Role of peptidoglycan in the pathogenesis of *Staphylococcus saprophyticus* in mice

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Abstract

The pathogenicity of *S. saprophyticus* was studied in mice. A group of white mice were injected transurethrally using a catheter with *S. saprophyticus* S_{67} cell suspension in a concentration reached 10^9 CFU/ml. concomitantly, the role of its peptidoglycan in the pathogenicity was studied by injecting another group of mice with 0.3 mg/0.2 ml of partially purified *S. saprophyticus* S_{67} peptidoglycan extract. After autopsy, kidneys and urinary bladder showed several histopathological changes both in cells and peptidoglycan injected mice, included: hydropic degeneration, glomerulus shrinkage, congestion of renal vessels, infiltration of inflammatory cells, and dekeratinization in urinary bladder.

Introduction

Staphylococcus saprophyticus is a member of coagulase negative staphylococci group that cause urinary tract infections; cystitis, urethritis and pyelonephritis in young women (1). Also it may cause Bacteremia (2), endophthalmitis (3), wound infection, eczema (4) and respiratory tract infections (5). This bacteria has the ability to produce urease which considered one of the important factor in establishing urinary tract infections (6).

The rigidity of bacterial cell wall is attributed to a macromolecule known as peptidoglycan; a complex polymer composed of alternating series of two major subunits, N-acetylmuramic (NAM) and N-acetylglucosamin (NAG), attached to each NAM a string of tetrapeptide chain, cross linkages can form between those chains (7).

Peptidoglycan extracted from staphylococci and streptococci is known to cause septic shock in lab animals characterized by fever, inflammatory reactions, thrombocytopenia and multiple organ dysfunction syndrome (8,9).

Recently, it was demonstrated that the intranasal administration of peptidoglycan from *S. aureus* to mice resulted in acute pulmonary inflammation (10).

The present study aimed to investigate the role of peptidoglycan extracted from *S. saprophyticus* and its pathogenicity in *vivo*.

Materials and methods

Animals

Nine female Swiss white mice from the animal house of Department of Biology, College of Science, University of Baghdad were used in this study, divided into 3 groups as triplicates.

Isolation

Staphylococcus saprophyticus was isolated as mentioned earlier and the isolate *S. saprophyticus* S_{67} was chosen because of its multi drug resistance capacity (11).

Peptidoglycan extraction

The peptidoglycan of *S. saprophyticus* S_{67} was extracted in accordance to the

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method described by De-Jonge (12), partially purified according to Amako *et al.* (13) and the purity was confirmed by the method of Umeda *et al.* (14).

***In vivo* study**

According to the method described by Mctaggart *et al*. (15) the animals were transurethrally injected via catheter (0.6 mm in diameter) after applying a gentle pressure on animal abdomen in order to empty the urinary bladder from urine. The first group (group A) was injected with 0.2 ml of *S. saprophyticus* cell suspension in a concentration reached 10^9 CFU/ml (phosphate buffer saline pH 7.2), the second group (group B) was injected with 3 mg/0.2 ml of partially purified *S. saprophyticus* S_{67} peptidoglycan extract, while the third group (group C) was injected with 0.2 ml of phosphate buffer saline pH 7.2 and considered as control group.

Four days later, all groups were sacrificed, kidney and urinary bladder were submitted to histopathological study (16).

Results and discussion

***In vivo* study**

1- kidney:

The histopathological study of mice kidney injected with cell suspension of *S. saprophyticus* S_{67} (1 X 10^9 CFU/ml) showed several pathological changes in comparison to control group (figure 1) represented by hydropic degeneration, glomerulus shrinkage, increase the glomerulus interstitial space as illustrated in figure 2., infiltration of inflammatory cells (figure 3).

While kidneys of mice injected with 3 mg/ml of peptidoglycan developed hydropic degeneration, glomerulus shrinkage(figure 4), infiltration of inflammatory cells, increase in glumerular cell size in addition to accumulation of body fluids inside the renal tissue, consequently, led to edema formation (figure 5) and vessel congestion (figure 6).

2- urinary bladder

Figure 7 showed the cross section of urinary bladder of control mice while the urinary bladder of group A showed dekeratinization, necrosis in urothelial layer, in addition to infiltration of inflammatory cells in the lumen (figure 8).

As illustrated in figure 9, similar histopathological changes; dekertinization, necrosis and infiltration of inflammatory cells were the characteristic features of the group B urinary bladder cross section.

Staphylococcus saprophyticus has high affinity to attach to renal cells by means of the extracellular materials (15,17). Also some strains of *S. saprophyticus* produce proteins called surface associated proteins bind to renal cells (17) and slime materials aid in its attachment to eukaryotes (18).Gatermann and his coworkers (17) have pointed out that *S. saprophyticus* can cause necrosis to renal tubules, infiltration of inflammatory cells and urolithiasis.

