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RESEARCH ARTICLE

Complete Genome of Multi-Drug Resistant *Staphylococcus Aureus* in Bovine Mastitic Milk in Anhui, China

Junjun Liu[1], Xinglin Zhang[2], Jianrui Niu[2], Zhaoqing Han[2], Chongliang Bi[2], Khalid Mehmood[3], Dunia A Al Farraj[4], Inshad Alzaidi[4], Rashid Iqbal[5] and Jianhua Qin[1]*

[1]College of Veterinary Medicine/Traditional Chinese Veterinary Medicine, Hebei Agriculture University, Baoding 071001, People's Republic of China; [2]College of Agriculture and Forestry, Linyi University, Shuangling Road, Linyi City, Shandong, 276005, PR China; [3]Department of Clinical Medicine and Surgery, Faculty of Veterinary & Animal Sciences, The Islamia University of Bahawalpur 63100, Bahawalpur, Pakistan; [4]Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia; [5]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur 63100, Bahawalpur, Pakistan.
*Corresponding author: qjhqqq@126.com

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A B S T R A C T

Staphylococcus aureus is a critical pathogen causing serious mastitis with huge economic losses in bovines. To reveal drug-resistant genes, a complete genome sequence of a multi-drug-resistant bacterium *S. aureu*s derived from bovine mastitic milk was performed via High-throughput sequencing. Results revealed that the genome length of current *S. aureu*s was 2.85 Mbp consisting of 2656 coding sequence genes, 60 tRNA, 19 rRNA, and 5 genomic islands. Functional annotation of *S. aureu*s encoded proteins showed that 2090 (COG), 1586 (KEGG), 1600 (GO), 2648 (Refseq), 2300 (Pfam), 964 (SwissProt), and 1826 (TIGRFAMs) proteins were annotated. Antibiotic-resistant genes of tet, mepR, mepA, mgrA, norA, *blaZ*, arlS, arlR and LmrS in *S. aureu*s genome were found via CARD annotation. More than 60 bacterial virulence factors including commonly known clfA, hla, hlb, fnbB, fnbA in *S. aureu*s genome were uncovered through VFDB annotation. The current study may contribute towards effective treatment and developing new antibiotics against mastitis caused by *S. aureu*s.

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INTRODUCTION

Antibiotic-resistant bacteria are gaining attention worldwide and are regarded as one of the biggest threats to human health (Chen *et al*., 2021; Mohamed *et al*., 2022). The transmission of antibiotic-resistant genes is typically observed in humans, animals and community environments (Thapa *et al*., 2020), and unified methods based on one health principles are compulsory to inhibit the increasing emerging of resistant bacteria (Aslam *et al*., 2021; Swar and Shnawa, 2021). Among them, *Staphylococcus aureus* is a typical antimicrobial resistant bacterium causing globe public and animal health issues (Gelbíčová *et al*., 2022), and been found in humans, livestock and even in sell-through meat (Verkade and Kluytmans, 2014; Li *et al*., 2019; Gelbíčová *et al*., 2022). *S. aureus* is responsible for causing various infections including skin disease, bacteremia, pneumonia, myelitis, meningitis, bacterial endocarditis, toxic shock syndrome (Hurni *et al*., 2022), and food poisoning due to contamination (Tong *et al*., 2015). *S. aureus* usually stands out as a serious pathogen in dairy cows causing bovine mastitis (Gonçalves *et al*., 2023). Additionally, *S. aureus* infections are expensive for cattle owners and substantially lower their revenue due to reductions in milk production of about 2.3 kg per day, expensive diagnosis and treatment of infected cows, and the need for early culling or replacement of animals (Heikkilä *et al*., 2018).

The development of next-generation sequence platforms has made it possible to analyze variations in drug resistance-related gene sequences using high-throughput sequencing techniques (Li *et al*., 2013). Previously, the complete genome characteristics of *S. aureus* strains were revealed from the skin infection of a person (Chua *et al*., 2010), pneumonia patient (Li *et al*., 2013), raw chicken, turkey, and pork products from Denmark (Li *et al*., 2019), and milk samples from Belgian and Norwegian dairy farms (Fergestad *et al*., 2021). In our previous studies, the high prevalence of *S. aureus* infection was found in bovines and

multi-drug resistant bacteria were isolated from their milk samples (Liu *et al*., 2022).

As a popular consumer goods, millions of people enjoy milk and milk products daily (Khalaf *et al*., 2021). Therefore, monitoring *S. aureus* in milk to uncover the pathogen's genetic characteristics is important and meaningful to the industry. Though this bacterium poses resistance to eleven antibiotics, only four resistant genes were successfully amplified. To further explore the existence of resistant genes and the genome characteristics of pathogenic *S. aureus* derived from fresh milk in Anhui, China, this research was carried out to perform a complete genome sequencing of a resistant bacterium of *S. aureus* isolated from cattle's mastitic milk in Central China.

