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First whole genome sequencing of *Staphylococcus aureus* isolates from Iraq: Insights into zoonotic relations and biofilm-related genes

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ABSTRACT

Background: *Staphylococcus aureus* is a significant zoonotic pathogen capable of causing infections in both humans and animals. The bacterium's capacity to develop biofilms and resistance to many different antibiotics has raised significant concerns for public health. Furthermore, studies have demonstrated that horizontal gene transfer enables the transfer of deleterious features between strains found in humans and animals, consequently rendering treatment and control efforts more challenging.

Aim: This study aimed to investigate the relationships between human and animal isolates and biofilm-associated genes in local *S. aureus* strains using whole genome sequencing technique.

Methods: We examined 111 suspected cases of *S. aureus* infection in humans and in animals and screened all *S. aureus* -positive isolates (11 isolates) for biofilm formation and antimicrobial profiles. Additionally, we sequenced and studied five *S. aureus* genomes isolated from humans, cows, sheep, cats, and dogs for significant biofilm-related genes and predicted their loci following annotation and deposition in the NCBI database.

Results: The study showed that the isolates have genome sizes between 2.7 and 2.8 megabases, a GC content of 32.8%–33.1%, and a coding sequence count between 2,718 and 2,838. The cow isolate (MHB) and cat isolate (MHF) exhibited substantial genomic similarities with human isolates of *S. aureus* (N315) and the type strain of *S. aureus* (DSM 20231). The genomes of the human isolate (MHH) and the dog isolate (MHC) were comparable to *S. aureus* (N315). The sheep isolate (MHO) showed lesser genomic similarity and was closely related to *S. aureus* subsp. anaerobius. The genomes were submitted to the NCBI database with the following accession numbers: MHB (GCA_040196135.1), MHH (GCA_040196155.1), MHO (GCA_040195495.1), MHF (GCA_040195555.1), and MHC (GCA_040195445.1). The isolates were categorized by PubMLST typing into MHC (ST-1156), MHB (ST-6), MHF (ST-6), and MHO (a unique ST). We identified the accession numbers, locations, and lengths of biofilm-associated genes and regulators within the studied genomes.

Conclusion: The study is the first to conduct complete genome sequencing of *Staphylococcus aureus* in Iraq, allowing analysis of biofilm-associated genes in local isolates. It provides the first large-scale genomic investigation of genetic relationships among animal and human isolates in Iraq.

Keywords: Biofilm formation, *Staphylococcus aureus,* Whole genome sequencing, Zoonotic diseases.

Introduction

Staphylococcus aureus is a significant pathogen known for causing a wide range of infections, from minor skin conditions to severe diseases such as *pneumonia*, endocarditis, and sepsis. Studies demonstrates that horizontal gene transfer and mutations enhance the virulence characteristics of human and animal strains, consequently complicating treatment and control measures (Narongpun *et al*., 2023). *Staphylococcus aureus* ability to develop resistance to antibiotics (especially methicillin resistant *S. aureus* (MRSA) and biofilms formation makes it particularly challenging threat (Archer, 1998; Becker, 2018; Ebani, 2020). Biofilms are organized communities of bacteria which are encased in a self-produced polymeric matrix, that adhere to surfaces and provide protection against hostile environments, including antibiotic treatment (Idrees *et al*., 2021). The biofilm-related genes in *S. aureus* play a central role in its ability to cause persistent in both hospital and community settings, contributing to chronic infections and increased resistance to conventional therapies (Arciola *et al*., 2012; Hong and Roh, 2018; Thiran *et al*., 2018). In order to counter diverse immune responses inside the host, pathogens need a wide range of sophisticated mechanisms that are associated with fast and efficient regulatory capacities (Thamer and Shareef, 2022). As a result, the regulatory network of *S. aureus* is quite complex and consists of

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many regulators that are instrumental in determining the best adaptive responses. Of these important regulators are the two-component systems (TCS) regulators. TCS are essential for bacterial survival and adaptation in various conditions. These TCSs are composed of a sensor kinase and a response regulator that can sense and react to different kinds of environmental signals, such as biofilm formation systems, antibiotic resistance, and virulence (Beier and Gross, 2006).

Humans have been historically recognized as the primary transmission hub for *S. aureus* to other species. However, a variety of host jump events have facilitated the emergence of endemic livestock strains, and subsequent host switches back into humans have led to the expansion of global epidemic *S. aureus* clones. Genetic modifications (genetic drift) in the genomes of the adapted *S. aureus* isolates facilitated these host jumps (Rodrigues *et al*., 2022). Genetic drift, a mechanism of neutral diversification or adaptive evolution, may influence the host adaptability of *S. aureus* by favoring advantageous mutations and reducing detrimental ones in the new host. These genomic modifications require not only the ability to evade the immunity of the new host but also to be able to transmit the new clone infection between the new host group members (Rodrigues *et al*., 2022). For instance, single nucleotide polymorphisms and insertion-deletion mutations (indels) might broaden the host range of a *S. aureus* isolate, while the incorporation of mobile genetic components may introduce new genes into its genome, facilitating infection and survival in other hosts (Viana *et al*., 2015; Bacigalupe *et al*., 2019). These genomic modifications could occur in key host adaptation genes such as biofilm genes or antimicrobial genes (Aboud and Khudaier, 2018; Shareef *et al*., 2023). The immune system's selective pressure and antimicrobial treatment may facilitate these genetic modification events during the infection course. Nevertheless, these alterations could only increase virulence, and they would not inherently alter host adaptation or host tropism, which may require *in vivo* experimental validation (Paharik and Horswill, 2016; Howden *et al*., 2023). In *S. aureus* infection, biofilm formation is a key process in adaptation against the host's defense mechanisms, allowing this bacterium to colonize and adapt. Therefore, genetic variations in the genes and regulators associated with biofilm formation may expand the virulence and even host tropism, enabling the infection of new hosts (Howden *et al*., 2023). This recurrent genetic drift necessitates continuous monitoring and analysis of pathogen genomes. It is therefore important to understand the pathogen genomes as well as coding sequences (CDs) within the community for developing effective therapeutic approaches (Kwong *et al*., 2015; O'Connor *et al*., 2018; Humphreys and Coleman, 2019). Furthermore, it would be advantageous to examine the biofilm-associated genes in various host species of *S. aureus* isolates to identify potential genetic variations that may influence interspecies infection or the transmission of illnesses among animals (Howden *et al*., 2023). The efficiency and cost-effectiveness of next-generation sequencing have significantly improved, resulting in a transformational effect on several genomics-related fields (Brlek *et al*., 2024; Bagger *et al*., 2024). Consequently, we have annotated the genomes of local isolates to track any alterations in the local *S. aureus* isolates in forthcoming genomic investigations. This study aimed to investigate the relationships between human and animal isolates and biofilm-associated genes in local *S. aureus* strains using whole genome sequencing technique. We sequenced the genomes of five *S. aureus* isolates from clinical cases in humans and animals. These isolates exhibited a robust capacity for biofilm formation. We employed bioinformatic approach to determine the phylogenetic relationship among these isolates and subsequently identified the biofilm-associated genes within their genomes. This study represents the first application of whole genome sequencing (WGS) on *S. aureus* isolates in Iraq, potentially offering significant insights for further research endeavors.

Materials and Methods

Samples

We obtained samples of suspected *S. aureus* infections (N = 111) from affected animals at veterinary clinics and hospitals in the Basrah governorate. This included pus and mastitis in animals. We obtained five human isolates from the Basrah Directorate Health Central Laboratory (identified *S. aureus* from pneumonia and pus cases). We focused on cases in animals exhibiting symptoms indicative of *S. aureus* infection, including pus and mastitis. The cases included: cows (25 cases of mastitis), sheep (20 cases of mastitis and five cases of pus), dogs (13 cases of pus), cats (12 cases of pus), camels (15 cases of pus), and chickens (15 cases of pus). We processed and cultivated each sample using standard microbiological techniques for isolation and identification, including growth on Mannitol salt agar, CHROMagarTM *S. aureus*, and Gram staining, before submitting it to the Vitek2 identification system.

Molecular identification

Using the universal primers 27F 5 ′-AGAGTTTGATCCTGGCTCAG-3 ′ and 1492R 5′-TACGGTTACCTTGTTACGACT-3′) (Frank *et al*., 2008; Srinivasan *et al*., 2015), the 1500 base pair PCR product of the 16 s RNA gene was sequenced using the internal sequencing primers the 27F and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', the 785F 5'-GGATTAGATACCCTGGTA-3' and 1492R, respectively. All the sequencing processes were carried out at Macrogen , Korea. Blast analysis throughout the NCBI database was conducted to confirm the identity of each isolate (Marchler-Bauer *et al*., 2015; Tatusova *et al*., 2016; Yang *et al*., 2020).

