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UDC 577.2:579.637.0 **Research article**

Microbiological and molecular genetic characteristics of *Staphylococcus aureus* isolated from raw horse meat

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Abstract

Background and Aim. Staphylococcal food poisoning is caused by the consumption of food contaminated with *Staphylococcus aureus* and represents a global public health concern. Food products of animal origin can serve as reservoirs for multi-drug resistant strains of *S. aureus*. This study aimed to characterize the microbiological and molecular genetic properties of *S. aureus* isolated from raw horse meat.

Materials and Methods. Species identification of *S. aureus* was performed using 16S rRNA gene analysis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The sensitivity of the isolate to antibiotics was investigated using the disc diffusion method. Genetic determinants of resistance were identified using whole-genome sequencing. High-throughput sequencing was performed on an Illumina MiSeq platform. DNA quantity was assessed spectrophotometrically, and quality was evaluated by electrophoresis on a 1% agarose gel. Genome assemblies based on short reads were obtained using SPAdes v. 4.0.0. Assembly quality assessment, organism verification, and initial annotation were performed using FastQC v.0.11.9. Antibiotic resistance genes were identified using the CARD and PATRIC databases.

Results. The *S. aureus* 76_KZ strain was isolated from a raw horse meat sample. Morphologically, it is a Gram-positive coccus arranged in irregular clusters resembling grape bunches. The identified antibiotic resistance profile of *S. aureus* 76_KZ characterizes this strain as a multidrug-resistant isolate with sensitivity to a limited spectrum of antibacterial drugs. This study presents the results of whole-genome sequencing of *S. aureus* isolated from raw horse meat in the Republic of Kazakhstan. The sequencing yielded a genome coverage of 173×. The sequenced genome of the *S. aureus* 76_KZ isolate, consisting of 2.616.354 bp, has been deposited in the GenBank genetic database under accession number JBNBZR000000000.1.

Conclusion. The whole-genome data obtained for *S. aureus* 76_KZ enable the assessment of the isolate's resistance to antimicrobial drugs and facilitate the identification of genetic features relevant to epidemiological typing.

Keywords: food products; resistance; *Staphylococcus aureus*; whole genome sequencing.

Introduction

Food contamination by pathogenic microorganisms remains a key issue in food safety and public health [1]. Foodborne infections affect both men and women of various age groups living in both rural and urban areas alike, and can occur sporadically or in the form of epidemics. Every year, foodborne infections affect approximately 20% of the population in industrialized countries [2]. To date, over 250 different foodborne diseases have been identified worldwide, two-thirds of which are caused by bacteria.

Staphylococcus aureus is the third most common foodborne pathogen globally [3]. Staphylococcal infections cause significant morbidity and mortality in both developing and developed countries.

Various foods, such as meat and meat products, milk and dairy products, poultry, eggs, fish, vegetable salads, and cream-filled pastries, have been implicated in staphylococcal food poisoning. Unhygienic food handling is an important source of staphylococcal contamination [2]. The main sources of staphylococcal contamination in food products are humans and animals with purulent inflammatory processes (such as abscesses, furuncles, or purulent wounds) who carry these microorganisms. Staphylococci can be transferred from humans to food products via airborne droplets, direct contact, or during processing activities such as equipment handling, slaughtering, and carcass cutting.

S. aureus is a gram-positive, non-motile, non-spore-forming, facultatively anaerobic, commensal, and opportunistic pathogen that can cause a wide range of infections, from mild skin infections to life-threatening conditions, such as bacteremia, endocarditis, necrotizing pneumonia, toxic shock syndrome, and food poisoning [4]. Food poisoning results from ingesting preformed staphylococcal enterotoxins. Five serologically distinct enterotoxins (A, B, C, D, and E) have been identified, with enterotoxin A being the most common cause of food poisoning outbreaks. An estimated 30% to 80% of the global population carries *S. aureus*, and 50% of these carrier's harbor variants associated with food poisoning. *S. aureus* can grow over a wide range of temperatures (7 to 48.5 °C; optimum 30 to 37 °C), pH (4.2 to 9.3; optimum 7 to 7.5), and sodium chloride concentrations up to 15% NaCl. It is resistant to desiccation and can survive in potentially dry and stressful conditions, such as on the human nose and skin, and on surfaces such as clothing. These characteristics facilitate the growth of organisms in many food products [2].

Contamination of food products with antibiotic-resistant bacteria poses a serious threat to public health because antibiotic resistance determinants can be transmitted to other bacteria of clinical importance. Monitoring the spread of antibiotic-resistant bacteria and their resistance genes is a key factor in efforts to prevent antibiotic resistance. It has been established that *S. aureus* strains have developed resistance mechanisms to virtually all antimicrobial drugs used in treatment. The most important factor is resistance to drugs most often used in the treatment of gram-positive infections, such as beta-lactams, glycopeptides, and oxazolidinones [5].

