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URINARY TRACT INFECTION CAUSED BY STAPHYLOCOCCUS SAPROPHYTICUS ISOLATED FROM ANIMAL

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Abstract:

This study was carreid out to isolate *Staphylococcus Saprophyticus* from animals (cow , sheep) in AL-Shatraa city as causative agent of a urinary tract infection. ,observe their antibiotic sensitivity pattern and test their pathgensity in rats as model .

To reach this goal 75 urine sample were gathered from animal (cow and sheep) with urinary tract infection in Thi-Qar city all these sample were cultured on different media (blood agar ,mannitol salt agar, nuterint agar) incubated in 37° C for 24 hours the isolates were diagonsed according to cultured and microscopic and many biochemical test, 14 isolate obtained (18.66%) ,the sensitivity of *S.saprophyticus* for antibiotic also tested and the result showed that the isolated *S.saprophyticus* highly sensitive to gentamcin (85.71%) and cefriaxone (78.57%) also most isolate were sensitive Ciprofloxacin with a percentage (71.42%) isolates. Other antibiotic sensitivity ranged from 64.28% to as low as 7.14%.

The results of the pathogenesis study of *S.saprophyticus* in rats (after two day of injection of *S.saprophyticus* with a dose 240 colony forming unit in urethra) showed acute histopathological change.

Keywords: Staphylococcus Saprophyticus , pathogenesis.

INTRODUCTION:

Staphylococcus saprophyticus, a Gram-positive bacterium, is responsible for 5 to 15% of simple UTIs. (1). Additionally, S. Saprophyticus has been found in 7% of rectal swabs obtained from the carcasses of pigs and cattle. The bacteria is a frequent food sample contamination, especially in raw beef and pork. (2). The organism may appear briefly and in tiny numbers on healthy skin, in the periurethral area, and in the urethral region. (3). According to several investigations, S. saprophyticus rectal, vaginal, and urethral colonization was linked to UTIs brought on by this With the exception of nalidixic acid, S. saprophyticus is often responsive to antibiotics that are frequently recommended to patients with UTI. Furthermore, empirical therapy without urine culture is frequently employed in patients with acute uncomplicated cystitis. The foundation for this methodology is a highly predictable spectrum of the etiologic agents that cause UTI and their patterns of antibiotic resistance. However, the prevalence of antibiotic resistance among uropathogens that cause pyelonephritis and cystitis in community-acquired UTIs is rising.(1)

bacterium. The bacteria is capable of adhering specifically to human urothelium, which results in direct hemagglutination. A combination of extracellular enzymes produced by this staphylococcal species has the ability to prevent the growth of gram positive and gram negative bacteria like Neisseria gonorrhoeae and S. aureus. (3 ,4). The virulence factors of S. Saprophyticus include the generation of extracellular slime, hemagglutinin that binds to fibronectin, a hemolysin, and adhesion to urothelial cells via a surface-associated protein, lipoteichoic acid. (5).

MATERIAL AND METHOD:-

Urine sample cather from animals (cows and sheep) suffer from UTI in different region of Al-Shatraa city. After collection The samples were processed as soon as they arrived at the laboratory in thermal boxes while being transported under refrigeration..

The sample were seeded onto blood agar with 5% sheep blood and MacConky agar mannitol salt agar then stained by the Gram method. By using the catalase test, Gram-positive organisms were discovered., the coagulase test were done in order to differentiate the coagulase negative staphylococcus CoNS (*S.Saprophyticus* and *S.epidermidis*) from coagulase negative staphylococcus (*Staphylococcus aureus*) as recommended by (6).

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The *S.saprophyticus* was identified according to different biochemical test such as mannitol fermentation, hemolysin production ,urease production, nitrate reduction, novobiocin resistance .
Novobiocin test (5 µg) Susceptibility was defined as the existence of an inhibition halo > 16 mm, whereas resistance was defined as the presence of an inhibition halo ≤12 mm or the absence of a halo. (6).Resistant strains were identified as *S. saprophyticus* and susceptible strains were considered to be *Staphylococcus epidermidis* (7).
By using the Mueller-Hinton agar and the modified Kirby-Bauer technique, the isolated organisms' sensitivity pattern was identified. (8). Amoxycillin (10 gm), cloxacillin (5 gm), co-trimoxozole (25 gm), gentamicin (10 gm), ciprofloxacin (5 gm), cephalexin (30 gm), ceftriaxone (30 gm), ceftazidime (30 gm), nalidixic acid (30 gm), and nitrofurantoin were the antibiotic discs (300gm).

PATHOGENSIS STUDY:-
Preparation of animal: Six rats (185-210 Body Weight and 2-3 mouth), these rats put in two group 3 in each one.
Group 1:three rats infected with a dose 240 cfu/ml ,uretheral injection.
Gruop 2:three rats injected with phosphate puffer saline, injected in urethera.
After 48 hours kidney and Bladder harvested from each each rat and put in 10% formalin for histopathological examination .

