Outbreak Reports

A Foodborne Outbreak Associated with ST59-*spa* t441-SCC*mec* IVa Methicillin-resistant *Staphylococcus aureus* Producing Enterotoxins A and B — Puyang City, Henan Province, China, September 2024

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Summary

What is already known about this topic?

Staphylococcus aureus (*S. aureus*) represents a clinically significant pathogen and serves as a common causative agent of foodborne intoxication. The *S. aureus* strain ST59 constitutes the predominant clone associated with both community-associated methicillin-resistant *S. aureus* (CA-MRSA) and hospital-associated MRSA (HA-MRSA) infections. However, staphylococcal food poisoning (SFP) outbreaks attributed to ST59 MRSA have been documented in only a limited number of Chinese cities through retrospective investigations.

What is added by this report?

This report documents the first recorded outbreak of staphylococcal food poisoning (SFP) in Henan Province, which was attributed to the ST59-*spa* t441-SCC*mec* IVa CA-MRSA strain producing enterotoxins A and B. The confirmed source of the outbreak was contamination of donkey and goose meat with *S. aureus* enterotoxins A and B. Additionally, comprehensive genomic analysis identified multiple virulence genes and antibiotic resistance genes within the outbreak-related strains.

What are the implications for public health practice?

The identification of foodborne clones of ST59 CA-MRSA in this outbreak underscores the prevalence and transmission risks associated with this hypervirulent lineage. These findings highlight the critical need to strengthen surveillance measures for CA-MRSA among food industry workers and implement enhanced food safety protocols.

ABSTRACT

Introduction: On September 16, 2024, the Puyang City CDC received a report of a suspected foodborne disease outbreak involving 14 individuals who developed nausea, vomiting, and diarrhea following attendance at a hotel banquet. Upon notification, the District CDC immediately deployed a specialized investigation team to characterize the epidemiological features of the outbreak, identify the causative pathogen, assess potential transmission risks, and implement effective control and prevention measures.

Methods: We integrated comprehensive on-site epidemiological investigations, clinical symptom analyses, and laboratory diagnostics to isolate and identify pathogenic agents from retained food samples, environmental specimens, and anal swabs collected from affected cases. The recovered isolates underwent enterotoxin-virulence-gene profiling, antimicrobial-susceptibility testing, and phylogenetic analyses. Additionally, we characterized the architecture of the enterotoxin-A-linked pathogenicity island vSaβ.

Results: A total of 4 *S. aureus* strains were successfully isolated from 22 leftover food samples, 2 environmental swabs, and 2 patient anal swabs. Contaminated donkey and goose meat was identified as the outbreak source. All isolates harbored *sea* and *seb* enterotoxin genes, exhibited PEN-OXA-ERY-CLI resistance patterns, and were identified as clonal ST59-spa t441-SCC*mec* IVa CA-MRSA strains. Phylogenetic analysis positioned the outbreak strains within the Asia-Pacific clade, distinguishing them from the North American ST59 sublineage. Comprehensive analysis of the *sea*-associated virulence island vSaβ identified a novel structural arrangement containing a type A IEC cluster (*sea-sak-chp-scn*).

Conclusions: The detection of foodborne ST59 CA-MRSA clones in this outbreak underscores the prevalence and transmission risks associated with this hypervirulent lineage. These findings emphasize the

critical need to strengthen surveillance measures for CA-MRSA among food industry workers.

On September 16, 2024, a district CDC in Puyang City received notification of a suspected foodborne disease outbreak involving 14 individuals who developed nausea, vomiting, and diarrhea following attendance at a hotel banquet. Upon receiving this report, the District CDC immediately deployed a specialized investigation team to characterize the epidemiological features of the outbreak, identify the causative pathogen, evaluate potential transmission risks, and implement comprehensive control and prevention measures.