S. aureus is able to develop antibiotic resistance through various mechanisms, including efflux pumps, biofilm formation, and enzymatic modification of antibiotics [6, 7, 8]. Whole-genome sequencing is increasingly used to analyze the genetic profiles of drug-resistant strains and the mechanisms of resistance gene transfer.

Identifying antibiotic-resistant foodborne *S. aureus* strains and assessing their pathogenic potential are urgent issues in food safety research. Currently, there are results on the study of phenotypic and genotypic resistance to resistance to antibacterial medications (ABMs) in S. aureus strains isolated from the milk of cattle in Northern Kazakhstan. The genotypic study of strains targeted genes related to resistance to β-lactam antibiotics (blaZ, 193 bp), macrolides (ermC 142 bp), and tetracyclines (tetK, 167 bp) [9]. In the study by *R. Rychshanova* et al., the results of the research shown were that *S. aureus* isolates obtained from cows' milk samples at the stage of subclinical mastitis were resistant to many antibiotics of the tetracyclines and *β*-lactam groups which are commonly used to treat mastitis [10]. There are also studies investigating the complete genomes of clinical *S. aureus* isolates obtained from hospitals in Kazakhstan [11, 12].

In this regard, the study aimed to perform microbiological and molecular genetic characterization of an S. aureus isolate obtained from raw horse meat. The novelty of this study lies in the implementation of whole-genome sequencing of non- clinical *S. aureus* isolates, providing information on the clustering of resistance genes, as well as pathogenicity, adhesion, and invasion factors.

Materials and Methods

Research object

A culture of *S. aureus*, isolated from a raw horse meat sample, was obtained from the Republic of Kazakhstan in 2025. Isolation, identification, and determination of the sensitivity of the bacterial isolates to antimicrobial drugs were performed at the National Scientific Laboratory of Biotechnology for Collective Use of the National Centre for Biotechnology LLP (Astana, Kazakhstan).

Species identification and storage of the microorganism

Species identification of the bacterium was performed based on analysis of the nucleotide sequence of the 16S rRNA gene, as well as by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using MALDI Biotyper 3 software. 1 (Bruker Daltonics, Germany). The criterion for reliable species identification by MALDI TOF MS was a score ≥ 2.0. The bacterial isolate was stored at -80 °C in Luria-Bertani broth supplemented with 50% glycerol.

Determination of sensitivity to antimicrobial drugs

The antimicrobial susceptibility profiles of the *S. aureus* food isolate were determined using the disc diffusion method on Mueller-Hinton agar for the following antibiotics: moxifloxacin (5 μg), ciprofloxacin (5 μg), rifampicin (5 μg), clindamycin (2 μg), erythromycin (15 μg), tobramycin (10 μg), amikacin (30 μg), and tetracycline (30 μg). Antimicrobial susceptibility was determined based on the growth inhibition zone breakpoints established by EUCAST (version 15.0 dated 01.01.2025). Quality control of sensitivity testing was performed on *S. aureus* ATCC29213 cultures.

Double disk approximation test (D-test)

The isolates that were resistant to erythromycin were tested for inducible clindamycin resistance by double disk approximation test (D-test) as per EUCAST 15.0 guidelines. In this test, a 0.5 McFarland's standard suspension of *S. aureus* was prepared and plated onto MHA plate (Mueller Hinton agar). An erythromycin disk (15 μg) and clindamycin (2 μg) were placed 15 mm apart edge-to-edge on the MHA plate. Plates were analyzed after 18 h of incubation at 37 °C [13].

DNA extraction and sequencing

Genetic determinants of resistance were identified in a multidrug-resistant *S. aureus* isolate from raw horse meat by whole-genome sequencing. DNA was extracted using cetyltrimethylammonium bromide/ NaCl. Bacteria grown on solid nutrient medium were collected with a bacterial loop and transferred to a clean test tube with 1.5 μL of TE buffer, and a uniform suspension was prepared. The mixture was centrifuged at 10.000 rpm for 3 min. The supernatant was then removed. DNA was extracted using the Kate Wilson method [14]. A centrifuge (Eppendorf 5415 D, Germany) was used at a centrifugation force of 12,000 × g.

Total DNA was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific) at a wavelength of 260 nm. RNase A (Thermo Fisher Scientific) was used to remove RNA impurities. For a more accurate determination of DNA concentration, a Qubit 2.0 fluorometer (Invitrogen/Life Technologies, Carlsbad, USA) was used. Qualitative characterization of total DNA was performed by electrophoresis on a 1% agarose gel (Applichem, Darmstadt, Germany).

