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STUDY OF *STAPHYLOCOCCUS LENTUS* ISOLATED FROM END STAGE RENAL FAILURE PATIENTS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS

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ABSTRACT

During the period from beginning of June 2014 tell end of December 2015, isolation and identification of *S.lentus* from end stage renal failure patients under haemodialysis therapy. A total of 400 clinical samples from 100 patients included (100 for each Skin, Urine, Blood, arterial Fistula, While 200 hospital environmental specimen included dialysis device (100), bed (100) from Nephrology department AL-Sadder Medical City in AL-Najaf province. Out of these samples, 50 isolates were confirmed as S.lentus by conventional bacteriological methods and vetik 2 compact system. Rest of these samples showed growth of other microorganism like as *Klebsiella* species, *Staphylococcus* species, *E.coli* etc. The antibiotic resistance of *S.lentus* bacteria has profound clinical implications. Hence, this research was aim for the first time in Iraq to isolation and study prevelance of *S.lentus*. From clinical and hospital environmental sources then determines their antimicrobial susceptibility patterns, the results revealed that *S.lentus.* isolated from Skin10 Urine 5, Blood 4 and arterial Fistula10. While 200 hospital environmental specimen included dialysis device12, bed 9. At the same time the results found that *S.lentus* were greatly resistant to antibiotics that most commonly used, so regard as multi-drug resistant (MDR), *S.lentus* isolates exhibited high resistance to Penicillin, Ceftazidime, Erythromycin, Clindamycin and Methicillin. While other antibiotics exhibited different activity against isolates. Furthermore, most isolates appeared low resistance to Tetracycline well Sulfamethoxazole / Trimthoprim and Levofloxacin while the isolates were sensitive to Vancomycin. **Conclusion:** The increase number of *S.lentus* isolated from humans indicates that *S.lentus* is an opportunistic pathogen and consider as one of nosocomial

infection in Iraq and These organisms are found to be resistance to the routinely used antibiotics. Appropriate antimicrobial drugs should be prescribed after detected antibiogram. The patients should also be prevent stop taking the drugs in the middle. This will help in minimising the complications, and help in preventing the emergence of resistant strains.

KEYWORD: *S.lentus*, identification, haemodialysis, antibiotic sensitivity.

INTRODUCTION

Chronic kidney disease (CKD) is a worldwide public health problem.[1] The rising prevalence of treated ESRD can be attributed primarily to the increasing numbers of patients who start renal replacement therapy (RRT) each year, and to a lesser extent, to the improving survival of patients with ESRD. End stage renal disease (ESRD) represents a clinical state in which there has been an irreversible loss of endogenous renal function and considar a major health problem resulting in increased mortality, decreased quality of life of people affected and high costs from renal replacement therapy.[2] The incidence of ESRD has been increasing relentlessly at an annual rate of about 6-8% in most European countries. In the Middle East a survey based on retrospective data reported an annual incidence for ESRD of 90-110 per million populations.[3]

S. lentus is a member of the *S. sciuri* group, which are facultative anaerobic Gram-positive, coagulase - negative staphylococci, oxidase-positive, and coagulase-negative non-motile non-sporforming, 0.7-1.2µm occur as single or in pairs and tetrads cocci.[4] *S. lentus* are isolated from different environments, such as soil, sand , water and the hospital environment.[5] It has commonly been isolated from food-producing animals, including poultry and dairy animals where founded that *S.lentus* causes metastasis in sheep and goate[6]

Several studies have shown that *S.lentus* cause a variety of diseases to humans, **Karachalios *et al.*, 2006**[7] revealed that *S. lentus* isolated from spleanic sample from patiens suffering sapsis. Also, significant increase in nosocomial infections has been reported in different unites like Intensive Care , haemodialysis and oncology departement this lead to Sepsis as a complicated this epsisodes have been associated with *S. lentus.*[8,9,10]

The evolution of antibiotic impedancein bacteria next foreword of antimicrobial agents has appear as an important failure therapy in infection anywhere in the world. It is a great challenge for clinician to treat these bacteria. In Iraq, there is no information available on the

occurrence of antibiotics resistance in *S.lentus* In addition, there are very little information regarding the role of *S.lentus* in human bacterial infections. So, this study was conducted to isolate and identify *S.lentus* from different sources clinical and environmental by different methods and detect the susceptibility of bacteria to different type of antibiotic.

