



Prevalence of Nasal Carriage *Staphylococcus aureus* and methicillin-resistant (MRSA) *S. aureus* among some type 2 diabetes mellitus patients in Baghdad city

Jassep Abdulnabi Thajeel¹, Ashwak Bassem Al-Hashimy²

1: Ministry of Health, Baghdad, Iraq.

2: Institutes of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Abstract

About one-third of humans have the bacterium *S. aureus*, which is recognized for its opportunistic tendency of invading the anterior nares *S. aureus* and/or (MRSA) can colonize the anterior nares and go on to colonize the skin and other anatomical areas. As such, these pathogens Eve the capacity to cause a wide variety of endogenous diseases. In order to examine the frequency of nasal carriage of *S. aureus* or MRSA among some diabetic patients in Iraq/Baghdad, nasal swabs were collected from 50 Type 2 diabetic patients and 50 control subjects. A 50 blood samples were collected (patient with T2DM) collected from different age groups and duration of the disease in patients who attended "The Specialist Center for Diseases of Endocrine and Diabetes" in Baghdad. Another 50 blood samples collected from normal healthy Entrols at different ages and genders. The period of study was from February 2021 and April 2022. The outcomes demonstrated that T2DM patients and healthy controls differed significantly. in FBG and HbA1c at (P≤0.01).

To find evidence of *S. aureus* colonization, a nasal swab was taken. Every patient with type 2 diabetes and the healthy controls provided a repeat swab for the purpose of estimating the persistence of *S. aureus* carriage. According to the cultures morphology and biochemical characteristics and through used API staph, to confirmed the diagnosis. and molecular detection using nuc gene, the S. aureus isolates were identified MRSA isolates were identified by PCR using specific primer of *mecA* gene Persistent carriage was seen in 30% of cases and *S. aureus* nas colonization in 52% of cases. from every Type 2 diabetic patient, and Nasal colonization by *S. aureus* was prevalent at 32%. and of recurrent carriage Healthy controls had 12%, with 24% of the total patients being colonized specifically with MRSA.

Keywords: Staphylococcus aureus, MRSA, Type Two Diabetes Mellitus, Nasal Carriage





Introduction

It is commonly acknowledged that diabetes mellitus (DM) poses a serious threat to public health. The phrase "diabetes mellitus" refers to a group of common metabolic and pathological conditions marked by high blood glucose levels. This disorder develops either as a result of the pancreas producing insufficient amounts of insulin or the body's inability to properly use the insulin produced. High blood glucose levels are the hallmark of hyperglycemia, a disease caused by uncontrolled diabetes mellitus. Extended periods of high blood sugar have negative impacts on the body's physiological systems, specifically on blood vessels and neurons. (Al, Haneen and Essam.. 2022; Fayyadh and ALThwani, 2023). Two primary features of the metabolic disorder known as (T2DM) are diminished insulin production by pancreatic B-cells and decreased responsiveness of insulin-sensitive tissues (Galicia-Garcia et al., 2020). The global incidence of diabetes is experiencing an upward trend. It is prevalence is increasing most rapidly in low and middle-income countries (Abdul-Hasan and Yassin 2018). According to Aljulifi (2021), global estimates indicate that over 415 million individuals were affected by diabetes mellitus in 2019. Furthermore, projections suggest that this figure is anticipated to rise to 642 million by the year 2040. In 2017, Iraq recorded a total of 1,411,500 cases of diabetes within its adult population (IDF, 2018). The pathogenic microbe Staphylococcus aureus is in charge of a wide variety of human illnesses that have a major effect on public health. The anterior nares is an essential reservoir for S. aureus, and the development of staphylococcal illness has been linked to the presence of the bacteria in the nasal cavity (Luzzago et al., 2014; Al-Shammary et al., 2016; Bitrus et al., 2018a; Oliveira et al., 2018; Cheung et al., 2021; Kawada-Matsuo et al., 2021). Due to its unusual capacity to evade the innate immune response, including mechanisms such as phagocytic . complementary, or antimicrobial peptide (AMP)-mediated death. This unique ability enables the pathogen to survive in various tissues, including the bloodstream. Therefore, Staphylococcus aureus is considered to be one of the major microorganisms responsible for causing many diseases. (Kaspar et al., 2016; Ansari *et al.*, 2019). The virulence of *S. aureus* is attributed to the presence of toxins and enzymes that have the ability to inflict significant harm on tissues and organs. Additionally, these toxins and enzymes are capable of regulating the immune response to these infections (Akrae *et al.*, 2021). The rise in antibiotic resistance, particularly (MRSA), has caused S. aureus to become a major public health concern (Mussa and AlMathkhury,2018; Shamkhi et al.,2019; Cheung et al.,2021; Omar and Mohammed, 2021). It has been proposed that a variety of processes underlie S. aureus colonization of the nasal passages, with the host's immunological response being the most important component. (Sakr et al., 2018; Ceccarelli et al., 2019). It is widely acknowledged that those diagnosed with diabetes are often regarded as being more vulnerable to infections, which





tend to manifest with greater severity compared to individuals without diabetes (Cockram and Wong, 2017; Chávez-Reyes *et al.*, 2021). According to Berbudi *et al.* (2020), the presence of hyperglycemia in individuals with diabetes exerts a significant impact on the immune response to microbial invasion. Previous research has demonstrated that elevated blood glucose levels, known as hyperglycemia, have a detrimental effect on the activation of immune cells' inflammatory genes. Consequently, this impairs their ability to mount an effective inflammatory response against microbial infections (Akbari and HassanZadeh, 2018). Numerous investigations carried out across many nations have revealed that people with diabetes have a higher incidence of nasal colonization by (MRSA) or Staphylococcus aureus than People who are deemed healthy (Tamer *et al.*, 2006; Lin *et al.*, 2017; Stacey *et al.*, 2019). Therefore; Investigating the occurrence of *S. aureus* or MRSA nasal colonization among Iraqis with type 2 diabetes was the goal of this investigation.

