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Whole-genome characterization and phenotypic antimicrobial resistance of *Staphylococcus aureus* isolates from children with food poisoning in Kazakhstan

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Abstract

Background. Staphylococcal food poisoning is one of the leading causes of foodborne intoxication in children worldwide. In Kazakhstan, *Staphylococcus aureus* strains responsible for such poisoning have so far been studied mainly by phenotypic methods and by PCR detection of individual genes, whereas whole-genome sequencing data are lacking. This study aimed to perform whole-genome and phenotypic characterization of *S. aureus* isolates recovered from children with food poisoning.
Methods. Five clinical isolates obtained in 2024–2025 from hospitalized children under 16 years of age (Pavlodar and Karaganda regions, Kazakhstan) were studied. Antimicrobial susceptibility was determined by broth microdilution and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria, version 16.0. Whole-genome sequencing, multilocus sequence typing, screening for antimicrobial resistance and virulence genes, and phylogenetic analysis were performed.
Results. The five clinical isolates belonged to four sequence types (STs): ST22 (n = 2), ST5, ST45, and ST15. Resistance to penicillin was detected in 4/5 isolates (80%), to cefoxitin in 2/5 (40%), and to linezolid in 1/5 (20%). Concordance between *blaZ* and penicillin resistance and between *mecA* and cefoxitin resistance was complete. Both methicillin-resistant ST22 isolates were multidrug-resistant and carried *lnu*(A) and *tsst-1*. Hemolysin genes were detected in all isolates.
Conclusions. Toxigenic *S. aureus* lineages associated with food poisoning in children, including multidrug-resistant MRSA ST22, circulate in the food chain of Kazakhstan. These findings support the integration of whole-genome sequencing and genetic screening for enterotoxin gene cluster (*egc*) genes and *tsst-1* into food safety surveillance.
Data deposition. Genome assemblies have been deposited in GenBank (National Center for Biotechnology Information, USA) under accession numbers JBQWQG000000000, JBQWQH000000000, JBQWQI000000000, JBQWQJ000000000, and JBQWQK000000000.

Keywords: *Staphylococcus aureus*; food poisoning; whole-genome sequencing; methicillin resistance; enterotoxin; phylogeny

Introduction

Staphylococcus aureus is a Gram-positive, facultatively anaerobic coccus. It is part of the normal microbiota of humans and animals and, at the same time, one of the leading causative agents of food poisoning [1]. A substantial proportion of the population is asymptomatically colonized by *S. aureus*. The pathogen is transmitted through contact with carriers and infected animals, as well as through contaminated food. Staphylococcal food poisoning (SFP) is caused not by the bacteria themselves but by enterotoxins accumulated in the food. Consequently, the disease has a rapid onset, within a few hours of ingestion, and manifests as vomiting, abdominal pain, and diarrhea. Children are particularly vulnerable, and institutional catering in childcare and school settings creates conditions for large outbreaks. For example, in 2025 an outbreak among high school students in Zhejiang Province, China, was caused by an *S. aureus* strain carrying the *sea*, *seq*, and *sek* genes [2].

Staphylococcal enterotoxins and toxic shock syndrome toxin-1 (TSST-1) act as superantigens, triggering a «cytokine storm» that leads to tissue damage, increased vascular permeability, and multiple organ failure [3]. In addition to superantigens, *S. aureus* produces proteases, hemolysins, leukocidins, and immunomodulatory factors. The genes encoding many of these toxins are located on plasmids, prophages, and pathogenicity islands and can be transferred horizontally [4]. Given the role of these virulence factors in food poisoning outbreaks, genomic surveillance of these determinants has become a key tool for assessing the pathogenic potential of *S. aureus* [5].

SFP has traditionally been attributed to the classical enterotoxins SEA–SEE, and commercial immunochemical assays target precisely these toxins. However, recent studies have shown that outbreaks can be caused by strains carrying only genes of the enterotoxin gene cluster (*egc*) — *seg*, *sei*, *selm*, *seln*, *selo*, and *selu* — in the absence of classical enterotoxins. For instance, poisoning of 46 people in China was caused by an *egc*-positive strain of the novel sequence type ST7591 (CC72) [6]. Such cases escape routine diagnostics, and genetic methods are required for their detection.

At the same time, antimicrobial resistance (AMR) in *S. aureus* is of growing concern. *S. aureus* is one of the six ESKAPE pathogens (*Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, and *Enterobacter*), and methicillin-resistant *S. aureus* (MRSA) accounted for more than 100,000 deaths in 2019 — more than any other pathogen-drug combination [7].

