

## PATHOLOGICAL STUDY ON *STAPHYLOCOCCUS XYLOSUS* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS

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### Abstract

One hundred and fifty urine specimens were collected from urinary tract infected patients, visiting different hospitals in Baghdad, for the period the first of September to the first of December, 2005.

Out of 61 staphylococci isolates, 13 isolates were diagnosed as coagulase positive staphylococci, 48 isolates as coagulase negative staphylococci (CoNS), 10 of the latter were identified as *Staphylococcus xylosus*. This identification was confirmed by biochemical tests and api 20 staph system.

All strains of *S. xylosus* isolates were able to produce protease and haemolysin, and unable to produce lipase, whereas urease activity was variable.

*S. xylosus* isolates showed high susceptibility toward ciprofloxacin and high resistant toward erythromycin.

Several pathological changes have been caused by *S. xylosus* isolates in mice represented by glomerulus shrinkage, haemorrhage, congestion, and infiltration of inflammatory cells in kidney, while the urinary bladder suffered from hydropic degeneration, dekeratinization, as well as infiltration of inflammatory cells.

It was clear that the urease producing isolate *S. xylosus* S7 was more virulent than the non urease producing isolate *S. xylosus* S12.

### Introduction

Coagulase-negative staphylococci (CoNS) are common inhabitants of the skin, skin glands and mucous membranes of various mammals and birds (1,2). Although these organisms are part of the normal flora and are generally considered to be of low virulence, they appeared to be implicated in the aetiology of a variety of human and animal infections, and they have established themselves as important pathogens showing increasing trends towards antibiotic resistance in the last decade (3).

*Staphylococcus xylosus* is a gram positive, coagulase negative, novobiocin resistant and xylose fermenter (4). Some strains of *S. xylosus* are opportunistic pathogens. They have been isolated from nosocomial infections, and described as multi-resistant to diverse antibiotics (5).

It was mentioned that this bacteria is the causing agent of urinary tract infections (UTI)

in women (6) and cutaneous lesion when injected in mice (7) due to possessing several virulence factors like hemolysin (8), urease (9), proteases (10), and lipase (11).

Unlike *Staphylococcus saprophyticus* less attention was paid toward *S. xylosus* as causing agent of UTI since its percentage was around 1% (12, 13, 14). However, recently, it was isolated with high percentage (25%) from UTI patients (15). Hence the purpose of this work was to study the pathogenicity of *S. xylosus* as causative agent of UTI *in vivo*.

### Materials and methods

#### Isolation and identification

One hundred and fifty mid stream urine specimens were collected from patients complaining from UTI aged from 15 to 50 years referring several hospitals in Baghdad from September to December 2005.

All specimens were cultured on mannitol salt agar (Biolife, England) cultivated at 37 °C

for 24 h. Mannitol non fermentor colonies were transferred to nutrient agar (Oxoid, England), identified in accordance to (16) and Bergey's manual (4), by conducting necessary biochemical tests (17), in addition to api 20 staph system. The ability of *S. xylosus* strains to produce hemolysin; urease, DNase, proteases and lipase (17) was investigated.

#### Antibiotic sensitivity

Sensitivity to gentamicin, ciprofloxacin, cephalothin, amoxicillin, and erythromycin (Oxoid) was done to all *S. xylosus* strains (18).

#### Animals

Female mice (*Mus musculus*), aged 8 weeks, weighing 25–30 gm were divided into 9 groups (A – I), three mice per group.

Urease producing *S. xylosus* S7 (SU+) strain was injected in A, B, C, and D groups in concentration of  $10^2$ ,  $10^4$ ,  $10^6$ , and  $10^8$  cfu / ml, respectively. Groups E, F, G, and H were injected with Urease non producing *S. xylosus* S12 (SU-) using the same concentration mentioned earlier. While group I was injected with phosphate buffer saline (PBS) and served as a control group.

#### Inoculation protocol

Inoculation was performed by introducing the bacterial suspension or PBS transurethraly. After sterilization of the periurethral area with 70% ethanol a soft polyethylene catheter (outer diameter 0.6 mm) was inserted through the urethra and 0.2 ml of the bacterial suspension or PBS was slowly introduced into the bladder over a period of not less than 30 s to avoid vesicourethral reflux (19). 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. The animals were left without water for 24 hours.

