



Staphylococcus aureus: Molecular Genetic Bioinformatics and Evolutionary Relationships

Mohja Aref Sadkhan¹, Hawraa Riyadh Abd Alhassan², Muhammad Hammoud Nasser³, Ghufran Ali Soued⁴, Adian Ali Kadhim⁵, Basim Mohammed Karim⁶, Khetam Ali Abd⁷, Zainab Hussein Hazam⁸, Zahraa Naeem Hashem⁹, Safa Ali Hadi¹⁰

¹University of Thi_Qar, College of Science/ Department of pathological analyzes

²Thi Qar University/ College of Science/ Department of Pathological Analysis

³Dhi Qar University/ College of Science/ Department of Pathological Analysis

⁴Dhi Qar University/ College of Science/ Department of Pathological Analysis

⁵Dhi Qar University/ College of Science/ Department of Pathological Analysis

⁶Dhi Qar University/ College of Science/ Department of Pathological Analysis

⁷University of Thi-Qar, College of Science/ Department of pathological analyzes

⁸University of Thi-Qar, College of Science/ Department of pathological analyzes

⁹University of Thi-Qar, College of Science/ Department of pathological analyzes

¹⁰University of Thi-Qar, College of Science/ Department of

Abstract

Staphylococcus aureus is a type of Gram-positive bacteria, usually living on human skin, in the nasal cavity or in the respiratory tract. This type of bacteria has several characteristics: coagulation-positive, DNA decoding, and its consumption of mannitol-type sugar. Although it does not always cause disease, one of the diseases caused by this type of germ is toxic shock syndrome, which leads to severe illness accompanied by fever, a widespread red rash with the effect of other organs in the body. Recently, new types of antibiotic-resistant Staphylococcus aureus have emerged, the most important of which is methicillin-resistant Staphylococcus aureus, which is an RNA component of the 30s subunit of the prokaryotic ribosome (SSU rRNA). It is related to the TTS of Shine-Dalgarno tuberculosis and provides most of the SSU structure. The genes encoding it are referred to as the 16S rRNA gene and are used in reconstructing strains due to the slow evolutionary rates of this region of the gene. Multiple sequences of the 16S rRNA gene can exist within a single bacterium. To identify the bacteria in a given sample, this procedure uses 16S rRNA gene sequencing, which is currently the most cost-effective method for comprehensive determination of bacterial taxonomy and relative quantitative performance. Alternative methods include targeted.

Keywords: Bioinformatics, Staphylococcus aureus, Genetic, Relationships



Copyright: © 2024 The Authors. Published by Publisher. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction:

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations.[1] Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-

Resistant Staphylococcus aureus). [2] [3] S. aureus is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals.[1] S. aureus does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a

variety of potentially serious infections.[1] Transmission is typically from direct contact. However, some infections involve other transmission methods.[4] The emerging clinical importance of *Staphylococcus aureus* and coagulase-negative staphylococci (5) in connection with the expanding number of staphylococcal subspecies described requires accurate identification to the subspecies level. Currently, the genus *Staphylococcus* is divided into 36 species and 21 subspecies. Staphylococcal subspecies not included in the databases of commercial identification systems, as well as phenotypic variants (e.g., small-colony variants), are often misidentified (6). The usefulness of genotypic identification of staphylococcal subspecies by using partial 16S rDNA sequences in comparison with phenotypic tests (7). However, the partial 16S rDNA sequences used were not discriminative enough to differentiate all staphylococcal subspecies. This study was aimed to show the genetic relatedness among several *S. aureus* strains collected from GenBank.

Genetic bioinformatics

With the widespread adoption of next generation sequencing technologies by the genetics community and the rapid decrease in costs per base, exome sequencing has become a standard within the repertoire of genetic experiments for both research and diagnostics. Although bioinformatics now offers standard solutions for the analysis of exome sequencing data, many challenges still remain; especially the increasing scale at which exome data are now being generated has given rise to novel challenges in how to efficiently store, analyze and interpret exome data of this magnitude. In this review we discuss some of the recent developments in

bioinformatics for exome sequencing and the directions that this is taking us to. With these developments, exome sequencing is paving the way for the next big challenge, the application of whole genome sequencing (8).

Bioinformatic tools for identifying disease gene and SNP candidates

As databases of genome data continue to grow, our understanding of the functional elements of the genome grows as well. Many genetic changes in the genome have now been discovered and characterized, including both disease-causing mutations and neutral polymorphisms. In addition to experimental approaches to characterize specific variants, over the past decade, there has been intense bioinformatic research to understand the molecular effects of these genetic changes. In addition to genomic experimental assays, the bioinformatic efforts have focused on two general areas. First, researchers have annotated genetic variation data with molecular features that are likely to affect function. Second, statistical methods have been developed to predict mutations (9).