MATERIALS AND METHODS

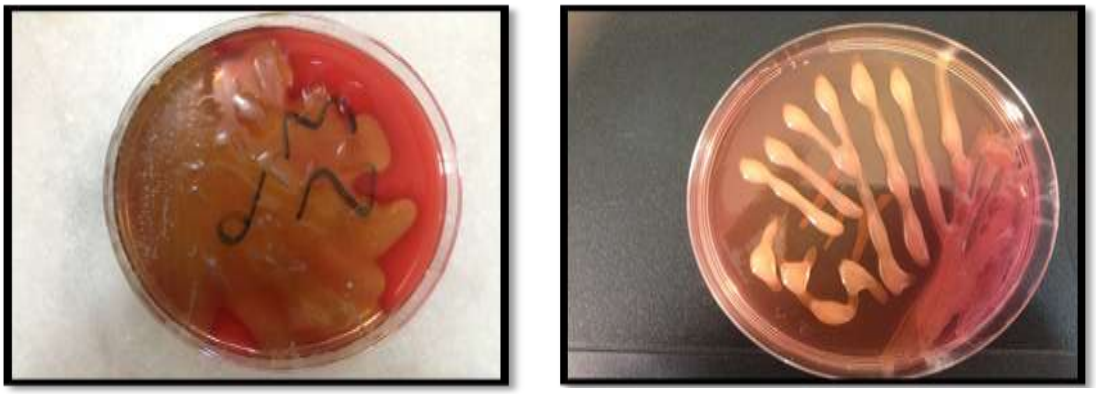
Isolation and Identification A total of 400 clinical samples from 100 patients which included 100 samples for each urine, blood, fistula and peri-fistula skin swabs (by pre-moistened cotton swab - in a solution of calcium alginate), in addition to 200 specimens (beds and systems) These samples were collected from patients attending to Nephrology department Al-Sadder Medical City, during the period from June 2014 and up to December - 2015. All samples were cultured on the Blood agar and Mannitol salt agar and incubated at 37 C˚under aerobic condition for 24 hour, Gram's stain was used to examine the isolated bacteria for studying the microscopic properties such as gram reaction and shape. While Biochemical tests used according to MacFaddin,[11] then finally confirmed by using Vitek-2 compact system gram– positive (G+ve).

Antibiotic susceptibility testing

Antimicrobial susceptability testing was accomplished by the "Kirby-Bauer disc diffusion method using Mueller-Hinton agar"as recommended by Clinical Laboratory Standard Institute.[12] The antimicrobial agents tested and their corresponding concentrations were as follows: Penicillin (10µg), Ceftazidime (30µg), Erythromycin (15µg), Clindamycin (2µg), Methicillin (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Tetracycline(30µg), Sulfamethoxazole / Trimthoprim (5µg), Levofloxacin (5µg), Vacomycin(30µg), Amoxillin/Clavulanicacid (30µg). A small inoculum of *S.lentus* suspension which prepared by inoculate 5 isolated grown on BHI agar to 5 ml of tryptic soy broth then incubated for 2hr. to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready–made 0.5 McFaerland tube standard) was inoculated on Mueller-Hinton plates and antibiotic discs were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. After incubating the inoculated plates aerobically at 37 C° for 18 to 24h, the susceptibility and resistance of the *S.lentus* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with criteria provided by.[12]

RESULTS

The result clarified that the positive result for any bacterial isolation was 392 and 208 which were the rest of the samples consider negative results. Some isolates had the ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones and turned the colour of the medium from pink to yellow, others were non mannitol-fermenter and appeared as small white colonies and no colour change was observed on the medium. *S.lentus* appeared as cocci, middle in size, mucoid , white or cream colonies on blood agar and usually B hemolytic colonies Fig.(1,A). While on Mannitol salt agar *S.lentus* produce smooth, convex, mucoid, and non-pigmented or yellow, pale beige to pale reddish yellow colonies with slow growth in 10%NaCl and growth poor in 15% NaCl Fig. (1,B).



A **B**

Fig. (1): *S.lentus.* on culture media such as (A) Blood agar, (B) Mannitol salt agar.

