

Brief Report

Large-Scale *Staphylococcus aureus* Foodborne Disease Poisoning Outbreak among Primary School Children

Hao Hong Thi Le ¹, Anders Dalsgaard ^{2,3} , Paal Skytt Andersen ^{2,4} , Huong Minh Nguyen ⁵ , Yen Thi Ta ¹ and Trung Thanh Nguyen ^{1,*}

¹ National Institute for Food Control, Hanoi 100000, Vietnam; lethihonghao@yahoo.com (H.H.T.L.); yenta84@gmail.com (Y.T.T.)

² Faculty of Health and Medical Sciences, University of Copenhagen, 2200 København, Denmark; adal@sund.ku.dk (A.D.); PSA@ssi.dk (P.S.A.)

³ School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 639798, Singapore

⁴ Department of Bacteria, Parasites and Fungi, Statens Serum Institute, 2300 København, Denmark

⁵ Institute of Biotechnology, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam; nguyenvh@vab.ac.vn (H.M.N.); nguyenvh@gmail.com

* Correspondence: trungnt@nifc.gov.vn or nguyenthanchung83@gmail.com

Abstract: A large-scale food poisoning outbreak happened at a school canteen in Ninh Binh Province, Vietnam, in 2018, resulting in the hospitalization of 352 students with clinical symptoms indicative of a staphylococcal food poisoning. A subsequent laboratory investigation detected *Staphylococcus aureus* in two food items—deep-fried shrimp and chicken floss—at up to 10³ CFU/mL, and staphylococcal enterotoxins (SEs) in chicken floss at ≥0.211 ng SEs/g. *S. aureus* was also isolated from patients' vomit and stool samples, and kitchen workers' stool samples, as well as in frozen chicken meat, but not on the kitchen workers' hand surfaces, suggesting the cause of this food poisoning outbreak was *S. aureus* contamination of the chicken meat. Molecular characterization revealed the *S. aureus* strains isolated from all samples were closely related; all belonged to sequence type (ST) ST6 and *spa* type t701 and carried both *sea* and *sec* genes. This SE-producing strain was resistant to penicillin and tetracycline, while still susceptible to oxacillin, erythromycin, gentamicin, methicillin, and vancomycin. Since *S. aureus* food poisonings are often underreported, our investigation added to the sparse qualitative and quantitative data of pathogenic *S. aureus* monitoring and surveillance in Vietnam, providing needed knowledge to guide preventative measures for future outbreaks.

Keywords: *Staphylococcus aureus*; enterotoxins; staphylococcal food poisoning; antibiotic resistance; MLST; *spa* typing



Citation: Le, H.H.T.; Dalsgaard, A.; Andersen, P.S.; Nguyen, H.M.; Ta, Y.T.; Nguyen, T.T. Large-Scale *Staphylococcus aureus* Foodborne Disease Poisoning Outbreak among Primary School Children. *Microbiol. Res.* **2021**, *12*, 43–52. <https://doi.org/10.3390/microbiolres12010005>

Academic Editor:

Beniamino T. Cenci-Goga

Received: 26 December 2020

Accepted: 8 February 2021

Published: 11 February 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The Gram-positive *Staphylococcus aureus* is a major bacterial pathogen often involved in food poisoning due to their high rate of human skin and nasal carriage, efficient airborne spread, and strong survival in fomites, which allow them to eliminate competing microorganisms that are less able to endure elevated temperatures, high osmotic pressure, and relatively low humidity [1–4]. *S. aureus* foodborne infections occur via a toxigenic mechanism caused by heat-stable staphylococcal enterotoxins (SEs) being produced in foodstuffs, most commonly in dairy products (for example, milk, cheese, and cream), as well as meat and fish. The resulting toxins, which are thermostable and resistant to digestive enzymes, are ingested preformed, thus causing sudden vomiting, diarrhea, nausea, malaise, abdominal cramps, pain and, sometimes, prostration, in which case hospital admission may become necessary after a short incubation period of one to seven hours [2]. So far, more than 20 types of SE have been identified; among these the five classical SEs (SEA to SEE) account for more than 95% of confirmed food poisoning cases [5,6].

The past two decades saw an alarmingly high occurrence of foodborne illnesses and outbreaks in Vietnam, mainly as the result of overall poor hygiene and sanitation, although public reports on these cases are few. The leading cause of foodborne illnesses in Vietnam is bacterial pathogens, and among these, *S. aureus* is commonly observed [7]. Sources of contamination are diverse, from ready-to-eat products, undercooked chicken, pork, and beef, to traditional cakes and sandwiches [7]. In Vietnam, *S. aureus* is monitored by local medical units and recorded in their monthly reports together with other gut pathogens such as *Salmonella* spp., *Escherichia coli*, and *Vibrio cholerae* under the category “foodborne diseases with diarrhea syndromes”. According to the Vietnam Food Administration, during the period from 2014 to 2018, in total there were 71 outbreaks caused by *S. aureus* and staphylococcal toxins nationwide, most of them were large-scale, causing a total of 3858 people to become ill and 3615 hospitalizations. Little is known about the characteristics of the *S. aureus* strains implicated in these outbreaks.

