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STUDY THE PATHOLOGIC EFFECT OF *Staphylococcus aureus* POST INFECTION IN RABBITS

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ABSTRACT

Staphylococcus aureus (*S.aureus*) is a versatile opportunistic pathogen that causes a wide spectrum of pathologies. In rabbits, this bacterium infects dermal lesions causing suppurative dermatitis, and invades subcutaneous tissues, causing different well-known disease conditions such as mastitis, abscesses (subcutaneously or affecting internal organs) and pododermatitis. The aim of the study is to update the knowledge on rabbit Staphylococcosis by focusing mainly on the different histopathologic changes. Twenty rabbits were used as laboratory animal models in an experiment designed to study the pathogenesis of *S. aureus*. A dose of ($1x10^8$ CFU/ml) of a Rifampicin-resistant *S.aureus* was given through the following routes: Intratracheal (IT), andsterile distilled water was given by same route to a group of rabbits used as control.There were difference in temperature and body weights among treated animals and control,but it were statistically significant($P<0.05$).

INTRODUCTION

Staphylococcus aureus is a pathogen capable of infecting humans and a wide variety of animals. This bacterium affects rabbits of different ages, infects dermal lesions and invades subcutaneous tissues (1), resulting in different pathologies including suppurative dermatitis, mastitis, multisystemicabscessation and pododermatitis (2). The ability of *S. aureus* to cause disease is due to a combination of virulence factors. Bacteria are considered major risk factors for diseases of respiratory system (3), skin (4), the eye (5) and other septicemia in rabbits (4, 6) as seen in this study. Bacterial infection following ulceration of paws and urine burns (7,4) was the likely cause to Sore hocks/chronic ulcerative pododermatitis and arthritis and osteomyelitis encountered in this study.

International dissemination of high virulent *S. aureus* clone in rabbits has been reported (8). Clinical diseases caused by *S. aureus*infections in rabbits are dermal lesions, pododermatitis, abscesses and mastitis; consequently lead to poor production and mortality in young and infertility of the breeders (9 ,10). As a result of agalactia in the doe caused by *S. aureus,* suckling rabbits acquire infection during lactation that lead to high mortality rates in offspring. Natural infection of the mammary gland of rabbits with *S. aureus* can cause acute gangrenous or chronic purulent mastitis (11,9). In the acute gangrenous form, which is rapidly fatal, the mammary tissue becomes oedematous, haemorrhagic and necrotic.Rabbit *Staphylococcosis*: Difficult solutions for serious problems

Staphylococcus aureus infections are a major problem in rabbits. The main manifestations are subcutaneous abscesses, mastitis, pododermatitis and septicaemia. Two patterns of infection can be distinguished. In the first type, clinical signs remain limited to a small number of rabbits in a flock. This type has little economic importance and is caused by low-virulence *S. aureus* strains. In the second type, the disease shows an epidemic spread. Consequences are poor production results, infertility and death. This leads to chronic problems and a subsequent decline in production. The latter type is caused by high-virulence strains. Biotyping, phage typing and RAPD typing contribute to the characterization of high-virulence *S. aureus* strains. Administration of antibiotics, disinfection of the environment and vaccination are not able to solve the problems. Therefore, the only effective measure is to cull the entire flock and to restart with a new rabbit population after thorough disinfection. Limiting the introduction of new rabbits in existingrabbitsand reducing contacts between rabbits to an absolute minimum are currently the only way to face this most difficult problem,(12) showed that staphylococci based mastitis was one of the main factors of culling of adult rabbits.

MATERIALS AND METHODS

At this study (15) male and female rabbits with body weight ranged between (2090-1080gm.) were used. The animals were raised and bred in the animal house of College of veterinary medicine/ University of Baghdad where the research was done. The animals were kept in cages of (20*30*50) cm^3 dimensions in average of three rats in each cage one month before study for acclimatization in optimum conditions of breeding at (22±3) ºC with a (14/10) hours (Light/Dark) cycle.Commercial feed pellets and drinking water were given all the time of experiment(13).

Inoculum:

*S. aureus*strain used in this research isolated from mastitis case diagnosis at medicine department/ College of veterinary medicine/ University of Baghdad.The dose prepared as (14).

Experimental design:

Fifteen rabbits (white newzeland), were used as laboratory animal models in an experiment designed to study the pathogenesis of *S. aureus* and divided randomly into *infected group*(n=12) with a dose of $1x10^8$ CFU/ml of a Rifampicin-resistant *S.aureus*Intratrachial (I/T), and *controlgroup* (n=3)with sterile distilled water was given by same route.