Biofilm formation

We investigated the biofilm formation as previously described, with slight modifications (Mashruwala *et al*., 2017a; Mashruwala *et al*., 2017b). We diluted aerobic cultures that were cultivated overnight in brain heart broth (obtained from individual colonies) into fresh sterile brain heart broths until the final optical density reached 0.05 (A630). 200 µl of diluted cultures were added to Costar® 96-well cell culture plates with a flat bottom (Corning , USA). The plates were incubated without agitation at 37°C for 22 hours. We evaluated the optical density (A630) of the cultures prior to harvesting the biofilm. The plates were washed three times with distilled water and then heat fixed at a temperature of 60°C for 1 hour. Finally, the plates were left to cool to room temperature before staining the biofilm mass with a 0.1% solution of crystal violet. Plates were washed three times with deionized water after staining. The biofilms were subsequently de-stained using a 33% acetic acid solution. After mixing the plates, the absorbance of the resulting solution was measured at 570 nm using BioTek 800TS microplate reader. The absorbance data were normalized to a blank acetic acid solution, and then to the harvested culture's optical densities. *Staphylococcus aureus* MRSA ATCC 43300 and *S. aureus* ATCC 29213 were used as controls.

Whole genome sequencing

After the initial screening, we selected five *S. aureus* isolates for whole genome sequencing based on the intensity of their biofilm phenotypes. The isolates were named as follows: the *S. aureus* MHB from a mastitis case in a cow, the *S. aureus* MHO from a mastitis case in an ewe, the *S. aureus* MHF from ear infections in a cat, the *S. aureus* MHC from skin infections in a dog, and the *S. aureus* MHH from an upper respiratory infection in a human. The genomic DNA (gDNA) from these isolates was extracted using DNA extraction kit (QIAamp Mini Kit HB-0329 from Qiagen company, USA, catalog number 51304-51306) according to the manufacturer instruction. Prior to sequencing, the quality of the gDNA samples was assessed using nanodrop and gel electrophoresis. All the sequencing processes were carried out at Macrogen, Korea including the whole genome sequencing which was carried out using Illumina novaseq sequencer. The sequencing library was prepared by random fragmentation of the gDNA samples, followed by 5' and 3' adapter ligation. Adapter-ligated fragments are then PCR amplified with a PCR primer solution which anneals to the ends of each adapter (Keats *et al*., 2018; Maljkovic Berry *et al*., 2019). The library was loaded onto a flow cell at which fragments are seized on a lawn of surface-bound oligos corresponding to the library adapters. Each fragment was amplified into distinct clonal clusters via bridge amplification. the templates resulted from cluster generation were submitted for sequencing using Novaseq sequencer which generated raw reads in FASTQ files format. FastQC (0.11.7) was used to check the quality of raw sequence data(Andrews, 2010). The total number of bases, reads, GC (%), and Phred quality score Q20 (%), and Q30 (%) were calculated. Applying the Phred quality score at each cycle's Q20 (%) and Q30 (%) helped assess the quality of the nucleobase identification provided by automated DNA sequencing, which in turn helped choose the quality of the data that was produced (Ewing and Green, 1998). Before the analysis, adapter sequences and low-quality bases were trimmed away from the reads using Trimmomatic program- version 0.38 (Bolger *et al*., 2014). In order to map the reads obtained from sequencing, *S. aureus* (NZ_AP014921.1) was used as a reference genome using BWA—Burrows-Wheeler Aligner (Li, 2013). After read mapping, Picard and SAMTools were utilized to remove duplicate reads and to determine variant information. Contigs were created by *de novo* assembly of the raw reads using the bioinformatics program SPAdes v.3.5. (Bankevich *et al*., 2012).

Genome submissions to NCBI GenBank

The assembled genome sequences were deposited at GenBank Bio project PRJNA1121204. The annotation was added by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova *et al*., 2016).

Multi locus sequence typing (MLST)

We utilized the MLST server, software version 2.0.9 (2022-05-11) and database version 2023-06-19, provided by the Center for Genomic Epidemiology at https://www.genomicepidemiology.org. This server determines the sequence types (STs) of bacteria from the whole genomes sequence data (Larsen *et al*., 2012). Furthermore, the bacterial population genomics BIGSdb (PubMLST website) (Jolley and Maiden, 2010; Jolley *et al*., 2018) was used to confirm and index the sequence types of our isolates.

Genome analysis and comparison

We used PATRIC's comprehensive genome analysis tool to examine the sequencing reads as previously described (Wattam *et al*., 2017). This helped to detect both conserved sequence features and produced approximate circular representationss of the genome using the circular genome viewer at the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) website (Olson *et al*., 2023). Additionally, we further investigated the assembled genomes for detailed information on functional genes categorized within the subsystem groups using the RASTtk server (Brettin *et al.*, 2015) and the SEED tool (Overbeek *et al.*, 2014). For consistency, the minimal cutoff for similarity in all annotation and comparison methods was 95% identity. We used the SEED tool to compare and analyze the genomes of the animals to the human MHH strain, which served as the reference genome.

Phylogenetic analysis

We submitted the genome sequences to the type (Strain) genome server (TYGS), a free bioinformatics platform at https://tygs.dsmz.de, for a whole genome taxonomy

analysis. The List of Prokaryotic Names withstanding in Nomenclature (LPSN) database (available via https://lpsn.dsmz.de), offered information on nomenclature, synonymy, and relevant taxonomic references (Meier-Kolthoff *et al*., 2022). Results were provided by TYGS on 2024-07-15. Based on PATRIC's comprehensive genome analysis phylogenic initial results, we included *S. aureus* subsp. *aureus* NCTC 8325 and *S. aureus* subsp. *aureus* N315 as controls in the TYG phylogenetic analysis, aiming to increase the resolution.

Ethical approval

Sampling from animals was conducted according to the ARRIVE guidelines and in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The human samples were collected from local hospitals' laboratories under the supervision of the Basrah Directorate of Health and a written consent was signed by each patient. The research ethics committee of Basrah College of Veterinary Medicine granted this research the permission number 40/37/2024 on May 22, 2024.

Results

Isolation of S. aureus

After identification, we evaluated 11 confirmed positive samples for *S. aureus* for biofilm production and antimicrobial properties, selecting five isolates for whole genome sequencing based on their biofilm-forming capacity and antibiotic resistance. We recognized these isolates as *S. aureus*, derived from human (MHH), cow (MHB), sheep (MHO), cat (MHF), and dog (MHC) origins.

Genomes information

Phred scores of 20 (Q20) quantify the precision of the DNA sequencing technique. The sequencing quality was excellent ranging between Q20 (%) 98.1 to 98.3%. The genome sizes ranged from 2.7 to 2.8 megabase (Mb), the GC content percentage was 32.8-33.1 %, and the number of CDs ranged from 2,718 to 2,838 (Fig. 1, Table 1). The assembled genome sequences were deposited at GenBank Bio project PRJNA1121204. The annotation was added by the NCBI PGAP (Tatusova *et al*., 2016). In addition, the annotation process included an analysis of the subsystems unique to these genomes. The genomes were submitted to the NCBI database with the following accession numbers: MHB (GCA_040196135.1), MHH (GCA_040196155.1), MHO (GCA_040195495.1), MHF (GCA_040195555.1), and MHC (GCA_040195445.1). Figure 2 presents an overview of the *S. aureus* subsystem categories for MHH, MHB, MHC, MHF, and MHO strains. The bioinformatic analysis revealed no plasmids in any of the tested isolates.

Multi locus sequence typing

Using the bacterial population genomics BIGSdb software and the PubMLST website (Jolley *et al*., 2018) we determined that the isolates belong to MHC ST-1156, MHB ST-6, MHF ST-6, whereas MHO is a new novel ST that is close to ST 522.

Genome comparison and phylogenetic analysis

The RASTtk server and Seed Viewer sequence-based comparison tool revealed that while there were variations in the genes of the animals' isolates, their genomes exhibited significant similarities (Fig. 3) and formed a cluster as species and subspecies in the phylogenetic tree based on the TYGS result for the local *S. aureus* whole-genome data set (Fig. 4). The genomes of cow isolate MHB and cat isolate MHF showed significant homology to the human isolates of *S. aureus* (N315) MRSA (Kuroda *et al*., 2001) and the type strain of *S. aureus* (DSM 20231) (Shiroma *et al*., 2015). The genomes of the human isolate MHH and the dog isolate MHC were remarkably comparable to those of *S. aureus* (N315). Nevertheless, the MHO strain exhibited a lesser degree of similarity in comparison to the human and other animals isolates and was close to *S. aureus* subsp. anaerobius (Elbir *et al*., 2013). The findings of our study support the presence of a strong phylogenetic connection between the human and animal isolates that were investigated. These findings provide further evidence that these isolates have a zoonotic origin. Upon comparing the genomes of animal isolates to the human strain MHH, we noticed dispersed groups of genes that either did not have any similarity or exhibited lower similarity when compared to the human isolate MHH (Fig. 3, marked with green, purple, blue, and orange arrows).

