

Outbreak Reports

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Outbreak Reports

A Foodborne Outbreak Caused by ST8(CC8)-spa t024 Methicillin-Sensitive *Staphylococcus aureus* Harboring *sea*, *seq*, and *sek* — Taizhou City, Zhejiang Province, China, February 2025

Weiwei Shen^{1,8}; Xiaoyue Wei^{2,8}; Haiyan Hu³; Guiwei Zhu³; Ying Sheng¹; Ning Wang¹; Tianlan Pang³; Luwei Wang³; Lingbo Wang⁴; Li Zhan⁴; Zhangnyu Yang⁴; Xiaomei Yan^{2,9}; Haijiang Lin^{1,9}

Summary

What is already known about this topic?

The *Staphylococcus aureus* (S. aureus) sequence type 8 (ST8) clone has emerged as a dominant epidemic clone strongly associated with community-associated methicillin-resistant *S. aureus* (CA-MRSA) infections worldwide. However, documentation of this clone in China remains sparse.

What is added by this report?

This report documents the first staphylococcal food poisoning outbreak in Zhejiang Province, China, caused by methicillin-susceptible *S. aureus* (MSSA) of the ST8 (CC8)-spa t024 lineage harboring enterotoxin genes *sea*, *seq*, and *sek*. Whole-genome phylogenetic analysis revealed that the outbreak strains belonged to a distinct evolutionary subclade (Clade II.1). These strains demonstrated close genetic relatedness to previously reported European lineages.

What are the implications for public health practice?

The identification of the ST8-spa t024 MSSA strain carrying *sea*, *seq*, and *sek* genes underscores the substantial public health risk in China. This finding highlights the emerging threat of foodborne illnesses caused by novel or under-recognized *S. aureus* ST8 strains and emphasizes the urgent need for enhanced surveillance of CA-S. aureus among food handlers to prevent future outbreaks.

Methods: Comprehensive epidemiological and laboratory investigations were conducted, encompassing the isolation of pathogenic microorganisms from food samples, environmental swabs, and patient anal swabs. Recovered isolates underwent antimicrobial susceptibility testing, enterotoxin detection, whole-genome sequencing, and phylogenetic and prophage characterization.

Results: Five *S. aureus* isolates were successfully recovered from seven specimens. All isolates were identified as ST8 (CC8)-spa t024, harbored enterotoxin genes (*sea*, *seq*, and *sek*), and demonstrated penicillin resistance. Rice balls contaminated with the pathogen were identified as the outbreak source. Phylogenetic analysis revealed that the outbreak ST8 strains clustered within a distinct evolutionary subclade (Clade II.1) and exhibited close genetic relatedness to European lineages. The *sea*, *seq*, and *sek* genes were localized on a prophage carrying a type D immune evasion cluster that also encoded the *sak* and *scn* genes.

Conclusions: This represents the first documented ST8-spa t024 methicillin-susceptible *S. aureus* food poisoning outbreak in China, involving a toxigenic clone with characteristics associated with hypervirulent lineages, thereby highlighting an emerging public health threat. Enhanced surveillance of ST8 strains among food products and food handlers is urgently needed.

ABSTRACT

Introduction: The hypervirulent community-associated methicillin-resistant *Staphylococcus aureus* (S. aureus, CA-MRSA) sequence type 8 (ST8) clone has disseminated globally yet remains only sporadically documented in Chinese clinical settings. To date, staphylococcal food poisoning outbreaks attributable to ST8 strains have not been reported in China.

On February 14, 2025, the County CDC in Taizhou City, Zhejiang Province, received notification of a suspected foodborne illness outbreak at a local high school involving two students and one food handler who developed nausea, vomiting, and diarrhea after consuming food on campus. Public health investigators from the County CDC immediately launched an epidemiological investigation to

characterize the outbreak, identify the causative agent, and trace potential transmission pathways, and implement comprehensive control and prevention measures.

INVESTIGATION AND RESULTS

On February 14, 2025, at 10:00, the County CDC received a report from a township health center regarding a suspected foodborne illness outbreak that had occurred at a local high school at 09:00 that same day. Initial investigation identified three cases: one kitchen staff member who experienced symptoms before 16:30 on February 13, and two students. A case was defined as any student or faculty member who dined at the school between February 13 and 14 and subsequently experienced ≥ 3 episodes of diarrhea with altered stool consistency within 24 hours, ≥ 2 episodes of vomiting within 24 hours, or other associated gastrointestinal symptoms. Primary clinical manifestations included nausea, vomiting, abdominal pain, and diarrhea, with an estimated incubation period of approximately 2 hours.

Seven specimens were collected for laboratory diagnosis based on a comprehensive assessment of patients' clinical symptoms, suspected contaminated foods, and corresponding food processing procedures. These specimens comprised one leftover food sample, three environmental swabs, and three patients' anal swabs. All specimens underwent enrichment culture, strain isolation, Gram staining, and plasma coagulase testing in accordance with GB 4789.10–2016 "National Food Safety Standard for *Staphylococcus aureus*" (1) and WS/T 80–1996 "Diagnostic Criteria for *S. aureus* Food Poisoning" (2) guidelines. Five *S. aureus* strains were isolated through this process and verified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis (Supplementary Table S1, available at <https://weekly.chinacdc.cn/>). The remaining two specimens tested negative, including one patient's anal swab and one environmental swab. Polymerase chain reaction assays demonstrated that all five strains harbored the *sea* gene. Furthermore, secreted enterotoxin A was confirmed in all strains via enzyme-linked immunosorbent assay. Antimicrobial susceptibility testing using the broth microdilution method revealed that all five strains exhibited resistance only to penicillin (PEN, Supplementary Table S2, available at <https://weekly.chinacdc.cn/>).

Whole-genome sequencing was performed on the

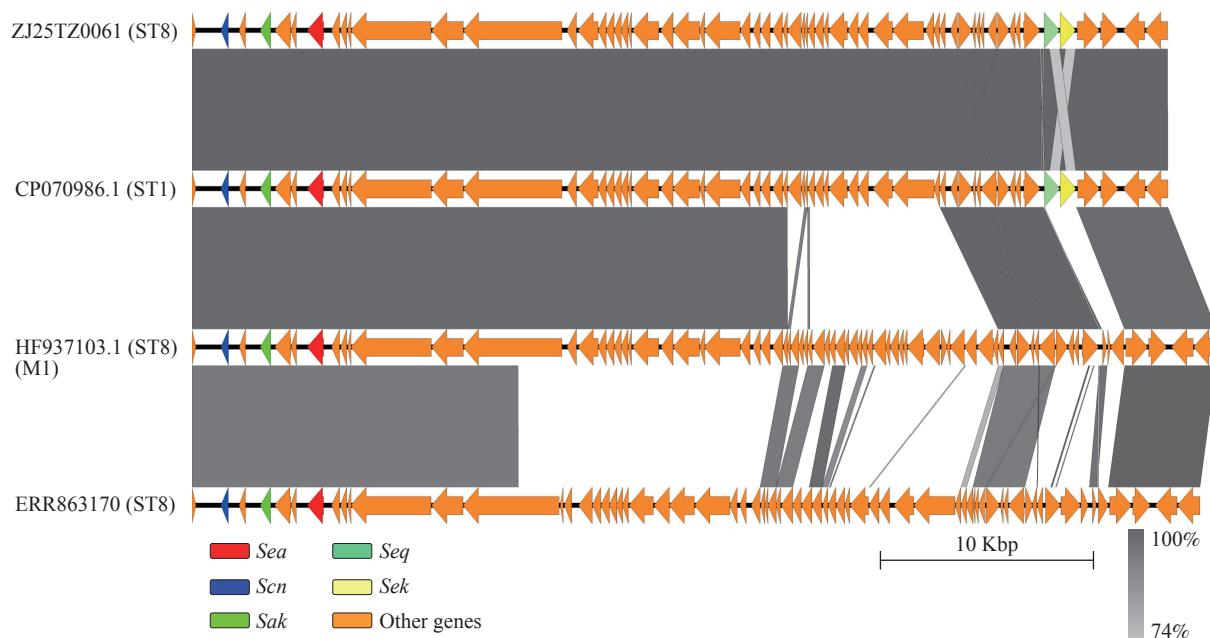
five *Staphylococcus aureus* (*S. aureus*) isolates, and the resulting sequence data were assembled using CLC Genomics Workbench (QIAGEN, Germany). Multilocus sequence typing, *spa* typing, and antibiotic resistance gene analysis demonstrated that all strains belonged to the ST8-*spa* t024 clone. These strains possessed two resistance genes: *blaZ* and *fosB*. A broad spectrum of virulence genes was detected across all isolates, including *sea*, *seq*, *sek*, and *lukDE* (Supplementary Table S3, available at <https://weekly.chinacdc.cn/>). Additionally, all strains harbored an identical prophage from the $\Phi Sa3int$ integrase group containing a type D immune evasion cluster (IEC) with the *sea-sak-scn* gene arrangement. To characterize this prophage, the structure in strain ZJ25TZ0061 was compared with the most homologous prophage (GenBank accession no. CP070986.1, belonging to the ST1/CC1 lineage) and two other *sea*-positive prophages derived from ST8 strains through collinearity analysis using Easyfig software (Version 2.2.5, Brisbane, Australia) (Figure 1). BLAST alignment between the ZJ25TZ0061 prophage and CP070986.1 yielded a maximum score of 102,200 bits with 99.52% sequence identity. Comparative analysis further revealed that the prophage-encoded protein profiles differed partially between the ZJ25TZ0061 prophage and the two *sea*-positive prophages from ST8 strains (M1 and ERR863170, respectively) (3–4).

Furthermore, whole-genome single-nucleotide polymorphism (wgSNP) analysis revealed minimal genetic variation (3–12 SNPs) among the five *S. aureus* isolates (Table 1). A core-genome SNP (cgSNP)-based phylogenetic tree incorporating 76 global ST8 sequences placed all five outbreak strains on a distinct evolutionary branch (Clade II.1), separate from other isolates (Figure 2). These genomic analyses confirmed that the outbreak isolates originated from a common source.

Integration of clinical manifestations, epidemiological data, laboratory findings, and genomic sequencing results identified the causative agent of this foodborne illness outbreak as ST8-*spa* t024 methicillin-susceptible *S. aureus* (MSSA) harboring enterotoxin genes *sea*, *seq*, and *sek*.

PUBLIC HEALTH RESPONSE

Following outbreak identification, the County CDC implemented comprehensive control measures. These interventions included thorough disinfection of all food preparation surfaces and tableware, enforcement

FIGURE 1. Comparative analysis of the prophage structure in strain ZJ25TZ0061 and other *S. aureus* prophages.

Note: Arrows and arrowheads represent ORFs and transcriptional direction, respectively. Gray shading indicates nucleotide sequence similarity across the compared regions.

