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Molecular detection of some virulence genes of *Staphylococus saprophyticus* isolated from women with cystitis cases

التوصيف الجزيئي لبعض جينات الضراوة لبكتريا *Staphylococcus saprophyticus* المعزوله من النساء المصابات بالتهاب المثانة

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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 .

الخلاصة

تضمنت هذه الدراسة جمع وزراعة (140)عينة من منتصف تيار عينة الادرار التي جمعت من (140) مريضة مصابات بالتهاب المثانة وممن احيلوا الى مستشفى الحلة التعليمي العام ومن العيادة الخاصة للفئه العمريه من 16- 55 سنة (من اكتوبر 2014 الى يناير2015) وقد تم عزل وتشخيص 14 عزلة فقط من بكتريا *Staphylococcus saprophyticus*.وقد تم تشخيص هذه البكتريا باستخدام الاوساط الزرعيه الانتخابية والاختبارات البايوكيميائية إضافة الى الاختبارات الجزيئية. من ناحية الكشف والتشخيص الجزيئي فقد تم استخدام بادئات نوعيه (Primer) في تقنية تفاعل البلمرة المتسلسل PCR للتحري عن الجينات الخاصة بإنتاج *(uafA)* بروتين وقد أظهرت النتائج بأن جميع العزلات البكترية تمتلك هذا الجين الخاص بالبروتين المسؤول عن الالتصاق بالخلايا الطلائية للقناة البولية. أيضا بواسطة نفس التقنية تم التحري عن الجين المسؤول عن إنتاج المحفظة في هذه البكتريا *capD* وقد أظهرت النتائج بأن عزلة واحدة فقط من بكتريا *S.saprophyticus* تمتلك هذا الجين .علاوة على ذلك، تم الكشف عن الجين المسؤول عن انتاج البروتين الالتصاقي المحلل *Aas* من قبل هذه البكتريا وقد بينت النتائج بأن جميع العزلات أظهرت نتائج إيجابيه لهذا الجين وبنسبة 100% .كما تم التحري عن الجين المنتج للبروتين المسؤول عن الارتباط بالكولاجين في هذه البكتيريا وأظهرت هذه الدراسة أن عزلة واحدة فقط تمتلك هذا الجين.بالإضافة إلى ذلك، في جميع العزلات البكتيرية تم الكشف عن الجين المسؤول عن تشفير وانتاج انزيم اللايبيز *Ssp* في بكتريا *S.saprophyticus* بإستخدام بادئات نوعية خاصة . وقد أظهرت نتائج البحث في هذه الدراسة بأن 12(85٪) من العزلات تمتلك هذا الجين

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 little 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 with cystitis.

Materials and methods

1-Urine specimen collection

The 140 mid sream urine specimen were collected from women suffering from cystitis. The mid stream urine specimens were collected in sterile disposable cup to avoid any possible contamination .The urine were taken to the laboratory during first half hour of taking. Each specimen was immediately inoculated on the mannitol salt agar medium, blood agar medium and nutrient agar using 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 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 fwdGGATAAAAATAGCACAATCGACGAA/rev CAAGGCTATATTTAGGTGTT, 1624 bp; *ssp* fwdAAATTCAGAGAATTAGTAGCC/rev-ATGAAGAGTTACGTTCACAC, 3164 bp; *uafA* fwd CGCGGATCCCCAACATCAGAAGTATATGG/ rev-GCGAAGCTTGTGTCAGAAACTAAACCAGC, 2267 bp; *capD* fwd-CGTTCAAGATAAAGAGCG/ rev TTCACCAGATCTAATGCC, 604 bp; *aas*fwd-CAGGTACCGTTAAAGTAC/rev-GATACAACTA ACTTGGCAG, 505 bp.

