ISSN: 0067-2904

Staphylococcus aureus Nasal Carriage and Obesity among Patients with Type Two Diabetes Mellitus

Sarah L. Hamad*, Alice K. Agop Melconian
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.

Abstract

The study aimed to find an association between Type two diabetes mellitus (T2DM) patients, obesity and the rate of nasal carriage of *Staphylococcus aureus* (NCSA) producer of TSST-1 in patients with T2DM compared with non-diabetic control groups. T2DM patients and control subjects were selected from outpatient of "The Specialist Center for Diseases of Endocrine and Diabetes" in Baghdad. The subjects were divided into 4 groups: Group I included 21 obese T2DM patients; Group II included 20 lean T2DM patients; Group III included 20 obese as control group and Group IV included 21 lean as control group. The study included sample with size (n= 82), male and female, with the ages ranged from 35 to 75 years, and the patients were not on any kind of anti-diabetic treatment. A total number of the nasal carriage *S. aureus* isolates were 38, of them 23 *S. aureus* (56.1 %) were isolated from the groups of patients with T2DM and 15 *S.aureus* isolates (38.46 %) were isolated from the control groups. Molecular method was used to detect the presence of *tstH* gene in *S. aureus* isolates indicating that the presnce of toxic shock syndrome toxin-1. The results revealed the presence of this gene in 12 (63.16%) *S. aureus* isolates collected from T2DM patients and 7 (36.84 %) isolates collected from control groups.

Keywords: *Staphylococcus aureus* Nasal Carriage, Obesity, Type Two Diabetes Mellitus, Toxic Shock Syndrome Toxin-1.

المكورات العنقودية الذهبية المعزولة من الأنف والسمنة لدى مرضى السكري من النمط الثاني

ساره لفته حمد*، اليس كريكور اغوب ملكونيان
قسم التقنيات الاحيائيه، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

هدفت هذه الدراسة إلى إيجاد علاقة بين مرض السكري من النمط الثاني والسمنة ومعدل بكتيريا المكورات العنقودية الذهبية *Staphylococcus aureus* المعزولة من الانف و المنتجة لذيفان متلازمة الصدمة السمية-1 (TSST-1) لدى مرضى السكري من النمط الثاني مقارنةً مع الاشخاص الأصحاء غير المصابين بالسكري. تم اختيار كل من مرضى السكري من النمط الثاني و الاشخاص الأصحاء من "المركز التخصصي لأمراض الغدد الصم والسكري" في بغداد. وقد تم تقسيمهم إلى 4 مجاميع: المجموعة الأولى تتضمن 21 شخص سمين من مرضى السكري من النمط الثاني و المجموعة الثانية تتضمن 20 شخص من مرضى السكري من النمط الثاني بأوزان طبيعية و المجموعة الثالثة تتضمن 20 شخص سمين من الأفراد الأصحاء وشملت المجموعة الرابعة 21 شخصمن من الأفراد الأصحاء بأوزان طبيعية. شملت الدراسة على

*Email: Sarahlafta20@gmail.com

عينة بحجم (n= 82) من الذكور والاناث حيث تراوحت اعمارهم من 35 الى 75 سنة علماً ان مرضى السكري من النمط الثاني لم يتلقوا اي نوع من العلاج. كان العدد الكلي لبكتيريا المكورات العنقودية الذهبية *S. aureus* المعزولة من الانف 38 عزلة، منها 23 عزلة (56.1 %) معزولة من مرضى السكري من النمط الثاني و 15 عزلة *S. aureus* (38.46%) معزولة من الاشخاص الاصحاء. استعملت الطريقة الجزيئية للكشف عن وجود الجين *tstH* في بكتيريا العنقودية الذهبية *S. aureus* للإشارة الى وجود ذيفان متلازمة الصدمة السمية-1 (TSST-1). أظهرت النتائج وجود هذا الجين في 12 عزلة *S. aureus* (63.16%) التي تم جمعها من مرضى السكري من النمط الثاني و 7 عزلات *S. aureus* (36.84%) المجموعة من الاشخاص الأصحاء.

