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# CHARACTERIZATION OF VIRULENCE FACTORS OF *STAPHYLOCOCUS SAPROPHYTICUS* ISOLATED FROM WOMEN WITH CYSTITIS

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## ABSTRACT

The study included isolation and identification of uropathogenic *Staphylococcus saprophyticus* which responsible for cystitis occurrence in young women. A total of 140 mid stream urine sample were taken from women suffering from urinary tract infection that attended to the Hilla general teaching hospital during a period from November 2014 to February 2015. The patients age ranged from 16 to 55 years. Only 14 isolates were identified as *Staphylococcus saprophyticus* which have been diagnosed by using selective medium, biochemical tests and by using molecular assays. Molecular detection of Ubiquitous uro-adherence factor A was done by using specific PCR primer. This marker was shown to be present in all isolates of this bacterium, while the molecular detection of the capsular polysaccharides gene *capD* has shown that only one isolate (7%) has this gene and gave positive result for this primer. Moreover, Autolysin-adhesin surface protein *Aas* was also detected in *S.saprophyticus*. It was shown that all isolates 14(100%) gave positive result for this gene. The collagen binding protein gene *sdrI* was also investigated in this bacteria and this study has shown that only one isolate possess this gene.In addition to that, the surface associated protein gene of *S.saprophyticus Ssp* was detected in this study by using specific primer in 12(85%) of isolates, this gene is responsible for lipase encoding. Some virulence factors were studied by phenotypic method in *S.saprophyticus* isolates. The capsule was present in only one isolate out of 14 whereas the other 13 isolates in this study did not possess the capsule. Colonization factor antigens were also detected in all isolates. It was found that 13(92%) of isolates had CFAI, while 1(7%) of them had CFAII and 3(21%) had the ability to produce CFAIII. The results also showed that all isolates of *S. saprophyticus* did not produce hemolysin, while all

isolates were able to produce urease enzyme.Extracellular protease were also investigated. It was found that 2(14%) of this bacterium were able to produce extracellular protease, whereas the other (86%) didn't have the ability to produce this enzyme. The ability of *S.saprophyticus* to produce lipases was identified and the results showed that 4(28%) of *S.saprophyticus* were able to produce lipase enzyme. Also the investigation of bacteriocin production in *S.saprophyticus* revealed that the isolates had shown different results for this test against different types of bacteria that include (*E.coli* or *P.auroginosa*, *S.epidermidis*, *S. penumoniae*, and *Klebsiella*) but no one of them had bacteriocin against each of *E.coli* and *P.auroginosa*, while 4( 28%) of them had produced bacteriocin against *Klebsilla* and 5(35%) of the isolates had bacteriocin against *S.epidermidis* while 1(7%) against *S .pneumoniae*.

**KEY WORDS:** *S. saprophyticus*, cystitis, PCR**,** virulence factors.

## INTRODUCTION

Cystitis is the medical term for inflammation of the bladder. Most of the time, the inflammation is caused by a bacterial infection, and it's called a urinary tract infection (UTI) Urinary tract infection is considered as common illness among causes for physicians visiting worldwide with *Staphylococcus saprophyticus* being the cause in 5–10% . It is estimated that *S. saprophyticus* causes up to one million UTI each year and it is the second most common cause of uncomplicated UTI in sexually active women.[1][2] This uropathogenic bacterium is characterized by its being coagulase negative which is the most important factor to distinguish between pathogenic *Staphylococcus aureus* and other pathogenic coagulase negative staphylococci. *S.saprophyticus* can cause varity of infections including cystitis, pyelonephritis, urithritis and more sever septicemia, nephrolithiasis, and endocarditis.[3][4] The disease -associated strains often promote infections by producing urease that hydrolyze urea and mediate the bacterial survival in the urinary tract ,expressing cell-wall anchored proteins that play an important role in bacterial virulence for adherence to uroepithelial cells. These proteins include uroepithelial adherence factor A (uafA) which act as adhesin for binding to the bladder cells, another cell wall anchored proteins aresurface -associated lipase (Ssp) which produce fimbria -like appendages and staphylococcal collagen- binding protein (sdrI) which is binding to the collagen of host tissues, these surface proteins mediate *S.saprophyticus* binding to the bladder cells. *S.saprophyticus* has two genes products play a role in mediation of the infection these include a hemagglutinin-autolysin adhesin (Aas) that binding to fibronectin and human ureters and the second is urease which play the most

