311–340.

13. Idbeis, H. I., & Khudor, M. H. (2019). Detection of Intracellular Adhesion Gene (Icaa And Icad) and Biofilm Formation *Staphylococcus aureus* Isolates from Mastitis Milk of Cow. *Kufa Journal For Veterinary Medical Sciences*, *10*(1).

14. Khudor. M. H., Basil ,B.A. and Idbeis, H. I.(2012) Detection of enterotoxin genes of *Staphylococcus aureus* isolates from raw milk . *Bas.J.Vet.Res.Vol.11,No.1,2012*..

15. Park, H. J., Oh, S., Vinod, N., Ji, S., Noh, H. B., Koo, J. M., Lee, S. H., Kim, S. C., Lee, K. S., & Choi, C. W. (2016). Characterization of chemically-induced bacterial ghosts (BGs) using sodium hydroxide-induced *Vibrio parahaemolyticus* ghosts (VPGs). *International Journal of Molecular Sciences*, *17*(11).

16. Anad, T. I., A. Abbas, B., & Y . Khudaier, B. (2014). Isolation of staphylococcus aureus from buffalo milk in basra governorate and detection of their antibiotic susceptibility. *Basrah Journal of Veterinary Research*, *13*(1).

17. Zuluaga, A., Arango-Bustamante, K., Caceres, D. H., Sánchez-Quitian, Z. A., Velásquez, V., Gómez, B. L., Parra-Giraldo, C. M., Maldonado, N., Cano, L. E., de Bedout, C., & Rivera, R. E. (2018). Concordance analysis between different methodologies used for identification of oral isolates of *Candida* species. *Colombia Medica*, *49*(3).

18. Monteiro, A. C. M., Fortaleza, C. M. C. B., Ferreira, A. M., Cavalcante, R. de S., Mondelli, A. L., Bagagli, E., & Cunha, M. de L. R. de S. (2016). Comparison of methods for the identification of microorganisms isolated from blood cultures. *Annals of Clinical Microbiology and Antimicrobials*, *15*(1).

19. Ramandinianto, S. C., Khairullah, A. R., Effendi, M. H., Tyasningsih, W., & Rahmahani, J. (2020). Detection of enterotoxin type B gene on methicillin resistant staphylococcus aureus (MRSA) isolated from raw milk in East Java, Indonesia. *Systematic Reviews in Pharmacy*, *11*(7).

20. Wangai, F. K., Masika, M. M., Maritim, M. C., & Seaton, R. A. (2019). Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa: Red alert or red herring? *BMC Infectious Diseases*, *19*(1).

21. Hu, M., Zhang, Y., Xie, F., Li, G., Li, J., Si, W., Liu, S., Hu, S., Zhang, Z., Shen, N., & Wang, C. (2013). Protection of piglets by a *Haemophilus parasuis* ghost vaccine against homologous challenge. *Clinical and Vaccine Immunology*, *20*(6).

22. Park, H. J., Oh, S., Vinod, N., Ji, S., Noh, H. B., Koo, J. M., Lee, S. H., Kim, S. C., Lee, K. S., & Choi, C. W. (2016). Characterization of chemically-induced bacterial ghosts (BGs) using sodium hydroxide-induced Vibrio parahaemolyticus ghosts (VPGs). *International Journal of Molecular Sciences*, *17*(11).