INVESTIGATION AND RESULTS

At 19:50 on September 16, 2024, the district CDC received notification from the Puyang City Health Commission regarding a suspected foodborne illness outbreak that had occurred during a hotel banquet at 12:00 that day. Investigation revealed that 70 individuals attended the banquet, of whom 14 subsequently developed illness. Among the affected individuals, 2 patients required hospitalization, 10 received outpatient treatment (2 with intravenous infusions and 8 with oral medication), and 2 with mild symptoms required no treatment. All patients exhibited symptom onset before 23:00 on September 16, 2024. The estimated incubation period was 4 hours, with detailed case onset times presented inSupplementary Table S1 and Figure S1 (available athttps://weekly.chinacdc.cn/). The affected population comprised 10 males and 4 females, with ages ranging from 6 to 55 years; notably, 71.4% of cases occurred in individuals aged 7–14 years. A total of 26 samples (Supplementary Table S2, available at https://weekly.chinacdc.cn/) were collected for laboratory analysis, including 22 leftover food samples, 2 environmental samples, and 2 anal swabs from patients, all submitted to Puyang CDC on September 17.

Rapid screening via multiplex fluorescence polymerase chain reaction (PCR) identified *S. aureus*-specific nucleic acids in 4 samples (Supplementary Table S2): 2 food samples and 2 anal swabs from patients (patient 1 and patient 5). Following the guidelines of GB 4789.10-2016 (National Food Safety Standards, Food Microbiology Inspection, *Staphylococcus aureus* Test) (*1*) and WS/T 80-1996 (Diagnostic Criteria and Principles of Management for Food Poisoning of *Staphylococcus aureus*) (*2*), these 4 samples underwent enrichment culture, strain isolation, Gram staining, and plasma coagulase testing. The suspected strains were confirmed as *S. aureus* through MALDI-TOF MS (Supplementary Table S2). Four *S. aureus* strains were isolated from the 4 positive samples. Fluorescent PCR assay identified the *sea* and *seb* enterotoxin genes in all 4 strains. Additionally, enzyme-linked immunosorbent assay (ELISA) detected the secreted forms of enterotoxins A and B in the culture medium (BHI) of these strains. Antimicrobial susceptibility testing using VITEK 2 Compact revealed that all 4 strains exhibited resistance to penicillin (PEN), oxacillin (OXA), erythromycin (ERY), and clindamycin (CLI). The results are presented in Figure 1A.

Whole-genome sequencing was conducted on 4

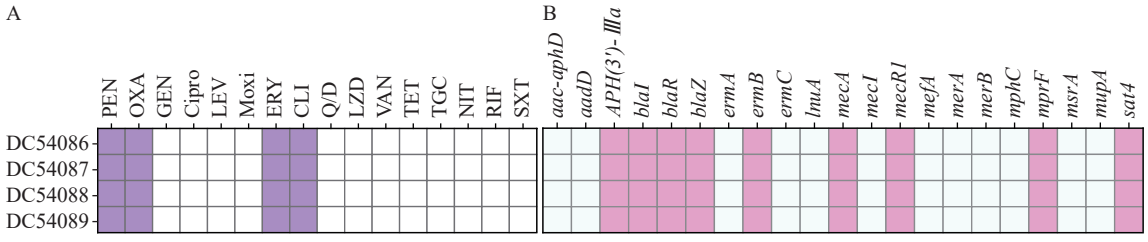

FIGURE 1. Distribution of antimicrobial resistance genes and virulence factors in four ST59 *S. aureus* isolates. (A) Heat maps display antibiotic susceptibility profiles across all strains; (B) Antimicrobial resistance gene distributions across all strains.

Note: White blocks indicate antibiotic susceptibility or gene absence, while colored blocks represent antibiotic resistance or gene presence.