important role in bacterial colonization to the kidney and bladder, establishment of inflammation in the bladder and also mediates the dissimination of the infection to the spleen.[5][6] *S.saprophyticus* is a causative agent of cystitis in women, which is inhabit the vagina and female genital tract as a normal flora and also found as a normal inhabitant in female perineum which act as an oppurtunistic pathogen that sexual intercourse promotes its spread. This bacterium possess many virulence factors that enabiling it to cause cystitis, The genome sequence of *S.saprophyticus* revealed a number of virulence factors were carried by this bacterium *S.saprophyticus* has gene encoding a cell wall anchored protein called uro-adherence factor A (uafA) which play as an adhesin that mediate adherence and hemagglutination to the cells of human bladder.[5] *S.saprophyticus* has another uro-adherence factor which is (uafB) for attachment to host tissues. The uafb is expressed on the cell surface of *S.saprophyticus* and regarded as major cell surface hydrophobicity factor it is glycosylated serine-rich repeat protein, Its role in virulence revealed by generating an isogenic mutant of uafb in *S.saprophyticus*. The uafB mutant strains showed reduced ability for fibrinogen and fibronectin binding. SdrI is another cell wall anchored protein characterized in *S.saprophyticus*, it is one member of serine –aspartate rich proteins family the function of which is binding to collagen.[7][8] On the other hand surface associated lipase (Ssp) forms surface appendages like fimbria and it play important role in persistence but not for initial colonization[, this protein present in high amount on the cell surface of *S.saprophyticus* strains. *S.saprophyticus* has D-serine deaminase enzyme which catalyses the metabolism of D-serine that is prevalent in urine, the metabolism of D-serine is one of the virulence factor this organism posses, *S.sapophyticus* is the only species among staphylococci that is uropathogenic because it has (D-sdA), D-serine present in urine as bacteriostatic or toxic to many bacteria deserine deaminase enzyme found in the genome of most uropathogens. It has been suggested that the ability to respond and resistance to metabolize D-serine or the posses of (D-sdA) is important for virulence. *S.saprophyticus* metabolism of D-serine has not been described . However it is able to grow in presence of high concentration of D-serine.[9] The surface proteins of staphylococci have been shown to be virulence factors in different infection models, although the mode of the action of many of them has not yet been revealed. The virulence factors known to date include surface structures (adhesions), exoprotiens, some biochemical properties increase staphylococcal survival such as catalase has the ability to convert hydrogen peroxide to water and oxygen, the production of super oxidize ions can also leads to tissue necrosis.[10][11] S.saprophyticus bacterium is the second cause of UTI occurrence after *E.coli*. For this reason and the few studies about *S.saprophyticus* and

because of the critical importance of this bacterium has dealt with this topic, search that aimed to study the phenotypic and molecular characteristics of virulence factors and antibiotic resistance of *Staphylococcus saprophyticus* that isolated from women with cystitis.

## MATERIALS AND METHODS

### 1-Urine specimen collection

The 140 mid stream urine specimen was collected from women suffering from cystitis. The midstream urine specimens were collected in a sterile disposable cup to avoid any possible contamination. The urine was taken to the laboratory during the first half hour of taking. Each specimen was immediately inoculated on the mannitol salt agar medium, blood agar medium and nutrient agar usinga sterile platinum loop and incubated aerobically for 24 hrs. at 37°C.

### 2-Identification of *S. saprophyticus* isolates

The urine sample was cultured on to selective medium which is Mannitol salt agar ,then incubated at 37C° for 24 hrs. Each isolate of *S.saprophyticus* was identified depending on morphological and biochemical tests that recommended by.[12][18]

### 3-PCR analysis

Genomic DNA of the *S. saprophyticus* isolates was extracted using the DNA purification Kit provided by the manufacturing company (Viogene,Taiwan). Suspending the bacteria in the recommended buffer for gram-positive bacteria and the addition of 100 µg lysostaphin at the lysis step. For the amplification of the genes three different PCR-programs were used with an initial denaturation at 94°C for 5 min and final extension at 72°C for 7 min, and 35 cycles. Program 1 (sdrI) : 94°C 30 s, 50°C 30 s, 72°C 1 min; Program 2 (ssp, uafA): 94°C 30 s,55°C 30 s, 72°C 2 min; Program 3 (capD, Aas): 94°C 30 s, 50°C 30 s, 72°C 30 s. The following primers were used: *sdr*I fwd GGATAAAAATAGCACAATCGACGAA/rev-CA AGGCTATATTTAGGTGTT, 1624 bp; *ssp* fwdAAATTCAGAGAATTAGTAGCC/rev-A TGAAGAGTTACGTTCACAC,3164bp;*uafA*fwdCGCGGATCCCCAACATCAGAAGTAT ATGG/ rev-GCGAAGCTTGTGTCAGAAACTAAACCAGC, 2267 bp; *capD* fwd-CGT TCAAGATAAAGAGCG/ rev TTCACCAGATCTAATGCC, 604 bp; *aas*fwd- CAGGTAC CGTTAAAGTAC/rev-GATACAACTA ACTTGGCAG, 505 bp.