Introduction

Staphylococcus aureus is a Gram positive cocci, facultative anaerobic and an opportunistic pathogen that colonizes the skin and mucosal surface of the human host [1, 2]. Hence considering a major human pathogen that cause a variety of infections in both healthy and immunocompromised individuals and this can lead to numerous complications and in some cases to death [3]. Carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. Earlier studies recorded the mean carriage rate in the general population is 37.2 % and in non-insulin dependent diabetic patients is 29.0 % [4]. In other study conducted by Kutlu *et al*. [5] was found that the *S. aureus* colonization in DM patients is 41.9 %. Persistent nasal colonization of *S. aureus* (NCSA) was range from 20–30% of healthy adults indicates a major risk factor for infections with the bacterium [6]. This should be considered seriously known that this bacterium produces several virulence factors and superantigens from which is the Toxic Shock Syndrome Toxin-1 (TSST-1) [7]. TSST-1 is a protein encoded by *tstH* gene, which is a part of the mobile genetic elements staphylococcal pathogenicity island 1 [8]. A mature TSST-1 protein possesses a coding sequence of 559 base pair [9]. Long time exposure to SAgs, such as TSST-1, possibly happening through recurrent *S. aureus* colonization and infection in obese individuals, may result in impaired glucose metabolism and the development of T2DM in the overweight and obese [10]. It is known that there is a strong evidence betweenT2DM and genetics, but obesity contributes 55 % of T2DM cases [10, 11]. Therefore; the aim of this study was to investigate the association between obesity and *S. aureus* nasal colonization and type two diabetes mellitus (T2DM).

Materials and Methods

Subjects

Nasal swabs were collected from a sample (n = 82), which consisted of T2DM patients and controls with the age range from 35 – 75 years. The samples were collected from outpatients of "The Specialist Center for Diseases of Endocrine and Diabetes" in Baghdad during the period of 16th November 2015 to 9th February 2016.

Diagnosis of diabetes

Diagnosis of diabetes was based on the criteria indicated by the American Diabetes Association (ADA), (2012) [12], fasting blood sugar level (FBS) ≥126 mg/dL. Subjects in healthy control groups have FBS below 110 mg/dl.

Measurement of Body Mass Index (BMI)

The BMI is defined as the individual's weight divided by the square of their height. According to the World Health Organization (WHO) [13] the BMI ≥ 30.0 kg/m^2 considered as obese.

Isolation and identification of Nasal carriage *S. aureus*

Nasal samples were collected from each subject under aseptic conditions using a sterile nasal swab. The nasal swab was inserted into the nose at approximately 1-inch and rotated three times clock-wise and three times anticlock-wise [3]. The swabs were immediately cultured onto mannitol salt agar and incubated at 37°C for 18-24 hours. Biochemical identification was carried out according to Bergey's manual [14] and confirmed using API Staph system (Bio-Merieux).

Detection of Toxic Shock Syndrome Toxin-1 (TSST-1)

Determination of TSST-1 was carried on using molecular method. *S. aureus* DNA were extracted and purified using G-spin™ Total DNA Extraction Kit (Promega, USA) according to the protocol stated by the kit manufacturer. The DNA concentration and purity were estimated using the Nanodrop. About 2µl of each sample was taken and the optical density (O.D) at wave length of 260 nm and 280

nm was measured which lead to estimate DNA purity ratio according to this formula: DNA purity = O.D at 260 / O.D at 280 [15].

Primer

The *tstH* gene was amplified using primer design by Monday and Bohach [9] Table-1.

Table 1- The sequence and product size of the primer used in this study.

Gene	Primer	Nucleotide sequences (5' ⟶ 3')	Gene Size (bp)
tstH	Forward	5'-GCTTGCGACACCTGCTACAG-3'	559
	Reverse	5'-TGGATCCGTCATTCATTGTTAT-3'	

Polymerase Chain reaction (PCR) Amplification Mixture and Conditions

Each 25 µl PCR mix contained the ingredients listed in Table- 2. DNA amplification was carried in a thermal cycler (Multi gene optimax-USA) according to Monday and Bohach [9]. A negative control without template DNA was used in each reaction to reveal the hazardous cross contamination of the PCR components.

Table 2- The Master Mix components.

Item	Volume
Nuclease free water	8.5 µl
Master mix	12.5 µl
Forward Primer (10pmol/ µl)	1 µl
Reverse Primer (10pmol/ µl)	1 µl
Total volume	23 µl
Then added genomic DNA 2 µl (100ng/µl) + mix 23 µl = 25 µl	

PCR Program

A PCR program was adopted according to Monday and Bohach [9] as shown in Table-3.

Table 3- PCR Program.