Abbreviation: ORF=open reading frame.

TABLE 1. Overview of whole-genome single-nucleotide polymorphism differences among the five *S. aureus* isolates.

| Isolates number | ZJ25TZ0061 | ZJ25TZ0063 | ZJ25TZ0064 | ZJ25TZ0062 | ZJ25TZ0065 |
|-----------------|------------|------------|------------|------------|------------|
| ZJ25TZ0061 | 0 | 3 | 3 | 5 | 7 |
| ZJ25TZ0063 | 3 | 0 | 6 | 8 | 10 |
| ZJ25TZ0064 | 3 | 6 | 0 | 6 | 10 |
| ZJ25TZ0062 | 5 | 8 | 6 | 0 | 12 |
| ZJ25TZ0065 | 7 | 10 | 10 | 12 | 0 |

Note: The red-to-green color gradient represents increasing single-nucleotide polymorphism differences between isolates.

of proper food storage protocols and cooking temperature requirements, mandatory school cafeteria self-inspection procedures, intensive training programs for food handlers emphasizing personal hygiene practices and environmental disinfection protocols, and enhanced medical surveillance systems to enable rapid detection and response to potential secondary cases.

DISCUSSION

Data from China's National Foodborne Disease Outbreak Surveillance System indicate that *S. aureus* ranks as the third-most common bacterial cause of foodborne illness (5). This pathogen produces various staphylococcal enterotoxins (SEs) and SE-like toxins that mediate disease. Among these toxins, *SEA*, *SEB*,

SEC, *SED*, and *SEE* represent the most prevalent enterotoxins; notably, prophages encode the *SEA* and *SEB* enterotoxins.

Multiple lines of evidence establish that contaminated rice balls prepared by an infected kitchen staff member caused this outbreak. First, all affected individuals consumed rice balls at breakfast and subsequently developed symptoms following a short, consistent incubation period characteristic of *S. aureus* food poisoning (SFP). Second, *S. aureus* strains isolated from the kitchen staff member, environmental surfaces, and rice ball samples confirmed the contamination source. Third, all five isolated strains produced enterotoxin A. Fourth, whole-genome phylogenetic analysis demonstrated that the outbreak strains clustered closely within evolutionary Clade II.1 of the ST8 genomes. Furthermore, the 3–12 single-nucleotide polymorphism (SNP) differences among the

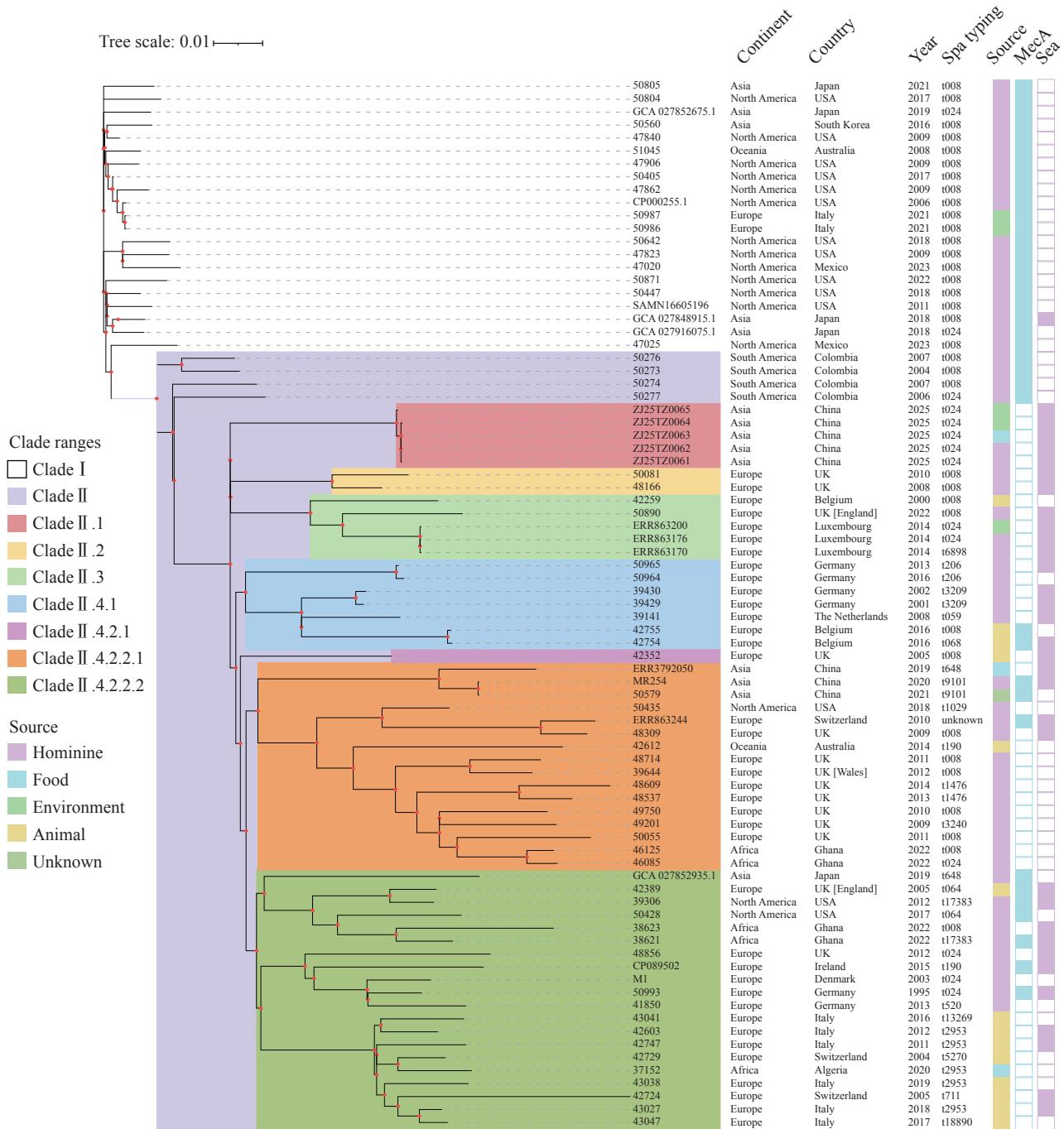


FIGURE 2. Phylogenetic reconstruction of ST8 lineages based on core-genome single-nucleotide polymorphisms.

Note: This phylogenetic analysis incorporated 81 ST8 sequences, comprising 5 isolates from the present study and 76 reference sequences retrieved from the PubMLST, NCBI, DDBJ, and ENA databases. The Taizhou ST8 isolates are highlighted in rose pink for visual distinction. The fully sequenced ST8 *S. aureus* strain NCTC 8325 (GenBank accession no. NC_007795.1) was used as the reference genome for comparative analysis. Filled squares denote the presence of specific genes, while empty squares indicate their absence, as detailed in the column headers. Clade designations and geographic origins of all 81 isolates are displayed on the left side of the phylogenetic tree.

Abbreviation: USA=United States of America; UK=United Kingdom; NCBI=National Center for Biotechnology Information; DDBJ=DNA Data Bank of Japan; ENA=European Nucleotide Archive.

five isolates fell well below the 28 SNP threshold for defining outbreak-related strains (6), confirming their common origin.

The predominant pathogenic clonal complexes (CCs) associated with SFP in China include CC7, CC1, CC5, CC398, CC188, CC59, CC6, CC88,

CC15, and CC25, with MSSA representing the majority of these strains (7). Our investigation identified the causative agent as an ST8-*spa* t024 MSSA strain harboring the *sea*, *seq*, and *sek* genes. Previous epidemiological studies have documented substantial geographic variation in ST8 *S. aureus* distribution: this clone predominates among CA-MRSA isolates in the United States, South America, Africa, and Europe. Conversely, ST8 *S. aureus* reports from Asia and the Asia-Pacific region remain limited, with only sporadic cases documented in Japan, South Korea, Pakistan, and Australia (8).

cgSNP-based phylogenetic analysis revealed that the outbreak ST8 strains formed a distinct evolutionary Clade II.1. These strains showed no close genetic relationship to the three previously reported domestic Chinese strains (ERR3792050, MR254, and ID 50579) in Clade II.4.2.2.1 (9–10); rather, they demonstrated close genetic relatedness to isolates from the United Kingdom (ID 48166 and ID 50081) in Clade II.2. These UK isolates were recovered from human blood samples in Europe in 2008 and 2010, respectively. This phylogenetic proximity suggests that the outbreak strains may have originated in Europe, potentially introduced through international human travel or global food supply chains.

The ST8 clone represents a globally disseminated, hypervirulent lineage predominantly associated with CA-MRSA, typically harboring virulence factors including enterotoxin genes, the Panton-Valentine leukocidin (PVL) gene, and the staphylococcal cassette chromosome *mec* (SCC*mec*) element. In this outbreak, however, the ST8-*spa* t024 strains were MSSA carrying enterotoxin genes *sea*, *seq*, and *sek*, along with IEC genes *scn* and *sak*, as well as the toxin gene *lukDE*. This virulence gene profile demonstrates that these MSSA strains possess characteristics typical of hypervirulent clones and may therefore pose a substantial public health threat.

Structural analysis of the prophage in strain ZJ25TZ0061, which harbors the type D IEC cluster (*sea*–*sak*–*scn*), revealed high sequence homology to prophages found in ST1(CC1) strains (represented by accession no. CP070986.1). This finding suggests that bacteriophages in SFP-associated strains may facilitate horizontal gene transfer across diverse ST/CC lineages. To elucidate this process more comprehensively, additional research should address two critical questions: how these genomic elements influence *S. aureus* pathogenicity, and what molecular mechanisms enable the horizontal transfer of the *sea*-prophage.

This outbreak represents the first documented emergence of a foodborne ST8-*spa* t024 MSSA clone in China, underscoring both the expansion of this lineage and its considerable pathogenic potential. These findings highlight the need for enhanced surveillance of CA-MSSA in asymptomatic carriers, particularly among food handlers, to strengthen food safety risk assessments and prevent future outbreaks.

Conflicts of interest: No conflicts of interest.

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY TABLE S1. Basic characteristics of the 5 *S. aureus* isolates.

| Isolate name | Origin | Sex | Age | Specimen |
|--------------|----------------|--------|-----|--------------------|
| ZJ25TZ0061 | Kitchen staff | Female | 46 | Anal swab |
| ZJ25TZ0062 | Student | Male | 17 | Anal swab |
| ZJ25TZ0063 | Rice ball | - | - | Leftover food |
| ZJ25TZ0064 | Chopping board | - | - | Environmental swab |
| ZJ25TZ0065 | Kitchen knife | - | - | Environmental swab |

Note: “-” means the sample type is not applicable for age and sex information.

