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RESEARCH ARTICLE

Phylogenetic tree and Submission of *Staphylococcus aureus* Isolate from Skin Infection

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Abstract

In this study, sixty specimens were collected of *Staphylococcus aureus* isolation from several sources but found twenty-five specimens of *Staphylococcus aureus* isolated from skin from External laboratories in Baghdad, Iraq, during the period of December to May 2018. Then the samples were inoculated on culture media (Mannitol agar). The results of the sequencing showed congruence with isolation *Staphylococcus aureus* of amplified product of 16S rRNA gene appeared 99%compatibility. The results as shown After alignment of product amplification of 16S rRNA having seven Transition(A>G, A>G, T>C, T>C, G>A, C>T and A>G, and having three Transversion (T>G, G>C and A>T) from the Gene Bank. Registration of Iraqi isolate for *Staphylococcus aureus* bacteria in National Center for Biotechnology Information and under the accession number MH 145371.1. and it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/ MH 145371.1. The aim of the study was to determine the phylogenetic tree of *Staphylococcus aureus* based on 16S rRNA sequencing.The local Iraq isolate was registered after the correspondence of (NCBI), and obtained accession number and became a reference to Iraq and the Middle East and the world found variations of the local strain on the world.

Keywords: *Staphylococcus aureus*, 16SrRNA gene, Skin infections.

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INTRODUCTION

Staphylococcus aureus is a ubiquitous organism responsible for widespread infections both in community as well as in hospital settings[1]. These infections include pneumonia, skin and soft tissue infections, osteomyelitis, endocarditis, toxic shock syndrome etc.[2]. Skin and soft tissue infections ranged from furuncle, carbuncle, boil, impetigo, erysipelas, cellulitis, and abscesses to surgical site infections etc. Incision and drainage is the primary therapy for abscesses[3]. *Staphylococcus aureus* is a Gram-positive coccus bacterium, facultative anaerobic, catalase-positive, with single cells aggregate in clusters. Its responsible for many infection in human for example skin lesions (impetigo, furunculosis, folliculitis, hidradenitis-suppurativa, mastitis and Staphylococcal scalded skin syndrome.), in addition to infections of the subcutaneous tissue[4]. its colonizes the mucous membranes of nasopharynx, it is presented in apportion 50-60% in human as carriers[5]. The carrier state is a major risk factor for staphylococcal food poisoning, folliculitis and furunculosis[6]. *S. aureus* is transmitted mostly *via* contaminated hands of patients and medical staff and this lead to nosocomial infection. *S. aureus* has multiple factors and mechanisms of antibiotics resistance thereby is able to cause health and life-threatening infections[4]. The major virulence factors in this bacteria include enzymes, peptidoglycan and toxins. Other factors which lead to damaged tissue include (protein A and *clumping factor*)[7]. Hemolysin and Panton-Valentine leukocidin cause killing of white blood cells and red blood cells, whereas hyaluronidase, lipases, proteases, DNA, coagulase and fibrinolysin facilitate spreading the toxins within the human body[8]. PCR amplification from protected regions of the bacterial genome, especially the 16S rRNA gene followed by sequence analysis, is a steadfast technique for the identification of Enterobacter cloacae[9]. 16SrRNA gene sequence are considered as perfect standard for conclusion the phylogenetic relationship of microorganism[10,11]. Aim of the study was to determine the phylogenetic tree of *Staphylococcus aureus* based on 16S rRNA sequencing.

MATERIAL AND METHODS

Bacterial isolates

In this study, sixty specimens were collected of *Staphylococcus aureus* isolation from several source but found twenty five specimens of *Staphylococcus aureus* isolation from skin. Then the samples were inoculated on culture media (Mannitol agar). Microscopic examination was done by using Gram stain [12]. Mannitol salt agar was used as selective medium[13].

PCR amplification of 16S rRNA genes

Partial 16S rRNA gene sequences were amplified by PCR using primers 16S rRNA Forward strand primers 5'- AGAGTTTGATCCTGGCTCAG -3' and Reverse strand primers 5'- GGTTACC-TTGTTACGACTT -3' [14]. The PCR reaction mixture included 1.5 ml of bacterial DNA, 16.5 of δdH_2O, 5 ml Master Mix PCR (intron, korea), and l ml each primer in a final reaction volume of 25 ml. Amplifications were performed as follows: initial denaturation at 5 min at 95°C, followed by 40 cycles of denaturation 95°C for 45 sec, annealing at 52°C for 1 min, extension at 72°C for 1 min and a final extension of 72°C for 7 min. The PCR products were separated on 1% agarose gel. The gel is left to run for 90min with a 70volt/65Am current. Following electrophoresis, visualization was conducted with a UV trans illuminator after red stain staining.