Abbreviation: PEN=penicillin; OXA=oxacillin; GEN=gentamicin; Cipro=Ciprofloxacin; LEV=levofloxacin; Moxi=moxifloxacin; ERY=erythromycin; CLI=clindamycin; Q/D=quinupristin/dalfopristin; LZD=linezolid; VAN=vancomycin; TET=tetracycline; TGC=tigecycline; NIT=nitrofurantoin; RIF=rifampin; SXT=trimethoprim/sulfamethoxazole.

isolates: 3 strains underwent next-generation sequencing (NGS) analysis, while 1 strain (DC54089) was characterized using third-generation sequencing (TGS). The DC54089 genome comprised 3,444,506 bp (GC content: 36.05%) and contained 1 circular chromosome (harboring the *sea* and *seb* genes) along with 2 circular plasmids, collectively encoding 3,104 coding DNA sequences (CDS). All 4 strains shared identical origins and were confirmed as ST59-*spa* t441-SCC*mec* IVa clones, exhibiting matching antibiotic resistance and virulence gene profiles. Multiple resistance genes were detected, including *mecA*, *ermB*, *blaZ*, and *APH(3')-IIIa* (Figure 1B). Additionally, virulence genes encoding hemolysins, capsule synthesis proteins, adhesion factors, Panton–Valentine Leukocidin (PVL) toxin, exoenzymes, enterotoxins (*sea*, *seb*, *seq*, *sek*), and immune evasion cluster (IEC) components (*scn*, *sak*, and *chp*) were identified (Figure 2), demonstrating substantial pathogenic potential.

Core genome single nucleotide polymorphisms (SNPs) were identified across the 4 strains, and phylogenetic analysis was performed on the genomic sequences of these outbreak strains alongside 103 *S. aureus* ST59 reference strains using Snippy (version 4.6.0, Melbourne, Australia) (*3*). The phylogenetic tree (Figure 3) revealed that all outbreak strains isolated from food matrices clustered together with those from human samples within the same evolutionary branch (Clade China-II-I). Moreover, the staphylococcal food poisoning (SFP) strains from Henan Province formed a distinct branch (Clade Henan MRSA). Core-genome multilocus sequence typing (cgMLST) analysis demonstrated that strain DC54087 clustered tightly with the other 3 strains (DC54086, DC54088, and DC54089), differing by only a single allelic locus (≤10 alleles). These findings collectively confirm that the 4 outbreak strains originated from a common source. Detailed analysis of the *sea*-associated virulence island *vSaβ* revealed structural divergence compared to 6 reference ST59 SCC*mec* variants (Figure 4). Collinearity analysis using Easyfig (version 2.2.5, Brisbane, Australia) identified a type A IEC gene cluster (*sea-scn-sak-chp*) with partial deletions in the *ΦSa3* gene. Two copies of *scn* and distinct rearrangements of the *chp* and *scn* loci revealed a novel gene sequence within the *vSaβ* region. Based on clinical symptoms, epidemiological evidence, laboratory findings, and genomic analysis, this outbreak was determined to be a foodborne illness caused by ST59-*spa* t441-SCC*mec* IVa MRSA producing enterotoxins A and B.

PUBLIC HEALTH RESPONSE

Following these findings, the District CDC implemented comprehensive public health interventions: 1) conducted thorough disinfection of cold dish storage areas, cutting boards, and knives; 2) ensured safe disposal of all remaining food items; 3) mandated immediate self-inspection protocols within the affected hotel; 4) provided enhanced food safety training for all employees, emphasizing proper food processing techniques, rigorous hand hygiene practices, and effective disinfection protocols.

DISCUSSION

Staphylococcus aureus represents a clinically significant pathogen frequently implicated in foodborne intoxication events. This organism produces over 29 distinct staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like (SEl) toxins. Among these, SEA, SEB, SEC, SED, and SEE constitute the most prevalent enterotoxins, collectively accounting for approximately 95% of SFP outbreaks globally (*4*).

The SFP outbreak documented in Puyang City resulted from contamination of donkey and goose meat (served as cold mixed dishes) with *S. aureus*