Steps	Temperatures	Time	Number of Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	1 min	30
Annealing	60°C	1 min	
Extension	72°C	1 min	
Final extension	72°C	7 min	1

Determination of PCR Specificity

The quantity and amplification size of PCR products were confirmed by Agarose gel electrophoresis of 10µl of amplified DNA with 100-10000 bp Ladder (promega) on 2% agarose gel for 1 hour at 90 voltages. The agarose gel was stained with ethidium bromide and visualized by UV

transilluminator at 320 nm and then were photographed by gel Documentation System. The specific size of PCR products were estimated by comparing with the ladder bench top PCR markers [16].

Results and Discussion

A sample (n = 82) were enrolled in the present study. The results revealed four groups: Group I included 21 obese untreated T2DM patients Table-4, Group II included 20 lean untreated T2DM patients Table-5, Group III included 20 obese control individuals Table-6 and Group IV included 21 lean control individuals Table-7. The duration of T2DM disease was ranged from 10 days to 3 years for the patient groups and from those 15 out of 41 T2DM patients (36.58%) were observed to have a family history of T2DM disease (father or mother or both).

BMI values for T2DM patients groups were at range 22-38.6 kg/m^2 while in healthy control groups the BMI values were at range 20.8-53 kg/m^2. Tables- 4, 5, 6 and 7 illustrate the differences between the study groups.

Table 4- Obese T2DM patients group with different ages, FBS and BMI values.

Obese T2DM patients No.	**BMI kg/m^2**	**Age**	**FBS (mg/dL)**	**Obese T2DM patients No.**	**BMI kg/m^2**	**Age**	**FBS (mg/dL)**
1	31.2	45	136.8	12	32	58	135
2	32	53	207	13	35.9	49	154.8
3	32.4	56	207	14	36.7	41	136.8
4	33.3	41	250.2	15	31.14	35	147.6
5	37.7	61	340.2	16	30.5	53	153
6	34.1	48	135	17	32.4	50	135
7	31.2	38	140.4	18	34.9	60	203.4
8	35	36	144	19	34.6	45	140.4
9	31.25	38	131.4	20	33.9	44	135
10	35.9	48	196.2	21	37.5	55	185.4
11	38.6	35	185.4				

BMI= Body Mass Index; FBS= fasting blood sugar.

Table 5- Lean T2DM patients group with different ages, FBS and BMI values.

Lean T2DM patients No.	BMI kg/m^2	Age	FBS (mg/dL)	Lean T2DM patients No.	BMI kg/m^2	Age	FBS (mg/dL)
1	25.3	54	277.2	11	22	38	352.8
2	29.4	67	199.8	12	24.4	38	139.6
3	25.3	73	253.8	13	26.8	45	154.8
4	26.7	41	133.2	14	25.7	50	149.4
5	25.3	41	266.4	15	27.5	75	142
6	24.2	37	145.8	16	27.3	57	139.6
7	28.3	35	142.2	17	29.7	69	131.2
8	23.4	52	136.8	18	27.3	38	198
9	29.6	37	136.8	19	25.7	45	198
10	27.7	40	262.8	20	24.1	68	144

BMI= Body Mass Index; FBS= fasting blood sugar.

Table 6- The measurement of BMI, ages and FBS for obese control group.

Obese control No.	BMI kg/m^2	Age	FBS (mg/dL)	Obese control No.	BMI kg/m^2	Age	FBS (mg/dL)
1	33.2	53	84.6	11	31	35	102.6
2	34.9	40	88.2	12	45.26	58	81
3	33.8	41	97.2	13	35.8	48	90
4	50.1	37	100.8	14	31.72	39	100.8
5	42.2	35	108	15	49	40	110
6	32.1	45	95.4	16	35.5	42	82.8
7	37.1	40	81	17	33.2	51	110
8	53	43	102.6	18	36.7	54	93.6
9	32.9	40	106.2	19	33	36	99

10	31.6	45	88.2	20	30	40	104.4

BMI= Body Mass Index; FBS= fasting blood sugar.

Table 7- The measurement of BMI, ages and FBS for lean control group.