Biofilm associated genes

We conducted a comprehensive review of the literature on *S. aureus* biofilm-associated genes and identified the sequences of 24 confirmed genes. Utilizing the NCBI BLAST analysis tool, we compared these sequences against the genomes of MHH, MHB, MHC, MHF, and MHO strains. Table 2 provides the accession numbers for the biofilm-associated genes in the studied genomes. Figure 5 shows a crystal violet assay for biofilm formation in *S. aureus* local clinical isolates MHB, MHC, MHH, MHF, and MHO.

Discussion

We selected five *S. aureus* isolates for whole genome sequencing based on the intensity of their biofilm phenotypes. The isolates were named as follows: *S. aureus* MHB from mastitis in cows, *S. aureus* MHO from mastitis in sheep, *S. aureus* MHF from ear infections in a cat, *S. aureus* MHC from skin infections in a dog, and *S. aureus* MHH from upper respiratory infections in a human. Prior to the start of this work, there were no genomic data sets available for Iraqi *S. aureus* isolates. The sole exceptions were *S. xylosus* (Al-Tameemi *et al*., 2023) and *S. epidermidis* (Talat *et al*., 2020). The purpose of this study was to sequence the whole genome of five local *S. aureus* isolates in order to identify and label genes involved

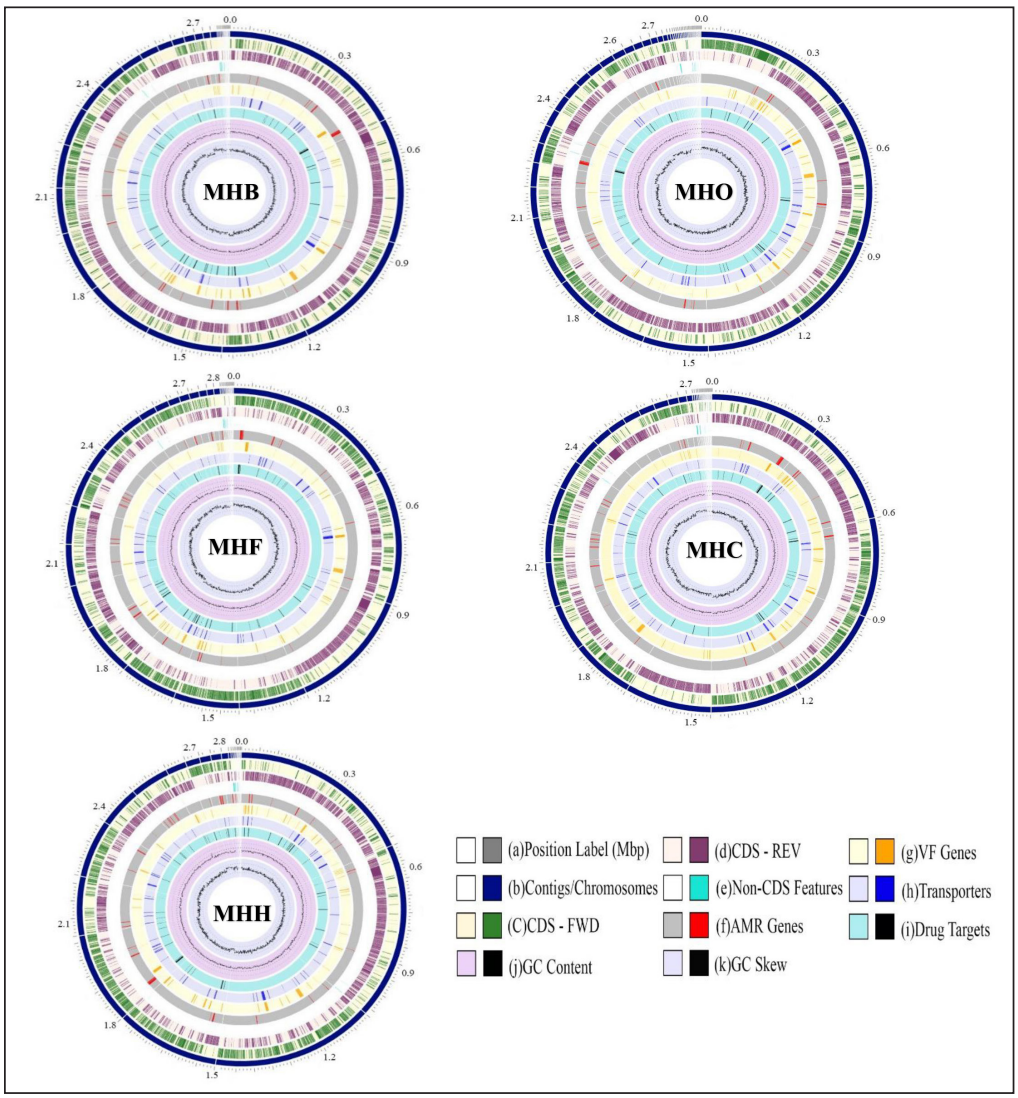
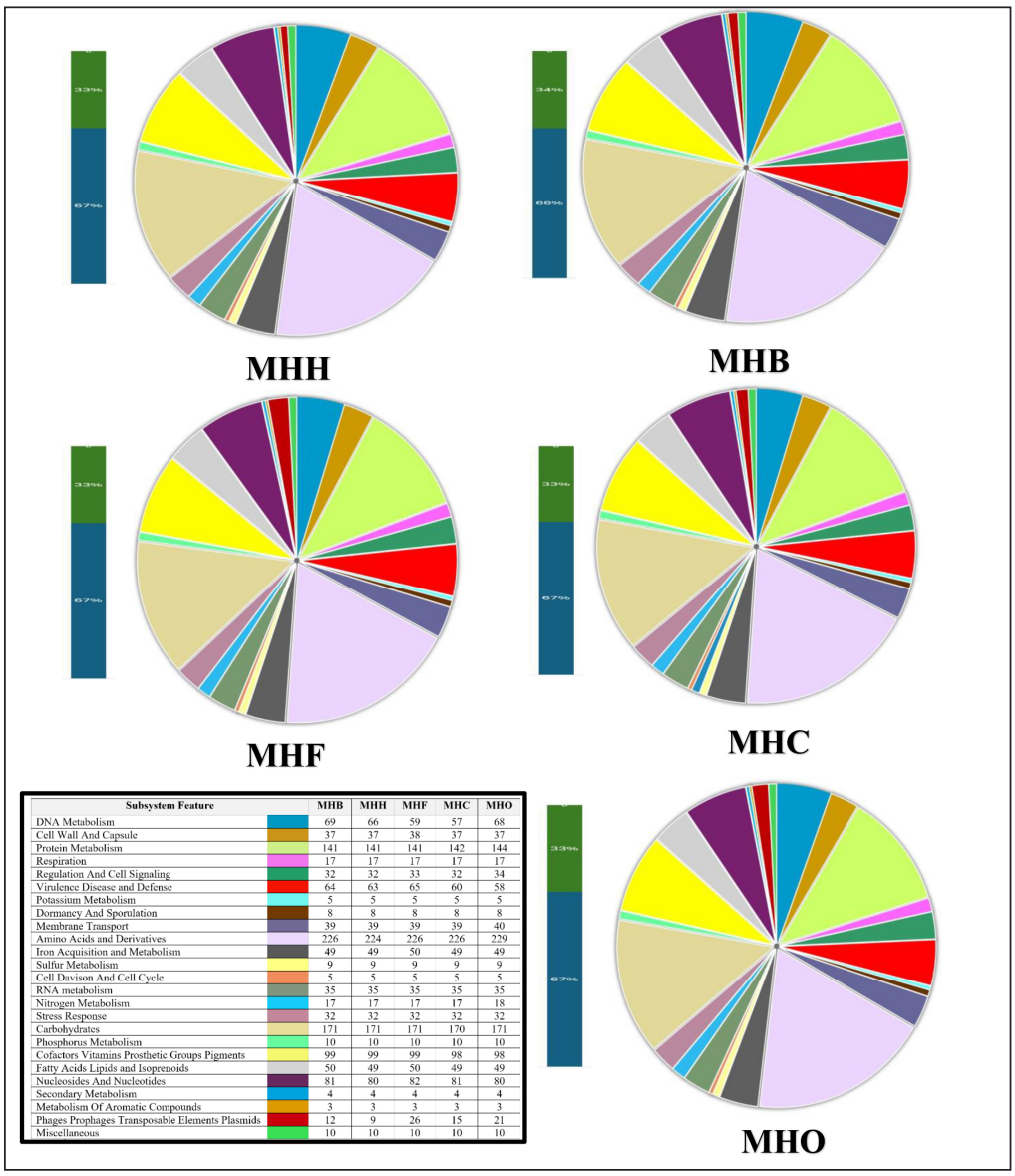