Whole-genome sequencing (WGS) now provides, in a single analysis, a complete profile of enterotoxin, resistance, and virulence genes and establishes the relatedness of isolates at high resolution [8]. WGS-based comparison of patient isolates with food isolates makes it possible to identify the source of infection.

We previously used WGS to characterize 42 isolates recovered from foods in Northern Kazakhstan [9]. Apart from this work on food isolates, studies of *S. aureus* in Kazakhstan have been limited to phenotypic typing and PCR detection of individual resistance and toxin genes, and no whole-genome data on strains causing food poisoning in children have been published. The aim of the present study was therefore to characterize the genomic profiles and phenotypic antimicrobial susceptibility of *S. aureus* isolates recovered from cases of food poisoning in children in Kazakhstan.

Materials and Methods

Study Isolates

The study included five clinical *S. aureus* isolates (K1–K5) obtained between June 2024 and June 2025 from children under 16 years of age hospitalized with clinically and laboratory-confirmed SFP (acute

gastroenteritis with isolation of *S. aureus* from feces and vomitus). Each isolate was obtained from a different patient. The isolates were submitted to the laboratory by the Departments of Sanitary and Epidemiological Control of the Pavlodar and Karaganda regions as part of routine epidemiological investigations of food poisoning cases. Samples were transported in sealed insulated containers at 4 °C, and microbiological analysis was performed within 24 h of collection.

For phylogenetic comparison, we used the genomes of 42 *S. aureus* isolates previously recovered by our group from raw and ready-to-eat foods (Astana and the Kostanay region) during the same period [9]. Data on the food isolates were used solely to determine the phylogenetic position of the clinical isolates.

Isolation and Identification of S. aureus

Nutrient broth and Luria–Bertani broth (HiMedia Laboratories, Mumbai, India) were used for primary enrichment, and meat-peptone agar and Luria–Bertani agar (HiMedia Laboratories, Mumbai, India) for cultivation. Selective isolation was performed on Chromatic Staph. aureus chromogenic medium (Liofilchem, Teramo, Italy), on which *S. aureus* formed purple colonies, and on Baird-Parker agar (HiMedia Laboratories, Mumbai, India), on which typical black colonies surrounded by a clear zone were observed. Isolates were also characterized by Gram staining and hemolysis type on blood agar.

Species identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a MALDI Biotyper system with a microflex LT mass spectrometer (Bruker Daltonik, Bremen, Germany). Colonies were spotted onto an MSP 96 steel target and overlaid with 1 µL of matrix (saturated α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). Spectra were acquired in linear positive mode over the range of 2,000–20,000 Da; calibration was performed with the Bacterial Test Standard (Bruker Daltonik, Bremen, Germany). Identification was carried out in MALDI Biotyper RTC 4.0 software with the BDAL-12.0.0.0 library; a log(score) ≥ 2.0 was considered reliable. Species identity was additionally confirmed by sequencing a 16S rRNA gene fragment amplified with primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 806R (5′-GGACTACCAGGGTATCTAAT-3′) on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), followed by BLAST analysis against GenBank.

Antimicrobial Susceptibility Testing

In vitro susceptibility was determined by broth microdilution using the OCHA Sensilla Test Staphy kit (Erba Lachema, Brno, Czech Republic) according to the manufacturer's instructions. Twelve agents were tested: β-lactams (penicillin, cefoxitin), macrolides (erythromycin), lincosamides (clindamycin), oxazolidinones (linezolid), phenicols (chloramphenicol), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin), folate pathway antagonists (trimethoprim/sulfamethoxazole), aminoglycosides (gentamicin), glycopeptides (vancomycin), and nitrofurans (nitrofurantoin). Minimum inhibitory concentrations (MICs) were read after incubation at 35 °C for 20 h (24 h for vancomycin). Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints for *S. aureus*, version 16.0 (1 January 2026). *S. aureus* ATCC 29213 was used as the quality control reference strain.

Isolates resistant to at least one agent in three or more antimicrobial categories were classified as multidrug-resistant (MDR) according to international criteria [10]. In accordance with these criteria, penicillins, anti-staphylococcal β-lactams (cefoxitin), and oxazolidinones were treated as separate categories, and all MRSA isolates were classified as MDR regardless of their resistance to other agents [10]. The multiple antibiotic resistance (MAR) index was calculated as the ratio of the number of agents to which an isolate was resistant to the total number of agents tested [11].