MATERIALS AND METHODS

Bacteria: Previously we isolated and identified a MDR bacterium strain of *S. aureus* (ON138914) from cow mastitic milk (Liu *et al*., 2022). A total of 31 (24.8%) bacteria were identified from cattle with clinical mastitis on a dairy farm in Anhui, China, of which one isolate with the strongest antibiotic resistance was selected and contained at -80 ^{o}C for further processing and study.

DNA extraction, library construction and quality inspection: Before DNA extraction, the stored *S. aureus* was recovered with 30 mL fresh Luria-Bertani medium (Haibo Biological, China) via incubation at 37℃ in a constant temperature shaker (160 rpm/min) for 10 hours. Then bacteria were harvested through centrifugation at 4000 g for 16 min, and washed by sterile phosphate buffer saline twice. The total DNA was isolated using a commercial QIAamp DNA Microbiome Kit (Qiagen, German). The DNA products quality and purity of *S. aureus* were examined and measured by 0.75% agarose electrophoresis, Nanodrop 2000 (Invitrogen, USA) with 1.8<OD260/280<2.0 and 2.0<OD 260/230<2.2, and Qubit 4.0 (Invitrogen, USA). Finally, the products were purified utilizing BluePippin automatic nucleic acid glue cutter (Sage Science, USA) and library was constructed via SMARTer™ PCR cDNA Synthesis Kit (Takara Bio Inc, Japan). The library products were quantified through Qubit 4.0 (Invitrogen, USA).

Sequencing and genome assembly analysis: A single-molecule real-time sequencing was performed via PromethION sequencer (Oxford Nanopore Technologies, Oxford, UK) in Baiyi Huineng Biotechnology Co., LTD (Wuhan, China). Clean data was generated by filtering the received PromethION sequencing raw readings with an average quality score of ≤ 7.0. Then the genome sequences were assembled via unicycler (v0.4.8) (https://github.com/rrwick/Unicycler), and examined via pilon (v1.24) (https://github.com/broadinstitute/pilon) and circulator (v1.5.5). After assembly, minimap2 (v2.18-R1015) and stools (v1.12) were used to count the sequencing depth of each site, and the average sequencing depth was calculated and plotted. Genome sequence blast was piloted to compare *S. aureus* genome sequence to the plasmid database and calculate the length of the comparison to the database to perform plasmid analysis. When a comparison length accounted for more than 20% of the total *S. aureus* sequence length and the comparison length was less than 1 Mb, that sequence was considered as a plasmid.

We employed prodial (v2.6.3) for forecast coding genes, tRNAscan-SE (v2.0) for tRNA prediction, infernal (v1.1.4) (http://eddylab.org/infernal/) for ncRNA prediction, minced (v0.4.2) for CRISPR prediction, Island Path-DIMOB (v1.0.6) (https://github.com/brinkmanlab/islandpath) for genomic island prediction, PhiSpy (v4.2.19) (https://github.com/linsalrob/PhiSpy) for prophage region prediction, Tandem Repeats Finder and RepeatMasker (http://www.repeatmasker.org/RepeatMasker/) for prediction the repeated sequences in *S. aureus* genome, respectively. Circos (v0.69.8) (http://circos.ca/) was used to plot the nuclear genome circle map of present *S. aureus.*

Phylogenetic analysis: Genome evolutionary study of current *S. aureus* was implemented based on available reference genomes, including *S. aureus* (CP011526.1), *S. aureus* (CP035101.1), *S. aureus* (CP040999.1), *S. aureus* (CP064365.1), *S. aureus* (CP018629.1), *S. epidermidis* (CP035288.1), *S. lugdunensis* (CP014022.1), *S. capitis* (CP092857.1) and *Streptococcus* (GCA_016712795.1).

Functional annotation analysis: Exploring of *S. aureus* encoded proteins were carried out by employing Interproscan (v5.30-69.0), Clusters of Orthologous Groups of proteins, KEGG (https://www.kegg.jp/kegg/), Gene Ontology (http://geneontology.org/), Refseq (https://www.ncbi.nlm.nih.gov/refseq/), Pfam (https://pfam.xfam.org/), SwissProt (http://ftp.ebi.ac.uk/pub/databases/swissprot/release/) (v20211117) and TIGRFAMs databases, respectively. Other annotation including Carbohydrate-Active Enzymes Database (CAZy) and Pathogen Host Interactions (phi) were also performed through blast with Carbohydrate-Active enzymes Database PHI-base and (http://www.phi-base.org/index.jsp), respectively. Secretory protein prediction was performed by piloting SignalP (v4.1) (https://services.healthtech.dtu.dk/service. php? SignalP-4.1) to predict signal peptide (Nielsen, 2017) and TMHMM (v2.0) to predict trans-membrane domain (Krogh *et al*., 2001), respectively. Abricate (v1.0.1) was utilized to perform the comprehensive antibiotic resistance database, and the virulence factor database (http://www.mgc.ac.cn/ VFs/main.htm) annotation. The codons usage of *S. aureus* was calculated as: codon numbers / total numbers x 100%.

