Molecular Identification of *Staphylococcus spp* Isolated from Clinical samples

عزل وتشخيص المكورات السريريه باستخدام الاوساط الزرعيه والجزيئيه (دراسه مقارنه)

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Abstract

The analysis of 16S rRNA gene sequences has been the technique generally used to study and confirm the identification and taxonomy of staphylococci. However, bacterial species cannot always be distinguished from each other using cultural methods. Thus, clinical samples were collected from 190 cases only 31 positive for staphylococcal infections with Urinary Tract Infection, Wounds, Burns, Otitis media, diarrhea infections, were applied for microbiological analysis which include: cultures on Manitol salt agar and HiCrome UTI Agar medium all the isolates gave positive golden yellow and identify as *Staphylococcus* spp. DNA was extracted from *Staphylococcus* spp and the 16srRNA gene were amplified by using specific primer, then sequencing of nucleic acid of genes was performed by machine is AB13730XL, Applied Biosystem, Macro gen company, the DNA sequencing results of flank sense of 16srRNA gene from 31 strains of *Staphylococcus* was confirm the identification into species level: *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Staphylococcus epidermidis And Staphylococcus sciuri*. Analysis of the sequences appeared that there two substitution(Transversion, Transition) in the *Staphylococcus aureus* strains with Sequence ID LC090540.1 location at Range of nucleotide from 4 to 636, 100% compatibility with NCBI while no substitution appeared in the *Staphylococcus haemolyticus* strains which have the sequence ID LN998078.1, 99% compatibility with NCBI also the sequence ID KR812401.1 which related to the strain *Staphylococcus sciuri* not appeared any substitution after sequencing analysis. Types of substitution detected in partial 16srRNA gene in *Staphylococcus epidermidis* strains 13 Transversion and 5 transition substitution location at range of nucleotide from 6 to 1026 *have the* Sequence ID KF575160.1 compared with data obtained from Gene Bank, these finding lead to conclusion, our assay allows rapid and confirm the detection to avoid possibility of misidentification of *Staphylococcus* species based on cultural analysis, the study aimed to propose the partial sequencing of the gene as an alternative molecular tool for the analysis of *Staphylococcus* species and for decreasing the possibility of misidentification. New submission of local Iraqi *Staphylococcus* clinical isolated during the current study show successfully record of four isolate Staphylococcus *sciuri*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Staphylococcus haemolyticus* with GenBank accession number: KY938530.1 ,KY938529.1, ,KY938528.1, and GenBank: KY938527.1respectivelly.

Key words: *Staphylococcus Spp*, HiCrome UTI Agar, Manitol salt Agar, and Sequencing, Gen Bank.

الملخص

استخدم تتابع الجين الرايبوسومي كتقنيه لتأكيد تشخيص وتصنيف المكورات لأن العزلات البكتيريه لانستطيع تمييزها عن بعضها باستخدام الطرق المزرعيه لذلك هدفت الدراسه الحاليه الى تشخيص الأنواع التابعه للمكورات السريريه باستخدام الطرق المزرعيه والجزيئيه ولتحقيق الهدف تم جمع 190 عزله بكتيريه أظهرت النتائج ان 31 فقط منها تابعه لجنس المكورات تم عزلها من أخماج السيل البولي، التهاب الاذن الوسطى، الحروق، الجروح، والاسهال، اخضعت العزلات للتحليل الكيموحيوي المتضمن زرعها على وسط أكار ملح المانيتول و الكروم أكار أظهرت جميع العزلات اللون الأصفر الذهبي مما يعني انها تابعه لأنواع جنس المكورات، عزل الدنا المجيني من المكورات ومضاعفته باستخدام باديء متخصص وعمل تسلسل للحامض النووي للجينات وأظهرت نتائج تسلسل الحامض النووي انه تم تأكيد تشخيص من بين 31 عزله من المكورات هنالك 21 عزله تابعه للمكورات العنقويه الذهبيه و 8 عزلات تابعه للمكورات العنقوديه الحاله للدم و عزله واحده تابعه للمكورات العنقوديه البشرويه وعزله تابعه للمكورات العنقوديه السنجابيه بعد اجراء تحليل تسلسل الحامض النووي تبين ان هنالك 16 موقع استبدال في الجين 16 الرايبوسومي من خلال تحليلنا تبين ان هنالك امكانيه لعمل فحص سريع و أكيد للتعرف على انواع المكورات العنقوديه لتجنب امكانيه الخطأ في التشخيص بالاعتماد على الفحوصات المزرعيه لذلك نقترح باستخدام تحليل الحامض النووي كوسيله جزيئيه لتشخيص المكورات العنقوديه وهذا يقلل امكانية الوقوع في الخطأ أثناء التشخيص. سجلت اربع