Biochemical tests revealed that all isolates were positive results for catalase, oxidase and negative results to produce indole and citrate and produced other biochemical reactions as shown in (Table 1).

Table: (1) Biochemical test for identification of *S.lentus*.

	Test	Result
1	Catalase	+
2	Oxidase	+
3	Coagulase	-
4	Bacitracin	Resistance
5	Hemolysis	B
6	Indole	-
7	MR	-
8	VP	-
9	Citrate	-
11	Gram stain	+
12	Mannitol salt agar	+

The final identification was performed with the automated VITEK-2 compact system using GP-ID cards for precise and accurate identification of the isolates at generic and species level. This test was applied on one hundred bacterial isolates that showed previously results as suspected CoNS.

The results demonstrate that only 50 samples were obtained from clinical and environmental sources back to *S.lentus* with ID message confidence level ranging between very good to excellent (Probability percentage from 93 to 99).The *S.lentus*. have been identified to be 50 (12. 7%) out of the total number of growth positive bacteria on blood agar (n =392) shown in Table (2) or Fig. (2).

Table (2): Distribution of *S.lentus* recovered from various clinical sample

No.	(%)	No.
Clinical sample		
Fistula-hemodialysis	10	2.5%
Skin	10	2.5%
Hospital environmental sample		
System	12	3.6%
Bed	9	2.2%
Total	50	12.7%

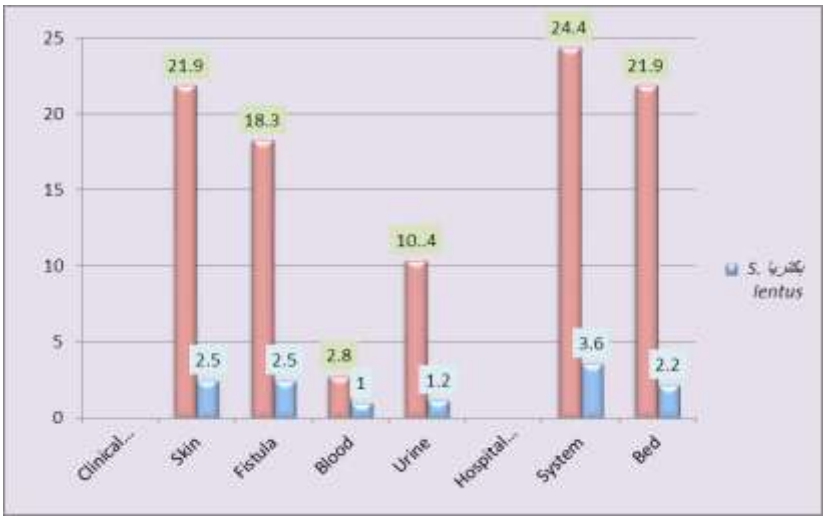


Fig. (2).Shown ratio *S.lentus*. of the total number of Positive bacteria on Mannitol salt agar

As shown in Table (3) the results of antibiotic sensitivity test for *S.lentus*. Most bacterial isolates showed high resistance towards most widespread antibiotics, This called multidrug resistant(MDR). Hence, isolates exhibited high resistance to Penicillin (100%), Ceftazidime (100%µg), Erythromycin (96%), Clindamycin (96%), Methicillin (94%), Cefotaxime (82%),

While other antibiotics exhibited different resistant activity against isolates Clavulanicacid / Amoxillin(78) , Ceftriaxone (76%), Furthermore, most isolates appeared less resistance to Tetracycline (28%), Sulfamethoxazole / Trimthoprim (20%), Levofloxacin (5%), sensitive to Vacomycin (0%),

Table (3): Antimicrobial susceptibility patterns of *S.lentus* (N = 50)

Antibiotic	Total
Penicillin	(%100) 50
Methicillin	(% 94) 47
Levofloxacin	(% 5) 10
Tetracycline	(%28)14
Clindamycin	(%96) 48
Trimthoprim/Sulfamethoxazole	(% 20) 10
Erythromycin	(%96) 48
Vacomycin	(% 0) 0
Ceftriaxone	(%76) 38
Cefotaxime	(% 82) 41
Ceftazidime	(%100) 50
Amoxillin / Clavulanicacid	(%78)39