Lean control No.	BMI kg/m^2	Age	FBS (mg/dL)	Lean control No.	BMI kg/m^2	Age	FBS (mg/dL)
1	28.13	47	110	12	27.5	41	97.2
2	29.4	45	106.6	13	29.3	36	102.6
3	26.5	42	100.8	14	23	46	91
4	29.7	43	90	15	26.3	35	108
5	29.2	44	102.6	16	22.5	37	84.6
6	27.7	51	93.6	17	20.8	37	91
7	27.3	50	104.4	18	24.5	37	106
8	28.8	60	86.4	19	27	36	102.6
9	26.1	40	90	20	24.3	36	93.6
10	27.3	58	99	21	24.1	35	84.6
11	28.5	35	99				

BMI= Body Mass Index; FBS= fasting blood sugar.

According to the traditional results (culture, biochemical and API Staph system), NCSA was accounted 56.1 % in T2DM patients groups (obese and lean), while in control groups (obese and lean) accounted 38.46 % Table-8. In T2DM patients groups, NCSA were found in 12 (60 %) of obese individuals and in 10 (62.5 %) of overweight individuals.

Table 8- *S. aureus* Nasal Carriage percentage in T2DM patients compared with control individuals.

Nasal Carriage of *S. aureus*	T2DM Patients groups		Healthy control groups	
	No.	%	No.	%
Number	23	56.10	15	38.46
Total number	41	---	39	---

Molecular method for detection of 44 *S. aureus* isolates from both T2DM patients and controls for the production of TSST-1 revealed that only 19 isolates were found to be positive for *tstH* gene Figure-1 of them, the high percentage (63.16 %) of the producer were isolated from T2DM patients

groups, while lower percentage (36.84%) of *S. aureus* TSST-1 producers were isolated from control groups Table-9.

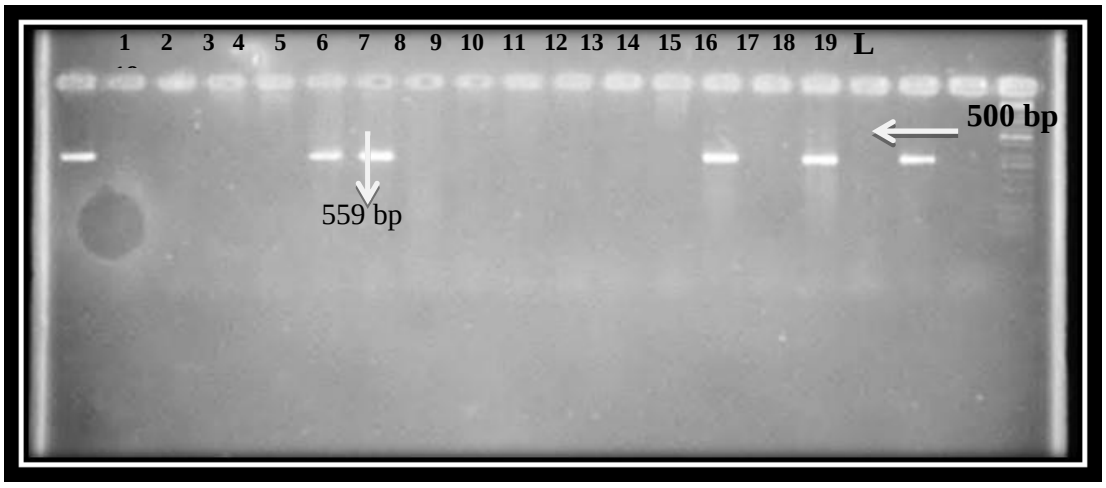

Figure 1- Agarose gel electrophoresis of the PCR products for *tstH* genes.

Fragments were fractioned by electrophoresis on 2% agarose gel (1hr/ 90V), 1X Tris-borate buffer and visualized using UV transilluminator after staining with Ethidium Bromide. Ladder (L) = 100-10000bp. Samples 1, 7 and 18 isolated from obese T2DM patients (Group I No. 1, 18 and 21 respectively), Sample 6 isolated from lean T2DM patient (Group II No. 2), Sample16 isolated from obese control (Group III No. 6) and Sample 14 isolated from lean control (Group IV No. 6). The other samples do not have *tstH* gene.

Table 9- *S. aureus* isolates producer of TSST-1 percentage in groups of T2DM patients compared with control groups.

