INFLAMMATORY EFFECT OF ANTIBIOTIC-KILLE STAPHYLOCOCCUS XYLOSUS ON MURINE RENAL SYSTEM

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Abstract

One hundred and fifty urine specimens were collected from patients with urinary tract infection, visiting Alyarmouk, Alkarama, and Madinat Altib hospitals in Baghdad. *Staphylococci* were isolated from 51 urine specimens, 39 isolates were coagulase-negative *Staphylococci*. Seven isolates (4.5%) were identified as *Staphylococcus xylosus*. Most of isolates are multiresistant to more than one antibiotic; all the isolates were susceptible to ciprofloxacin, and resistant to erythromycin, the isolate *S. xylosus S4* was elected because of its susceptibility to more than one antibiotic. In order to testify the pathogenicity of antibiotics-killed *S. xylosus S4* in murine urinary tract system, mice were injected with *S. xylosus* S4 supernatant which previously exposed to Ampicillin, Cefotaxime, Gentamicin, Rifampin, Erythromycin, Co-Trimoxazole, or Ciprofloxacin at concentration of 200, 600, 200, 100, 300, 100, 500 µg / 0.2 ml respectively via intraurethral catheter. Organs of mice (kidneys and bladders) treated with beta-lactam-killed *S. xylosus* S4 showed different pathological changes in kidneys included infiltration of inflammatory cells, haemorrhage and vaculation of blood vessels, whereas the bladders developed dekeratinazation and infiltration of inflammatory cells. However, kidneys and bladders maintained normal state after exposure to supernatant of *S. xylosus S4* with antibiotics other than beta-lactam.

التاثير الالتهابي لبكتريا Staphylococcus xylosus المقتولة بمضادات الحياة في الجهاز البولي للفئران

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الخلاصة

جمعت 150 عينة ادرار من مرضى مصابين بخمج المجاري البولية من مستشفيات اليرموك والكرامة ومدينة الطب في مدينة بغداد .اذ تم عزل وتشخيص 51 عزلة تابعة لجنس المكورات العنقودية، وتم اثبات عائدية 39 عزلة الى المكورات العنقودية السالبة لاختبار الكواكيوليز ، وكانت 7 عزلات منها تابعة للنوع *S. xylosus* ، كما تم اختبار حساسية عزلات *S. xylosus* لمجموعة منتخبة من مضادات الحياة ، حيث ابدت معظم العزلات مقاومة متعددة لاغلب المضادات المستعملة ، وكانت العزلات جميعها حساسة للمضاد الحياتي Ciprofloxacin ، ومقاومة للاريثرومايسين. وقد انتخبت العزلة S4 *S. xylosus* لكونها العزلة الحساسة لاكثر من مضاد ، ولمعرفة التأثير المرضي لهذه العزلة المقتولة بمضادات الحياة في الجهاز البولي للفئران ، حقنت الفئران براشح S4 *S. xylosus* المعرضة مسبقا لمضادات الحياة Ampicillin ، و Cefotaxime ، و Gentamicin ، و Rifampin ، و Erythromycin ، و Co–Trimoxazole ، و Ciprofloxacin وبالتراكيز : 200 ، 600 ، 200 ، 100 ، 300 ، 100 ، 500 مايكروغرام/ 0.2 مليلتر على التوالي ،

حيث حقنت الفئران عن طريق الاحليل وباستخدام قثطرة بولية . اظهرت الاعضاء الكلى والمثانة للفئران المحقونة براشح *S. xylosus* S4 والمعرضة مسبقا لمضادات الحياة التي تثبط بناء الجدار الخلوي Ampicillin , Cefotaxime تغييرات نسيجية ومرضية واضحة في الكلى تمثلت بارتشاح الخلايا الالتهابية ، والنزف ، وظهور الوذمة ، وتفجي الوعاء الدموي. والتغييرات النسيجية للمثانة تمثلت بفقدان الطبقة الشمعية ، وارتشاح الخلايا الالتهابية . في حين حافظت الكلى والمثانة على الحالة الطبيعية لها عند حقن الفئران براشح بكتريا *S. xylosus* S4 والمعرضة مسبقا لمجموعة مضادات الحياة الاخرى غير β–lactam .

Introduction

The genus *Staphylococcus* has at least 35 species. The coagulase-negative staphylococci -(CoNS) are normal human flora and sometimes cause infection, often associated with implanted appliances and devices, especially in very young, old, and immunocompromised patients. Approximately 75% of these infections caused by coagulase-negative staphylococci [1].

Staphylococcus xylosus is a Gram positive bacterium with a low G + C content. It belongs to the coagulase-negative group of staphylococci. It is a commensal bacterium of the skin which is of major interest for several reasons [2,3]. *Staphylococcus xylosus* are able to form biofilm on both hydrophilic and hydrophobic surfaces [4].