Whole-Genome Sequencing and Bioinformatic Analysis

DNA was extracted using the cetyltrimethylammonium bromide (CTAB)/NaCl method. DNA quantity was assessed with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE,

USA) and a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA), and DNA quality by electrophoresis in a 1% agarose gel. Libraries were prepared with the Collibri PS DNA Library Prep Kit for Illumina (Invitrogen, Carlsbad, CA, USA), and sequencing was performed on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) using the MiSeq Reagent Kit v3 (600 cycles, paired-end reads).

Read quality was assessed with FastQC v0.11.9, and adapters and low-quality bases were removed with Trim Galore v0.6.5. De novo assembly was performed with Unicycler v0.4.8 (minimum contig length 300 bp, minimum coverage 5×) with two rounds of Pilon polishing (mapping with Bowtie2 v2.4.4 and processing with SAMtools v1.20). Assembly metrics were evaluated with QUAST v5.2.0, and annotation was performed with RASTtk; the presence of plasmid replicons was also assessed during annotation.

Molecular Typing and Phylogenetic Analysis

Multilocus sequence typing (MLST) was performed with mlst v2.23.2 using the PubMLST *S. aureus* scheme (loci *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*). A maximum-likelihood phylogenetic tree based on core-genome single-nucleotide polymorphisms (SNPs) of 47 isolates (5 clinical and 42 food) was constructed with CSI Phylogeny v1.4 using the following filters: minimum depth 10×, minimum relative depth 10%, SNP pruning distance 10 bp, minimum SNP quality (Phred) 30, minimum mapping quality 25, and Z-score threshold 1.96.

Analysis of Resistance and Virulence Genes

Antimicrobial resistance genes were identified in the assemblies using ResFinder and the Comprehensive Antibiotic Resistance Database (CARD) with default parameters. Virulence factors — genes encoding enterotoxins and enterotoxin-like proteins, superantigens, hemolysins, leukocidins, adhesins, and immune evasion factors — were identified using the Virulence Factor Database (VFDB).

Data Analysis and Presentation

This was a descriptive study; owing to the small sample size (n = 5), no statistical hypothesis testing was performed, and results are presented as absolute frequencies (number of isolates out of five). The gene presence/absence matrix for Fig 1 was compiled from ResFinder, CARD, and VFDB outputs and phenotypic susceptibility data, and visualized in Python v3.13 with matplotlib v3.11.2.

Ethics Statement

This study used isolates from the collections of the Departments of Sanitary and Epidemiological Control obtained during routine activities of the sanitary and epidemiological surveillance authorities. No personal patient data were provided to the investigators. Under the legislation of the Republic of Kazakhstan, ethics committee review was not required.

Results

General Characteristics of the Clinical Isolates

All five isolates were Gram-positive cocci; species identity was confirmed by MALDI-TOF MS (log(score) ≥ 2.0) and 16S rRNA gene sequencing. All five isolates exhibited β-hemolysis on blood agar. Assembled genome sizes ranged from 2,733,046 to 2,842,486 bp, and mean coverage ranged from 117× to 183× (Table 1; S1 Table). No plasmid replicons were detected during annotation.

MLST assigned the isolates to four STs: ST22 (K1 and K4), ST5 (K2), ST45 (K3), and ST15 (K5). Both ST22 isolates were MRSA, whereas the remaining three were methicillin-susceptible *S. aureus* (MSSA).

Table 1. Characteristics of clinical *S. aureus* isolates from children with food poisoning

Table 1.

Isolate	Genome size, bp	Coverage, ×	ST[4]	Hemolysis	Resistance (phenotype)[1]	Category[2]	MAR[3]
K1	2 842 486	125	ST22	β	PEN, FOX, LZD	MDR, MRSA	0.25
K2	2 740 426	183	ST5	β	PEN	R, MSSA	0.08
K3	2 733 046	149	ST45	β	—	S, MSSA	0
K4	2 754 519	117	ST22	β	PEN, FOX	MDR, MRSA	0.17
K5	2 754 942	121	ST15	β	PEN	R, MSSA	0.08

[1] PEN = penicillin; FOX = cefoxitin; LZD = linezolid. All isolates remained susceptible to all other agents among the 12 tested.

[2] MDR = multidrug-resistant; R = resistant; S = susceptible to all agents tested; MRSA/MSSA = methicillin-resistant/methicillin-susceptible *S. aureus*.

[3] MAR = multiple antibiotic resistance index.

[4] ST = sequence type. GenBank accession numbers are provided in the Data Availability Statement.