Four days later, urine specimen was collected and cultured on blood agar to confirm the infection. Animals were killed; bladders and kidneys were removed aseptically for histopathological study (20).

#### Results and discussion

Sixty one staphylococcal isolates (40.6 %) were obtained from 150 urine specimens. All isolates were glucose fermentor (anaerobically), bacitracin resistant and oxidase positive which differentiate them from micrococci and they were catalase positive which distinguish them from streptococci (17).

Out from 61 staphylococcal isolates, 48 (78.8 %) were coagulase negative and 31 (21.3 %) were coagulase positive.

From the coagulase negative isolates, 10 (16.3 %) isolates suggested being *S. xylosus* as they able to grow at 15 and 45 °C, novobiocin resistant, grow in 10 % and 15 % NaCl containing media and ferment xylose (16). Api 20 staph was employed to confirm the results. Also *S. cohnii*, *S. lentus*, and *S. saprophyticus* were isolated with percentages 5.6, 2.4 and 13.5 % respectively Fig. (1).

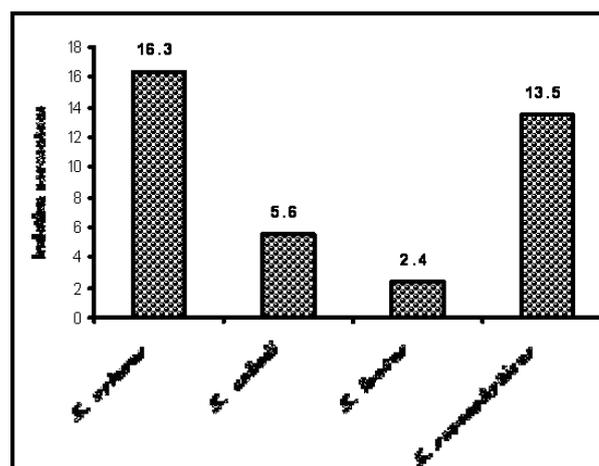


Fig. (1) : Isolation percentages of coagulase negative staphylococci.

*S. xylosus* represented 5 strains (3.4 %) from 145 staphylococcal strains in a study done by (21). while Al-Kanani (22) isolated it in a percentage reached 25 % out of 71 staphylococcal isolates.

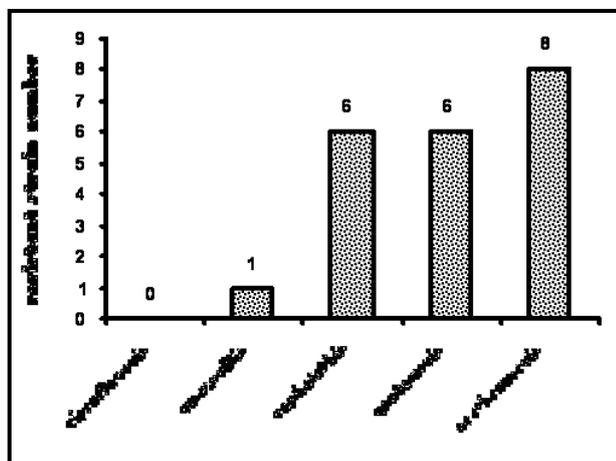
All of *S. xylosus* strains were unable to produce DNase and lipase and able to produce proteases and haemolysin. However seven of them were urease producers Table (1).

**Table (1)**  
**Enzymes produced by *S. xyloso*s strains.**

| Enzyme     | <i>S. xyloso</i> s strains |      |      |      |      |      |      |      |      |      |
|------------|----------------------------|------|------|------|------|------|------|------|------|------|
|            | S 7                        | S 12 | S 24 | S 25 | S 31 | S 39 | S 42 | S 49 | S 53 | S 55 |
| DNase      | -                          | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| Ureases    | +                          | -    | +    | +    | -    | +    | +    | +    | -    | +    |
| Proteases  | +                          | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Hameolysin | +                          | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| Lipase     | -                          | -    | -    | -    | -    | -    | -    | -    | -    | -    |

