Original Article

Urinary tract infections caused by *staphylococcus aureus* DNA in comparison to the *candida albicans* DNA

Harith Jabbar Fahad Al-Mathkhury, Saba Nazeih Abdul-Ghaffar

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

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Abstract

Background: Bacterial DNA released upon bacterial autolysis or killed by antibiotics, hence, many inflammatogenic reactions will be established leading to serious tissue damage. **Aim**: the present work aimed to elucidate the histopathological changes caused by prokaryotic (bacterial) DNA and eukaryotic (candidal) DNA. **Materials and Methods**: twenty one *Staphylococcus aureus* and 36 *Candida albicans* isolates were isolated from UTI patients. Viable cells and DNA of the highest antibiotic sensitive isolates were injected, intraurethraly, in mice. Results were evaluated via histopathological examination. **Results:** Mildest reactions were obtained from mice challenged with viable *C. albicans* compared with those challenged with viable *S. aureus*. Dose-dependent histological changes were observed for both eukaryotic and prokaryotic DNA. However, the eukaryotic *C. albicans* DNA developed less intense histological changes than *S. aureus* DNA. **Conclusion:** microbial DNA has the ability to cause damage in murine renal system. Nevertheless, bacterial DNA caused more intense damage than candidal DNA.

Keywords: Staphylococcus aureus, candida albicans, UTI, DNA.

Correspondence to: Harith Jabbar Fahad Al-Mathkhury, PhD., Assistant Professor, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. Tel.: 009647712277025. Email: harith_fahad@yahoo.com

Introduction

Microbial components signal the presence of foreign pathogens in the innate immune system. They are key players in infectious diseases and implicate toll-like receptors (TLR) in the activation of inflammation [1].

Bacterial DNA causes potent immune response stimulation. The first report on the immunostimulatory properties of bacterial DNA date back to Tokunaga and colleagues in 1984 due to the presence of unmethylated CpG dinucleotides in a particular base sequence context termed "CpG motif". Importantly, the immunostimulatory activity is found in bacterial, yeast, viruses, insect and nematode DNA [2]. In contrast, eukaryotic DNA is nonstimulatory; this lack of immunostimulatory potential has been explained by CpG suppression, CpG methylation, inhibitory motifs, and saturable DNA uptake in combined [3].

Microbial DNA activates innate immune cells via TLR 9 leading to secretion of pro-inflammatory cytokines and directs the development of adaptive immunity, suggesting that microbial DNA is an important virulence determinant and inflammatory stimulus during infections [4]. Deng *et al.* [5] reported that when the microbe degraded or autolysed for any reason such as killing by antibiotics or processing by the antigen presenting cells, the DNA will be released, hence, it will evoke the inflammatogenic reactions causing serious problems.

For the critical role of DNA in pathogenesis and the absence of *in vivo* study tracing the comparison between prokaryotic unmethylated DNA and eukaryotic slightly methylated DNA in the respect of the urinary system, this work aimed to conduct a comparative histopathological study between DNA extracted from *S. aureus* and *C. albicans* in urinary tract of mice.

Materials and Methods

Isolation and Identification

Mid-stream urine specimens were collected from 308 patients (15-50 years), presented with urinary tract infections visiting Baghdad hospitals.

All specimens were inoculated on mannitol salt agar and sabouraud dextrose agar, incubated at 37ºC and 30ºC, respectively for 24hrs. Staphylococci were identified depending on biochemical tests according to Forbes *et al.* [6]. The classification was achieved according to Holt *et al.* [7]. API-Staph system was employed to confirm the identification. *Candida albicans* was identified depending on the morphological features on culture medium and germ tube formation in addition to API-Candida system.

Antibiotic Susceptibility

The modified Kirby-Bauer method [8] was used to investigate the antibiotic susceptibility against amoxicillin, ampicillin, cefotaxime, methicillin, oxacillin, and vancomycin. A standard *S. aureus* strain (*S. aureus* ATCC 6538P) was used as the quality control strain.

DNA Extraction

Staphylococcus aureus DNA were prepared using a Wizard genomic DNA purification kit (Promega). The method of Harju *et al.* [9] was followed to extract the DNA from *Candida albicans*. DNA preparations were stored at 2-8ºC.

Animals

Female white BALB/C mice (age 6-8 weeks, weight 20-25 gm) were used in this study. Mice were housed in plastic cages and fed *ad libitum* with a conventional diet.