The library was obtained using PS DNA Library Prep Kit with UD Indexes (Thermo Fisher Scientific) per the manufacturer's instructions. The genome sequencing of *S. aureus*, strain 76_KZ was performed using Illumina Miseq platform and Miseq kit v3 (Illumina, Cambridge, UK) which allows to obtain 300 bp long paired-end reads.

Bioinformatic analysis

Genome assemblies based on short reads were obtained using SPAdes v. 4.0.0 [15].

Assembly quality assessment, organism verification, and initial annotation were performed using FastQC v.0.11.9 software [15]. Antibiotic resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) and PATRIC (http://patricbrc.org) databases.

Statistical processing of results

Statistical processing of the research results was performed using standard descriptive statistical methods in Microsoft Office Excel 2010. The statistical significance of differences in the proportion of resistant cultures was assessed using Student's t-test at a significance level of $\alpha < 0.05$.

Results and Discussion

Isolation and primary identification of Staphylococcus spp. isolated from fresh horse meat

For the isolation and primary identification of Staphylococcus spp. from the samples studied, we used the selective chromogenic medium, Chromatic S. aureus agar (Liofilchem, Teramo, Italy) (Figure 1). Figure 1 shows that *S. aureus* colonies were round with a smooth surface and a pinkish-purple color.

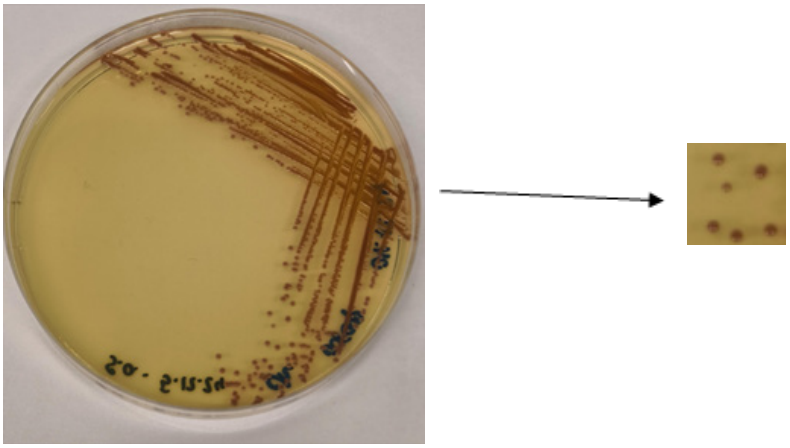

Figure 1 – Growth pattern of the studied isolate on Chromatic S. aureus agar (Liofilchem, Teramo, Italy)

The isolate was identified using MALDI-TOF MS by comparing the spectra of the constant proteins of the microorganisms with the MALDI Biotyper database (Bruker Daltonics GmbH, Bremen, Germany). The isolated strain was identified as *S. aureus* 76_KZ.

Next, the morphological and biochemical properties of the horse meat isolate were studied, namely, Gram staining and hemolytic activity of the isolated culture. The studied isolate, *S. aureus* 76_KZ, is a gram-positive coccus (Figure 2).

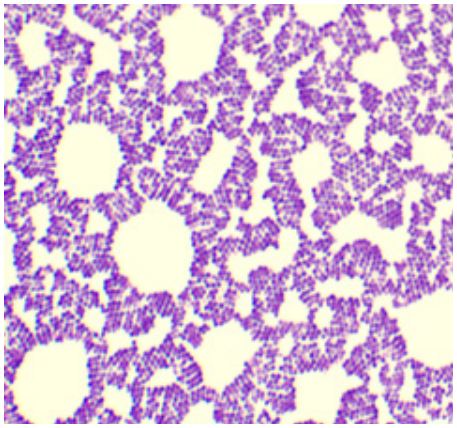

Figure 2 – Gram staining of *S. aureus* isolate 76_KZ

Figure 3 illustrates the growth of *Staphylococcus aureus* on the Columbia blood agar.

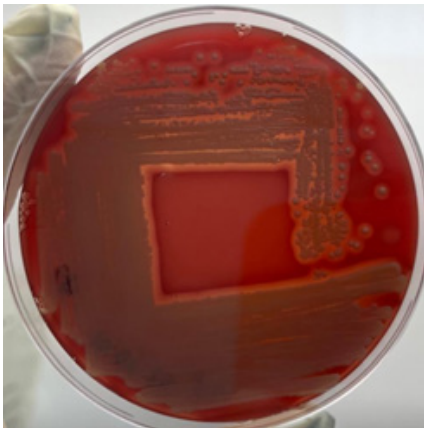

Figure 3 – Growth of *S. aureus* 76_KZ on a solid nutrient medium (Columbia blood agar, Liofilchem, Teramo, Italy)

As shown in Figure 3, the growth of the isolate on blood agar was marked by the formation of round colonies with a convex surface and smooth edges; a distinctive feature of the growth was the demonstration of β-hemolysis, which manifested itself in the formation of a transparent zone of lysis around the colonies.

