# Experimental Study of Prepared Killed Vaccine For *S. Aureus* in Local Rabbits

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## Abstract

This study was performed to estimate the *S. aureus* prepared killed vaccine in rabbits. The isolate of *S. aureus* that used in the present study were obtained from the college of veterinary medicine, University of Fallujah which were confirmed by using gram stain and other biochemical tests. A total of 30 local rabbits were used in this study, these were divided into 3 groups, each group contain 10 rabbits, the group 1 used as control negative, group 2 given 1 ml antigen contained 20 mg according to the pilot study that performed, group 3 serve as control positive. At day 21 post-immunization, a skin test was done, at the day 28 post first immunization, the rabbits of group 2 and 3 were challenged intra peritoneal with (1ml) of bacterial suspension containing $1\times10^8$ cfu/ml of viable virulent *S. aureus*. At day 33 post immunization, the rabbits were sacrificed then postmortem examination was carried out to all sacrificed rabbits, pieces from internal organs (liver, spleen and kidney).

The results showed that all animals in G2 showed positive reaction after 24 and 48 hrs. for skin test, the result of bacterial isolation after challenge with virulent *Staphylococcus aureus* showed the isolation of heavy bacterial isolation in control positive group from different organs of body, while immunized groups showed significant decrease in bacterial isolation as compared with control positive. The result of histopathological study of G2 showed PMNs infiltration in liver, spleen and kidney, with vacuolar degeneration of epithelial lining the renal tubule, also, the spleen showed hemorrhage, congestion of red pulp.

In conclusion, the killed vaccine that prepared showed a higher effectively when given to the rabbits; furthermore in our conclusion some bacteria were spread from the site of inoculation toward internal organs and during the course of the experiment, activated macrophages engulfed and destroyed most of them.

***Key words:*** *killed, S. aureus, ra*bbits.

## Introduction

It is a major pathogen in both community-acquired and nosocomial infections [(1)]. Many studies have reviewed the clinical infection of Staphylococcus infection which considered to be the 2nd most public cause of wound infection after *E. coli* .The wide spread use of antibiotics has led to result in multiple resistance strains [(2)].

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*Staphylococcus aureus* is regarded as a main pathogen for humans as well as animals importance due to the rise in antibiotic resistance being a highly adaptable organism, it has the ability to create infections in a wide-ranging in most body positions. The type of infections which caused by this species were to be frequently acute and pyogenic, if untreated may transmit to the adjacent deeper tissues or organs, may result in spread or deep-seated infections which are life threatening [(3)].

The important of this strain was due to multiple drug resistance as reported by [(4)].

Different type of *S. aureus* antigens were used previously as a vaccine but these Ags produced partial protection [5], however, Increasing of *S. aureus* resistance to the latest line of drugs, which is vancomycin may places of interest for seeking a new as well as novel antibiotics [6].

To this time the vaccine for *S. aureus* till unavailable to encourages the active immunity against infections from result from this bacteria [7].

A study designed to investigate the clumping factor as well as FnBPA for *S. aureus* as a vaccine, the authors showed a highly immunogenicity of the vaccine with a significant protection in mice [8].

A vaccine was developed which would offer protection against a wide-ranging of *S. aureus* strains and possibly other gram +ve bacteria. The use of peptidoglycan was studied, because it is the structure which has been exposed to play a main part in the opsonic recognition of Staphylococcal and also has a huge ability to activate both the classical as well as the alternative pathways for human complements [9].

The aim of current study was to estimate the *S. aureus* prepared killed vaccine in rabbits.

## Materials and Method

**Strain of *S. aureus*:**

The isolate of *S. aureus* used in this study were obtained from the college of veterinary medicine, university of Fallujah. This strain was ensured by using many tests including gram stain as well as the biochemical tests according to [10].

**Preparation of killed antigen:**

*Staphylococcus aureus* cultured on brain heart infusion agar, then incubated at 37 °C for 24 hrs., the harvesting was done by using PBS 7.2, then centrifuged at 3000 rpm for thirty minutes then washed for 3 times with sterilized PBS, and the precipitate was re-suspended by using PBS. This suspension were heated till boiling and then cultured on agar to ensure that the bacteria killed and preserved till used.