RESULTS: -
Different morphological and biochemical tests were done to identified *Staphylococcus saprophyticus* table no.(1) This table also showed that *S.saprophyticus* isolate was (100%) resistant to the antibiotic novobiocin when used as diagnosed method. In laboratories with limited resources, the novobiocin disk provides a workable substitute for identifying S. saprophyticus in urine samples

Table (1)Morphological and biochemical tests of S.saprophyticus

Bacteria	**Morphological examination**		**Biochemical tests**		**Novobiocin test**
Staphylococcus saprophyticus	**Gram stain**	**+**	**Urease**	**+**	**100% sensative**
	Blood agar	**Non hemolysis**	**Motility test**	**-**	
	MacConkey agar culture	**No growth**	**Catalase test**	**+**	
	Mannitol Salt agar culture	**Clear colonies (not ferment manitol)**			

14(18.66%) isolate of *s.saprophyticus* obtained from 75 sample from animal (cow,sheep) with UTI. The result of antibiotic sensitivity tests for the 14 isolate have been shown that most strian of *S.saprophyticus* high sensitivity was observed to gentamcin (85.71%) and cefriaxone(78.57%) .71.42% of the isolates sensitive to Ciprofloxacin. Other antibiotics were varied in their activity against the isolates from 64.28% to as low as 7.14% table No. 2.

Table (2)antibiotic sensitivity test for S.saprophyticus

Antibiotic	**Sensitive**	**Resistant**
Gentamicin	**12(85.71%)**	**2(14.28%)**
Ceftriaxone	**11(78.57%)**	**3(21.42%)**
Ciprofloxacin	**10(71.42%)**	**4(28.57%)**
Ceftazidime	**9(64.28%)**	**5(35.71%)**
Cephalexin	**8(57.14%)**	**6(42.85%)**
Cloxacillin	**6(42.85%)**	**8(57.14%)**
Nitrofurantoin	**6(42.85%)**	**8(57.14%)**
Cotrimoxazole	**5(35.71%)**	**9(64.28%)**
Nalidixic acid	**3(21.42%)**	**11(78.57%)**
Amoxycillin	**1(7.14%)**	**13(92.85%)**

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The histopathological section of the bladder tissue after 2 days injection with virulent *S.saprophyticus* isolate with a dose 240 coloy forming unit injected in urethra Were showed lesion represented by dilatation of renal tubules with vaccuolation & increase in size of the cell lying renal tubules in kidney figure (1) while showing infiltration of inflammatory cells in subepithelial layer, inflammatory cells, in particular neutrophils, attaching to the endothelial cell in the urinary bladder, alsothere is oedema and congestion in the blood vessels. Figure(2) in compare with normal tissue fig.(3) and (4).

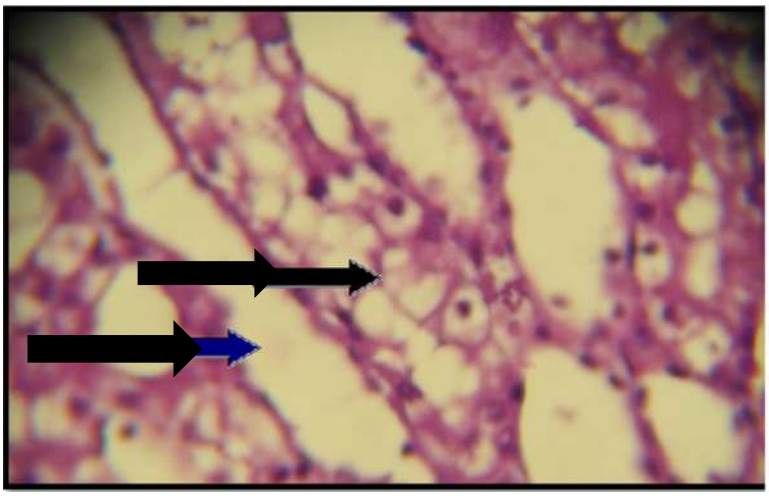

Figure (1): Two days after S. saprophyticus infection, a histological section of the kidney reveals dilated renal tubules with vacuolation and enlarged cell-lying renal tubules. (H&E stain 40X)

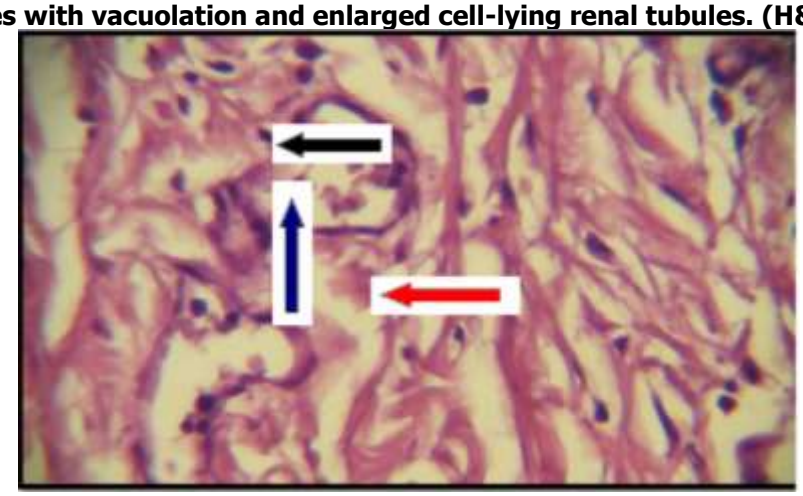

Figure (2). Two days after S. saprophyticus infection, a histological section of the urinary bladder of one rat reveals oedema with mononuclear cells in the subepithelial layer, congested blood vessels, and inflammatory cells in their lumen (neutrophils). (H&E stain 40X)