**Antibiotic sensitivity**

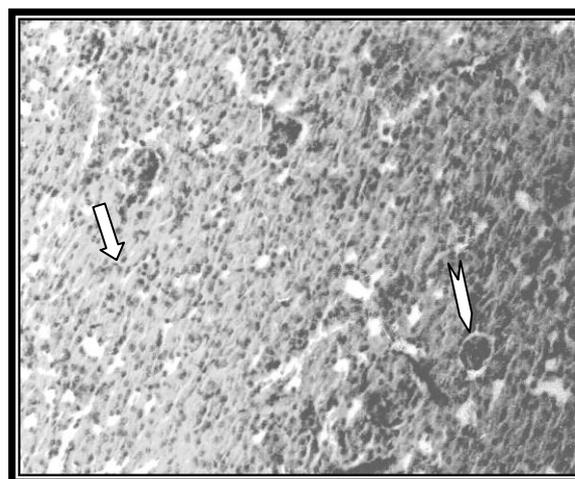
As shown in Fig.(2), most of *S. xyloso*s strains (8) developed resistant to erythromycin, while ciprofloxacin developed high efficiency since no strain were able to resist it. However *S. xyloso*s S7 (SU+) showed the highest number of antibiotics in comparison with other urease producing strains while the strain *S. xyloso*s S12 (SU-) showed the highest number of antibiotics in comparison with other urease non producing strains hence both strains were chosen for the *in vivo* study.



**Fig. (2) : Antibiotic resistance of *S. xyloso*s strains.**

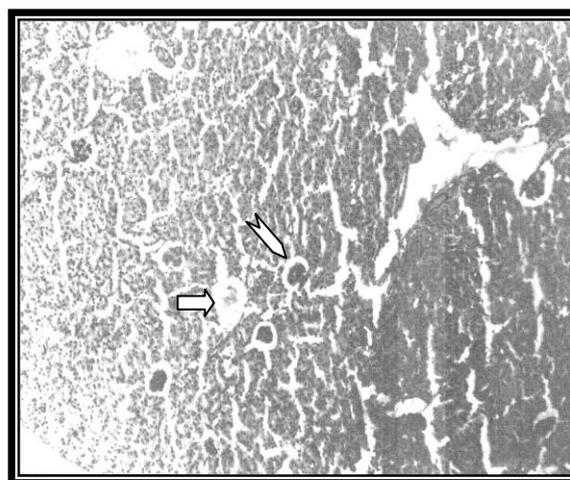
**In vivo study**  
***S. xyloso*s S12**  
**Kidney**

In comparison with control Fig.(3), injection with concentrations  $10^2$  and  $10^4$  cfu / ml did not cause any pathological changes.

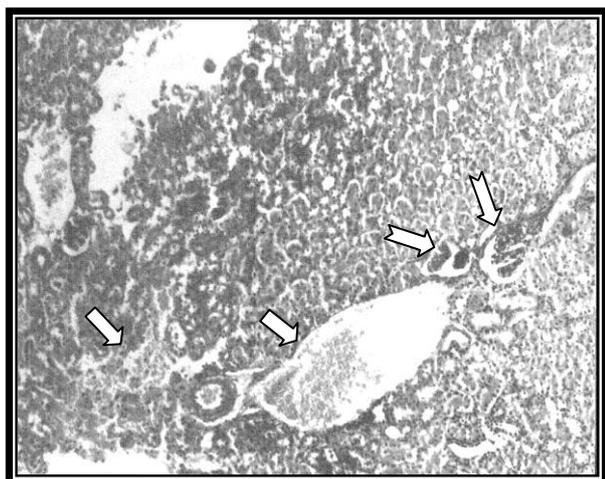


**Fig.(3) : Cross section of mice kidney (control) shows glomerulus (  $\approx$  ) and tubules (  $\Rightarrow$  ) X400 H &E.**

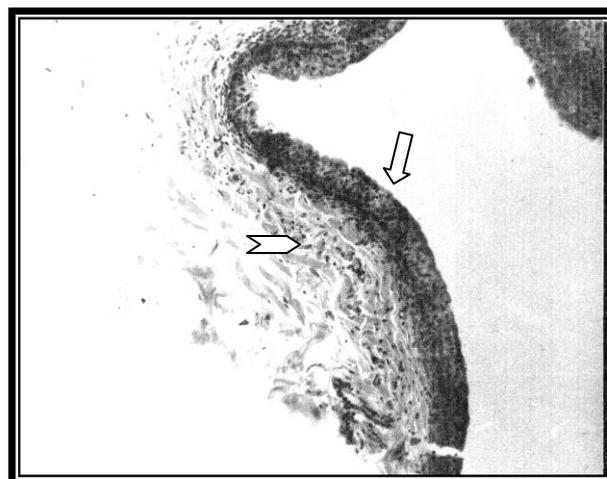
while several histopathological changes has been noticed in animals treated with  $10^6$  cfu/ml represented by glomerulus shrinkage, infiltration of inflammatory cells inside renal tissue Fig.(4), segmental corpuscles degeneration, haemorrhage Fig.(5). It can be noticed from figure 6 that the changes due to treatment with  $10^8$  cfu/ml were more severe which are characterized by shrinkage of glomerulus and increase of mesangial cells numbers, increase the interstitial spaces, degeneration of the nuclei of epithelial cells lining the tubules, oedema, as well as infiltration of inflammatory cells.