Methods:

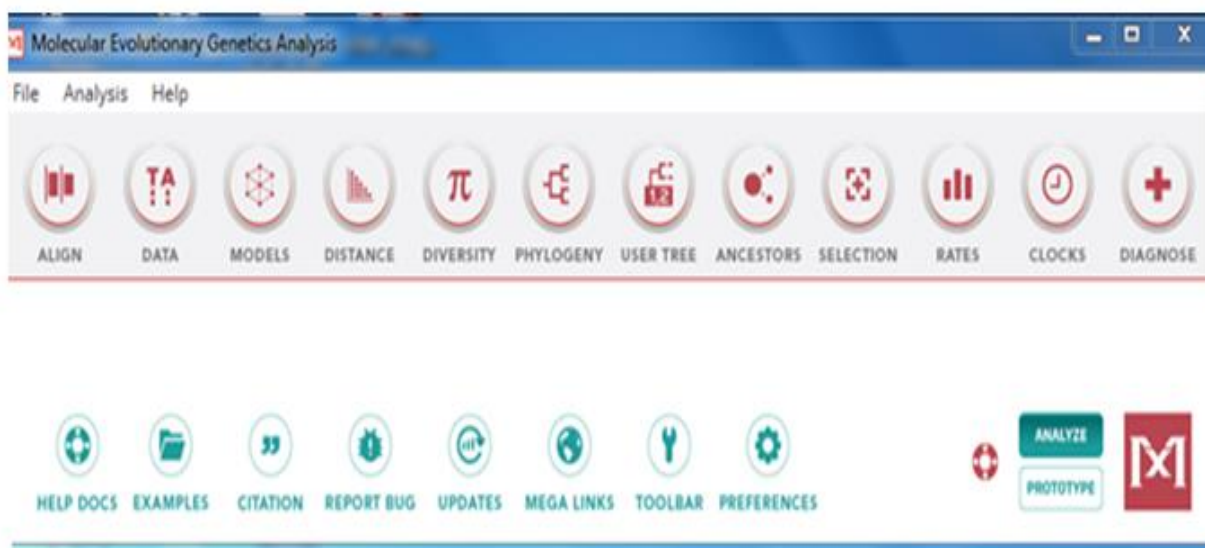
Samples collection

S. aureus samples were collected from GenBank in form of Fasta file. Key words used to select the strains were “*Staphylococcus aureus*”, “16sRNA gene”, “2019 or 2020 or 2021” and “Iraq- Basrah, Thi-Qar”. Nucleotides sequences of *S. aureus* strains were implemented in Mega X software and analyzed using UPGMA tools to construct the phylogenetic tree. The pairwise relatedness was calculated to show the relationships between the strains using numerical data.

Fig. 1 website of NCBI and the search strand of GenBank.

The image shows a screenshot of the NCBI GenBank website. At the top, the NIH logo and 'National Library of Medicine' are visible. The search bar contains the query 'Staphylococcus aureus 16S rRNA Iraq Basrah'. Below the search bar, there are filters for 'Species' (Bacteria (28)), 'Molecule types' (genomic DNA/RNA (28)), and 'Source databases' (INSDC (GenBank) (28)). The search results are displayed in a table with columns for 'Summary', '20 per page', and 'Sort by Default order'. The first result is '16S RIBOSOMAL RNA' for 'Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA, complete sequence'. The result includes a link to the sequence, a 'FASTA' button, a 'BioProject' link, and a 'Targeted Loci Overview' link. There are also 'BLAST' and 'Download' buttons. On the right side, there are sections for 'Find related data', 'Search details', and 'Recent activity'.

Fig. 2 the main screen of Mega x software



Results:

Eight strains of *S. aureus* were selected from both Thi-Qar and Basrah provinces. A strain of Baghdad was added as control (out-group).

Results from Mega x software showed that there is a genetic relatedness between the strains of both Basrah and Thi-Qar Fig. 3, Except one strain from Thi-Qar appeared down the tree as out-group.