SUPPLEMENTARY TABLE S2. Antimicrobial susceptibility profiles of the 5 *S. aureus* isolates.

| Antimicrobial agent | MIC (μg/mL) | S/R |
|---------------------|-------------|-----|
| Penicillin | >2 | R |
| Oxacillin | 0.25 | S |
| Cefoxitin | 4 | S |
| Gentamicin | 0.5 | S |
| Erythromycin | ≤0.25 | S |
| Clindamycin | 0.25 | S |
| Levofloxacin | 0.25 | S |
| Vancomycin | 1 | S |
| Teicoplanin | ≤0.5 | S |
| Linezolid | ≤1 | S |
| Co-trimoxazole | ≤0.25/4.75 | S |
| Rifampicin | ≤0.06 | S |
| Nitrofurantoin | ≤16 | S |
| Daptomycin | 0.25 | S |
| Tetracycline | ≤0.25 | S |

Abbreviation: MIC=Minimum Inhibitory Concentration; S=susceptibility; R=resistance.

SUPPLEMENTARY TABLE S3. Virulence gene profiles of the 5 *S. aureus* isolates.

| Virulence factors | Gene names |
|-------------------|--|
| Adhesion | <i>isdG, srtB, isdF, isdE, isdD, isdC, isdA, isdB, icaR, icaA, icaB, icaC, icaD, sdrC, sdrD, sdrE, clfA, clfB, fnbA, fnbB, ebp</i> |
| Capsule | <i>cap8A, cap8B, cap8C, cap8D, cap8E, cap8F, cap8G, cap8L, cap8M, capN, cap8O, cap8P</i> |
| Secretion system | <i>esxA, esaA, essA, esaB, essB, essC, esxC, esxB</i> |
| Exoenzymes | <i>aur, sspA, sspB, sspC, sak, lip, geh</i> |
| Hemolysins | <i>hla, hlgA, hlgB, hlgC</i> |
| Enterotoxin | <i>sea, seq, sek</i> |
| Leukotoxins | <i>lukDE</i> |
| Others | <i>map, sbi, scn, coa, adsA, hysA, spa, vWbp</i> |

Multi-City Outbreak of *Staphylococcus aureus* Infections Linked to Durian Mille-Feuille Cakes — Shandong Province, China, May–June 2025

Maoqiang Zhuang¹; Xinpeng Li¹; Peirui Xiao¹; Zhengqiao Kong¹; Jian Song¹; Zunhua Chu¹; Liansen Wang^{1,2}

Summary

What is already known about this topic?

Staphylococcus aureus (*S. aureus*) is a Gram-positive, halotolerant bacterium. Enterotoxin-producing strains of *S. aureus* can cause foodborne outbreaks in humans, with incubation periods typically ranging from 1 to 6 hours.

What is added by this report?

Investigation identified 22 cases aged 9–61 years. All patients experienced diarrhea. Durian mille-feuille cakes were identified as the suspected food vehicle. cgMLST analysis demonstrated that clinical isolates, food samples, and food handler isolates exhibited minimal genetic divergence.

What are the implications for public health practice?

Comprehensive hygiene control measures throughout the production chain are critical for cold-processed pastries, encompassing thorough fruit sanitation, rigorous microbial monitoring of raw ingredients, robust employee health surveillance, and uninterrupted cold chain maintenance with storage temperatures consistently below 4 °C. Franchise food operations demand enhanced regulatory oversight despite decentralized supply chains, underscoring the necessity for uniform food safety protocols implemented across all retail outlets.

ABSTRACT

Introduction: Foodborne disease incidence peaks during summer months when elevated temperatures and humidity create optimal conditions for pathogen proliferation. Cold-processed pastries represent particularly high-risk foods due to their susceptibility to bacterial contamination. Between May and June 2025, multiple foodborne disease outbreaks occurred across Shandong Province, all epidemiologically linked

to durian mille-feuille cakes from a single franchise brand.

Methods: Centers for Disease Control and Prevention in three cities (Qingdao, Yantai, and Tai'an) conducted comprehensive epidemiological investigations from May to June 2025. Investigations included structured patient interviews, supply chain traceback analyses, and laboratory testing employing whole genome multilocus sequence typing (wgMLST) for high-resolution pathogen characterization.

Results: Twenty-two confirmed cases were identified across the three cities, with a median age of 30 years; 64% of cases were female. Laboratory analysis identified *Staphylococcus aureus* producing enterotoxins A through D as the causative pathogen. wgMLST analysis demonstrated that clinical isolates, food samples, and food handler isolates differed by only 0–3 alleles across gene loci, indicating exceptionally high genetic relatedness. Epidemiological and molecular evidence identified food handlers as the probable contamination source rather than raw materials.

Conclusion: This multi-city outbreak resulted from durian mille-feuille cakes contaminated by food handlers carrying *S. aureus*. The franchise business model, characterized by decentralized production and independent ingredient sourcing, contributed to the prolonged temporal span and geographic dispersion of the outbreak. We recommend strengthening hygiene controls throughout the cold-processed pastry production chain, including enhanced employee health surveillance, standardized hand hygiene protocols, and strict temperature control during storage and transport.

On June 18, 2025, during routine surveillance of the foodborne disease outbreak reporting system, we identified a foodborne disease outbreak reported by Pingdu CDC on June 16 that involved consumption

of durian mille-feuille cakes from a specific franchise brand. Recognizing the potential for multi-city transmission through this franchise network, we immediately contacted Qingdao CDC to evaluate preliminary findings and coordinate response measures. We subsequently expanded case searches through the foodborne disease surveillance system and initiated comprehensive investigations to determine the outbreak's etiology and extent, and to implement appropriate control measures.

INVESTIGATION AND RESULTS

Case Definition

Individuals who developed one or more of the following symptoms — nausea, vomiting, diarrhea (≥ 3 bowel movements per 24 hours with altered stool consistency), or abdominal pain — after consuming food from a franchise outlet between May 28 and June 23, 2025. A total of 22 cases were confirmed across three cities (Qingdao, Yantai, Taian). During May and June, CDCs in these cities conducted standardized questionnaire surveys of affected patients.

The Qingdao outbreak involved a single family who purchased durian mille-feuille cake on May 27. One family member developed illness on May 28 but self-medicated without seeking medical attention. Three additional family members developed symptoms on May 29.

The Yantai cases comprised five distinct exposure groups. Group 1 consisted of a three-member household who purchased and consumed the cake on June 7; all three subsequently developed illness. Group 2 involved work colleagues who purchased the cake on June 7 and shared it; both consumers became ill. Group 3 included employees from another company who purchased the cake on June 8; one employee who

consumed the cake became ill, while two colleagues who also consumed it remained asymptomatic. Groups 4 and 5 each involved separate households who purchased cakes on June 8. In both groups, patients consumed refrigerated leftover cake on June 9 and subsequently developed symptoms. In Group 4, the patient consumed the remaining half of the cake. In Group 5, the patient's son consumed a small portion on June 8 without becoming ill, while the patient consumed the remaining cake the following day and developed symptoms.

The Taian cases involved three separate families. In Family 1, three members became ill after consuming durian mille-feuille cake purchased on June 21. In Family 2, three members developed symptoms following consumption of cake purchased on June 22. In Family 3, five members fell ill after eating cake also purchased on June 22. Notably, all household members who consumed the durian mille-feuille cake became ill, while those who did not consume it remained healthy.

The median patient age was 30 years (range: 9–61 years), with 14 cases (64%) being female. Illness onset dates spanned from May 28 to June 23, 2025 (Figure 1).

Clinical manifestations are detailed in Table 1. No severe cases or deaths occurred. A total of 16 patients sought medical care, while 6 self-managed their symptoms at home. Food exposure investigation revealed that all 22 cases had consumed durian mille-feuille cakes. Among family members and friends who shared meals with cases, 7 individuals consumed the cakes but did not develop illness, while all individuals who did not consume the cakes remained healthy.

Traceback Investigation

Qingdao, Yantai and Tai'an CDC investigators

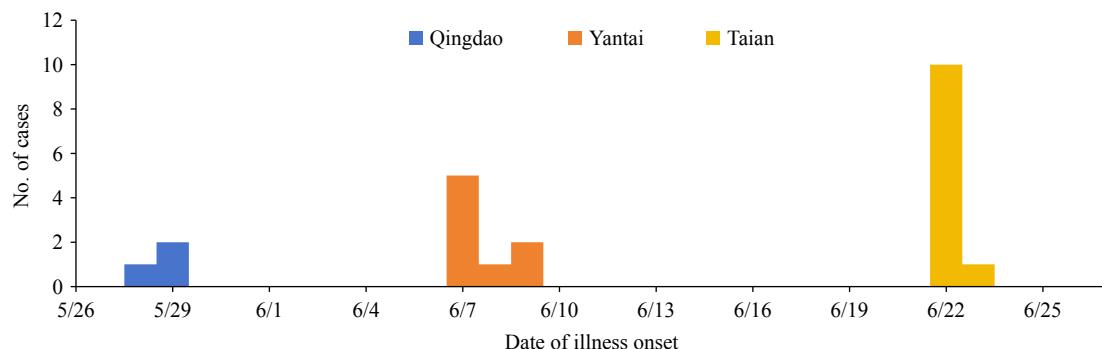


FIGURE 1. Number of persons (N=22) by date of illness onset — three cities, Shandong Province, China, May 28–June 23, 2025.

TABLE 1. Clinical manifestations of cases in an outbreak of *Staphylococcus aureus* infections — Shandong Province, May–June 2025 (N=22).

| Symptom | No. | Percentage (%) |
|----------------|-----|----------------|
| Diarrhea | 22 | 100.00 |
| Abdominal pain | 21 | 95.45 |
| Vomiting | 18 | 81.82 |
| Nausea | 16 | 72.73 |
| Headache | 5 | 22.73 |
| Dizziness | 2 | 9.09 |
| Chills | 1 | 4.55 |

conducted site visits to cake shops where patients had purchased and consumed durian mille-feuille cakes. During these visits, they collected environmental swabs, equipment samples, and employee specimens, while performing comprehensive traceback investigations to identify the sources of remaining cakes and their constituent raw materials, including all ingredient distributors. This brand operates through a franchise model in which individual stores receive training at headquarters before opening outlets under the brand name. Critically, headquarters does not centrally distribute raw materials or finished products; instead, each franchise store independently procures all required ingredients. Supply chain analysis revealed that three franchise stores across Qingdao, Yantai, and Weihai cities sourced pitted durian flesh from Thailand through the same supplier, while all other ingredients came from different sources. Although all franchise stores were required to purchase a specified cream brand from New Zealand, each store obtained this cream through different distributors, with no common supplier identified.