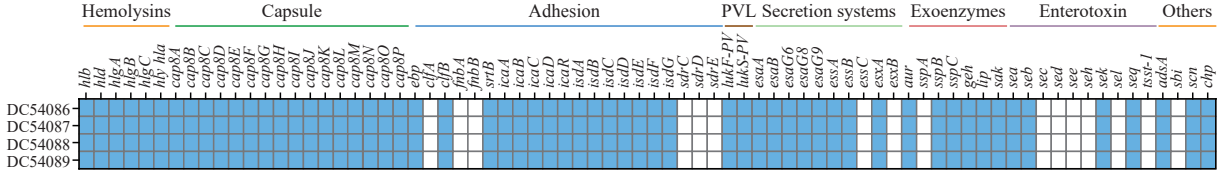

FIGURE 2. Virulence gene profiles and genetic variations across the four outbreak strains.
Note: White blocks denote gene absence, while colored blocks indicate gene presence. The horizontal color bar (left to right) represents genes associated with hemolysins, capsule formation, adhesion factors, PVL, secretion systems, exoenzymes, enterotoxins, and additional virulence determinants.
Abbreviation: PVL=panton-valentine leucocidin.

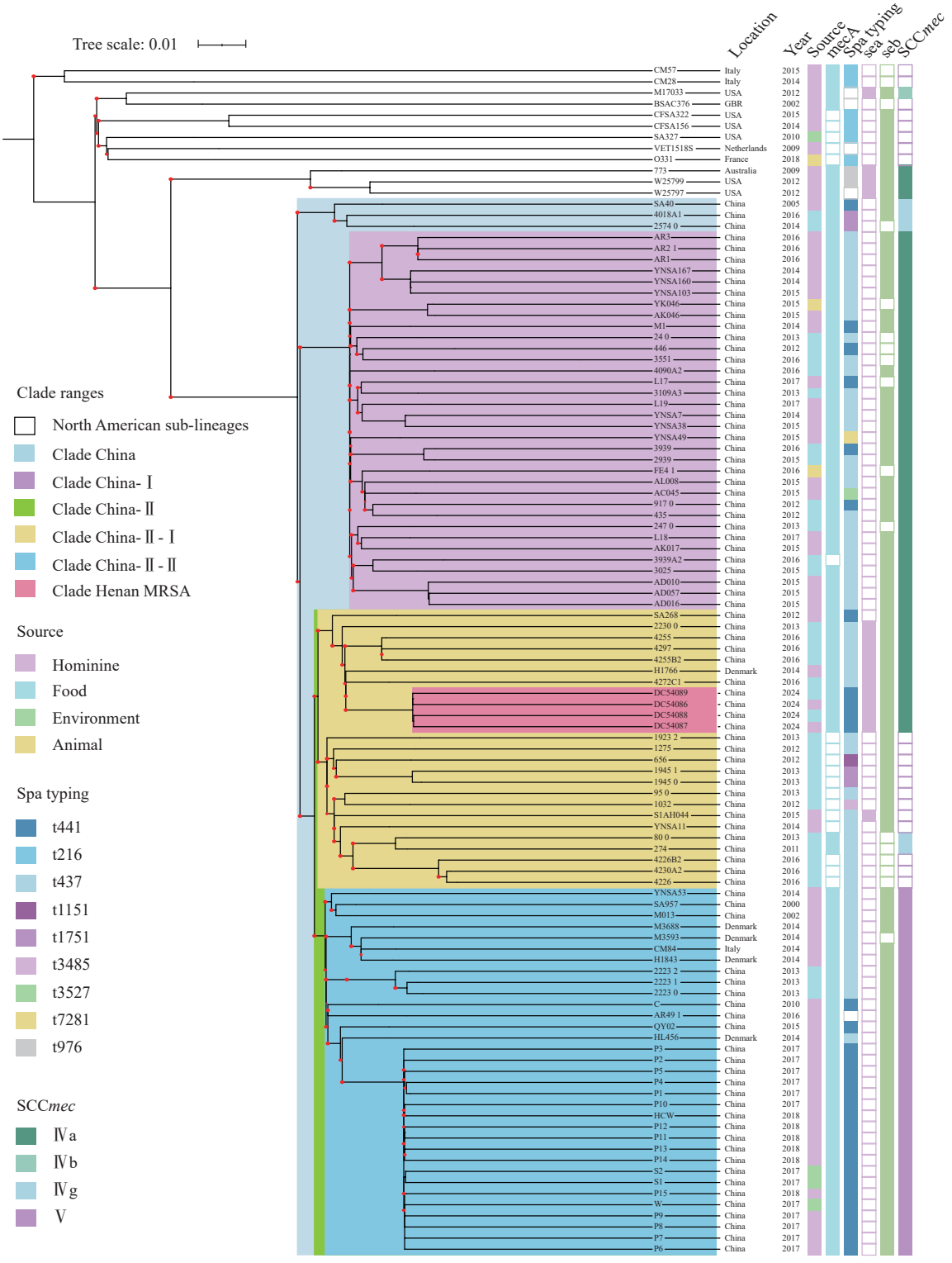

FIGURE 3. Phylogenetic reconstruction of the ST59 lineages.
Note: A total of 107 ST59 sequences (4 from this study and 103 from the NCBI database) were analyzed. The Henan MRSA ST59 isolates are shown in red. The reference genome is the completely sequenced ST59 MRSA isolate M013 (NCBI