عزلات محليه للمكورات في المركز العالمي لمعلومات التقنيه الحيويه المعزوله خلال الدراسه للعزلات المكورات العنقودية سسيوري، المكورات العنقودية البشروية، المكورات العنقودية الذهبية والمكورات العنقودية المحلله للدم واللتي تم اعطائها رقم وصول لبنك لجينات العالميه: KY938527.1 ,KY938528.1, ,KY938529.1, KY938530.1 بالتتابع.

الكلمات المفتاحيه: المكورات العنقوديه، أكارملح المانيتول، الكروم أكار، تتابع الحمض النووي، بنك الجينات

Introduction

Staphylococcus Spp. is a versatile, opportunistic pathogen able to cause a wide range of diseases in humans. It is considered to be a major pathogen that colonizes and infects both hospitalized patients with decreased immunity, and health immuno-competent people in the community. This bacterium is found naturally on the skin and in the nasopharynx of the human body. It can be cause local infection of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening [1]. *S. aureus* is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis [2,3,4]. Staphylococcal infections in humans result in a transient increase in anti-staphylococcal antibody levels. Nevertheless, protective immunity is not observed and recurrent infections occur frequently [5]. Basically *Staphylococcus aureus* causes a broad range of human disease, and can infect almost any organ system. Recently, assays based on PCR technology were employed to detect the presence of *Staphylococcus Spp* using several gene for example the 16S rRNA gene sequence is about 1,550 bp long and is composed of both variable and conserved regions. The gene is large enough, with sufficient interspecific polymorphisms of 16S rRNA gene, to provide distinguishing and statistically valid measurements. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the whole sequence (about the 1,550-bp region), and the sequence of the variable region in between is used for the comparative taxonomy [6,7]. The current study aimed to Molecular identification of *Staphylococcus Spp* isolated from clinical samples.

Materials and Methods

Sample collection

From June, till November, 2016, samples were taken from different infections (males and females) admitted to the Baghdad Teaching Hospital who were clinically suspected diagnosed by the physician in the hospital, the study includes a total of 190 cases (Swabs, Urine, Stool, Biopsy samples were obtained from 190 patients) from Urinary Tract Infection , Wounds, Burns, Otitis media , Diarrhea infections. All samples were inoculated on suitable culture media HiCrome UTI Agar, Modified, M1418 (Himedia/India), Modified is chromogenic differential medium for identification, differentiation and confirmation of enteric bacteria from specimens and incubated for 24 hrs. at 37°C [8]. Manitol salt Agar also used as a semi selective media during isolation of Staphylococci.

Extraction of genomic DNA

A single colony of cultivated bacteria, which had been incubated overnight, suspended into 1ml of distilled water, centrifuged at 14000xg for 2min., then the supernatant discarded, after that 120µL of lysostaphin (10 mg/L; Sigma) was added. DNA extracted using mini DNA extraction kit (G- spin dna extraction kit , intron biotechnology , cat.no. 17045) according to manufacture instructions [4]. The concentration and purity of the isolated DNA samples were measured by the Nano Drop spectrophotometer before the performance of PCR, for DNA isolated by the commercial kit technique and by the manual technique. Nano drop is highly sensitive and directly provides us with the concentration of DNA, A260/A280 ratio, and A260/A230 ratio. First 2µl of the elution buffer (TE) that was used in DNA isolation was applied on the highly sensitive micro-detector of Nano drop as blank, then 2µL of the sample was applied and results (both concentrations and absorbance) were obtained from the operating software on the computer that installed to Nano drop device [6].