Fig. 1. A circular view of the genomes of *S. aureus* MHB, MHF, MHH, MHO, and MHC isolates generated by PATRIC (Wattam *et al*., 2017; Olson *et al*., 2023) shows the physical map of its important features. From outside in: (a) position in Mbp; (b) order of contigs (shown in navy); (c)/(d) distribution of forward and reverse CDs in plus and minus strands (shown in green and purple, respectively); (e) distribution of noncoding elements (shown in blue); (f) distribution of genes involved in antibiotic resistance (shown in red); (g) distribution of virulence factors (shown in orange); (h) distribution of genes encoding transmembrane proteins (shown in dark blue); (i) distribution of genes encoding drug targets (shown in black); (j) distribution of GC content along plus and minus strands; (k) GC skew (most inner two circles, respectively).

Table 1. Genomes information for *S. aureus isolates* from human (MHH), cow (MHB), sheep (MHO), cat (MHF), and dog (MHC).

Strain ID	Host	Size of genome (bp)	Contigs count	Genes count	GC %	Bio sample nO.	Accession nO.	Assembly accession nO.
MHH	Human	2,801,742	23	2,763	32.9	SAMN41769231	JBEGCO000000000	GCF_040196155.1
MHB	Cow	2,737,048	34	2,718	33.1	SAMN41769230	JBEGCN000000000	GCF_040196135.1
MHO	Sheep	2,746,731	49	2,743	32.9	SAMN41769234	JBEGCR000000000	GCA_040195495.1
MHF	Cat	2,822,615	32.5	2,838	33	SAMN41769233	JBEGCQ000000000	GCA_040195555.1
MHC	Dog	2,742,206	37	2,748	32.8	SAMN41769232	JBEGCP000000000	GCA_040195445.1



Subsystem Feature	MHB	MHH	MHF	MHC	MHO
DNA Metabolism	69	66	59	57	68
Cell Wall And Capsule	37	37	38	37	37
Protein Metabolism	141	141	141	142	144
Respiration	17	17	17	17	17
Regulation And Cell Signaling	32	32	33	32	34
Virulence Disease and Defense	64	63	65	60	58
Potassium Metabolism	5	5	5	5	5
Dormancy And Sporulation	8	8	8	8	8
Membrane Transport	39	39	39	39	40
Amino Acids and Derivatives	226	224	226	226	229
Iron Acquisition and Metabolism	49	49	50	49	49
Sulfur Metabolism	9	9	9	9	9
Cell Davison And Cell Cycle	5	5	5	5	5
RNA metabolism	35	35	35	35	35
Nitrogen Metabolism	17	17	17	17	18
Stress Response	32	32	32	32	32
Carbohydrates	171	171	171	170	171
Phosphorus Metabolism	10	10	10	10	10
Cofactors Vitamins Prosthetic Groups Pigments	99	99	99	98	98
Fatty Acids Lipids and Isoprenoids	50	49	50	49	49
Nucleosides And Nucleotides	81	80	82	81	80
Secondary Metabolism	4	4	4	4	4
Metabolism Of Aromatic Compounds	3	3	3	3	3
Phages Prophages Transposable Elements Plasmids	12	9	26	15	21
Miscellaneous	10	10	10	10	10

Fig. 2. The distribution of *Staphylococcus aureus* subsystem categories for MHH, MHB, MHC, MHF, and MHO strains. The genomes were annotation using the Rapid Annotation System Technology (RASTtk) tool. The pie chart displayed the number of each subsystem feature, while the SEED viewer showed the degree of subsystem coverage. The green bar in the subsystem coverage indicates the proportion of proteins that are part of the subsystems, whereas the blue bar reflects the proportion of proteins that are not part of the subsystems.

in biofilm formation and to examine the potential zoonotic relationship between these organisms and animals. The findings will provide a strong basis for future research in this area. Therefore, our findings will expand the pool of genomes accessible from the Middle East, possibly explaining the variety of strains present in the region.

The relationship between humans and animals has resulted in a significant rise in global incidences of zoonotic infections caused by bacteria that were previously known to infect only specific hosts (Bartels *et al*., 2021). *Staphylococcus aureus* is able to infect new hosts and cross species barriers, despite the fact that humans are considered its primary reservoir. Host-jumping events might occur between humans and animals and vice versa. Horizontal gene transfer of genetic elements that confer survival characteristics in new hosts has been associated with host-species transformations (Richardson *et al*., 2018). These transmissions between species have led to the rise of

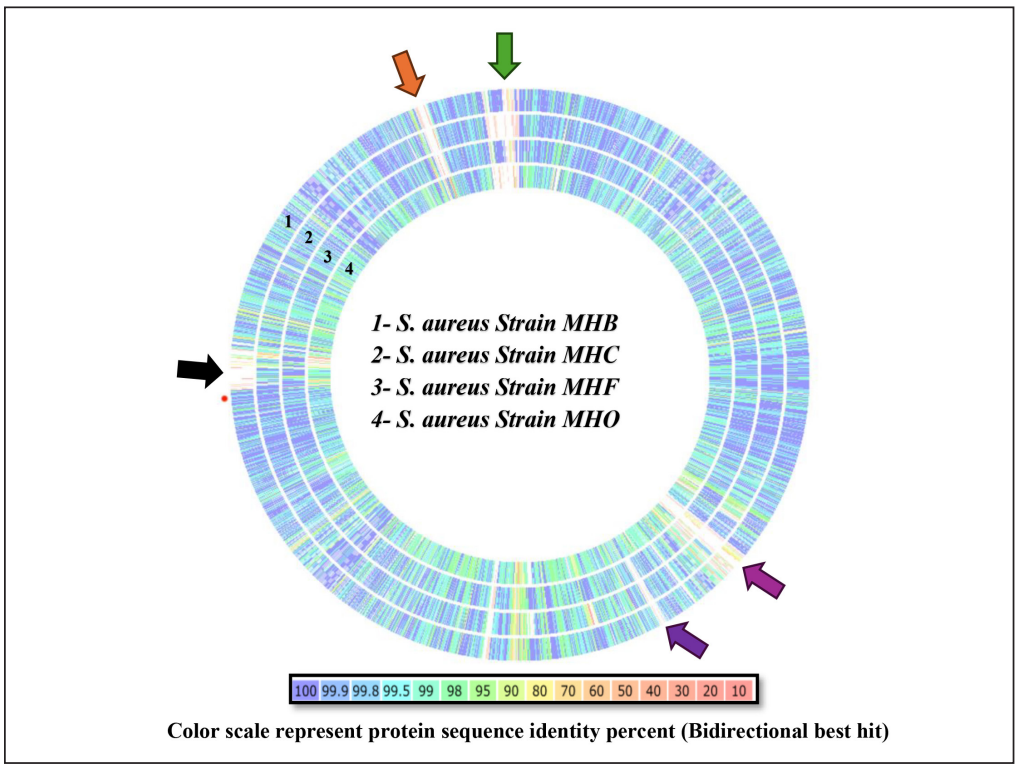


Fig. 3. An interactive genome comparison map between the human strain MHH (reference) and four animal strains was created using the RASTtk server's Seed Viewer sequence-based comparison tool. The strains are (1) strain MHB, (2) strain MHC, (3) strain MHF, and (4) strain MHO, sorted from outer to inside rings. Colors range from 100% purple to 10% pale red, signifying the degree of amino acid similarity to the reference genome. The MHH reference strain's genome is not shown in the graphic. Arrows (green, black, orange, purple, and blue) represent low-identity areas of CDs in the isolates compared to the MHH human strain.