RESULTS

Raw data deposit: The sequenced *S. aureu*s genome and plasmid were preserved in the database assigned with NCBI accession numbers: chromosome (PRJNA855545) and plasmid (OP004824).

Sequencing results and Quality control analysis: In the current study, 1486592870 bp raw reads with mean length 4876.28 bp were obtained, while 1391118660 bp filtered reads with average length of 4,904.25 bp were generated by quality control (Fig. 1 a & b). Data contamination assessment of *S. aureu*s sequences was performed by randomly blasting 5,000 filtered reads with Nucleotide Sequence Database by employing blastn. Blasting results found that 95.83% sequences were identified belonging to *S. aureu*s while there may have been some other bacteria and viruses' contamination (Fig. 1c).

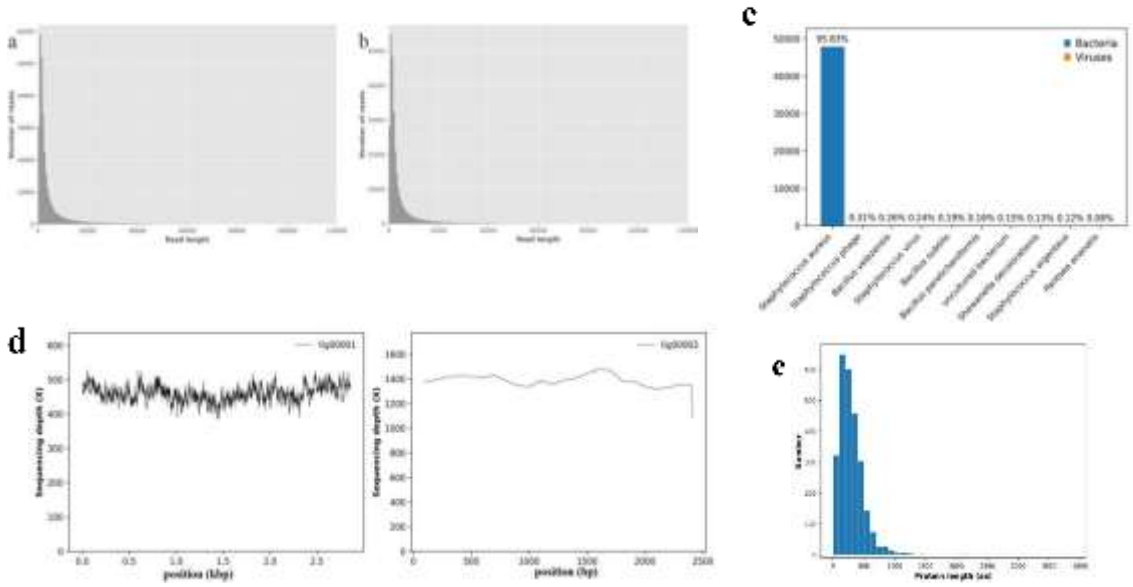

Fig. 1: Genome sequencing information of *S. aureus* sequencing data. a: Original sequencing data, b: Filtered sequencing data, c: distribution map, d: sequencing depth, e: Protein length distribution.

Genome assembly: The current assembled genome of *S. aureus* had 2856975 bp with GC content of 32.91%. The A, T, C, G content was 953480, 963185, 470107 and 470203 bp, respectively. There were two contigs in the genome of *S. aureus* named tig00001 (genome) and tig00002 (plasmid). To verify the integrity of the assembly results and the uniformity of sequencing, the filtered data was compared to the genome to generate a sequencing depth distribution map, results showed that both genome (> 400 X) and plasmid (> 100 X) of the present *S. aureus* depicted high sequencing depth (Fig. 1d). The coding sequences of current *S. aureus* had 2656 CDS region encoded 2735 proteins with most protein length having less than 500 amino acids (Fig. 1e). The codons usage was shown in Table 1, Glu (GAA, 54.44‰), Leu (TAA, 53.97‰) and Ile (ATT, 52.02‰) were the three highest frequency usage codons, while Arg (CGG, 0.44‰) and stop codon (TAG, 0.54‰; TGA, 0.37‰) were the least usage codons. The present *S. aureus* genome contained 60 tRNA, 19 rRNA, 25 regulatory and 83 other RNAs. In the tig00001 sequence 5 genomic islands were predicted. The genome structure containing CDS, rRNA, tRNA, CRISPR, gene island and phage was predicted (Table 2). TRF and RepeatMasker were used to predict the repetitive sequences on the genome. The results showed that there were 393 repeat sequences with average length of 101.94 bp in tig00001, while there were only 2 repeat sequences in tig00002. The nuclear genome circle map of present *S. aureus* was generated via Circos (v0.69.8) and showed in Fig. 2a. The plasmid circle map was drawn through SnapGene Viewer (Fig. 2b). Genome phylogenetic analysis found that the current *S. aureus* was highly similar to *S. aureus* (CP018629.1) (Fig. 3).