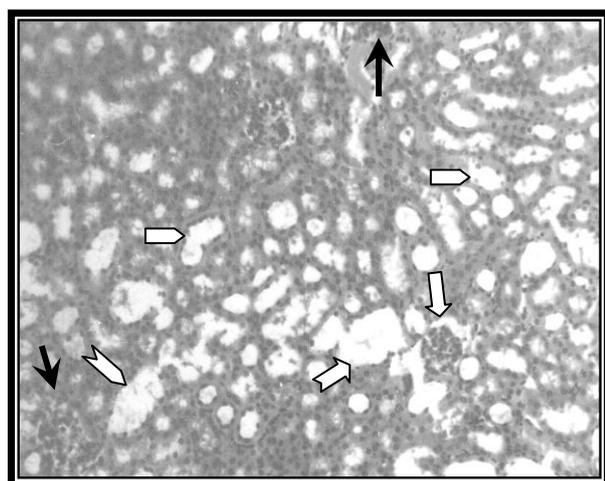
**Fig.(4) : Cross section of mice kidney treated with  $10^6$  cfu /ml *S. xyloso*s S12 shows shrinkage of glomerulus (  $\approx$  ) and infiltration of inflammatory cells (  $\Rightarrow$  ) X400 H &E.**



**Fig.(5) :** Cross section of mice kidney treated with  $10^6$  cfu /ml *S. xylosus* S12 shows segmental corpuscle degeneration of glomerulus (⇔) and haemorrhage (→) X400 H &E.



**Fig.(7) :** Cross section of mice urinary bladder (control) shows epithelium layer (⇔) and submucosa layer (→) X 400 H &E.



**Fig.(6) :** Cross section of mice kidney treated with  $10^8$  cfu /ml *S. xylosus* S12 shows shrinkage of glomerulus and increase of mesangial cells numbers (⇔), degeneration of the nuclei of epithelial cells lining the tubules (◻), oedema (⇔), increase the interstitial spaces (⇔), as well as infiltration of inflammatory cells (→) X400 H &E..

#### Urinary bladder

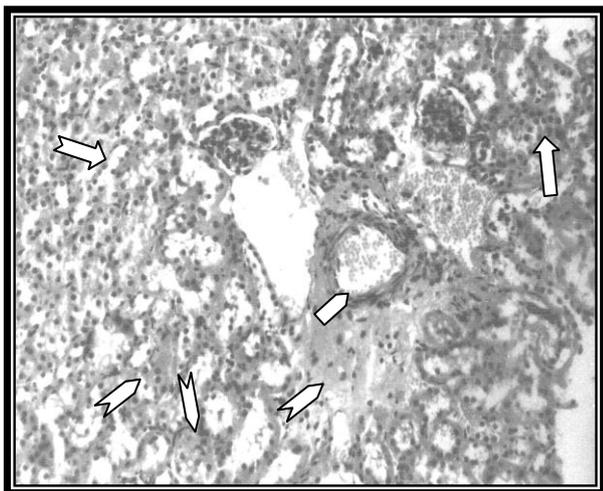
Regarding the control Fig.(7), No marked changes were observed in animals treated with  $10^2$ ,  $10^4$  and  $10^6$  cfu /ml, whereas injection with  $10^8$  cfu/ml led to degeneration of epithelial cells lining the bladder tissue, dekeratinization in addition to infiltration of inflammatory cells Fig.(8).



**Fig.(8) :** Cross section of mice urinary bladder treated with  $10^6$  cfu/ml *S.xylosus* S12 shows degeneration of the nuclei of epithelial cells lining the (⇔), dekeratinization (⇔) and infiltration of inflammatory cells (⇔) X400 H &E.