Laboratory Investigation

Investigators collected 50 samples for analysis: 13 patient samples (9 anal swabs, 3 vomitus samples, and 1 fecal sample), 10 food handler samples (6 anal swabs and 4 nasal swabs), 16 food samples, and 11 environmental swabs. All samples underwent polymerase chain reaction (PCR) testing and bacterial isolation for *Staphylococcus aureus* (*S. aureus*). PCR testing yielded positive results for 5 patient anal swabs, 6 food samples, and 3 food handler nasal swabs. Pulsed-field gel electrophoresis (PFGE) analysis revealed that all isolates exhibited identical banding patterns. Further characterization using core genome multilocus sequence typing (cgMLST) demonstrated that clinical isolates, food sample isolates, and food

handler isolates differed by only 0–3 alleles across gene loci, indicating high genetic relatedness and strongly suggesting a common contamination source (Figure 2). Virulence gene testing detected staphylococcal enterotoxins A and D in isolates from patient, food, and food handler samples. Notably, the genetic sequence of *S. aureus* isolated during this outbreak exhibited low homology with strains previously detected through Shandong Province's food safety risk monitoring programs.

PUBLIC HEALTH RESPONSE

On June 20, 2025, the Shandong CDC reported the investigation findings to the Shandong Provincial Health Commission regarding a suspected foodborne outbreak linked to durian mille-feuille cakes from a specific brand. Municipal CDCs communicated the investigation results to local market regulation authorities and collaborated with regulators to conduct additional sampling and testing, further investigating to identify contamination sources and transmission pathways. Local market regulation authorities sealed and disinfected affected facilities, enhanced training for operators and staff, and standardized food processing procedures. No additional foodborne illness cases linked to this brand's cake shops were reported after June 23, 2025.

DISCUSSION

Summer represents the high-incidence season for foodborne diseases, a pattern supported by clear epidemiological evidence globally. Elevated temperature and humidity conditions significantly accelerate the growth and reproduction of foodborne pathogens, thereby increasing outbreak risk following food contamination. Rising temperatures demonstrate a positive correlation with foodborne disease incidence rates. Specifically, within the 20 °C to 45 °C range, various foodborne pathogens can rapidly multiply, enabling pathogen concentrations in food to reach infectious levels within hours. In China, foodborne disease surveillance data demonstrates that June through September constitutes the peak period for bacterial foodborne diseases, with *Salmonella*, *S. aureus*, and diarrheal *E. coli* (such as O157:H7) representing the most common causative agents (1).

The epidemiological investigation revealed that some cases shared only durian mille-feuille cake as their

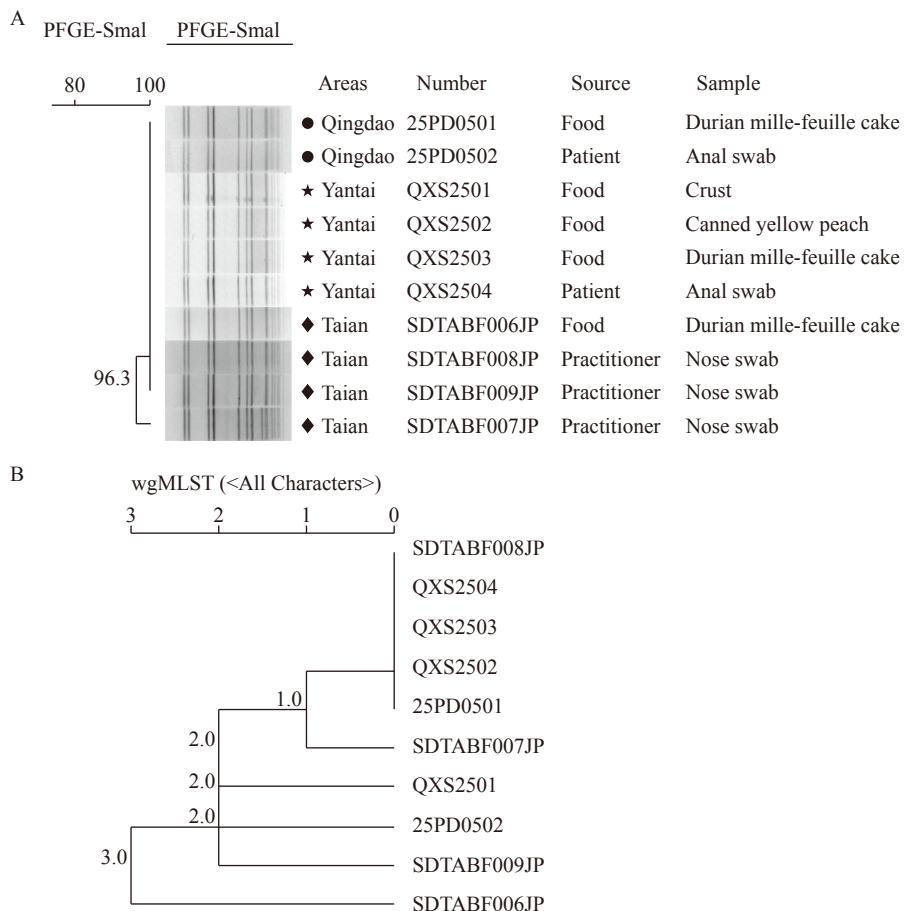


FIGURE 2. Electrophoresis and whole genome sequences analysis of *Staphylococcus aureus*. (A) Pulsed-field gel electrophoresis; (B) Whole genome multilocus sequence typing.

Abbreviation: PFGE=Pulsed-Field Gel Electrophoresis; wgMLST=Core genome multilocus sequence typing.

common dietary exposure, while family members or friends who had not consumed the cakes remained healthy. Traceback investigation results demonstrated that, except for cream which must be sourced from a specified brand, no other raw materials required by franchise outlets were subject to brand specifications. The headquarters did not provide unified distribution of raw materials or finished products; instead, all required ingredients were purchased independently by individual franchise stores. Franchise stores in different cities procured supplies from different suppliers, with no common source identified. Strong genetic relationships existed between clinical isolates and food isolates, which, combined with epidemiological and traceback evidence, confirmed that durian mille-feuille cakes were the likely source of these outbreaks.

Regarding the contamination source, epidemiological investigation could not identify common raw material suppliers, and no pathogens were detected in raw materials. However, the same *S.*

aureus strain was isolated from food handlers, remaining food samples, and patient specimens. These findings strongly suggest that food handlers carrying *S. aureus* served as the contamination source, particularly during cake preparation steps involving direct product handling, such as cream spreading and durian placement. Cross-contamination represents another critical factor contributing to *S. aureus* foodborne outbreaks, especially when cleaning protocols in food preparation areas are inadequate. Furthermore, *S. aureus* enterotoxin production requires specific temperature and time conditions. Without proper temperature control during storage and distribution, even minimal initial contamination can generate sufficient enterotoxin levels to cause illness within hours.

Laboratory testing, particularly molecular typing techniques, proved decisive in establishing epidemiological links between outbreaks. PFGE and cgMLST analyses successfully connected three

apparently independent events, confirming they originated from the same *S. aureus* strain and demonstrating the essential role of laboratory data in outbreak investigations. Whole-genome sequencing has increasingly replaced PFGE as the preferred molecular typing method for foodborne disease investigations, including listeriosis outbreaks, with its importance continuing to grow. This technology offers substantial advantages for analyzing microbial genetic characteristics, tracking strain evolution, and tracing outbreak sources, thereby providing enhanced technical support for foodborne disease surveillance and investigation. In this outbreak, genome sequencing not only confirmed the epidemiological connections between events but also generated scientific evidence to guide contamination source identification and control strategy development, underscoring that rapid, accurate pathogen characterization is fundamental to protecting public health during foodborne disease investigations.

This outbreak exhibited an atypical epidemiological pattern warranting detailed discussion. Despite originating from a common food vehicle sold under the same franchise brand, cases were distributed across three cities over a 27-day period. This pattern differs markedly from typical point-source foodborne outbreaks, which characteristically present with sharp epidemic curves and cases clustered both temporally and geographically.

The franchise business model directly explains this unusual temporal and spatial distribution pattern. Unlike centralized production systems where a single contaminated batch simultaneously affects multiple consumers, this franchise operated with completely decentralized production — each outlet independently purchased raw materials and produced cakes locally. Consequently, contamination events occurred independently at different times and locations, generating scattered outbreak waves rather than a single concentrated epidemic peak.

The franchise training system inadvertently facilitated multi-city pathogen spread. Although headquarters provided standardized production training, it failed to establish unified hygiene protocols, regular health screening requirements, or occupational health management systems for franchise staff. This critical gap allowed asymptomatic carrier status among food handlers to persist undetected across multiple outlets. The manual-intensive production process, particularly steps requiring direct hand contact with ready-to-eat products, created numerous

contamination opportunities that were amplified by inadequate hand hygiene practices.

This outbreak underscores the critical importance of preventing contamination in cold-processed pastries to mitigate foodborne disease risks. Durian mille-feuille cakes and similar cold-processed pastries represent typical high-risk foods whose ingredients and processing characteristics render them highly susceptible to pathogenic bacterial colonization. First, cake cream and cake bases are rich in proteins, carbohydrates, and moisture, providing an optimal nutritional substrate for microbial proliferation. *S. aureus* can rapidly multiply in cream-based foods under favorable temperatures and produce enterotoxins (2). Second, durian and other fruits used as decorative or filling materials typically undergo only simple rinsing with clean water, which inadequately removes surface-attached microorganisms. Research has demonstrated that fresh fruit surfaces may harbor *E. coli* or *Listeria* (3–4). Furthermore, the production process for such pastries involves multiple manual operations, including pastry layering, cream spreading, and fruit placement. When operators maintain inadequate hand hygiene or harbor asymptomatic infections, direct contact readily contaminates final products. Multiple foodborne disease outbreak investigations have confirmed that carrier operators frequently serve as contamination sources in pastry-related incidents (5).

In high-temperature summer environments, comprehensive hygiene controls must be strengthened throughout the entire production chain — from raw material handling and processing through storage and transportation — for cold-processed pastries with high moisture and nutrient content. We recommend the following measures: 1) effective cleaning and sanitization of fruit raw materials; 2) strict microbiological control of raw materials such as cream and egg liquid; 3) enhanced employee health management and rigorous hand hygiene training; 4) maintaining cold chain continuity, with finished product storage temperatures consistently below 4 °C to inhibit pathogen growth (6). Only through systematic risk control across all production stages can the likelihood of such foods causing foodborne disease outbreaks be effectively minimized.

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A Local Chikungunya Fever Outbreak Field Investigation — Fujian Province, China, 2025

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Summary

What is already known about this topic?

In China, both imported and locally transmitted Chikungunya fever (CHIKF) cases have been documented in southern provinces. The urban transmission chain comprises three critical links: viremic humans, the extrinsic incubation period (EIP, 2–10 days), and the intrinsic incubation period (IIP, 1–12 days).

What is added by this report?

This outbreak in Fujian Province demonstrated that in a non-endemic area for CHIKF, the introduction of an imported viremic case combined with the presence of local *Aedes* mosquitoes can establish local transmission within approximately 9 days through mosquito vectors.