Materials and Methods

Patients

Fifty Iraqi patients (females and males) with Type 2 DM (mean age 42.97 ± 4.02 years), and 50 apparently healthy individuals as controls (mean age 46.07 ± 3.61 years) who's their gender and age matched the patients group, enrolled in this study. From February 2021 until April 2022, they were enrolled in "The Specialist Center for Diseases of Endocrine and Diabetes" in Baghdad. All patients were chosen based on the diabetes criteria, which were determined by measuring body mass index (BMI) in both patients and controls in accordance with the American Diabetes Association's 2012 guidelines (ADA, 2012).

Sample collection

1- Blood Samples Collection

Blood samples were obtained from gel tubes and EDTA tubes for the purpose of conducting biochemical investigations. The serum was utilized for the quantification of glucose. The evaluation of glucose levels was conducted using the methodology described in reference (Barham and Trinder 1972), with the reagents utilized in the analysis being supplied by Biotek, Spain. The determination of HbA1C is performed using blood taken in an EDTA tube. NycoCard is a Norwegian company.

2- Bacterial Isolation: Nasal specimens were taken under aseptic conditions from the anterior nares of both patients and controls using sterile nasal swabs. After the cotton swab for nose was placed into the nasal cavity at a depth of about one inch, it was rotated three times in a clockwise direction and three times counterclockwise direction. Immediately after, the swabs





were streaked onto blood agar plates and incubated for twenty-four hours at 37°C. A minimum of three months had passed before obtaining a second nasal swab culture. Subjects were categorized as intermittent carriers when one culture was positive and the other was negative, as non-carriers when both cultures were negative, and as persistent carriers when both cultures were positive. Those who only had one nose swab available were not included in the ongoing investigation for persistent carriage.

3- Based on their colonial morphology and gram-stained film, the colonies that were thought to be present were sub culturing on mannitol salt agar that contained 7.5% sodium chloride. After that, the subcultures were maintained at 37°C for eighteen to twenty-four hours.

Detection of Methicillin-resistant S. aureus (MRSA)

Antibiotic susceptibility tests Muller-Hinton (MH) agar was used to test the antibiotic susceptibility of Staphylococcus aureus isolates by the disk diffusion (Kirby-Bauer) method. For this study, the antibiotic methicillin (Bioanalyses, Turkey) was used. After measuring the inhibitory zone's diameter, the results were compared to the National Committee for Clinical Laboratory Standard institute's chart (CLSI, 2022).

Genomic DNA extraction from S. aureus bacteria: -

The DNA extraction was performed for the chromosomes of S. aureus isolates. Isolates were grown first on the blood agar to get a prolific growth and then one colony of each strain cultured was then inoculate in 5 ml of brain heart infusion broth. Incubated at 37°C for 24h. Chromosomal DNA was purified from bacterial cells used as templates for all PCR experiments. To attempt the optimize of the DNA extraction from staphylococcal isolates, the classical method using Genomic DNA mini kit, (Geneaid, Korea) protocol for gram positive bacteria was used.

Estimation of DNA concentration and purity: -

The Nano drop technique was utilized to estimate the DNA concentration of the samples. 0.5µl of the extracted DNA was placed in the instrument to detect concentration in ng, and the O.D. 260/280 ratio was observed to identify the purity of the DNA samples including protein. For pure DNA, a ratio of 1.6 to 2 was considered acceptable. A 1% agarose gel electrophoresis might be used to evaluate the quality of DNA (Sambrook and Russell, 2001).



Gel Electrophoresis

Agarose gel electrophoresis was used to verify the existence and integrity of the isolated genomic DNA following its extraction.

Detection of S. aureus Using Specific genes by polymerase chain reaction technique:

The polymerase chain reaction method was used to amplify particular regions inside the target gene in order to identify S. aureus utilizing that particular gene. The DNA sample (template) and a master mix reagent containing Taq polymerase, PCR buffer, MgCl2, and dNTPs were combined with particular sets of primers selected for each target gene to conduct the experiment. The deionized water was the last component. To achieve a final volume of 25μ l, the reaction mixture was mixed and centrifuged for 3 seconds to collect any droplets from the walls. It was then transferred to a thermal cycler to initiate the reaction in accordance with the program's instructions.

Primers preparation

The primers were supplied by the integrated DNA Technologies Company (Alpha DNA) as a lyophilized product of different Picomole concentrations. Primers were resuspended according to company policy, which calls for bringing the final primer concentration to 10 pmol/ μ l of TE buffer and storing it at -20°C until needed Table (1).

Genes	Primer	Primor		
Genes			Size (bp)	
N 110	Forward	CGATTGATGGTGATACGGTT	279	
nuc	Reverse	ACGCAAGCCTTGACGAACTAAAGC	2/9	
MecA Forward		GTAGAAATGACTGAACGTCCGATAA	310	
MecA	Reverse	CCAATTCCACATTGTTTCGGTCTAA		

Table (1): Name, sequence, product size and reference of primers used in this study.

Working solution

The PCR Pre Mix was accomplished after several trials. Thus the following mixture was adopted, Table (2).





Table (2): The PCR master-mix Volume for (nuc and mecA) genes.

Item	Master mix	Target DNA	Forward Primer (10pm/ µl)	Reverse Primer (10pm/ μl)	Nuclease free water	Total volume
Volume	12.5 μ	3 µl	1 µl	1 µl	7.5 μl	25 µl

PCR program to detect the (*nuc*) gene amplification, the PCR program was adopted as in Table (3).

Step	Temperature °C	Time	No. of cycles
Initial denaturation	95	5min	1
Denaturation	94	1min	
Annealing	56	1 min	35
Extension	72	1min	
Final Extension	72	10min	1
Hold	4	8	-

Table (3): Optimized polymerase chain Reaction (PCR)cycling program.

To detect the (mecA) gene amplification, the PCR program was adopted as in Table (4).

