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RESEARCH ARTICLE
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16S rRNA Profiling of Nine Global New Strains of Staphylococcus aureus Isolated from Clinical Specimens in Basrah Province, Iraq

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ABSTRACT

Background: Staphylococcus aureus is familiar as a common pathogen. It is found worldwide and is a leading cause of disease. Many methods have been used for identifying the associated strains in clinical specimens. Sequencing by 16SrRNA methods is achieved in identification.

Methods: A total of 300 clinical swab samples were collected from diabetic foot infected and hemodialysis patients who were admitted to Basrah General Hospitals during the period from March-2018 to February 2019. Bacterial isolates were identified and sequencing was done by using a Vitek®2 system and 16SrRNA with specific primers for Staphylococcus genus.

Results: Thirty-six Staphylococcus species comprised 21 (58.3%) isolates determined in diabetic foot patients, while 15 (41.7%) were identified from hemodialysis patients. The 24 isolates were identified as S. aureus. The 16SrRNA showed the nine strains of Staphylococcus aureus isolates (No.1,2,4,6,8,9,17,25 and 26) had many differences when compared with their reference strains. These isolates were reported as new global separated strains and published by the National Center for Biotechnology Information (NCBI) and European Nucleotide Archive (ENA), as Staphylococcus aureus TSH-Basrah 01, TSH-Basrah 02, TSH-Basrah 04, TSH-Basrah 06, TSH-Basrah 08, TSH-Basrah 09, TSH-Basrah 17, TSH-Basrah 25 and TSH-Basrah 26 strain under accession number (MN982864.1, MN982865.1, MN982866.1, MN982867.1, MN982868.1, MN982869.1, MN982870.1 MN982871.1, and MN982872.1) respectively.

Conclusion: Nine global strains of S. aureus were detected from clinical specimens in Basrah. The result of 16S rRNA has given a clear picture of the significance of the Vitek ® 2 system that was used for the identification of S.aureus from clinical samples.

Keywords: *Staphylococcus aureus, 16SrRNA, new isolates, diabetic foot, hemodialysis.*

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INTRODUCTION

In recent years, methicillin-resistant Staphylococcus aureus (MRSA) infections have grown at an alarming rate. Infection with MRSA is an increasingly growing global risk to health (Singh et al., 2019). In patients with hemodialysis and diabetes with complicated foot disease, for example, S.aureus could lead to life-threatening bacterial infections if the host is immunocompromised or if physiological barriers are breached. (Abed et al., 2018;Stacey et al., 2019).

The 16SrRNA gene sequencing and PCR-restriction fragment length polymorphism analysis had been categorized for Staphylococcus species identification (Figueras et al., 2012). Clinical laboratories of microbiology and public health agencies have historically relied on phenotypic approaches to classify bacterial pathogens, but for a long time, traditional culture was recognized as a gold standard for the identification of bacteria. However, it can require days or even weeks to evolve bacteria successfully, as some clinically important bacteria grow gradually, are hard to grow, are fastidious, or often uncultivable (Winand et al., 2019).

A possible alternative is the use of 16S rRNA gene sequencing, which has been used traditionally to classify known and new bacteria irrespective of cultivability and phenotype (Woo et al., 2008). This study is aimed to identify and characterize Staphylococcus aureus strains from clinical specimens by using some traditional, Vitek ® 2 system and molecular methods with 16SrRNA.

MATERIALS AND METHODS

Sample collection

From March 2018 to February 2019, a total of 300 clinical swab samples were collected from patients with diabetic foot infections and hemodialysis who were admitted to Basrah General Hospitals, all patients were enrolled and the research was accepted by the ethical committee of the College of Science, University of Basrah. Swabs cultivation and Identification by Vitek®2 system (Vitek®2 GP ID-P Reference number 21342, bioMérieux, USA) .