Phenotypic Antimicrobial Resistance

Resistance to penicillin was detected in 4/5 isolates (80%; K1, K2, K4, K5), to cefoxitin in 2/5 (40%; K1, K4), and to linezolid in 1/5 (20%; K1) (Fig 1A). Isolate K3 (ST45) was susceptible to all agents tested. All isolates remained susceptible to erythromycin, clindamycin, tetracycline, chloramphenicol, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, vancomycin, and nitrofurantoin.

Isolate K1 (ST22, MRSA) was resistant to agents from three categories — penicillins, anti-staphylococcal β-lactams, and oxazolidinones — and was classified as MDR (MAR index 0.25). Isolate K4 (ST22, MRSA), resistant to penicillin and cefoxitin (MAR index 0.17), was also classified as MDR because, under the criteria described in the Antimicrobial Susceptibility Testing section, any MRSA isolate is considered multidrug-resistant [10]. The proportion of MDR isolates among the clinical isolates was 40% (2/5). The MAR index was 0.08 for isolates K2 and K5 and 0 for isolate K3 (Table 1).

Antimicrobial Resistance Genes

ResFinder detected the β-lactamase gene *blaZ* in 4/5 isolates (80%; K1, K2, K4, K5), and its presence fully matched phenotypic penicillin resistance. The *mecA* gene was detected in both ST22 isolates (40%), likewise in complete agreement with cefoxitin resistance. Both ST22 isolates also carried the lincosamide nucleotidyltransferase gene *lnu*(A), although they remained susceptible to clindamycin. No acquired genes accounting for linezolid resistance in isolate K1 (*cfr*, *optrA*, *poxtA*) were identified.

CARD analysis revealed the global regulator and efflux system genes *mgrA*, *arlR*, and *mepR* in all five isolates (100%), and *arlS* in 4/5 isolates (80%). The efflux pump genes *norA* (K2, K5) and *sdrM* (K5) and the fosfomycin thiol transferase gene *fosB* (K2) showed a lineage-specific distribution. With CARD, *blaZ* was identified only in isolate K2 (Fig 1A).

Toxin, Adhesion, and Immune Evasion Genes

The classical enterotoxin gene *sea* was detected only in isolate K2 (ST5). Genes of the *egc* cluster were found in 4/5 isolates: *seg*, *selm*, *seln*, and *selo* in K1–K4, and *sei* in K1 and K4. Thus, both ST22 (MRSA) isolates carried the *egc* gene set (*seg-sei-selm-seln-selo*). The *selk* gene was detected in K2 and K3, *yent2* in K1–K4, and *yent1* only in K2. Isolate K5 (ST15) carried no classical enterotoxin or enterotoxin-like genes (Fig 1B).

The *tsst-1* gene was detected in both ST22 (MRSA) isolates, K1 and K4 (40%). The hemolysin genes *hla*, *hld*, and *hlgA/B/C* were present in all five isolates (100%). Among leukocidin genes, only *lukD* was detected (K2, K5); *lukE* and *lukM* were absent. The number of staphylococcal superantigen-like protein genes (*set*/*ssl*) ranged from 7 to 11 per genome (Fig 1B).

The adhesin genes *atl*, *ebp*, *fnbA*, *icaA*, *icaC*, and *spa* were detected in all isolates, and *icaB*, *icaR*, *sdrC*, *sdrD*, *sdrE*, and *efb* in 4/5. Variable genes included *fnbB* (K2, K3, K5), *cna* (K1, K3, K4), *ebh* (K2, K5), *eap*/*map* (K3, K4), and *clfA* (K2 only). The immune evasion genes *chp*, *scn*, and *sbi* and the capsular operon were present in all isolates, and *adsA* in all except K2 (Fig 1C).