Staphylococcus saprophyticus attachment to urothelial cells lining the urinary bladder is achieved via oligosaccharides receptors on these cells (4) can result in edema formation, swelling of cells, rupture of intracellular connections leading to dekartinization of urothelial layer and exposing the cells to bacterial colonization and immigration of macrophages and polymorphonuclear cells and other immunocompetent cells (17).

The peptidoglycan polymer was characterized by having many bioactivities, it can activate leukocytes, generate proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, and cause systemic inflammatory response syndrome (18). Peptidoglycan was also shown to mediate platelet aggregation in *Staphylococcus aureus* septicemia, induce tissue factor (TF) in monocytes, display procoagulant activity (19), stimulation of

polymorphonuclear cells, induce mast cells to produce histamine, and increase the permeability of blood vessels (20). Due to its high molecular weight, it has antigenic capacity hence elicit acute and chronic immune response (21).

The present study showed that the peptidoglycan extracted from *S. saprophyticus* has the ability to cause damage to the renal system in mice similar to whole bacteria, therefore it can be concluded that this polymer may play an important role in its pathogenicity.

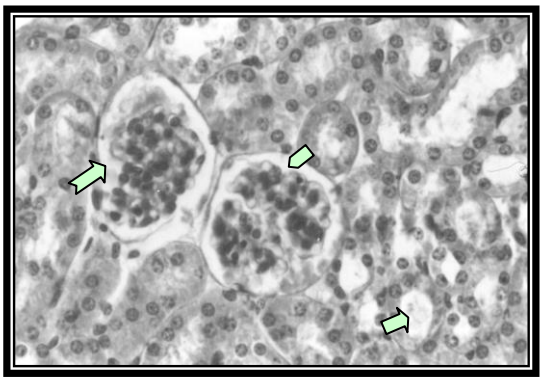

Figure 1: cross section in mouse kidney (control) shows; glumerulus ( ), interstitial space ( ) and the renal tubule ( ) at X400 magnification. H&E.

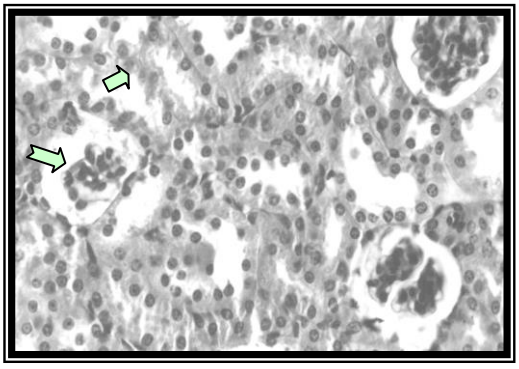

Figure 2: cross section in mice kidney injected with S. saprophyticus (1 x 109 CFU/ml) shows; hydropic degeneration ( ), glumerulus shrinkage and increase of interstitial space ( ) at X400 magnification. H&E

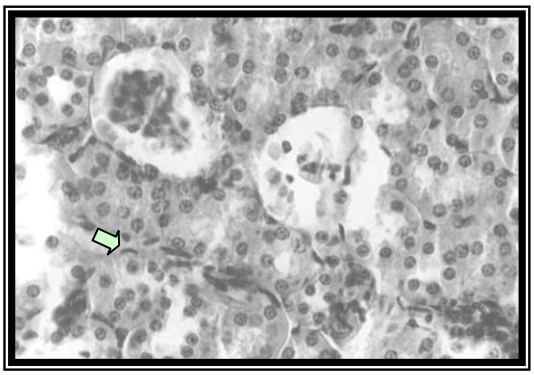

Figure 3: cross section in mice kidney injected with S. saprophyticus (1 x 109 CFU/ml) shows the infiltration of inflammatory cells ( ) at X400 magnification. H&E

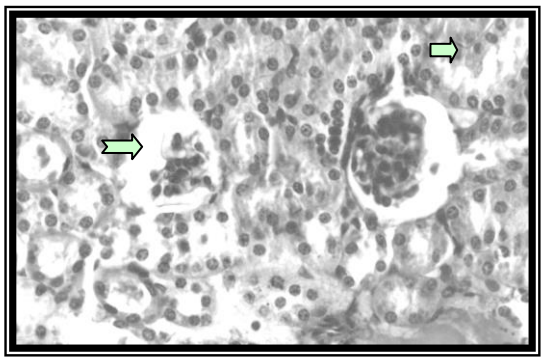

Figure 4: cross section in mice kidney injected with S. saprophyticus peptidoglycan (3 mg/ml) shows; hydropic degeneration ( ), glumerulus shrinkage and increase of interstitial space ( ) at X400 magnification. H&E

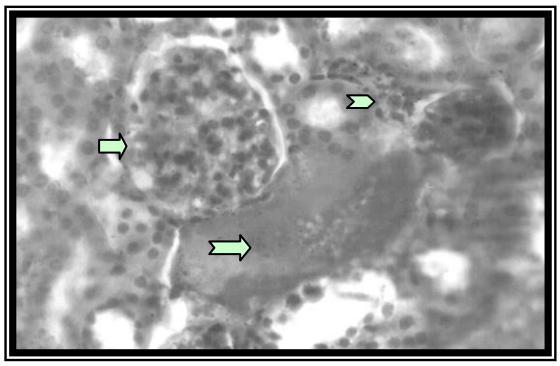

Figure 5: cross section in mice kidney injected with S. saprophyticus peptidoglycan(3 mg/ml) shows; infiltration of inflammatory cells ( ), edema ( ) and increase in glumerulus size ( ) at X400 magnification. H&E