 Table (4): Optimized polymerase chain Reaction (PCR)cycling program.

Step	Temperature °C	Time	No. of cycles
Initial denaturation	94	5min	1
denaturation	94	45sec	
Annealing	50	45sec	35
Extension	72	1min	
Final Extension	72	5min	1
Hold	4	8	-

On accomplishment of PCR for the *nuc* and *mecA* genes, In 2% agarose, the product was electrophoresed. Eight microliters of the PCR product were applied to each well of a 2% agarose gel for gel electrophoresis. Next, to act as a marker, 7µl of a 100 bp promega DNA ladder with loading dye was added to the first well of the agarose gel. Approximately two hours were spent



doing the electrophoresis at 70 Volts. After the gel was removed, bands on the gel were visible with a UV transilluminator. The image of the bands was captured by using a camera.

Results and Discussions:

Comparison of Age and sex in Patients with T2DM and Healthy Group:

Fifty Iraqi, T2DM patients were selected for this study and fifty healthy control. The average age in the patient group was (42.97 ± 4.02) , while in the control group it was (46.07 ± 3.61) . As shown in Table (5), there wasn't no statistically significant variation between the groups with a P value of 0.511. This result agreed with the result of Alturki and his colleagues, who did not document any significant difference between the diabetic group and the non-diabetic group (Alturki *et al.*, 2022). Al-Darraji *et al.*, 2017, find did not document any significant difference between the diabetic group which consistent with the result of the current study.

Parameters	Control (n=50)		Patient ((n=50)	t – test	
rarameters	No.	%	No.	%	P-Value	
Sex				·	·	
Male	25	0.50	25	0.50	NIC	
Female	25	0.50	25	0.50	——NS	
Age				·	·	
Age (mean±SD)	46.07 ±3.	61	42.97 ±4	.02	0.511 NS	
	NS: Non		Significa	nt		

Table (5): Statistical analysis of Age and sex for patients and healthycontrols.

Comparison of FBS and HbA1C in Patients with T2DM and Healthy Group:

The levels of Fasting blood sugar were estimated in serum of all T2DM patients and controls. In this study, diabetic patients have abnormal levels in blood glucose (FBS) compared to the healthy control group. Table (5) shows that a high significant difference was observed in FBS mean level (p<0.001) in the patient group (259.67±12.72) and in the control (89.00±1.72).

The HbA1c was measured in specimen of all T2DM patients and apparently healthy controls. The results showed in Table (6) that there were highly significant differences (P=0.0001) between the patients and healthy controls. The mean average percentage and standard deviation of patients were (9.09 \pm 0.20). The mean average percentage and standard deviation of healthy controls were



 (5.23 ± 0.08) which was lower than the percentage of patient. According to American Diabetes Association HbA1c can be used as a diagnostic biomarker for detecting T2DM (American Diabetes Association, 2021).

The present study's findings corroborated those of two other studies conducted among Iraqis, which showed a highly significant difference between T2DM patients and healthy controls. The FBG went up in T2DM patients whereas it went down in healthy controls. Additionally, the patients' HbA1c % showed a significantly higher increase than that of the healthy controls. (Alturki *et al.*, 2022; Al-Darraji *et al.*, 2017). The identical results were found in a research conducted among Egyptians, which demonstrated that T2DM patients had significantly higher FBG levels and HbA1c percentages than controls. (Fathy *et al.*, 2018; El Gendy *et al.*, 2019).

These findings concur with those of Sherwani *et al.* (2016), who showed that the physiological effective cycle normally includes the synthesis of glycated hemoglobin. Schiff base is formed when glucose in the open branch binds to the beta chain's N-terminal. The Schiff base is transformed into Amadori products during the reformation, the most well-known of which being HbA1c. This is a non-enzymatic process that often manifests in vivo as higher HbA1c levels in plasma and average blood glucose.

nearly controls.							
Biochemical	Control	Control (n=50)		n=50)			
Parameters	No.	%	No.	%			
FBS (mg/dl)	89.00	± 1.72	259.67 ± 3	12.72	0.0001**		
HbA1C (%)	5.23 ± 0.0	08	9.09 ±0.2	20	0.0001**		

 Table (6): Statistical analysis of FBS and HbA1C parameters in T2DMpatients and healthy controls.

Isolation and identification of *S. aureus* bacteria: Morphological examination

All 100 nasal swabs from T2DM patients and control were cultured on blood agar. There were 72 specimen gave positive culture under aerobic condition and 128 swabs specimen with negative growth .72 of suspected bacterial isolate growth on MSA were characterized by the appearance of yellow colonies due to mannitol fermentation.

All the positive MSA were termed Staphylococci because they were yellow; the high concentration of salt induces Staphylococci to generate carotenoid pigments and can transform the medium around the colony into yellow owing to mannitol fermentation. Appendix (2). Whereas others white colonies would not ferment mannitol (Thakur *et al.*, 2017).





The colonies of *Staph. spp. Species* appeared on the blood agar in various shapes, from a white to creamy or golden colonies, convex surface, smooth. *S. aureus* usually appeared alight to golden yellow pigment with Beta-hemolysis (B-hemolysis) on blood agar. This results agreed with the results of other researchers, including (Ifeanyi *et al.*, 2014) and (Almwafy, 2020).

Biochemical identification

To identify the microorganisms mentioned in Table (7), a number of biochemical assays were carried out. These tests made it possible to swiftly identify the unidentified isolates using color changes that were seen in the different tested staph. When a hydrogen peroxide reagent was added to the colonies, all of the suspicious isolates in the catalase test produced gas bubbles, indicating a positive result. On the oxidase and coagulase tests, however, they tested negative and positive, respectively. These traditional biochemical assays were performed in the current investigation, and the outcomes were compared to benchmark data recorded by (Brooks, 2010; Kateete *et al.*, 2010; El-Hadedy and Abu El-Nour, 2012).