Genome annotation: Functional annotation of *S. aureus* encoded proteins showed that 2090 (COG), 1586 (KEGG), 1600 (GO), 2648 (Refseq), 2300 (Pfam), 964 (SwissProt) and 1826 (TIGRFAMs) proteins were annotated by different databases, respectively (Table 3). COG annotation found that the current S. *aureus* had encoded genes mainly related to amino acid transport (9.61%), translation, ribosomal structure and biogenesis (9.18%), carbohydrate transport and metabolism (7.19%), inorganic

Table 1: Statistics of the codon's usage of *S. aureus*.

Amino acid	Codon	Number	‰	Amino acid	Codon	Number	‰
Gly	GGG	3388	4.25	Thr	ACG	7500	9.41
Gly	GGA	11246	14.12	Thr	ACA	23016	28.89
Gly	GGT	25887	32.49	Thr	ACT	13412	16.83
Gly	GGC	7524	9.44	Thr	ACC	2112	2.65
Glu	GAG	8511	10.68	Trp	TGG	6034	7.57
Glu	GAA	43372	54.44	*	TGA	297	0.37
Asp	GAT	36557	45.89	Cys	TGT	3958	4.97
Asp	GAC	10076	12.65	Cys	TGC	968	1.22
Val	GTG	7516	9.43	*	TAG	427	0.54
Val	GTA	18112	22.73	*	TAA	1932	2.43
Val	GTT	21700	27.24	Tyr	TAT	24395	30.62
Val	GTC	5794	7.27	Tyr	TAC	6729	8.45
Ala	GCG	7503	9.42	Leu	TTG	10648	13.37
Ala	GCA	23970	30.09	Leu	TTA	42999	53.97
Ala	GCT	16183	20.31	Phe	TTT	25864	32.46
Ala	GCC	3606	4.53	Phe	TTC	9494	11.92
Arg	AGG	1130	1.42	Ser	TCG	3135	3.94
Arg	AGA	9512	11.94	Ser	TCA	16161	20.29
Ser	AGT	13337	16.74	Ser	TCT	10301	12.93
Ser	AGC	4108	5.16	Ser	TCC	1320	1.66
Lys	AAG	11500	14.43	Arg	CGG	351	0.44
Lys	AAA	48658	61.08	Arg	CGA	3872	4.86
Asn	AAT	34258	43.00	Arg	CGT	10568	13.27
Asn	AAC	10937	13.73	Arg	CGC	2515	3.16
Met	ATG	20724	26.01	Gln	CAG	3988	5.01
Ile	ATA	14862	18.66	Gln	CAA	29135	36.57
Ile	ATT	41445	52.02	His	CAT	14731	18.49
Ile	ATC	11723	14.71	His	CAC	3552	4.46
Leu	CTG	1825	2.29	Pro	CCG	3177	3.99
Leu	CTA	6789	8.52	Pro	CCA	12931	16.23
Leu	CTT	8484	10.65	Pro	CCT	8609	10.81
Leu	CTC	1552	1.95	Pro	CCC	756	0.95

Table 2: Statistics of genomic structure prediction results of *S. aureus*.

Type		Number	Length (bp)	% genome
tRNA		60	4,630	0.16
rRNA	16S	6	9,312	0.33
rRNA	23S	6	17,538	0.61
rRNA	5S	7	805	0.03
CDS		2,656	2,390,028	83.66
CRISPR		0	0	0.00
genomic island		5	114,471	4.01
prophage region		0	0	0.00

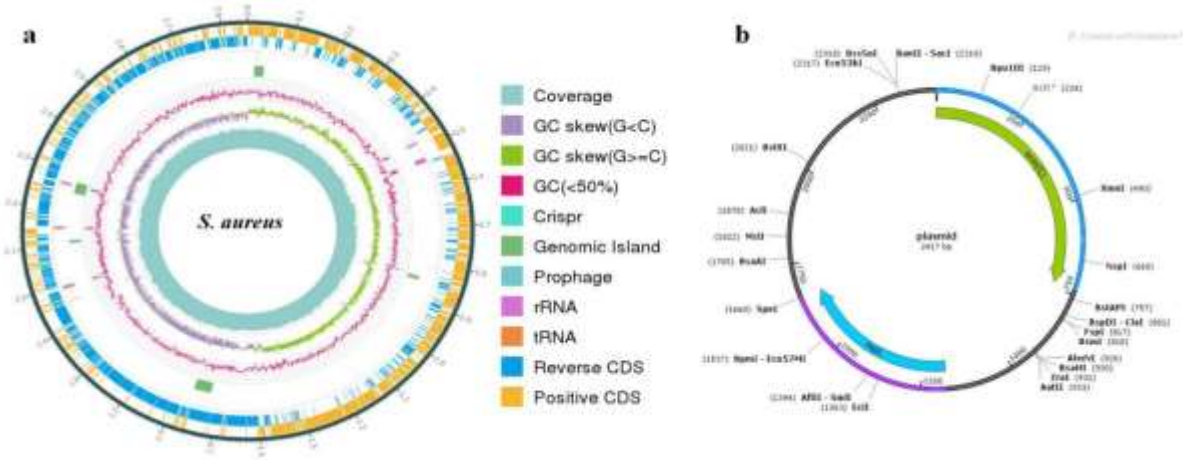

Fig. 2: The nuclear genome (a) and plasmid (b) circle map of *S. aureus*.