What are the implications for public health practice?

In regions with established *Aedes* mosquito populations, imported CHIKF cases may trigger import-driven local transmission if vector control measures are delayed or inadequately implemented. During epidemic seasons, upon identifying imported cases, public health authorities should immediately map the patients' viremic-phase activity areas, delineate high-risk zones, and implement targeted mosquito elimination measures.

ABSTRACT

Introduction: In late August 2025, a locally transmitted CHIKF outbreak was detected in Licheng District, Quanzhou City, Fujian Province. On September 3, 2025, two locally acquired cases without travel history to Licheng District were reported in the adjacent Nan'an City, Quanzhou City. This study aimed to identify the infection source of the local chikungunya cases in Nan'an City.

Methods: Field epidemiological investigations were conducted to collect case-related information and trace

the infection source. *Aedes* mosquito density was monitored in the core epidemic area. Whole-genome amplification and sequencing were performed on chikungunya virus (CHIKV) in the serum samples of the cases. The obtained sequences were aligned with those from GenBank, and a phylogenetic tree was constructed for viral genotypic analysis.

Results: Nine days before the onset of the two local cases, an imported chikungunya case from Licheng District had received treatment at Clinic E near their residential area. Whole-genome sequencing revealed complete identity among the CHIKV strains from the two local cases and the one imported case, all belonging to the ECSA genotype.

Conclusion: Epidemiological link between the locally acquired CHIKF cases in Nan'an City, Quanzhou and the imported case from Licheng District, Quanzhou. In a previously CHIKV-free non-endemic area with *Aedes* mosquitoes, secondary local cases may emerge approximately 9 days after the introduction of imported viremic cases.

CHIKF is an acute mosquito-borne infectious disease caused by CHIKV, characterized by fever, rash, and severe arthralgia (1). Global climate change, increased international travel and trade, have expanded it from tropical/subtropical to temperate zones, posing a persistent global public health threat (2). In southern China, widespread *Aedes albopictus* has facilitated imported cases and subsequent local transmission (3–5).

The urban CHIKV transmission cycle involves three key epidemiological links: viremic human hosts, infected mosquito vectors (with a typically 2–10 days EIP), and susceptible individuals (with a typically 1–12 days IIP) (6). While laboratory studies have defined EIP and IIP ranges, empirical field data on the

complete transmission cycle (EIP+IIP) during real-world remain scarce. Such data are critical for establishing evidence-based timeframes to guide grassroots public health interventions.

In late August 2025, a locally transmitted CHIKV outbreak was reported in Licheng District, Quanzhou City, Fujian Province. On September 3, two locally acquired cases involving siblings were identified in the adjacent Nan'an City, Quanzhou City. Notably, neither case reported travel to Licheng District or other CHIKV-endemic areas within the 12 days preceding symptom onset. This study aimed to identify the infection source of the local chikungunya cases in Nan'an City, and provide empirical evidence to inform prevention and control strategies against importation-driven local transmission in non-endemic regions.

The Nan'an CDC collected four datasets: 1) clinical manifestations and laboratory results of the two index cases and their co-exposed individuals; 2) detailed activity trajectories and exposure histories during the 12 days preceding symptom onset; 3) entomological surveillance data (Breteau Index measurements for *Aedes* larvae and Adult Biting Index assessments for adult mosquito density) within the 100-meter radius around Clinic E and the patients' residences; and 4) epidemiological linkage data to trace potential infection sources.

Viral nucleic acids were extracted using a magnetic bead-based nucleic acid extraction kit (ZYHJ, 2507003) and an automated extraction system (EXM 3000). Serum samples underwent CHIKV nucleic acid testing with a triple detection kit for dengue virus, Zika virus, and chikungunya virus using fluorescent PCR on a real-time quantitative PCR system (QuantStudio 7 Flex), in accordance with the Diagnosis and Treatment Protocol for CHIKV (2025 Edition).

Viral RNA was extracted from patient serum samples and reverse transcription followed by whole-genome targeted amplification of CHIKV was performed using the BK-CHIKV024 kit (Hangzhou Baiyi). Library construction was conducted using a no-amplification barcoding kit, and pathogen genome sequencing was conducted on a GridION X5 nanopore sequencing platform. Baiyi Analysis Software (version 5.2, Hangzhou Baiyi Technology Co., Ltd., Hangzhou, China), was used to assess sequencing data quality and assemble the CHIKV whole genome with GCF_002889555.1 as the reference sequence.

Sequences obtained from this study (Cases 1 and 2) were aligned with sequences from the suspected linked case, the first case strain from Licheng District, and representative CHIKV genotype sequences retrieved from the GenBank database. Multiple sequence alignment was performed using MAFFT software (version 7.487, Advanced Industrial Science and Technology, Tokyo, Japan) and the best-fit nucleotide substitution model (GTR+F+I) was determined with IQ-TREE (version 2.4.0, Bui Quang Minh, Department of Evolutionary Biology of Vienna, Vienna, Austria). A Maximum Likelihood phylogenetic tree was then constructed with 1,000 bootstrap replicates.

INVESTIGATION AND RESULTS

Epidemiological Investigation

Case 1: A 10-year-old male primary school student developed red maculopapular rashes on both thighs with mild wrist joint pain on the afternoon of September 2, the rash subsequently spread systemically. He presented to D Community Health Center the same day with a temperature of 37.8 °C. Laboratory tests revealed negative dengue virus NS1 antigen. He revisited D Community Health Center on September 3 with a temperature of 37.6 °C. A blood sample was sent to the Nan'an CDC laboratory for testing, with positive CHIKV nucleic acid (Ct=27.3).

Case 2: A 16-year-old female middle school student (elder sister of Case 1) developed ankle joint pain in September 3, followed by scattered red maculopapular rashes on her limbs, accompanied by wrist and finger joint pain. At 20:00, her temperature was 38.0 °C. Following the of Case 1's diagnosis, local health staff collected her blood sample for testing, with positive CHIKV nucleic acid (Ct=23.3).

Both cases had the typical clinical triad of CHIKV infection and were confirmed by RT-PCR, representing the first locally transmitted CHIKV cases reported in Nan'an City, Quanzhou. Neither case had traveled outside Nan'an City in the 12 days prior to symptom onset. Environmental investigations on September 3 showed that the Breteau Index of 11.3 and Adult Biting Index of 1.6 mosquitoes per person per hour within a 100-meter radius of Clinic E and the patients' residences.

Contact Tracing of Co-exposed Individuals and Infection Source Investigation

Three co-exposed individuals (parents and grandmother) were identified and evaluated. None exhibited clinical symptoms, and all tested negative for both CHIKV nucleic acid and antibodies.

Active case finding was conducted across all households (1,655 households, 3,993 individuals) within the core exposure areas (defined as a 100-meter radius surrounding Clinic E and the patients' residences) and at Community Health Center D. A retrospective investigation covering the period from August 15 to September 3 was implemented using questionnaire surveys combined with symptom screening (fever, rash, arthralgia). This investigation identified 13 febrile cases and 2 cases presenting with

rash. Serum samples from all identified cases tested negative for CHIKV nucleic acid.

Since August 20, Health Center D has implemented strict fever clinic protocols and triage systems while requiring village clinics within its jurisdiction to enhance screening for patients presenting with fever, rash, arthralgia, and relevant epidemiological history. Suspected cases were promptly referred for further evaluation. As of September 3, Health Center D had submitted specimens from 12 suspected cases to the Nan'an CDC for testing. With the exception of 3 cases, all results were negative.

Activity trajectory mapping demonstrated that the sibling pair resided approximately 840 meters from Community Health Center D and only 70 meters from Clinic E (Figure 1). Further investigation revealed that on August 25, Clinic E had treated a

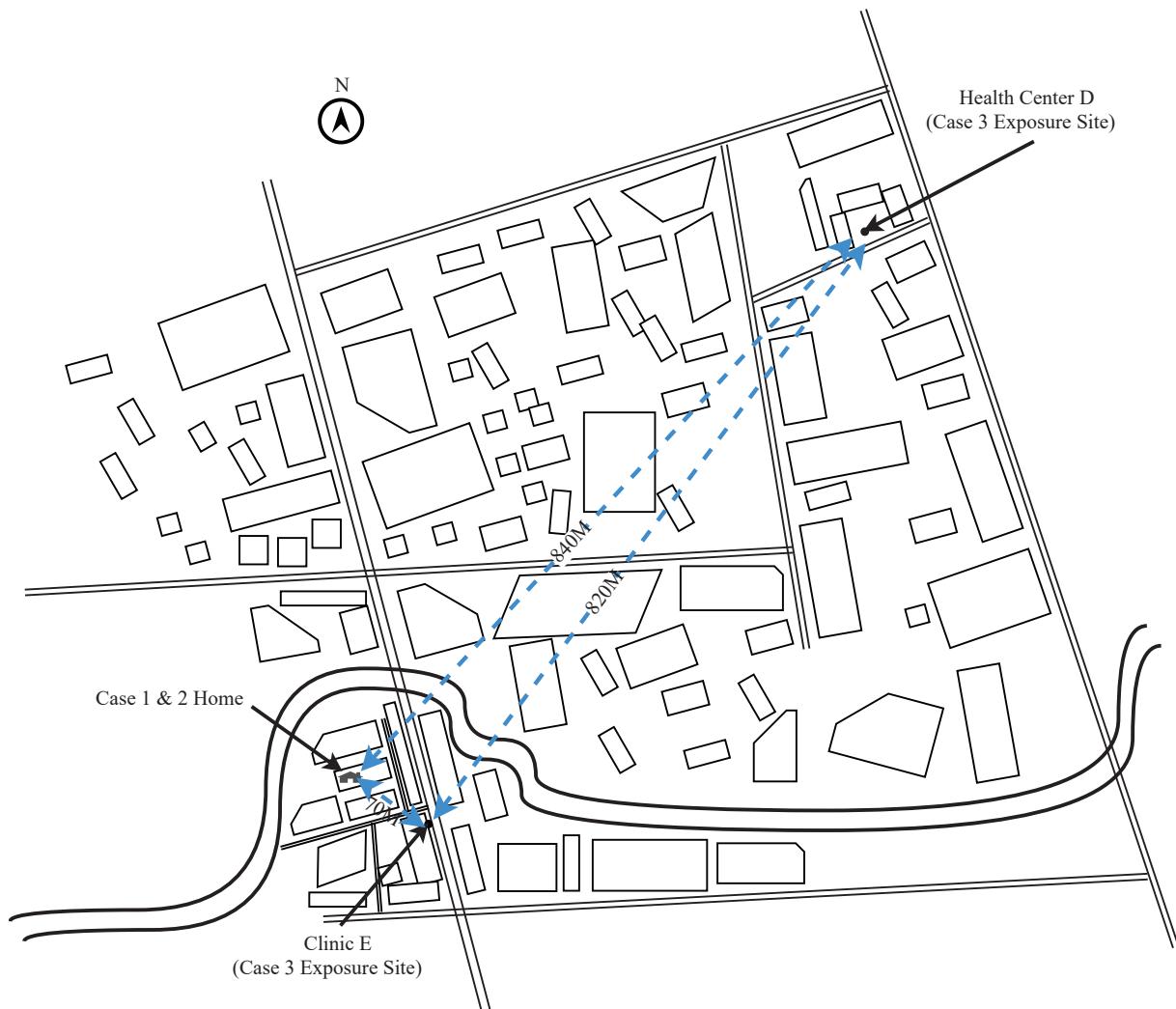


FIGURE 1. Spatial relationship between residential locations of Cases 1 and 2 and exposure sites of the imported case.