Injection Protocol

The bladder was emptied from urine by pressing on abdominal area. Urethra and surrounding area were sterilized with 70% ethanol then a polyethylene tube (0.6 mm in diameter) was introduced to urinary bladder via urethra; the inoculums (1.5×10^8 CFU/ml and 1×10^8 CFU/ml for *S. aureus* and *C. albicans*, respectively) was injected by 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 [10].

Mice were divided into fourteen groups, two animals per group:

- First, second, third, fourth and fifth groups were injected with 0.1ml of *S. aureus* S_{11} DNA at concentrations 10, 20, 30, 40 and 50 µg/100µl, respectively.
- Sixth, seventh, eighth, ninth and tenth groups were injected with 0.1ml of *C. albicans* C_3 DNA at different concentrations 10, 20, 30, 40 and 50 µg/100µl respectively.
- Eleventh and Twelfth groups were injected with 0.1ml of *S. aureus* S_{11} and *C. albicans* C_3 suspensions, respectively.
- Thirteenth and fourteenth groups (control groups) were injected with normal saline and TE buffer respectively.

All groups were injected with two repeat to each injection and kept in their cages without water for 24hrs. 3 days later, they were sacrificed; kidneys and bladders were removed for histopathological study.

Results

Out of 72 staphylococcal isolates, 51 were identified as coagulase negative staphylococci and 21 as *S. aureus*. 36 specimens were identified as *C. albicans*. The isolates *S. aureus* S_{11} and *C. albicans* C_3 showed the highest antibiotic susceptiblity, hence, they were selected for *in vivo* study.

Histopathology

No histological changes were observed in kidney and bladder of control mice as shown in Figure 1.

Histopathological examination of kidney from mice challenged with viable *S. aureus* S_{11} revealed congestion, inflammatory cells infiltration and degenerative changes of renal tubules (Figure 2a), while its bladder suffered from edema, moderate inflammatory cells infiltration and focal mucosal surface epithelial ulceration (Figure 2b). Whereas those of mice challenged with viable *C. albicans* C_{11} showed congestion (Figure 2c). The bladder developed mild infiltration of inflammatory cells with edema in subepithelial lining layer (Figure 2d).

Kidney challenged with 50µg/100µl of *S. aureus* S_{11} DNA showed congestion, degenerative and necrosis of renal tubule with severe infiltration of inflammatory cells, the bladder revealed damage of mucosal wall with severe inflammatory cells infiltration (Figure 3a, b). At 40 µg/100µl histological sections of kidney showed congestion of blood vessel with degenerative changes of renal tubules, the bladder developed mild edema and infiltration of inflammatory cells with sloughing of mucosal epithelial surface (Figure 3c, d). While at 30 µg/100µl histological sections of the kidney revealed congestion and mild degenerative changes of renal tubules with mild inflammatory cells infiltration, the bladder showed edema with infiltration of inflammatory cells (Figure 3e, f). At 20 µg/100µl and 10 µg/100µl, histological sections showed normal structure appearance of the kidney and the bladder.

Sections of kidney challenged with 50 µg/100µl of *C. albicans* C_3 DNA showed congestion with moderate inflammatory cells infiltration, the bladder revealed edema with mild inflammatory cells infiltration (Figure 3g, h). At 40 µg/100µl kidney showed congestion of blood vessels, the bladder developed mild inflammatory cells infiltration and edema (Figure 3i, j). While at 10, 20 and 30 µg/100µl, histological sections showed normal kidney and bladder structure.