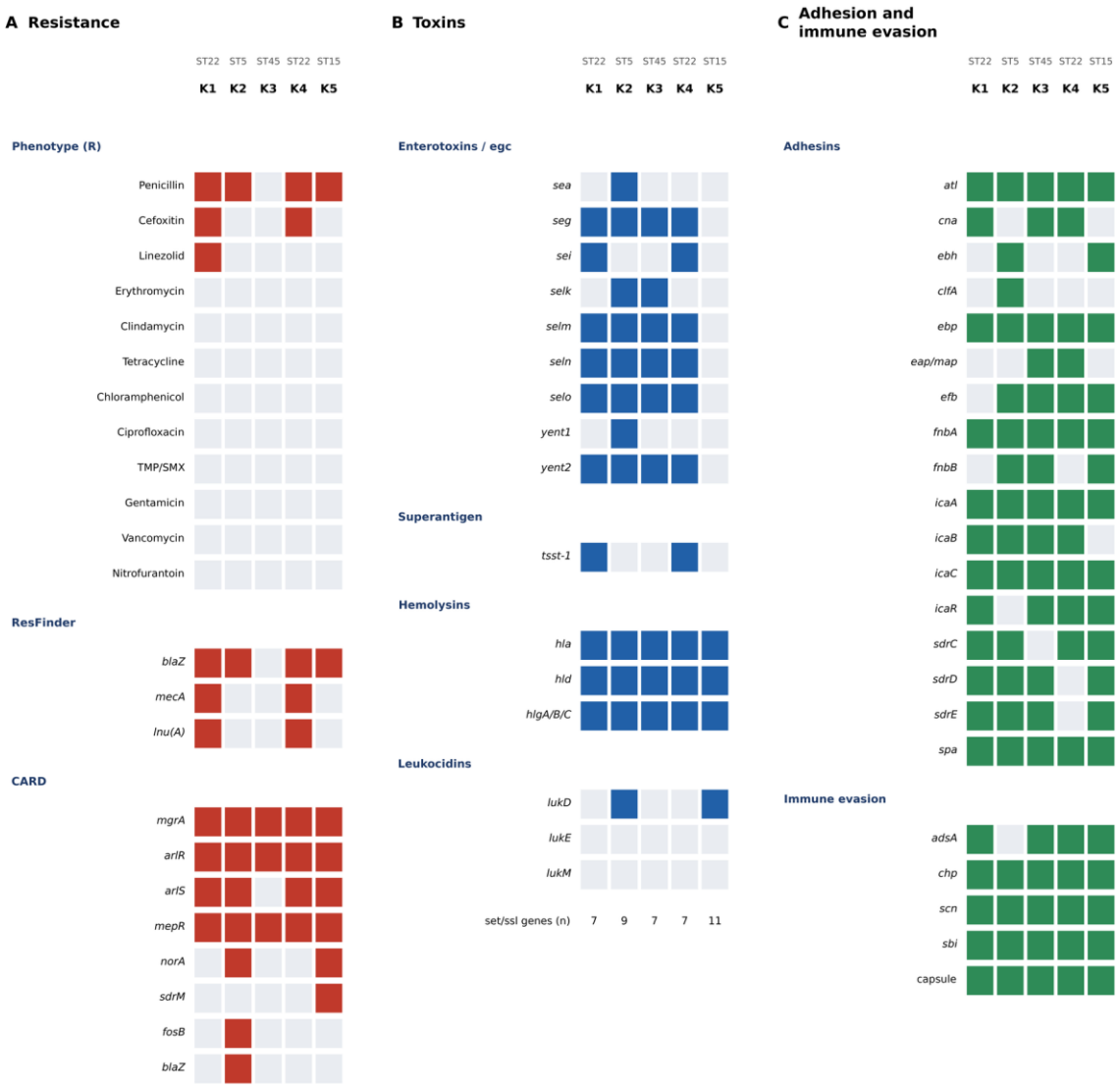


Figure 1. Fig 1. Phenotypic resistance and genetic profiles of five clinical *S. aureus* isolates (K1–K5) from children with food poisoning. (A) Phenotypic resistance to 12 antimicrobial agents, acquired resistance genes (ResFinder), and chromosomal resistance-associated determinants (CARD). (B) Genes encoding enterotoxins and the *egc* cluster, the superantigen TSST-1, hemolysins, and leukocidins; the bottom row shows the number of staphylococcal superantigen-like protein genes (*set*/*ssl*) per genome. (C) Adhesion and immune evasion genes. Filled cells indicate gene presence or phenotypic resistance; light cells indicate absence. The sequence type is shown above each isolate. No statistical comparisons were performed for these data; complete profiles of individual isolates are shown (n = 5). TMP/SMX, trimethoprim/sulfamethoxazole.

Phylogenetic Relatedness of Clinical and Food Isolates

In the phylogenetic tree based on core-genome SNPs of 47 isolates, each clinical isolate was positioned within a cluster of food isolates of the same ST with minimal branch lengths (Fig 2; Table 2).

Isolates K1 and K4 (ST22, MRSA) clustered with eight food isolates from Astana recovered from raw milk, chicken meat, chicken pâté, ready-to-eat dishes (Olivier salad, Alfredo pasta, and choux pastries), canned tuna, and beef dumplings (pelmeni). All isolates in this cluster carried the *blaZ-mecA-lnu*(A) gene combination and were resistant to penicillin and cefoxitin; in six of the eight food isolates, the toxin gene profile (*seg*, *sei*, *selm*, *seln*, *selo*, *yent2*, *tsst-1*) was identical to that of the clinical isolates.