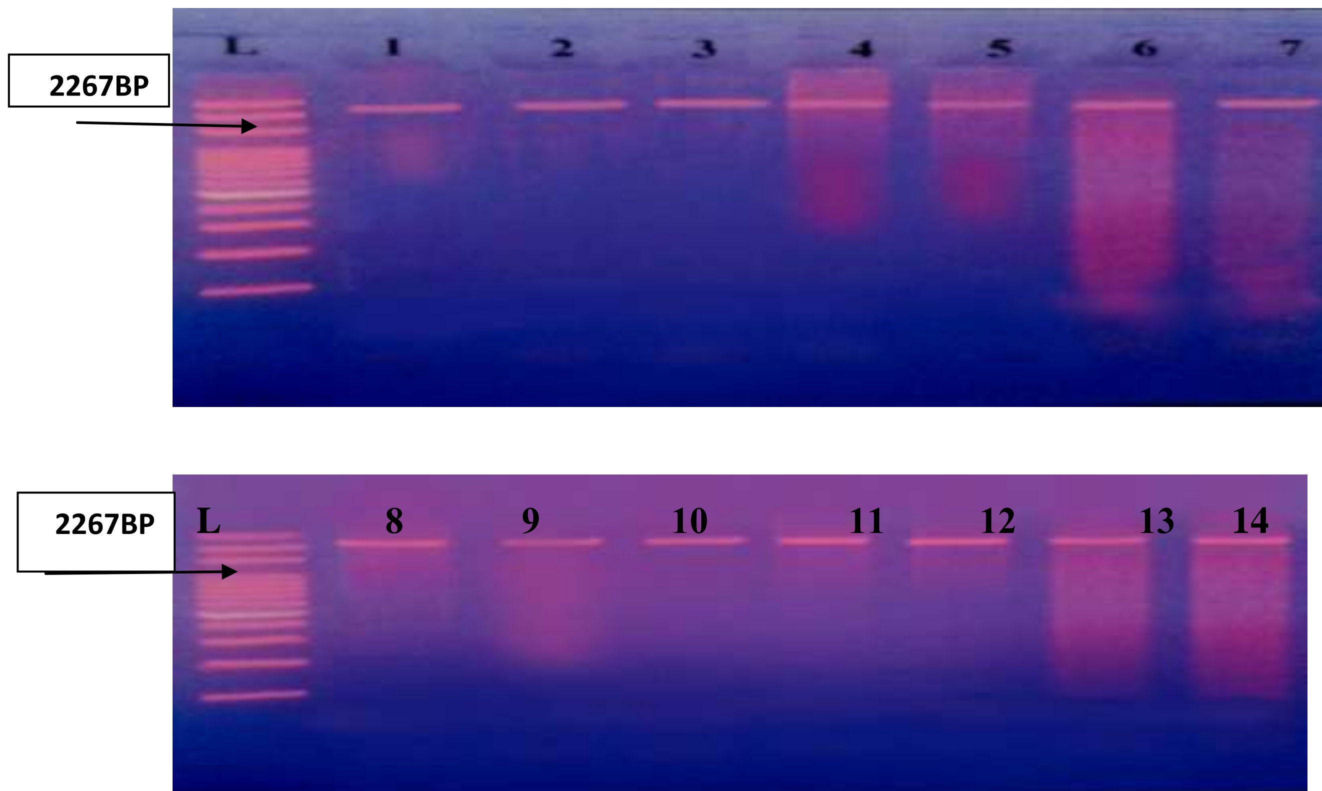
Results and discussion:

Five virulence genes were studied in *S.saprophyticus* isolates using molecular technique. That are 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

specific PCR primer was used for 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).

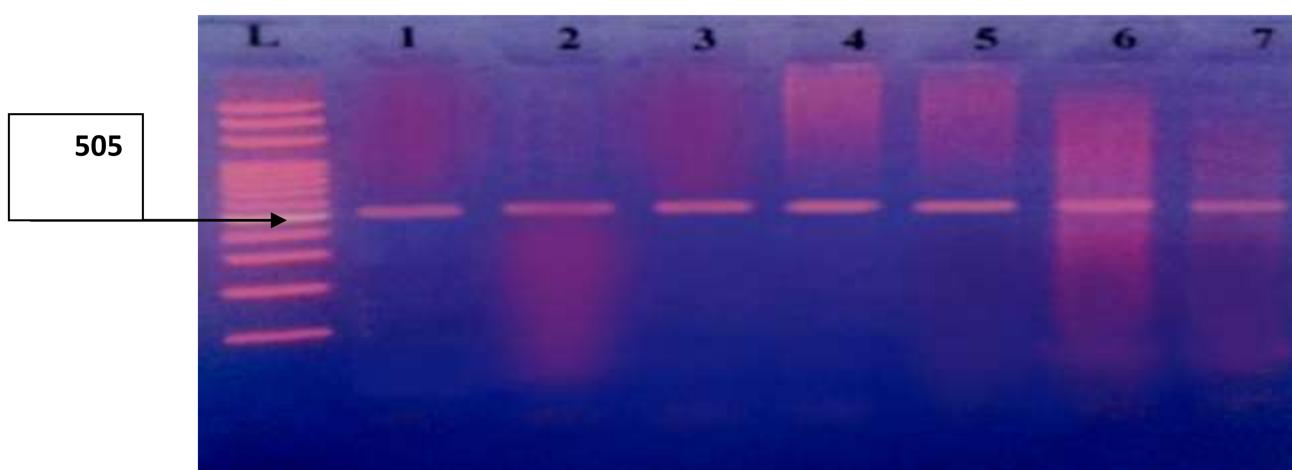
Figure (1) Gel electrophoresis of PCR product of *UafA* (The isolates No. 1, 2, 3, 4, 5, 6,7, 8, 9, 10 , 11 ,12 ,13 ,14) were positive for *UafA* , L=Ladder