**Laboratory animals:**

Thirty (30) local breed healthy rabbits male and female were used in the present study. They were fed on pellet and green grass ad libitum. They were reared for two weeks for adaptation, these were divided into two groups:

G1: this include 10 rabbits were used as control negative.

G2: this include 10 rabbits and used as treated group which given 1 ml antigen contained 20 mg according to the pilot study that performed.

G3: this include 10 rabbits which serve as control positive

At day 21 post-immunization, a skin test was done according to [11]. At day 28 post first immunization, the rabbits of groups 2 and 3 were challenged intra peritoneal with (1ml) of bacterial suspension containing $1\times10^8$ cfu/ml of viable virulent *S.aureus* [12].

**Histological examination:**

At day 33 post immunization, the rabbits were sacrificed then postmortem examination was carried out to all sacrificed rabbits, 1 cm pieces from the internal organs (liver, spleen & kidney) were taken for bacterial isolation on Nutrient, blood, mannitol salt agars and other pieces were fixed in 10% formalin at (72)hrs. for histological examination which is done according to [13].

**Statistical analysis:**

This is done by using SPSS software according to [14].

## Results and Discussion

All animal in G2 showed a positive reaction after 24 and 48 hours of skin test, this positive reaction represented by presence of a circular erythema and increasing skin thickness against used Ag, the control groups give negative reaction (erythema & thickness) (Table 1).

**Table (1) Mean values of skin test in immunized Group (G2)**

| Animal No. | Erythema Diameter (cm) | | Skin Thickness( mm) | |
|---|---|---|---|---|
| | 24 hrs | 48hrs | 24hrs | 48hrs |
| 1 | 2.3 | 1.3 | 9.2 | 5.1 |
| 2 | 2.4 | 1.2 | 8.3 | 4.4 |
| 3 | 1.7 | 0.9 | 7.1 | 4.7 |
| 4 | 2.4 | 1.8 | 9.8 | 3.8 |
| 5 | 2.0 | 1.5 | 8.4 | 5.4 |
| 6 | 2.3 | 1.3 | 8.7 | 4.2 |
| 7 | 2.1 | 1.1 | 7.9 | 5.1 |
| 8 | 1.8 | 0.9 | 6.8 | 3.4 |
| 9 | 1.8 | 1.0 | 7.0 | 4.6 |
| 10 | 2.6 | 1.4 | 10.2 | 5.9 |
| Mean ± SE | 2.2±0.14 a | 1.4±0.26 a | 8.1±0.55 b | 5.40±0.22 c |

In the skin test, remaining macrophages in the skin epidermis collect the supernatant $Ag_s$ that pass from the skin and carrying into the local lymph nodes, where T-cells are activated by Ags and rapidly differentiate to Th-1 cells, which produce various cytokines and chemokine's like IL8, so that macrophages and other cells are activated and attracted to the site of skin test [15].

All rabbits in G2 gave different reaction this is due to the different degrees of immune response the same results were recorded by [16]. In general positive reaction of all Ags refer the ability of smooth strains to give immune response higher than rough strains due to their consisting of O-side chain [17].

The result of bacterial isolation after challenge with virulent *Staphylococcus aureus* showed the isolation of heavy bacterial isolation in control positive group from different organs of body, while immunized groups showed significant decrease in bacterial isolation as compared with control positive (Table 2).

**Table (2) Bacterial isolation from the internal organs of groups animals infected with virulent *S.aureus* at day 33 showed number of colonies in 1gm from each organs**.

| **Group of animal** | **Spleen** | **Liver** | **Heart** | **Kidney** |
|---|---|---|---|---|
| G3 | 42 | 49 | 23 | 26 |
| G1 | 0 | 0 | 0 | 0 |
| G2 | 1 | 1 | 0 | 0 |

O'Riordan and Lee [18] explained that the phagocytic response of PMNs is the body's first line of defense against invasion by *S.aureus* and critical determinant in the outcome of staphylococcal infections.

These findings indicated that some bacteria were spread from the site of inoculation toward internal organs and during the course of the experiment, activated macrophages engulfed and destroyed most of them. This evidence was consent with several previous studies that explained the opsonization of *S.aureus* is critical for

uptake and killing of bacteria by professional phagocytic cells, complement and Abs, are the principle serum opsonin and had been shown to play an important role in opsonophagocytic killing to *S.aureus* [19].

