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The Action of Ciprofloxacin on Bacterial Infection Caused by *Staphylococcus epidermidis* on Wounds

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Abstract

Staphylococcus epidermidis is a member of the skin microflora that can develop in hospitals and become a pathogen, especially in immunocompromised patients. Although it has fewer exoenzymes and virulence factors than *Staphylococcus aureus*, it occasionally causes infection. This study aimed to study the effect of *S. epidermidis* on induced hamster wound healing and compare it with ciprofloxacin antibiotics used to treat pathogen bacteria, in addition to studying physiological and some immunological parameters. *S. epidermidis* isolates were taken from Tikrit Teaching Hospital and identified using a VITEK 2 compact system. Hamsters were obtained and kept under typical conditions at the veterinary college and divided into third groups during each stage of the study. Briefly, the induced wounds without contaminated bacteria healed more slowly than those in the contaminated group. The therapy group achieved good outcomes, with wounds healing rapidly. Hematological data showed constant white blood cells (WBCs) in G1, whereas G2 raised WBCs, indicating the presence of pathogen bacteria. The treatment group's WBCs dropped, and red blood cell (RBC) levels remained almost unchanged. Hemoglobin levels improved to normal in the G3, and platelet levels remained stable in the treated group. C-reactive protein (CRP) levels were stable in animals with wounds but increased in contaminated wounds. Medically treated wounds had lower CRP levels. Kidney and liver health indices remained unchanged, and immunoglobulin levels remained within the normal range. The results conclude that pathogen bacteria affect the healing rate of induced wounds, which take longer to cure compared with therapy and non-therapy wounds.

Keywords: *Staphylococcus epidermidis*, wounds, ciprofloxacin, healing, hamsters

Резюме

Staphylococcus epidermidis принадлежи към кожната микрофлора, който може да се развие в болници и да стане патоген, особено при имунокомпрометирани пациенти. Въпреки че има по-малко екзоензими и вирулентни фактори от *Staphylococcus aureus*, понякога причинява инфекция. Това проучване има за цел да проучи ефекта на S. *epidermidis* върху индуцираното зарастване на рани при хамстери и да го сравни с антибиотиците ципрофлоксацин, използвани за лечение на патогенни бактерии, в допълнение към изучаването на физиологични и някои имунологични параметри. Изолатите на *S. epidermidis* са взети от болница за обучение в Тикрит и са идентифицирани с помощта на компактна система VITEK 2. Хамстерите са получени и държани при типични условия във ветеринарния колеж и разделени на трети групи по време на всеки етап от изследването. Накратко, в групата на предизвиканите рани, но без допълнителна инфекция с бактерии, зарастват по-бавно от тези в групата с допълнителна инфекция. Терапевтичната група постигна добри резултати, като раните зарастват бързо. Хематологичните данни показват постоянни бели кръвни клетки (WBC) в G1, докато G2 се повишава броят на белите кръвни клетки, което показва наличието на патогенни бактерии. Белите кръвни клетки в лекуваната група спадат и нивата на червените кръвни клетки (RBC) остават почти непроменени. Нивата на хемоглобина се подобряват до нормални в G3, а нивата на тромбоцитите остават стабилни в третираната група. Нивата на С-реактивния протеин (CRP) са стабилни при животни с рани, но се повишават при замърсени рани. Медицински лекуваните рани имат по-ниски нива на CRP. Индексите на здравето на бъбреците и черния дроб остават непроменени, а нивата на имуноглобулините остават в нормалните граници. Резултатите заключават, че патогенните

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бактерии влияят върху скоростта на заздравяване на индуцираните рани, чието излекуване отнема повече време в сравнение с терапевтичните и нетерапевтичните рани.

Introduction

Staphylococcus is a genus of bacteria that includes several species that are prevalent in the skin, epidermis, and other areas of the body such as *Staphylococcus epidermidis*. One member of the natural skin microflora is *S. epidermidis*. This species has been recognized as a major cause of nosocomial infections connected to artificial materials (catheters, shunts, prosthetic heart valves, cardiac pacemakers, and so on) placed into patients' bodies (Costerton *et al*., 1999). More than 36% of catheter-related bloodstream infections are caused by these bacteria (Sherertz *et al*., 1990). Because of poor hygienic conditions, wound infections are most common in poorer countries. Some of the most common species that cause wound infection comprise *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* (Mertz and Ovington, 1993). Wound healing is a dynamic physiological process that attempts to restore the cutaneous barrier function in response to cutaneous damaging stressors (Leonel *et al*., 2019).