Biochemical test	Results
Mannitol salt agar	Yellow colonies
Gram stain	Gram positive cocci
Blood agar	white to creamy or golden colonies
Coagulase test	(+)
Catalase	Babbles (+)
Oxidase	No Purple color (-)
(+): Positive; (-): Negative	

Table (7): Biochemical tests and their results of S. aureus bacteria

Identification of S. aureus by analytical profile index Staph and VITEK 2 system

The VITEK 2 and API Staph. compact systems were utilized to precisely and accurately identify S. aureus isolates that were linked to the species of isolates that had previously been recognized by traditional biochemical testing. In the current investigation, the outcomes from this method matched those from biochemical identification.

Molecular identification of *staphylococci*: DNA extraction



Genomic DNA was extracted from 72 bacterial isolates that diagnosed by API staph. Total DNA extracted using DNA extraction kit. The concentration and the purity of extracted DNA was measured by Nano drop and the purity is ranged to(1.7-2.04) then detected by gel electrophoresis as shown in Fig (1).

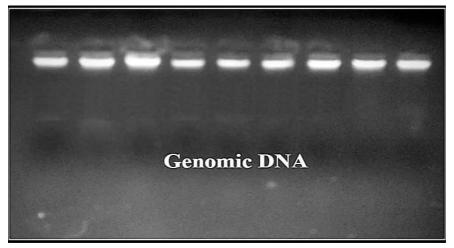


Figure (1): Gel electrophoresis of whole genome DNA of suspected *S. aureus*. Agarose 1%, 70 V for 1hrs., stained with Safe Gel Stain and visualized on a UV transilluminator.

Molecular diagnosis of S. aureus bacteria:

For isolates that had already been confirmed as *S. aureus* by morphological and biochemical characteristics, as well as by the API staph and Vitek 2 systems, a second diagnosis was made using the PCR technique. This involved amplifying a conserved region of the nuc gene encoding thermostable nuclease (TNase) using gene-specific primers for confirmation up to the species leve. The whole collection of *S. aureus* staphylococci was identified using the nuc primer set. (Ihab and Atef, 2008).

Seventy-two of the isolates were identified as *S. aureus* based on the molecular diagnostic. The single DNA band that represented the amplified PCR product had a molecular size of around 279 bp. (Fig.2) When compared to the size Marker 100bp Ladder.

This finding is comparable to the earlier published study (Sutejo *et al.*, 2017) that found nuc genes in every isolate of S. aureus. Additionally, it transpired in line with the discovery of (Al-



Ugailiity, 2013), Who observed that: the molecular method using *nuc* gene to detect *S. aureus* is of high specificity and sensitivity to all the isolate used.

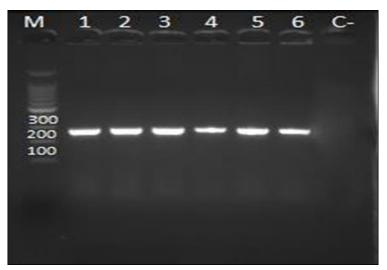


Figure (2): Agarose gel electrophoresis of PCR amplification products of *S. aureus, nuc* gene, line M: DNA molecular Wight marker (100 bp), lines (1-6)positive amplification (279 bp) for *nuc* gene, line (C-) negative control.

The *S. aureus* bacteria was found in 26 out of 50 (52%) of the individuals with T2DM whose nasal swabs were taken in the initial round. Compared to the 50 individuals from which a second nasal sample was obtained for culture, 15 (or 30%) were classified as persistent carriers, 11 (or 22%) as intermittent carriers, and 24 (or 48%) as non-carriers. In contrast, 16/50 (or 32%) of the individuals from whom the first nasal swab sample was obtained were found to be colonized by S. aureus. Based on the data displayed in Table (8), it was determined that, out of the 50 persons from whom a second nasal sample for culture was collected, 6 (12%), were carriers of *S. aureus*, were intermittent (20%), and 34 (68%), were not carriers.

Table (8): S. aureus Nasal Carriage percentage in T2DM patients compared with control individuals.

S. <i>aureus</i> Patients groups Healthy control gr	oups
---	------





	(N = 50)	$(\mathbf{N}=50)$		(N = 50)	
	No.	%	No.	%	
Total Number	26	52	16	32	
Persistent carriers	15	30	6	12	
Intermittent carriers	11	22	10	20	
Non-carriers	24	48	34	68	

Panierakis *et al.*, (2009) stated that nearly 31.2% of *S. aureus* were isolated from initial nasal swab sample which was taken from patients with T1DM, was obtained 19 (25%) were defined as persistent *S. aureus* carriers, 21 (27.6%) as intermittent carriers, and 36 (47.4%) as non-carriers for *S. aureus*.

In a previous study, it was found that the nasal carriage of *S. aureus* bacteria in T2DM patients was (19.7%), 10 (6.2%) were classified as intermittent S. aureus carriers, eighty-six (8.6%) as persistent carriers, and eighty-two (85.2%) as non-carriers. (Messaritakis *et al.*, 2014). Also, another study found that the percentage of nasal carriage of

The *S. aureus* (NCSA) in T2DM patients from Iraq was found in 56.10% of the patients, compared to 36.58% in the control groups (Hamad et al., 2018). According to AL-Kazaz (2014), 40% of nasal samples *S. aureus* was present. Another study indicated that the rate of NCSA in diabetic patients was greater than that in the control group, as in the study of Ahluwalia et al (2000), who discovered that the prevalence of NCSA in DM patients was 56.6%, whereas in the control group, it was 14.8%.