The animals sacrificed at the end of experiment post infection (1, 3,7,14days), after post-mortem examination the tissue specimens were preserved in formalin 10% to prepare hematoxylene-eosin tissue slides (15).

RESULTS

1- Clinical signs:

The clinical signs appeared in rabbits injected via (I/T) were characterized by chock & cough for a while after injection, difficulty in breathing and then death of two animals from (I/T) group after one day of injection.

A significant decrease ($P > 0.05$) in body weight in infected group I/T along the experimental periods comparing with control group,also the body temperature showed significant increase ($P > 0.05$) ininfected group along the experimental periods comparing with control group (Figure-1).

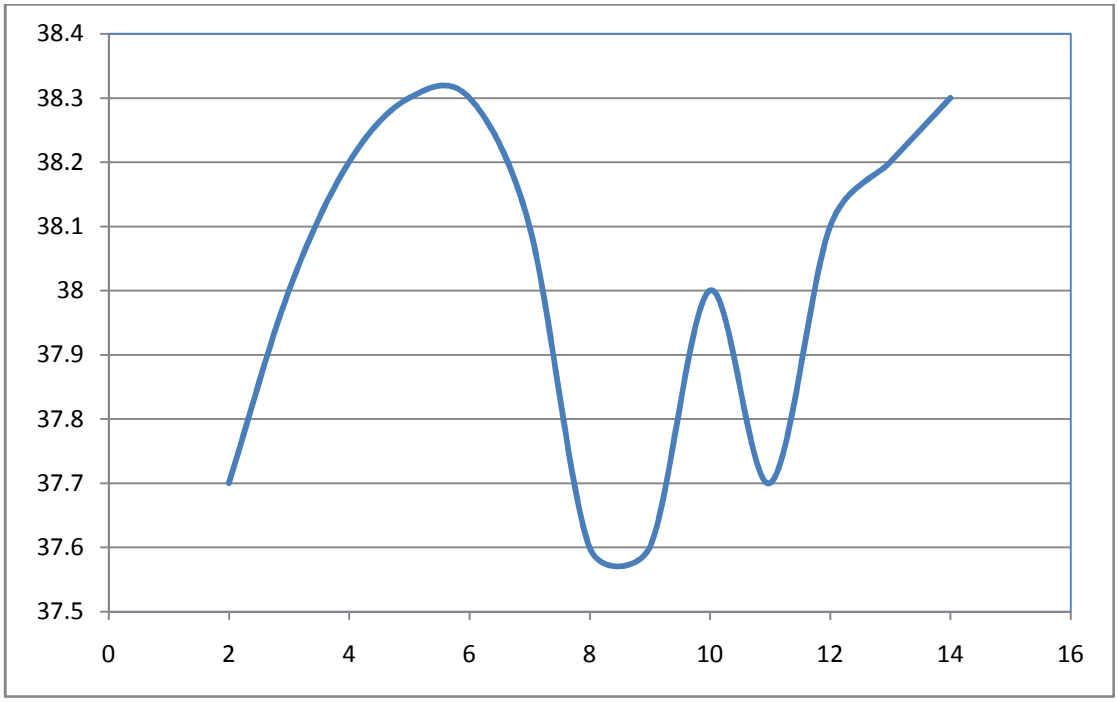

Figure- 1: illustrated temperature changes at(I/T) group,while the normal body temperature was 37.7 °C.

2- Isolation of bacteria and Grossappearance:

Isolation of bacteria from lung and liver after 1day post infection was positive while at (3, 7,14days)after infection the isolation of bacteria was positive for lung,liver,brain,blood,and kidneys.

Gross lesion were characterized by hemorrhageand presence of blood on the lumen of trachea,hydropercardium,paleness due to emphysema of some lobes of lung **,** pulmonary consolidation and liver congestion with pale area (1-3) mm in diameter on its surface.There were congestion of most visceral organs of treated rabbits and congestion of brain**(Figure - 2).**

3-Histopathologic examination:

Histopathological changes were characterized by suppurativetrachietis in(I/T) groupas sloughing of lining epithelia of trachea with focal area of (PMNS) cells infiltration especially neutrophils instead of normal tissue**(Figure-3).**Abscesses formed consisted of dead andlive tissue cells.This lesion was diagnosed as suppurativetrachietis with congestion of blood vessels.In pulmonary tissue the lesion mainly PMNs infiltration, congestion of alveolar capillaries caused thickening of alveolar walls **(Figure-4).**

The **liver** in all infected animals were characterized by hemorrhage, congestion and vacuolar degeneration of hepatocytes; different in severity with focal areas of (PMNS) accumulation especially neutrophils(**Figure-5)**

Hemorrhage, congestion of blood vessels in all layers of the **brain** were seen with edema of neuroglial cells and degeneration or loss of nuclei of some Purkinje cells (chromatolysis),with thickness of pia mater layer due to infiltration of (PMNS) cells and lymphocytes(**Figure -6)**.