Genetic profiling

The genomic DNA was extracted from the bacterial isolates by using DNA Presto Mini g DNA Bacteria kit (Geneaid, USA) was used for genomic DNA extracted. The DNA sample was considered pure when the rate was 1.8 - 2.0 ng.

16S ribosomal RNA (16SrRNA)

The specific primers for Staphylococcus genus with product size 756 bp according to McClure et al. (2006). The PCR reagents that are used in amplification are DNA template: 1 µl (3ng), forward primer: 1 µl (10 pmol), reverse primer:1 µl (10 pmol), Promega master mix (Promega, USA):12.5 µl, nuclease-free water: 9.5 µl and with total volume: 25µl. It was performed with: a 1 cycle denaturation step (5 min. at 95°C) and ended with a final extension step (5 min. at 72°C), 35(30s at 95°C; 30s at 55°C;1 min. at 72°C). Amplified products electrophoresis in 1.5% agarose gel and visualized with ethidium bromide by using a UV gel documentation system.

Preparation of 16SrRNA products for sequencing

The 16SrRNA products of (n=24) bacterial isolates were prepared according to the manual of the sequencing company and the tubes were labeled with the name of the patient and number of the sample, then were sent to the Macrogen Company (https://dna.macrogen.com/) in South Korea.

Analysis of the sequencing results

The treatment sequencing was put in the box of the "BLAST" program (http://blast.ncbi.nlm.nih.gov). The similarities of ≥ 99% were dependent on the diagnosis (Drancourt et al., 2000).

RESULT & DISCUSSION

The present study focused exclusively on Staphylococcus aureus isolated from patients with diabetic foot and hemodialysis Infections. S. aureus is associated with significant increases in hospitalization, infections, and treatment for patients, and morbidity and mortality rates among patients with diabetic foot and hemodialysis (Stacey et al., 2019)

Out of 300 samples, Thirty-six of Staphylococcus spp. divided into two different groups of patients were isolated. The first group was diabetic foot patients consisting of 21 (58.3%) isolates which include 18(85.7%) S.aureus and 3(14.3%) Staphylococcus spp. While the second group was hemodialysis (HD) patients, which consisted of 15 (41.7%) isolates distributed in 6(40%) S.aureus and 9(60%) Staphylococcus spp. according to results of Vitek ® 2 (Table 1).

TABLE 1: Frequency of Staphylococcus spp in clinical specimens

Source of bacterial isolates	Types of bacterial isolates				Total of percentage
	Staphylococcus aureus		Staphylococcus spp.		
	No	%	No	%	
Diabetic foot patients	18	85.7	3	14.3	100
Hemodialysis patient	6	40	9	60	100
Total	24		12		
	36				

Vitek®2 is an important tool for the proper management of staphylococcal bloodstream and other clinical infections, but it is also necessary for a better understanding of the pathophysiological factors affecting the clinical outcome and for epidemiological surveillance (Sanguinetti et al., 2003; Layer et al., 2006). Delmas et al., (2008) study showed highlighted the interesting performance of the colorimetric Vitek ® 2 Gram-positive GP card, which can be used in clinical samples, also Nimer et al., (2016) results indicate that the Vitek® 2 method is an appropriate tool for fast, direct identification and sensitivity testing of Gram's negative rods and Gram's positive cocci. However, the results of the Vitek® 2 system have been given a perfect match with the results obtained by using the biochemical identification tests, but with the advantage of effort and time and provide a greater opportunity to diagnose the pathogen faster.

Genetic profiling of bacteria

16S rRNA

Single 16S rRNA band viewed in (756 bp) when compared to a typical molecular DNA ladder (2000 bp) Figure-1.

