

Colonization of *Staphylococcus xylosus* in the kidneys and bladder of mice

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Abstract

Two isolates of *Staphylococcus xylosus* (urease producer and non urease producer) were injected in mice at a dose of 2×10^8 colony-forming units (CFU) intraurethrally. Results showed that both isolates were able to colonize kidney and bladder of the injected mice, regardless of their urease production. Moreover, there were insignificant differences between the two groups. These results emphasized the pathogenicity of this bacteria in UTI.

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 have been isolated from nosocomial infections, have been described as multi-resistant to diverse antibiotics (5).

These organisms may contribute to development and stabilization of color, inhibition of rancidity, and improvement of the flavor of cured ham by reductases, catalases, and lipases (6)

It was mentioned that this bacteria is the causing agent of urinary tract infections (UTI) in women (7), cutaneous lesion when it injected in mice (8). This bacterium has several virulence factors

like hemolysin (9), urease (10), proteases (11), and lipase (12).

Unlike *Staphylococcus epidermidis* and *S. saprophyticus*, less attention was paid toward *S. xylosus* as causing agent of UTI since its percentage was around 1% (13,14,15). However, recently, it was isolated with high percentage (25%) (16) and 16.3 % (17) from UTI patients, hence the purpose of this study was to investigate the colonization of *S. xylosus* as causative agent of UTI in kidney and urinary bladder.

Material and Methods

Microorganism

Two isolates of *S. xylosus* (one is urease positive and the other one is urease negative) were isolated from UTI patients on mannitol salt agar (Himedia), both are susceptible to ciprofloxacin and resistant to erythromycin, gentamicin and amoxicillin. They were cultured in Brain heart infusion (Himedia) at 37 °C for 24 h. then cultures were collected by centrifugation at 6,000 x g for 10 min and resuspended in phosphate-buffered saline (PBS).

Animals

Nine female mice (*Mus musculus*), aged 8 weeks, weighing 25 – 30 gm were

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divided into three groups. Group one and two were challenged with 2×10^8 colony-forming units (CFU)/ml of urease producing and non urease producing *S. xylosus*, respectively, suspended in phosphate buffer saline (PBS), while the third group was injected with (PBS) which referred to as control group, using an ascending UTI model as described below.

Inoculation protocol

The inoculation was performed by introducing the bacterial suspension or PBS transurethrally. 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 (18). Four days after challenge, mice were euthanised by cervical dislocation. Bladders and kidneys were removed aseptically and separately homogenised in 10 ml of PBS using a Lab Blender. Homogenised tissues were quantitatively cultured on nutrient agar. The number of CFU per bladder or kidney was determined after 24 h of incubation at 37 °C (19).

Bacteriological analysis

To determine the number of viable bacteria in organs, 50- μ l portions were spread onto the nutrient agar (Difco). The plates were incubated aerobically at 37 °C for 24 h. Results are expressed as the mean number of bacteria (CFU) in the entire organ (20).

Statistical analysis

Statistical differences between the control group and the treated group were evaluated with Student's *t* test for. A *P* value of <0.05 was considered significant.

Results and Discussion

S. xylosus was able to colonize both kidney and bladder of the injected mice regardless of their urease production. Moreover, there were insignificant differences between group one and two, while there were significant differences between bacterial counts in kidney and bladder as the former was higher than the latter (table 1).

Table 1: Bacterial counts (CFU) in mice renal system challenged with 2×10^8 CFU/ml of *S. xylosus**

Organ	<i>S. xylosus</i> urease positive	<i>S. xylosus</i> urease negative
Urinary bladder	5.6×10^3	5.2×10^3
Kidney	4.8×10^4	5×10^4

* Each datum is a mean of triplicate

As it can be seen from table (1) bacterial counts in kidney is more than in bladder and that could be due to clearance of non adherent bacteria from the bladder by mechanical forces of urine flow and other innate defenses. The higher counts of *S. xylosus* in the kidneys indicated that the bacteria in the kidney were able to establish residence capable of evading innate defenses. Nevertheless, this assumption is needed to be investigated intensively.

The present study results have an agreement with the study of Kau and his coworkers as they used a mouse model of urinary tract infection, they have shown that *Eenterococcus faecalis* is capable of persisting in the kidneys of mice for at least 2 weeks. In contrast, bacterial titers from the bladders of the same mice were inconsistent and tended to be much lower than those recovered from the kidney. This preference for the kidney over the bladder is also observed in other clinical *E. faecalis* strains (21).

These results agreed with Al-Ghairy (17) as he mentioned that *S. xylosus* caused several histopathological changes in mice kidney and bladder. Furthermore, the urease producer caused damage more than non urease producer. According to the present results this damage could be attributable to urease production or to another virulence factors rather than to bacterial colonization density inside the organ.

Scavone and his coworkers (19) mentioned that challenging mice with 2×10^8 colony-forming units (CFU) of *Proteus mirabilis* Pr2921, resulted in harboring the kidney with 6.3×10^6 while urinary bladder showed 5×10^5 .

Asahara *et al* found that *Escherichia coli* strain HU-1 at an inoculum size of 2×10^6 CFU into the bladder induced a local transitory infection in the mice, as the bladder harbored 3×10^6 CFU and kidneys harbored 10^4 CFU. However, the number of polymorphonuclear leukocytes and myeloperoxidase activity in the urine were markedly elevated during the infection period (20).

Regarding the study of Al-heety (16) and Al-Ghairy (17) which isolated *S. xylosus* with high percentages and considering the studies of Scavone and his coworkers (19) and Asahara *et al.* (20) it can be suggested that the *S. xylosus* is considered as an important UTI pathogen since it has the ability to colonize the urinary system and cause severe damage.

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استيطان *Staphylococcus xylosus* الكلى و المثانة في الفئران

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الخلاصة:

حققت عزلتان من بكتريا *Staphylococcus xylosus* (عزلة منتجة لليورياز واخرى غير منتجة له) في الفئران بجرعة مقدارها 2×10^8 وحدة مكونة للمستعمرة عبر الاحليل. بينت النتائج ان كلا العزلتين تمكنتا من استيطان الكلى و المثانة بغض النظر عن انتاج اليورياز. فضلا عن ذلك كانت الفروق غير معنوية بين المجموعتين. هذه النتائج تؤكد على امراضية هذه البكتريا للجهاز البولي.