Cloudy swelling of lining epithelia of the **renal tubules** appeared in star- shaped with some necrotic tubules, dilation of bowman's space with mild infiltration of inflammatory cells especially neutrophils and its severity continuousalong the experimental periods.

Heart,showed intramuscular fibers odemaand congestion of blood vessels with mild infiltration of inflammatory cells especially neutrophils some muscle fibers loss its disc due to inflammation became enlarged, swollen.

Marked Rifampicin–resistant *Staphylococcus aureus s*train used in all the inoculated animals was recovered and identified permanently from the samples taken which included:blood, heart, lung, liver, kidney and brain.

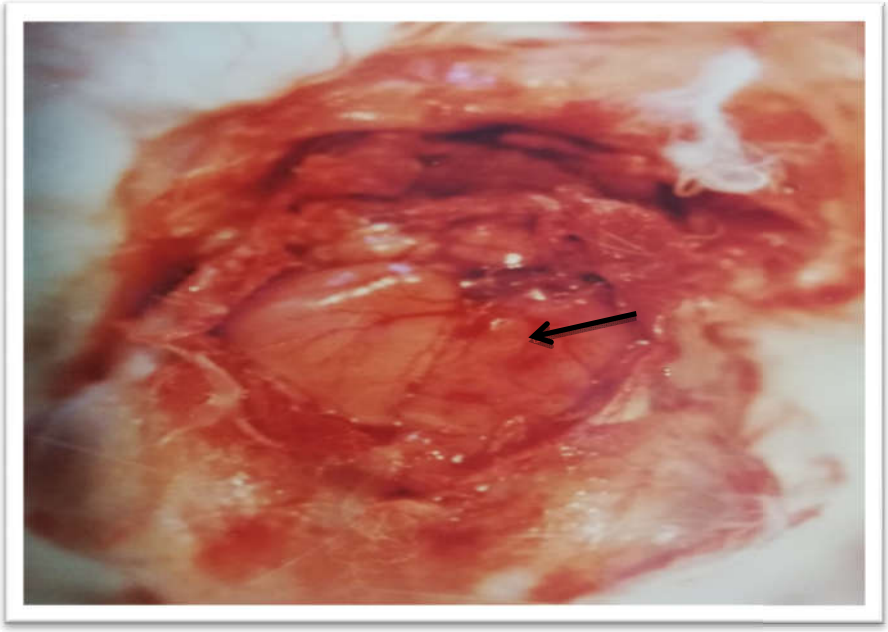

Figure- 2: rabbit brain of (I/T- 3 days); congestion blood vessels and enlargement of the brain.

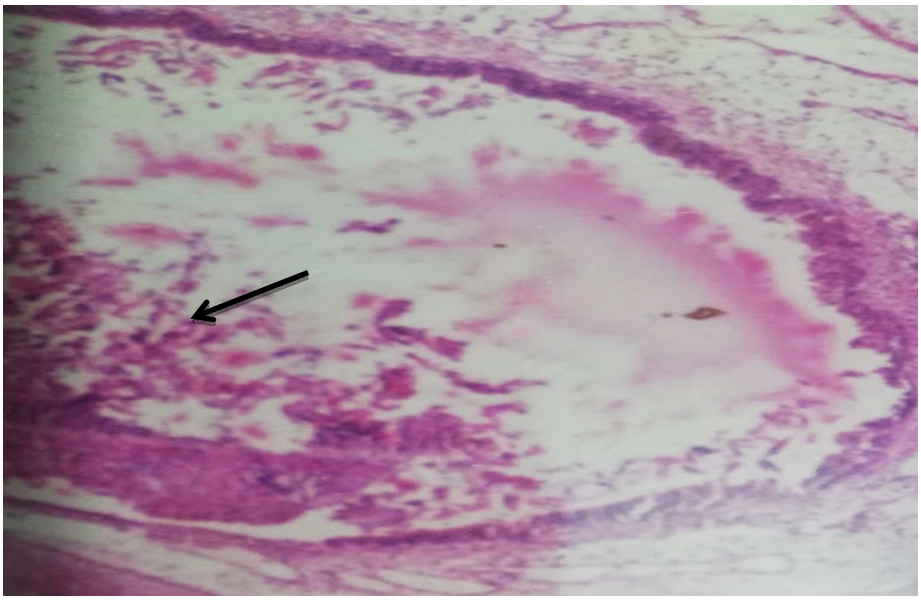

Figure- 3: Histopathological section of rabbit treachea (I/T-14days); showedsuppurativetrachietis, PMNs infiltration and necrotic epithelial debris, (H&E, stain 10X)