## RESULTS AND DISCUSSION

Five virulence genes were studied in *S. saprophyticus* isolates using molecular technique. That is uroadherence factor A (*UafA),* autolysin *Aas,* the surface-protein and lipase gene *Ssp* were present in 100% of *S. saprophyticus* isolates. The capsular gene (*cap*D) could only be found in one clinical isolates(7%). Another collagen binding gene *SdrI* was present in (7%) of the clinical isolates.

**Molecular detection of *uafA* gene**

The specific PCR primer was used in the molecular finding of ubiquitous surface hemagglutinin protein uafA among *S.saprophyticus* isolates. It was found that *uafA* gene was observed in 100% of bacterial isolates as shown in Figure (1).

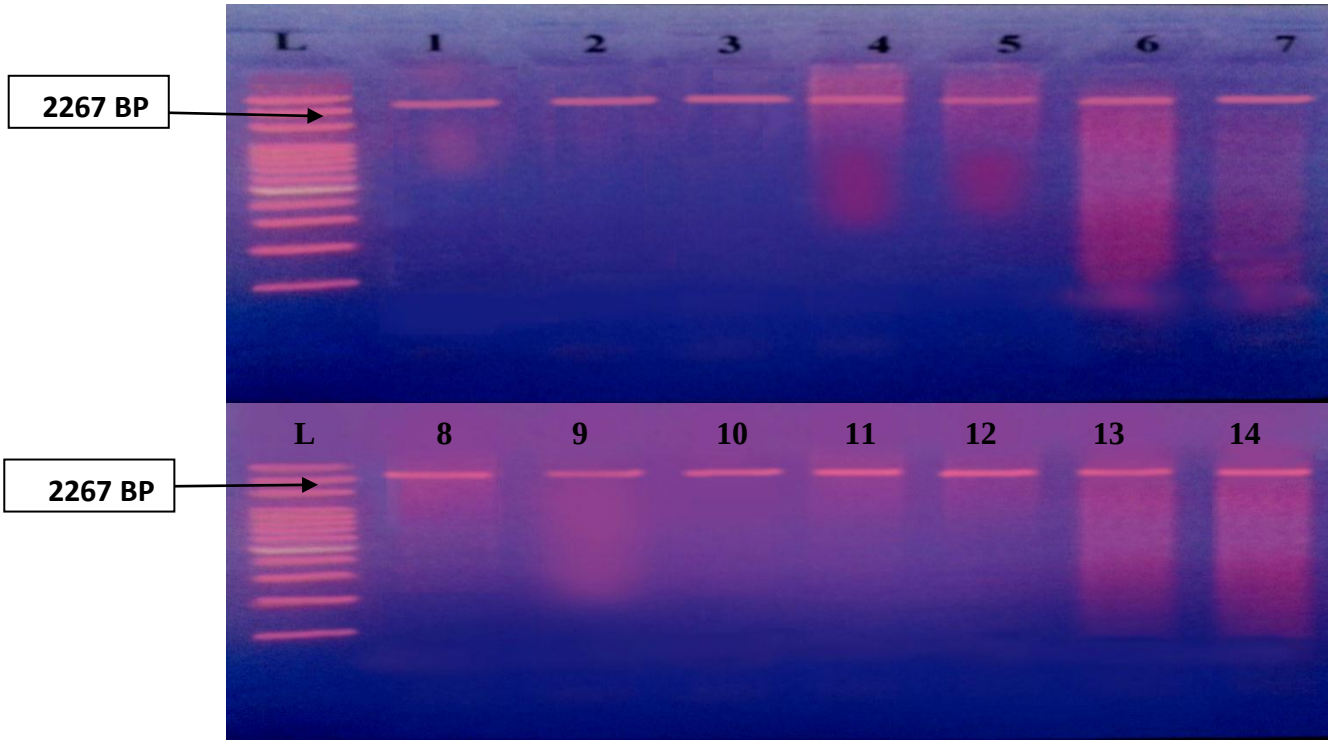



**Figure (1) Gel electrophoresis of PCR product of *UafA* (The isolates No. 1, 2, 3, 4, 5, 6,7, 8, 9, 10 , 11 ,12 ,13 ,14) were positive for *UafA* , L=Ladder**

The positive result of the *UafA* marker will allow the bacteria to attach to the mucosal surfaces of the host uroepithelial cells, which is a crucial step in colonization. It was shown that the *UafA* mutants were assayed for the adherence, it showed reduced capabilities to binding to human uroepithelial cells that resolved the important function of UafA in the binding activity.[4] Our results are identical to the results obtained by Kleine and his co-worker.[13]

**Molecular detection of *Aas* gene**

In this study specific marker was used for detection of Aas gene,all the isolates of *S.saprophyticus* gave positive result when screened for this gene (as shown in Figure 2).

