

## **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 ( $1 \times 10^8$  CFU/ml) of a Rifampicin-resistant *S.aureus* was given through the following routes: Intratracheal (IT), and sterile 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, multisystemic abscessation 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 existing rabbits and 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<sup>3</sup> dimensions in average of three rats in each cage one month before study for acclimatization in optimum conditions of breeding at (22±3) °C 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<sup>8</sup> CFU/ml of a Rifampicin-resistant *S. aureus* Intratracheal (I/T), and *control group* (n=3) with sterile distilled water was given by same route.

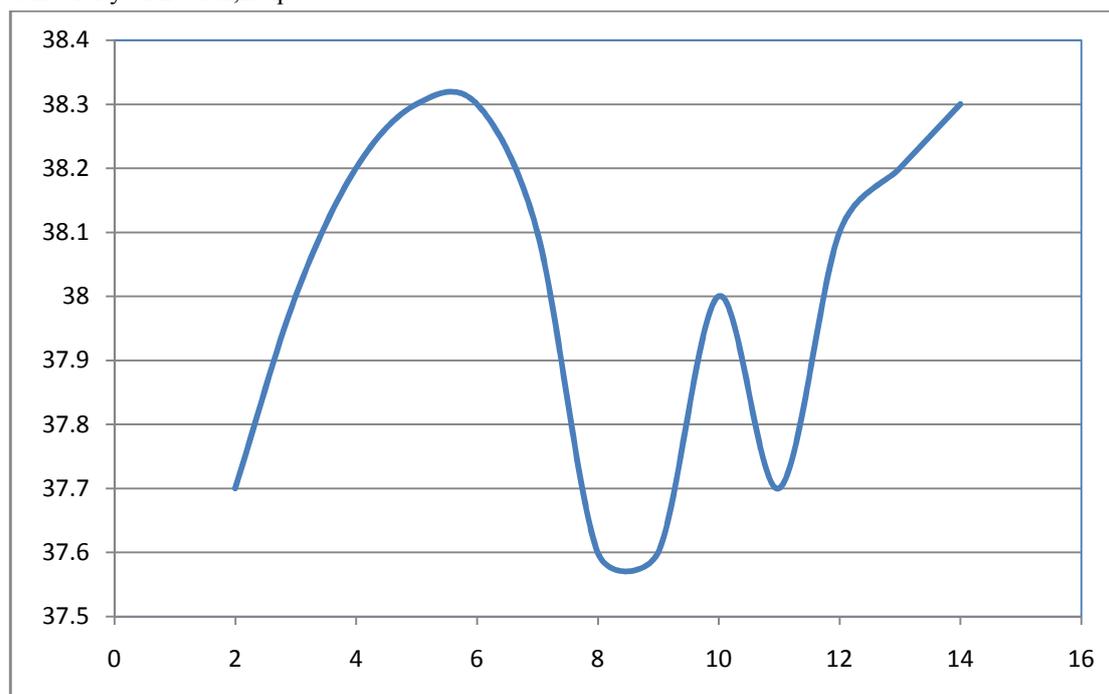
The animals sacrificed at the end of experiment post infection (1, 3,7,14days), after post-mortem examination the tissue specimens were preserved in formalin 10% to prepare hematoxyline-eosin tissue slides (15).

## **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) in infected 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 Gross appearance:**

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

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

## **3-Histopathologic examination:**

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

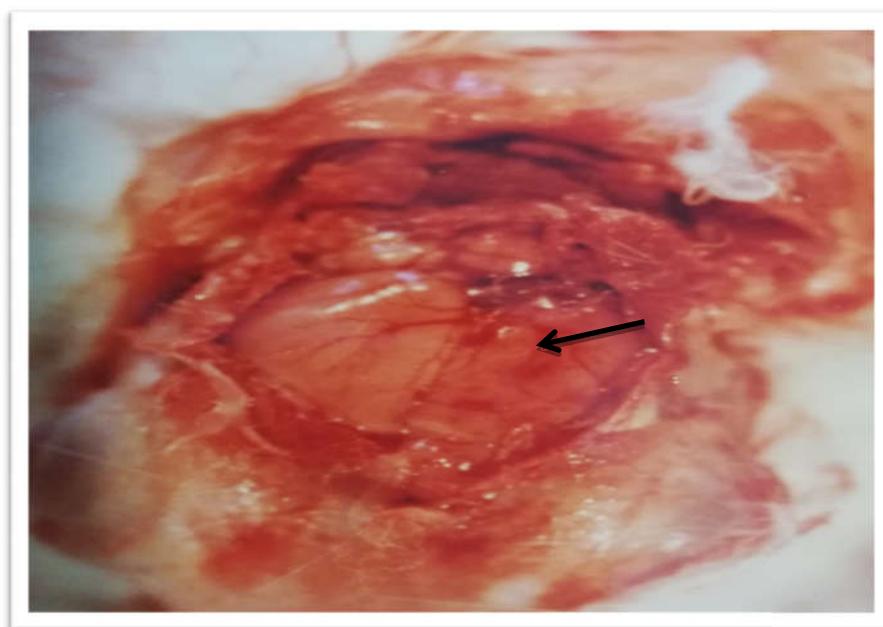
The **liver** in all infected animals were characterized by hemorrhage, congestion and vacuolar degeneration of hepatocytes; different in severity with focal areas of (PMNS) accumulation especially neutrophils(**Figure-5**)

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**).

