Phylogenetic Characterization of *Staphylococcus aureus* isolated from the women breast abscess in Al-Qadisiyah Governorate, Iraq

***Maitham Ghaly Yousif** **Mohammed Hassan Al-Mayahi**

*matham.yousif@qu.edu.iq husssaindaik@gmail.com

*Biology Department College of Science University of Al-Qadisiyah, Iraq

Abstract

Objective: The main objective of the study was to isolate and detect the phylogenetic Characterization of *Staphylococcus aureus* from women with breast abscess

Methods: *Staphylococcus aureus* was isolated from 85 samples of women with breast abscess Using agricultural biochemical methods .polymerase chain reaction (PCR) was performed for the detection of virulence gene and gene 16srRNA followed by DNA Sequence analysis for this gene

Results :A total of 23 isolates of Staphylococcus aureus were isolates from 17 (29.3%) lactating women,6(22.2%) in non lactating women ,16srRNA gene was detected in 23 (100%) , Luks gene 21(91.3%) ,blaz gene 20 (86.9%) and eta gene 4 (4.3%) of *Staphylococcus aureus* isolates .

Conclusion: the isolates that proved positive test for these Luks and eta gene are very important in the study of S.aureus infection and and pathogen city .

Keywords: Staphylococcus aureus , Phylogenetic ,polymerase chain reaction ,16srRNA gene sequence

Abstract :

The study aimed at isolating and diagnosing Staphylococcus aureus from breast abscesses in women using some morphological and molecular methods as well as investigating some of the virulence factors of the same bacteria. The clinical samples were collected from women with abscesses in the breast and different age groups under the supervision of a specialized medical staff Diwaniyah Education and maternity hospital and children in the province of Diwaniyah for the period from September 2017 to April 2018, which included the number of samples taken 85 sample.

Results of biochemical tests, microscopy, Api Staph and MASSTSTAP showed 23 isolates of *S.aureus* bacteria . All isolates were diagnosed with a 16srRNA gene (100%), as well as some genes were detected using PCR technique. The results showed that 21 isolates of the Luks gene (91.3%) And 20 isolates possessed of the blaz gene (86.9%), and isolated one possessing a eta gene (4,3%).DNA sequencing and Phylogenic Tree Analysis were used for three samples of

S.aureus isolates based on the sequence of the 16srRNA gene, which is the diagnostic gene for S.aureus .

Introduction:

Abscesses are still the major and common infection among patients and are often caused by a bacterial infection. The abscess is shaped like an infected tissue containing pus. The breast appears to be "functional" and is not complex but in fact it is a group of critical pathological changes and breast abscess is one of these changes Pathogenesis where the abscess is formed under the skin and comes from a bacterial infection to be a painful mass within the breast which is abscess and is more likely to develop in women and can be replicated by 40-50% [1], and more frequent "in women aged 15 to 60 years, especially in Lactating women where the infection is formed either because The stagnation of the remaining milk and the breastfeeding of a new baby, where the bacteria are transmitted to the breast tissue through the cracks on the nipple, causing blockage in the duct and the breast abscess occurs in women who are not breastfed smokers, who suffer from weakness of immunity, but not common and this type of abscess should be differentiated from breast cancer because one of the symptoms of breast cancer is a fluid exit from the breast[2] ,that patients with abscess may be accompanied by several signs including fever, an increase in the number of white blood cells and irregular heartbeat may be a swollen, painful and reddish breast It has free complications Such as septicemia and toxic shock syndrome and may affect infant health and early separation of the infant from breastfeeding[3]

Many studies have shown that Staphylococcus aureus is a common cause of female abscess in women [4] noted that staphylococcus aureus is a common cause of abscess in lactating and other women non- Lactation. .

Staphylococcus aureus is one of the most important and prevalent pathogens found in human and animal skin. It is associated with a wide range of diseases, ranging from minor infections of the skin and soft tissue to life-threatening injuries. It is common in hospitals and acquired in The severity of these staphylococcus aureus with its virulence factors, which are associated with the virulence factors of a particular disease and the early detection of these virulence factors is very important for the purpose of conducting appropriate treatment interventions[5]. This bacteria is widespread in Europe and comes after E. coli and is responsible for more than 55% of individuals[6]. The most prominent places in the cavity of the nose and under the armpit, thigh, hair and head and pubic may penetrate the defense mechanisms of the body and cause diseases and the most important cases associated with These bacteria are food poisoning, infections of the cellular tissue and inflammation of the heart and heart Ear, ear, nose, urinary tract infection, septicemia and abscesses including abscess breast[7].