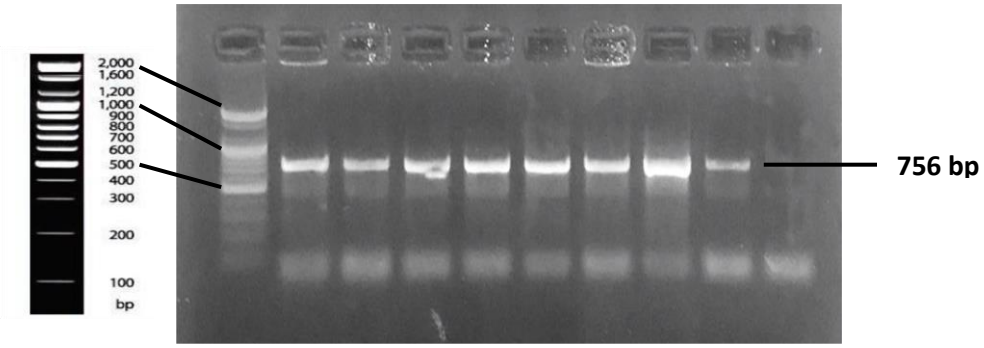

FIGURE 1: Gel electrophoresis of 16SrRNA products. Lane L:(2000bp DNA ladder) , Lane:(no. 2-8) 16SrRNA band of bacterial isolates.

The 16S rRNA, a gene has proved to be an excellent molecular target and is strongly conserved in a population over time (Petti, 2007; Sabat et al., 2017). The 16S rRNA sequencing has therefore played a very important role in the accurate detection of bacterial isolates and the discovery of novel bacteria in laboratories of clinical microbiology (Connor et al., 2019).

Identification of new global strains

Sequencing, widely used for the 16S rRNA gene, is difficult in large samples of polymicrobial (Deurenberg et al., 2017). Next-generation developments sequencing (NGS) in sequencing technology over the past decade provides many advantages over Sanger sequencing, including increased resolution and precision in microbial pathogen detection (MacCannell,2016; Motro and Moran-Gilad, 2017).

In addition, this technology has allowed independent culture analysis of complex polymicrobial samples in parallel for the detection and identification of multiple pathogens (Peker et al., 2019). The present study was focused on the use of the16S rRNA to detect the rate of accurate conventional biochemical tests and the Vitek® 2 system used in this study to identify S.aureus. The 16S rRNA result gives a clear picture of the significance of the Vitek® 2 system that was used for the identification of S.aureus from clinical samples.

Nine bacterial isolates (No.1,2,4,6,8,9,17,25 and 26) from 24 isolates differ from their reference strain in several nucleotide sequence positions. All these isolates were recorded as new strains by the National Biotechnology Information Center (NCBI) which is a part of the United States National Library of Medicine and European Nucleotide Archive (ENA), the distribution of these nine new global strains was six from diabetic foot (No.1, 2,6,8,25 and 26), while three strains isolated from hemodialysis patients (No.4,9 and 17).

The isolate (No.1,2and 6) S.aureus isolated from diabetic foot patients was recorded as S.aureus TSH-Basrah 01 (GenBank: MN982864.1) strain, S.aureus TSH-Basrah 02 (GenBank: MN982865.1) strain and S. aureus TSH-Basrah 06 (GenBank: MN982867.1) strain respectively with strongly linked (99.86%),(99.72%) and (99.59%) respectively with reference S.aureus strain CPO 4.236 with a point mutation: pyrimidine (C) replaces by a purine (A) in strain (No.1), but with a two-point mutation: first: insertion the pyrimidine (T) and second: insertion the pyrimidine (C) in strain (No.2), while with a three-point mutation: first, insertion the pyrimidine (C) While, the second: the pyrimidine (C) replaces by a purine (A), and the third: the purine (A) replaces by a pyrimidine (T) respectively in different location Figure-2,3and 4.