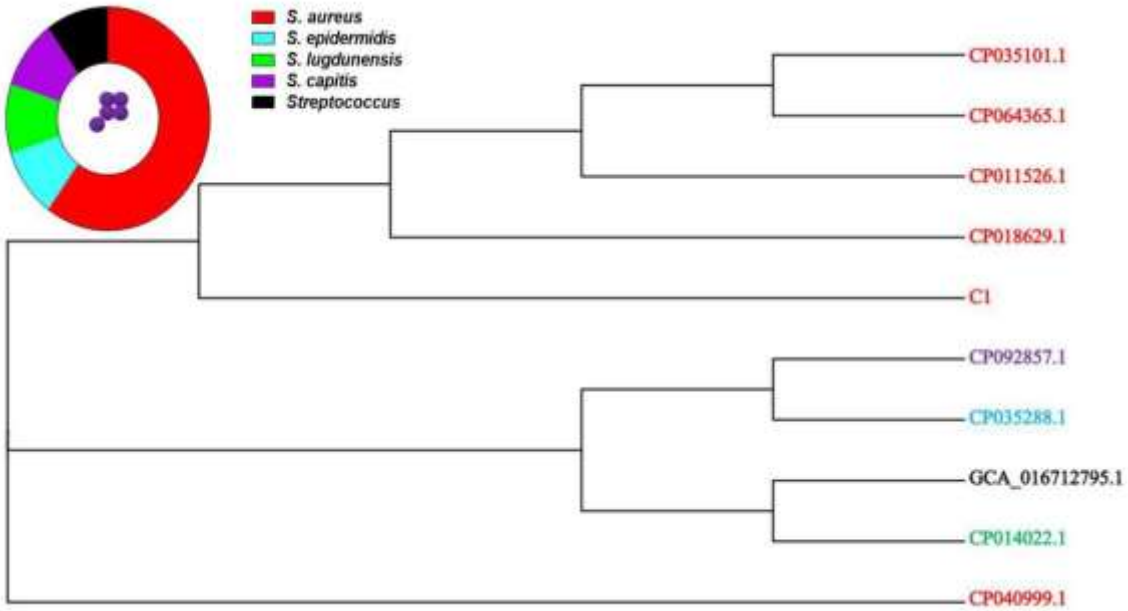

Fig. 3: Genome phylogenetic analysis of current *S. aureus* with available reference genomes via ggtree.