Unlike *S. saprophyticus* less attention was paid toward *S. xylosus* as a causing agent of UTI since its incidence was around 1%. However, in local previous studies this bacteria was isolated with relatively high percentage from Iraqi patients presented with UTI [5,6,7]. What's more, this bacteria was able to colonize the kidney and the urinary bladder of mice when injected intraurethrally [8].

Tawfiq [6] found that the peptidoglycan (PG) extracted from *S. xylosus* caused damaged to murine renal system. van Langevelde *et al.* [9] reported that the exposure of Gram-positive bacteria to antibiotics

can lead to the release of stimulatory cell wall fragments such as lipoteichoic acid (LTA) and peptidoglycan. DNA extracted from bacteria play an important pathogenic role in urinary tract infections [10].

The aim of the present study was to investigate whether supernatants of *S. xylosus* cultures, exposed to different classes of antibiotics, have an inflammatory effect on murine renal system.

Materials and Methods

Specimens collection

One hundred and fifty mid stream urine specimens were collected in sterile containers from patients aged 15-50 yrs, presented with UTI and referring Baghdad city hospitals (Al-Karama, Al-Yarmouk and Madinat altib).

Isolation and identification

Immediately, all specimens were cultured on blood agar than on Mannitol salt agar (HiMedia, India) and incubated aerobically at 37° C for 24 hrs. Thereafter, non mannitol fermentor colonies were transferred onto nutrient agar (HiMedia, India) in order to obtain pure colonies for morphological and biochemical study. Identification was performed according to [11,12]. While, morphological and biochemical tests were achieved according to Forbes *et al.*[13]. Api staph 20 (BioMerieux, France) was employed to confirm the identification results.

Antibiotic susceptibility test

Susceptibility of the isolates toward the antibiotic listed in table 1 was accomplished according to Bauer *et al.* (14). For quality control, *S.aureus* ATCC 25923 (a kind gift from The Central Public Health Lab/ ministry of Health) was used.

Table 1: Antibiotics used in the present study

Antibiotic	Code	Potency µg/disc	Company, origin
Ampicillin	AM	10	Bioanalyse, Turkey
Cefotaxime	CTX	30	Bioanalyse, Turkey
Ciprofloxacin	CF	5	Bioanalyse, Turkey
CO-trimaxazole	SXT	25	Bioanalyse, Turkey
Gentamicin	GM	10	Bioanalyse, Turkey
Rifampin	RA	5	Bioanalyse, Turkey
Tobramycin	TM	10	Bioanalyse, Turkey

Preparation of *S. xylosus* S4 supernatant

Several well isolated colonies of *S. xylosus* S4 were transferred on seven test tubes

containing 10 ml of Mueller Hinton broth (HiMedia, India). The turbidity of these tubes was adjusted according to McFarland tube No. 0.5 to obtain about 1.5×10^8 cfu/ml of bacterial concentration. All tubes were incubated for 2 hrs at 37° C to reach the logarithmic phase. Subsequently, the following antibiotics were added to the tubes: Ampicillin, Cefotaxime, Gentamicin, Rifampin, Erythromycin, Co-Trimoxazole and Ciprofloxacin in a final concentration reached 20 times the disc potency. Extra two tubes were added one served as negative control since it contained Mueller Hinton broth only, while the other one contained the broth and *S. xylosus* S4 to represent the positive control. All nine tubes were incubated for four hours at 37° C. Afterward; the tubes were centrifuged at 1000 rpm for three min and filtered through 0.45 µm membrane filter. All resultant supernatants were cultured on nutrient agar at 37° C for 24 hours for the sterility check [9].

In vivo study

Animals

Female white mice *Mus musculus* aged 6-8 weeks and weighing 22-26 gm obtained from national center for drugs supervision and researches were used in this study. Mice were housed in plastic cages and fed *ad libitum* with a conventional diet. The animals were divided into nine groups (3 animals per group) and injected with previously prepared antibiotic exposed supernatants. The control group animals were injected with non antibiotic exposed supernatant.

Injection protocol

First of all the bladder was emptied from urine by pressing on abdominal area. Urethra and surrounding area were sterilized with 75 % ethanol then a polyethylene tube (0.6 mm in diameter) was introduced to urinary bladder via urethra; the inoculums (20 µl) was injected by the aid of this catheter. Thereafter the catheter was withdrawn immediately, animals were returned to their cages with their lower end directed upward to avoid effusion of the inoculum outside [15].

All animals kept in their cages without water for 24hrs. After 2 days of injection they were sacrificed, the left kidneys and bladders were aseptically removed, for histopathological study according to Humason [16].