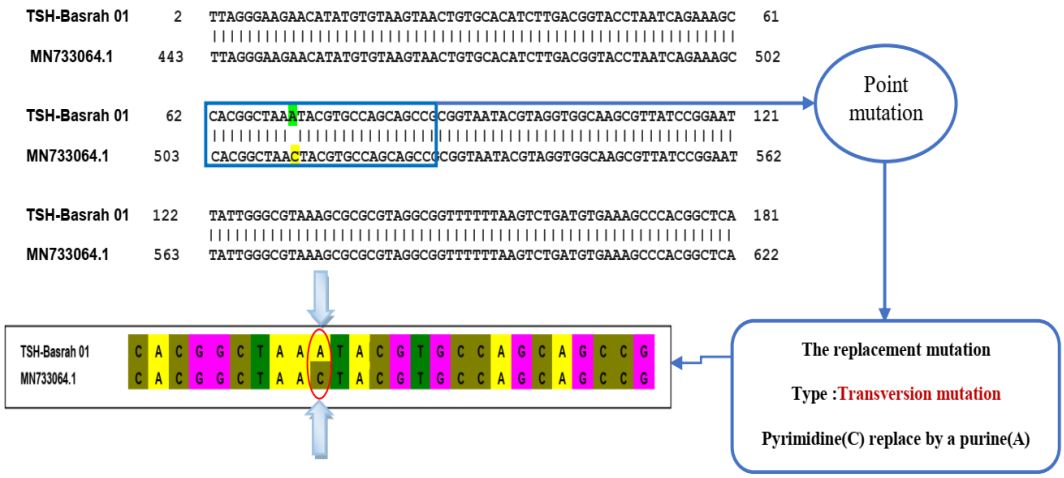

FIGURE 2: 16S rRNA nucleotide sequences (730bp) compared S. aureus TSH-Basrah 01 strain, (No.1) from the present study with reference strain S. aureus CPO 4.236.

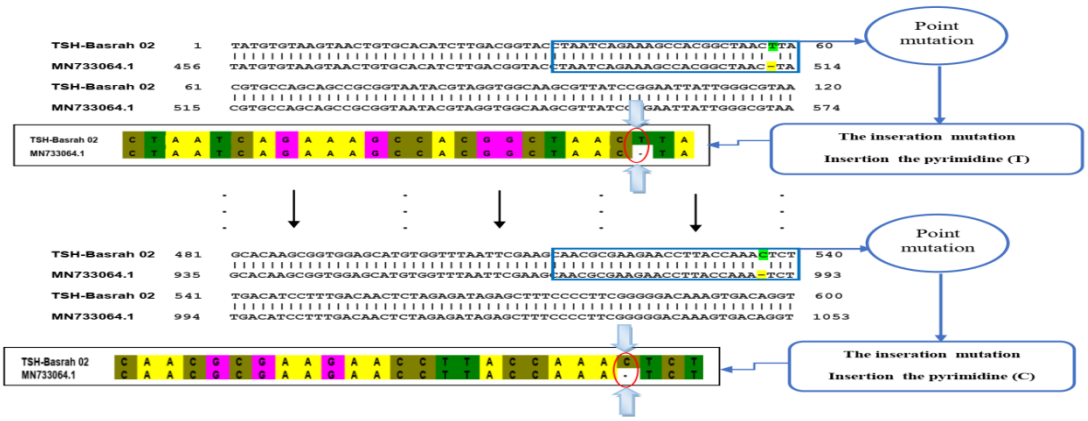

FIGURE 3: 16S rRNA nucleotide sequences (719bp) compared S. aureus TSH-Basrah 02 strain,(No.2) from the present study with reference strain S. aureus CPO 4.236.