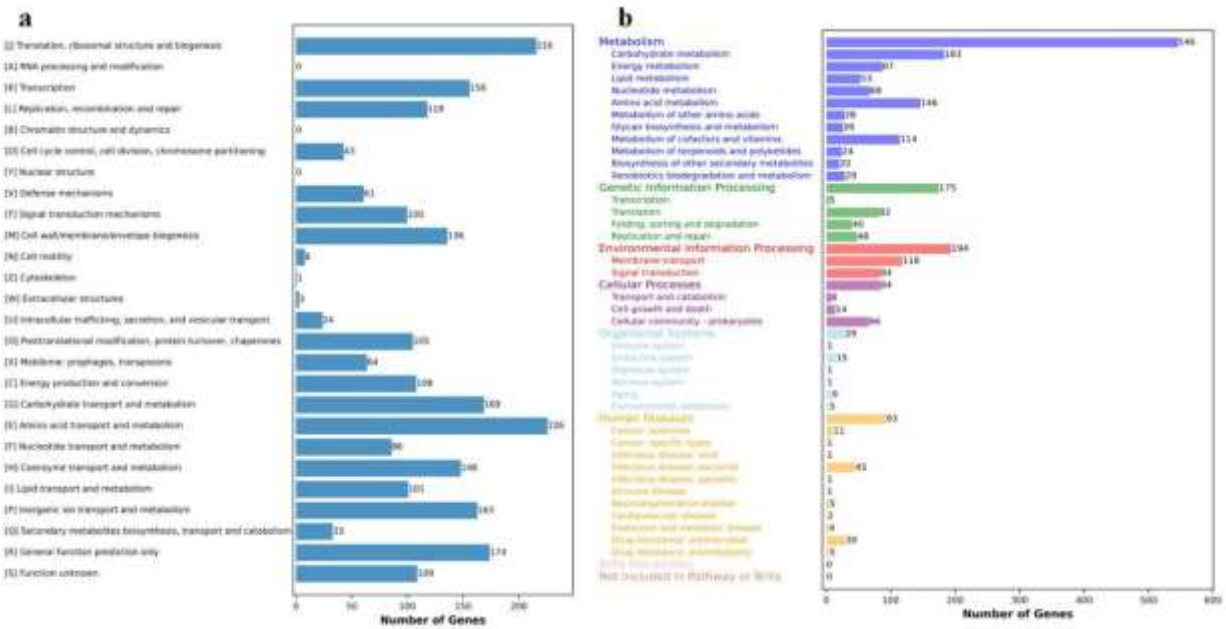

Fig. 4: Statistics of COG (a) and KEGG (b) function classification of genome coding protein of *S. aureus*.

Table 3: Functional annotation of *S. aureus* encoded proteins by different databases.

Database	Annotated number	% all
COG	2090	78.69
KEGG	1586	59.71
GO	1600	60.24
Refseq	2648	99.70
Pfam	2300	86.60
TIGRFAMs	964	36.30
SwissProt	1826	68.75
all databases	756	28.46
at least one database	2648	99.70
Overall	2656	100

transport and metabolism (6.93%) (Fig. 4a). KEGG annotation uncovered that the present *S. aureus* had encode genes mainly contributed to metabolism, carbohydrate and amino acid metabolism, genetic information for processing, environmental information processing, membrane transport, cellular processes, cellular community, organismal systems, human diseases, infectious disease, and drug resistance (Fig. 4b). GO annotation discovered that *S. aureus* had encode genes regarding to molecular function (catalytic activity, binding, transporter activity), biological process (metabolic process, cellular process, single-organism process, localization and biological regulation), and cellular component (cell, cell part, macromolecular complex and membrane) (Fig. 5). CAZy encoding proteins annotation found that *S. aureus* genome had genes mainly related to glycoside hydrolases (GH, 43.71), glycosyl transferases (GT, 36.38), carbohydrate-binding modules (11.44%) (Fig. 6a), while phi annotation reported increased virulence genes (hypervirulence, 10.57%), lethal genes (1.82%) and resistance chemistry target genes (0.24%) (Fig. 6b). There were 174 proteins with signal peptide, 2000 proteins without transmembrane domain and 122 secreted proteins in *S. aureus* genome. CARD annotation revealed that antibiotic resistant genes of tet, mepR, mepA, mgrA, norA, blaZ, arlS, arlR and LmrS in *S. aureus* genome (Table 4). VFDB annotation showed that 66 bacterial virulence factors including commonly known clfA, hla, hlb, fnbB, fnbA in *S. aureus* genome (Table 4).

DISCUSSION

Due to *S. aureus*'s growing antibiotic resistance, there are fewer therapeutic choices available. The complex mechanisms behind this germ's pathogenicity and drug resistance can be studied using genome sequencing (Li *et al*., 2013). The genome length of current *S. aureus* was 2.85 Mbp, which is longer than *S. aureus* S94 (2.69 Mbp) isolated from bloodstream infected patient in France (Aziz *et al*., 2008), *S. aureus* MSSA463 (2.77 Mbp) isolated from a pneumonia patient in China (Li *et al*., 2013) and *S. aureus* LCT-SA112 (2.79 Mbp) isolated in China (Wang *et al*., 2012). There were 2656 coding sequence genes, 60 tRNA, 19 rRNA and 5 genomic islands in the present *S. aureus*

Table 4: Statistics of CARD and VFDB annotation results of *S. aureus* genome.