Amplification of 16S rRNA

Specific primers were designed for amplification by using a forward primer (16S rRNA F: 5′- AGA GTT TGA TCC TGG CTC AG -3′) and a reverse primer (16S rRNA R:5′ GGT TAC CTT GTT ACG ACT T -3′) (Primers set supplied by IDT (Integrated DNA Technologies company, Canada) [9]. PCR reaction was conducted in 25µl of a reaction mixture containing 2µl of DNA, 12.5 µl *GoTaq0T® Green* Master (Promega, CA), (0.5 µl) 25mM MgCl2, 2µl of (10 Pmol\ µl) of each primer, 2µl of distilled water.

Amplification program was 1 cycle at 94°C for 1 min; 35 cycles of 94°C for 1min, 63°C for 1min, 72°C for 1min; 72°C for 10min, using the Master cycler (Eppendorf). The amplified product was subjected to 1.5% agarose gel electrophoresis, and visualized under UV (Imagemaster VDS, Pharmacia Biotech, USA) after Red safe staining. Positive PCR product samples were sent for sequence analysis; nd 25 µl (10 pmol) from the forward primer. The samples were treated with AB13730XL APPLIED BIOSYSTEMS machine in national instrumentation centre for environmental management NICM/USA Company. The result of the sequence analysis was analysed by blast in the National Centre Biotechnology Information (NCBI).

Results and Discussion

The specimens used in present study were obtained from different clinical cases. The enrolled cases included infections, chronic otitis media (ear discharge) infections, urinary tract infections, wounds and burns. Samples were only taken if infection was suspected and depending on the clinical condition of patient. The specimens were directly inoculated onto plates of mannitol salt agar and incubated at 37ºC for 24 h, of 190 specimens only 31 specimens of bacterial isolates were Staphylococcal positive from the total number of collected isolates, obtained from patients between 17 and 50 years old. These specimens included Swabs, Urine, stool, Biopsy samples culture and identified based on cultural, morphological and biochemical in order to verify their ability to produce acid on this medium, the colonies which exclusively fermented the Mannitol appeared green colony in the side of plate with Mannitol salt agar identified as *S. aureus* isolates were produce acid on this medium as show in figure (1 A). whereas identification based on HiCrome UTI Agar, Modified, M1418 medium appeared as raised, golden yellow as show in figure (1 B) the biochemical tests identified the isolate at the species level as *Staphylococcus* spp. but the bacterial isolates exhibited different morphological appearances [2]. These variable morphologies suggested the presence of many different species related to *Staphylococcus* genus [6].

Fig. (1): Growth of *Staphylococcus* cultures on different media: A: manitol salt Agar, B: - , + HiCrome UTI Agar medium incubated at 37 °C for 24 hrs supported 100% growths.

HiCrome UTI agar was more useful as primary urine culture medium in both higher rate of isolation and presumptive identification of uropathogens in comparison to conventional media. Its inherent characteristics in demonstrating polymicrobial growth and ease of rapid identification by distinct colony color are unique the HiCrome UTI agar also reigned over the conventional media by providing high isolation rate as well as specific identifying characteristics of the organisms in mixed growth thus enabling microbiologists to assess more accurately the clinical relevance of urine culture results and clinical samples [5]. The study employed a total of 31 staphylococcal isolates. Among those that were recovered, *S. aureus* had been considered as the most significant species recording 21 isolate which represent 76 % and the rest were diagnosed as other species of staphylococci. To distinguish between *Staphylococcus* spp based on molecular level and confirm the identification of the isolates, DNA extracted successfully from 31 isolates as show in figure (2) to use it in polymerase chain reaction (PCR) application. The concentration and purity of total DNA isolates in the samples were measured spectrophotometrically at wavelengths of A260 and A280. It was performed in a Nano Drop machine (Thermo Scientific). The yield of the DNA extracted from *Staphylococcus* spp isolates was in range of (65-210) ng/µl with purity of (1.5-1.9). 16srRNA gene was successfully amplified using specific PCR primer amplification of 16srRNA gene of 31 strains of *Staphylococcus* spp collected in the present study to confirm the presence of 16S rRNA gene in the strains as show in figure (2), appeared that molecular weight of 16S rRNA gene was 1500 bp in the PCR product of *Staphylococcus* spp strains was