***S. aureus* isolates producer of TSST-1**	**T2DM Patients groups**		**Healthy control groups**	
	No	%	No	%
Number	12	63.16	7	36.84
Total number	19	---	19	---

Infections correlated with *S. aureus* are frequently observed in diabetic patients and lead to significant morbidity and death [17]. It has been shown that the NCSA have an increased risk for acquiring *S. aureus* infections [18]. In the present study, the percentage of NCSA in the T2DM patients (56.1 %) was found to be considerably higher than that in the controls (38.46 %). AL-Kazaz [19], found that the percentage of *S. aureus* in nasal samples was 40%. Also, another study was found that the mean NCSA In the general population was 37.2% [4]. However, in few studies has been reported that the rate of NCSA in diabetic patients is higher than that in the control group, as in the study of Ahluwalia *et al*. [20], who found that the prevalence of NCSA in DM patients was 56.6 %, while in the control group it was 14.8 %. Data from the study of Alizargar *et al*. [21], performed on healthy population shows lesser rates of NCSA in a healthy individuals. This presence may be due to; being one of the main causes of hospital (Nosocomial infections) and community-acquired infections which can lead to serious consequences [22]; as one of the important pathogens with potential to cause opportunistic infection for being a member of the normal flora in the body [23]; in addition of possessing many virulence factors which enables it to penetrate body tissue and participate in pathogenesis of infection [24]. In T2DM patients groups, NCSA for individuals with obesity and excess body weight were 12 (60 %) and 10 (62.5 %) respectively, this may be an indication of increased susceptibility to bacterial colonization. Considering the strong correlation between obesity and T2DM, and proposed roles of microbes in the pathophysiology of obesity, it is possible that *S.*

aureus existence in obese group might have an effect on the obesity progress, resulting in the T2DM development [6]. High percentage (63.16 %) of *S. aureus* isolates producer of TSST-1 were isolated from the T2DM patients, while lower percentage (36.84%) of *S. aureus* TSST-1 producers were isolated from the controls. The presence of negative results by the other isolates for TSST-1 production can be due to the absence of *tstH* gene. These results are close to the results of Neill *et al*. [25] who found that TSST-1 is usually produced by some strains of *S. aureus* associated with toxic shock syndrome. Most of the *S. aureus* isolates that were TSST-1 positive were collected from the study groups who were suffering from obesity (high BMI values).This is due to the role of this bacterium in obesity and T2DM disease. Since all the pathogenic *S. aureus* isolates in human produce superantigens, obese individuals have a high risk of frequent exposure to superantigens. The TSST-1 of *S. aureus*, an essential exotoxin in pathogenesis, induces inflammation, lipolysis, and chronic insulin resistance in multiple tissues results in impaired systemic glucose tolerance, the hallmark finding in T2DM in humans, suggesting a role of *S. aureus* and its SAg in the progression of T2DM [10].

References

1. Werckenthin, C., Cardoso, M., Martel, J. L. and Schwarz, S. **2001**. Antimicrobial resistance in staphylococci from animals with particular reference to bovine. *S. aureus*, porcine *S. hyicus* and Canine *S. intermedius. Journal of Veterinary Research*, 32 (3-4), pp: 341-362.
2. William, B.W. , Paul, D.V. , George, M. G., Dorothy, J. , Noel, R. K. , Wolfgang, L., Fred, A. R. and Karl, S. **2009**. Bergey's Manual of Systematic. 2nd edition. Com., pp:392-433.
3. Tamer, A., Karabay, O. and Ekerbicer, H. **2006**. *Staphylococcus aureus* Nasal Carriage and Associated Factors in Type 2 Diabetic Patients. *Japanese journal of infectious diseases*, 59 (1), pp:10-14.
4. Kluytmans, J., van Belkum, A. and Verbrugh, H. **1997**. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews*, 10 (3), pp: 505–520.
5. Kutlu, S. S., Cevahir, N., Akalin, S., Akin, F., Dirgen Caylak, S., Bastemir, M. and Tekin, K. **2012**. Prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* colonization in a diabetic outpatient population: a prospective cohort study. *American Journal of Infection Control*, 40 (4), pp: 365-368.
6. Olsen, K., Danielsen, K., Wilsgaard, T., Sangvik, M., Sollid, J. U., Thune, I., Eggen, A. E., Simonsen, G. S. and Furberg, A. S. **2013**. Obesity and *Staphylococcus aureus* nasal colonization among women and men in a general population. *PloS One*, 8 (5), e63716, pp: 1-9.
7. Spaulding, A. R., Salgado-Pabon, W., Kohler, P. L., Horswill, A. R., Leung, D. Y. and Schlievert, P. M. **2013**. Staphylococcal and streptococcal superantigen exotoxins. *Clinical microbiology reviews*, 26 (3), pp: 422–447.
8. Dinges, M. M., Orwin, P. M., and Schlievert, P. M. **2000**. Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*, 13 (1), pp: 16-34.
9. Monday, S. R. and Bohach, G. A. **1999**. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in Staphylococcal isolates. *Journal of Clinical Microbiology*, 37**,** pp: 3411-3414.
10. Vu, B. G., Stach, C. S., Kulhankova, K., Salgado-Pabon, W., Klingelhutz, A. J. and Schlievert, P.M. **2015**. Chronic Superantigen Exposure Induces Systemic Inflammation, Elevated Bloodstream Endotoxin, and Abnormal Glucose Tolerance in Rabbits: Possible Role in Diabetes. *MBio*, 6 (2), e02554-14.
11. Olokoba, A. B., Obateru, O. A. and Olokoba, L. B. **2012**. Type 2 diabetes mellitus: a review of current trends. *Oman Medical Journal*, 27 (4), pp: 269-273.
12. American Diabetes Association (ADA) **2012**. Standards of medical care in diabetes—2012. Diabetes Care. 35 (Supp 1), pp: 11- 63.