CARD Name	Annotated gene	Annotated number	CARD name	Annotated gene	Annotated number
S. aureus norA	CI 00681	1	tet(38)	CI 00085	1
PC1 beta-lactamase (blaZ)	CI 00786	1	mepR	CI 00342	1
arlS	CI 01325	1	mepA	CI 00343	1
arlR	CI 01326	1	mgrA	CI 00672	1
S. aureus LmrS	CI 02185	1			
VFDB Name	Annotated gene	Annotated number	VFDB name	Annotated gene	Annotated number
adsA	CI 00024	1	cap8H	CI 00105	1
Spa	CI 00059	1	cap8I	CI 00106	1
cap8A	CI 00097	1	cap8J	CI 00107	1
cap8B	CI 00098	1	cap8K	CI 00108	1
cap8C	CI 00099	1	cap8L	CI 00109	1
cap8D	CI 00100	1	cap8M	CI 00110	1
cap8E	CI 00102	1	cap8N	CI 00111	1
cap8F	CI 00103	1	cap8O	CI 00112	1
cap8G	CI 00104	1	cap8P	CI 00113	1
Coa	CI 00170	1	essA	CI 00225	1
esaB	CI 00226	1	esaC	CI 00229	1
essB	CI 00227	1	esxB	CI 00230	1
essC	CI 00228	1	geh	CI 00261	1
sdrC	CI 00546	1	sdrD	CI 00547	1
sdrE	CI 00548	1	vWbp	CI 00778	1
clpP	CI 00757	1	sspC	CI 00952	1
clfA	CI 00777	1	sspB	CI 00953	1
sspA	CI 00954	1	isdC	CI 01035	1
isdB	CI 01033	1	isdD	CI 01036	1
isdA	CI 01034	1	isdE	CI 01037	1
isdF	CI 01038	1	ebp	CI 01392	1
srtB	CI 01039	1	hysA	CI 01767, CI 02222	2
isdG	CI 01040	1	lukF-PV	CI 01781	1
hly/hla	CI 01064	1	map	CI 02001	1
Hlb	CI 02002	1	hlgC	CI 02432	1
Hld	CI 02039	1	hlgB	CI 02433	1
Sbi	CI 02430	1	fnbB	CI 02509	1
hlgA	CI 02431	1	fnbA	CI 02510	1
clfB	CI 02644	1	icaA	CI 02684	1
Aur	CI 02652	1	icaD	CI 02685	1
icaR	CI 02683	1	icaC	CI 02687	1
icaB	CI 02686	1	lip	CI 02688	1
cna	CI 02712	1			

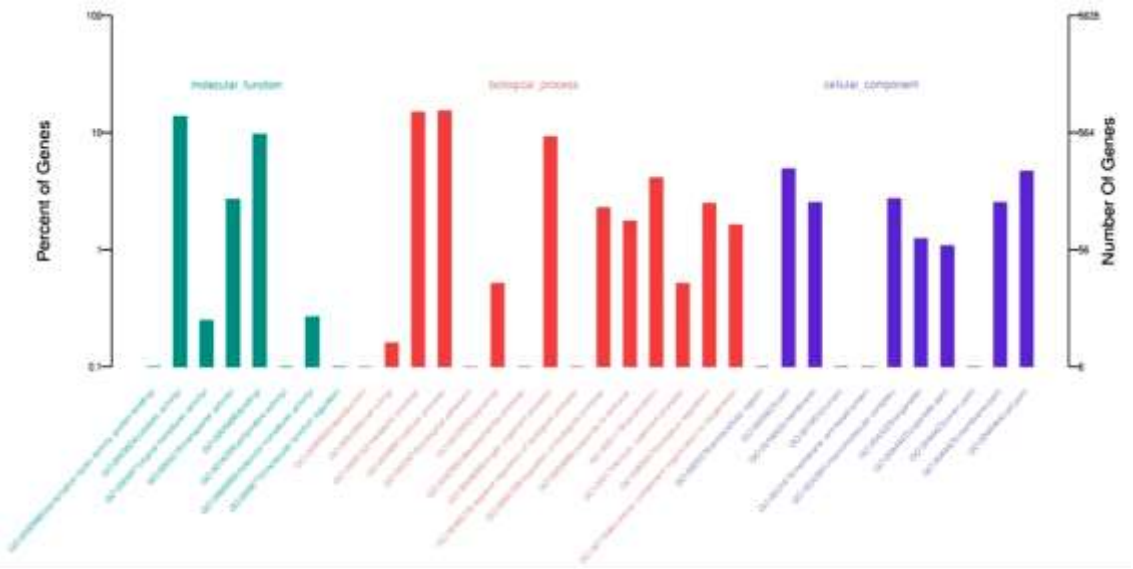

Fig. 5: Statistics of GO function classification of genome coding protein of *S. aureus*.

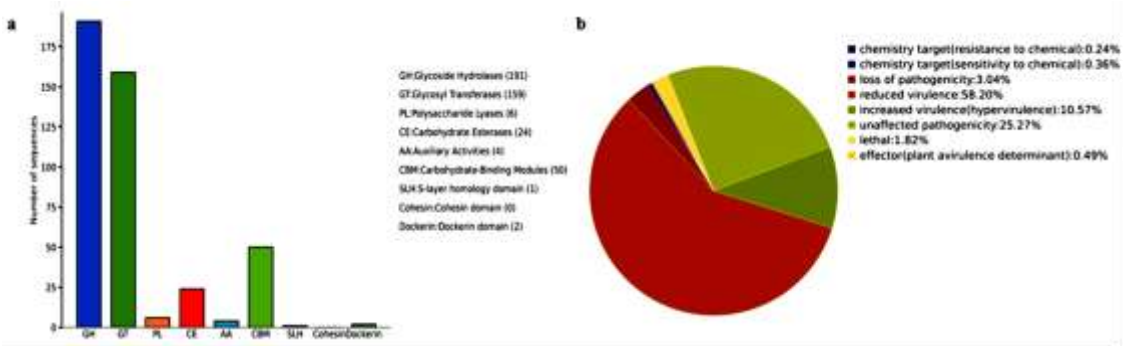

Fig. 6: Functional classification statistics of CAZy encoding proteins (a) and phi encoding proteins (b) in *S. aureus* genome.