exclusively used to proceed for the sequencing analysis assay to confirm the identification of *Staphylococcus* strains and also detect the polymorphism in gene content.

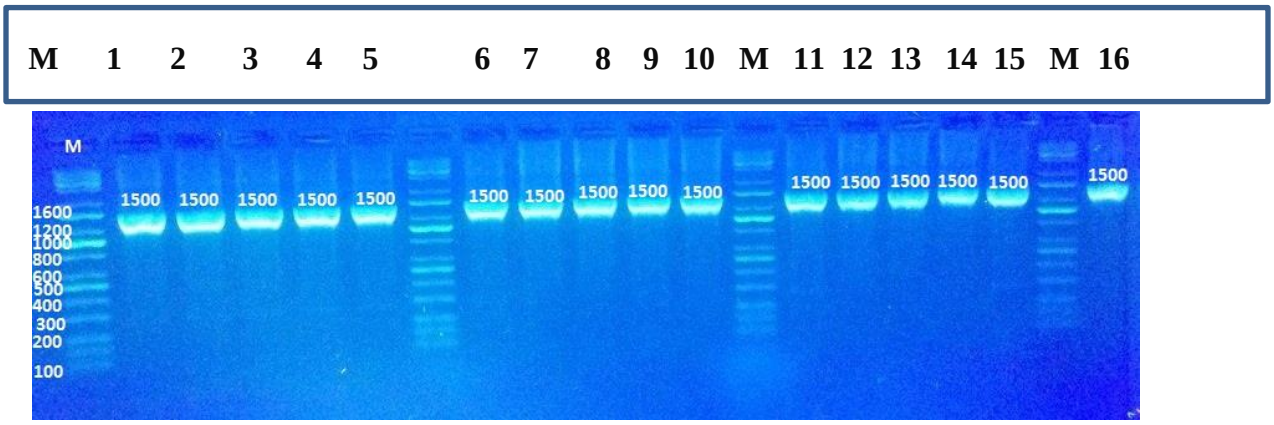

Fig. (2): PCR product the band size 1500bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100), lane (1-15) PCR product of band size 1500 bp, visualized under U.V light.

Sequencing of 16srRNA gene was performed to confirm the identification of *Staphylococcus* spp strains based on species level as appeared in Table (1), from the result of sequencing molecular identification appeared that 21 strains from the total number related to *Staphylococcus aureus*, 8 strains related to *Staphylococcus haemolyticus*, and only one of each *Staphylococcus epidermidis* and *Staphylococcus sciuri*.

Table (1): Identification of *Staphylococcus* spp strains isolated during the present study by 16S rRNA sequencing.