BioSample accession: CP003166). The filled squares indicate the presence of individual genes, while the empty squares indicate their absence, as specified at the top of each column. The clade ranges, sources, *spa* types, and SCC*mec* types of 107 strains have been presented on the left side.
Abbreviation: MRSA=methicillin-resistant *Staphylococcus aureus*; SCC*mec* types=*Staphylococcal* cassette chromosome *mec* types.

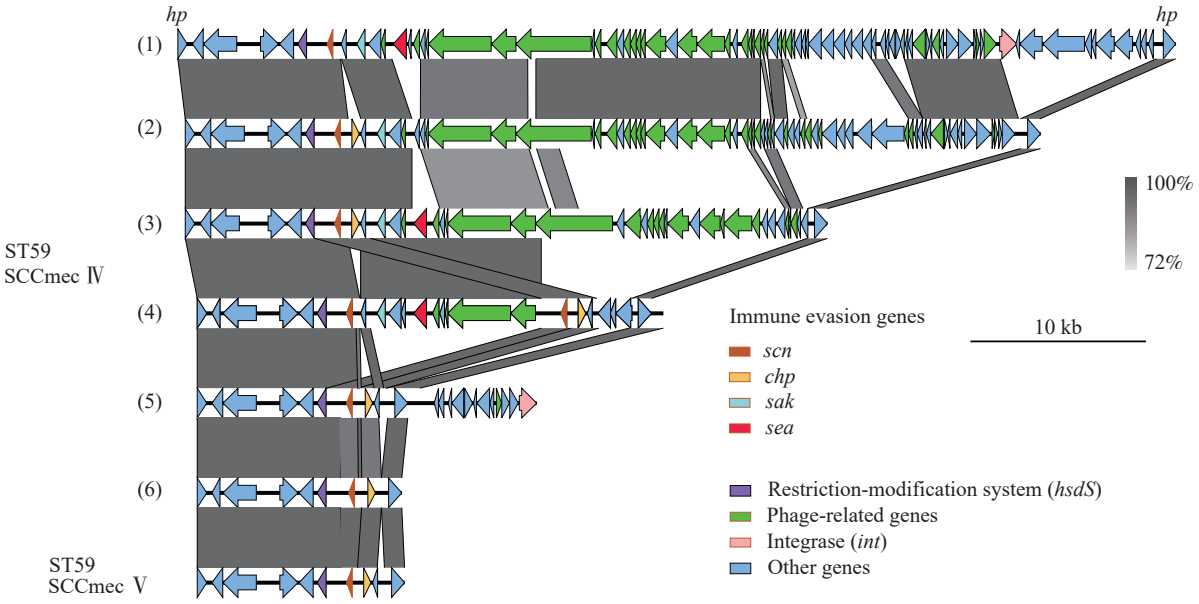

FIGURE 4. Comparison of genomic island (*vSaβ*) among ST59 isolates with different SCC*mec* types.
Note: Arrows and arrowheads represent open reading frames (ORFs) and the direction of transcription. The nucleotide similarities in the structure are indicated by grey shading. No. (4) represents the Henan representative strain DC54089.