The result of histopathological study of G2 showed few number of leucocytic infiltration and small amount of fibrin deposition in the liver (Fig. 1), there's also PMNs infiltration with vacuolar degeneration of epithelial lining the renal tubule (Fig. 2). The spleen showed hemorrhage, congestion of red pulp as well as neutrophilic infiltration (Fig. 3).

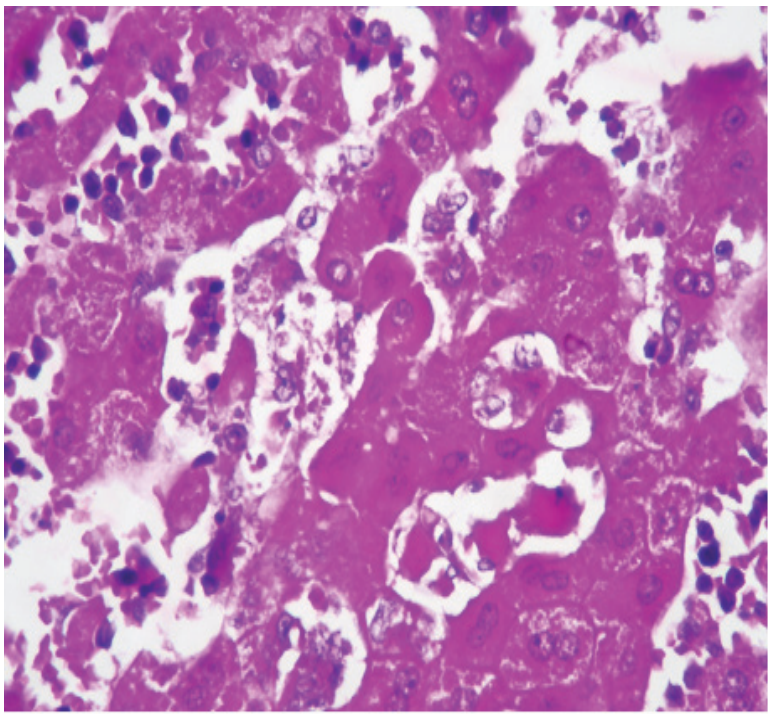

**Figure (1). Histological section in the liver of immunize group with killed *S. aureus* Ag showed few number of PMN leucocyte infiltration (H&E stain X40) .**

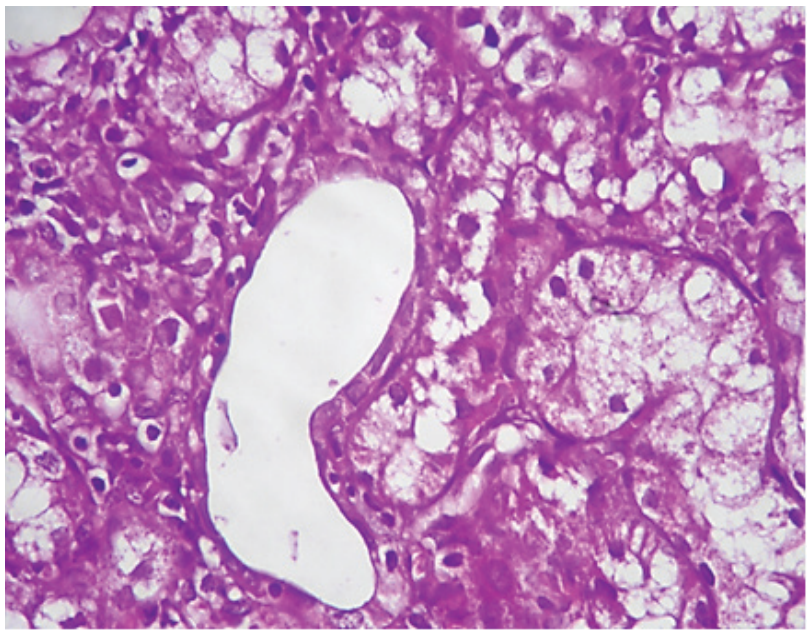

**Figure (2). Histological section in the kidney of immunize group with killed *S. aureus* Ag showed neutrophilic infiltration and vacuolar degeneration of epithelia lining of renal tubules (H&E stain X40).**