13. World Health Organization (WHO) .**2013**. Global Database on Body Mass Index-World Health Organization. Available: http://apps.who.int/bmi/index.jsp?intro. Page=intro_1.html.Accessed 2013 April 12. Geneva.

14. Holt, J.G., Krieg, N.R., Sneath, P.H., Staley, J.T. and Williams, S.T. **1994**. Bergy's Manual of Determination Bacteriology. 9th ed. Williams and Wilkins, Baltimore, USA. Com., pp:141-156.

15. Maniatis, T., Fritsch, E. F. and Sambrook, J. **1982.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, N. Y.**,** 11 (2), p 82.

16. Xu, X., Zhao, H., Shi, Y., You, L., Bian, Y., Zhao, Y. and Chen, Z. J. **2011**. Family association study between INSR gene polymorphisms and PCOS in Han Chinese. *Reproductive Biology and Endocrinology*, 9 (76), pp: 1-5.

17. Breen, J. D. and Karchmer, A. W. **1995**. *Staphylococcus aureus* infections in diabetic patients. *Infectious Disease Clinics of North America*, 9 (1), pp: 11-24.

18. Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A. and Nouwen, J. L. **2005**. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet. *Infectious Diseases*, 5 (12), pp: 751–62.

19. AL-Kazaz, E. J. N. **2014**. Biochemical and Molecular Study of Staphyloxanthin Extracted from Clinical Isolates of *Staphylococcus aureus*. M.Sc. Thesis, College of Science, University of Baghdad, Baghdad, Iraq.

20. Ahluwalia, A., Sood, A., Sood, A., Lakshmy, R., Kapil, A. and Pandey R. M. **2000**. Nasal colonization with *Staphylococcus aureus* in patients with diabetes mellitus. *Diabetic Medicine*, 17 (6): 487-488.

21. Alizargar, J., Sharif, M. and Sharif, A. **2013**. Risk Factors of Methicillin-Resistant *Staphylococcus aureus* Colonization in Diabetic Outpatients, A Prospective Cohort Study. *International Journal of Microbiology Research*, 4 (2), pp: 147-151.

22. Diekema, D. J., Pfaller, M. A., Schmitz, F. J., Smayevsky, J., Bell, J., Jones, R. N. and Beach, M. **2001**. Survey of infections due to *Staphylococcus species*: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, the Western Pacific region for the SENTRY Antimicrobial Surveillance Program 1997–1999. *Clinical Infectious Diseases*, 32 (2), pp: 114–132.

23. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. and Fowler, V. G. **2015**. "*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management". *Clinical Microbiology Reviews*, 28 (3), pp: 603–661.

24. Zadik, P. M., Davies, S., Witaker, S. and Muson, C. **2001**. Evaluation of new selective medium for methicillin – resistance *Staphylococcus aureus*. *Journal of Medical Microbiology*, 50, pp: 476 – 479.

25. Neill, R. J., Fanning, G. R., Delahoz, F., Wolff, R. and Gemski, P. **1990**. Oligonucleotide Probes for Detection and Differentiation of *Staphylococcus aureus* Strains Containing Genes for Enterotoxins A, B, and C and Toxic Shock Syndrome Toxin-1. *Journal of Clinical Microbiology*, 28 (7), pp: 1514-1518.