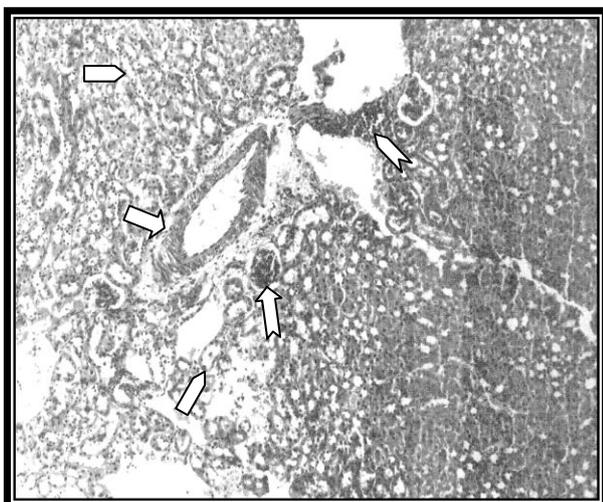
#### *S. xylosus* S7 Kidney

Mild changes were observed in mice treated with  $10^4$  cfu/ml demonstrated by congestion of vessels, infiltration of inflammatory cells, and precipitation in interstitial spaces that led to oedema formation. Most of tubules were in normal shape with the exception of some of them which underwent of hydropic degeneration Fig.(9).



**Fig.(9) : Cross section of mice kidney treated with  $10^4$  cfu /ml *S. xylosus* S7 shows congestion of vessels (  $\rightarrow$  ), infiltration of inflammatory cells (  $\uparrow$  ), precipitation in interstitial spaces (  $\searrow$  ), and hydropic degeneration (  $\Sigma$  ) X400 H &E.**

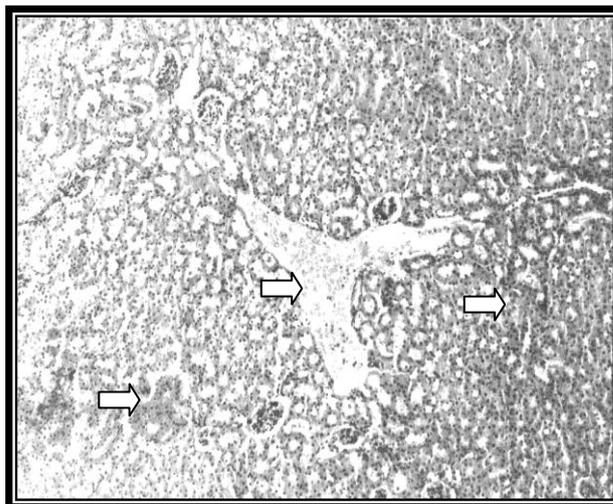
Injection with  $10^6$  cfu /ml led to congestion of vessels, increase in mesangial cells numbers, infiltration of inflammatory cells, most of tubules presented with of hydropic degeneration Fig.(10).



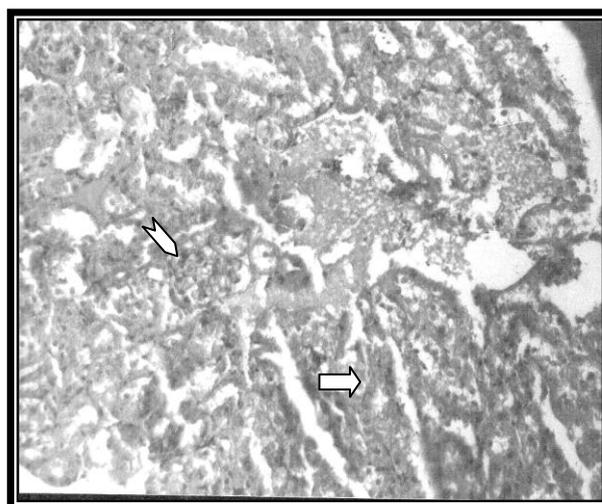
**Fig.(10) : Cross section of mice kidney treated with  $10^6$  cfu /ml *S. xylosus* S7 shows congestion of vessels (  $\rightarrow$  ), increase in mesangial cells numbers (  $\searrow$  ), infiltration of inflammatory cells (  $\searrow$  ), and hydropic degeneration of tubules (  $\Sigma$  ) X400 H &E.**