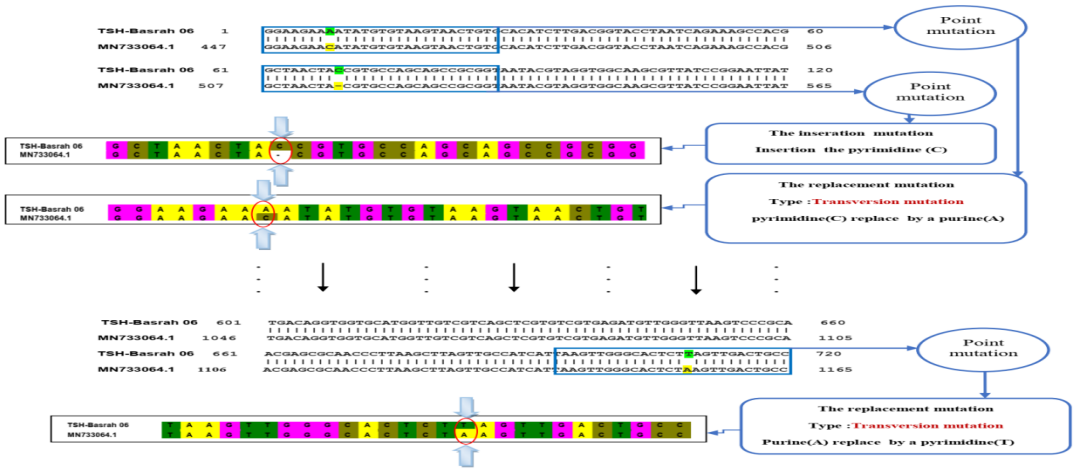

FIGURE 4: 16S rRNA nucleotide sequences (729bp) compared S. aureus TSH-Basrah 06 strain, (No.6) from the present study with reference strain S. aureus CPO 4.236.

Furthermore, isolate (No.8) was recorded as S. aureus TSH-Basrah 08 (GenBank: MN982868.1) strain was related closely (99.04%) with S.aureus strain ST20130939, but with a five-point mutation, first: the pyrimidine (C) replaces by a purine (A), second: insertion the purine (G), third: insertion the purine (G), fourth: insertion two pyrimidine (T) respectively in different locations While, fifth: insertion pyrimidine (T) Figure-5.

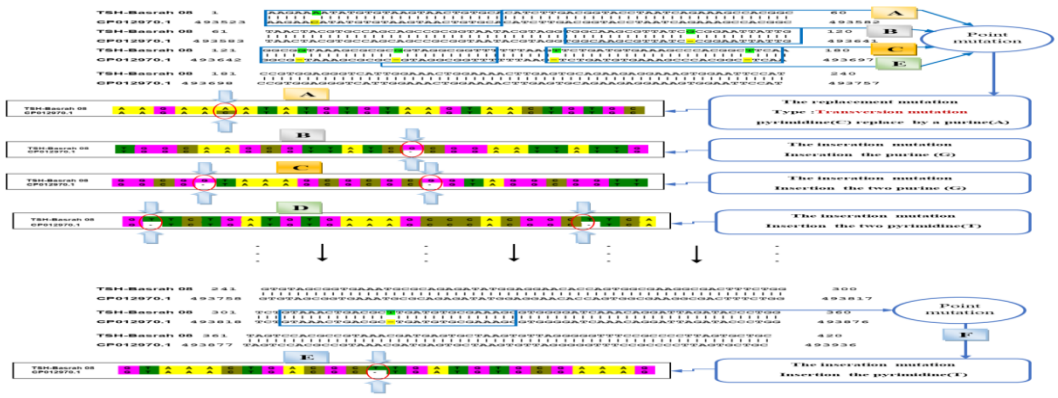

FIGURE 5: 16S rRNA nucleotide sequences (727bp) compared S. aureus TSH-Basrah 08 strain, (No.8) from the present study and reference strain S. aureus ST20130939.

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Moreover, isolate (No.25) was recorded as S. aureus TSH-Basrah 25 (GenBank: MN982871.1) strain was related closely (99.86%) with S. aureus strain LKY-Strp01, but with a one-point mutation: deletion the purine (A) Figure-6.