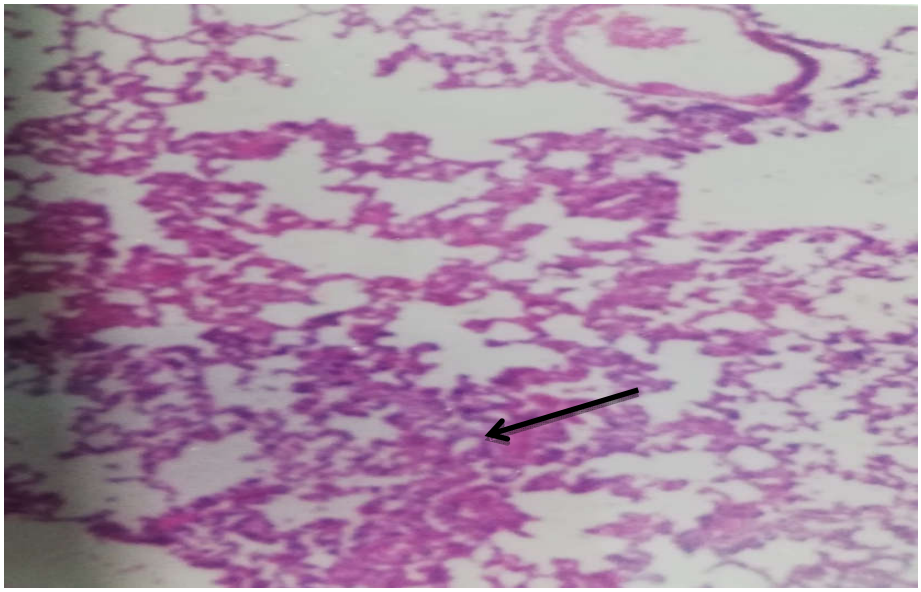

Figure-4: Histopathological section of rabbit lung (I/T-1day); characterized by thickening of alveolar wall, infiltration of neutrophils cells, congestion of blood vessels with emphysema (H&E, stain 4X).

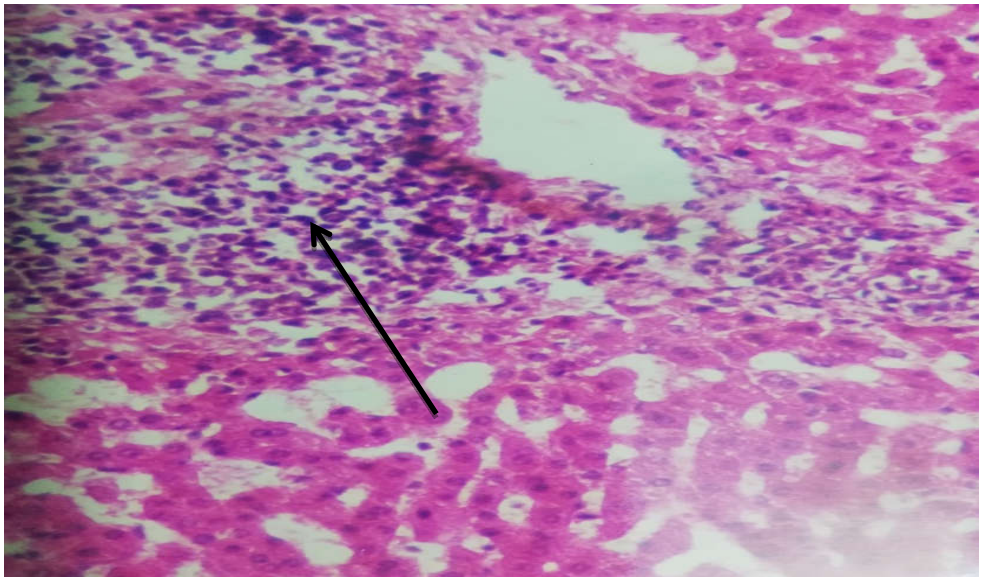

Figure- 5: Histopathological section of rabbit liver (I/T-14days);suppurativehepatitis, infiltration of (PMNs) cells, mainly neutrophils in the liver parenchyma (H&Estain, 20X).