Also haemorrhage has been noticed, aggregation of RBCs in interstitial space and renal tubules (i.e. haematuria) Fig.(11), hence

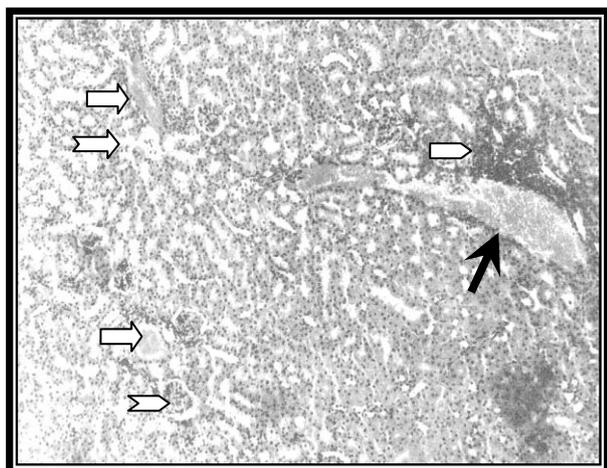
the changes were more severe compared with animals treated with  $10^4$  cfu/ml. Obviously, treatment with  $10^8$  cfu/ml caused several changes more severe than those caused by  $10^4$  and  $10^6$  cfu / ml demonstrated by lacking the normal morphology of tubular and glomerular cells Fig.(12), increase in mesangial cells numbers, infiltration of inflammatory cells, increase the interstitial spaces, haemorrhage and oedema Fig.(13).



**Fig.(11) : Cross section of mice kidney treated with  $10^6$  cfu /ml *S. xylosus* S7 shows haemorrhage aggregation of RBCs in interstitial space and renal tubules (  $\rightarrow$  ), X400 H &E.**



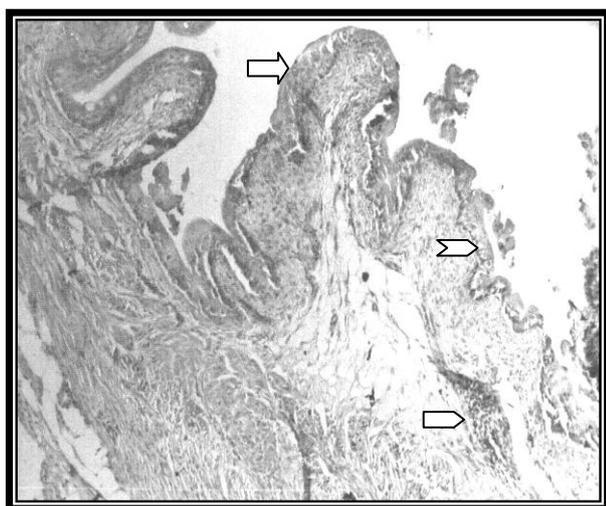
**Fig.(12) : Cross section of mice kidney treated with  $10^8$  cfu /ml *S. xylosus* S7 shows lacking the normal morphology of tubules (  $\searrow$  ), and glomeruli (  $\searrow$  ) X400 H &E.**



**Fig.(13) :** Cross section of mice kidney treated with  $10^8$  cfu /ml *S. xylosus* S7 shows increase in mesangial cells numbers (  $\Sigma$  ), infiltration of inflammatory cells (  $\square$  ), increase the interstitial spaces (  $\Sigma$  ), and oedema (  $\square$  ) and haemorrhage (  $\blacktriangleright$  ) X400 H &E.

#### Urinary bladder

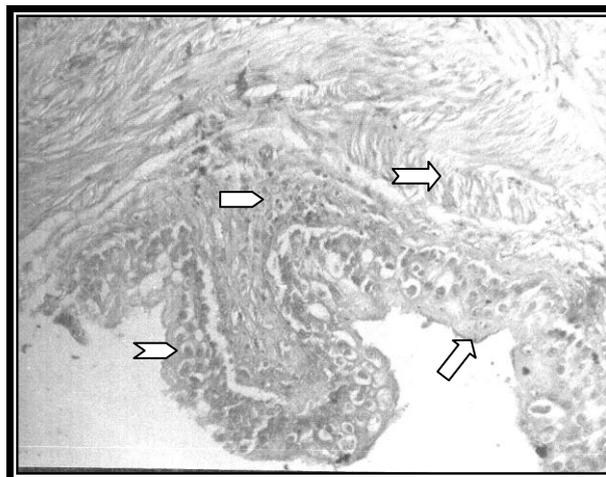
Treatment of animals with  $10^2$ ,  $10^4$  and cfu/ml did not cause any apparent changes, while treatment with  $10^6$  cfu/ml caused several changes characterized by hydropic degeneration, dekeratinization, and infiltration of inflammatory cells Fig.(14).