DISCUSSION

Generally little information are available about *S.lentus* especially in Iraq. *S.lentus* was considered as an uncommon cause of infections in human beings.[13,14] The results of morphology showed that *S.lentus*. appeared as round, smooth convex mucoid, glistening white, white or cream colonies on Mannitol salt agar this results in agreement with (deVisscher *et al*.,[15] In biochemical test the positive results to oxidase test and this test performed to differentiate between *Staphylococcus* from genus *Micrococcus* (Faller and Schleifer,[16] The negative result for Indole test are because inability of the bacteria to produce tryptophanase that deamination of tryptophan to produce indole resistance. The methyl red test was negative because inability of the bacteria to production of the sufficient acid during the fermentation of glucose. Voges-Proskauer was negative because inability of the bacteria to produce acetoin from the pyruvic acid. All these results (morphology and biochemical) were identical with.[11]

Results of this study explain that Staphylococci most common cause in haemodialysis infection and this agree with study done **by Fluck *et al*.,[17]** who explain that most common cause for hemodialysis-associated infections was coagulase-negative staphylococci (CoNS). in the same line **Mermel *et al*.,[18]** revealed that infection in blood stream (BSIs) of hemodialysis patients were common by gram positive cocci. At the same time study done in

Turkey by **Koksal *et al.*,(19)** who reported that *S.lentus* were one caused nosocomial bacteremia. Also agree with study by **Mayhall, *et al.*,**[20] they found that *S.lentus* was responsible for the outbreak of blood stream infection occur in the NICU. **Winn *et al.*,**[21] found that Coagulase negative bacteremia occurs as a result of long term usage of indwelling central venous catheters, in haempdialysis.

Infection is a frequent cause of rehospitalization as well as remains one of the greatest risk factors to morbidity and mortality for the dialysis population. ANZDATA illustrated that 11% of all deaths in the dialysis dependent population in 2010 were due to infection.[22] The arteriovenous fistula (AVF) is comprise about 4.5% rate for AVF infection in the first year of follow up by The Canadian Morbidity Study.[23] **Kaplowitz *et al.*,**[24] investigated that *Staph. aureus* carriage in the nose and on the skin found to be a major pathogen in chronic haemodialysis patients and consider as the causative agent of access site infections.

Results of this study revealed that *S.lentus* one of causative agents in UT infection in HD patients this agree with study by **Stepanovic *et al.*,**[13] who indicated that *S.lentus* isolated from a urinary tract infection, with presented 0.79 % of the total number of CONS isolated.

The results of this study in skin frequent nearly agreement with **Rivera *et al***[25] who explain that isolated *S.lentus* from both renal failure and urinary tract infections patients also they concluded that was the common agents associated with UTI patients.[14] As show **Alcaraz *et al***[26] they revealed that *S.lentus* play a clinically significant role in UTI.

The usage of antibiotics without antibiotics sensitivity testing, is the most important factor promoting the emergence of multi-drug resistance which lead to selection and dissemination of antibiotic resistant pathogens in clinical medicine.[27] The result revealed that all isolates were resistance to pencillen These results are correlated well with those obtained by **Yasuda *et al.*,**[28] and with **Al-Hasani**[29] who found rate resistance to Penicillin were 100%. Also, the results explain that resistance to third generation Cephalosporin's was caused mainly by mutations in the common *mecA* gene which extended the hydrolytic spectrum of the enzyme to these antibiotics.[30] The reason of β-lactam resistance isolates is may be because of the production of β-lactamase, which may be heritably localized on the chromosome or on a plasmid, The *S.lentus*. has ability to produce β-lactamase enzyme that has given the bacteria high resistance to several β-lactam antibiotics.[31,32]

S. lentus presented consider able resistance to the erythromycin and Clindamycin. this result Similar to study by Kokasl *et al*,.[19] Furthermore in many studies, high resistance ratios against erythromycin, clindamycin, tetracycline and ciprofloxacin were reported[33,34] *S. lentus* has the ability to acquire antibiotic resistance genes including erythromycin ribosome methylase (erm) genes which confer resistance to macrolide, lincosamide, and streptogramin B (MLSB) antibiotics.[35] These results in the same line with Kehrenberg and Schwarz,[36] *S.lentus* was shown to be resistant to chloramphenicol, clindamycin, erythromycin, florfenicol , streptomycin ,and tetracycline by agar disk diffusion . Omran,[37] illustrated that antibiotic resistance profile must be determined from time to time since the pathogenic bacteria change their ability in response to the antibiotics used to treatment the infections.