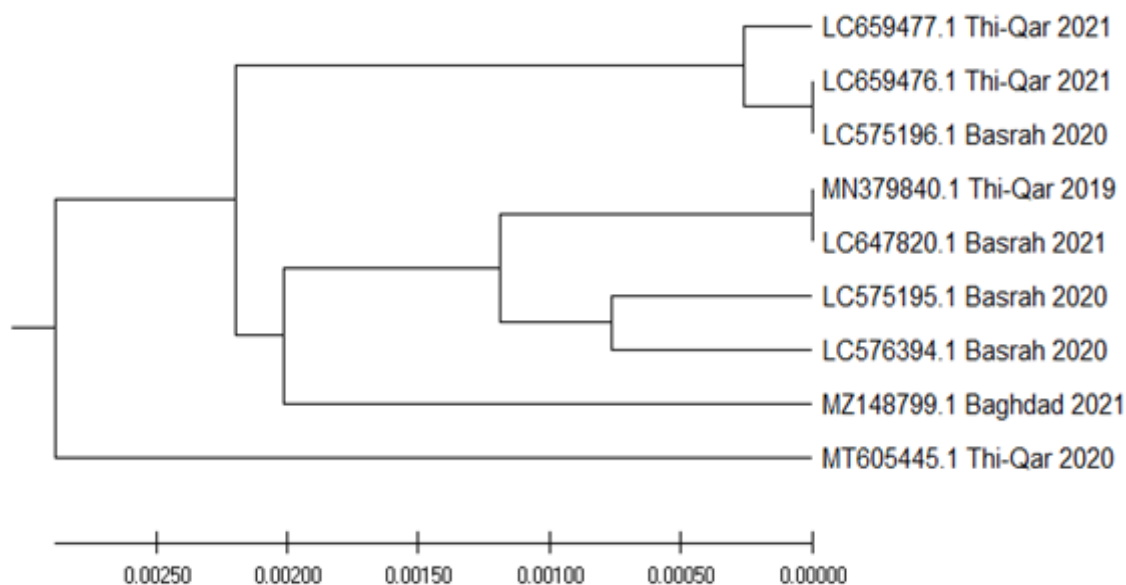


Fig. 3 Evolutionary relationships of *S. aureus*

The evolutionary history was inferred using the UPGMA method [1]. The optimal tree with the sum of branch length = 0.01218485 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the

Maximum Composite Likelihood method [2] and are in the units of the number of base substitutions per site. The analysis involved 9 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1460 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [3].

Table 1. The pairwise of genetic relatedness

LC659477.1_Thi-Qar_2021								
LC659476.1_Thi-Qar_2021	0.0010							
MT605445.1_Thi-Qar_2020	0.0078	0.0071						
MN379840.1_Thi-Qar_2019	0.0015	0.00045	0.0064					
LC647820.1_Basrah_2021	0.0015	0.00045	0.0055	0.0000				
LC575196.1_Basrah_2020	0.0000	0.0000	0.0062	0.0006	0.0209			
LC575195.1_Basrah_2020	0.0054	0.0037	0.0046	0.0028	0.0022	0.00355		
LC576394.1_Basrah_2020	0.0040	0.0029	0.0034	0.0022	0.00220	0.00636	0.00153	
MZ148799.1_Baghdad_2021	0.0040	0.0032	0.0047	0.0025	0.00563	0.00612	0.00269	0.00521

Discussion:

In Phylogenetic analysis, alignment of nucleotide sequences is a major consideration, particularly in studies of genes from divergent taxa. It seems obvious to state that the phylogenetic analysis of sequences begins with the appropriate alignment of the data themselves, yet alignment remains one of the most difficult and poorly understood facets of molecular data analysis (10). In this study, we found that the *S. aureus* isolates transfer between the populations of Basrah and Thi-Qar located southern of Iraq.

References:

1. Lowy FD. Staphylococcus aureus infections. *N Engl J Med.* 1998 Aug 20;339(8):520-32. [PubMed]
2. Centers for Disease Control and Prevention (CDC). Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections--Los Angeles County, California, 2002-2003. *MMWR Morb Mortal Wkly Rep.* 2003 Feb 07;52(5):88. [PubMed]
3. Boucher HW, Corey GR. Epidemiology of methicillin-resistant Staphylococcus aureus. *Clin Infect Dis.* 2008 Jun 01;46 Suppl 5: S344-9. [PubMed]
4. Rasigade JP, Vandenesch F. Staphylococcus aureus: a pathogen with still unresolved issues. *Infect Genet Evol.* 2014 Jan; 21:510-4. [PubMed]
5. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis.* 2002; 2:677-85. 10.1016/S1473-3099(02)00438-3 [PubMed] [CrossRef] [Google Scholar]
6. Seifert H, Wisplinghoff H, Schnabel P, von Eiff C. Small colony variants of *Staphylococcus aureus* and pacemaker-related infection. *Emerg Infect Dis.* 2003; 9:1316-8. [PMC free article] [PubMed] [Google Scholar]
7. Becker K, Harmsen D, Mellmann A, Meier C, Schumann P, Peters G, et al. Development and evaluation of a quality-controlled ribosomal sequence database for 16S ribosomal DNA-based identification of *Staphylococcus* species. *J Clin Microbiol.* 2004; 42:4988-95. 10.1128/JCM.42.11.4988-

4995.2004 [PMC free article] [PubMed]
[CrossRef] [Google Scholar]

8. Lelieveld SH, Veltman JA, Gilissen C. Novel bioinformatic developments for exome sequencing. Hum. Genet. Springer Verlag; 2016. p. 603–14 .
9. Mooney SD, Krishnan VG, Evani US. Bioinformatic tools for identifying disease gene and SNP candidates. Methods Mol. Biol. Humana Press Inc.; 2010. p. 307–19.
10. Hsu HW, Su HY, Huang PH, Lee BL, Liu HJ, Sequence and phylogenetic analysis of P10- and P17-encoding genes of avian reovirus. Avian Dis. 2005; 49(1):36-42.