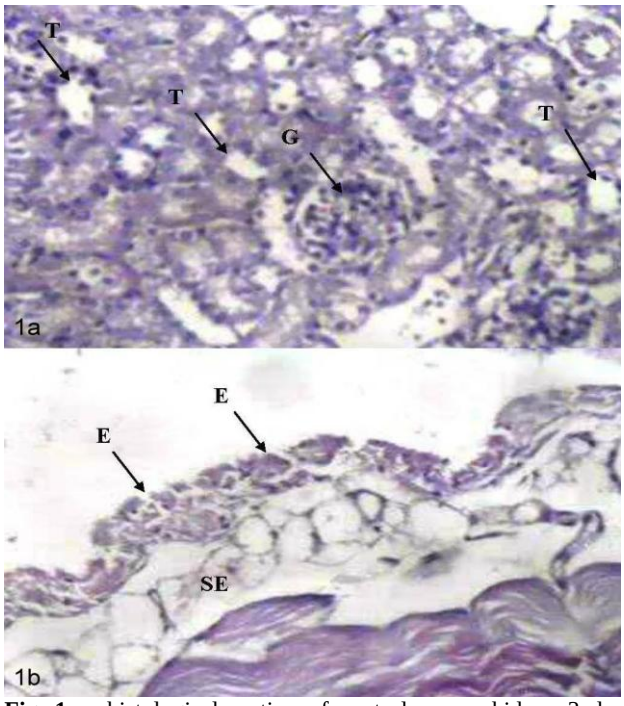

Fig. 1 a: histological section of control mouse kidney 3 days after challenged with 0.1ml of saline shows a normal structure appearance of glomeruli (G) and renal tubules (T). **b:** The histological section of control mouse urinary bladder 3 days after challenged with 0.1ml of saline shows a normal structure appearance of urinary epithelial lining mucosa (E) and subepithelial lining layer (SE). H & E (X200).

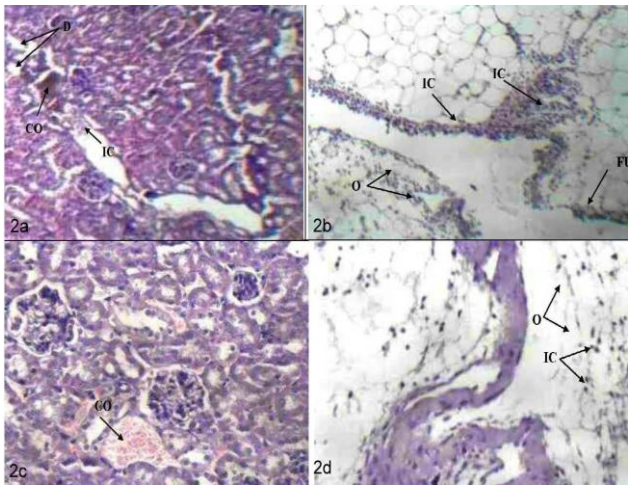

Fig. 2 a: Histological section of mouse kidney 3 days after challenged with 0.1 ml of 1.5×10^8 cfu/ml of *S. aureus* S11 shows congestion (CO)), inflammatory cells infiltration (IC) and degenerative changes of renal tubules (D). **b:** The histological section of mouse urinary bladder 3 days after challenged with 0.1ml of 1.5×10^8 cfu/ml of *S. aureus* S11 shows edema (O) with moderate inflammatory cells infiltration (IC) and focal mucosal surface epithelial ulceration (FU). **c:** The histological section of mouse kidney 3 days after challenged with 0.1ml of 1×10^8 cfu/ml of *C. albicans* C3 shows congestion (CO) and no obvious histological changes. **d**: The histological section of mouse urinary bladder 3 days after intraurethrally challenged with 0.1ml of 1×10^8 cfu/ml of *C. albicans* C3 shows mild infiltration of inflammatory cells (IC) with edema (O) in subepithelial lining layer. H & E (X200).