When cultured on Bayer-Parker medium, it forms shiny grey-black colonies surrounded by a zone of medium clarification. This is because Staphylococcus aureus reduces tellurium from potassium tellurite, staining the colonies black, and also has lecithinase activity, which causes the surrounding medium to lighten due to the breakdown of egg yolk (Figure 4).

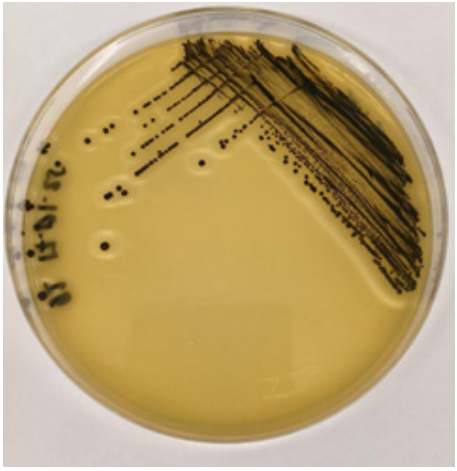

Figure 4 – Growth of *S. aureus* 76_KZ on Bayer-Parker medium

Study of antibiotic resistance

The antibiotic resistance profile of the S. aureus 76_KZ isolate was assessed using the disc diffusion method in accordance with the current EUCAST 15.0 (2025) interpretation criteria (Table 1).

Table 1 – Antibiotic susceptibility of *S. aureus* strains 76_KZ and ATCC 29213 determined by the disk diffusion method according to EUCAST standard v.15.0 (2025)

№	Antibiotic	Name of microorganism				Clinical breakpoints (EUCAST 2025-01-01)
		S. aureus 76_KZ		*S. aureus* ATCC 29213		
		Inhibition zone diameter (mm)	Interpretation (S/R)*	Inhibition zone diameter (mm)	Interpretation (S/R)*	
1	Tobramycin	18.3±0,6	S	18.0 ± 2.8	S	≥18: S; <18: R
2	Moxifloxacin	19.3±1,2	R	18.5 ± 3.5	R	≥25: S; <25: R
3	Rifampicin	22.7±2,1	R	21.5 ± 5.0	R	≥26: S; <26: R
4	Ciprofloxacin	16.0±0,0	R	15.0 ± 1.4	R	≥50: S; <17: R
5	Erythromycin	20.3±0,6	R	19.0 ± 1.4	R	≥21: S; <21: R
6	Clindamycin	19.7±0,6	R	17.0 ± 4.2	R	≥22: S; <22: R
7	Amikacin	17.7±1,2	S	15.5 ± 6.4	S	≥15: S; <15: R
8	Tetracycline	21.7±1,5	S	19.0 ± 1.4	R	≥22: S; <22: R
Note: *Interpretation (S – susceptible, R – resistant)						

The average inhibition zone diameter of the *S. aureus* 76_KZ strain ranged from 16.0 ± 0.0 mm (ciprofloxacin) to 22.7 ± 2.1 mm (rifampicin). Similarly, the average inhibition zone diameters of the S. aureus ATCC 29213 strain ranged from 15.0 ± 1.4 mm (ciprofloxacin) to 21.5 ± 5.0 mm (rifampicin).

Unlike the control strain S. aureus ATCC 29213, the clinical strain *S. aureus* 76_KZ showed susceptibility to tetracycline, whereas the control strain was resistant to it. For the other antibiotics

tested, the clinical strain *S. aureus* 76_KZ demonstrated similar susceptibility and resistance patterns as the control strain S. aureus ATCC 29213.

The experimental data demonstrated a heterogeneous pattern of sensitivity of the studied strain to antimicrobial drugs from various pharmacological groups. Analysis of the growth inhibition zones revealed resistance to several key antibacterial agents. Thus, the *S. aureus* 76_KZ strain showed resistance to fluoroquinolones, moxifloxacin, and ciprofloxacin, indicating the overexpression of NorC efflux systems, including resistance to moxifloxacin. This is consistent with the data of Que Chi Truong-Bolduc [16], who found that overexpression of *norC* contributes to the development of a quinolone resistance phenotype in the *mgrA* mutant. In our opinion, the overexpression of *norA* efflux systems may also be associated with ciprofloxacin resistance, which is consistent with the data of the authors [17]. In addition, pronounced resistance to rifampicin was observed, indicating potential modifications in the β-subunit of bacterial RNA polymerase encoded by the *rpoB* gene [18].