**Fig.(14) :** Cross section of mice urinary bladder treated with  $10^6$  cfu /ml *S. xylosus* S7 showed hydropic degeneration (  $\Sigma$  ), dekeratinization (  $\square$  ) and infiltration of inflammatory cells (  $\square$  ) X400 H &E.

Fig.(15) shows the changes in animals treated with  $10^8$  cfu / ml, which are represented by hydropic degeneration, dekeratinization,

infiltration of inflammatory cells, as well as vacuolation of epithelial cells lining the bladder.



**Fig.(15) :** Cross section of mice urinary bladder treated with  $10^8$  cfu /ml *S. xylosus* S7 shows hydropic degeneration (  $\Sigma$  ), dekeratinization (  $\square$  ) vacuolation of epithelial cells (  $\Sigma$  ) and infiltration of inflammatory cells (  $\square$  ) X400 H &E.

Concerning these results, it can be suggested that the urease producer isolate *S. xylosus* S7 is more virulent than the non urease producer isolate *S. xylosus* S12 and that could be attributed to the former one caused pathological changes at  $10^4$  cfu /ml while the latter started the damage at  $10^6$  cfu / ml. In addition to the damages caused by the urease producer were more severe than the non urease producer. Urease has a very crucial role in pathogenesis since most of the above changes are caused by this enzyme (e.g. haemorrhage, oedema, shrinkage of the glomerulus, and infiltration of inflammatory cells). Moreover, these effects are dose dependent (22).

Nevertheless, the isolate *S. xylosus* S12 caused damages to the urinary system despite urease activity, therefore it is highly recommended to study this bacterium (*S. xylosus*) and investigate the virulence factors, toxins, and enzymes that might participate in its pathogenicity.