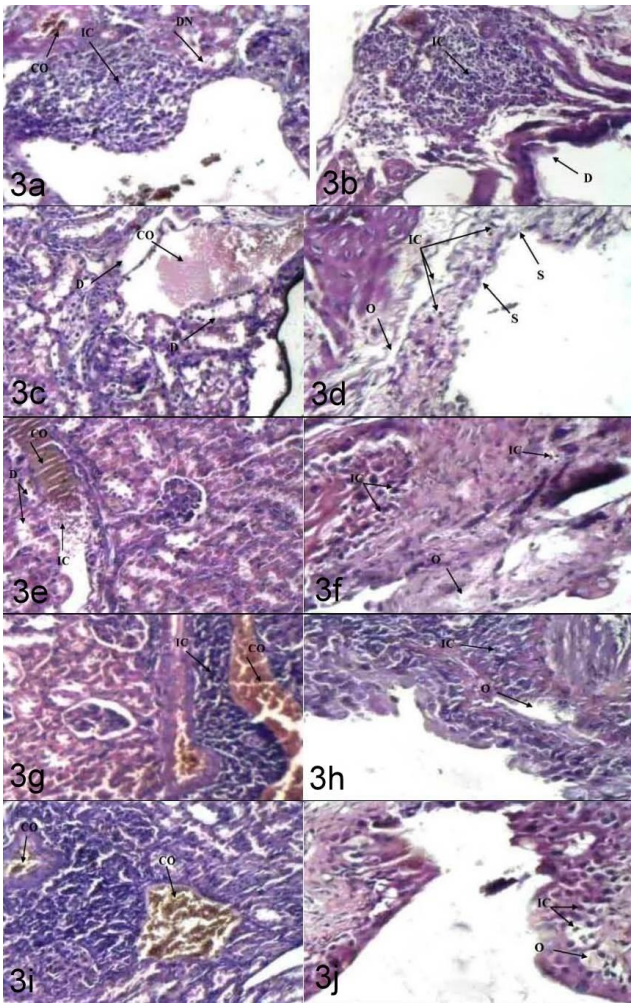

Fig. 3 a: The histological section of mouse kidney 3 days after challenged with 0.1ml of 50 µg/100 µl of *S. aureus* S11 DNA showing congestion (CO), degenerative and necrosis (DN) of renal tubule with severe infiltration of inflammatory cells (IC). **b**: The histological section of mouse urinary bladder 3 days after challenged with 0.1ml of 50 µg/100 µl of *S. aureus* S11 DNA showing damage of mucosal wall (D) with severe inflammatory cells infiltration (IC). **c**: The histological section of mouse kidney 3 days after challenged with 0.1ml of 40 µg/100 µl of *S. aureus* S11 DNA showing congestion (CO) of blood vessel with degenerative changes (D) of renal tubules. **d**: The histological section of mouse urinary bladder 3 days after challenged with 0.1ml of 40 µg/100 µl of *S. aureus* S11 DNA showing mild edema (O) and infiltration of inflammatory cells (IC) with sloughing (S) of mucosal epithelial surface. **e:** The histological section of mouse kidney 3 days after challenged with 0.1ml of 30 µg/100 µl of *S. aureus* S11 DNA showing congestion (CO) and mild degenerative changes (D) of renal tubules with mild inflammatory cells infiltration (IC). **f:** The histological section of mouse urinary bladder 3 days after challenged with 0.1ml of 30 µg/100 µl of *S. aureus* S11 DNA showing edema (O) with infiltration of inflammatory cells (IC). **g**: The histological section of mouse kidney 3 days after challenged with 0.1ml of 50 µg/100 µl of *C. albicans* C3 DNA showing congestion (CO) with moderate inflammatory cells infiltration (IC). **h:** The histological section of mouse urinary bladder 3 days after challenged with 0.1ml of 50 µg/100 µl of *C. albicans* C3 DNA showing edema (O) with mild inflammatory cells infiltration (IC). **i:** The histological section of mouse kidney 3 days after challenged with 0.1ml of 40 µg/100 µl of *C. albicans* C3 DNA showing congestion (CO) of blood vessels. **j:** The histological section of mouse urinary

bladder 3 days after challenged with 0.1ml of 40 µg/100 µl of *C. albicans* C3 DNA showing mild inflammatory cells (IC) infiltration and edema (O). H & E (X200).

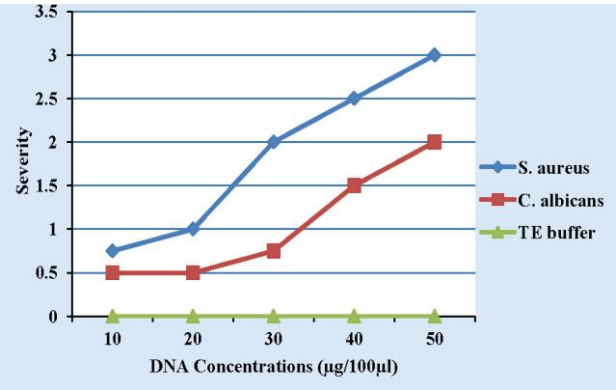

Fig. 4 Effect of DNA concentrations on histological changes severity

Discussion

Tissue damage was developed with features relatively less intense in mice challenged with *C. albicans* than those whom challenged with *S. aureus*. That may be due various virulence factors produced by *S. aureus* of [11] and *C. albicans* [12]. From the previous findings it was concluded that the DNA extracted from *S. aureus* S_{11} bacteria and *C. albicans* C_3 yeast may function as a virulence factor of pathogenic microbes via histopathological effects that caused to kidneys and bladders treated by both DNA.

The inflammatory effects of DNA may result from the production of cytokines and chemokines [13]. Recognition of microbial DNA by TLR 9 in mammalian phagocytic cell triggers an immunostimulatory cascade that culminates in the maturation, differentiation, and/or proliferation of multiple cell types. Together, these cells secrete cytokines and chemokines that create proinflammatory immune stimuli and other mediators of inflammation in addition to reactive oxygen species generation [14-17]. Thus, this cell population and its soluble products are crucial to the observed inflammatory response to DNA [18], and might participate, directly or indirectly, in the pathogenesis of urinary tract infection.