The positive result of the *UafA* marker will allow the bacteria to attach to the mucosal surfaces of the host uroepithelial cells , which is an 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 with 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).



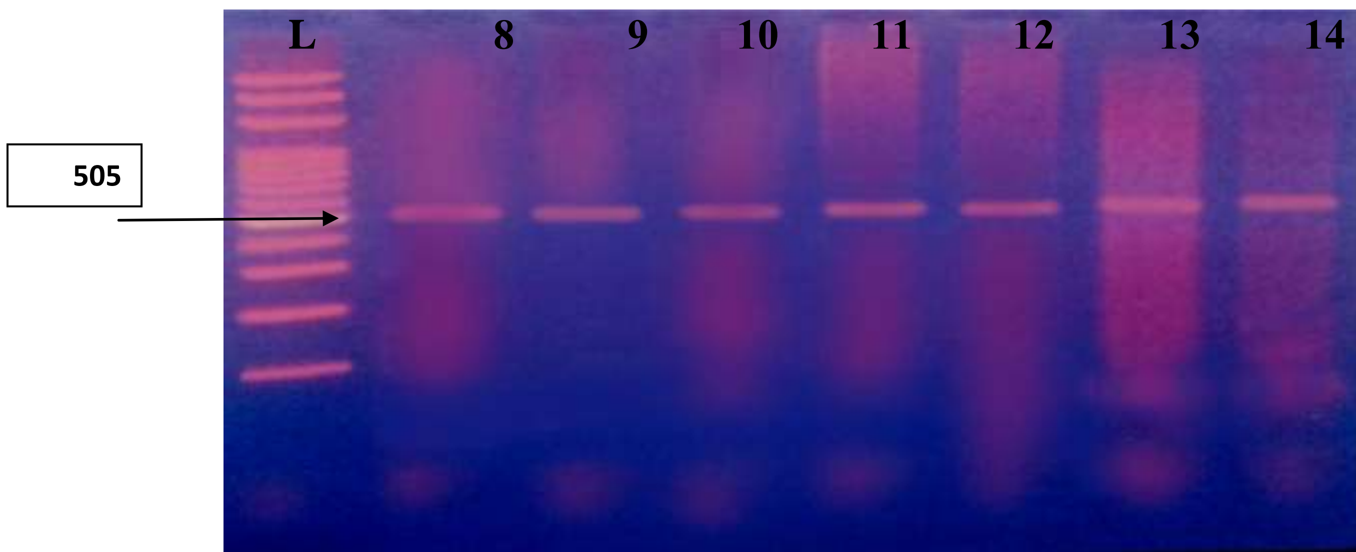
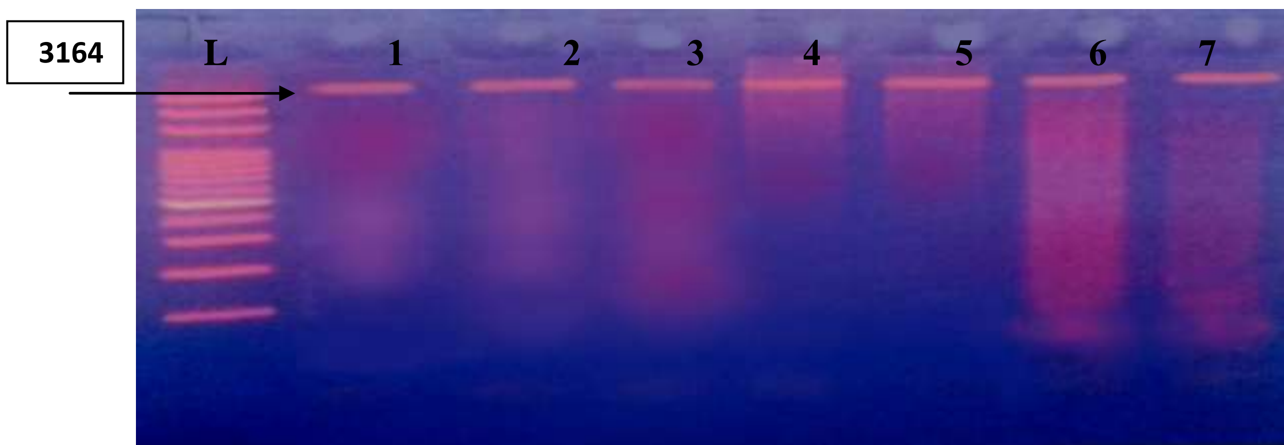