Additional research revealed that the rate of non-communicable diseases (NCSA) among patients with diabetes was 41.78% in Turkey (Kutlu et al., 2012); 42.5% (210/494) among 494 DM patients in Iran (Biedenbach et al., 2004); 72.41% (42/58) among long-term hemodialysis patients in Saudi Arabia (Saxena et al., 2002); and 56.67% (34/60) among hospitalized diabetic patients in India (Ahluwalia et al., 2000). Diabetes patients in China (20.50%, 41/200) (Junhua et al., 2005), Australia (39.09%, 258/660) (Hart et al., 2015), and China (10.11%, 43/417) (Kutlu et al., 2012) are the countries with the highest percentage of diabetic patients. In a related study, Tamer et al. (2006) found that among type II diabetes patients, the nasal carriage rate and associated factor were 22.2% (33/148).

From the above statistics, it was known that the prevalence of *S. aureus* nasal carriage among diabetic population were different in different countries and regions, and diabetic population might be more likely to carry *S. aureus*.





This variation of nasal carriage rate of *S. aureus* in studies might be due to difference in the characteristics of the study population, quality of sampling, geographical distribution, culturing and diagnostic techniques (Tigabu *et al.*, 2018). This presence may result from the fact that it is one of the primary causes of community-acquired and hospital-acquired infections (nosocomial infections), both of which can have serious repercussions. It is also one of the important pathogens that have the potential to cause opportunistic infections because it is a normal part of the body's flora and has a number of virulence factors that allow it to penetrate body tissue and contribute to the pathogenesis of infection. (Tong *et al.*, 2015; Kumar *et al.*, 2020).

In this investigation, the nasal swab culture total nasal carriage rate of MRSA for S. aureus was positive in 12/50 (24.0%) of T2DM patients and 9/50 (18.0%) of healthy controls. Table (9).

The prevalence of MRSA nasal colonization among the diabetes population in this study was higher than those of type 2 diabetes patients in China (5.28%, 22/417) (Yan *et al.*,2015), Diabetes patients receiving long-term hemodialysis in Saudi Arabia (18.97%, 11/58) (Saxena et al., 2002), Turkey (9.87%, 30/304) (Kutlu et al., 2012), and China (0.50%, 1/200)Twenty Australians with diabetes (1.21%, 8/660)Hart and colleagues, 2015). However, it fell short of Iranian research findings, which showed that 24.6% of all DM patients met this criteria (Biedenbach et al., 2004).This variation of nasal carriage rate of *S. aureus* in studies might be due to difference in the characteristics of the study population, quality of sampling, geographical distribution, culturing and diagnostic techniques (Tigabu *et al.*, 2018).

Type of Diabetes mellitus	~ .		Healthy control groups N=50	
	No.	%	No.	%
T2DM	12	24	9	18

Table (9): MRSA nasal carriage rate among T2DM Patients and Healthy controls.

Molecular detection of Methicillin Resistance S. aureus (MRSA)

In addition to their resistance to Methicillin antibiotic disk on plate, MRSA isolates were diagnosed by Molecular method using specific primer for*mecA* gene (310 bp), from total 72 *S. aureus* isolates only 41 isolates (56.9%) were found to be positive for mecA gene Figure (3).



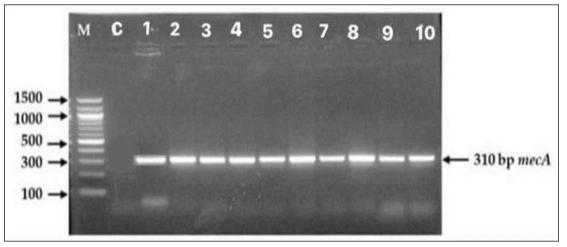


Figure (3): Gel electrophoresis of amplified PCR products of *MecA* gene (310bp) of *S. aureus* isolates in PCR technique Agarose gel (2%).

The results of the current study is in with accordance Nwaogaraku *et al.*, (2019) who found that 56% of *S. aureus* were methicillin resistant *S. aureus* harboring the *mec A* gene while another study done by Kali *et al.*, (2014) found that *mecA* gene presented (90.1%) from all the isolate. In addition, other studies by (Abulreesh *et al.* 2017, Ibrahim *et al.*, 2017) and (Sherfi and Badri, 2018) demonstrated that the percentage of *mecA* gene were (44%, 55.5% and 42%) respectively. The *S. aureus* becomes methicillin resistant by the acquisition of the *mecA* gene which encodes a penicillin binding protein 2a (PBP2a) with a low affinity for β -lactams, the strains that produce PBP2a are resistant to all β -lactams (Vishnu *et al.*, 2018).

The prognosis of S. aureus infection greatly depends on the precise and timely identification of methicillin resistance. Even though there are several techniques for detecting this resistance, they are frequently too slow, sensitive, or specific to guarantee that patients with MRSA infections receive the proper care. The individual using the procedures and the approach they employ affect the methods' sensitivity and specificity values.

The most accurate way to identify MRSA isolates is to identify the *mecA gene*; however, not all labs are able to integrate molecular biology techniques into their standard clinical practice. Due to technological and financial limitations, the detection of mecA, which is the gold standard for identifying MRSA strains, was not possible. For this reason, it is critical that phenotypic techniques capable of quickly and accurately identifying MRSA isolates be made available in order to guarantee appropriate antibiotic treatment and prevent the spread of MRSA isolates.