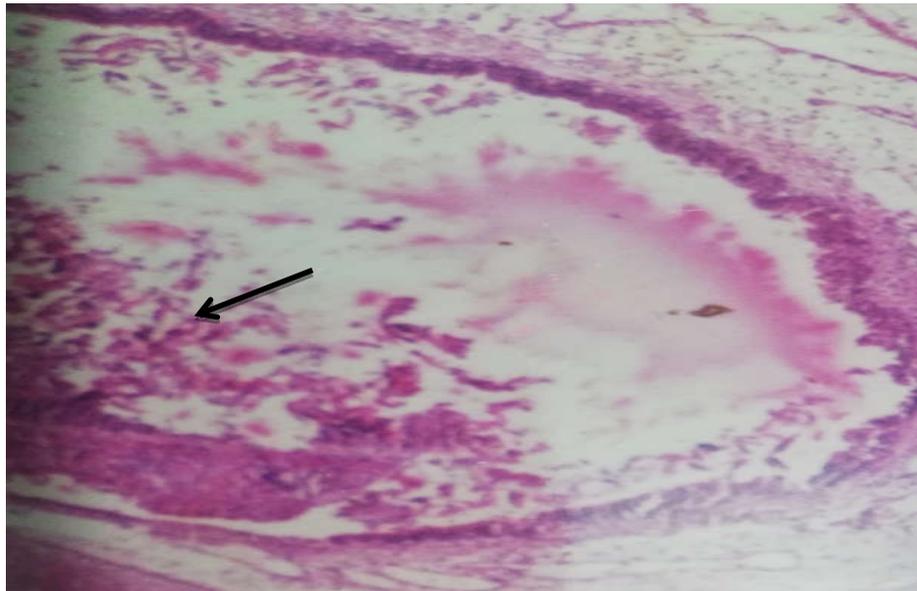
Cloudy swelling of lining epithelia of the **renal tubules** appeared in star- shaped with some necrotic tubules, dilation of bowman's space with mild infiltration of inflammatory cells especially neutrophils and its severity continuous along the experimental periods.

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

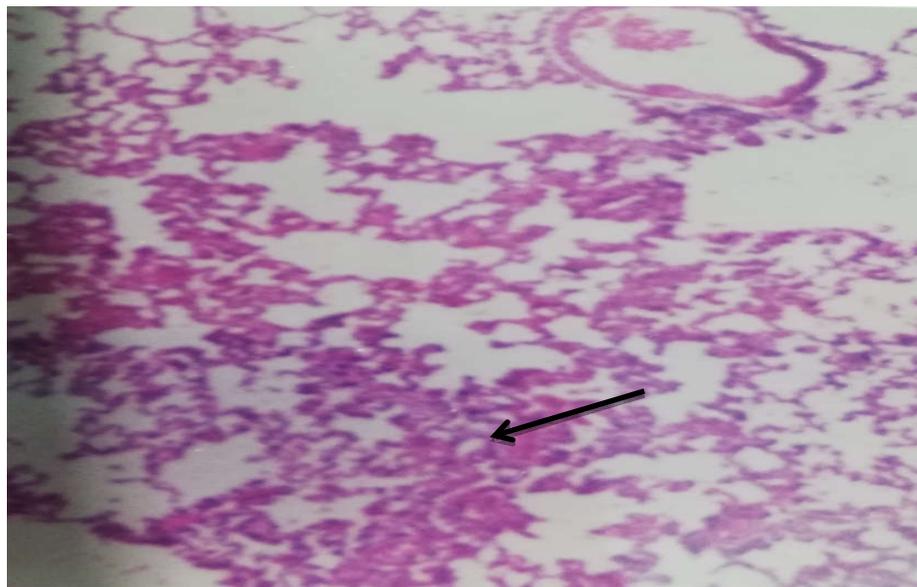
Marked Rifampicin-resistant *Staphylococcus aureus* strain used in all the inoculated animals was recovered and identified permanently from the samples taken which included: blood, heart, lung, liver, kidney and brain.