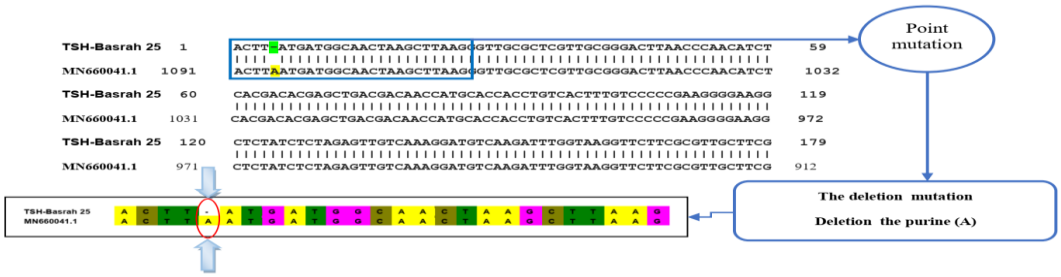

FIGURE 6: 16S rRNA nucleotide sequences (701bp) compared for isolate S. aureus TSH-Basrah 025 strain, (No.25) from the present study and reference strain S. aureus LKY-Strp01.

While the last isolate (No.26) was recorded as S. aureus TSH-Basrah 26 (GenBank: MN982872.1) strain was closely related (99.23%) with S. aureus strain INBio_4062P, but with a five-point mutation: first, the purine (G) replaces by a purine (A), second: (G) replace by a purine (A), third: purine (G) replaces by a pyrimidine (C), fourth: pyrimidine (C) replaces by a purine (A) and also fifth: pyrimidine (C) replaces by a purine (A) Figure-7.

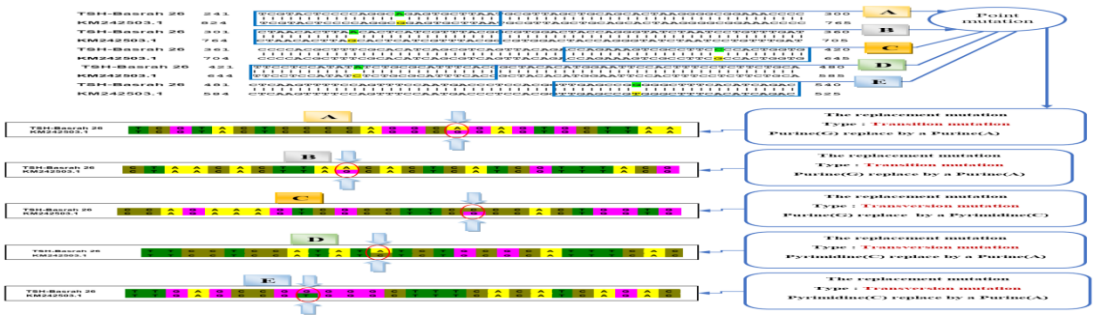

FIGURE 7: 16S rRNA nucleotide sequences (653bp) compared S. aureus TSH-Basrah 026 strain,(No.26) from the present study and reference strain S. aureusINBio_4062P.

On the other hand, the isolated from hemodialysis patients (No.4, 9, and 17) also showed point mutations and differences when compared with reference strains. The isolate (No.4) was recorded as S. aureus TSH-Basrah 04 (GenBank: MN982866.1) strain was related closely (99.86%) with S. aureus strain LKY-Strp01, but with a point mutation: deletion pyrimidine (T) Figure-8.

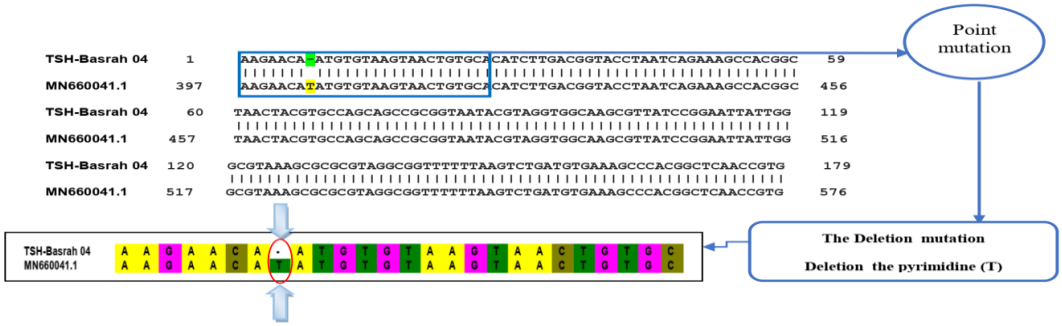

FIGURE 8: 16S rRNA nucleotide sequences (718bp) compared S. aureus TSH-Basrah 04 strain,(No.4) from the present study with reference strain S. aureus-Strp01.