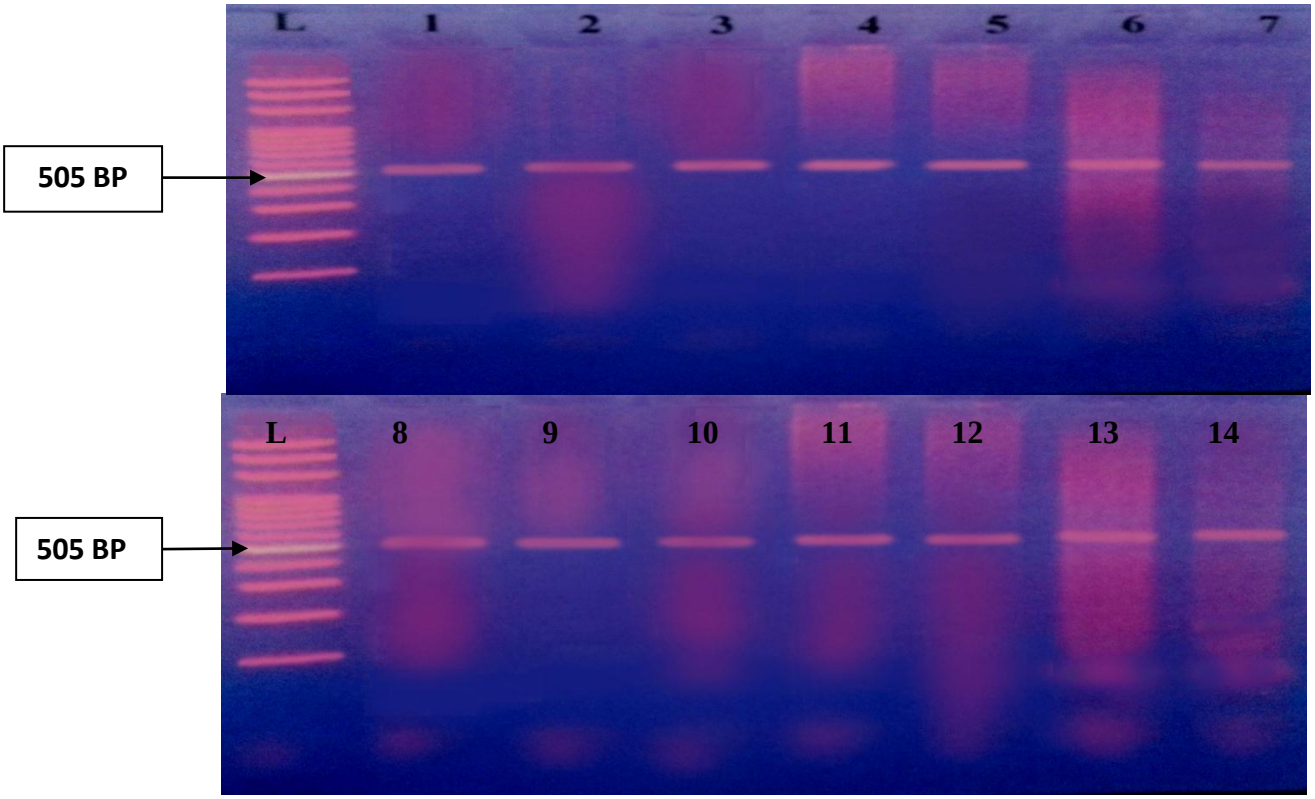
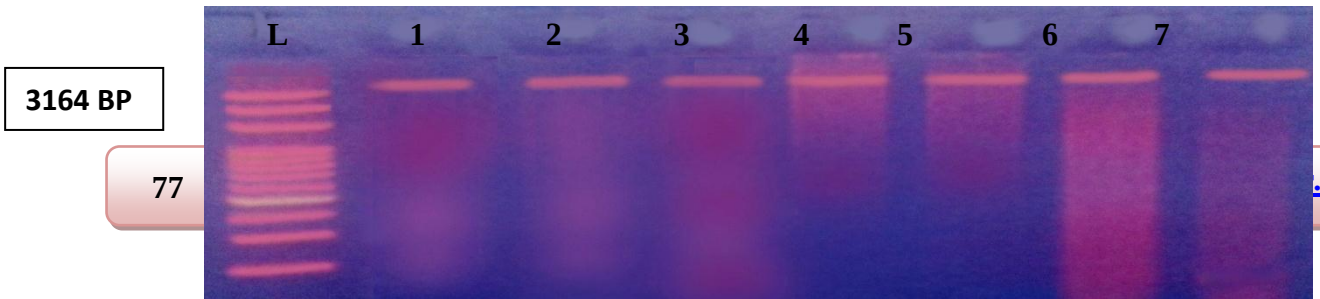