enterotoxins A and B. This determination was substantiated through comprehensive epidemiological evidence, clinical symptom analysis, and laboratory findings. Multiple lines of evidence supported this conclusion: 1) all affected individuals shared exposure to a single hotel banquet; 2) symptom onset occurred synchronously within a 12-hour window (Supplementary Figure S1), consistent with the characteristic incubation period for SFP; 3) patients presented with uniform clinical manifestations, predominantly nausea and vomiting with or without diarrhea, which are pathognomonic for SFP; 4) multiplex fluorescence PCR and bacterial culture successfully identified *S. aureus* in specimens from two patients and two food samples. The four isolated strains demonstrated the capacity to produce enterotoxins A and B. Phylogenetic analysis of these SFP strain genomes (Clade Henan MRSA) alongside 103 ST59 sequences revealed that the outbreak strains clustered within Clade China-II-I, a lineage predominantly comprising strains of food origin. The two contaminated food items were identified as chilled donkey meat and chilled Cantonese-style roast goose. However, the absence of specimens from kitchen staff or the processing environment precluded definitive identification of the contamination source.

Currently, ST6, ST7, ST943, ST5, ST2315, ST15, ST59, and ST7591 represent the predominant causative strains of SFP in China, with most demonstrating methicillin sensitivity (*5*–*8*). Our investigation identified the ST59-t441 SEA/SEB-positive MRSA strain as the causative agent, marking the first documented SFP outbreak in Henan Province. This finding aligns with retrospective analyses demonstrating that ST59 constitutes the primary MRSA type responsible for food poisoning outbreaks across multiple Chinese cities, including Shijiazhuang, Suzhou, and locations throughout Sichuan Province (*6*–*8*). Furthermore, ST59 has emerged as the most prevalent CA-MRSA strain isolated from Eastern Asian communities and has established dominance in healthcare settings over the past two decades (*9*).

Phylogenetic analysis demonstrated that the SFP outbreak strains exhibited distant genetic relationships with North American sub-lineages while clustering closely with ST59 MRSA strains isolated from food and human samples throughout China, confirming their classification within the Asia-Pacific clone. Within the phylogenetic tree, most Chinese ST59 strains carried *seb*, whereas only strains within Clade China-II-I and North American sub-lineages harbored both *sea* and *seb* genes (Figure 3). All ST59-t441 MRSA isolates detected in this investigation carried the characteristic human IEC genes *scn*, *sak*, and *chp*, indicating their potential association with human hosts. Unfortunately, the absence of specimens from kitchen staff prevented determination of the outbreak source.

ST59 strains commonly exhibit elevated prevalence of the *seb*, *seq*, and *sek* genes. In this outbreak, the ST59-t441 MRSA strains harbored both *sea* and *seb* genes and actively secreted enterotoxins A and B *in vitro*. The synergistic action of these dual toxins likely accounts for the severe clinical manifestations and subsequent hospitalization observed in several patients. Structural analysis of the *sea*-associated virulence island *vSaβ* identified a previously unreported configuration featuring a type A IEC cluster (*sea-sak-chp-scn*). Consistent with earlier findings, genetic environment analysis indicated that the ST59-t441 MRSA strains from this outbreak lacked the characteristic *ν Saβ* structure due to staphylococcal prophage ΦSA3 insertion (*10*). Within these ST59-t441 MRSA strains, certain ΦSA3-related genes were absent, while two copies of the *scn* gene were detected. The functional implications of these genomic alterations on strain pathogenicity and horizontal *sea* transfer warrant additional investigation.

The detection of foodborne ST59 CA-MRSA clones in this outbreak underscores both the widespread distribution and transmission potential of this hypervirulent lineage. To support comprehensive risk assessment efforts, we recommend strengthening surveillance protocols for CA-MRSA among healthy populations, with a particular emphasis on food industry workers.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. The basic information, clinical symptoms and treatment of 14 patients by onset time.