S. aureus lineages that can cause outbreaks and spread widely in both humans and animals. Understanding the population and genomic patterns of MRSA across various hosts is crucial for advancing a One Health strategy and securing optimal health for humans, domestic animals, wildlife, and the environment (Rodrigues *et al*., 2022). In *S. aureus* infection, biofilm formation is a key process in adaptation against the host's defense mechanisms, allowing this bacterium to colonize and adapt (Mazaal *et al*., 2021). Therefore, genetic variations in the genes and regulators associated with biofilm formation may expand the virulence and even host tropism, enabling the infection of new hosts (Howden *et al*., 2023) . Consequently, we have annotated the genes in the genomes of local *S. aureus* isolates to track any alterations in the local *S. aureus* isolates in forthcoming genomic or epidemiological investigations. Due to the constant transfer of virulence genes through horizontal gene transfer among *S. aureus* populations, the flow of genomic information plays a crucial role in understanding and controlling this pathogenic bacterium. The prolonged instability and conflicts in Iraq have hindered the ability to carry out extensive research that can track alterations in the genetic composition of persistent pathogenic infections such as *S. aureus*. To the best of our knowledge, only a two gram-positive Staphylococcus whole genome sequencing studies have been conducted in Iraq, such as *S. xylosus* (Al-Tameemi *et al*., 2023) and *S. epidermidis* (Talat *et al*., 2020).

MLST has been used to categorize *S. aureus* isolates into clonal complexes (CCs) and STs. Certain pathogenic *S. aureus* complexes (CCs) and STs are particularly important as they affect humans and animals. The MLST server showed only seven registered Iraqi isolates, all of which are human isolates belonging to the clonal complexes CC1 (ST-1), CC5, and CC15 (Jolley *et al*., 2018). These types are consistent with our MHH human strain, which is also a member of the ST-1group. However, the isolates from the animals were identified as MHC ST-1156, MHB ST-6, and MHF ST-6, while MHO, a new novel ST is closely related to ST 522. Many studies have reported these clonal complexes in humans and animals which may indicate zoonotic relationships. For example, researchers have reported the presence of ST-1156 in rodents (Ge *et al*.,

2019), surgical wards (Aklilu *et al*., 2012), camel meat (Raji *et al*., 2016) , and humans (Abrudan *et al*., 2023; Roy *et al*., 2024). Whereas ST-6 were detected in humans (Roy *et al*., 2024) and in domestic animals and nonhuman primates (Schaumburg *et al*., 2015). However, the genome wide analyses provide better resolution compared to MLST as it provides detailed information to better understand strain-to-strain relationships (Rodrigues *et al*., 2022; Al-Tameemi *et al*., 2023).

The RASTtk server sequence-based comparison tool and the TYGS demonstrated that while there were minor variations between the human strain MHH and the animals' isolates, their genomes exhibited substantial similarity and formed a distinct cluster (Figs. 3 and 4). These findings may indicate a zoonotic relationship between human and animal isolates. The genomes of cow isolate MHB and cat isolate MHF showed significant homology to the human isolates of *S. aureus* (N315) (Kuroda *et al*., 2001) and the type strain of *S. aureus* (DSM 20231) (Shiroma *et al*., 2015). The genomes of the human isolate MHH and the dog isolate MHC were remarkably comparable to those of *S. aureus* (N315). Nevertheless, the MHO strain exhibited a lesser degree of similarity in comparison to the human and other isolates and was close to *S. aureus* subsp. anaerobius which was isolated originally from an abscess of a sheep with Morel's disease in Khartoum state, Sudan (Elbir *et al*., 2013). It is worth mentioning that unlike the human strain MHH (which was used

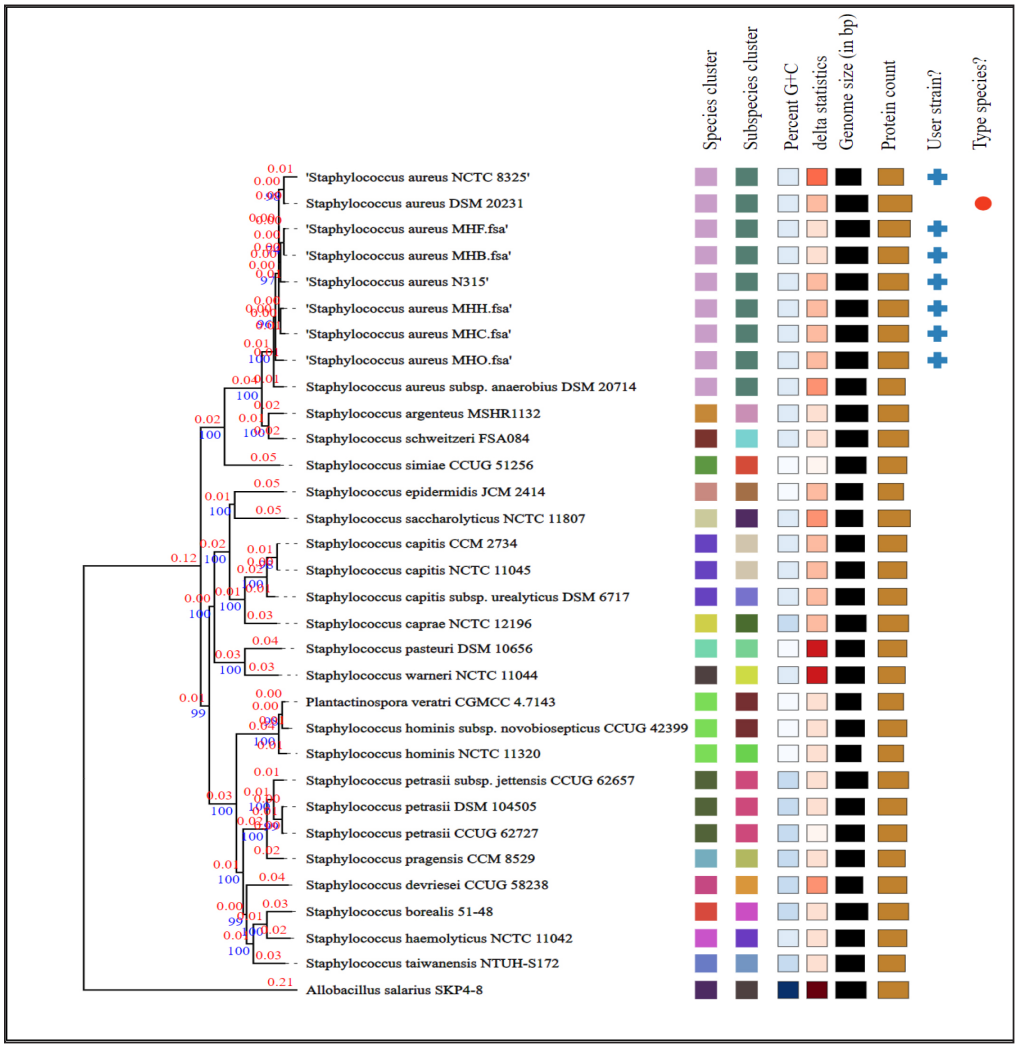


Fig. 4. Phylogenetic tree based on TYGS results for the local *S. aureus* whole-proteom. FastME 2.1.6.1 (Lefort *et al*., 2015) generated the tree using whole-proteome-based GBDP distances (genome BLAST distance phylogeny method). The lengths of the branches are scaled using the GBDP distance formula *d5*. The branch values (numbers above branches) are GBDP pseudo-bootstrap support values that are above 60% from 100 replications, with an average branch support of 92.0%. The tree was midpoint-rooted (Farris, 1972).

Table 2. Biofilm associated genes in *S. aureus* MHH, MHB, MHF, MHC, and MHO isolates.