Isolate K2 (ST5) grouped with four ST5 food isolates from chicken meat and sour cream (Kostanay region) and beef (Astana). It shared with the beef isolate the *sea* gene and the *seg*, *selk*, *selm*, *seln*, *selo*, *yent1*, and *lukD* genes; unlike three of the ST5 food isolates, K2 lacked *tsst-1* but carried *blaZ*. Isolate K3 (ST45) was nearly identical to an isolate from dumplings (Kostanay region) and fully matched it in toxin genes and CARD regulators, differing only in the absence of *blaZ* and in penicillin susceptibility. Isolate K5 (ST15) paired with an isolate from chicken fillet (Astana) with an identical set of virulence genes and CARD determinants; unlike the clinical isolate, the food isolate additionally carried *erm*(C) and was multidrug-resistant.

Table 2. Food-derived *S. aureus* isolates phylogenetically closest to the clinical isolates

Table 2.

Clinical isolate (ST)[1]	Food isolates in the cluster: source, region	Shared determinants	Differences	Ref.
K1, K4 (ST22, MRSA)	Sa8, raw milk; 118, chicken meat; 119, chicken pâté; 120, Olivier salad; 126, choux pastries; 127, canned tuna; 142, Alfredo pasta; 570, beef dumplings (all from Astana)	PEN-R, FOX-R; *blaZ*, *mecA*, *lnu*(A); *seg*, *sei*, *selm*, *seln*, *selo*, *yent2*, *tsst-1* (in 6 of 8 food isolates)	K1, additionally LZD-R; 120 lacks enterotoxin genes and *tsst-1*; 142 lacks *tsst-1* and *hld*	[9]
K2 (ST5)	Sa7, chicken fillet; 149, sour cream; 2142, chicken meat (Kostanay region); 529, beef (Astana)	*fosB*, *norA*, *arlS*; *seg*, *selk*, *selm*, *seln*, *selo*, *yent1*, *lukD*; *sea* shared with 529	K2: *blaZ*+, *tsst-1*−; Sa7, 529, 2142: *tsst-1*+; 149: MDR, *erm*(C)	[9]
K3 (ST45)	325, dumplings (Kostanay region)	Identical toxin gene set (*seg*, *selk*, *selm*, *seln*, *selo*, *yent2*, 7 *set* genes) and CARD determinants	325, *blaZ*+, PEN-R; K3, susceptible to all agents	[9]
K5 (ST15)	Sa2, chicken fillet (Astana)	PEN-R; *blaZ*; *norA*, *sdrM*, *arlS*; *lukD*, 11 *set* genes; no enterotoxin genes	Sa2, *erm*(C), MDR (PEN, ERY, CLI); K5, PEN-R only	[9]

[1] PEN = penicillin; FOX = cefoxitin; LZD = linezolid; ERY = erythromycin; CLI = clindamycin; R = resistant; MDR = multidrug-resistant; MRSA = methicillin-resistant *S. aureus*; ST = sequence type.