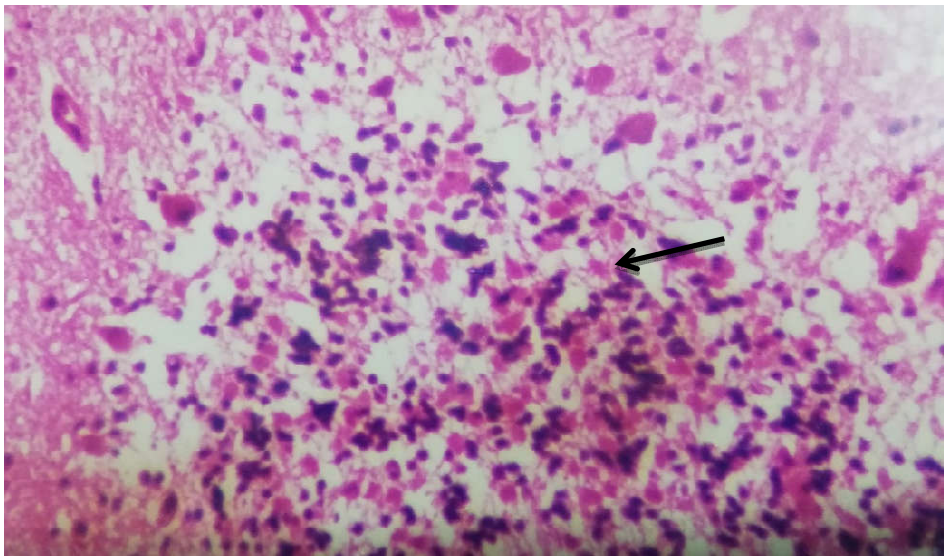

Figure-6: Histopathological section of rabbit cerebellum(I/T-1day); characterized by chromatolysis of perkunji cells,(H&Estain,10X).

DISCUSSION

The body weight along the period of the study which showed a significant decrease ($P<0.05$)was due to systemic reaction against the bacterial infection and it toxins on enteral organs which confirmed by positive isolation of bacteria and the effect of bacterial toxin on the digestive system which effect the absorption of food from intestine(16).While the body temperature showed significant increasecomparing with control group due to the Neutrophiles ability to produce endogenous pyrogenes which it is responsible to increase the body temperature or due to the effect of bacteria(causing lesions) on hypothalamus it is the part of brain responsible to regulate body temperature lead to increase the body temperature (17).the clinical sings that experiment animals show (chock & cough for a while after injection, difficulty in breathing) it is consider as normal reaction to the irritant injected material .on the other hand, the death of animals occur due to the lethal toxin effect of *Staphylococcus aureus*which it kill with in (2-24 h) (18).However, the congestion of some organs was due to bacterial toxins harmful effects on endothelial blood vessels while lead to cause disturbance in the permeability of these blood vessels(19,20).

The typical inflammation response to bacterial infection of *Staphylococcus aureus*wascharacterized byaccumulation of inflammatory cells at different part of the organ mainly (neutrophils, macrophage and lymphocytes) which lead to abcessation or suppurative inflammationat different organs such as trachea, heart, lung, liver, kidney andbrain(21,22) with degeneration changes(19).

The*Staphylococcus aureus*bacteria were isolated along the hall period of experiment from blood, as trachea, heart, lung, liver, kidney and brain,may due to the distribution of bacteria via the blood stream (bacteremia)(23, 17).

CONCLUSIONS:

This study proved that the rabbit is a good laboratory animal for specific *Staphylococcus aureus* tests, the clinical signs and histopathological changes (grossly and microscopically) were variable in different internal organs. The bacteria label technic was suitable way to study the same bacteria without mixing with others from same type.

REFERENCES

1. Malachowa N, Kobayashi SD, Porter AR, Braughton KR, Scott DP, Gardner DJ, et al. (2016) Contribution of *Staphylococcus aureus* Coagulases and Clumping Factor A to Abscess Formation in a Rabbit Model of Skin and Soft Tissue Infection. PLoS ONE 11(6): e0158293.

2. Corpa,K.Hermans,E.Valencia,J.M.(2009).Main pathologies associated with Staphylo coccus aureusinfections in rabbits: Vol 17, No 3 .