Wound healing is a series of events that occur in a logical attempt to reestablish the wounded tissue's integrity: aggressive, proliferation, and remodeling steps (Kokane *et al*., 2009). After an injury, the inflammatory stage begins with vasoconstriction, which promotes homeostasis and releases inflammatory mediators. Granulation tissue proliferation, mostly created by fibroblasts, and angiogenesis characterize the proliferative phase. Reformulations and improvements in collagen fiber components characterize the remodeling stage, which boosts the tensile strength (Varoglu *et al*., 2010). Healing is performed through the secretion of eicosanoids, prostaglandins, leukotrienes, and Reactive Oxygen Species (ROS) (Houghton *et al*., 2005).

For the treatment of wound infections in laboratory animals, several antibiotics are used (Aftab *et al*., 2014). Because of the huge widespread use of antibiotics in the treatment of infected wounds in recent years, pathogen bacteria have become more resistant to chemotherapy, ciprofloxacin antibiotic, a chemotherapy agent still works against pathogen bacteria.

S. epidermidis is a common nosocomial infection that occurs in immunocompromised patients and animals, but it is usually a commensal dweller in healthy human skin and mucosa tissue of animals. *S. epidermidis* has developed unusual methods to establish the hospital environment as a novel ecological niche and become a notorious pathogen while living on the brink of commensalism and pathogenicity (Ziebuhr *et al*., 2006). Although the isolate has less exoenzymes and virulence factors than *S. aureus* sometimes causes infection, the current study examines the impact of *S. epidermidis* on wound healing activity and compares it with ciprofloxacin antibiotic results in laboratory hamsters through tests on blood parameters, including the some complete blood count, some liver enzymes, urea, creatinine, and some immunoglobulins.

Materials and Methods

Isolation of pathogen S. epidermidis

The isolates of *S. epidermidis* were obtained from the Teaching Hospital of Tikrit, Iraq. Twenty-two isolates of *S. epidermidis* were obtained from 72 inflamed human wound samples after conforming to the identification through macroscopic and microscopic characterization on blood agar, mannitol salt agar, and MacConkey agar, at condition 37 ºC for 16-24 hours, as well as some biochemical tests (Schleifer and Bell, 2009). In addition, for further confirmation, the VITEK 2 compact system (BIOMÉRIEUX. USA) was used.

Influence of ciprofloxacin antibiotics on pathogen bacteria

The inhibitory effect of ciprofloxacin antibiotic on Mueller Hinton agar plates was tested using the disk diffusion method. The antibiotic concentration employed was determined to be 10 µg/mL. Using a sterilized cotton swab, 0.1 ml of fresh *S. epidermidis* suspension (1.5×10^8 CFU/mL) from a log-phase bacterial culture obtained after overnight cultivation on nutrient broth media was spread. The inhibitory zone diameter was then measured (Lehrer *et al*., 1991).

In vivo assay

Animals were obtained from the Veterinary College at Tikrit University when they were 4–5 months old and weighed between 210 and 230 mg. They were divided into three groups and housed in polypropylene cages.

Laboratory hamsters were kept in standardized laboratory conditions throughout the study, with air circulation and a constant temperature of 27±3°C according to the international guiding principles for biomedical research involving animals

developed by the Council for International Organizations of Medical Sciences (CIOMS) (Giles, 1987). Kondrup *et al.* (2003), provided guidelines for the diet. We employed nine laboratory hamsters in this study. Three groups of animals were kept. As repeated, each group had three hamsters. G1: Wounds are only used on control animals. G2: *S. epidermidis*-infected wounds in animals. G3: *S. epidermidis* infection of animal wounds followed by treatment with the antibiotic ciprofloxacin (PHARMADA ILAC A.S. Turkey).