Materials and methods

Collection Sample

samples were collected from Diwaniyah city hospitals, specifically in the breast and early detection consultations at Diwaniyah Teaching Hospital and Women's Hospital and Children from September 2017 to April 2018. The study included the collection of clinical samples from one place, specifically from women's breasts and all ages. . The samples were collected under the supervision

of the specialist doctor and the surgeon. They were transferred sterilely by cotton swabs to the laboratory for a period not exceeding 30 minutes.

Culture of specimens

The swabs were transferred directly to the laboratory within 20-30 minutes. The samples were cultured on media of blood and MacConkey agar, then incubated at 37°C for 24 hours, as well as incubation of media that did not show growth within 24 hours for another 24 hours before being negative.

Test System Diagnosis System Analytical Profile Index (API)

This test was used to give a precise diagnosis of S.aureus bacteria. This test was carried out in accordance with the instructions of the French company Biomerieux, where sterile water droplets were placed on the groove on which the tape was placed to create wet conditions and then the suspension was prepared by transferring isolated and pure colonies In the center of the 24-hour nut medium to the center of the Staph medium liquid. Then the 20-hole ribbons were vaccinated with a sterile pipette. Then close the tape to the dedicated folder and incubate at 37 ° C for 24 hours. Then compare the results with the API Staph [8] .

serological identification

This test was used for the ability of S.aureus bacteria to produce Protein A. The MASSTSTAPH solution was used according to the instructions of the US company MAST Diagnosic Virginia.USA by placing a drop of solution on a black piece or a clean glass slide and mixing with it a colony of developing bacteria during 24 (30 - 60 seconds), it is observed that coagulation is more like intermittentmilk and this is evidence that the result is positive.

Polymerase chain reaction test

the test was conducted to investigate some of the causes of virulence and antibiotic resistance in *Staphylococcus aureus* (as follows):

1. Bacterial genomic DNA extraction according to the genomic DNA Extraction Kit (Presto ™ Mini g DNA Bacteria Kit) and instructions supplied by the American Generaid Company.
2. DNA Examination by the Nano drop device for the detection and measurement of nucleic acid concentrations. It is detected by determining the concentration of the DNA (ng/µl) and measuring its purity by reading the absorbance at a wavelength of 260-280 nm.
3. Preparation of PCR Master Mix by using the AccuPower® PCR PreMix kit and its processed instructions from Bioneer (South Korea).
4. Thermocycler Program to DNA amplified was programmed device according to the Townsend et al. (19) method for each primer. The thermocyclers for each primer were repeated to 30 cycles.
5. Preparation of agarose gel according to method of Sambrook et al. [9].

6. Agarose gel electrophoresis (1.5%) under 100 volts and 80 mA at 60 minute for detection of extracted DNA bands and amplified DNA representing amplified sizes or PCR products, according to method of Sambrouk et al. [9].

Statistical analysis:

The results of the present study were statistically analyzed and Statistical Results for Social Sciences (SPSS) were used for this purpose. The statistical test was used to compare the percentages of all study variables. The confidence interval was equal to 95% and the probability level is less than 0.05 ($P < 0.05$) [10] .

DNA Sequencer method

The DNA Sequencer method was carried out to perform the definitive diagnosis S.aureus was diagnosed by the PCR examination ,by conducting the phylogenetic tree analysis based on 16srRNA. After the PCR reaction ,the PCR reaction was sent to macrogen in South Korea for a procedure sequence of DNA using the AB sequencing system.

Results

Isolation and diagnosis

The present study aimed at isolating and diagnosing S.aureus bacteria from women's breast abscess. In this study, 23 bacterial isolates of the staphylococcus aureus were found in 85 samples collected from women's abscesses. The study was carried out by studying characteristics microscopic and biochemical tests and using the MASTSTAPH and APi. Twenty-three isolates belonging to S.aureus were isolated from the total number of samples of 85 cases of breast abscess in women with a 27% isolation rate. The highest percentage of S.aureus isolates was from lactating women with 29.31% followed by isolation rate of non lactating women (22.22%) as shown in Table 1.