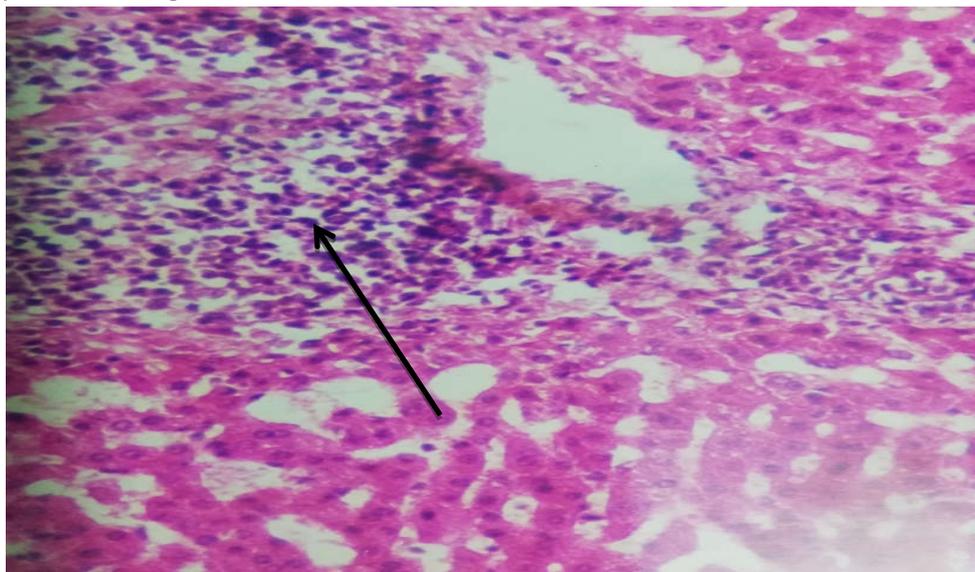
**Figure- 2: rabbit brain of (I/T- 3 days); congestion blood vessels and enlargement of the brain.**



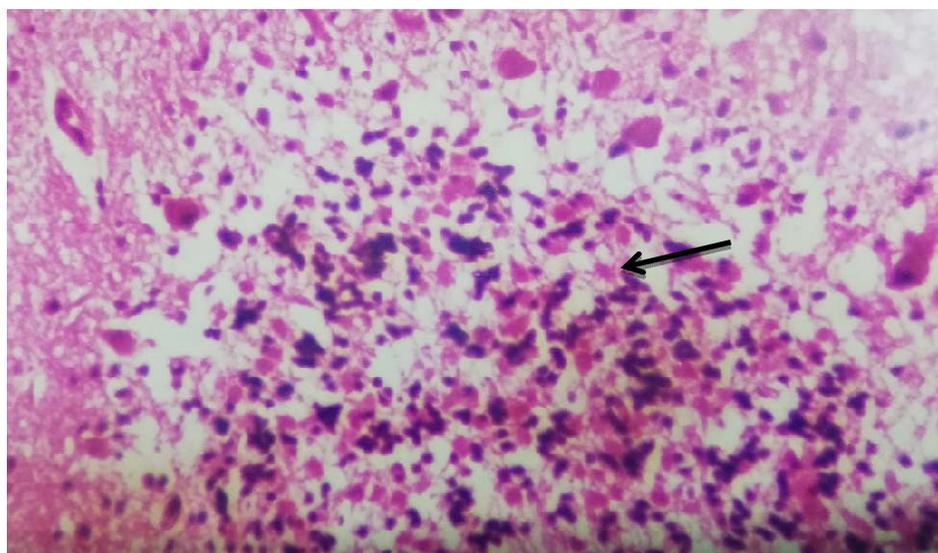
**Figure- 3:** Histopathological section of rabbit trachea (I/T-14days); showed suppurative tracheitis, PMNs infiltration and necrotic epithelial debris, (H&E, stain 10X)



**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); suppurative hepatitis, infiltration of (PMNs) cells, mainly neutrophils in the liver parenchyma (H&E stain, 20X).



**Figure-6:** Histopathological section of rabbit cerebellum (I/T-1day); characterized by chromatolysis of Purkinje cells, (H&E stain, 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 its toxins on enteral organs which confirmed by positive isolation of bacteria and the effect of bacterial toxin on the digestive system which affects the absorption of food from the intestine (16). While the body temperature showed significant increase comparing with control group due to the Neutrophils ability to produce endogenous pyrogens which it is responsible to increase the body temperature or due to the effect of bacteria (causing lesions) on the hypothalamus, which is the part of the brain responsible to regulate body temperature, leading to increase the body temperature (17). The clinical signs that experiment animals show (chock & cough for a while after injection, difficulty in breathing) it is considered as normal reaction to the irritant injected material. On the other hand, the death of animals occurs due to the lethal toxin effect of *Staphylococcus aureus* which it kills within (2-24 h) (18). However, the congestion of some organs was due to bacterial toxins harmful effects on endothelial blood vessels which lead to cause disturbance in the permeability of these blood vessels (19,20).

The typical inflammation response to bacterial infection of *Staphylococcus aureus* was characterized by accumulation of inflammatory cells at different parts of the organ mainly (neutrophils, macrophages and lymphocytes) which lead to abscessation or suppurative inflammation at different organs such as trachea, heart, lung, liver, kidney and brain (21,22) with degeneration changes (19).

The *Staphylococcus aureus* bacteria were isolated along the whole period of experiment from blood, as trachea, heart, lung, liver, kidney and brain, may be 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 technique was a suitable way to study the same bacteria without mixing with others from the same type.

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