REFERENCES

1. Levey,m.A. S, Atkins,R.; Coresh, J.; Cohen, E. P.; Collins, A. J. and Eckardt, K . U. Chronic kidney disease as a global public health problem: Approaches and initiatives -a position statement from Kidney Disease Improving Global Outcomes. Kidney International, 2007; 72: 247-259.
2. Brown, W. The Stages of chronic kidney disease. PKD National Convention, 2014. http://kidney.niddk.nih.gov/kudiseases/pubs /polycystic/.
3. United States Renal Data System. USRDS. Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2013.
4. Stepanovic, S.; Dakic,I.; Morrision,D.; Hauschild,T. and Devriese. A comparative evaluation of phenotypic and molecular methods in the identification of members of the *Staphylococcus sciuri* group. Syst. Appl. Microbiol, 2005a; 28: 353–357.
5. Dakic, I.; D. Morrison, D.; Vukovic, B. Savic; A. Shittu, P.; Jezek,T. Hauschild and S. Stepanovic. Isolation and molecular characterization of Staphylococcus sciuriin the hospital environment. J. Clin. Microbiol, 2005; 43: 2782–2785.
6. Kunz, F. Giannatale, E.; Salinetti, AP.; Salandra, G. and Bartoli, M.. Antimicrobial resistance of Staphylococcus aureusand coagulase negative staphylococci isolated from mastitis milk samples from sheep and goats. Schweiz. Arch. Tierheilkd, 2011; 153: 63–69.

7. Karachalios, G.N. ; Di Giannatale, E. ; Salinetti , AP. ; Salandra, G. and Bartoli , M. Splenic abscess due to *Staphylococcus lentus*:a rare entity. Scand. J. Infect. Dis., 2006; 38: 708–710.
8. Horii, T., Y.; Suzuki, T. ;Kimura, T. ; Kanno, and M. Maekawa. Intravenous catheter-related septic shock caused by *Staphylococcus sciuri* and Escherichia vulneris.Scand. J. Infect. Dis., 2001; 33: 930–932.
9. Hedin, G., and M. Widerstrom. Endocarditis due to *Staphylococcus sciuri*.Eur. J. Clin. Microbiol. Infect. Dis., 1998; 17: 673–675.
10. Shittu, A., J. ; Lin, D. ; Morrison,D. and Kolawole, M. Isolation and molecular characterization of multiresistant *Staphylococcus sciuri* and *Staphylococcus haemolyticus* associated with skin and soft-tissue infections. J. Med.Microbiol, 2004; 53: 51–55.
11. Macfaddin, J.F. Biochemical test for identification of medical bacteria."3rd ed" The Williams and Wilkins. Baltimor. USA, 2000.
12. CLSI (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing, 22nd informational supplement M100-S22. 2012; 31. Wayne, PA: CLSI.
13. Stepanovic, S.; Jezek, P.; Vukovic, D., Dakic, I. And Petras. Isolation of members of the Staphylococcus scuiri group from urine and their relationship to Urinary tract infection. J. Clin. Microbiol, 2003; 41(11): 5262-5264.
14. Guirguitzova, B., D.; Chankova, and B. Zozikov. Staphylococci asuropathogens—frequency of isolation in hospitalized patients and sensitivityto antimicrobial agents. Ann. Urol. (Paris), 2002; 36: 341–347.
15. de Visscher, A.; Haesebrouck, F.; Piepers, S.; Vanderhaeqhen, W.; Supre, K.; Leroy, F.; Van Coillie, E, and De Vliegher S. Assessment of the suitability of mannitol salt agar for growing bovine-associated coagulase-negative staphylococci and its use under field conditions. Res Vet Sci., 2013; 95(2): 347-51.
16. Faller A and Schleifer K-H. Modified oxidase and benzidine tests for separation of staphylococci from micrococci. J. Clin. Microbiol, 1981; 13: 1031-1035.
17. Fluck, R.; Wilson, J. and Tomson, C.R. epidemiology of methicillin resistant *Staphylococcus aureus* bacteraemia amongst patients receiving dialysis for established renal failure in England. *Nephron Clin Pract*., 2010; 115(1): c261–270.
18. Mermel ,L.A; Allon, M. and Bouzam E. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect*, 2009; 49(1): 1–45.