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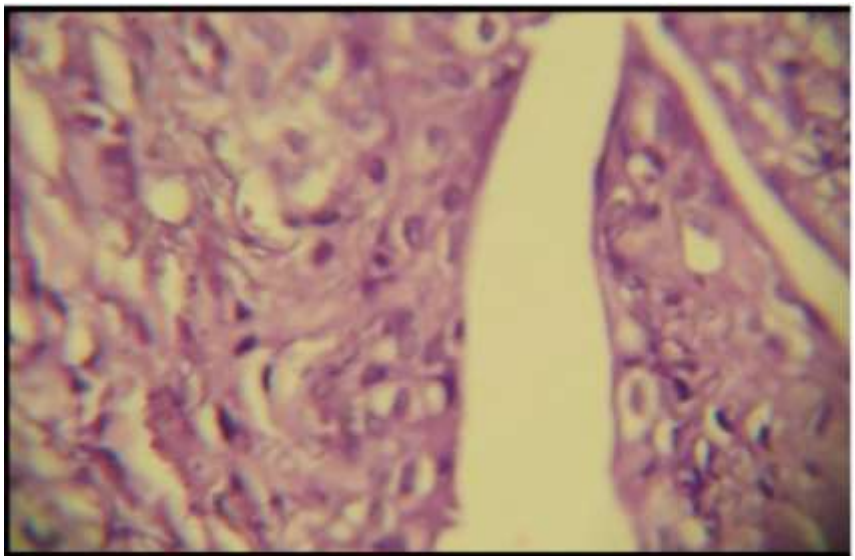

Figure (3): Histological section in normal urinary bladder showed no pathological changes and normal structure of uriny bladder (H&E stain 40X)

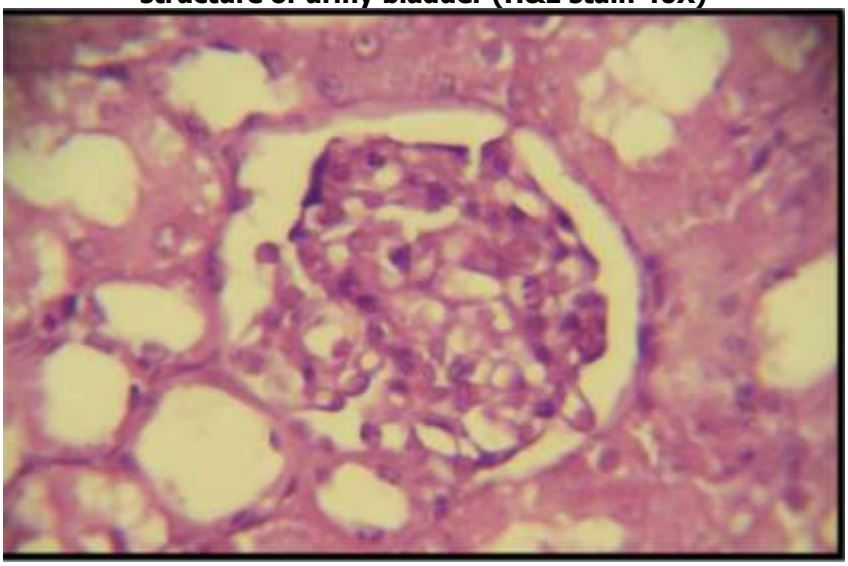

Figure (4): Histological section in normal animal showed no histopathological change in structure of kidney (E&H Stain 40X)

DISSCUSION:

The result showed that *Staphylococcus saprophyticus* found in (18.66%) of the isolate these result was disagree with(9) who isolate the *S.saprophyticus* from animal specimen (taken from urine, rectal and vaginal swabs) with a percentage (5.33%).

In the last three decade , there have been many reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections (10).The results of antibiotic sensitivity tests isolate have been shown that most strian of *S.saprophyticus* were resistant to nalidixic acid this is agree with(11) who found that *S.saprophyticus* was resistant to nalidixic acid in india. This may be properly due to indiscriminate use of antibiotics in our society.The adhesion of bacteria is the first step in pathogensis of UTI following the second step in colonize of bacteria to the gut, perineum, urethra, bladder, renal pelvicalyceal system and renal interstitium (12).The pathogensis experiment in this study showed that the *S.saprophyticus* was able to produce histopathological chane in kidney and urinary bladder these result was agreed with (13) who discovered S.saprophyticus causes oedema, degeneration, and hyperplasia of epithelial lumen cells in the bladder.

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