RESULTS AND DISCUSSION

Genetic Detection of Staphylococcus aureus by 16S-rRNA

The present research findings pertain to the isolation of *Staphylococcus aureus* ,samples were confirmed as on the basis of morphological, and molecular characterization . PCR is a rapid, sensitive, specific and inexpensive method. After gradient PCR has been performed ,bands has been obtained as presents in Figure 1. Showing that the best annealing a temperature to give 16S-rRNA was (52°C, 45s) depending on results of gradient PCR amplification procedure Several studies have used PCR for the detection of 16S rRNA by PCR method more rapidly and reliably. The results of current study agree with result of a same study in Iran reporte[15]. Who noticed that amplification of 16s-rRNA confirmed all the staphylococcal isolates as *Staphylococcus aureus*. Utilizing the 16S rRNA gene instead from whole genome information is not only computational efficient but also economical[16] however, will provide an analysis that demonstrates that at least in the context of oral

microbial communities, the 16S rRNA gene retains sufficient information to allow us detect unknown bacteria[17]. Figure 1 showed PCR amplification of the *16SrRNA* where a specific product at 1250bp was observed.

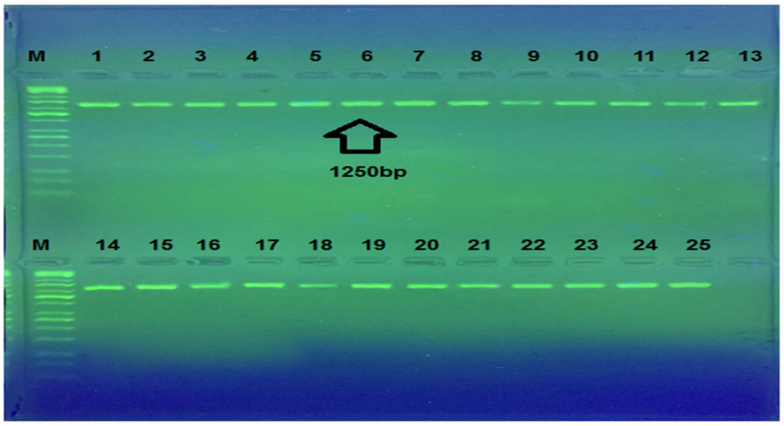

Fig. 1. Agarose gel electrophoresis for *16SrRNA* gene (1250bp) amplification of *Staphylococcus aureus*. electrophoresis on a 2% agarose gel and visualized under U.V. light after staining with red safe staining. Lane: 1 (M: 100bp ladder), Lane: 1to 25 product PCR positive.

Sequencing

The sequencing and BLAST analysis of partial *16S rRNA* gene figure 2 and 3 . The results of the sequencing showed congruence with isolation *Staphylococcus aureus* of amplified product of 16S*rRNA* gene appeared 99% compatibility. (87 to 1178) number of nucleotide from gene of gene bank, and have number score (1925) bits, expect (0), and corresponds to the global number ID: NR_118997.2. The results as shown After alignment of product amplification of 16S rRNA having seven Transition (A>G, A>G, T>C, T>C, G>A, C>T and A>G, and having three Transversion (T>G, G>C and A>T) from the Gene Bank. 16*SrRNA* gene was successfully amplified using specific PCR primers for gene.

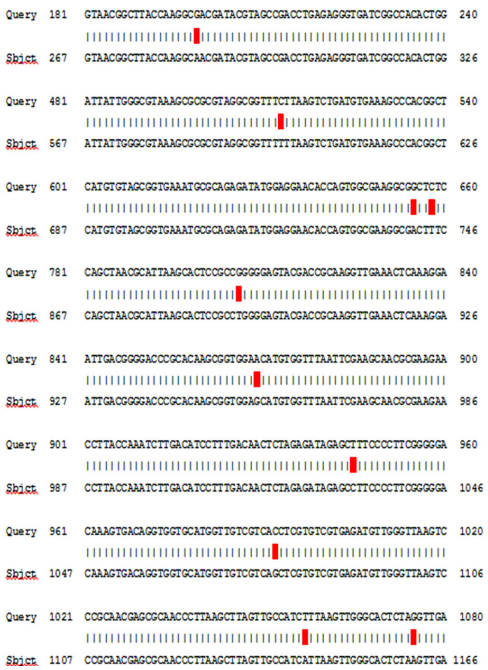

Fig. 2. Sequencing of *Staphylococcus aureus* obtained from Gene Bank.