Results and Discussion

Out of 155 urine specimens, 51 (32.9%) isolates were identified as the genus *Staphylococcus*. Thirty nine (25.1%) isolates were coagulase positive while 12 (7.7%) isolates were identified as CoNS. Seven isolates were belonged to *S. xylosus* (4.5%).

Nicolle *et al.* [17] isolated 5 isolates of *S. xylosus* out of 145 isolates from UTI patients. Al-Kanani [18] recorded 18 isolates of *S. xylosus*. Tawfiq [6] and Al-Mathkhury *et al.* [7] reported that they isolated 10 isolates of *S. xylosus* out of 150 urine specimens collected from Iraqi UTI patients.

Antibiotic susceptibility

(Figure 1) illustrates the antibiotic susceptibility of the seven isolates of *S. xylosus*. All isolates were susceptible to ciprofloxacin, while five isolates were resistant to ampicillin and erythromycin. One isolate was resistant to cefotaxime. Table (2) represents the multidrug resistance of *S. xylosus* isolates and it shows that the isolate S4 is the most sensitive one, therefore, it was chosen for the further experiments.

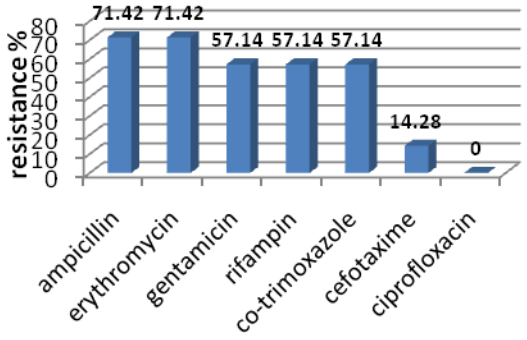


Figure 1: Antibiotic susceptibility of *S. xylosus*

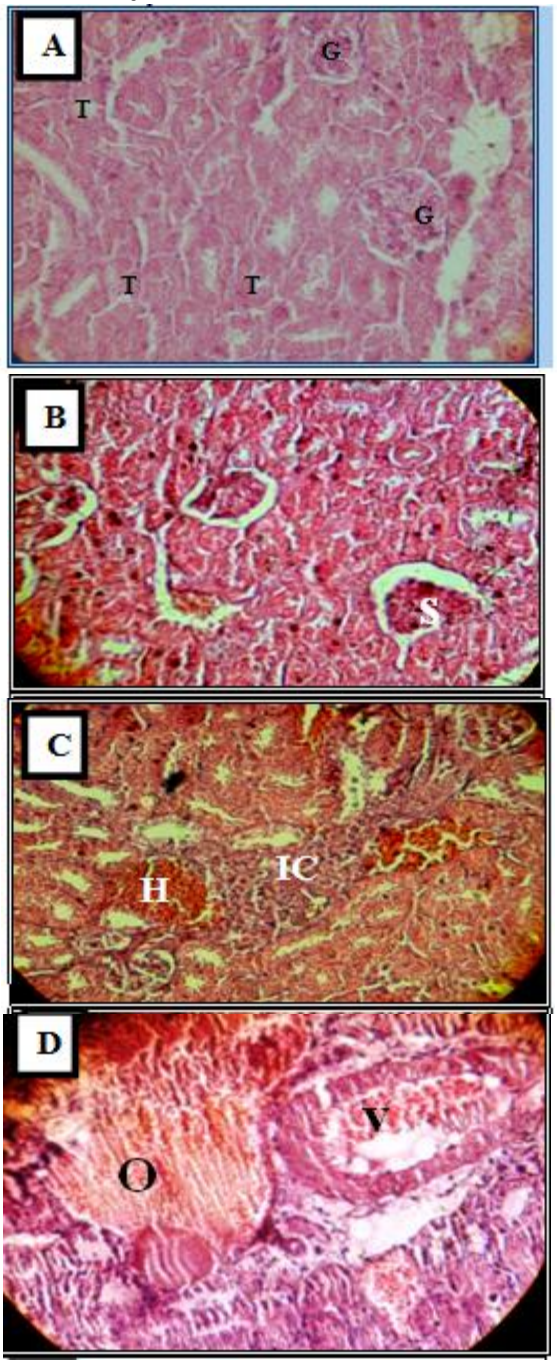
Table 2: Multidrug resistance of *S. xylosus.*

antibiotic	isolates *S. xylosus*
AM, GM, E, SXT	S1
RA, AM, GM	S2
SXT, RA, E	S3
RA, E	S4
AM, SXT, E	S5
GM, RA, AM	S6
SXT, AM ,GM, CTX, E	S7

In vivo study

kidneys and urinary bladders taken from mice injected with *S. xylosus* exposed to

antibiotics other than beta lactams show normal features. While figures 2 and 3 show the kidneys and urinary bladders, respectively, of mice injected with supernatant of *S. xylosus* killed with beta lactam antibiotics. Kidneys developed shrinkage in glomerulus, infiltration of inflammatory cells, hemorrhage, vacculation in blood vessels and edema, whereas the bladders suffered from dekeratinization and infiltration of inflammatory cells.