	Infections	identification by hychrom media	Molecular identification
1	Urinary tract infection	golden yellow	*Staphylococcus aureus*
2	Urinary tract infection	golden yellow	*Staphylococcus aureus*
3	Urinary tract infection	golden yellow	*Staphylococcus aureus*
4	Urinary tract infection	golden yellow	*Staphylococcus aureus*
5	Urinary tract infection	golden yellow	*Staphylococcus aureus*
6	wounds	golden yellow	*Staphylococcus aureus*
7	wounds	golden yellow	*Staphylococcus aureus*
8	wounds	golden yellow	*Staphylococcus aureus*
9	wounds	golden yellow	*Staphylococcus aureus*
10	wounds	golden yellow	*Staphylococcus aureus*
11	wounds	golden yellow	*Staphylococcus aureus*
12	Otitis media	golden yellow	*Staphylococcus aureus*
13	Otitis media	golden yellow	*Staphylococcus aureus*
14	Otitis media	golden yellow	*Staphylococcus aureus*
15	Otitis media	golden yellow	*Staphylococcus aureus*
16	Otitis media	golden yellow	*Staphylococcus aureus*
17	burns	golden yellow	*Staphylococcus aureus*
18	burns	golden yellow	*Staphylococcus aureus*
19	burns	golden yellow	*Staphylococcus aureus*
20	burns	golden yellow	*Staphylococcus aureus*
21	burns	golden yellow	*Staphylococcus aureus*
22	Burns	golden yellow	*Staphylococcus haemolyticus*
23	Burns	golden yellow	*Staphylococcus haemolyticus*
24	Otitis media	golden yellow	*Staphylococcus haemolyticus*
25	Otitis media	golden yellow	*Staphylococcus haemolyticus*
26	Otitis media	golden yellow	*Staphylococcus haemolyticus*
27	Stool	golden yellow	*Staphylococcus haemolyticus*
28	Urinary tract infection	golden yellow	*Staphylococcus haemolyticus*
29	wounds	golden yellow	*Staphylococcus haemolyticus*
30	Burns	golden yellow	*Staphylococcus epidermidis*
31	Burns	golden yellow	*Staphylococcus sciuri*

Sequencing of 16srRNA gene was performed to confirm the identification of *Staphylococcus* spp strain isolated during the current study, Sequences alignment using BLAST and Bio Edit showed that the strain

Staphylococcus haemolyticus accession number : LN998078.1, 100% compatibility with NCBI, score 2026 and expect 0.0 of the 16srRNA gene the compatibility of *Staphylococcus haemolyticus* strain with the strain mammoth-14 isolated in France, Staphylococcus *aureus* accession number: LC090540.1, 99% compatibility with NCBI, score 1158 and expect 0.0 of the 16srRNA gene the compatibility of *Staphylococcus aureus* with the strain N7_261 isolated in Brazil, *Staphylococcus epidermidis* accession number : KF575160.1 , 98% compatibility with NCBI, score 1775 and expect 0.0 of the 16srRNA gene the compatibility of *Staphylococcus epidermidis* with the strain C0181 isolated in UK, at the last *Staphylococcus sciuri* accession number : KR812401.1, 100% compatibility with NCBI, score 1868 and expect 0.0 of the 16srRNA gene the compatibility of *Staphylococcus sciuri* in the present study with the strain EA14 isolated in Saudi Arabia. As show in Table (3) and figure (3). After the analysis of the sequences results different type of substitution appeared in the Staphylococcus strains as listed in Table (3). There are two substitution (Transversion, Transition) in the *Staphylococcus aureus* strains with Sequence ID LC090540.1 location at Range of nucleotide from 4 to 636, while no substitution appeared in the *Staphylococcus haemolyticus strains which have the* Sequence ID LN998078.1, also the Sequence ID KR812401.1 which related to the strain *Staphylococcus sciuri* not appeared any substitution after sequencing analysis.

Types of substitution detected in partial 16srRNA gene in *Staphylococcus epidermidis* strains 13 Transversion and 5 transition substitution location at Range of nucleotide from 6 to 1026 *have the* Sequence ID KF575160.1 compared with data obtained from Gene Bank as show in Table (2) and figure (3).

Table (2): Types of substitution detected in partial 16srRNA gene in *Staphylococcus* spp strains.