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
1.	*icaA*	Intercellular Adhesion Genes (*icaA, icaD, icaB*, and *icaC*). The *icaADBC* operon is a group of genes responsible for synthesizing polysaccharide intercellular adhesin (PIA) or polymeric N-acetyl-glucosamine (PNAG), which play a vital role in the creation of biofilms in staphylococci. The operon has four genes, namely *icaA, icaD, icaB*, and *icaC*, which collaborate to produce and transport PIA/PN (O'Gara, 2007; Cue *et al*., 2012; Kalantar-Neyestanaki *et al*., 2023).	4	1,239	MHB	MEQ7660391
			4	1,239	MHH	MEQ7733616
			2	1,239	MHO	MEQ7740879
			7	1,239	MHF	MEQ7620428
			3	1,239	MHC	MEQ7670133
2.	*icaD*		4	306	MHB	MEQ7660392
			4	306	MHH	MEQ7733615
			2	306	MHO	MEQ7740878
			7	306	MHF	MEQ7620429
			3	306	MHC	MEQ7670132
3.	*icaB*		4	873	MHB	MEQ7660393
			4	873	MHH	MEQ7733614
			2	873	MHO	MEQ7740877
			7	873	MHF	MEQ7620430
			3	873	MHC	MEQ7670131
4.	*icaC*		4	1,053	MHB	MEQ7660394
			4	1,053	MHH	MEQ7733613
			2	1,053	MHO	MEQ7740876
			7	1,053	MHF	MEQ7620431
			3	1,053	MHC	MEQ7670130
5.	*icaR*	The *icaR* gene. Situated upstream of the *icaADBC* operon, encodes a repressor essential for the formation of biofilms. This repressor works by binding to the up element of the *icaADBC* promoter region (Yu *et al.*, 2012).	4	561	MHB	MEQ7660390
			4	561	MHH	MEQ7733617
			2	561	MHO	MEQ7740880
			7	561	MHF	MEQ7620427
			3	561	MHC	MEQ7670134
6.	*tcaR*	Teicoplanin-Associated Locus *tcaR*. TcaR is a transcriptional regulator that inhibits the *icaADBC* operon expression which influences biofilm formation. When *tcaR* is deleted biofilm production increases demonstrating that it plays an important role in controlling biofilm processes (Jefferson *et al*., 2004; O'Gara, 2007).	7	456	MHB	MEQ7661055
			7	456	MHH	MEQ7734376
			7	456	MHO	MEQ7741731
			18	456	MHF	MEQ7621310
			9	456	MHC	MEQ7671452
7.	*atl*	Autolysin Mediated Adherence *atl*. Atl is an important autolysin that mediate bacterial attachment to host tissues by binding to fibronectin gelatin and heparin. Such adhesion is necessary for the early stages of biofilm development (Porayath *et al*., 2018).	1	3,771	MHB	MEQ7659205
			1	3,771	MHH	MEQ7732518
			1	3,762	MHO	MEQ7741040
			1	3,771	MHF	MEQ7619148
			4	3,771	MHC	MEQ7670482
8.	*sarA*	Staphylococcal accessory regulator-A s*arA*. SarA is a global transcriptional regulator that controls transcription of the *icaADBC* operon gene cluster as well as other genetic components involved in biofilm formation serving an essential role in initiating and sustaining biofilm communities (Cheung *et al*., 2008).	1	375	MHB	MEQ7659552
			5	375	MHH	MEQ7733896
			5	375	MHO	MEQ7741540
			1	375	MHF	MEQ7618803
			1	375	MHC	MEQ7669663

(Continued)

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
9.	*codY*	GTP-Sensing Pleiotropic Transcriptional Regulator *codY*. CodY modulate gene expression based on nutrient levels (Pohl *et al.*, 2009). When nutrients are abundant CodY represses the *icaADBC* operon impacting the creation of biofilms in response to environmental cues. This allows *S. aureus* to strategically determine biofilm formation relative to accessible resources (Wu *et al*., 2022).	6	774	MHB	MEQ7660845
			1	774	MHH	MEQ7732315
			1	774	MHO	MEQ7740519
			5	774	MHF	MEQ7620090
			2	774	MHC	MEQ7669980
10.	*lytR*	Response Regulator Transcription Factor *lytR*. LytR is part of the LytSR two-component system that controls cell wall metabolism and autolysis. It affects biofilm generation by controlling the expression of genes involved in cell lysis and death. Controlling these processes is critical for biofilm maturation (Sharma-Kuinkel *et al*., 2009).	3	741	MHB	MEQ7660126
			3	741	MHH	MEQ7733333
			2	741	MHO	MEQ7740643
			2	741	MHF	MEQ7619380
			5	741	MHC	MEQ7670623
11.	*lytS*		3	1755	MHB	MEQ7660127
			3	1755	MHH	MEQ7733332
			2	1755	MHO	MEQ7740644
			2	1755	MHF	MEQ7619381
			5	1755	MHC	MEQ7670622
12.	*lytM*	Glycine-Glycine Endopeptidase *lytM* : LytM is an autolysin that cleaves glycine-glycine link in the peptidoglycan layer. This process facilitates cell wall alteration and is important for the dispersion of biofilms and the release of extracellular DNA (eDNA). Extracellular DNA is a critical component of the biofilm matrix (Lehman *et al*., 2015).	3	951	MHB	MEQ7660111
			3	951	MHH	MEQ7733348
			2	951	MHO	MEQ7740627
			2	951	MHF	MEQ7619365
			5	951	MHC	MEQ7670638
13.	*lytN*	Peptidoglycan-links Domain-Containing Protein *lytN* : LytN binds to peptidoglycan and contributes to cell wall integrity and biofilm stability. LytN contributes to the adhesion of bacterial cells to surfaces and to one another hence promoting the formation of biofilms (Frankel *et al*., 2011; Cue *et al*., 2015).	6	1,119	MHB	MEQ7660853
			1	1,119	MHH	MEQ7732323
			1	1,119	MHO	MEQ7740527
			5	1,119	MHF	MEQ7620082
			2	1,119	MHC	MEQ7669988
14.	*fnBa*	Fibronectin-Binding Proteins *fnBa* and *fnBb* . Are proteins on the microbial surface that can interact with a range of relevant mammalian extracellular proteins. These proteins help the attachment of *S. aureus* to host tissues via binding to fibronectin. Such binding process is critical for the initial colonization and the production of biofilms (Arciola *et al.*, 2005; Gries *et al.*, 2020).	12	3,132	MHB	MEQ7661530
			4	3,048	MHH	MEQ7733788
			4	3,048	MHO	MEQ7741275
			13	3,132	MHF	MEQ7621116
			4	3,048	MHC	MEQ7670306
15.	*fnBb*		12	2,790	MHB	MEQ7661531
			4	2,874	MHH	MEQ7733789
			9	1,442	MHO	MEQ7621121
			13	2,790	MHF	MEQ7621117
			5	2,874	MHC	MEQ7670307

(Continued)

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
16.	*sasG*	G5 Domain-Containing Protein *sasG*. SasG are surface protein that enhances bacterial attachment to both host tissues and nonliving surfaces. This binding has a significant role in the bacterial accumulation phase of biofilm formation. The G5 domains within SasG bind to N-acetylglucosamine increasing its adhesive properties (Hasani *et al*., 2023).	12	1,353	MHB	MEQ7661535
			4	1,385	MHH	MEQ7733793
			4	1,812	MHO	MEQ7741271
			9	1,442	MHF	MEQ7621121
			3	1,385	MHC	MEQ7670311
17.	*sasC*	LPXTG Anchored Aggregation Protein *sasC* . SasC has a role in the process of cell aggregation and production of biofilms. The motif LPXTG serves as an anchor to attach SasC to the cell wall facilitating cell to cell interactions and enhancing the stability of biofilm (Schroeder *et al*., 2009).	11	6,561	MHB	MEQ7661419
			2	6,561	MHH	MEQ7733028
			24	6,552	MHO	MEQ7742695
			8	6,561	MHF	MEQ7620531
			11	6,561	MHC	MEQ7671605
18.	*cidA*	Holin Like Murein Hydrolase Modulator *cidA*. CidA controls the process of cell lysis and release of extracellular DNA (eDNA). Extracellular DNA is a critical component of the biofilm matrix (Grande *et al*., 2014).	12	396	MHB	MEQ7661489
			4	396	MHH	MEQ7733748
			4	396	MHO	MEQ7741315
			13	396	MHF	MEQ7621075
			3	396	MHC	MEQ7670266
19.	*sarX*	HTH-Type Transcriptional Regulator *sarX*. The SarX protein controls the activity of genes related to biofilm development. SarX protein is involved in the formation and stability of biofilms (Hao *et al*., 2021).	1	360	MHB	MEQ7659502
			5	360	MHH	MEQ7733946
			5	360	MHO	MEQ7741491
			1	360	MHF	MEQ7618853
			1	360	MHC	MEQ7669613
20.	*luxS*	S-Ribosylhomocysteine Lyase *luxS*: LuxS plays a role in quorum sensing, which is a process of cell-cell communication that controls the production of biofilms. LuxS influences the expression of biofilm-related genes in response to population density (Kuehl *et al*., 2009).	10	471	MHB	MEQ7661328
			9	471	MHH	MEQ7734608
			8	471	MHO	MEQ7741891
			11	471	MHF	MEQ7620908
			10	471	MHC	MEQ7671505
21.	*sspC*	Staphostatin B Protein *sspC*. The *sspC* gene produces a protease inhibitor that regulates the function of proteases involved in the process of breaking down biofilms. As a result, SspC controls proteolytic activity of biofilms proteins which helps to preserve biofilm structure. Inactivating SspC improves bacterial adhesion to solid surfaces and biofilm formation (Shaw *et al*., 2005).	1	330	MHB	MEQ7659212
			1	330	MHH	MEQ7732525
			3	330	MHO	MEQ7741033
			1	330	MHF	MEQ7619143
			4	330	MHC	MEQ7670475
22.	*clfA*	Microbial surface components that recognize adhesive matrix molecules are known as the MSCRAMMs family proteins. ClfA and ClfB are MSCRAMMs proteins that help bacteria bind to fibrinogen and other proteins in host. Therefore, they play a vital role in the process of biofilm formation during the attachment phase (O'Brien *et al*., 2002).	1	2,871	MHB	MEQ7659376
			10	2,859	MHH	MEQ7734657
			9	2,820	MHO	MEQ7741996
			1	2,913	MHF	MEQ7618979
			1	2,772	MHC	MEQ7669486
23.	*clfB*		4	2,808	MHB	MEQ7660355
			4	2,700	MHH	MEQ7733653
			4	2,730	MHO	MEQ7741411
			7	1,943	MHF	MEQ7620392
			3	2,742	MHC	MEQ7670169