References

- 1. Abdul-Hasan AA, Yassin BA (2018). Health Literacy of Diabetic Patients and its Impact on Disease Outcome. Journal of the Faculty of Medicine Baghdad, Vol. 60 No. 1.
- Abulreesh,H. H.; Organji, S. R.; Osman, G. E.; Elbanna, K.; Almalki, M. H., and Ahmad, I. (2017). Prevalence of antibiotic resistance and virulence factors encoding genes in clinical Staphylococcus aureus isolates in Saudi Arabia. Clinical Epidemiology and Global Health, 5(4): 196-202.
- 3. Ahluwalia, A., Sood, A., Lakshmy, R., Kapil, A., & Pandey, R. M. (2000). Nasal colonization with Staphylococcus aureus in patients with diabetes mellitus. Diabetic medicine: a journal of the British Diabetic Association, 17(6), 487-488.
- 4. Akbari, M. and Hassan-Zadeh, V. 2018. Hyperglycemia affects the expression of inflammatory genes in peripheral blood mononuclear cells of patients with type 2 diabetes. Immunological Investigations 47(7): 654–665.
- Akrae, D. K., Al-Ahmer, S. D., & Ghareeb, A. M. (2021). Association Of Biofilm Production Involved Icaa Gene And Antibiotic Resistance Profile With Ocular Infections Incidence Caused By Staphylococcus Aurous. Biochemical & Cellular Archives, 21(1).
- 6. Al, Haneen A. Abd & Essam F. (2022). The Relationship between Some Biochemical Parameters and Type 2 Diabetes Mellitus among Iraqi Patients. Iraqi journal of biotechnology, 21(2).
- 7. Al-Darraji,S.; Al-Azzawie, H. and Al-Kharsani, A. (2017). Vitamin D status and its receptor genes BsmI, FokI, ApaI, TaqI polymorphism in relation to glucose metabolism in obese Iraqi type 2 diabetes mellitus patients. Journal of Molecular and Genetic Medicine, 11(2): 1747-0862.
- Aljulifi, M. Z. (2021). Prevalence and reasons of increased type 2 diabetes in Gulf Cooperation Council Countries. Saudi Medical Journal, 42(5), 481. IDF.https://www.idf.org/our-network/regions-members/middle-east-andnorthafrica/members/36-iraq.html. 2018
- 9. AL-Kazaz, E. J. N. (2014). Biochemical and Molecular Study of Staphyloxanthin Extracted from Clinical Isolates of Staphylococcus aureus (Doctoral dissertation, M. Sc. Thesis, College of Science, University of Baghdad, Baghdad, Iraq
- 10. Almwafy,A. (2020). Preliminary Characterization and Identification of Gram Positive Hemolysis Bacteria. Al-Azhar Journal of Pharmaceutical Sciences, 62(2), 96-109.





- Al–Shammary, A. A., Rasheed, M. N., Hasan, O. M., & Nader, M. I. (2016). Study The Coagulase Gene in Staphylococcus Aureus Isolated from Different Sources by Using PCR Amplification. European Journal of Biomedical, 3(1), 42-45.
- 12. Alturki, A. D.; Allami, R. H. and Fadhel, Z. A. (2022). Association of vitamin D deficiency with type 2 diabetes mellitus in Iraqi population: A case-control study. In AIP Conference Proceedings,2547(1).
- 13. Al-Ugailiity, D. N. (2013). Bacteriological and Genetic Studies on Oxacillin Resistant Staphylococcus aureus Isolated from Some Hospital in Baghdad. Thesis Ph.D.Nahrain University .
- 14. American Diabetes Association (ADA). classification and diagnosis of diabetes: standards of medical care in diabetes Diabetes Care 2012; 41(1), S13- S27.
- 15. Ansari S, Nepal HP, Gautam R, *et al.* Staphylococcus aureus: methicillin resistance and small colony variants from pyogenic infections of skin, soft tissue and bone. J Nepal Health Res Counc. 2015;13(30):126–132.
- 16. Barham, D. and Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst, 97(151): 142–145.
- 17. Berbudi, A., Rahmadika, N., Tjahjadi, A.I. and Ruslami, R. 2020. Type 2 diabetes and its impact on the immune system. Current Diabetes Review 16(5): 442–449.
- Biedenbach, D. J.; Moet, G. J. and Jones, R. N. (2004). Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002). Diagnostic microbiology and infectious disease, 50(1): 59-69.
- Bitrus, A., Peter, O., Abbas, M., & Goni, M. (2018). Staphylococcus aureus: a review of antimicrobial resistance mechanisms. Veterinary Sciences: Research and Reviews, 4(2), 43-54.
- 20. Brooks, G. F. (2010). Jawetz, Melnick, and Adelberg's medical microbiology/Geo. F.Brooks. New York; Chicago: McGrawHill Medical . 30-75.
- Ceccarelli, F., Perricone, C., Olivieri, G., Cipriano, E., Spinelli, F. R., Valesini, G., & Conti, F. (2019). Staphylococcus aureus nasal carriage and autoimmune diseases: from pathogenic mechanisms to disease susceptibility and phenotype. International Journal of Molecular Sciences, 20(22), 5624.
- 22. Chávez-Reyes, J., Escárcega-González, C. E., Chavira-Suárez, E., LeónBuitimea, A., Vázquez-León, P., Morones-Ramírez, J. R., ... & MarichalCancino, B. A. (2021).





Susceptibility for some infectious diseases in patients with diabetes: the key role of glycemia. Frontiers in public health, 9, 559595.

- 23. Cheung, G. Y., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. Virulence, 12(1), 547-569.
- Cockram, C. S., & Wong, B. C. (2017). Diabetes and infections. Textbook of Diabetes, 799-818
- 25. El Gendy,H.I.; Sadik, N.A.; Helmy, M.Y. and Rashed, L.A. (2019). Vitamin D receptor gene polymorphisms and 25 (OH) vitamin D: Lack of association to glycemic control and metabolic parameters in type 2 diabetic Egyptian patients. Journal of clinical and translational endocrinology, 15: 25-29.
- 26. El-Hadedy,D. and Abu El-Nour, S. (2012). Identification of Staphylococcus aureus and Escherichia coli isolated from Egyptian food by conventional and molecular methods. Journal of Genetic Engineering and Biotechnology, 10(1): 129–135.
- 27. Fathy,W.; Tawfeek, G.; Tawfeek, A. and Aboelyazeid Ellayen, S. (2018). Vitamin D receptor (BsmI) gene polymorphism and type 2 diabetes mellitus in an Egyptian population. Menoufia Medical Journal, 31(2): 557-563.
- Fayyadh, Z., &ALThwani, A. N. (2023). Molecular and Demographic study about (T1DM) and associated with HLA typing Class II in sample of Iraqi children. Journal of Survey in Fisheries Sciences, 10(3S), 1465- 1473.
- Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences, 21(17), 6275.
- Hamad, S. L., & Melconian, A. K. A. (2018). Bacterial endotoxin, Staphylococcus aureus nasal carriage and obesity among type two diabetes mellitus patients. Karbala International Journal of Modern Science, 4(1), 93-99.
- Hart, J., Hamilton, E. J., Makepeace, A., Davis, W. A., Latkovic, E., Lim, E. M., ... & Davis, T. M. (2015). Prevalence, risk factors and sequelae of Staphylococcus aureus carriage in diabetes: the Fremantle Diabetes Study Phase II. Journal of Diabetes and its Complications, 29(8), 1092-1097.
- 32. Ibrahim, O. M. A.; Bilal, N. E.; Osman, O. F., and Magzoub, M. A. (2017). Assessment of methicillin resistant Staphylococcus aureus detection methods: analytical comparative study. The Pan African Medical Journal, 27: 281.