patient from Licheng District (Case 3), who had onset of symptoms on August 25 and was laboratory-confirmed on August 27. The patient received intravenous infusion therapy at the clinic for 2 hours (15:00–17:00) without effective mosquito prevention measures. On August 27 at 8:30, the patient returned to Clinic E for an additional 15 minutes. Case 3 resided in Licheng District, where he had acquired the infection, making him an associated case of the local transmission outbreak in that district. The straight-line distance between Case 3's residence and the sibling pair's residence was approximately 7 km, and approximately 6.8 km from Clinic E. Due to his travel history to Licheng District, the patient was immediately referred to the fever clinic of Community Health Center D. His CHIKV nucleic acid test returned positive ($Ct=25.1$), and the case was reported as an imported case linked to the Licheng District outbreak. The epidemiological relationships among the three cases are illustrated in Figure 2.

Case 3's activity trajectory during the viremic period (August 25–31) was confirmed through medical records, transportation record (ride-hailing orders, walking trajectories), and interviews. He had visited only three locations: his residence in Licheng District, Clinic E, and Community Health Center D, with no other potential exposure sites identified. He visited Clinic E on August 25 and 27, remaining at home for

the remainder of the period without going out and his trajectory was complete and verifiable.

Molecular Epidemiological Analysis

Whole-genome sequencing revealed complete identity among the CHIKV strains from all three cases, with 99.99% similarity to the first case strain from Licheng District. Phylogenetic analysis demonstrated that these three sequences clustered within the same branch as the first Licheng District case strain, the 2025 Foshan sequences (PX216391.1-PX216394.1), and the 2024 Réunion Island sequence (PV593524.2), all belonging to the East/Central/South African (ECSA) genotype. Furthermore, the sequences within this cluster exhibited complete identity and high homology in the E1 and E2 gene regions, forming a branch clearly distinct from other genotypes (Figure 3). This molecular evidence confirms a direct epidemiological link between the two locally acquired cases in Nan'an and the imported case from Licheng District.

PUBLIC HEALTH RESPONSE

Upon outbreak identification, targeted measures were initiated per mosquito-borne disease guidelines (6–7): medical institutions enhanced surveillance to detect and report suspected cases; all confirmed cases

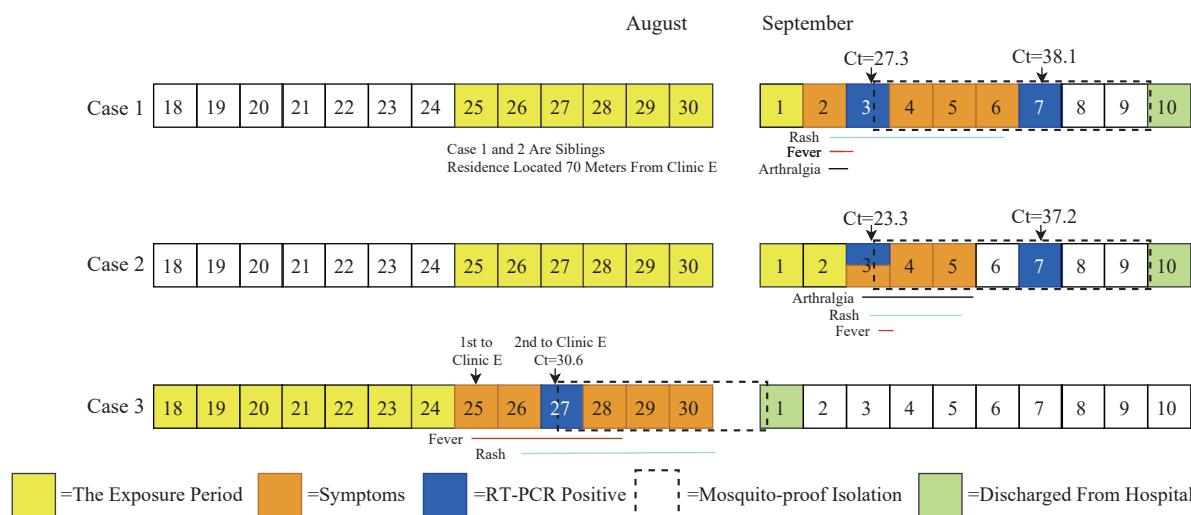


FIGURE 2. Clinical course and laboratory results of three chikungunya virus cases in Fujian Province, China, 2025.

Note: An amplification curve displaying a characteristic S-shape with a Ct value ≤ 38 indicates a positive result; a Ct value > 41 or no detection indicates a negative result. Suspected positive results exhibit a typical S-shaped amplification curve with $38 < Ct \leq 41$, requiring retesting. If retest results are consistent, the sample is classified as positive; if the Ct value > 41 or remains undetected, the sample is classified as negative.

Abbreviation: CHIKV=chikungunya virus; RT-PCR=real-time polymerase chain reaction; Ct =cycle threshold.

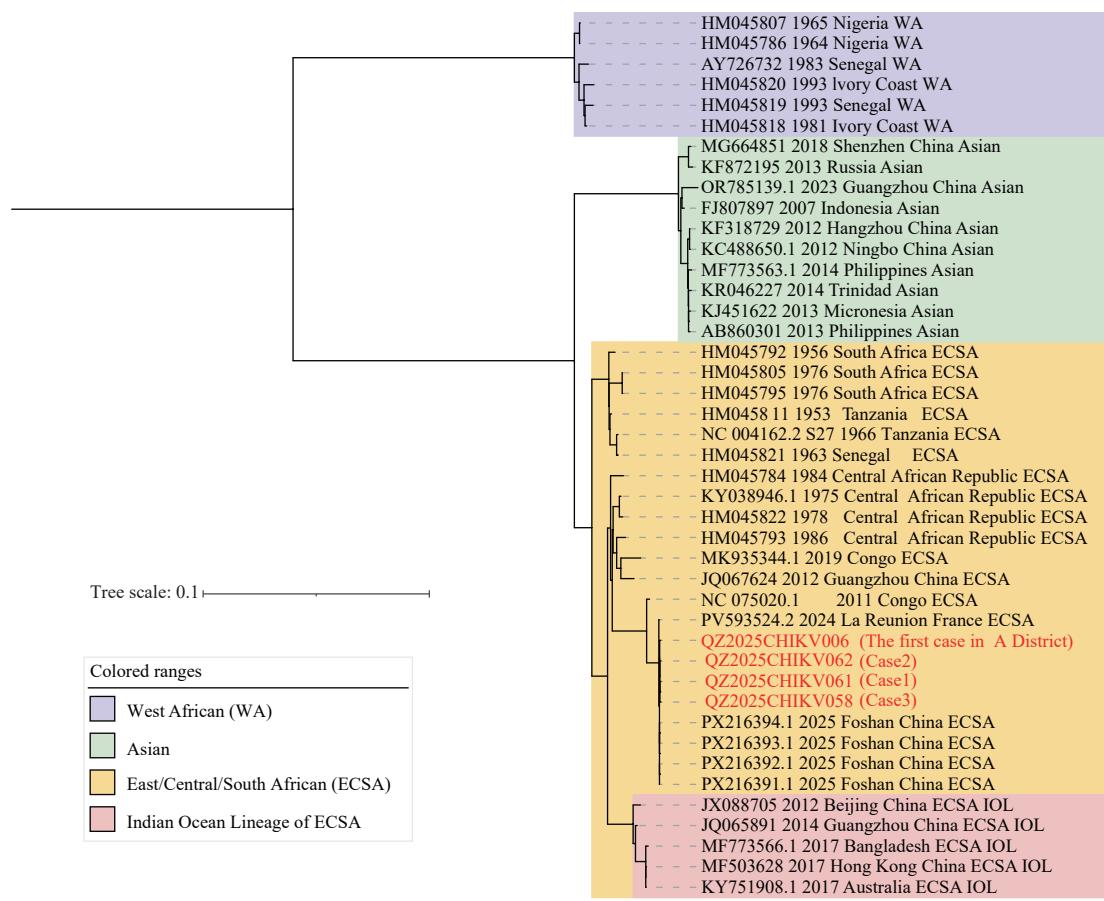


FIGURE 3. Phylogenetic tree of chikungunya virus strains based on Whole-Genome Sequences.

Note: Viral strain sequences downloaded from GenBank were aligned using MAFFT software. Purple denotes the WA lineage, green represents the Asian lineage, yellow indicates the ECSA lineage, and pink represents the Indian Ocean Lineage of the ECSA genotype.

Abbreviation: WA=West African; ECSA=East/Central/South African.

received mosquito-proof isolated treatment to block transmission. Individual epidemiological investigations focused on 12-day pre-onset activity/travel history to clarify infection source (imported/local), with 12-day health monitoring for 3 exposed contacts. Three-tiered zones were delineated (core zone: 100-meter radius, warning zone: 200-meter extension from the core, monitoring zone: involved communities). Core zone completed household surveys, adult mosquito killing and breeding site cleaning in 3 days. Community education promoted “no stagnant water, no mosquitoes”, mobilizing residents to eliminate Aedes breeding sites (8). Emergency control terminated with 22 consecutive days of no secondary cases and standard core zone mosquito density (6). These measures effectively controlled spread and safeguarded public health.

DISCUSSION

This investigation confirmed that local CHIKV cases in Nan'an City, Quanzhou, were triggered by an imported case from the adjacent Licheng District, Quanzhou. Both epidemiological and molecular evidence established a clear transmission link among the three reported cases. As of September 3, Case 3 was the first and only imported CHIKV case reported in Nan'an City. Epidemiological investigation confirmed that he acquired the infection in Licheng District, a known outbreak area. Subsequently, the high genetic homology of the viral strains from Cases 1 and 2 (the first locally transmitted cases in Nan'an City) with the strain from Case 3 — particularly the 100% identity in the E1 and E2 genes — provided definitive molecular evidence for this transmission link. Furthermore, active case searching within the core exposure areas revealed

no additional cases, further confirming that this outbreak was limited and focal in nature.