Name of strain	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID
Staphylococcus haemolyticus		----------		**84 to 1180**	**LN998078.1**
Staphylococcus sciuri		--------		**48 to 1058**	**KR812401.1**
Staphylococcus aureus	**Transversion**	**171**	**G>T**	**4 to 636**	**LC090540.1**
Staphylococcus aureus	**Transition**	**407**	**C>T**		
	Transversion	**145**	**G>T**	**6 to 1026**	**KF575160.1**
Staphylococcus epidermidis	**Transversion**	**151**	**T>G**		
	Transversion	**192**	**T>G**		
	Transversion	**200**	**G>T**		
	Transversion	**237**	**T>G**		
	Transversion	**379**	**T>G**		
	Transversion	**493**	**C>G**		
	Transversion	**496**	**T>G**		
	Transversion	**543**	**C>G**		
	Transversion	**546**	**A>T**		
	Transversion	**595**	**C>G**		
	Transition	**602**	**A>G**		
	Transition	**612**	**A>G**		
	Transversion	**622**	**T>G**		
	Transition	**830**	**G>A**		
	Transition	**856**	**T>C**		
	Transversion	**857**	**C>A**		
	Transition	**871**	**G>A**		
	Transversion	**931**	**T>A**		
	Transversion	**981**	**A>T**		

Table (3): NCBI information of Molecular Identification of *Staphylococcus* spp strains Iraq isolated during the present study based on 16srRNA gene sequencing.

ACCESSION	Strain	Strain	country	Compatibility	Expect	score	Range
LN998078.1	Staphylococcus haemolyticus	mammoth-14	France	100%	0.0	2026	84 to 1180
KR812401.1	Staphylococcus sciuri	EA14	Saudi Arabia	100%	0.0	1868	48 to 1058
LC090540.1	Staphylococcus aureus	N7_261	Brazil	99%	0.0	1158	4 to 636
KF575160.1	Staphylococcus epidermidis	C0181	UK	98%	0.0	1775	6 to 1026

Group 1

Staphylococcus haemolyticus partial 16S rRNA gene, strain mammoth-14 Sequence ID: LN998078.1

Score	Expect	Identities	Gaps	Strand
2026 bits(1097)	0.0	1097/1097(100%)	0/1097(0%)	Plus/Plus

```
Query 2
CTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGA 61
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 84 CTTTGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGGGA
143
Query 62
TAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTCGAACCGCATGGTTCGATAGTG 121
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 144 TAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTCGAACCGCATGGTTCGATAGTG
203
Query 122
AAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAGCTAGTTGGTAAGGT 181
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 204 AAAGATGGTTTTGCTATCACTTATAGATGGACCCGCGCCGTATTAGCTAGTTGGTAAGGT
263
```

Group 2

Staphylococcus aureus gene for 16S ribosomal RNA, partial sequence, isolate: N7_261
Sequence ID: LC090540.1