The wounds were made on the animal's back near the end of its dorsal region after anesthetizing the animal with a chemical agent (Isoflurane). The sterilized operating blades were used to create two symmetrically aligned thick skin incisions with a diameter of approximately 6 mm. Each animal's dorsal end created both wounds. In the dorsal end of the hamster, 6 mm diameter skin wounds at the subcutaneous fat level were formed (Leitzel *et al*., 1985).

The daily dose for wounds with infection was administered until the pus inflammation had been shown. Using ciprofloxacin at 10 µg/mL as a cure. Fifty µL of ciprofloxacin solution was taken and put on the infected wounds.

Healing time

The healing time was considered to be healed when no scab was clear and full epithelialization had occurred (Leitzel *et al*., 1985). At the beginning and end of the experiment, 2 mL of blood was taken from a vein and maintained in two tubes (EDTA anticoagulant, without EDTA for serum collection) by special veterinarians for some blood and serum tests. The auto-analyzer Hematology (CBC Mindray BC-3000Plus, China) was used to screen WBCs, RBCs, hemoglobin Hb, and platelet counts. In addition to the serum parameters that were studied: CRP, glutamic pyruvic transaminase GPT, glutamic oxaloacetic transaminase GOT, B. urea, and creatinine. Finally, IgA and IgG immunoglobulin antibodies were also tested using the ELISA technique.

Analytical statistics

The data were analyzed using Bonferroni's two-way ANOVA test (GraphPad Prism 7). The significance probability value was 0.05.

Results and Discussion

Isolation of S. epidermidis

The isolates are capable of producing small pink or red colonies with no colour change to the medium. These isolates are referred to as *S. epidermidis* on mannitol salt agar. While, *S. epidermidis*, forms white, raised, cohesive colonies approximately 1–2 mm in diameter after overnight incubation and is not haemolytic on blood agar as shown in Fig. 1.

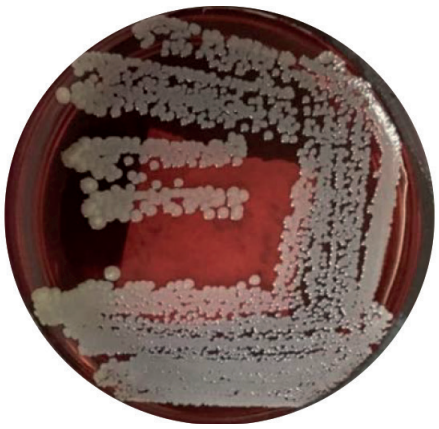

Fig. 1. *S. epidermidis* on blood agar plate.
A single isolate colony was streaked on the surface of blood agar and incubated for 18 hours at 37°C.

Ciprofloxacin susceptible

After 20 h of incubation, twenty-two isolates were examined for resistance to ciprofloxacin antibiotics, as indicated in Fig. 2. Susceptibility of *S. epidermidis* to antibiotics ranged from resistant to intermediate and sensitive, with ten isolates showing sensitivity which appeared at a high rate at 32 mm, while the other isolates were divided between two isolates intermediate to antibiotics and 10 isolates resistant. Many factors contribute to antibiotic resistance in isolates, as published by Suood *et al*. (2021) who reviewed the problem of resistance in some topics of his paper. The species of *S. epidermidis* widely spread on skin and other areas of skin such as sweat glands. The inflammation of the epidermis and dermis comes from the pathogenic bacteria *S. epidermidis*. The widespread use of sup-lethal concentrations of ciprofloxacin antibiotics has revealed some isolates that can resist antibiotic therapy.

Many factors play essential roles in the development of pathogen bacteria to fierce bacteria (Tsang, 2017). In the last decade, *S. epidermidis* became the third most common chronic non-health wound infection after *S. aureus* and *P. aeruginosa* (Watters *et al*., 2015). The activity of ciprofloxacin antibiotics is still active against various pathogen bacteria, except in the current study where some *S. epidermidis* isolated from chronic wounds showed resistance to antibiotics. This may be a result of the isolates having some plasmid genes that are resistant to the studied antibiotics.