(Continued)

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
24..	*sdrE*	Serine aspartate repeat family proteins family *sdrE*. SdrE is an adhesin that binds to host proteins. This binding facilitates bacterial adherence, aggregation, and biofilm formation (O'Brien *et al*., 2002).	1	3,507	MHB	MEQ7659607
			5	4,116	MHH	MEQ7733839
			5	3,471	MHO	MEQ7741593
			1	3,507	MHF	MEQ7618748
			1	3,501	MHC	MEQ7669726
25.	*agrA*	The *agrBDCA* operon comprises four open reading frames: *agrA*, *agrB*, *agrC*, and *agrD*. The Agr system is a quorum-sensing regulator that controls the virulence of *Staphylococcus aureus* by activating many virulence genes, including certain genes involved in biofilm formation, when the cell density reaches a certain level. AgrC is a sensor kinase that "senses" the autoinducing peptide (AIP). AgrA, a response regulator protein, and AgrB play a vital role in signal transduction, while AgrD serves as a peptide precursor (autoinducing peptide) (Grande *et al*., 2014; Tan *et al*., 2022; Aubourg *et al*., 2022).	8	717	MHB	MEQ7661082
			6	717	MHH	MEQ7734210
			17	717	MHO	MEQ7742486
			14	717	MHF	MEQ7621147
			17	717	MHC	MEQ7671971
26.	*agrB*		8	570	MHB	MEQ7661085
			6	564	MHH	MEQ7734207
			17	570	MHO	MEQ7742483
			14	570	MHF	MEQ7621150
			17	570	MHC	MEQ7661084
27.	*agrD*		8	141	MHB	MEQ7661084
			6	141	MHH	MEQ7734208
			17	141	MHO	MEQ7742484
			14	141	MHF	MEQ7621149
			17	141	MHC	MEQ7671969
28.	*agrC*		8	1293	MHB	MEQ7661083
			6	1293	MHH	MEQ7734209
			17	1293	MHO	MEQ7742485
			14	1293	MHF	MEQ7621148
			17	1293	MHC	MEQ7671970
29.	*sigB*	RNA Polymerase Sigma Factor *sigB*. SigB is a stress response regulator that directs the transcription of genes such as the ones associated with the development of biofilms and resistance to antibiotics. *S. aureus* utilizes this mechanism to effectively adapt to various environmental pressures, hence enhancing the stability of biofilms (Xie *et al*., 2020)	10	771	MHB	MEQ7661398
			9	771	MHH	MEQ7734538
			8	771	MHO	MEQ7741960
			11	771	MHF	MEQ7620978
			10	771	MHC	MEQ7671575
30.	*mgrA*	HTH-Type Transcriptional Regulator *mgrA*. MgrA controls the activation of genes responsible for the development of biofilms. It functions as a global regulator, impacting the total disease-causing capacity of *S. aureus* (Trotonda *et al*., 2008).	1	444	MHB	MEQ7659483
			5	444	MHH	MEQ7733966
			5	444	MHO	MEQ7741472
			1	444	MHF	MEQ7618872
			1	444	MHC	MEQ7669594
31.	*walK*	The WalKR (or YycGF). In *Staphylococcus aureus* WalKR is a two-component regulatory system. This system plays a critical role in cell wall metabolism, regulation of autolysis, biofilm, and virulence (Monk *et al*., 2019)	4	1827	MHB	MEQ7660453
			4	1827	MHH	MEQ7733553
			2	1827	MHO	MEQ7740821
			7	1827	MHF	MEQ7620490
			3	1827	MHC	MEQ7670070
32.	*walR*		4	702	MHB	MEQ7660452
			4	702	MHH	MEQ7733554
			2	702	MHO	MEQ7740822
			7	702	MHF	MEQ7620489
			3	702	MHC	MEQ7670071

(Continued)

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
33.	*GraS*	The *graS* gene (also referred to as *apsS*). Is part of the GraRS (or ApsRS) two-component regulatory system. This system is important in biofilm formation, antimicrobial peptide resistance, and global stress response (Prieto *et al*., 2020)	1	1041	MHB	MEQ7659510
			5	1041	MHH	MEQ7733938
			5	1041	MHO	MEQ7741498
			1	1041	MHF	MEQ7618845
			1	1041	MHC	MEQ7669621
34.	*graR*		1	675	MHB	MEQ7659511
			5	675	MHH	MEQ7733937
			5	675	MHO	MEQ7741499
			1	675	MHF	MEQ7618844
			1	675	MHC	MEQ7669622
35.	*nsaS*	The NsaRS two-component regulatory system. Is involved in sensing and responding to cell envelope stresses. This system also plays a role in biofilm formation and antibiotic resistance (Kolar *et al*., 2011)	4	888	MHB	MEQ7660348
			4	888	MHH	MEQ7733660
			4	888	MHO	MEQ7741404
			12	888	MHF	MEQ7621050
			3	888	MHC	MEQ7670176
36.	*nsaR*		1	666	MHB	MEQ7660349
			4	666	MHH	MEQ7733659
			4	666	MHO	MEQ7741405
			12	666	MHF	MEQ7621051
			3	666	MHC	MEQ7670175
37.	*phoR*	The PhoR-PhoP two-component regulatory system. Is involved in regulating the response to environmental phosphate levels and plays a significant role in biofilm formation and virulence.	7	636	MHB	MEQ7661018
			12	636	MHH	MEQ7734745
			7	636	MHO	MEQ7741766
			4	636	MHF	MEQ7619858
			20	636	MHC	MEQ7672073
38.	*phoP*	PhoPR loss impairs a number of cellular activities that are crucial to the outcome of infection, such as biofilm formation, quorum sensing, capsule manufacturing, and response to environmental stresses like acidic or alkaline pH (Vuppada *et al*., 2018)	2	705	MHB	MEQ7659919
			2	705	MHH	MEQ7732960
			6	705	MHO	MEQ7741672
			3	705	MHF	MEQ7619798
			6	705	MHC	MEQ7671067
39.	*srrA*	The SrrAB two-component regulatory system. plays a crucial role in controlling the production of biofilms and the level of pathogenicity. This system facilitates the bacteria's ability to adjust to different environmental pressures, including hypoxia (insufficient oxygen levels), oxidative stress, and nitrosative stress (Mashruwala *et al*., 2017a; Tiwari *et al*., 2020)	2	726	MHB	MEQ7659726
			2	726	MHH	MEQ7732766
			1	726	MHO	MEQ7740289
			3	726	MHF	MEQ7619605
			6	726	MHC	MEQ7670871
40.	*srrB*		2	1752	MHB	MEQ7659725
			2	1752	MHH	MEQ7732765
			1	1752	MHO	MEQ7740290
			3	1752	MHF	MEQ7619604
			6	1752	MHC	MEQ7670870

(Continued)