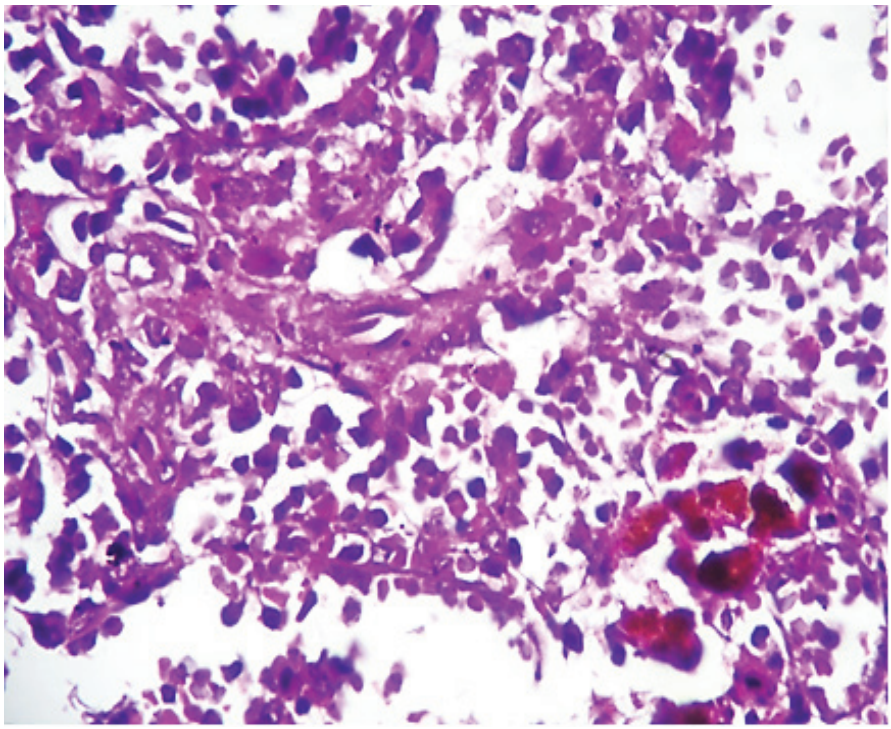

**Figure (3). Histological section in the spleen of immunize group with killed *S. aureus* Ag showed hemorrhage and congestion of red pulp and neutrophilic infiltration. (H&E stain X40) .**

The liver was the most affected organ, spleen, kidney, this is may be due to the nature of the function of the liver which mention nutritional metabolic balance in the body and this include all nutrient material and the liver considered to be the important filter which play potential role in elimination of toxins, body waste protected, metabolic substance and other foreign materials including pathogens more over the liver is major organ depended in portal circulation. These mean that all hard substance cross through the liver tissue [(20)].

The present neutrophils infiltration in organs of tissue included in this study the higher proportion of this infiltration in group 2 may related to the active production of TNF-α which proximal mediator of neutrophils chemo tactic factor [(21)] and related also to the fact that is the neutrophil represent essential cells for host define [(22)], neutrophil also can produce pro inflammatory mediators such as IL-12 [(23)].

In conclusion, the killed vaccine that prepared showed a higher effectively when given to the rabbits; furthermore in our conclusion some bacteria were spread from the site of inoculation toward internal organs and during the course of the experiment, activated macrophages engulfed and destroyed most of them.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**: The authors declare that they have no conflict of interest.

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## References

1. European Food Safety Authority The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union. The EFSA Journal, (2006). 94. 12-17
2. Ayliffe, G.A.J; Green, W. ; Livingston, R. and Lowbury, E.J.L. Antibiotic resistant Staphylococcus aureus in dermatology and burn wards. J Clinic Pathol, (1986): 30: 40-4.
3. Safdar, N. and Bradley, AThe risk of infection after nasal colonization with *Staphylococcus aureus* :Am . J . Med ., .(2008). V. 121, P.310-315.