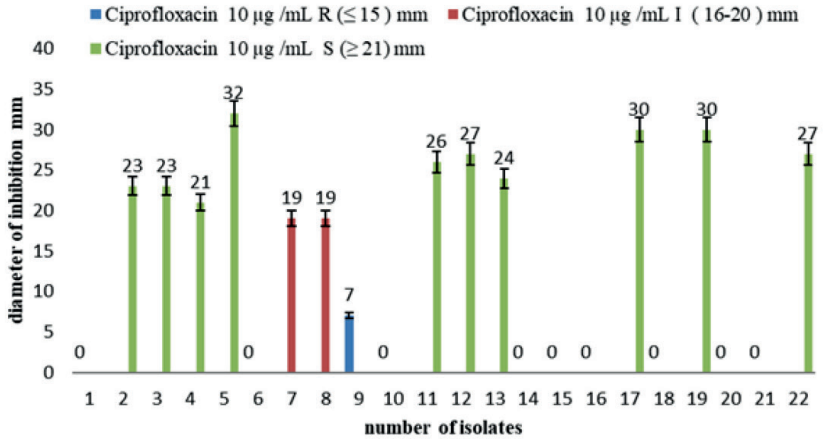

Fig. 2. Activity of ciprofloxacin against the studied *S. epidermidis* isolates

In vivo results

S. epidermidis number 5 was chosen because it exhibited a high rate of susceptibility to ciprofloxacin antibiotics.

Morphological wound healing

The results of the wound injury without infection and treatment showed natural healing with intermediate time in G1, the time healing depended on the activity of animal immunity. The healing wound in G2 was slowly healing due to infection using the pathogen bacteria. While in G3 showed perfect morphology with completed healing for induced wounds as shown in Fig. 3.

Wound healing is a complicated physiological process, which is a natural biological process in the human body and occurs in four distinct and highly organized stages: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal properly, all four phases must occur in the correct order and period. Many variables can disrupt one or more stages of this process, resulting in inappropriate or compromised wound healing. One of them is pathogen bacteria, which can express virulence factors and surface proteins that affect wound healing (Hasamnis *et al*., 2010).

Some hematological parameters.

The hematological assay gives a clear view of the health of the animal. As shown in G1 (Table 1) the count of leukocytes has been stable due to there being no infection therefore the count of WBCs doesn't change compared with the same group before the procedure, but the G2 when comparing the normal level of WBCs with infected wound noted there is increased in the level of WBCs that results from the infection of wounds (Suood *et al*., 2021).

The increase in WBC count was caused by inflammation in the body of animals, which is common after bacterial infection. The host's immune response acts as a line of protection against bacterial invasion. The increase in leukocytes has been attributed to the emergence of a severe bacterial infection, which prompted the immune system to produce defense cells. These cells play an important role in the body's immunity because of their ability to release immunological chemicals that destroy pathogens (Stites, 1987). After treatment, the wound that was contaminated with pathogen bacteria using antibiotics noticed a decreased level of WBCs compared with the results of G2. The lack of significance in WBC counts in *S. epidermidis*-infected animal groups treated with ciprofloxacin in G3 is attributable to the drug's successful removal of the bacteria's negative influence and restoration of normal parameter values by destroying their DNA component.

The level of RBCs in G1, G2, and G3 decreased because the bleeding came from induced wounds on the first day of the experiment. Hb is stable in G1. But at G2 the little increase has been noticed. In the treatment group using antibiotics, the level of Hb recorded a significant change due to the activity of antibiotics. RBCs and Hb are all

Table 1. Efficacy of *S. epidermids* on some factors of blood in induced wounds

Groups	WBCs (cells/mm^3)		RBCs (cells/mm^3)		Hb (g/dI)		Platelet (cells/mm^3)	
	Before	After	Before	After	Before	After	Before	After
G1	12.23 a	12.13 a	5.11 a	5.2 a	11.2 a	11.2 a	210 a	312.3 b
G2	12.6 b	14.1c	5.89 c	5.49 c	12.2 b	12.7 b	220 c	280.3 d
G3	12.2 e	13.03 e	5.11 e	5.09 e	11.93 c	12.5 c	215 d	259 d

*G1, 2 and 3 mean group one, two and three.