Of particular note is the presence of constitutive MLSB in this strain, a phenotype associated with simultaneous resistance to two classes of antibiotics: macrolides and lincosamides (to erythromycin (15 μg) and clindamycin (2 μg)) [19].

In this study, we investigated the inducible resistance of *S. aureus* 76_KZ to clindamycin (phenomenon D) (Figure 4). Some Streptococcus strains exhibit inducible resistance to clindamycin in the presence of erythromycin. This is because erythromycin activates the resistance mechanisms [20].

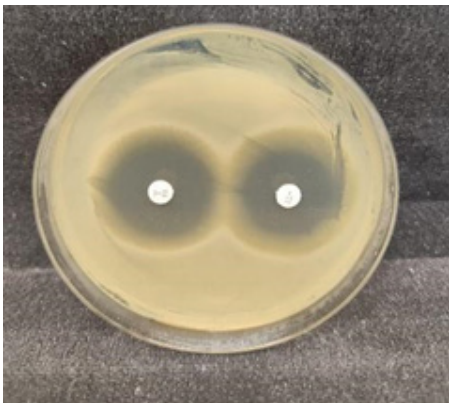

Figure 5 – Detection of inducible resistance of staphylococci to clindamycin
(D phenomenon, E: erythromycin 15 μg, CD: clindamycin 2 μg)

Figure 5 shows the results of a test to detect induced resistance to clindamycin (D-test) in the *S. aureus* 76_KZ strain. As can be seen in Figure 5, there is no characteristic D-shaped deformation of the inhibition zone, which indicates the absence of the D phenomenon and the presence of a constitutive resistance phenotype (cMLS phenotype) of resistance, which may be due to ribosomal mutations of the 23S rRNA gene, manifested by resistance to both erythromycin and clindamycin [21].

As shown in Figure 5 the test result was negative; that is, clindamycin was not inhibited by the antagonist erythromycin. Nevertheless, the S. *aureus* 76_KZ isolate retained its sensitivity to aminoglycosides, tobramycin, and amikacin, which may indicate the absence of aminoglycoside-modifying enzymes or mutations in the binding site in the 30S subunit of the ribosome. Moreover, sensitivity to tetracycline was preserved, indicating the absence of active resistance mechanisms such as tetracycline efflux proteins (Figure 6 and Figure 7).

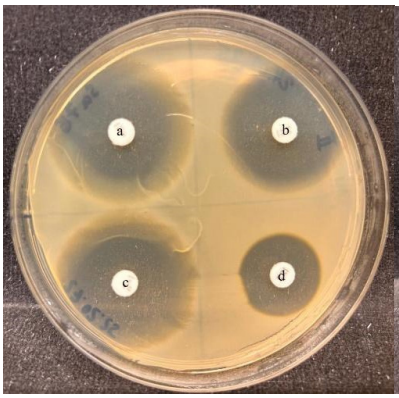

Figure 6 – Antibiotic resistance of *S. aureus* 76_KZ to erythromycin (a), tetracycline (b), clindamycin (c) and amikacin (d)

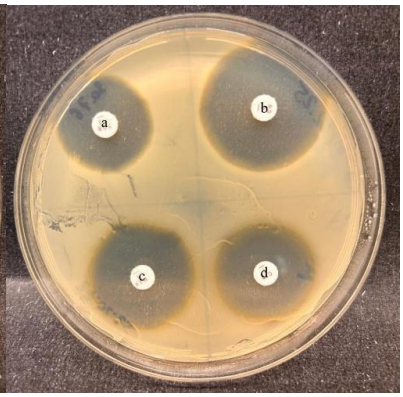

Figure 7 – Antibiotic resistance of *S. aureus* 76_KZ to tobramycin (a), rifampicin (b), ciprofloxacin (c) and moxifloxacin (d)

Therefore, the identified antibiotic resistance profile of *S. aureus* 76_KZ characterized this strain as a multidrug-resistant isolate with sensitivity to a limited range of antibacterial drugs.

Analysis of the whole genome of S. aureus 76_KZ

Whole-genome sequencing data were obtained using a MiSeq platform (Illumina, USA). The whole genome of the *S. aureus* 76_KZ isolate was 2.616.354 bp in length. The genome was sequenced with 173× coverage, an average read length of 150.24 bp, and a G+C content of 32.76%. A total of 899.768 reads were obtained. Read quality was checked using FastQC v.0.11.9. Sixteen contigs were assembled using SPAdes version 4.0.0. Final assemblies were annotated using the NCBI Prokaryotic Genome Annotation Pipeline v.6.10. Default parameters were used for all software packages. Annotation of the *S. aureus* 76_KZ genome revealed 2.577 genes, 2.488 of which encode proteins. In addition, 27 tRNAs and two complete rRNAs were detected.