A key empirical finding was the 9-day interval from the initial exposure of the imported case (Case 3) at Clinic E to the onset of the first local secondary case. This timeline is consistent with the combined EIP and IIP for CHIKV. Recent research provides context for this finding; Wan et al. (9) demonstrated that the viremic period lasts from day 0 to day 7 after symptom onset, representing the phase of highest infectiousness. Considering the timeline and spatial characteristics of this study, Case 3 spent 2 hours at Clinic E on August 25 without effective mosquito prevention measures. This scenario represented the period of highest probability for mosquito bites and subsequent infection. Therefore, the approximately 9 days interval from this exposure to the onset of the first local case is epidemiologically plausible. If exposure had occurred during his shorter visit on August 27, the interval would have been approximately 7 days. This range of 7–9 days aligns with the theoretical sum of the incubation periods, with the 9-day interval being better supported by the context of longer unprotected exposure. Although the 9-day interval is not universally applicable, it provides a crucial “action benchmark” for rapid response in non-endemic areas. The absence of symptoms and negative laboratory test results among all co-exposed household members excluded the possibility that family members served as a source of transmission to Cases 1 and 2.

This study has several limitations. First, the small sample size (3 cases) means that the observed 9-day interval represents an empirical finding from a single outbreak rather than a universally applicable transmission cycle. The EIP depends heavily on environmental temperature (10) and *Aedes* density, while the IIP varies with individual immunity. Consequently, this interval cannot be generalized to other regions, seasons, or environmental conditions. Second, the exact date of mosquito infection remains uncertain, yielding an estimated range of 7–9 days. Third, the absence of CHIKV-positive mosquito samples from the core exposure area means direct vector evidence is lacking. However, this finding aligns with the characteristically low detection rates observed in small-scale outbreaks. It is likely attributable to low viral infection rates and inherent sampling limitations, suggests that CHIKV failed to achieve effective

amplification within the local mosquito population.

In conclusion, this outbreak demonstrates that in non-endemic areas harboring competent vectors, CHIKV can rapidly establish local transmission following case importation. Public health efforts must prioritize the rapid detection of imported viremic cases, precise mapping of their exposure sites, and immediate implementation of intensive, targeted vector control measures within a critical time window to interrupt transmission. Future multi-center studies incorporating larger sample sizes and integrated entomological surveillance are essential to deepen our understanding of transmission dynamics across diverse settings.

Conflicts of interest: No conflicts of interest.

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First Human Case of *Streptococcus parasuis* Infection — Henan Province, China, 2025

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Summary

What is already known about this topic?

Streptococcus parasuis was historically classified as serotypes 20, 22, and 26 of *Streptococcus suis*. Limited availability of suitable detection methods has resulted in scarce case reports, which have predominantly focused on bacterial identification and pathogenic mechanisms rather than comprehensive epidemiological investigations.

What is added by this report?

This study confirmed the first documented human case of *Streptococcus parasuis* infection in Henan Province through comprehensive genetic sequencing. Phylogenetic analysis revealed that this strain exhibits substantial genetic divergence from previously isolated domestic strains. Notably, the patient reported no documented history of livestock contact, indicating that environmental exposure may represent a critical transmission route.

What are the implications for public health practice?

This investigation underscores the urgent need to strengthen environmental surveillance and implement rigorous disinfection protocols for *Streptococcus parasuis*, while expanding monitoring efforts to encompass both high-risk occupational groups and the general population. Development and deployment of rapid diagnostic reagents should be prioritized to prevent underdiagnosis in cases lacking documented livestock exposure history.

desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), real-time quantitative polymerase chain reaction (qPCR) targeting *Streptococcus suis*-specific nucleic acid sequences, and whole-genome sequencing.

Results: MALDI-TOF MS initially identified strain HN04 as *Streptococcus suis*; however, the *S. suis*-specific qPCR assay produced a negative result. Average nucleotide identity (ANI) analysis definitively identified HN04 as *Streptococcus parasuis*. Phylogenetic analysis revealed that HN04 is distantly related to previously reported clinical isolates from China. Epidemiological investigation found no documented history of livestock contact, particularly with pigs, suggesting environmental exposure as the likely transmission route.

Conclusions: This study documents the first confirmed human case of *Streptococcus parasuis* infection in Henan Province, suggesting widespread environmental distribution of this pathogen and highlighting the need for surveillance beyond occupational risk groups. We recommend including *S. parasuis* in the differential diagnosis for patients presenting with severe traumatic infections even in the absence of livestock contact history. Enhanced environmental surveillance systems and rapid diagnostic capabilities are urgently needed. These findings provide critical insights into the epidemiological characteristics and transmission dynamics of *S. parasuis*.

ABSTRACT

Introduction: On June 26, 2025, a hospital in Luoyang City reported a suspected case of *Streptococcus suis* infection. The local CDCs immediately initiated an epidemiological investigation upon notification.

Methods: Blood samples were collected from the patient for microbial culture. The isolated strain HN04 underwent species identification and phylogenetic analysis through matrix-assisted laser

On June 26, 2025, the Jinghua Campus of the First Affiliated Hospital of Henan University of Science and Technology reported a suspected case of *Streptococcus suis* infection. The Luoyang CDC immediately initiated an epidemiological investigation and submitted the isolated strain, designated HN04, for laboratory analysis. Through collaborative verification by the Henan Provincial CDC and the Luoyang CDC,

the strain was definitively identified as *Streptococcus parasuis*, representing the first documented human infection with this pathogen in Henan Province.

Investigation and Results

On June 23, 2025, at 9:00 AM, a 69-year-old male sanitation worker was admitted to the Jinhua Campus of The First Affiliated Hospital of Henan University of Science and Technology with a diagnosis of “a lower limb injury”. His left foot had been crushed by a garbage truck during work, resulting in significant hemorrhage. The patient reported good general health with no history of hypertension, diabetes, heart disease, or cerebrovascular disease. Upon admission, his body temperature was normal (36.8 °C). Complete blood count results revealed a white blood cell (WBC) count of $8.5 \times 10^9/L$, with a neutrophil percentage of 92.90% and a lymphocyte percentage of 4.40%. The C-reactive protein (CRP) concentration was 23.15 mg/L. A venous blood sample was collected for blood culture. At 13:00, prophylactic anti-infective therapy with cefoperazone sodium and sulbactam sodium was initiated and administered every 8 hours for a total of three doses. A left below-knee amputation was performed at 19:00, lasting 75 minutes. Postoperatively, the prophylactic anti-infective agent was changed to cefuroxime sodium, administered every 8 hours for three consecutive days. On June 26, the hospital cultured a bacterial strain designated HN04 and identified it as *Streptococcus suis* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The hospital reported the case on the same day, and the HN04 strain was sent to both the Luoyang CDC and the Henan Provincial CDC for verification. No microorganisms were isolated from the blood culture on July 1. On July 10, the complete blood count showed a WBC count of $6.85 \times 10^9/L$, with a neutrophil percentage of 77.60% and a lymphocyte percentage of 17.40%. With active treatment, the amputation wound healed well, and the patient was discharged on July 14.

On June 26, following the hospital report, the Luoyang CDC conducted an epidemiological investigation at both the patient's home and the hospital. The investigation revealed that the patient's household did not raise pigs or other livestock, no pig farms existed in the vicinity of his residence, and he had no routine contact with raw pork or sick pigs. From 2023 to the present, a total of 365 *Streptococcus* strains have been cultured and identified at the Jinhua

Campus of The First Affiliated Hospital of Henan University of Science and Technology, but no *S. suis* had been detected. No additional cases of *S. suis* infection were identified in the patient's ward or hospital-wide.

On June 27, the Luoyang CDC tested the HN04 strain using a *S. suis* nucleic acid detection kit, which yielded a negative result. Subsequently, the Henan Provincial CDC successfully amplified a 679 bp fragment using *S. parasuis* *recN*-specific primers (1). Whole-genome sequencing of strain HN04 (Accession number: SRR35358561) was completed on August 13, and Average Nucleotide Identity (ANI) analysis definitively identified the isolate as *S. parasuis*. Phylogenetic analysis based on whole-genome single nucleotide polymorphisms (wgSNPs) revealed that strain HN04 occupied a distinct phylogenetic branch from six other human-derived *S. parasuis* isolates previously reported in China, with ANI values ranging from 97.69% to 97.85% (Figure 1).

Public Health Response

Following receipt of the hospital report, the Luoyang CDC promptly initiated comprehensive investigation and control measures. Environmental disinfection was performed at the patient's residence and in surrounding areas, including garbage trucks, waste collection bins, and pig farms in Xiaolangdi Town. The animal disease control department was notified of the outbreak, and a coordinated joint investigation and response mechanism for zoonotic diseases was activated. The Luoyang CDC provided guidance to the hospital regarding patient isolation protocols, clinical management, and facility-wide infection prevention and control practices. Active case finding was conducted throughout the village and township where the index case resided, with no additional cases identified to date.

DISCUSSION

This study identified *S. parasuis* through whole-genome sequencing of a blood culture isolate from a patient injured in a traffic accident, representing the first documented human case of *S. parasuis* infection in Henan Province. Notably, the patient reported no history of contact with live pigs or pork products. Phylogenetic analysis revealed that the isolated strain was distantly related to previously reported clinical isolates from other regions of China.