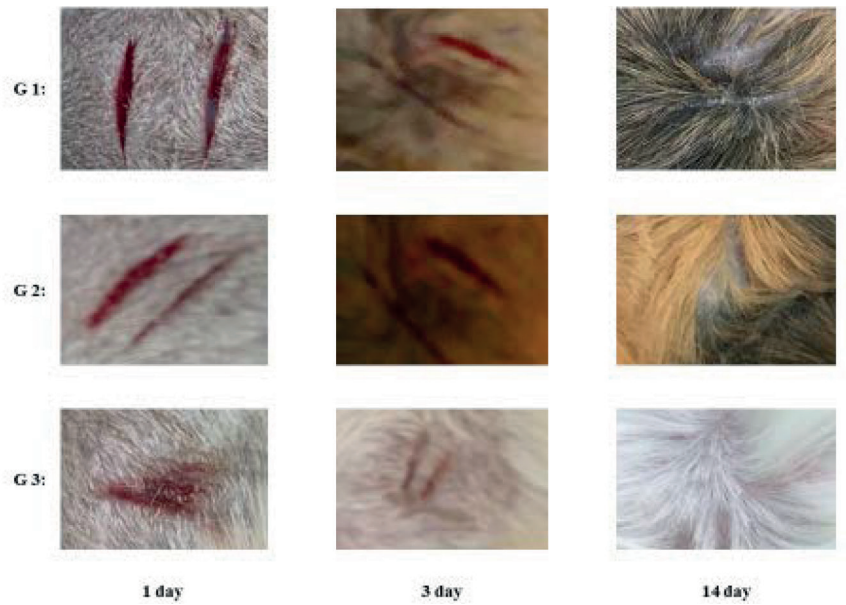

Fig. 3. Ciprofloxacin treat wounds infected with *S. epidermidis* for 14 days.
G1: 14 days for the wounds to heal without infection. G2: wounds infected with *S. epidermidis*. G3: *S. epidermidis*-infected wounds treated with a ciprofloxacin (The pictures at 1 day have shaved, therefore appearing different colors compared with others).

connected parameters, and it appears that the therapies using the study's components had an impact on the parameters' recovery. The platelets have been increased in induced wounds that do not receive treatment. The increase in G2 was small compared with G1. The treatment group with drugs does not show a significant change in the level of platelets. To stop bleeding, platelets, a major component of blood, create clots in blood vessels. As a result of the treatment solution's activity, the infection group's and other platelet levels increased. These healing benefits of ciprofloxacin can be attributed to the effects of their factors on the inhibitory actions of *S. epidermidis*, which resulted in the values returning to normal.

Some serum parameters

The serum parameters are considered indicators of the health of the animal and are therefore used in the test. The CRP is a protein secreted from the liver in the bloodstream at low levels but at high levels, which indicates inflammation in the body of animals (Clyne and Olshaker, 1999). The level in uncontaminated wounds has non-significant change, on the other hand, the level has increased in contaminated wounds that are considered indicators of infection wounds. The treatment on contaminated wound drugs noticed the level of CRP decreased at a lower level, which means there is no indicator of infection. The CRP assay is a measure of the acute phase response, which includes fever, leukocytosis, and changes in acute-phase protein liver synthesis (Black *et al*., 2004).

GPT and GOT are enzymes involved in the transfer of the amino group from the amino acid to alpha-ketoglutaric acid to produce glutamate and pyruvate (Huang *et al*., 2006). These enzymes are located in some organs such as the liver and kidney primarily. GPT increased activity of GPT is more specific for liver damage because of its more limited tissue distribution, therefore measurement of serum GPT concentration is preferable to hepatocyte injury. The control group noticed a stable concentration with change but in contaminated induced wounds with pathogens, the GPT concentration reached 51.33 U/L on the other hand in the treatment group the concentration decreased to 40.66 U/L (Table 2).

The GOT enzymes are less specific than GPT because of their greater distribution in most organs of animals' bodies or humans. Despite GOT test being used as an indicator of the health of the liver and kidney. Also, the value of GOT in the control sample has a non-significant change, whereas little increase is shown in G2. After treatment of the pathogen in the contaminated wound, the value of GOT decreased to 29.33 U/L.