Further, the isolate (No.9) was recorded as S. aureus TSH-Basrah 09 (GenBank: MN982869.1) strain was related closely (99.86%) with S. aureus strain FDAARGOS_40, but with a point mutation: insertion the purine (G) Figure-9.

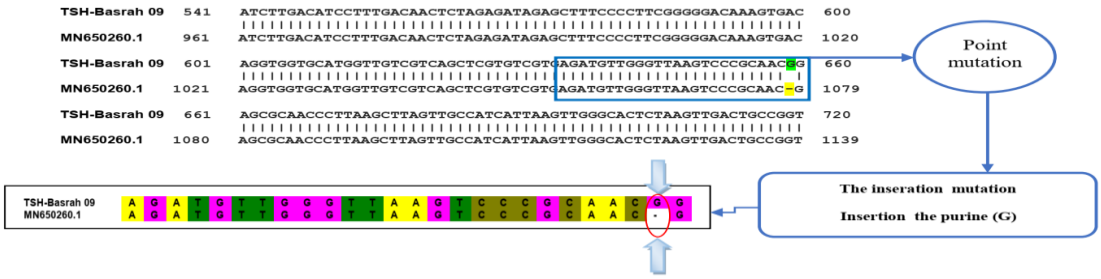

FIGURE 9: 16S rRNA nucleotide sequences (724bp) compared S. aureus TSH-Basrah 09 strain,(No.9) from the present study with reference strain S. aureus FDAARGOS_40

While the last one of isolating (No.17) was recorded as S. aureus TSH-Basrah 017 (GenBank: MN982870.1) strain was related closely (99.86%) with S. aureus strain DSM20231, with a point mutation: insertion pyrimidine (C) Figure-10.

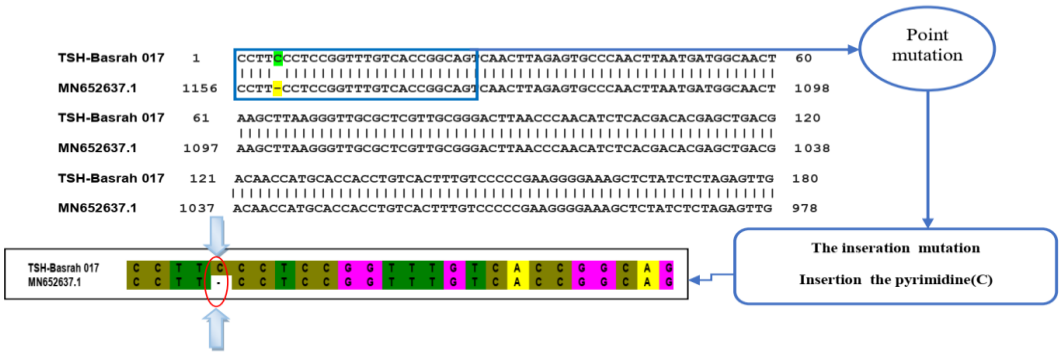

FIGURE 10: 16S rRNA nucleotide sequences (708bp) compared for isolate S.aureus TSH-Basrah 017 strain, (No.17) from the present study and reference strain S. aureus DSM20231.

CONCLUSION

Nine global strains of S. aureus were detected from clinical specimens in Basrah. The result of 16S rRNA has given a clear picture of the significance of the Vitek ® 2 system that was used for the identification of S.aureus from clinical samples.

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