- Ifeanyi, V. O.; Nwosu, S. C.; Okafor, J. O.; Onnegbu, C. P. and Nwabunnia, E. (2014). Comparative studies on five culture media for bacterial isolation. African Journal of Microbiology Research, 8(36): 3330–3334.
- Ihab, M. and Atef, S. (2008). Molecular characteristic of Methicillin resistant Staphylococcus aureus removed from outpatient clinic in Riyadh Saudi Arabia. Saudi Medical Journal. 30(5): 611 – 617.
- Inical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial 8 Susceptibility Testing; Thirty-second Informational Supplement. CLSI document M100, 2022
- 36. Junhua, M.; Junchang, C.; Rui, W. and Chunrong, Z. (2005). Nasal carriage of Staphylococcus aureus and drug resistance in patients with diabetes mellitus. Chin Journal Nosocomiol, 15: 830-831.
- 37. Kali,A.; Stephen, S. and Umadevi, S. (2014). Laboratory evaluation of phenotypic detection methods of methicillin-resistant Staphylococcus aureus. Biomedical journal, 37(6): 411-414.
- Kaspar U., Kriegeskorte A., Schubert T., Peters G., Rudack C., Pieper D. H., *et al.*. (2016). The culturome of the human nose habitats reveals individual bacterial fingerprint patterns. Environ. Microbiol. 18, 2130–2142
- Kateete, D. P.; Kimani, C. N.; Katabazi, F. A.; Okeng, A.; Okee, M. S.; Nanteza, A.,...*et al.* (2010). Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials, 9(1): 23.
- 40. Kawada-Matsuo, M., Le, M. N. T., & Komatsuzawa, H. (2021). Antibacterial peptides resistance in Staphylococcus aureus: Various mechanisms and the association with pathogenicity. Genes, 12(10), 1527.
- Kumar, S.; Singh, S.; Kumar, V.; Datta, S.; Dhanjal, D. S.; Sharma, P., *et al.* (2020). Pathogenesis and antibiotic resistance of Staphylococcus aureus. Model organisms for microbial pathogenesis, biofilm formation and antimicrobial drug discovery, 99-115.
- 42. Kutlu,S. S.; Cevahir, N.; Akalin, S.; Akin, F.; Caylak, S. D.; 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): 365-368.
- 43. Lin, J., Xu, P., Peng, Y., Lin, D., Ou, Q., Zhang, T., ... & Yao, Z. (2017). Prevalence and characteristics of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus





nasal colonization among a community-based diabetes population in Foshan, China. Journal of diabetes investigation, 8(3), 383-391.

44. Luzzago, C., Locatelli, C., Franco, A., Scac-cabarozzi, L., Gualdi, V., Viganò, R., Sironi, G., Besozzi, M., Castiglioni, B., Lanfranchi, P. and Cremonesi, P. 2014. Clonal diversity, virulence-associated genes and antimicrobi-al resistance pro le of Staphylococcus aureus isolates from nasal cavities and soft tissue infec-tions in wild ruminants in Italian Alps. Veteri-nary Microbiology, 170(1):157-61.

45. Messaritakis, I.; Samonis, G.; Dimopoulou, D.; Maraki, S.; Papadakis, J. A.; Daraki, V.,... et al.(2014). Staphylococcus aureus nasal carriage might be associated with vitamin D receptor polymorphisms in type 2 diabetes. Clinical Microbiology and Infection, 20(9): 920-925.

46. Mussa, A. A., & Al-Mathkhury, H. J. F. (2018). Incidence of Ciprofloxacin-Resistant of Methicillin Resistant Staphylococcus aureus isolated from Iraqi patients. Iraqi Journal of Science, 1225-1230.

47. Nwaogaraku,C.N.; Smith ,S.I. and Badaki, J.A. (2019). The Molecular Detection of mecA Genes of Staphylococcus aureus. Annals of Advanced Biomedical Sciences, 2(2):1-7.

48. Oliveira, D., Borges, A., & Simões, M. (2018). Staphylococcus aureus toxins and their molecular activity in infectious diseases. Toxins 10: 252.

49. Omar, N. N., & Mohammed, R. K. (2021). A Molecular Study of Toxic Shock Syndrome Toxin gene (tsst-1) in β -lactam Resistant Staphylococcus aureus Clinical Isolates. Iraqi Journal of Science, 825-837.

50. Panierakis, C., Goulielmos, G., Mamoulakis, D., Maraki, S., Papavasiliou, E. and Galanakis, E. (2009). Staphylococcus aureus nasal carriage might be associated with vitamin D receptor polymorphisms in type 1 diabetes. International Journal of Infectious Diseases, 13(6): 437-443.

51. Patricia, M. 2017. Bailey & Scott's diagnostic microbiology. Elsevier, St Louis, MO..

52. Sakr, A., Brégeon, F., Mège, J. L., Rolain, J. M., & Blin, O. (2018). Staphylococcus aureus nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. Frontiers in microbiology, 9, 2419.