genome. The highest amino acid codon usage were Lys (AAA, 6.108%), Glu (GAA, 5.444%), Ile (ATT, 5.202%), Leu (TTA, 5.397%), and Asp (GAT, 4.589%) in *S. aureus* genome, while stop codon (TGA, 0.037%) (TAG, 0.054%) and Arg (CGG, 0.044%) were the least usage codons (Table 1). Also, it was interestingly found that 53.79% amino acid codon were two or three repeat bases like AAA or AA-/-AA. There were 393 long repetitive sequences with evenness length of 101.94 bp and 2 short repeat sequences with average length of 52.50 bp found in the current *S. aureus* genome.

COG annotation showed that the present *S. aureus* genome encoded genes mainly related to E (amino acid transport), J (biogenesis, ribosomal structure, and translation), G (carbohydrate convey and supersession), and P (inorganic ion exchange and supersession) (Fig. 4a). In the current S. aureus, KEGG annotation revealed that 51.6% of the encoded genes contributed to metabolism, including carbohydrate, energy, lipid, and amino acid metabolism; 18.33% were associated with environmental information machining; and 16.54% of the genes were genes that functioned in genetic information machining. Interestingly, 8.79% genes were related to human diseases including cancer, neurodegenerative and immune problems, endocrine and metabolic disease, and drug fastness (Fig. 4b). GO annotation discovered that *S. aureus* encoded genes regarding to cellular component, molecular function and biological system (Fig. 5). CAZy annotation found that *S. aureus* genome encoded many enzymes of glycoside hydrolases (GH) and glycosyl transferases (GT) (Fig. 6a). GTs are enzymes contributing to oligosaccharides, polysaccharides, and glycoconjugates biosynthesis, while GHs are involved in carbohydrate chain degradation (Zhu *et al*., 2015; Lin *et al*., 2022), which may conclude that this bacterium is actively involved in carbohydrate metabolism. Phi annotation reported increased virulence genes (hypervirulence, 10.57%), lethal genes (1.82%) and resistance chemistry target genes (0.24%) (Fig. 6b), which may reveal the potential virulence and drug resistance of current *S. aureus* isolate. CARD annotation revealed that antibiotic resistant genes of tet, mepR, mepA, mgrA, norA, *blaZ*, arlS, arlR and LmrS in *S. aureus* genome (Table 4), which is partly in line with previous study reported *blaZ*, spc, ant(6)-Ia-like, tet(K), lnu(B), aph(30)-III, str, mecA, qnrB19, erm(A), erm(T), vga(E), lnu(A), mph(C), msr(A), tet(L), dfrA1, tet(M), erm(C), dfrK, erm(B), dfrG, aadD-like and catA1 in different *S. aureus* genomes (Li *et al*., 2019). The encoded drug resistant genes of tet, mepA, norA and *blaZ* may illustrate the multi-drug (Tetracycline, Penicillin, Methicillin, Ciprofloxacin) resistance of current *S. aureus* (Li *et al*., 2019; Papkou *et al*., 2020). Besides directly anti-drug against genes, efflux pump gene of mepA and its regulatory gene mepR, and LmrS were also found in the present *S. aureus*, which may enhance the ability to reduce susceptibility (Nava *et al*., 2020; Herrera *et al*., 2021).

MgrA and arlS-arlR are involved in regulating norA gene (Juárez-Verdayes *et al*., 2012), which may also explain the drug resistant mechanism of current isolated *S. aureu*s. VFDB annotation showed that over 60 bacterial virulence factors including commonly known clfA, hla, hlb, fnbB, fnbA in *S. aureu*s genome (Table 4), which is partly in accordance with *S. aureu*s genomes isolated from food products reported sei, sea/sep, scn, sec, lukF-PV, seg, seh, sek, sem, sen, seo, sel, seq, seb, seu and tst (Li *et al*., 2019). The multiple virulence factors found in the *S. aureu*s genome came from bovine mastitic milk products may reflect the high pathogenicity of this bacterium, as adhesion genes of clfA, fnbB and fnbA, hemolysin gene of hla and hlb were commonly found in isolates infection in animals and people (Salasia *et al*., 2011; Khan *et al*., 2021).

Conclusions: To sum up, in the current study, the whole genome of *S. aureu*s isolated from fresh bovine mastitic milk samples was sequenced, which revealed antibiotic resistant genes and virulence factors of this bacteria. This study may aid in the scientific use of antibiotics at this farm and other locations as some of the antibiotic resistance genes were not frequently reported in this region.

Data available statement: The sequence data in the current study were deposited in available database with the NCBI accession numbers: chromosome (PRJNA855545) and plasmid (OP004824).

Ethics statement: Experiments were performed guiding by LAR Centre of and the ethics committee of Hebei Agricultural University and Liyi University.

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