Figure (2) Gel electrophoresis of PCR product of *Aas* (isolates No.: 1,2,3,4,5,6,7,8,9,10,11,12,13,14) isolates with positive result for *Aas***.** L= ladder.

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 was 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 encode 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)**.**



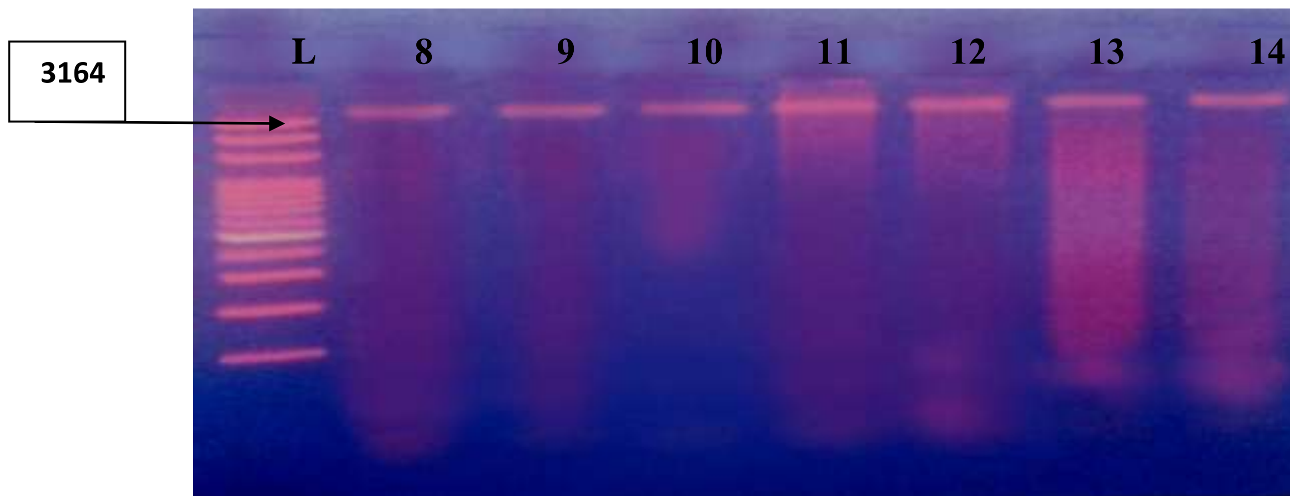


Figure (3) Gel electrophoresis of PCR product of *Ssp gene*
(1,2,3,4,5,6 ,7,8,9,10,11,12,13,14) isolates with positive result for *Ssp* gene ,L= ladder

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)