	Gene ID	Function	Contig	length (bp)	Strain	Protein accession no.
41.	*saeR*	The *saeRS*: SaeRS is a two-component system, which controls the activation of the formation of biofilm by regulating the activity of genes involved in the metabolism of the cell wall and the process of autolysis (Mashruwala *et al*., 2017b)	1	687	MHB	MEQ7659463
			5	687	MHH	MEQ7733986
			5	687	MHO	MEQ7741452
			1	687	MHF	MEQ7618892
			1	687	MHC	MEQ7669574
			1	1056	MHB	MEQ7659464
42.	*saeS*		5	1056	MHH	MEQ7733985
			5	1056	MHO	MEQ7741453
			1	1056	MHF	MEQ7618891
			1	1056	MHC	MEQ7669575
43.	*arlR*	The *arlRS*. ArlRS two-component system controls the activity of virulence genes, including those responsible for biofilm formation. It regulates the activity of genes involved in cell wall metabolism and autolysis, which affects the formation of biofilms (Fournier *et al*., 2001). Studies have demonstrated that mutations in the ArlRS system can modify the arrangement of biofilms and increase resistance to antimicrobial drugs (Walker *et al*., 2013).	20	660	MHB	MEQ7661775
			2	660	MHH	MEQ7732692
			1	660	MHO	MEQ7740360
			22	660	MHF	MEQ7621404
			3	660	MHC	MEQ7672052
44.	*arlS*		20	1356	MHB	MEQ7661774
			2	1356	MHH	MEQ7732691
			1	1356	MHO	MEQ7740361
			22	1356	MHF	MEQ7621403
			3	1356	MHC	MEQ7672053

as the reference genome in the RASTtk analysis), the genomes of the animal isolates featured regions of clustered genes that had no matches or had minimal similarities. Figure 3 illustrates these regions, marked by black, green, orange, and blue arrows. These gene clusters may suggest the occurrence of horizontal gene transfer or gene deletion events. The MHB strain, in contrast to the other four analyzed isolates, exhibits a region (about 5727 base pairs-data not shown) with less similarity to the other isolates. The RASTtk server identifies this region as a cluster of proteins related to phages, with a similarity of 98–100% (Fig. 3, black arrow). The orange arrow indicates a region of putative proteins linked to *S. aureus* pathogenicity islands (SaPIs). SaPIs are small genetic elements that play a crucial role in the pathogenesis and virulence of the bacteria. They carry genes for superantigens, toxins, and other virulence factors, including the gene for toxic shock syndrome toxin. SaPIs are mobilized by specific bacteriophages (phages) through a process called lateral transduction, which allows them to transfer not only their own genes but also additional virulence genes from the host chromosome (Tormo *et al*., 2008; Novick and Ram, 2017). Except for the MHO strain, there were no matches or significant similarity found for the SaPI-related genes in the MHF, MHC, and MHB isolates (data not shown). SaPI-related genes are critical for *S. aureus* pathogenesis and pathogenicity. These genes encode superantigens, toxins, and other features that contribute to *S. aureus* ability to cause disease, including the gene responsible for producing toxic shock syndrome toxin (Tormo *et al*., 2008; Novick and Ram, 2017). The green arrow refers to a region with hypothetical proteins that have low identity to the MHH strain; however, the MHF and MHB strains exhibited genes that are identical to the *mecA* gene cassette (data not shown). The blue arrow denotes a section of hypothetical proteins with unknown roles in the MHH human strain that are not present in animal isolates. We are currently conducting an additional study to investigate the antimicrobial characteristics and the mobile genetic components associated with these regions.

The process of biofilm formation in *S. aureus* is sophisticated and requires the coordinated expression of several genes. The ability to form biofilms differs among the various genetic lineages of *S. aureus* (Tasse *et al*., 2018). This is precisely what we noted, as illustrated in Figure 5. Our local isolates from diverse host sources displayed different levels of biofilm production, even though they shared the same biofilm-associated genes and regulators. The study by Croes *et al*., (2009) points out that some lineages, especially those assigned to MLST clonal complexes (like CC8),

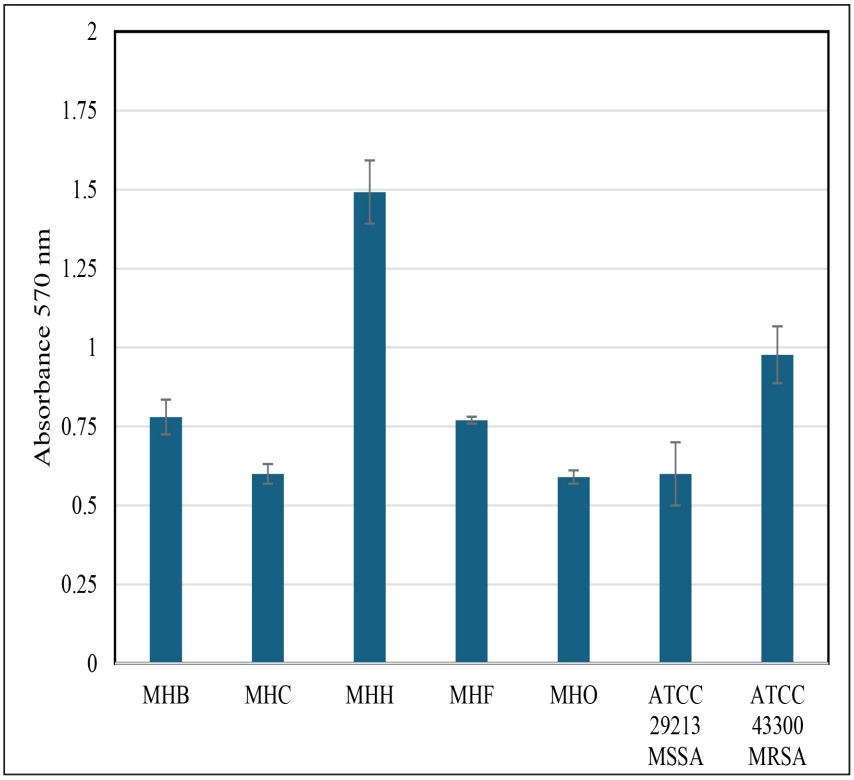


Fig. 5. Crystal violet binding assay showing biofilm formation ability of different *S. aureus* local isolates (MHB, MHC, MHH, MHF, and MHO) to the 96-well polystyrene plate surface in brain heart broth. Values represent the mean of two independent experiments; error bars indicate the standard deviation. *S. aureus* ATCC 29213, ATCC 43300 were used as a control.

are more likely to produce more persistent biofilms than others. The reasons for variability among different *S. aureus* lineages are not fully understood but could be related to differences in gene expression, the presence of virulence factors, or environmental conditions(Tasse *et al*., 2018). Whatever the cause, these findings signify the capacity of certain lineages of *S. aureus* to cause disease (Croes *et al*., 2009; Idrees *et al*., 2021).

The main limitation of this study is the number of WGS samples included from each targeted host and the difficulty in proving the impact of genetic drift, particularly in biofilm-related genes, on the ability of bacteria to adapt to new animal host species through *in vivo* experiments. Previous investigations into a few *in vivo* models, including human-rabbit, human-ovine, and human-murine models, yielded varying conclusions, indicating the occurrence of multiple adaptation pathways in these models. The understanding of adaptive mechanisms that underlie species-specific pathogenesis could be improved further through the exploitation of host-switching models of pathogenesis (Viana *et al*., 2015; Bacigalupe *et al*., 2019; Mrochen *et al*., 2020). Consequently, the small number of samples from five distinct hosts in our analysis made it challenging to identify specific host adaptation genes across our targeted genomes. Consequently, we utilized the phylogenetic relationships and the overall genome identity to demonstrate the zoonotic nature of the isolates under investigation, as has been done in other studies (Abouelkhair and Kania, 2024; Jablonska *et al*., 2024; Wokorach *et al*., 2024). Due to the fact that biofilm formation in *S. aureus* is a crucial virulence trait that helps in colonization and adaptation inside the host during infection, we believe that tracing these genes in future zoonotic and host tropism studies is a valued study proposal that can be performed on local genomes. Unfortunately, there are no *S. aureus* whole genomic investigations in Iraq that we can compare or contrast in order to confirm this hypothesis. The magnitude and molecular basis of host-switching events are still inadequately understood, and a large-scale, genome-based analysis of the evolutionary history of *S. aureus* in the context of its host ecology is lacking.

Our study is the first to include complete genome sequencing of *S. aureus* in Iraq, allowing us to annotate the biofilm-associated genes present in our local isolates. We conducted a thorough analysis of genes and regulators associated with biofilms in *S. aureus*,

as described in the literature. In conclusion, this study mapped the genomes of local isolates for the first time, underscored the importance of periodic WGS for local isolates, identified genomic similarities among *S. aureus* local isolates indicating potential zoonotic relationships, and presented a list of biofilm-related genes for further exploration as critical virulence factors and potential determinants of host tropism.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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Authors' contributions

Mohammed A. Al-Bukhalifa : Data curation; Formal analysis; Investigation, Methodology; review & editing. Hassan M. Al-Tameemi : Writing - original draft

Data availability

The genome sequence of *Staphylococcus aureus* (MHB, MHH, MHO, MHF, MHC) has been deposited at GenBank under the accession numbers listed below.

Bio Sample NO.	Accession NO.	Assembly Accession NO.	Isolates
SAMN41769230	JBEGCN000000000	GCA_040196135.1	MHB
SAMN41769231	JBEGCO000000000	GCA_040196155.1	MHH
SAMN41769234	JBEGCR000000000	GCA_040195495.1	MHO
SAMN41769233	JBEGCQ000000000	GCA_040195555.1	MHF
SAMN41769232	JBEGCP000000000	GCA_040195445.1	MHC

The annotation was added by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP-*https://www.ncbi.nlm.nih.gov/genome/annotation_prok/*).

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