4. Kalmus, P.; Aasmäe, B.; Kärssin, A.; Orro, T. and Kask, K. Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. Acta Veterinaria Scandinavica , (2011). 53: 4.
5. Stranger-Jones YK, Bae T, Schneewind O. Vaccine assembly from surface proteins of Staphylococcus aureus. Proc Natl Acad Sci U S A. Nov 7; ( 2006). 103(45):16942-7.
6. D'Costa, V.M.;McGrann, K.M.; Hughes, D.W.; Wright, G.D. Sampling the antibiotic resistome. Science; (2006). 311 (5759): 374–377.
7. Todar, K. Staphylococcus. In TODARSOnline Textbook of Bacteriology. (2005).
8. Arrecubieta, C. ; Asai ,T and Bayern, M. The role of Staphylococcus aureus adhesions in the pathogenesis of ventricular assist device- related infections Journal of infectious Diseases (2006) 193:1109-1119.
9. Esmaily, F. and Sharifi-Yazdi, A Modified Method for Preparation of Staphylococcus aureus Peptidoglycan Towards Making a Sub- Unit Vaccine. Iranian J. Publ. Health, (2002). Vol. 31, Nos. 1-2, PP. 37-44. Iran.
10. Markey, B. ; Leonard, F. ; Archambault, M.; Cullinane, A. and Maguire, D.Clinical veterinary microbiology. Second edition, Mosby, Elsevier.2013: 246-249.
11. Li, X.; Lockatel, C.V.; Lohnson, D.E.; Lane, M.C.; Warren, J.W.& Mobley, H.L.T. Development of intranasal vaccine to prevent urinary tract infection by *Proteus mirabilis*. Infect. Immun., Jan . (2004). 72(1) :66-75.
12. Go´mez, M.I.; Sordelli, D.O. Buzzola, F.R. and Garcia, V.E. Induction of cell-mediated immunity to Staphylococcus aureus in the mouse mammary gland by local immunization with a live attenuated mutant. Infect. Immun., (2002). 70: 4254–4260.
13. Luna, L.G. "Manual of Histologic Staining Methods of the Armed Force Institute of Pathology". 3rd Ed. McGraw-Hill, New York. (1968).
14. Snedecor, G. and Cochran, W. Statistical methods. 7th ed. The state university press American, Iowa (1989).
15. Kindt, T.J.; Goldsby, R.A. and Osborne, B.A. "Kuby Immunology". 6th Ed. Library of Congress, Publisher, S. Tenney, W.H. Freeman and Company, Printing and Binding, R.R. Donnelley New York, USA. (2007).
16. Laudat, P.; Limet, J.; Barrat, F.; Poncelet, S. ; Marquet, N.; Persong, J.M.; Audurier, A.; Gillant, J.C.; Lienarad, M. and Strady, A. Brucellosis among occupationally exposed people: Evaluation of immunological tests after vaccination. Pathol. Biol., (1993). 41(5):457-462.
17. Cheville, N.F.; Stevens, M.G.; Jensen, A.E.; Tatum, F.M. and Halling, S.M. Immune responses and protection against infection and abortion in cattle experimentally vaccinated with mutant strains of *Brucella abortus* .Am.J. Vet. Res., (1994). 54:1591-1597.
18. O'Riordan, K. and Lee, K.J. Staphylococcus aureus capsular polysaccharides. Clin. Microbiol. Rev., (2004). 17(1): 218–234.
19. Leijh, P.C.; van den Barselaar, M.T.; Daha, M.R. and van Furth, R. Participation of immunoglobulins and complement components in the intra-cellular killing of Staphylococcus aureus and Escherichia coli by human granulocytes. Infect. Immun., (1981). 33: 714-724.
20. Kumar, V.; Mitchell, R.M.; Abbas, A.K. and Fausto, N. Basic Pathology. 8thedition.pathology. bsd.uchicago.edu/faculty/V Kumar.html. (2009).
21. Netea, M.G.; VanTits, l.J.; Curfs, J.H.; Amiot, F.; Mers, J.F.;Vandermeer, J.W. and Kullberg, B.J. Increased susceptibility of tumor necrosis factor-α lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of Candida albicans. J. Immunol., (1999). 163: 1498-1505.
22. Herring, A.C. and Huffnagle, G.B. Innate immunity and fungal Infection. In Kaufmann S.H.E. , spher A., and Ahmed R. (eds).Immunology of Infectious Diseases. American Society for Microbiology, Washington, DC, (2001). PP.127-137.
23. Cassatella, M.A. The production of cytokines by polymorph nuclear neutrophils. Immunol. Today, (1995). 16: 21-25.