At 15:00 on 5 October, 2018, the Department of Food Safety and Hygiene in Ninh Binh Province, Vietnam, received information from the leader of the Ninh Binh Obstetrics and Pediatrics Hospital that a high number of children aged 6–10 years from the Dinh Tien Hoang Primary School had been admitted to the hospital mostly with signs of gastroenteritis after eating a chicken-based lunch prepared by kitchen workers. Relevant provincial health departments together with the city police immediately established teams to investigate the disease outbreak to assess the extent of the outbreak and to identify the mode and vehicle of transmission as follows: (1) checking of hygiene and food storage procedures as well as record keeping in the school canteen; (2) inspection of hygiene conditions at the slaughterhouse providing the chicken used to prepare the lunch; (3) interviewing patients and their parents admitted to three local hospitals and one medical center.

The aim of this study is to describe the epidemiological and laboratory investigation of this large-scale outbreak of food poisoning among school children in Ninh Binh Province, Vietnam. This will add to the scarce molecular data on *S. aureus* strains associated with food poisoning outbreaks in the country.

2. Materials and Methods

2.1. Study Hypothesis

It was postulated that the lunch meal served at the school canteen was the source of the food poisoning outbreak, and that the clinical profile and symptoms were suggestive of staphylococcal toxin and *S. aureus* intoxication. A retrospective cohort study was designed to test this hypothesis.

2.2. Study Population and Case Definition

All children who had eaten lunch in the school canteen on 5 October 2018 were included. A case was any person who had reported at least one of the following symptoms: vomiting, abdominal pain, nausea, fever or diarrhea after eating in the school canteen on 5 October 2018.

2.3. Outbreak Investigation

There were more than 900 children at the primary school. Only children had their lunch at the school canteen as teachers and other adults had lunch elsewhere. The established teams inspected the school canteen as well as the slaughterhouse that provided the chicken consumed by the children along the entire process of food preparation, conservation, and record keeping.

A standardized questionnaire was administered to a cohort of 89 cases who had eaten in the school canteen to ascertain and assess the following information: demographic data; food consumption on 5 October; presence of symptoms; hospitalization; laboratory data. All children completed the questionnaires. These inspections and interviews of patients and their parents were conducted from the 5 to the 7 of October, 2018.

2.4. Microbiological Analysis and Characterization of *S. aureus*

Vomit and stool samples from patients and swabs from the hands of kitchen workers were collected as well as food samples in (1) the school canteen, i.e., deep-fried shrimp, chicken floss, tomato soup, and cooked rice used to prepare the lunch dish and (2) two frozen chicken samples at a local abattoir. Swab samples were obtained about four hours after receiving news of the outbreak. All samples were transferred in an ice box at 4–5 °C to the National Institute for Food Control in Hanoi. All of the samples were analyzed within 6 hrs after collection at the National Institute of Food Safety and Hygiene, Hanoi (food and hand swab samples) or the Provincial Preventive Medicine Center in Ninh Binh Province (stool).

Samples were homogenized in sterile saline buffer at 1:10 ratio, diluted up to 10⁻⁴-fold, and 0.1 ml of each dilution was plated on Baird-Parker (BP) agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) according to standard method (International Organization for Standardization, ISO 6888-1:1999/AMD 1:2003). Plates were incubated at 37 ± 1 °C for 24–48 h, and black colonies were chosen for coagulase test. Coagulase-positive isolates were identified by Vitek[®]-MS (bioMérieux Clinical Diagnostics, Marcy l'Etoile, France) and confirmed *S. aureus* isolates were kept at –80 °C in Brain heart infusion (BHI, Difco, Franklin Lakes, NJ, USA) broth supplemented with 15% glycerol until further analyses. Hand swabs were analyzed as previously described to detect *Salmonella* spp. (ISO 6579-1:2017) and *E. coli* (ISO 16649-2:2001).

DNA was extracted from *S. aureus* isolates grown overnight in BHI broth at 37 °C using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions. DNA quality was assessed using an ABC NanoDrop 1000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). The presence of four classical staphylococcal enterotoxin genes *sea*, *seb*, *sec*, and *sed* [8] as well as two methicillin-resistance determinant genes *mecA* and *femA* were detected by PCR and amplicons visualized by agarose electrophoresis. The PCR primers used are listed in Table 1. The presence of classical *S. aureus* enterotoxin types A to E was confirmed by 3M[™]TECRA[™] Staph Enterotoxin Visual Immunoassay (VIA) kit (3M[™]TECRA[™], Australia) according to the manufacturer's instructions. Vomit samples were adjusted to pH 5–6 by adding 0.1N NaOH according to the ISO 6887-1:2019 method before enterotoxin detection. Our protocol can detect the presence of classical *S. aureus* enterotoxin types A to E at the threshold of 0.211 ng SEA/g meat-based food.

Table 1. PCR primers used for detection of staphylococcal enterotoxin genes, methicillin-resistance genes, and *spa* typing.