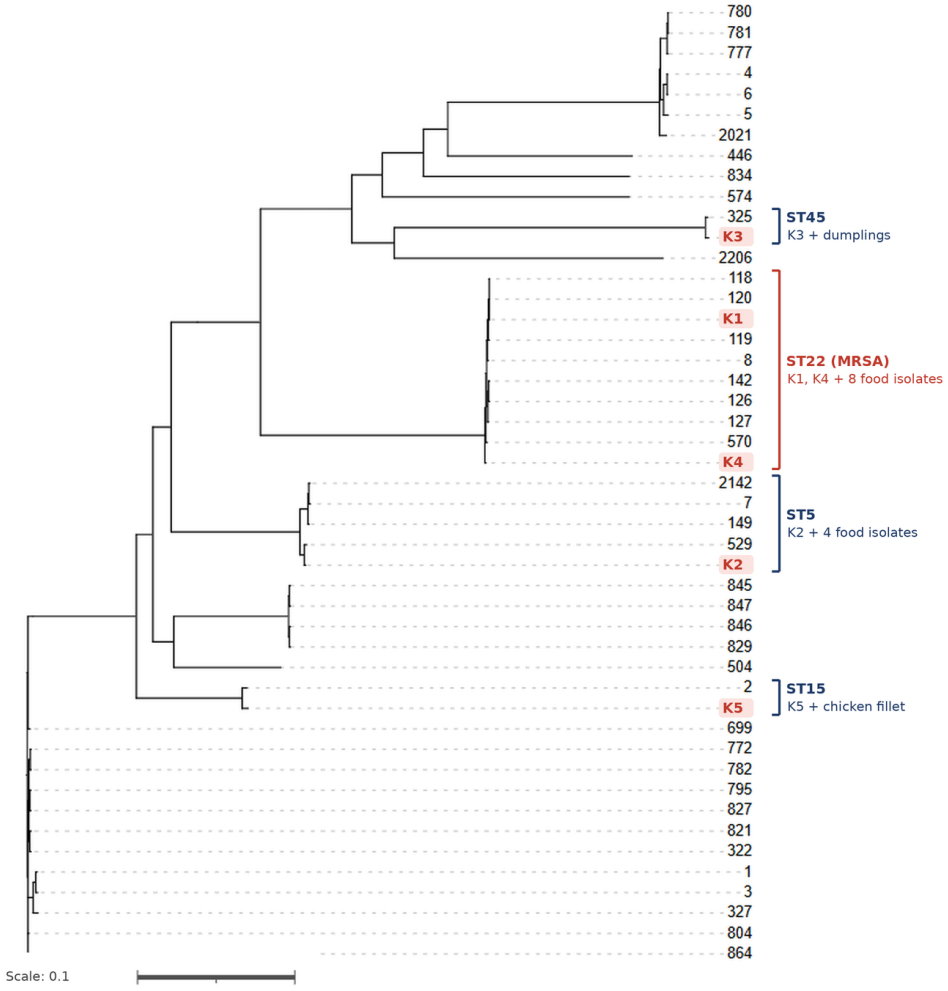


Figure 2. Fig 2. Maximum-likelihood phylogenetic tree of 47 *S. aureus* isolates (5 clinical and 42 food-derived) based on core-genome single-nucleotide polymorphisms (CSI Phylogeny v1.4). Clinical isolates (K1–K5) are shown in red; numerical labels denote food isolates [9]. Brackets mark clusters containing clinical isolates, with the sequence type indicated. Scale bar, nucleotide substitutions per site.

Discussion

This study provides the first whole-genome characterization of *S. aureus* isolates from children with food poisoning in Kazakhstan and compares them with food isolates from other regions of the country. Children are among the most vulnerable groups: SFP outbreaks in educational institutions affect dozens of people and are associated with breaches of sanitary and hygiene requirements in institutional catering [2,12].

The five clinical isolates belonged to four STs, suggesting that the cases were sporadic. Nevertheless, each clinical isolate clustered phylogenetically with food isolates of the same ST previously recovered from retail products [9]. Because the clinical isolates originated from the Pavlodar and Karaganda regions and the food isolates from Astana and the Kostanay region, the observed relatedness should be interpreted as evidence of widespread circulation of these lineages in the food chain of Kazakhstan rather than as direct identification of the source of infection. Identifying the primary source would require

concurrent sampling of suspected foods and epidemiological data, with WGS providing a resolution unattainable by conventional typing methods [8].

The most important epidemiological finding was the detection of two ST22 (MRSA) isolates that clustered with eight food isolates from raw milk, poultry meat, semi-processed meat products, and ready-to-eat dishes. The combination of methicillin resistance with *tsst-1* and the *egc* cluster is characteristic of the hypervirulent ST22 MRSA lineage described in China [13]. TSST-1 producers belong predominantly to clonal complexes CC5 and CC22 [3], and the gene itself is increasingly detected in food isolates [14] and in isolates associated with food poisoning [5]. A similar association of *tsst-1* with the ST22 MRSA clone has been reported for ready-to-eat food in Russia [15]. The frequency of *tsst-1* among the clinical isolates (2/5) was higher than that reported in food collections from the wider region, for example, in raw beef in Mongolia (17.1%) [16] and in raw shrimp in China (3.2%) [17].

The classical *sea* gene was detected only in the ST5 isolate (K2). SEA has historically been regarded as the most frequent cause of SFP, including large outbreaks [1,18]. The ST5 lineage is notable for its high adaptability and its capacity to acquire resistance and produce enterotoxins [19]; the food isolate from beef closest to K2 also carried *sea*. The remaining enterotoxigenic isolates (K1, K3, K4) carried only *egc* cluster genes and non-classical enterotoxin-like genes. A predominance of non-classical enterotoxin genes among food isolates has been reported in China and the USA [20,21], and outbreaks caused by *egc*-positive strains lacking classical enterotoxins have been documented [6]. Because commercial assays detect only SEA–SEE [18], such cases remain unrecognized in routine diagnostics. Isolate K5 (ST15) carried no enterotoxin genes; its etiological role in this poisoning case remains unclear and may reflect carriage or the involvement of other toxins or pathogens, which warrants phenotypic confirmation of enterotoxin production.