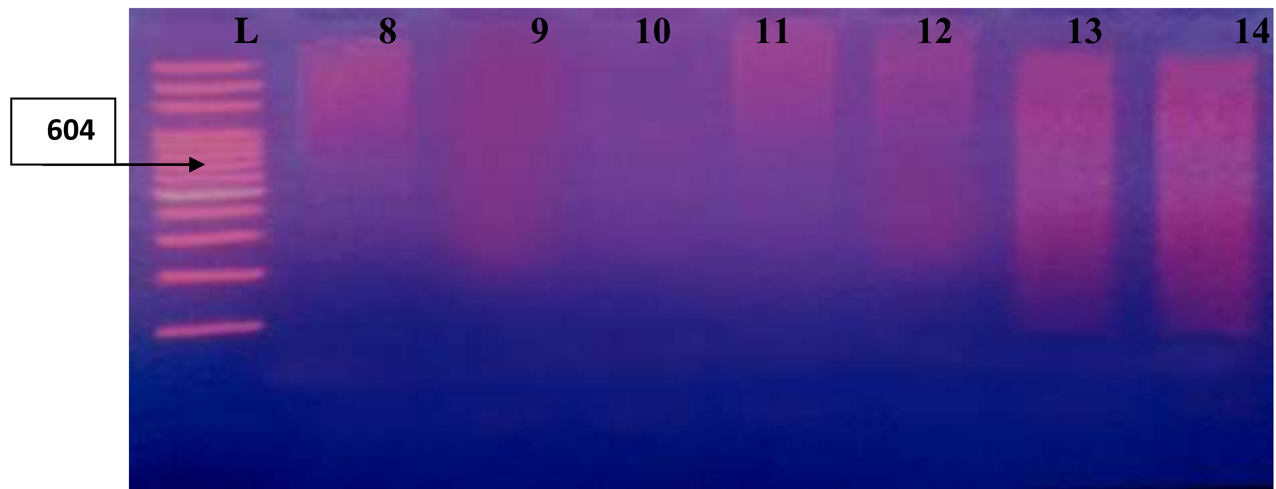


Figure (4) Gel electrophoresis of PCR product of *capD gene*
Only one isolate No.(1) with positive result (2,3,4,5,6,7,8,9,10,11,12,13,14) isolates were negative , L=Ladder

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 agreement with [13] results of this gene.

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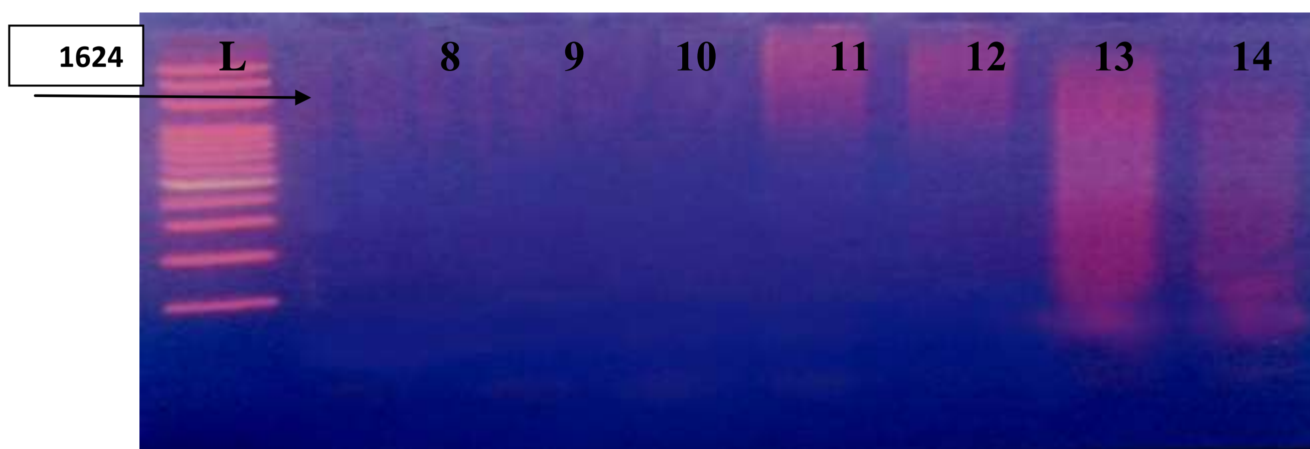

Figure (5) Gel electrophoresis of PCR product of *SdrI* :Isolate No.(3) with positive result for *SdrI***.**While (1,2,4,5,6,7,8,9,10,11,12,13, and 14) with negative result .

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].

Conclusions:

According to this study, the study conclude 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.