Next, we performed a phylogenetic analysis of the whole genome of the isolate using the complete genomes of representative strains from the *Staphylococcaceae* family available in the NCBI database (Figure 8).

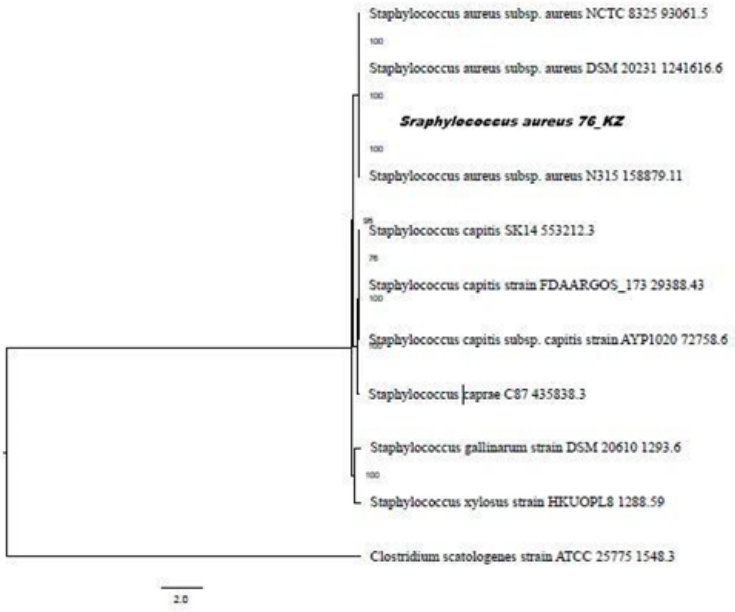

Figure 8 – Phylogenetic tree of representative *Staphylococcus spp*. genomes taken from the NCBI database using CSI Phylogeny v1.4

Figure 8 shows that the Kazakh isolate *S. aureus* 76_KZ forms a single branch with strains belonging to the species *S. aureus* and is located between the strain *Staphylococcus aureus* subsp. aureus DSM 20231 1241616.6, which was isolated from a human in 1953. *S. aureus* N315 is methicillin-resistant (MRSA) and was first isolated in 1982.

Antibiotic resistance genes

Antibiotic resistance genes were identified using the PathoSystems Resource Integration Centre (PATRIC) (https://www.patricbrc.org) (Table 2).

Table 2 – Antibiotic resistance genes

Resistance mechanisms	Genes
Antibiotic inactivation enzyme	FosB
Antibiotic resistance gene cluster, cassette, or operon	TcaA, TcaB, TcaB2, TcaR
Antibiotic target in susceptible species	Alr, Ddl, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, MurA, rho,rpoB, rpoC, S10p, S12p
Antibiotic target in susceptible species	BceA, BceB, NorA, Tet (38)
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, MprF, PgsA
Regulator modulating expression of antibiotic resistance genes	BceR, BceS, LiaF, LiaR, Lia

Table 2 demonstrates that the norA gene has been identified, which encodes a multi-component efflux pump in *S. aureus* and provides resistance to fluoroquinolones and other structurally unrelated antibiotics, such as acriflavine [21]. It should be noted that the isolate under study was phenotypically resistant to fluoroquinolones, such as moxifloxacin. The gyrA gene, which is resistant to ciprofloxacin, was also identified during the analysis of the inhibition zones with this antibiotic. Interestingly, despite the isolate's resistance to both clindamycin and erythromycin, the genes responsible for the resistance to these drugs were not detected. Coutinho et al. also described six *S. aureus* isolates that were resistant to both erythromycin and clindamycin but did not carry any resistance genes [22, 23]. In addition, the *S. aureus* 76_KZ isolate contains the rpoB gene, which causes resistance to the antibiotic rifampicin, and is phenotypically evident. Thus, the phenotypic profile data were correlated with the identified genetic determinants of resistance, except for clindamycin and erythromycin resistance.

Virulence genes

Virulence genes are specific DNA segments that encode proteins or molecules contributing to the pathogenicity of microorganisms. These genes play key roles in the ability of pathogens to cause disease in their hosts. The identification of S. aureus virulence genes is important for assessing disease development (Table 3).