Comparison between S. aureus DNA and C. albicans DNA

The present study indicates that histological changes caused by eukaryotic fungal pathogen *C. albicans* C_3 DNA were generally with potency lower than that of prokaryotic bacterial pathogen *S. aureus* S_{11} DNA.

They were of approximately similar GC content DNA organism (32.5 ٪ GC of *S. aureus* DNA and 35.1% GC of *C. albicans* DNA) in order to show that the differences observed in histological changes between both were not due to the difference in frequency of CG (fCG). As that the dinucleotide (CG) showed a frequency as expected from the individual G+C content and that an increase in fCG went along with increased immunostimulation [19].

Candida albicans DNA have mitogenic properties for immune cells that are similar to those of bacterial DNA, which correlated with the presence of nonmethylated CG dinucleotides and suggest that the CpG motif-containing DNA may contribute to the development of inflammatory responses after infection with *C. albicans*. In addition *C. albicans* DNA may contain as yet unidentified immunostimulatory structure other than CpG motifs that may be present at a higher frequency in this organism [20].

There are several explanations why *C. albicans* DNA may not be a strong inflammatory agent: 1) *C. albicans* DNA contains methylated DNA which diminishes the activity of CpG DNA via interaction with TLR9 [21, 22]; 2) the CpG frequency of *C. albicans* DNA may be less than that of *S. aureus* and may explain the difference in the activating capabilities of these two DNA; 3) it may be that candidal DNA has inhibitory motifs as that of vertebrate DNA which inhibit the immunostimulatory function of unmethylated CpG motifs [3]. Miyazato *et al.* [20] hypothesized that some inhibitory effect of *C. albicans* DNA could interfere with the DNA stimulation. This could be due to the inhibition of DNA uptake by some molecules or inhibitory DNA sequences that can bind TLR9 sufficiently and neutralized the CpG induced activation of immune cells. Finally, alternatively, stimulation by unmethylated CpG motifs require optimal flanking sequences and/or depend on localization of the unmethylated CpG motifs within the genomic DNA sequence. Hence, the high potency of bacterial DNA probably reflects the presence of optimal flanking sequences around CpG motifs which is known that the optimal flanking sequences higher for bacterial than for eukaryotic DNA [14]. In this respect, the optimal flanking sequences may be higher for *S. aureus* than for *C. albicans* DNA.

Effect of DNA concentration on histological changes

DNA from *S. aureus* S_{11} induced significant histopathological changes starting from 10 µg/100 µl and increasing up to 50 µg/100 µl. DNA from *C. albicans* C_3 was effective at concentrations above 30 µg/100 µl but developed less changes than observed with *S. aureus* S_{11} DNA. Most of these results agreed with many studies correlated with the important role of DNA in the pathogenesis of microbes [5, 14, 16, 20, 21].

The histological changes severity evaluated with regard to the extent of the inflammation and judged on an arbitrary scale (Figure 4); from Grade 0 (no signs of inflammation), Grade 1 (mild diffuse or focal inflammation in a single area), to grade 2 (moderate diffuse or focal inflammation), and Grade 3 (heavy diffuse and focal inflammation) [18]. Mölne *et al.* [18] reported that when *S. aureus* DNA was injected intradermally into mice, moderate to heavy inflammatory changes in skin and focal inflammation in an extended pattern were triggered. Tasaka *et al.* [23] mentioned that intratracheal instillation of CpG-ODN revealed acute lung injury in a dose-dependent effect.

In issue of periodontal disease Takeshita *et al.* [16] suggested that *Porphyromonas gingivalis* DNA may function as a virulence factor in periodontal disease through expression of inflammatory cytokine. The bacterial DNA markedly stimulated in a dose-dependent manner IL-6 production by human gingival fibroblasts. In a study done by Al-Mathkhury and Al-Zubeidy [15], *E. coli* DNA caused shrinkage of glomerulus and increased capsular space, edema and inflammatory cells infiltration in kidney tissue while the urinary bladder suffered from infiltration of inflammatory cells. Anders *et al.* [17] found that *E. coli* DNA increased serum DNA autoantibodies in association with progression of glomerulonephritis.

Conclusion

Bacterial DNA has the ability to cause damage in renal tissue in a dose dependent manner. Additionally, this damage intense was more than candidal DNA. Furthermore, even though the microbe is killed or auloysed the risk of inflammation is still need to be investigated.

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