Urea and creatinine are waste products produced during protein metabolism. All these waste products are carried to the kidneys and filtered into the urine. Both these products were measured to evaluate how well the kidney is working. the blood urea concentration in G1 and G2 showed little decrease, while the concentration of blood urea in G3 showed little increase compared with G1 and G2, perhaps due to the physiology of lab animals. The creatinine concentration in the control group showed no significant change but in the contami-

Table 2. Effect of *S. epidermids* on some serum biological parameters

Groups	CRP (mg/dI)		GPT (U/L)		GOT (U/L)		B. Urea (mg/dI)		Creatinine (mg/dI)	
	Before	After	Before	After	Before	After	Before	After	Before	After
G1	8.15a	8.13a	44.66a	44.66a	30a	32a	44a	42.33a	8.15a	8.13a
G2	8.68b	9.47c	45a	51.33b	38.66b	42c	40b	39b	8.68b	9.47c
G3	8.05c	5.76d	43c	40.66d	30c	29.33c	38.66c	41d	8.0c	5.76d

*G1: only wounds from the control group are represented. G2: represents the control group, wounds infected with *S. epidermidis*. G3: Ciprofloxacin is used to treat a wound infected with *S. epidermidis*. The different letters in the two adjacent columns indicate statistically significant differences at the 0.05 level.

nated group showed increasing in creatinine concentration, while in G3, the effect of antibiotics decreased the concentration of creatinine.

IgG and IgA levels

The body of animals produces a few different kinds of immunoglobulin antibodies, immunoglobulin plays an essential role in the immunity of animals. Antibodies are proteins that enable immune cells to fight off bacteria, viruses, and other harmful invaders (Woof and Kerr, 2006). There are five types of antibodies; this study focuses on IgG and IgA antibodies because IgA is the most abundant immunoglobulin in secretions and IgG is the most abundant glycoprotein in the body of animals compared with other immunoglobulins. IgG gets ready to multiply and attack when foreign substances get into the body and their activity lasts a long period after infection. IgA comprises a smaller amount compared with other immunoglobulins in healthy serum, IgA is weak complement-activating antibody. However, secretory IgA works together with lysozymes, which can hydrolyze carbohydrates in bacterial cell walls, thereby enabling the immune system to clear the infection (Macpherson *et al*., 2008). IgA appeared as quickly as the infection was found at a high level, then decreased quickly after a cure. The assay of immunoglobins was tested at 1 and 14 days of the experiment (Table 3).

Table 3. Effect of *S. epidermidis* on IgG and IgA levels in hamsters with experimentally produced lesions infected

Groups	IgG mg/dI		IgA mg/dI	
	Before	After	Before	After
G1	930	930	210	210
G2	951	982	201	196
G3	1007	1004	220	153

*G1: only wounds from the control group are represented. G2: represents the control group, wounds infected with *S. epidermidis*. G3: Ciprofloxacin is used to treat wounds infected with *S. epidermidis*.

The level of IgG in G1 showed no change in its level before and after the experiment. In contaminated group noticed little increase in the level of IgG. On the other hand, in the treatment group with antibiotics, the level of IgG decreased, which can be considered an indicator of the elimination of pathogen bacteria. As well as no level change in the control group of IgA, but G2 and G3 showed a decrease in the level of IgA antibodies, that come may from the removable of the used cure in G3, while in G2 back to less affected the pathogen bacteria.

Conclusions

The findings of the research approved the delay in the nature of healing processing of inducing wounds in studied animals that were contaminated and infected with *S. epidermidis* through the observation of morphological changes when compared with non-contaminated wounds and the treatment of infected wounds using ciprofloxacin antibiotics. The assay of some blood parameters showed an increase in WBCs resulting from pathogen bacteria, whereas other samples showed a decrease in the same cells. The other parameters did not change much. On the other hand, the parameters of serum began with the CRP test, which showed an increase in its number, as indicated on an infected wound, and the last sign of serum showed a slight change. The immunoglobulin IgG level appeared to be increased due to infection in the contaminated group, but the IgA level was decreased.

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