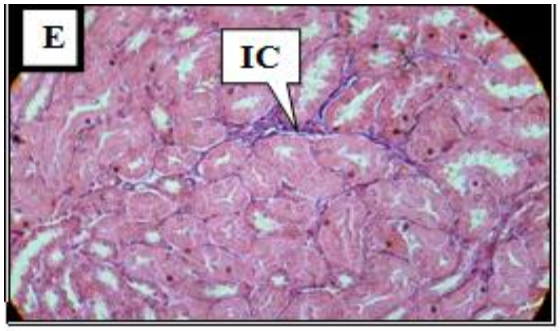


Figure 2: Cross section in mouse kidney. A) normal tissue. B, C, D and E) after injection with supernatant of cefotaxime killed *S. xylosus*; G=glomerulus, T=tubule, S=Shrinkage of glomerulus, H=hemorrhage, IC=infiltration of inflammatory cells, O=edema, V=vacculation of vessel.

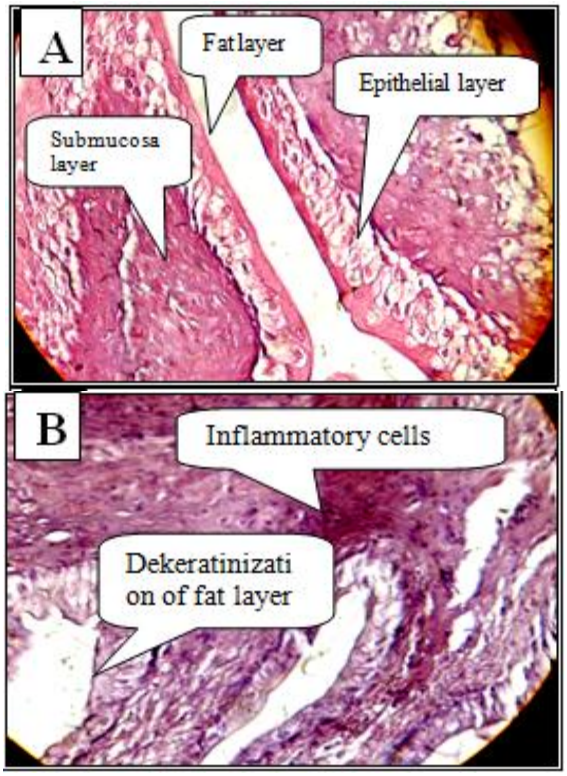


Figure 3: Cross section in mouse urinary bladder. A) normal tissue. B) after injection with supernatant of cefotaxime killed *S. xylosus*.

Such changes in the tissues of renal system could be attributed to several concepts:

1- When Gram positive bacteria exposed to beta lactam antibiotics, non cross-linked insoluble polymers of peptidoglycan will be librated [19].

2- Peptidoglycan can activate polymorphonuclear leukocytes to release hydrolytic enzymes, mast cells activation,

induce the acute and chronic immune response and participate in the attachment of bacteria to eukaryotic cell [19].

3- Gold *et al.*[20] found that the released peptidoglycan due to beta lactam exposure has a role in monocytes and macrophages activation and induce inflammatory reactions. Peptidoglycan released from treating *Streptococcus faecium* with penicillin, stimulate the release of IL-1 from leukocytes and colony-stimulating factor from macrophages in addition to inhibition the release of plasminogen activator from leukocytes and hyperproduction of granulocytes and fibrin precipitation. Moreover, prolonged treatment with beta lactam antibiotics led to release the non cross-linked insoluble polymers of peptidoglycan as well.

4- Peptidoglycan can induce coagulation via stimulation tissue factors on leukocytes; this activity has an important role in staphylococcal pathogenesis. Also peptidoglycan molecules are able to stimulate the production of proinflammatory cytokines from leukocytes [21].

5- In addition to peptidoglycan lipoteichoic acid may also released which led to the liberation of IL-10, TNF and monocyte chemotactic protein-1, consequently granulocytes and monocytes will be attracted and migrated to the infection site [9].

6- Injection of bacterial DNA, intraurethrally, in mice led to infiltration of inflammatory cells, shrinkage of glomerulus and increases the capsular space, as well as edema formation in kidney tissues. Whereas, urinary bladder sections showed infiltration of inflammatory cells [10].

As a conclusion, treating Gram positive bacteria; *S. xylosus* with beta lactam antibiotics led to serious histopathological changes in kidneys and urinary bladder of mice, such changes could be assigned to the release of inflammatory components like peptidoglycan, lipoteichoic acid and DNA. While treatment with antibiotics other than beta lactam failed in causing such changes.

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