## References

- [1] N.Nagase, A.Sasaki, K. Yamashita, A.Shimizu, Y.Wakita, S. Kitai, & J.Kawano. "Isolation and species distribution of staphylococci from animal and human skin". J. Vet. Med. Sci. Vol. 64, 2001, pp. 245–250.
- [2] T.Hauschild. "Phenotypic and genotypic identification of staphylococci isolated from wild small mammals". Syst. Appl. Microbiol. Vol. 24, 2001, pp. 411–416.
- [3] C.von Eiff, R. A.Proctor, & G. Peters, "Coagulase-negative staphylococci. Pathogens have major role in nosocomial infections". Postgrad. Med. Vol.110, 2001, pp. 63–76.
- [4] J.Holt, N.Kreig, P.Sneath, J.Staley, and S.Williams, "Bergey's manual of determinative bacteriology 9th ed" Williams and Willikins Maryland, USA. 1994.
- [5] C.Barreire, R.Bruncker, and R.Talon, "Characterization of single superoxide dismutase of *Staphylococcus xylosus*" J. Appl. Environ. Microbiol. Vol. 67, 2001, pp. 4096-4104.
- [6] M. E Rupp. D. E.Soper, and G. L.Archer. "Colonization of the female genital tract with *Staphylococcus saprophyticus*". J. Clin. Microbiol. Vol. 30 1992, pp. 2975-2979.
- [7] V.Thornton, J. DAVIS, S. Clair, and M.Cole, "Inoculation of *Staphylococcus xylosus* in JL/J mice to determine pathogenicity". Anim.Sci. Vol. 212, 2003. pp.49-52.
- [8] A.,Sawicka–Grezalak, A.,Szymanowska, A.Mlynarczyk, and G.Mlynarczyk, "Production of staphylolysin and hemolysin by coagulase negative staphylococci". Med. Bosw.Microbiol. Vol. 45, 1993, pp.7-10.
- [9] J.Jose, S.Christians, R.Rosenstein. F.Gotz, and H. Kultwasser, "Cloning and expression of various staphylococcal genes in coding urease in *Staphylococcus carnosus*" FEMS Microbiol. Lett. Vol.64 1991. pp. 277-281.
- [10] A. Casaburi, F. Villani, F. Toldra, and Y.Sanz, "Protease and esterase activity of staphylococci". Int.J.Food Microbiol. Vol.16, 2006, pp.216-219.
- [11] H.Mosbah, A.Sayari, S. Bezzine, and D.Garguairy "Expression, purification and characterization of His tagged *Staphylococcus xylosus* lipase wild type and its mutant Asp 290 Ala". Protein exprpuf.J.Clin. Vol. 3, 2006, pp. 189-195.
- [12] Tselenis-Kotsowilis, A., Koliomichalis, M.and Papvassiliou, J. (1982). Acute pyelonephritis caused by *Staphylococcus xylosus*. J. Clin.Micorbiol. 16:593.
- [13] Medscape "*Staphylococcus* endocarditis". Infec.Med. Vol. 21, 2004, pp. 68-72.
- [14] D.,Novacova, V., Stetina, A.,Ivosedleck, and P.Petras, "Characterization of presumptive *Staphylococcus xylosus* strain by SDS-PRGe protein profiles". J. Clin. Microbiol. Vol. 24, 2005, pp. 176-179.
- [15] A.Al-heety, "Role of peptidoglycan in pathogenicity of *Staphylococcus saprophyticus*" .MSc. thesis. University of Baghdad. College of Science. Baghdad, Iraq. 2005.
- [16] W. E. Kloos, and K. H. Schleifer, "Simplified scheme for routine identification of human *Staphylococcus* species". J.Clin.Microb. Vol. 1, 1975, pp. 82-88.
- [17] B.Forbes, D.Sahm, and A.Weissfeld, "Bailey & Scott's: Diagnostic microbiology, international edition, 12th ed", Mosby, Elsevier. Missouri, USA. 2007.
- [18] A. W.Bauer, W. M.Kirby, J. C, Sherris, and M.Turck, "Antibiotic susceptibility testing by a standardized single disk method". Am. J. Clin. Pathol. Vol.36, 1966, pp. 493-496.
- [19] B.,Kaijser, P.,Larsson, S. Olling, "Protection against ascending *Escherichia coli* pyelonephritis in rats and significance of local immunity", Infect. Immun. Vol.20, 1978. pp.78–81.
- [20] C.Humson, "Animal tissue techniques 3rd ed". W.H. Freeman company. 1972.
- [21] L. E.Nicolle, S. A. Hoban, and G. K. M. Harding, "Characterization of coagulase-negative Staphylococci from urinary tract specimens". J.Clin.Microbiol. Vol.17, 1982. pp.267-271.

[22] N.Alkanai, "Production and description of urease enzyme produced from *S. saprophyticus*" MSc. Thesis. University of Baghdad, College of Science, Baghdad, Iraq. (2005).

### الخلاصة

جمعت 150 عينة ادرار من المرضى الوافدين الى مستشفيات مختلفة في بغداد للفترة من الاول من ايلول الى الاول من كانون الاول 2005.

من مجموع 61 عزلة عائدة للعنقوديات شخصت 13 عزلة على انها موجبة للكواكيولاز و 48 سالبة له، و من الاخيرة اثبت عائدية 10 عزلات منها الى النوع *S. xylosus*. تم تاكيد التشخيص بوساطة الاختبارات الكيمائية و نظام *api 20 staph*. وكان لعزلات *S. xylosus* القدرة على انتاج البروتيناز و حال الدم و لم تتمكن من انتاج اللايباز في حين تغاير انتاجها لليوريزاز. اظهرت عزلات *S. xylosus* حساسية عالية تجاه المضاد الحياتي سبروفلوكساسين و مقاومة عالية للمضاد ارثرومايسين.

تسببت هذه البكتريا في عدة تغييرات مرضية في الفئران تمثلت بانكماش الكبيبة و النزف و الاحتقان و ارتشاح الخلايا الالتهابية في الكلى، اما المثانة فقد عانت من التنكس الاستسقائي و ازالة الطبقة المنقرنة فضلا عن ارتشاح الخلايا الالتهابية.

لقد بدا واضحا ان العزلة المنتجة لليوريزاز *S. xylosus* S7 كانت اشد فوعة من العزلة غير المنتجة له *S. xylosus* S12.