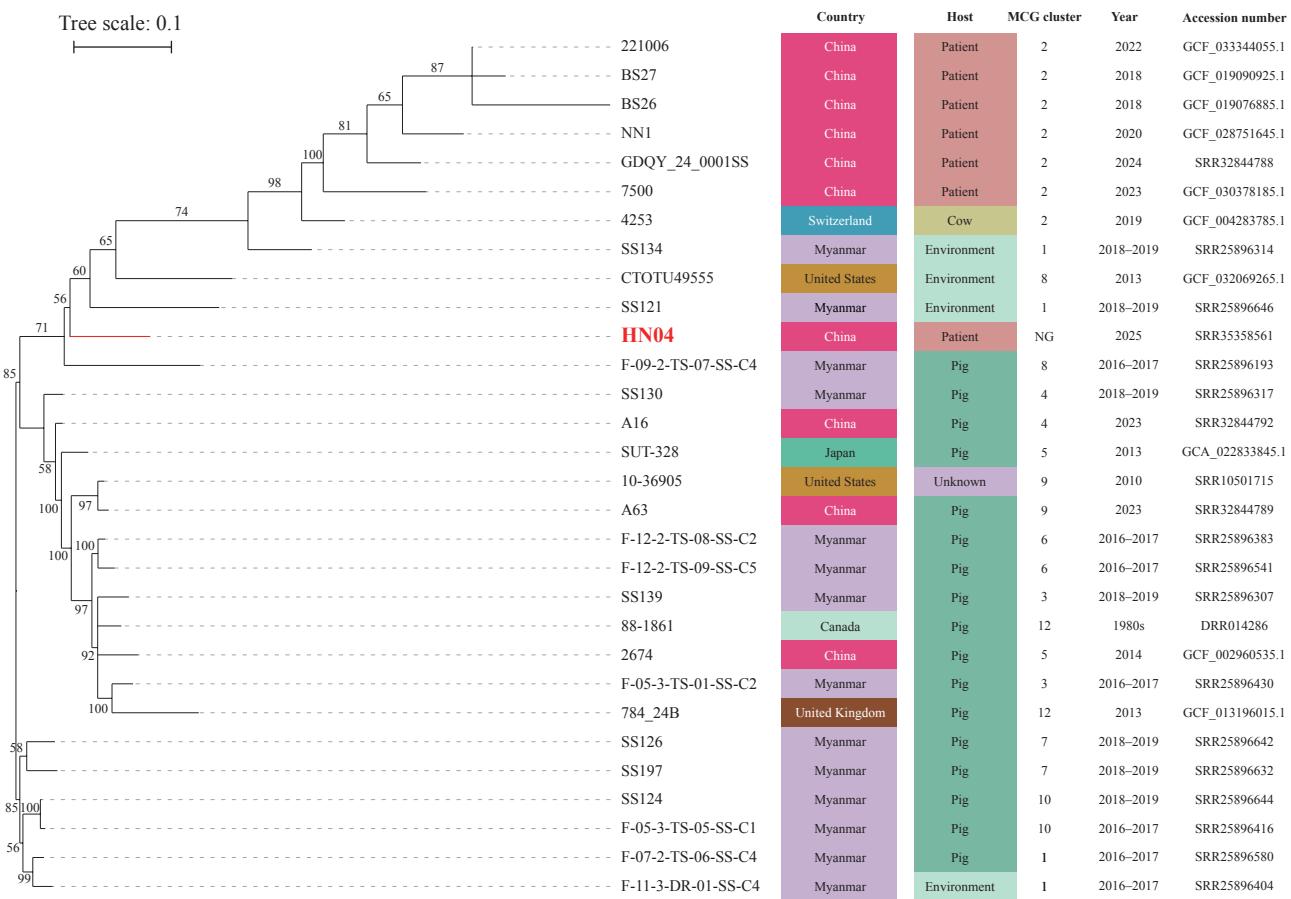


FIGURE 1. Whole-genome single nucleotide polymorphism (wgSNP) phylogenetic analysis of strain HN04 from Henan Province, China (June 2025), constructed using the Neighbor-Joining method.

Note: Following the minimum core genome (MCG) typing strategy developed by Zhang et al. (2), we constructed a phylogenetic tree using strain SUT-286 (GCA_021654455.1) as the reference genome. The analysis incorporated two representative strains from each MCG group, with the exception of MCG2, for which all seven available strains were included. Bootstrap analysis was performed with 1,000 replicates.

“NG” designates MCG non-groupable genomes.

Human infections with *S. parasuis* have been documented in Guangxi Zhuang Autonomous Region and Guangdong Province in China, with clinical presentations typically including fever accompanied by peritonitis, pneumonia, or meningitis, consistent with the pathogenic profile of *S. parasuis* (3–4). The index case reported here lacked these characteristic clinical manifestations, likely due to prompt initiation of prophylactic antibiotic therapy following the traumatic injury. Subsequently, two additional *S. parasuis* infections were identified in Luoyang City. The first involved a patient with severe burns from a liquefied gas explosion, in whom *S. parasuis* was detected in wound secretions; this patient’s critical condition resulted in death. The second case presented with fever and retching, with physical examination revealing extensive erythematous rashes and edema on the left leg, waist, and chest. This patient was diagnosed with

sepsis, and *S. parasuis* was isolated from blood culture. Following targeted antibiotic therapy, the patient recovered and was discharged. Importantly, neither of these subsequent cases reported contact with pigs or other livestock. Phylogenetic analysis of strains from all three cases demonstrated genetic diversity, indicating independent sporadic infections rather than a common-source outbreak. Current research on *S. parasuis* remains limited, with no evidence supporting human-to-human transmission. However, the etiological findings and exposure patterns observed in these cases suggest that *S. parasuis* may be widely distributed in the local environment. This environmental persistence raises concern for continued sporadic human and livestock infections, with the potential for localized case clusters in the future.

Given the limited clinical reports on *S. parasuis* and the scarcity of reference strain data in existing MALDI-

TOF MS databases, accurate differentiation from *S. suis* in clinical settings remains challenging (5–6). During this investigation, the hospital initially identified the pathogen as *S. suis* by MALDI-TOF MS, with confirmation as *S. parasuis* only achieved through whole-genome sequencing. This diagnostic limitation, combined with the lack of routine molecular testing, indicates that both the infection rate and public health risk of *S. parasuis* may be substantially underestimated (7). Although *S. parasuis* is phylogenetically related to *S. suis*, the two species differ significantly in antibiotic resistance gene profiles, pathogenic mechanisms, and the inflammatory responses they trigger in hosts. Furthermore, *S. parasuis* itself exhibits considerable genomic heterogeneity (3,8), complicating both identification and treatment. Accurate species-level identification is therefore essential for guiding empirical antibiotic therapy and ensuring appropriate clinical management. The transmission dynamics of *S. suis* are closely associated with occupational pig exposure. If *S. parasuis* cases are misclassified as *S. suis* in surveillance data, the true disease burden will be obscured, preventing public health systems from detecting emerging transmission patterns and identifying at-risk populations beyond traditional occupational groups. Moreover, given the limited current understanding of *S. parasuis* pathogenicity and pathophysiology, accurate differentiation and identification provide the foundation for conducting mechanistic research, developing targeted therapeutic strategies, and properly assessing its public health and clinical significance. Establishing and implementing rapid, species-specific laboratory detection systems for *S. parasuis* — such as PCR assays targeting unique genetic markers — represents a critical prerequisite for enhancing surveillance sensitivity and improving clinical outcomes.

Notably, none of the three cases identified in Luoyang City reported direct contact with live pigs or pork products, a finding that diverges substantially from the traditional epidemiological pattern of *S. suis* infection, which predominantly affects occupational groups with livestock exposure. All three patients presented with skin lesions prior to hospitalization. While this observation raises the possibility of environmental persistence of *S. parasuis*, the absence of environmental sampling during this investigation limits our ability to establish definitive transmission pathways. Future investigations should prioritize comprehensive environmental surveillance to identify potential contamination sources and elucidate

transmission routes. These findings carry important clinical implications: clinicians should consider *S. parasuis* in the differential diagnosis of patients presenting with fever, neurological symptoms, or sepsis, even without documented animal contact history. Antimicrobial selection should be guided by susceptibility testing when available. From a public health perspective, these cases underscore the need for environmental sampling and targeted disinfection protocols during outbreak response. Prevention and control strategies for *S. parasuis* infection must extend beyond occupational health frameworks to encompass population-wide surveillance and systematic investigation of environmental transmission pathways.

This report documents the first confirmed human case of *S. parasuis* infection in Henan Province and provides a valuable bacterial isolate for developing rapid diagnostic assays, conducting antimicrobial susceptibility studies, and investigating virulence mechanisms. Given the emerging recognition of *S. parasuis* as a human pathogen, strengthening surveillance systems is essential to better characterize its epidemiological patterns and pathogenic properties.

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Notifiable Infectious Diseases Reports

Reported Cases and Deaths of National Notifiable Infectious Diseases — China, December 2025*

| Diseases | Cases | Deaths |
|--|-----------|--------|
| Plague | 0 | 0 |
| Cholera | 0 | 0 |
| COVID-19 | 15,129 | 1 |
| SARS-CoV | 0 | 0 |
| Acquired immune deficiency syndrome [†] | 4,560 | 2,027 |
| Hepatitis | 128,222 | 585 |
| Hepatitis A | 1,204 | 0 |
| Hepatitis B | 109,580 | 39 |
| Hepatitis C | 14,159 | 545 |
| Hepatitis D | 33 | 0 |
| Hepatitis E | 2,543 | 1 |
| Other hepatitis | 703 | 0 |
| Poliomyelitis | 0 | 0 |
| Human infection with novel influenza virus | 3 | 0 |
| Measles | 90 | 0 |
| Epidemic hemorrhagic fever | 617 | 6 |
| Rabies | 28 | 30 |
| Japanese encephalitis | 1 | 0 |
| Dengue | 151 | 0 |
| Monkeypox [§] | 43 | 0 |
| Anthrax | 23 | 0 |
| Dysentery | 1,509 | 0 |
| Tuberculosis | 52,826 | 228 |
| Typhoid fever and paratyphoid fever | 349 | 0 |
| Meningococcal meningitis | 29 | 0 |
| Pertussis | 1,313 | 0 |
| Diphtheria | 0 | 0 |
| Neonatal tetanus | 0 | 0 |
| Scarlet fever | 3,058 | 0 |
| Brucellosis | 3,420 | 0 |
| Gonorrhea | 10,547 | 0 |
| Syphilis | 52,197 | 3 |
| Leptospirosis | 20 | 0 |
| Schistosomiasis | 3 | 0 |
| Malaria | 393 | 2 |
| Influenza | 6,727,614 | 14 |
| Mumps | 6,471 | 0 |

Continued

| Diseases | Cases | Deaths |
|----------------------------------|------------------|--------------|
| Rubella | 38 | 0 |
| Acute hemorrhagic conjunctivitis | 1,560 | 0 |
| Leprosy | 15 | 0 |
| Typhus | 65 | 0 |
| Kala azar | 31 | 0 |
| Echinococcosis | 373 | 0 |
| Filariasis | 0 | 0 |
| Hand, foot and mouth disease | 106,825 | 0 |
| Infectious diarrhea [†] | 118,529 | 0 |
| Total | 7,236,052 | 2,896 |

* According to the National Bureau of Disease Control and Prevention.

† The number of deaths of Acquired immune deficiency syndrome (AIDS) is the number of all-cause deaths reported in the month by cumulative reported AIDS patients..

§ Since September 20, 2023, Monkey pox was included in the management of Class B infectious diseases.

[†] Infectious diarrhea excludes cholera, dysentery, typhoid fever and paratyphoid fever.

The number of cases and cause-specific deaths refer to data recorded in National Notifiable Disease Reporting System in China, which includes both clinically-diagnosed cases and laboratory-confirmed cases. Only reported cases of the 31 provincial-level administrative divisions in the Chinese mainland are included in the table, whereas data of Hong Kong Special Administrative Region, Macau Special Administrative Region, and Taiwan, China are not included. Monthly statistics are calculated without annual verification, which were usually conducted in February of the next year for de-duplication and verification of reported cases in annual statistics. Therefore, 12-month cases could not be added together directly to calculate the cumulative cases because the individual information might be verified via National Notifiable Disease Reporting System according to information verification or field investigations by local CDCs.

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