Phenotypic β-lactam resistance was fully explained by genetic determinants: *blaZ* was detected in all penicillin-resistant isolates and *mecA* in all cefoxitin-resistant isolates. The high frequency of *blaZ* is consistent with its wide distribution in the global *S. aureus* population [22,23]. The proportion of MRSA among the clinical isolates (2/5) is comparable to the *mecA* frequency (38%) among clinical isolates from hospitals in Ghana [24]. The *lnu*(A) gene, rarely reported in *S. aureus* [25], proved to be a marker of the ST22 MRSA clone in our collection. Isolate K1 was phenotypically resistant to linezolid, a last-resort agent for the treatment of MRSA infections, despite the absence of the acquired genes *cfr*, *optrA*, and *poxtA*. Oxazolidinone resistance may result from mutations in 23S rRNA genes and in the genes encoding ribosomal proteins L3/L4 [26]; this finding requires confirmation by a reference method and targeted analysis of these loci. Given the global burden of MRSA [7], the detection of MDR ST22 MRSA isolates in children with food poisoning argues for AMR monitoring along the food chain.

The regulator genes *mgrA*, *arlR*, and *mepR* were detected in all isolates, consistent with their conserved chromosomal nature and their role in regulating virulence factors and efflux systems [27,28]. The distribution of *norA*, *sdrM*, and *fosB* was lineage-specific, and the discrepancy in *blaZ* detection between ResFinder and CARD most likely reflects differences in the identity thresholds of the databases rather than biological differences.

The hemolysin genes *hla*, *hld*, and *hlgA/B/C* were present in all isolates, consistent with their status as key virulence genes in both clinical and food isolates [23]. The *lukD* gene was detected in two isolates in the absence of *lukE*, although both subunits are required to form the functional pore-forming leukotoxin LukED [29,30]. The conserved set of adhesion genes (*atl*, *ebp*, *fnbA*, *icaA/C*, *spa*) and immune evasion genes (*chp*, *scn*, *sbi*, capsule) in all isolates, together with the lineage-specific *cna*, *clfA*, *ebh*, and *eap*/*map*, suggests that colonization potential is retained irrespective of ST.

The limitations of this study include the small sample size (n = 5) and their origin from only two regions, which precludes statistically supported generalizations; the lack of data on the specific foods consumed by the patients; the absence of phenotypic confirmation of enterotoxin and TSST-1 production; and the use of Illumina short reads only, which does not allow precise localization of mobile genetic elements.

Conclusions

Clinical *S. aureus* isolates from children with food poisoning in Kazakhstan are genetically heterogeneous (ST22, ST5, ST45, ST15), and each is phylogenetically close to isolates circulating in foods in other regions of the country. The ST22 MRSA clone is of greatest concern, as it combines multidrug resistance (*blaZ*, *mecA*, *lnu*(A); and, in one case, phenotypic linezolid resistance) with *tsst-1* and the *egc* cluster. Most enterotoxigenic isolates carried only non-classical enterotoxin genes that are not detected by standard assays.

We therefore recommend integrating WGS-based typing into epidemiological investigations of food poisoning in Kazakhstan, expanding screening panels to include *egc* cluster genes and *tsst-1*, and monitoring the ST22 MRSA clone in ready-to-eat food, dairy, and meat production chains.

Supporting Information

S1 Table. Genomic and phenotypic characteristics of clinical *S. aureus* isolates K1–K5. XLSX, download

Data Availability Statement

Genome assemblies of all clinical isolates have been deposited in GenBank (National Center for Biotechnology Information, USA) under accession numbers JBQWQG000000000 (K1), JBQWQH000000000 (K2), JBQWQI000000000 (K3), JBQWQJ000000000 (K4), and JBQWQK000000000 (K5). Accession numbers of the food isolates are provided in reference [9]. All other data are included in the article and its Supporting Information files (S1 Table).

Author Contributions

Conceptualization: E.Zh., S.K., A.B.; Investigation: D.B., A.B., A.T., S.K., E.Zh.; Methodology: D.B., A.B., A.T.; Project administration: A.B.; Software: A.D.; Validation: D.B., A.B., A.T.; Visualization: A.D.; Writing – original draft: S.K., E.Zh., D.B., A.B., A.T., A.D.; Writing – review & editing: S.K., E.Zh. All authors have read and approved the final version of the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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