**Figure (2) Gel electrophoresis of PCR product of *Aas* (isolates No.: 1,2,3,4,5,6,7,8,9,10, 11,12,13,14) isolates with positive result for *Aas*. L= ladder.**

The bacterial autolysins are powerful lethal enzymes, that hydrolyze some moieties of peptidoglycan. They play an important role in the bacterial cell separation. Aas exhibit adhesive properties and in addition to fibronectin binding, it can binds avidly to human uroepithelial cells and sheep erythrocyte surface protein to facilitate the cell entry.[14] Our results were in agreement with Kleine and his co-worker[13] result who had pointed that this gene is present in all isolates of this bacteria that were isolated from human.

**Molecular detection of *Ssp* gene**

The *Ssp* gene is the essential genetic factor that encodes the production of lipase enzyme. By using specific PCR primer this gene was detected. It was found that among 14(100%) isolates of *S.saprophyticus* gave positive result and revealed its possession of this gene (Figure 3).



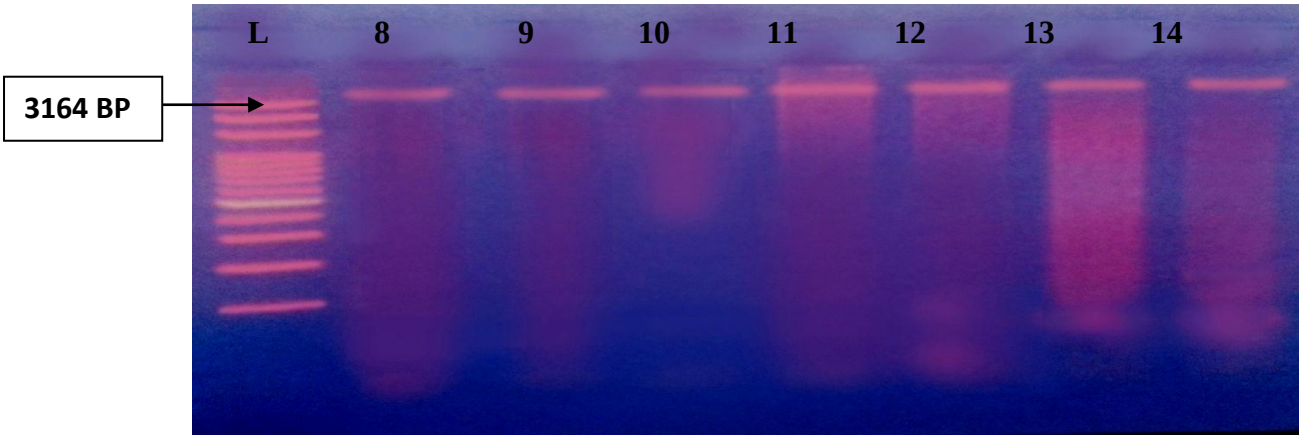



**Figure (3) Gel electrophoresis of PCR product of *Ssp gene* (1,2,3,4,5,6 ,7,8,9,10,11,12,13,14) isolates with positive result for *Ssp* gene, L= ladder**

Phenotypically high percent of *S.saprophyticus* isolates were found to be able to produce lipase enzyme extracellularly and many strains have a second type of lipase that will give an interpretation that this enzyme may be encoded by more than one genetic loci.[5] Lipase function regarded to the pathogenicity in the urinary tract has not been determined but its activity on the skin was reported by hydrolysis of triacylglycerols into glycerol and free fatty acid this may due to the absence of lipid in the urine.[6][17]

**Molecular detection of *capD* gene**

Molecular detection of *capD* gene among *S.saprophyticus* isolates was done by using specific marker. In this study we found that only one isolates gave positive result for this primer as shown in Figure (4).