References

1. Nicolle, L.E. (2008): Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am .**35 (1):** 1–12.
2. King, N.P., Sakinc, T., Ben Zakour, N.L., Totsika, M., Heras, B., Simerska, P., Shepherd, M., Gatermann, S.G., Beatson, S.A. and Schembri, M.A., (2012): Characterisation of a cell wall-anchored protein of *Staphylococcus saprophyticus* associated with linoleic acid resistance.BMC Microbiology. **8**(**12**):1-12.
3. Ryan, K. and Ray C.(2004): Sherris Medical MicrobiologyAn Introduction to Infectious Diseases. 4th edn. McGraw Hill Publishers.

4. Raz, R., Colodner, R. and Kunin. C.M. (2005): Who are you—*Staphylococcus saprophyticus*? Clin. Infect. Dis. **40**:896–898.
5. Kuroda, M., Yamashita, A., Hirakawa, H., Kumano, M., Morikawa, K., Higashide, M., Maruyama, A., Inose, Y., Matoba, K., Toh, H., Kuhara,S., Hattori, M., and Ohta, T. (2005):Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc. Natl. Acad. Sci.**102**:13272–13277.
6. Sakinc, T., Woznowski, M., Ebsen, M., and Gatermann, S.G. (2005): The surface-associated protein of *Staphylococcus saprophyticus* is a lipase. Infect. Immun. **73**:6419–6428.
7. King, N.P., Sakinc, T., Ben Zakour, N.L., Totsika, M., Heras, B., Simerska, P., Shepherd, M., Gatermann, S.G., Beatson, S.A. and Schembri, M.A., (2012): Characterisation of a cell wall-anchored protein of *Staphylococcus saprophyticus* associated with linoleic acid resistance.BMC Microbiology. **8(12):**1-12.
8. Sakinç, T., Kleine, B., Michalski , N., Kaase, M. and Gatermann, S.G. (2009): SdrI of *Staphylococcus saprophyticus* is a multifunctional protein: localization of the fibronectin-binding site. Microbiol Lett. **301**(1):28-34.
9. Berwanger, M.K., Sakinc,T., Kline, K., Nielsen, H. V., Hultgren, S. and Gaterman, S. G.(2013): Significance of the D-serine-deaminase and D-serine metabolism of *Staphylococcus saprophyticus* for virulence. American Society for Microbiology**.81(12):**13-50.
10. Zetola ,N., Francis, J., Nuermberger, and Bishai .(2005): Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect. Dis. **5**: 275–286.
11. Josefsson , E., Juuti , K., Bokarewa, M. and Kuusela . (2005): The surface protein Pls of methicillin-resistant *Staphylococcus aureus* is a virulence factor in septic arthritis. Infect. Immun. **73(5):** 2812–2817.
12. Collee, J. G., Fraser A. G., Marmino B. P. and Simons A. (1996):Staphylococci In: Mackie and McCartney Practical Medical Microbiology. 14th Ed. The Churchill Livingstone, Inc. UK.
13. Kleine, B., Gatermann, S. and Sakinc, T.(2010): Genotypic and phenotypic variation among *Staphylococcus saprophyticus* from human and animal isolates.BMC Research Notes.3:163-168.
14. Park, S., Kelley, K.A., Vinogradov, E., Solinga, R., Weidenmaier, C. and Misawa, Y.(2010): Characterization of the structure and biological functions of a capsular polysaccharide produced by *Staphylococcus saprophyticus.* J Bacteriol .**192**:4618-26.
15. Allignet, J., Aubert, S., Dyke, K.G. and ElSolh, N.(2001): *Staphylococcus caprae* strains carry determinants known to be involved in pathogenicity: a gene encoding an autolysin-binding fibronectin and the ica operon involved in biofilm formation. Infect. Immun. **69**: 712-718.
16. Sakinc, T., Kleine, B., and Gatermann, S.G. (2006): SdrI, a serine-aspartate repeat protein identified in *Staphylococcus saprophyticus* strain 7108, is a collagen-binding protein. Infect. Immun. **74**:4615–4623.
17. Hu, C., Xiong, N., Zhang, Y., Rayner, S. and chen, S. (2012): Functional characterization of lipase in the pathogenesis of *Staphylococcus aureus*. Biochem. Biophys. Rec. Commun. **419(4):**617-620.
18. MacFaddin, J. F. (2000): Biochemical tests for the identification of medical bacteria. 3rd Ed. The Williams and Wilkins- Baltimor, USA.