19. Koksal, F.; Yasar, H. and Samasti M. Antibiotic resistance patterns ofcoagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey. Microbiol. Res., 2009; 164: 404–410.
20. Mayhall, C.G. Hospital epidemiology and infection control, 3rd ed. Philadelphia: Lippincott Wiliam and Wilkins, 2004; 495–510.
21. Winn, W.C,; Allen, S.D.; Janda, W.M.; Koneman EW ;Procop GW. ;Schreckenberger, P.C.; Woods, G.L.and Koneman's. Color Atlas and Textbook of Diagnostic Microbiology, 2006; 6th ed.Philadelphia
22. ANZDATA, The Thirty Third Report, S.; McDonald, L.; Excell and B. Livingston, ditors. Australia and New Zealand Dialysis and Transplant Registry: Adelaide, South Australia, 2010;
23. Butterly, D.W and Schwab, S.J. Dialysis access infections. Current opinion in nephrology and hypertension, 2000; 9: 631.
24. Kaplowitz, L.; Comstock, J.; Flynn, Jr., N.; Unonius, I. U. and Landwehr D. Prospective study of microbial colonization of the nose and skin and infection of the vascular access site in hemodialysis patients. Journal of clinical microbiology, 1988; 26: 1257.
25. Rivera, M.; Dominguez,M.; Mendiola, N. and Roso,g., *Staphylococcus lentus* peritonitiis Acase report. Peritoneal Dialysis Int, 2014; 469-472.
26. Alcaraz, I.E;Satorres, S.E.; Lucero, R.M;Puig, S.K. and liu, T.K. Species identification slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens .Bras .J. Microbiol, 2003; 19: 875–879.
27. Neu, H.C. The crisis in antibiotic resistance. Science, 1992; 257(5073): 1064-1073.
28. Yasuda, R.; Kawano,H. and Anzai, A. Methicilin–resistan coagulase –negative staphylo cocci isolated from healthy horses in Japan Am J.Res., 2000; 61(11): 1451-5.
29. Al-Hasani, H.M. Comparative study between methicillin resistant coagulase positive and negative staphylococci.M.SC.Thesis of science. University of Baghdad, 2011.
30. Kitao, T. Survey of methicillin-resistant coagulase-negative staphylococci isolated from the fingers of nursing students. J. Infect. Chemother, 2003; 9: 30–34.
31. Dyke KGH. Resistance to b-lactam antibiotics: resistance mediated byb-lactamases. In: Crossley KB, Archer GL, eds. The Staphylococci in Human Diseases. New York: Churchill Livingstone Inc., 1997; 139–57.
32. Voladri, R.K and Kernodle D,S. Characterization of a chromosomal gene encoding type Bb-lactamase in phage group II isolates ofStaphylococcus aureus.Antimicrob Agents Chemother, 1998; 42: 3163–8.

33. Huber H. Sands, K.; Millerm E. and Mendiola, N. Prevalence and characteristics of methicillin resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. BMC Vet. Res., 2011; 7: 6.
34. Hauschild, T.; Lüthje, P. and Schwarz, S. Staphylococcal tetracyclineMLSBresistance plasmid pSTE2 is the product of an RSA-mediatedin vivo recombination. J. Antimicrob. Chemother, 2005; 56: 399– 402
35. Poehlsgaard, J.and Douthwaite, S. The bacterial ribosome as a target for antibiotics. Nat. Rev. Microbiol, 2005; 3: 870–881.
36. Kehrenberg, C. and Schwarz, S. fexA, a Novel *Staphylococcus lentus* Gene Encoding Resistance to Florfenicol and Chloramphenicol. Antimicrobial agents, 2004; 615–618.
37. Omran, L. and Askar, E. (2016). Antibiotic Sensitivity Patterns of the Most Common Bacteria Isolated from Al-Mouwasat University Hospitalin, Syria, International Journal of PharmTech Research, 2015; 9(1): 113-119.