3. Selva L, D Viana, JR Penadés, JM Corpa, G Xicato, ATrocino and SD Lukefahr, 2008. *Staphylococcusaureus*nasal carriage in rabbits. In: Proceedings of the 9th World Rabbit Congress, 10-13 June, Verona,Italy, pp: 1079-1084

4. Corpa JM, K Hermans and F Haesebrouck, 2010. Main pathologies associated with *Staphylococcus aureus*infections in rabbits: A review. World Rabbit Sci, 17:110-115

5. Hinton M, 1977. Treatment of purulent staphylococcal conjunctivitis in rabbits with autogenous vaccine.Lab Anim, 11: 163-164.

6. Cucarella C., Tormo M.A., Ubeda C., Trotonda M.P., Monzon M.,Peris C., Amorena B., Lasa I., Penades J.R. 2004. Role ofbiofilm-associated protein bap in the pathogenesis of bovine*Staphylococcus aureus*. *Infect. Immun., 72: 2177-2185.doi:10.1128/IAI.72.4.2177-2185.2004*

7. Blair J, 2013. Bumblefoot: A Comparison of Clinical Presentation and Treatment of Pododermatitis inRabbits, Rodents and Birds. Vet Clin North Am ExotAnimPract, 16: 715-735.

8.Vancraeynest D. 2006.International dissemination of a high virulence rabbit *Staphylococcus aureus*clone.. J. Vet. Med. B Infect. Dis. Vet. Public Health. ;53:418–422

9. Corpa J.M., Hermans K., Haesebrouck F.F. 2009. Main pathologies associated to *Staphylococcus aureus*infections in rabbits: A review. *World Rabbit Sci., 17: 115-125. doi:10.4995/wrs.2009.65*

10. Hermans K., De Herdt P., Devriese L.A., Godard C., Haesebrouck F. 2000. Colonisation of rabbits with *Staphylococcus aureus*after experimental infection with high and low virulencestrains. *Vet. Microbiol., 72: 277-284.*

11.Viana D., Selva L., Segura P., Penades J.R., Corpa J.M. 2007.Genotypic characterization of *Staphylococcus aureus*strainsisolated from rabbit lesions. *Vet. Microbiol., 121: 288-298*

12.Viana, D., Selva, L., Callanan, J. J., Guerrero, I., Ferrian, J., and Corpa, J. M.(2011). Strains of Staphylococcus aureus and pathology associated with chronic suppurative mastitis in rabbits. Vet. J. 190, 403–407.

13. Hafes ,E.S.E.(1970). Reproduction and breeding Techniques of Labortaory Animals.Lea and Febiges Philadelphia

14.Al shabaniZainab J.w.1996.Study of Pathological effect of*Staphylococcus aureus* in rabbits.Msc. thesis. College of veterinary medicine/ University of Baghdad

15.Luna, L. G. (1993**).** Manual of histologic staining methods of the Armed Forces Institute of Pathology.3thed., Mcgraw-Hill, NewYork

16.Emsile,K.R. and Nade,S.1983.Acute hematogenous*Staphylococc*al osteomyelitis.Am.J.Pathol.110:333-345.

17. Kobayashi SD, Malachowa N, DeLeo FR.2015. Pathogenesis of *Staphylococcus aureus*abscesses. Am J Pathol.;185: 1518–27.

18.Merchant,L.A.and Parker,R.A.1967:In "Veterinary bacteriology &virology,7th ed.PP.120-124.The Iowa state uni.press

19.Edelweijin,Z.Jeljaszewicz,J.Wadstron,T.Mollby,R.and Pulverer, G. (1976).Influance of *Staphylococcus aureus* alpha-hemolysin on cortical optical evoked potentiols in "*Staphylococc*i &*Staphylococc*al Disease".J .Jeljaszewicz,ed.PP.747-754,Karger,Basle

20.Landrum ML, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, *et al*. 2012. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005–2010. JAMA.;308: 50–9.

21.Adlam,C.;Thorley,C.M.;Ward,P.;Collins,M.;Lucken,R.N.andKnight,P.A.;1976.Naturala nd experimental *Staphylococcal mastitis in rabbits.J.Comp.path.86:581-593.*

22. Lowy, F.D. 1998. Staphylococcus aureus Infections. N. Engl .J .Med.;339: 520–532.

23.DeMaria and Frank A. Kapral .1978. Pulmonary Infection of Mice with *Staphylococcus aureus*.Infect Immun. Jul; 21(1): 114–123