Table 3 – Virulence genes of *S. aureus*

Name	Virulence factor	Identity	Position in the contig	Protein function	NCBI number
S.aureus_ exoenzyme	aur	100.0	27108..28637	aureolysin	BA000018.3
S.aureus_ exoenzyme	splA	100.0	548336..549043	serine protease splA	BA000018.3
S.aureus_ exoenzyme	splB	100.0	547489..548211	serine protease splB	BA000018.3
S.aureus_toxin	hlgA	100.0	37327..38256	gamma-hemolysin chain II precursor	BA000018.3
S.aureus_toxin	hlgB	100.0	39772..40748	gamma-hemolysin component B precursor	BA000018.3
S.aureus_toxin	hlgC	100.0	38823..39770	gamma-hemolysin component C	BA000018.3

Continuation of Table 3

S.aureus_toxin	lukD	100.0	553174..554157	leukocidin D component	BA000018.3
S.aureus_toxin	lukE	100.0	554159..555094	leukocidin E component	BA000018.3
S.aureus_toxin	lukE	100.0	554159..555094	leukocidin E component	CP001781.1
S.aureus_toxin	seg	99.87	560236..561012	enterotoxin G	CP001844.2
S.aureus_toxin	sei	100.0	563014..563742	enterotoxin I	BA000018.3
S.aureus_toxin	sei	100.0	563014..563742	enterotoxin I	CP011147.1
S.aureus_toxin	sem	100.0	563777..564496	enterotoxin M	BA000018.3
S.aureus_toxin	sen	100.0	561295..562071	enterotoxin N	BA000018.3
S.aureus_toxin	seo	100.0	564777..565559	enterotoxin O	BA000018.3
S.aureus_toxin	seu	99.61	562089..562860	enterotoxin U	HE681097.1
S.aureus_toxin	tst	100.0	94247..94951	toxic shock syndrome toxin-1	AP009324.1

The table 3 shows that the isolate under investigation contains several exotoxins, which are divided into three groups based on their known functions: cytotoxins, superantigens (SAgs), and cytotoxic enzymes. Cytotoxins affect the membranes of host cells, leading to the lysis of target cells and inflammation. Superantigens mediate cytokine production and induce T- and B-cell proliferation. The secreted cytotoxic enzymes damage mammalian cells. Collectively, these exotoxins modulate the host immune system and are crucial for infections caused by *S. aureus* [24]. *S. aureus* secretes a metalloproteinase known as aureolysin, encoded by the aur gene. Aureolysin stimulates T and B lymphocytes via polyclonal activators and suppresses the production of lymphocyte immunoglobulins [25]. The toxin γ-hemolysin is encoded by the hlgA, hlgB, and hlgC genes. γ-hemolysin mimics leukocidins, forming pores in the membranes of host cells, promoting bacterial survival and evading immunity [26]. The toxin known as Panton-Valentine leukocidin (PVL) belongs to the family of synergistic chemotactic toxins, which also includes γ-hemolysin and other leukocidins such as LukE-LukD. Leukocidins destroy leukocytes and inhibit phagocytosis [27]. Serine protease-like proteins (Spls), encoded by the *splA* to *splF* genes, were discovered nearly three decades ago; however, their pathophysiological basis and biological functions during infection remain largely unknown [28]. Enterotoxin-like (SEl) toxin, encoded by the genes *seg, sei, sem, sen, seo,* and *seu*, is the most significant virulence factor involved in food poisoning, toxic shock syndrome, and staphylococcal infectious diseases in humans. These toxins belong to a broad family of pyrogenic superantigens that stimulate nonspecific T-cell proliferation [29]. Thus, identifying S. aureus virulence genes is important for the evaluation of isolated strains and the subsequent development of the disease.

Phylogenetic analysis

A comparative genome analysis was performed using the complete genome of the studied isolate, *S. aureus* 76_KZ. The comparison included reference genomes of S. aureus strains circulating in Russia, Japan, South Korea, and China. An isolate circulating in Kazakhstan was also included in the comparison (Table 4, Figure 9).