Table 1 : percentages of isolating S.aureus bacteria by breast feeding status

Feeding status	Number of samples	Percentage	Number of positive isolates	Percentage
lactating women	58	%68.23	17	29.31
non lactating women	27	%31.76	6	22.22
Total	85	%100	23	27.05
X^2				0.469*
P value				0.493

X2 represents the value of the calculated Kai box

*There were no significant differences at the 0.05

All isolates of the S.aureus bacteria were identified in this study by several MASSTSTAPH and APi Staph. All isolates showed positive results and 100% in the susceptibility of these isolates to Protein A formation. All isolates were given 100% positive results of APi Staph and were given special colors

Detection of the virulence genes of S.aureus bacteria using a PCR

All isolation isolates were tested for isolating 16srRNA gene. All isolates and 100% contained 16srRNA gene. Some virulence genes for S.aureus isolates isolated from the 23 female breast abscess were also detected using PCR technique. The gene responsible for leukocidine, the gene responsible for the blaz and the skin responsible for skin flaking, showed 21 isolates with Luks (91.3%), 20 isolates (86.9%), , While in the eta gene the results showed only one isolation of This gene (4.3%) is shown in the table 2 .

Table2: Percentages to detect some virulence genes in S.aureus bacteria using a PCR device

Genes	Number of isolates	percentage
16srRNA	23	100%
Luks	21	%91.30
blaz	20	%86.95
Eta	1	%4.34
X^2		65.99*
P value		0

X2 represents the value of the calculated Kai box

*There are significant differences at the level of probability of 0.05

Phylogenetic tree analysis

The DNA sequence technique is used to analyze the phylogenetic tree for 3 samples of s.aureus based on the 16srRNA gene,using the NCBI blast program ,Mega 6, UPGMA tree as shown in fig 1 and 2 .

Fig. 1 Multiple sequence alignment analysis of S.aureus Isolated from the abscesses of women's breasts and represented by isolates from 1 to 3.Only variable sites are shown with different color.

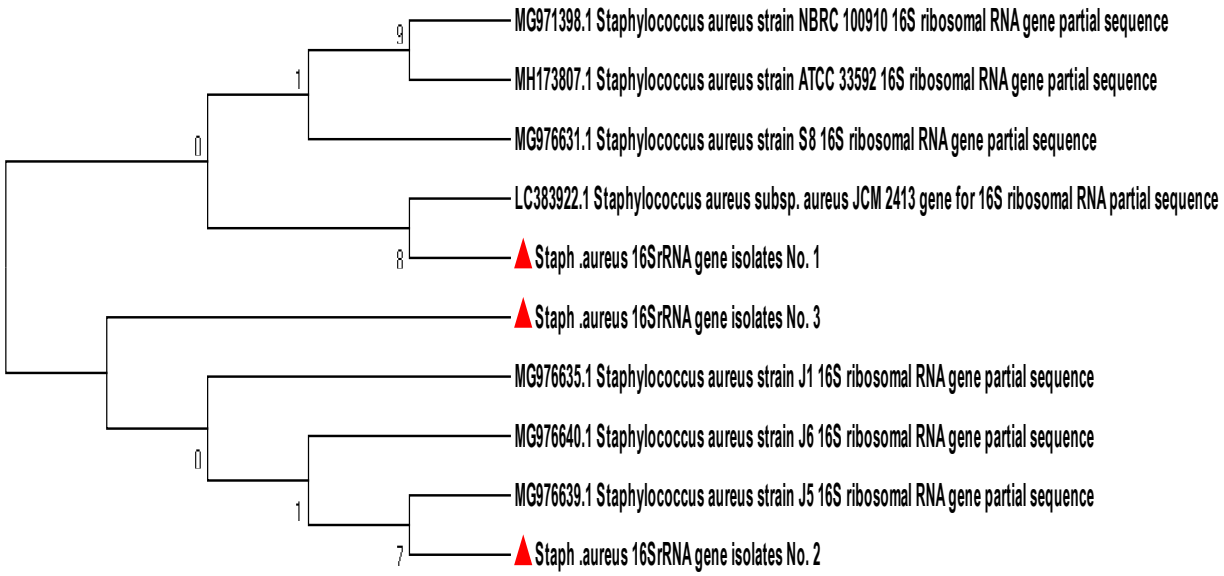

Fig .2 Phylogenetic tree analysis on the basis of 16srRNA using the MEGA 6 program where the results of the analysis showed a clear convergence in the isolates Staphylococcus aureus isolated from the breast abscess compared with the rest of the other species shown in the analysis of the phylogenetic tree.

Nucleotide accession number

Three sequences PCR samples used in this study based on 16srRNA gene sequence have been deposited in Gen Bank under accession number as shown in Table 3 .