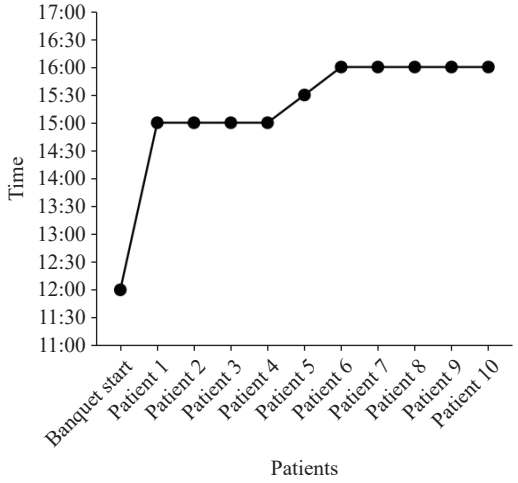
Patients	Sex	Age (years)	Onset time	Clinical symptoms	Treatment
Patient 1	Male	7	15:00, Sep 16	Vomiting (6 times), diarrhea (3 times)	Hospitalization,
Patient 2	Male	12	15:00, Sep 16	Vomiting (8 times), diarrhea (5 times)	Outpatient oral medication
Patient 3	Male	12	15:00, Sep 16	Vomiting (2 times)	Outpatient oral medication
Patient 4	Male	40	15:00, Sep 16	Nausea, diarrhea (4 times)	Outpatient oral medication
Patient 5	Male	7	15:30, Sep 16	Vomiting (8 times), diarrhea (3 times)	Hospitalization
Patient 6	Male	40	16:00, Sep 16	Vomiting (3 times)	Outpatient oral medication
Patient 7	Male	6	16:00, Sep 16	Nausea	Untreated
Patient 8	Female	12	16:00, Sep 16	Nausea, vomiting (1 time)	Untreated
Patient 9	Female	12	16:00, Sep 16	Nausea, diarrhea (3 times)	Outpatient oral medication
Patient 10	Male	13	16:00, Sep 16	Nausea, vomiting (5 times)	Outpatient oral medication
Patient 11	Female	37	−, Sep 16	Vomiting (multiple times), diarrhea (multiple times)	Outpatient intravenous infusion
Patient 12	Female	53	−, Sep 16	−	Outpatient intravenous infusion
Patient 13	Male	14	−, Sep 16	Vomiting (3 times), diarrhea (3 times)	Outpatient oral medication
Patient 14	Male	13	−, Sep 16	Vomiting (3 times), diarrhea (2 times)	Outpatient oral medication

Note: "−" means unrecorded.

SUPPLEMENTARY TABLE 2. The information and test results of the 26 samples.

Sample source	Sample name	Multiplex fluorescence PCR	MALDI-TOF MS	Enterotoxins gene	Enterotoxins protein	Strain ID
Patient	Anal swab 1 (Patient 5)	*S. aureus*	*S. aureus*	*sea*, *seb*	SEA, SEB	DC54086
	Anal swab 2 (Patient 1)	*S. aureus*	*S. aureus*	*sea*, *seb*	SEA, SEB	DC54087
Leftover food	Donkey meat	*S. aureus*	*S. aureus*	*sea*, *seb*	SEA, SEB	DC54088
	Cantonese roast goose	*S. aureus*	*S. aureus*	*sea*, *seb*	SEA, SEB	DC54089
	Five blessings platter, Scallion and beef tripe salad, Daokou braised chicken, Yam vermicelli, Crisp and refreshing celery, Secret recipe water chestnut, Dalian live abalone, Steam pot seafood shrimp, Steamed deep-sea flounder, Lucky braised pig's face, Beijing roast duck with fruit wood, Stewed turtle with old hen, Garlic-topped steamed scallops, Caterpillar fungus stuffed lion's head, Mildly spicy lamb with green peppers, Pumpkin wrapped eight treasures, Cantonese style morning glory, Guihe big steamed buns, Red date flavored steamed sponge cake, Small cake	Negative	No	No	No	No
Environment	Chopping board and Kitchen knife	Negative	No	No	No	No

Abbreviation: MALDI-TOF MS=Matrix-Assisted laser desorption/ionization time-of-flight mass spectrometry.



SUPPLEMENTARY FIGURE S1. Epidemic curve displaying patient onset times.
Note: Onset times for patients 11–14 were not recorded.