Table 4 – Presents the characteristics of the strains included in the phylogenetic analysis (country, year, and source of isolation)

№	Accession	Name strain	Country	Year	Source of isolation
1	AP017922.1	JP080	Japan	2005	human
2	NZ_BRBM00000000.1	JARB-OU1260	Japan	2018	human
3	NZ_JANVJN000000000.1	GD9M30A	China	2019	milk
4	NZ_CP113018.1	Taliyah	Taiwan	2019	environmental
5	NZ_WNKR00000000.1	1709	Russian Federation	2018	milk
6	NZ_JALJBR000000000.1	Crie-F374	Russian Federation	2019	Minced pork cutlet
7	NZ_JAJNOL010000000	SA201503,	China	2015	anal swab (human)
8	CP134071.1	4233	Kazakhstan	2022	water
9	CP030138.1	M48	China	2012	pig
10	CP121204.1	SA0907	China	2022	eccrine
11	NZ_PQWU00000000.1	0257-2201-2015	Russia	2015	Skin lesions (human)
12	CP080562.1	HL21008	South Korea	2017	Human blood
13	CP080560.1	HL17064	South Korea	2015	Human blood
14	JBNBZR000000000.1	76_KZ	Kazakhstan	2025	raw horse meat
15	CP082815	SCAID WND1-2021	Kazakhstan	2021	Swab from Wound (human)
16	CP082813	SCAID OTT1-2021	Kazakhstan	2021	Swab from ear (human)

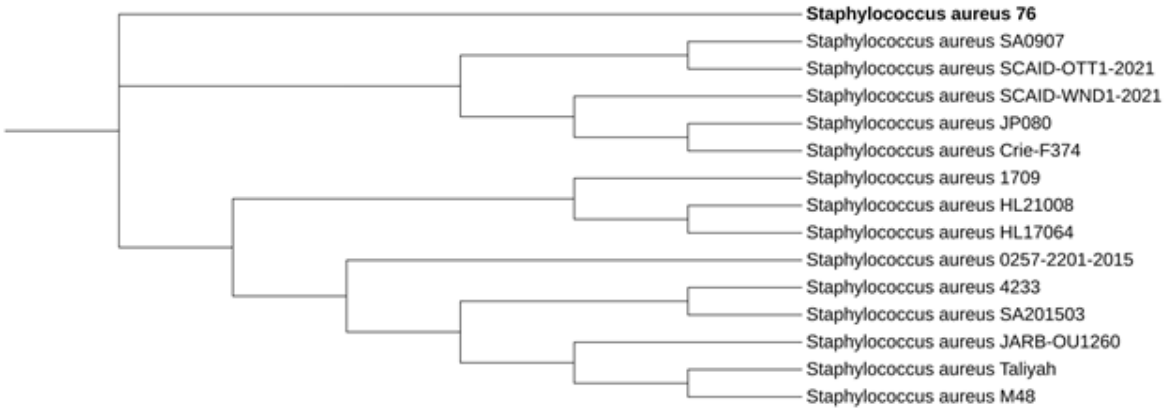

Figure 9 – Phylogenetic analysis of the genomes of the studied isolate *S. aureus* 76_KZ and complete genomes circulating in different countries using CSI Phylogeny v1.4

Figure 9 demonstrates that *S. aureus* 76_KZ is significantly distant from the complete *S. aureus* genomes included in the phylogenetic tree. The closest relatives are strain SA0907, isolated in China, and strain SCAID OTT1-2021, isolated in Kazakhstan.

Conclusion

Whole-genome sequencing of *S. aureus* isolates was performed to provide information on the clustering of resistance genes, as well as factors of pathogenicity, adhesion, and invasion, representing the novelty of this study. The identified antibiotic resistance profile of *S. aureus* 76_KZ characterizes this strain as a multidrug-resistant isolate with sensitivity to a limited spectrum of antibacterial drugs. Phenotypic resistance data were consistent with the identified genetic determinants of resistance. Notably, *S. aureus* 76_KZ isolate was resistant to erythromycin and clindamycin, but did not carry

known resistance genes associated with these antibiotics. This discrepancy requires further study to elucidate the underlying mechanism of resistance. Genes associated with pathogenicity that characterize the isolate under study were identified as pathogenic determinants.

The results obtained are important for the epidemiological monitoring of the spread of resistant clones of *S. aureus*. Findings related to the *S. aureus* 76_KZ isolate may be used to analyze the genetic characteristics of foodborne pathogenic strains circulating in Kazakhstan.

Authors' Contributions

SKh: Design and control of microbiological experiments, writing a publication on the microbiological part. EZh: Conceptualized and designed the research, processing, and interpretation of genetic data, writing the rest of the publication. DZh, AT, and AB: Conducted experimental work: microbiological experiments, preparation of DNA libraries, and DNA sequencing. All authors have read, reviewed, and approved the final manuscript.

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Based on the results of whole-genome analysis, the S. aureus 76_KZ genome sequence was deposited in GenBank with the accession number JBNBZR000000000.1. Raw data were obtained from BioProject No. PRJNA1245609 was submitted to the NCBI Sequence Read Archive under accession number SRX28236276. This research is funded by the Ministry of Agriculture of the Republic of Kazakhstan (BR22885795) We would like to express our gratitude to Professor A.K. Bulashev for his advisory assistance.

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