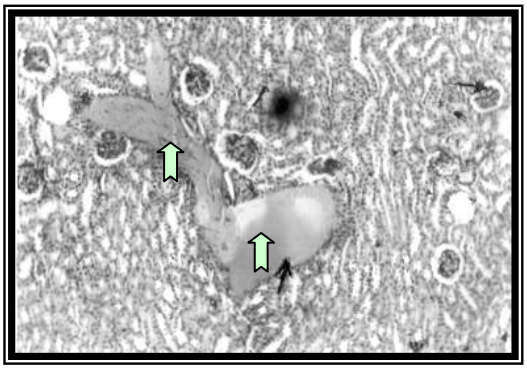

Figure 6: cross section in mice kidney injected with S. saprophyticus peptidoglycan (3 mg/ml) shows; congestion of blood vessel (⇨) at X100 magnification. H&E

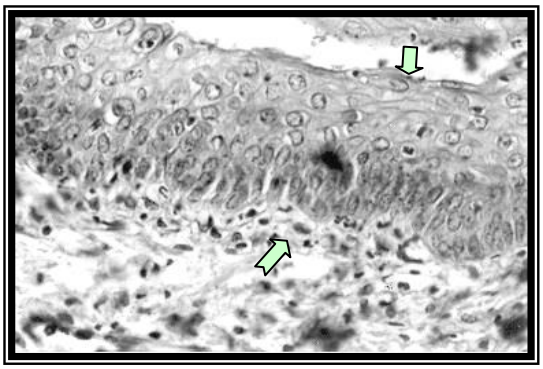

Figure 7: cross section in urinary bladder of mice (control) showed the urothelial layer (⇨) and submucosa layer (⇨) at X 400 magnification. H&E

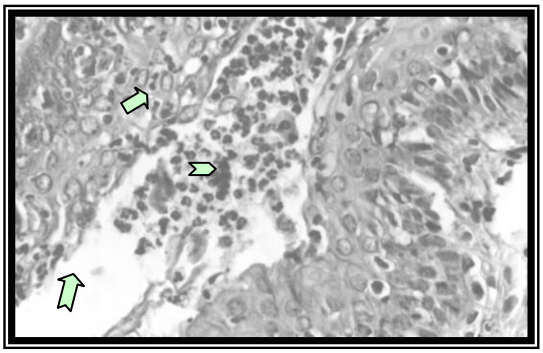

Figure 8: cross section in mice urinary bladder injected with S. saprophyticus (1 x 109 CFU/ml) shows the infiltration of inflammatory cells (⇨), necrosis (⇨) and dekertinization (⇨) at X400 magnification. H&E

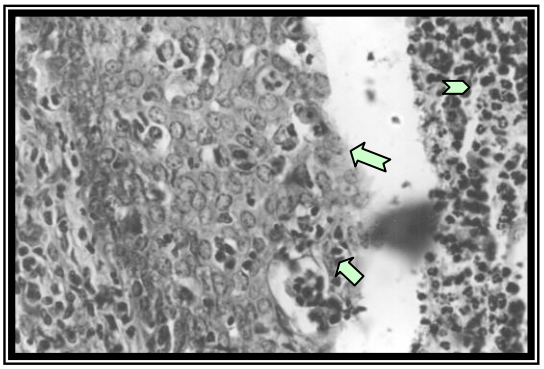

Figure 9: cross section in mice urinary bladder injected with S. saprophyticus peptidoglycan (3 mg/ml) shows the infiltration of inflammatory cells (⇨), necrosis (⇨) and dekertinization (⇨) at X400 magnification. H&E

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دور الببتيدوكلايكان في امراضية *Staphylococcus saprophyticus* في الفئران

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الخلاصة:

درست امراضية بكتريا *S. saprophyticus* في الفئران فقد تم حقن مجموعة من الفئران الاناث البيض عبر الاحليل باستخدام قثطرة بعالق بكتريا *S. saprophyticus* S_{67} و بتركيز 10^9 CFU/ml. كما درس دور البتيدوكلايكان العائد لها في الامراضية وذلك بحقن 0.3 mg/0.2 ml من هذا المستخلص المنقى جزئيا في مجموعة اخرى من الفئران. بعد التشريح اظهرت الكليتان والمثانة عدة تغييرات مرضية نسيجية سواء في الفئران المحقونة بعالق البكتريا او بالببتيدوكلايكان وقد تضمنت هذه التغييرات: تنكس استسقائي و انكماش الكبيبة و احتقان الاوعية الدموية و ارتشاح الخلايا الالتهابية و فقدان طبقة الكيراتين في المثانة.