53. Sambrook, J. and Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, CSH, New York, USA.





54. Saxena, A.K.; Panhotra, B.R.; Venkateshappa, C,K.; Sundaram, D. S.;Naguib, M.; Uzzaman, W., et al. (2002). The impact of nasal carriage of methicillin-resistant and methicillin-susceptible Staphylococcus aureus (MRSA and MSSA) on vascular access- related septicemia among patients with type-II diabetes on dialysis. Renal failure, 24(6), 763–777.

55. Shamkhi, G. J., Saadedin, S. M., & Jassim, K. A. (2019). Detection the prevalence of some chromosomal efflux pump genes in Methicillin resistant Staphylococcus aureus isolated from Iraqi patients. Iraqi journal of biotechnology, 18(3).

56. Sherfi, S. A. and Badri, A. M. (2018). Prevalence of mecA Gene in Methicillin Resistant Staphylococcus aureus Isolated from Different Clinical Specimens in Khartoum State, Sudan. EC Microbiology, 14: 444-449.

57. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A. and Sakharkar, M.K. (2016). Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomarker insights, 11: 95-104.

58. Stacey, H.J., Clements, C.S., Welburn, S.C. and Jones, J.D. 2019. The prevalence of methicillin-resistant Staphylococcus aureus among diabetic patients: a meta-analysis. ActaDiabetology 56(8): 907–921.

59. Sutejo, S. V. H., Amarantini, C. and Budiarso, T. Y. (2017). Molecular detection of Staphylococcus aureus resistant to temperature in milk and its products. In AIP Conference Proceedings ,1908(1).

60. Tamer, A., Karabay, O. and Ekerbicer, H. 2006. Staphylococcus aureus nasal carriage and associated factors in type 2 diabetic patients. Japan Journal of Infectious Diseases 59(1): 10–14

61. Thakur, P.; Nayyar, C.; Tak, V. and Saigal, K. (2017). Mannitol- fermenting and Tube Coagulase-negative Staphylococcal Isolates: Unraveling the Diagnostic Dilemma Journal of Laboratory Physicians, 9(1):65–66.

62.Tigabu, A., Tiruneh, M., & Mekonnen, F. (2018). Nasal carriage rate, antimicrobial susceptibility pattern, and associated factors of Staphylococcus aureus with special emphasis on MRSA among urban and rural elementary school children in Gondar, Northwest Ethiopia: A comparative cross-sectional study. Advances in preventive medicine, 2018, 1-11.





63.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.

64.Vishnu, V. R.; Vishal, P. K.; Arjun, B.; Rajendran, A.; Manakadan, A. A. and Saranya, T. S. (2018). A Review on Resistance of Antibiotics against Methicillin-Resistant Staphylococcus aureus and effect of Curcumin on MRSA. Journal of Pharmaceutical Sciences and Research, 10(5): 1198-1203.

65.Yan, L.; Ping, X.; Lin, D.; Ou, Q. and Yao, Z. (2015). Nasal colonization prevalence and risk factors of methicillin-resistant Staphylococcusaureus in type 2 diabetic patients from communities. Journal Practice Medical, 31: 4133-4135.





انتشار المكورات العنقودية الذهبية والمقاومة للميثيسيلين (MARS) بين بعض مرضى السكري من النوع

الثاني في مدينة بغداد

اشواق باسم الهاشمي 2, جاسب عبد النبي ثجيل 1

حوالي ثلث من البشر لديهم بكتريا S. aureus وهي معروفه بميلها الانتهازي لغزو الناريس الأمامي. ويمكن لها وللمقاومه منها للمثيسيلين (MRSA) استعمار المسامير الامامية والاستمرار في استعمار الجلد والمناطق التشريحية الاخرى على هذا النحو ، فإن مسببات الأمراض هذه لديها القدرة على التسبب في مجموعة واسعة من الأمراض الداخلية. من أجل فحص تواتر الحمل الأنفي لبكتريا لبكتريا العنقودية الذهبية والمقاومه للمثيسيلين بين بعض مرضى السكري في العراق / بغداد ، تم جمع مسحات الأنف من خمسون مريضا بالسكري من النوع الثاني و خمسون ظاهريا اصحاء كضوابط. تم جمع مسحات الأنف من مخلف الفئات العمرية ومدة المرض لدى المرضى الذين حضروا "المركز التخصصي لأمراض الغدد الصماء والسكري" في بغداد. تم جمع خمسون عينة دم أخرى من الضوابط الصحية الطبيعية في مخلف الأعمار والأجناس. كانت فترة الدراسة من بداية شباط 2021 الى نهاية نيسان 2022

أظهرت النتائج وجود فروق ذات دلالة إحصائية عالية بين مرضى T2DM والضوابط الصحية في FBG و HbA1c عند P≤0.01, تم الحصول على مسحة الأنف للكشف عن الاستعمار بواسطة بكتريا العنقودية الذهبيه. تم الحصول على مسحة متكررة من جميع المرضى المصابين بداء السكري من النوع الثاني والسيطرة لتقدير النقل المستمر للمكورات العنقودية الذهبية.

وفقا للمظاهر الزرعية والخصائص الكيموحيوية ومن خلال نتائج اختبارات المكورات العنقودية API المستخدمة ، لتأكيد التشخيص. والكشف الجزيئي باستخدام موروث nuc ، تم تحديد عز لات بكتريا العنقودية الذهبية تم تحديد عز لات عز لات بكتريا العنقودية الذهبية المقاومه للمثيسيلين بواسطة تفاعل السلسله المتبلمرباستخدام برايمر محدد لموروث mecA وكان انتشار استعمار الأنف S. aureus 52 والنقل المستمر 30٪. من جميع المرضى الذين يعانون من مرض السكري من النوع 2 ، و كان انتشار استعمار الأنف عموم S. aureus لموروث المستمر 12 ٪ في الضوابط الصحية. مع استعمار 24٪ من إجمالي المرضى على وجه التحديد معهم المحمي.