Score	Expect	Identities	Gaps	Strand
1158 bits(627)	0.0	631/633(99%)	0/633(0%)	Plus/Plus

```
Query 122
AAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCAGTAGCTAGTTGGT 181
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 124 AAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGT
183
Query 182
AAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC 241
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 184
AAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACAC 243
Query 242
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG 301
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 244
TGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG 303
Query 302
GGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTC 361
              ||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct 304
GGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTC 363
Query 362 TGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTCGACGGTACCTAATCAG 421
||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct 364 TGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAG
Group 3
Staphylococcus epidermidis strain C0181 16S ribosomal RNA gene, partial sequence, Sequence ID: KF575160.1

Score	Expect	Identities	Gaps	Strand
1775 bits(961)	0.0	1001/1021(98%)	0/1021(0%)	Plus/Plus

Query 781
GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAGGCACTCCGCCTGGGG 840
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 786
GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG 845
Query 841
AGTACGACCGTCAGGTTGAAACTCAGAGGAATTGACGGGGACCCGCACAAGCGGTGGAGC 900
|||||||||| |||||||||||| ||||||||||||||||||||||||||||||
Sbjct 846
AGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGC 905
Query 901 ATGTGGTTTAATTCGAAGCAACGCGTAGAACCTTACCAAATCTTGACATCCTTTGACAAC 960
|||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct 906 ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAAC 965
Query 961
TCTAGAGATAGAGCCATCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTC 1020
|||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct 966
TCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTC 1025
Query 1021 A 1021
|
Sbjct 1026 A 1026
Group 4
Staphylococcus sciuri subsp. sciuri strain EA14 16S ribosomal RNA gene, partial sequence Sequence ID: KR812401.1

Score	Expect	Identities	Gaps	Strand
1868 bits(1011)	0.0	1011/1011(100%)	0/1011(0%)	Plus/Plus

Query 1
TTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGG 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 48 TTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTATAAGACTGG 107
Query 61
GATAACTCCGGGAAACCGGGGCTAATACCGGATAATATTTTGAACCGCATGGTTCAATAG 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 108 GATAACTCCGGGAAACCGGGGCTAATACCGGATAATATTTTGAACCGCATGGTTCAATAG 167

Fig. (3): Sequencing of sense flanking the partial 16srRNA gene in *Staphylococcus* compared with recorded 16srRNA obtained from Gene Bank. Query represents of sample; Sbject represent of database of National Center Biotechnology Information (NCBI), Group1*: Staphylococcus aureus,* Group 2: *Staphylococcus haemolyticus,* Group 3: *Staphylococcus epidermidis* Group 4: *Staphylococcus sciuri*.

The 16S rRNA gene is used as the standard for classification and identification of microbes, because it is present in most microbes and shows proper changes. Type strains of 16S rRNA gene sequences for most bacteria and archaea are available on public databases such as NCBI. However, the quality of the sequences found on these databases is often not validated. Therefore, secondary databases that collect only 16S rRNA sequences are widely used [8]. Conventional methods to assess levels of Polymorphism of *Staphylococcus aureus* are based on culture in combination with agar dilution [4,11,12]. Since sequencing analysis seems to

be restricted to the occurrence of specific mutations in a small region of the 16S rRNA molecule [6, 13,14,15], molecular methods an attractive and alternative. In the present study a PCR-based on sequencing analysis was used to confirmation the identification based on 16S rRNA gene sequencing. This assay distinguishes the high-level of Polymorphism in isolates from the data from sequencing analysis of 16S rRNA genes in *H. pylori* strains. Since all the isolates show high-level of Polymorphism that's may be linked to therapy failure [16,17,18], this sequencing analysis approach is useful for the detection of clinically relevant levels of polymorphism in *Staphylococcus*. It is striking that all characterized *Staphylococcus* isolates contain mutations in the exact same 16S rRNA region, especially because these isolates were obtained from patients living in same geographic regions [19]. This observation suggests that *Staphylococcus aureus* require mutations within the 16S rRNA primary binding site for antibiotic resistance. Probably this resistance arises by mutations, although the acquisition of mutant 16S rRNA alleles through horizontal gene transfer cannot be excluded [20,21], molecular identification using 16S rRNA region gene was used for molecular confirmation of *Staphylococcus spp* and detection specie directly without the need to proceed the step of biochemical test and antibiotic disc diffusion methods as referred by Tang and Stratton, [22] who suggested that the specificity identification is based on the 16S rRNA gene. This assay allows detection and identification of *Staphylococcus spp* in less than 6 h after sample collection, based on genetic basis. these finding lead to conclusion, use the molecular teqnique in the hospital and laboratory in Iraq to confirm the identification that's lead to use the right antibiotic in treatment also we concluded from present result that the cultural and biochemical analysis method not sufficient in the identification of the bacterial species isolated from different sample, our sequencing assay in present result allows rapid detection of *Staphylococcus spp*. This study the same [23] that appeared variation in produce β-lactamase by *blaKPC* gene of local *K. pneumoniae* isolated from Iraqi patients.

New submission of local Iraqi *Staphylococcus* clinical isolated during the current study in the NCBI

Four from the total *Staphylococcus* isolated during the study recorded in the NCBI with the GenBank: KY938530.1 for Staphylococcus *sciuri* strain, also *Staphylococcus epidermidis* strain recorded with Gen Bank: KY938529.1, *Staphylococcus aureus* strain labeled with the GenBank number: KY938528.1, finally *Staphylococcus haemolyticus* strain isolated during the study and recorded with the GenBank: KY938527.1 as show in figure (4).

Stain (1): Staphylococcus sciuri strain EA111 16S ribosomal RNA gene, partial sequence

GenBank: KY938530.1