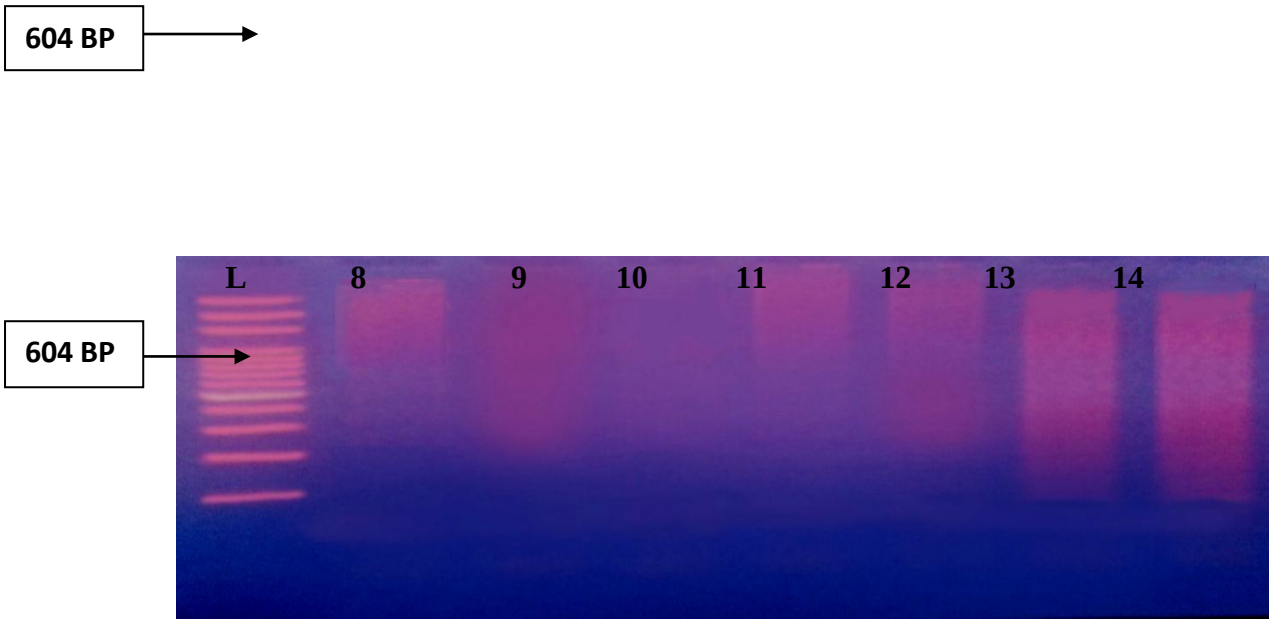



**Figure (4) Gel electrophoresis of PCR product of *capD gene* Only one isolate No.(1) with positive result (2,3,4,5,6,7,8,9,10,11,12,13,14 ) isolates were negative, L=Ladder**

Kuroda and his co.workers[5] has pointed that capsular polysaccarides envelop the surface of the bacteria, that resulting in the inhibition of bacterial adherence that mediated by UafA protein because the CP masks the UafA protein. In our study the prevalence of *CapD* positive gene was 1(7%) that disagree with Kleine and his co.workers[13] and Park and his co.workers[14] who had reported that about 1.3% and 78.5% ,respectively, of *S.saprophyticus* isolates were encapsulated. These differences in the sequence of this marker may give rise to these differences**.**

**Molecular detection of *SdrI* gene**

Collagen binding MSCRAMM *SdrI* gene was detected in *S.saprophyticus* isolates as shown in Figure 5. It was shown that in our study there is only one isolates gave positive result for *SdrI* gene. This result is an agreement with[13] results of this gene.