Phylogenetic tree structuring

The phylogenetic tree diagrammatic by (MEGA) software version 6.0 is shown in figure 3. This study take care for phylogenetic analysis for geographic genetic distances determination The isolated sequences have shown 99% compatibility. Neighbor-joining tree was constructed for phylogenetic analysis. These alignments appeared

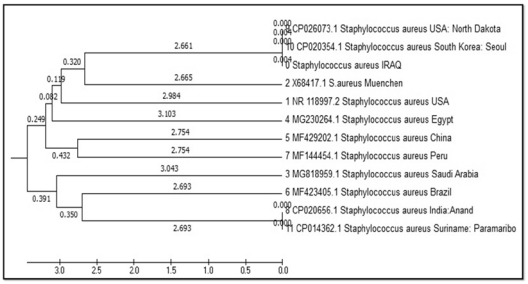

Fig. 3. Neighbor-joining tree *Staphylococcus aureus* of 16S rRNA gene.

the genetic distance and other global strains by partial sequence similarity in 16S ribosomal RNA gene for translating specific region. The genetic dimension between Iraq and the isolates of the world is detailed according to the Phylogenetic tree and the comparison table. hierarchical cluster analysis determine the following clusters: large Cluster divided into several neck: first root USA: North Dakota, South Korea: Seoul and IRAQ the genetic dimension was by 2.661, second root Muenchen the genetic dimension was by 2.665, third root USA the genetic dimension was by 2.984, fourth root Egypt the genetic dimension was by 3.103, and small Cluster divided into two root: China and Peru the genetic dimension was by 2.754. The last cluster is divided into two branches the first branch Saudi Arabia the genetic dimension was by 3.043), the second branch divided into two neck : India , Brazil and Suriname: Paramaribo the genetic dimension was by2.693).

Table 2 represents comparison between strain of *Staphylococcus aureus* isolated from skin, recorded in the National Center Biotechnology Information (NCBI) and isolated from different source *Avicenniae marina* (white mangrove), buffalo milk, Minas cheese surface, sputum, milk, fatal septicaemia and septic arthritis in a 16-month-old American-Indian girl who had not risk agents

Table 1. Represent type of polymorphism of 16S rRNA gene from *Staphylococcus aureus* isolate.

No. of Sample	Type of substitution	Location	Nucleotide	Sequence ID
isolates	Transition	285	A>G	ID: NR_118997.2
	Transition	599	T>C	
	Transition	741	A>G	
	Transition	744	T>C	
	Transversion	892	T>G	
	Transition	955	G>A	
	Transition	1031	C>T	
	Transversion	1078	G>C	
	Transversion	1143	A>T	
	Transition	1161	A>G	

Staphylococcus aureus strain ATCC 12600 16SrRNA, complete sequence Sequence ID:NR_118997.

Score	Expect	Identities	Gaps	Strand
1925 bits (2134)	0.0	1082/1092 (99%)	0/1092 (0%)	Plus/Plus

Table 2. Sequencing ID in gene bank, and compatibility of DNA sequences obtained from National Center Biotechnology Information (NCBI).

ACCESSION	isolate	country	Source	Compatibility
ID: NR_118997.2	strain="ATCC 12600"	USA	*Staphylococcus aureus*	99%
ID: X68417.1	strain="ATCC 12600"	Muenchen	*Staphylococcus aureus*	99%
ID: MG818959.1	strain="FA-1"	Saudi Arabia	*Staphylococcus aureus*	99%
ID: MG230264.1	strain="AM"	Egypt	*Staphylococcus aureus*	99%
ID: MF429202.1	strain="CAU1287"	China	*Staphylococcus aureus*	99%
ID: MF423405.1	strain="SABRC56"	Brazil	*Staphylococcus aureus*	99%
ID: MF144454.1	strain="FQXIII"	Peru	*Staphylococcus aureus*	99%
ID: CP020656.1	strain="K5"	India: Anand	*Staphylococcus aureus*	99%
ID: CP026073.1	strain="Mw2"	USA: North Dakota	*Staphylococcus aureus*	99%

associated with health care, blood and Perineum , and throat/groin but the same host (Homo sapiens) have under sequence(ID: NR_118997.2[18], ID: X68417.1[18]., ID: MG818959.1[19]., ID: MG230264.1[20]., ID: MF429202.1[21]., ID:MF423405.1[22], ID: MF144454.1[23].,ID: CP020656.1[24]., ID: CP026073.1[25]., ID: CP020354.1[26].,and ID: CP014362.1[27]. respectively with source of isolation and showed compatibility 99% and score (1925 and 1920), and expect 0.0 with gene bank.

Submission of local Iraq isolate in NCBI

Broad-range PCR targets the *16S rRNA* gene which is the most common housekeeping genetic marker because the ribosomal small subunit is present universally in all bacteria[28]. Registration of Iraqi isolates for *Staphylococcus aureus* bacteria in National Center for Biotechnology Information and under the accession number MH 145371.1. and it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/ MH 145371.1.

CONCLUSION

The efficiency of genetic methods in the detection of accurate and rapid bacterial isolates thus shortening the time and cost and giving the correct treatment and accurate treatment of disease without error and time loss. After obtaining the global number of Iraqi isolates proved near the Iraqi isolates of the isolates USA :North Dakota , South Korea: Seoul.

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