Table 3:Detalis of S.aureus isolates used in the present study with the accession number in GenBank from breast abscess

.Isolate No.	Genbank accession number	NCBI-Blast isolates
No.1	MH591764	MG971398.1
No.2	MH591765	MG976631.1
No.3	MH591766	MG976635.1

Discussion

The number of women infected with breast abscess was 68.2% and the percentage of S.aureus isolates was 29.13%. In non- lactating women, the number of infected women was 27 and 31.7%. These results were decreased with the results of [11] which indicated that breast abscess is common in lactating women. The percentage of S.aureus isolates was 74.6% and very rare among non-nursing women with 25.4% bacterial isolation. Staphylococcus aureus was reported by Abdul hadi and his group where he collected 139 samples of breast abscess E There were 103 cases of return for lactating women were isolated bacteria S.aureus 76%, while the proportion of 43 cases of women belonging to non-nursing mothers and isolate the bacteria were 24% The high incidence of S.aureus infection in lactation can be attributed to several causes, including infection through breastfeeding, through the transfer of bacteria to the breast tissue by scratches or cracks on the nipple during lactation, leading to obstruction in the lobe that is responsible for the transmission Milk, which leads to stagnation of the milk and emptying it, which causes inflammation of the breast and the occurrence of abscess if not treated quickly and poor personal hygiene[12] , According to Efem (1997)[13], breast abscesses in lactating women are 95% and in non-breast-feeding women 5%. The incidence of breast abscess in non-lactating women decreases with lower smoking because smoking leads to an increase in milk ducts and decreases in immunity as in diabetes, rheumatoid arthritis and trauma All this leads to the abscess of the breast in non- lactating women.

The results of our study showed the incidence of S.aureus bacteria for non- lactating women in the number of cases examined 27 with isolation rate of 26%. The highest percentage of isolates of bacteria In breastfed women with breast cancer and breast inflammation. This may be due to reduced patient immunity and age of the patient, as well as the reviews performed by women who are hospitalized .Diabetes may be lead to a decrease in the immunity of the patient, exposing her to S.aureus infection, which was consistent with what Verghese and Raviknath[14] , Breast abscess is associated with non lactating mothers with diabetes mellitus.

All isolates of Staphylococcus bacteria were tested in the current study for diagnosis of S.aureus bacteria using 16srRNA and all isolates contained this gene These results were similar to Saruta et al.[15] , which tested 28 isolates from Staphylococcus aureus and non-cluster staphylococcus aureus The results of the present study showed that there are 20 isolates with 86.9% having a blaz gene where the resistance to penicillin is shown by producing the enzymes of the home, which is encoded by the blaz gene, which contains three types A, B, and C). It is recommended to detect the blaz gene, S.aureus bacteria from cases requiring treatment with penicillin [16] .

The presence of the gene encoding the killer toxin of the white blood cells (Luks) in the current study may be due to the source of isolation of these bacteria, the abscess of the breast because of the close association between the gene and this type of infections and was the highest compared to other genes of virility of bacteria S.aureus The presence of this gene in the current study was higher than the rate of the presence of this gene in the study of Jawad [17], which was the percentage of the presence of this gene in isolates S.aureus bacteria of the abscess 33.3% .

The results showed that only one isolated S.aureus bacteria contained the exofilative toxin gene (4.3%) In this study, the results of the study revealed that Jawad [17] found that 40% of the isolates of S.aureus isolates isolated from different clinical samples were present in Diwaniyah, while Vigues et al [18] In the study of Staphylococcus aureus isolated from diabetics and osteitis, which did not receive this gene in isolation study may be due to the area of isolation of bacteria and the period of time in which the study was conducted.

The phylogenetic tree was analyzed based on the sequence of the 16srRNa gene Which is an optimal choice in diagnosis, has clear shown that S.aureus could be reliable sign to indicate the presence of S.aureus and this is confirmed by Johnson et al [19] in a study to diagnose S.aureus bacteria based on the sequence of 16srRNA gene .In our present study, we have diagnosed three isolates of S.aureus secluded from breast abscess women based on 16srRNA gene sequence.

Fig. 1 clarify the differences in the Sequences of three isolates (1-3) which we tested in comparison with the other Gene Bank isolates based on the analysis of the 16srRNAgene.

The results of the comparison between local isolates and isolates recorded globally showed that there was a significant genetic match between isolation No.1 with Indian isolation (MG971398.1) and isolation No.2 with Indian isolation (MG976631.1) and isolation No.3 with Indian isolation (MG976635.1) For 16srRNA gene.

Conclusion

Staphylococcus aureus isolates were higher in lactating women than in non-lactating women. The spread of the killer gene for white blood cells (LUKS) is more common than gene toxic shock syndrome toxin(Eta). The results of the genetic investigation were consistent with the results of the phenotypic investigation in terms of the isolates had the diagnostic 16srRNA gene, In addition, the 16srRNA gene sequence could be considered the best choice for diagnosis of S.aureus infection depending on the source of infection isolation and the type of clinical infection .

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