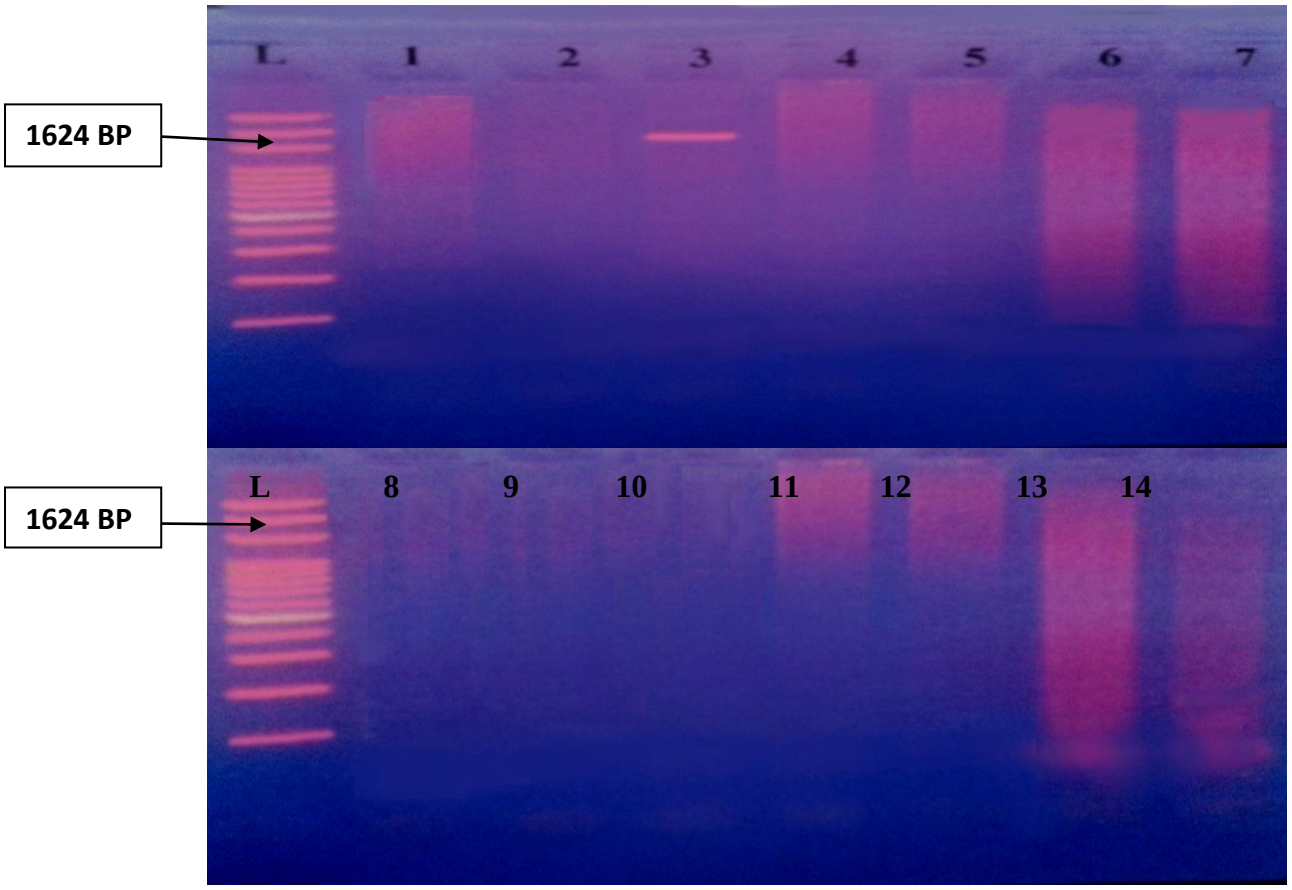



**Figure (5) Gel electrophoresis of PCR product of *SdrI*: Isolate No.(3) with positive results for *SdrI*.While (1,2,4,5,6,7,8,9,10,11,12,13, and 14) with negative result.**

*S.saprophyticus* collagen binding protein belongs to the serine aspartate repeat proteins which expressed on the bacterial cell surface. This protein was not necessary for initial colonization of this bacterium, but it has essential role in the persistence of the bacteria in the kidney and the bladder.[13][16]

**Detection of virulence factors *S.saprophyticus* isolates by phenotypic methods:**

| No. of *S. saprophyticus* Isolates | Gelatinase | Protease | Lipase | Urease | Sidrophore | Heamolycin |
|---|---|---|---|---|---|---|
| 1. | — | — | + | + | _ | γ |
| 2. | — | — | — | + | _ | γ |
| 3. | — | — | — | + | _ | γ |
| 4. | — | — | + | + | _ | γ |
| 5. | — | — | — | + | _ | γ |
| 6. | — | + | + | + | _ | γ |
| 7. | — | + | — | + | _ | γ |
| 8. | — | — | + | + | _ | γ |
| 9. | — | — | + | + | _ | γ |
| 10. | — | — | — | + | _ | γ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **11.** | — | — | — | + | _ | γ |
| **12.** | — | — | + | + | _ | γ |
| **13.** | — | — | + | + | _ | γ |
| **14.** | — | — | + | + | _ | γ |
| **%** | **0(0.0%)** | **2(14%)** | **8(57%)** | **14(100%)** | **14(100%)** | **14(100%)** |