```
LOCUS       KY938530               180 bp    DNA     linear   BCT 12-JUN-2017
DEFINITION  Staphylococcus sciuri strain EA111 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   KY938530
VERSION     KY938530.1
KEYWORDS    .
SOURCE      Staphylococcus sciuri
  ORGANISM  Staphylococcus sciuri
            Staphylococcaceae;
            Staphylococcus.
REFERENCE   1  (bases 1 to 180)
  AUTHORS   Taghreed,M.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-APR-2017) Institute of Medical Technology, Al Mansour
            University, Baghgdad 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="EA111"
                     /isolation_source="burns"
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/db_xref="taxon:1296"
/country="Iraq"
/collection_date="2016"
/collected_by="Taghreed"
<1.>180
/product="16S ribosomal RNA"

Stain (2): Staphylococcus epidermidis strain 40.1 16S ribosomal RNA gene, partial sequence

GenBank: KY938529.1

DEFINITION Staphylococcus epidermidis strain 40.1 16S ribosomal RNA gene, partial sequence.
ACCESSION KY938529
VERSION KY938529.1
KEYWORDS.
SOURCE Staphylococcus epidermidis
ORGANISM Staphylococcus epidermidis
Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus.
REFERENCE 1 (bases 1 to 1021)
AUTHORS Taghreed,M.K.
TITLE Direct Submission
JOURNAL Submitted (13-APR-2017) Institute of Medical Technology, Al Mansour University, Baghdad 00964, Iraq
COMMENT ##Assembly-Data-START##
Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
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/strain="40.1"
/isolation_source="burns"
/db_xref="taxon:1282"
/country="Iraq"
/collection_date="2016"
/collected_by="Taghreed"
/product="16S ribosomal RNA

Strain (3): Staphylococcus aureus strain SW-60 16S ribosomal RNA gene, partial sequence

GenBank: KY938528.1

DEFINITION Staphylococcus aureus strain SW-60 16S ribosomal RNA gene, partial sequence.
ACCESSION KY938528
VERSION KY938528.1
KEYWORD .
SOURCE Staphylococcus aureus
ORGANISM Staphylococcus aureus
Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus.
REFERENCE 1 (bases 1 to 900)
AUTHORS Taghreed,M.K.
TITLE Direct Submission
JOURNAL Submitted (13-APR-2017) Institute of Medical Technology, Al Mansour University, Baghdad 00964, Iraq
COMMENT ##Assembly-Data-START##
Sequencing Technology: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..900
/organism="Staphylococcus aureus"

/mol_type="genomic DNA"
/strain="SW-60"
/isolation_source="urinary tract infection"
/db_xref="taxon:1280"
/country="Iraq"
/collection_date="2016"
/collected_by="Taghreed"
/product="16S ribosomal RNA"

Strain (4): Staphylococcus haemolyticus strain G59 16S ribosomal RNA gene, partial sequence

GenBank: KY938527.1

DEFINITION Staphylococcus haemolyticus strain G59 16S ribosomal RNA gene, partial sequence.
ACCESSION KY938527
VERSION KY938527.1
KEYWORDS.
SOURCE Staphylococcus haemolyticus
ORGANISM Staphylococcus haemolyticus
Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus.
REFERENCE 1 (bases 1 to 1080)
AUTHORS Taghreed,M.K.
TITLE Direct Submission
JOURNAL Submitted (13-APR-2017) Institute of Medical Technology, Al Mansour University, Baghdad 00964, Iraq
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
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/mol_type="genomic DNA"
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/isolation_source="urinary tract infection"
/db_xref="taxon:1283"
/country="Iraq"
/collection_date="2016"
/collected_by="Taghreed"
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<1.>1080
/product="16S ribosomal RNA"

Figure (4): GenBank number of the *Staphylococcus spp* isolated from sample of iraq during the present study as display in the NCBI, Stain (1) : Staphylococcus sciuri; Stain (2): Staphylococcus epidermidis; Strain (3): Staphylococcus aureus; Strain (4): Staphylococcus haemolyticus.

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