**Production of adherence, biofilm, capsule and colonization factors among *S.saprophyticus* isolates**

| No .of *S. saprophyticus* isolates | Capsule | Biofilm | Adherence activity | CFA-I | CFA-II | CFA-III |
|---|---|---|---|---|---|---|
| **1.** | + | — | + | + | — | — |
| **2.** | — | — | + | + | — | — |
| **3.** | — | — | + | + | — | — |
| **4.** | — | + | + | + | — | — |
| **5.** | — | + | + | + | — | — |
| **6.** | — | — | + | + | — | — |
| **7.** | — | — | + | + | — | — |
| **8.** | — | — | + | + | — | — |
| **9.** | — | — | + | — | — | — |
| **10.** | — | — | + | + | — | + |
| **11.** | — | — | — | + | — | — |
| **12.** | — | — | + | + | — | + |
| **13.** | — | — | — | + | — | + |
| **14.** | — | — | + | + | — | — |
| **%** | **1(7%)** | **2(14%)** | **12(85%)** | **13(92%)** | **1(7%)** | **3(21%)** |

### 3.3.9. Bacteriocin production

Production of bacteriocin was detected by using all *S.saprophyticus* isolates as producers for bacteriocin against indicator strains. In this study five types of different Gram positive and Gram negative bacteria were used as indicators to see the influence of *S.saprophyticus* bacteriocin against them . These include (*Klebsiella*, *E.coli*, *S.epidermidis*, *S.pneumonie* and *P.auroginosa*). The 14 isolates of *S.saprophyticus* give different results but no one of them has bacteriocin against *E.coli* or *P.auroginosa* , while (28%) of them give positive bacteriocin against *Klebsiella* and (35%) of the isolates have bacteriocin against *S.epidermidis* and (7%) against *S. pneumoniae*, as shown in table 3-4). According to Al-Qassab and Al-Khafaji, (1992).[19] This test was done to detect the production of bacteriocin that is a biological material produced by one microorganism and affect the growth of the other bacteria. Bacteriocin is antimicrobial protein produced by bacteria that kill or inhibit the growth of other bacteria related to the same group or species.[20] Variety of inhibitory substances including bacteriolytic enzymes, phages, and antibiotics were known to produce by

Staphylococci and earlier investigators couldn't identify exactly the nature of the observed inhibitory effects.

The bacteriocins produced by Gram positive bacteria are a potent antimicrobial agent. They usually have no activity against Gram negative bacterial outer membrane. But when the Gram negative bacterial outer membrane is weak this bacteriocin has a potential effect on it.

The result of this study may be attributed to the fact that the outer membrane of *Klebsiella* was weak enough to degrade by *S. saprophyticus* bacteriocin.[21]

Microorganisms naturally produce a range of protein component from simple polypeptides to very complex macromolecules such as siderophores,toxins, pili, adhesions, flagella, etc. Bacteriocins are grouped under the term toxins and provide a means of defense against other microorganism in the same environment.[22] Tagg, *et al.*, (1976)[23] have defined bacteriocin, a subgroup of bacterial toxins, as proteinaceous compound that kill closely related bacteria. Although this is true for most bacteriocins, it is evident that these molecules take many forms and may have bactericidal actions beyond closely related species. Frequently bacterial species carry genes that encode both the production of one or more bacteriocin and immunity to them on a chromosome or on plasmids.

**Investigation of bacteriocin production among *S.saprophyticus* against *Klebsiella, E. coli, S. epidermidis, S. pneumoniae* and *P. auroginosa*:**

| ***S. saprophyticus*** | ***Klebsiella*** | ***E.coli*** | ***S.epidermidis*** | ***S.pneumoniae*** | ***P.auroginosa*** |
|---|---|---|---|---|---|
| **1.** | — | — | — | — | — |
| **2.** | — | — | — | — | — |
| **3.** | + | — | + | + | — |
| **4.** | + | — | + | — | — |
| **5.** | + | — | + | + | — |
| **6.** | + | — | + | — | — |
| **7.** | — | — | — | — | — |
| **8.** | — | — | + | — | — |
| **9.** | — | — | — | — | — |
| **10.** | — | — | — | — | — |
| **11.** | — | — | — | — | — |
| **12.** | — | — | — | — | — |
| **13.** | — | — | — | — | — |
| **14.** | — | — | — | — | — |
| **%** | **4(28%)** | **0(0.0 %)** | **5(35%)** | **2(14%)** | **0(0.0%)** |

## CONCLUSIONS

According to this study, the study concludes that *S.saprophyticus* was prevalent in 10% among cystitis cases in women and the most of isolates in this study have many types of virulence factors that responsible for pathogenicity, among these are virulence factor genes (*UafA*, *Aas* , and *Ssp*) are more common among isolates of *S.saprophyticus*.

### Recommendations

Molecular techniques should be used for detection of other genes those are responsible for the important virulence factors.

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