Gene	Primer	Primer Sequence (5'–3')	Reference
<i>sea</i>	SEA Fw	GCA GGG AAC AGC TTT AGG C	[8]
	SEA Rv	GTT CTG TAG AAG TAT GAA ACA CG	
<i>seb</i>	SEB Fw	GTA TGG TGG TGT AAC TGA GC	[8]
	SEB Rv	CCA AAT AGT GAC GAG TTA GG	
<i>sec</i>	SEC Fw	CTT GTA TGT ATG GAG GAA TAA CAA	[8]
	SEC Rv	TGC AGG CAT CAT ATC ATA CCA	
<i>sed</i>	SED Fw	GTG GTG AAA TAG ATA GGA CTG C	[8]
	SED Rv	ATA TGA AGG TGC TCT GTG G	
<i>femA</i>	FemA Fw	AAA AAA GCA CAT AAC AAG CG	[8]
	FemA Rv	GAT AAA GAA GAA ACC AGC AG	
<i>mecA</i>	MecA Fw	TGCTATCCACCCTCAAACAGG	[9]
	MecA Rv	AACGTTGTAACCAACCCAAGA	
<i>spa</i>	spa-1113f	TAA AGA CGA TCC TTC GGT GAG C	[10]
	spa-1514r	CAG CAG TAG TGC CGT TTG CTT	

MLST (<https://pubmlst.org/saureus/> (accessed on 27 November 2018)) and *spa* typing were performed as previously described [10–12]. Amplification of the seven housekeeping genes in the MLST scheme was referred from PubMLST with no modifications. The polymorphic region of the *spa* gene was amplified following a previously

described method [10]. PCR products of the housekeeping genes and the *spa* repeat were purified and sequenced by the Sanger method (1 Base DNA Sequencing Services, Singapore). Sequence type (ST) assignment and clustering were conducted using PubMLST [13] and eBURST [14,15], respectively. *Spa* types were assigned by the SpaServer website (<http://spaserver2.ridom.de> (accessed on 27 November 2018)) and clustered using “Based upon repeat pattern” BURP cluster analysis [16]. Geographical distribution and phylogeographic analyses were performed using Microreact [17].

Antimicrobial susceptibility tests were carried out by the disk diffusion method for oxacillin (OXA; 1 µg), erythromycin (ERY; 15 µg), gentamicin (GEN; 10 µg), tetracycline (TET; 30 µg), and penicillin (PEN; 10 µg). Susceptibility to methicillin (MET; 5 µg) and vancomycin (VAN; 30 µg) were determined by the minimum inhibitory concentration (MIC) method. Isolates were classified as sensitive, intermediate or resistant in accordance with Clinical and Laboratory Standard Institute (CLSI 2018) breakpoints for each tested antimicrobial. Multidrug resistance (MDR) was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories [18].

3. Results

3.1. Child Cases, Attack Rate and Clinical Manifestations

All 926 children at Ninh Binh primary school were part of the study cohort. All children began eating their lunch at the school canteen at around 10:30 a.m. on 5 October, 2018. At 2:00 p.m. four children showed signs of food poisoning including nausea, vomiting, abdominal pain, and headache. By 5:00 p.m., a total of 352 children showed similar clinical symptoms and were admitted to three local hospitals and one medical center for monitoring and treatment. Duration of hospitalization varied among the 352 children hospitalized and was a maximum of three days. All 926 children ate lunch with 352 children being admitted to the hospital before 5:00 p.m. As of 4:00 p.m. on 8 October, all 352 patients had recovered and were discharged.

The overall attack rate was 38.0% (352 out of 926 children). Among the 89 children participating in our interview, all provided answers including 42 girls and 47 boys (Table 2). Based on the responses provided during the interviews, the most frequent symptoms were vomiting (72; 80.9%), abdominal pain (64; 71.9%), nausea (38; 42.7%), fever (36; 40.4%), and diarrhea (4; 4.5%). No patients showed signs of skin rash and convulsions. In addition, some children had headaches, felt dizzy, and had cold limbs. Symptoms were seen from 2 to 6.5 h after eating. All children aged 6–7 (23 girls, 28 boys) and 94.7% of children aged 8–10 (18 girls, 18 boys) were reported to be affected.

Table 2. Attack rate stratified by age group and gender based on the results of questionnaire interviews.

Age (years) and Gender	Affected	Unaffected	Attack Rate (%)	Total
6–7 girls	23	0	100	23
6–7 boys	28	0	100	28
8–10 girls	18	1	94.7	19
8–10 boys	18	1	94.7	19
Total	87	2	98.0	89

3.2. Laboratory Analysis and Investigation

Based on case symptoms, it was postulated that the lunch meal served at the school canteen was the source of the food poisoning outbreak, and that the clinical symptoms were suggestive of *S. aureus* infection. Thus, vomit ($n = 6$) and stool ($n = 27$) samples were collected from students and kitchen workers admitted to the hospital (six). A total of six swabs were collected from the hand and nail surfaces of kitchen workers. All samples were analyzed for coagulase-positive *S. aureus*, *Salmonella* spp. and *E. coli*.

Of the six vomit samples collected from affected children, all samples contained coagulase-positive *S. aureus* at levels of 6.0×10^1 to 3.3×10^3 CFU/mL. None of the vomit samples showed enterotoxin activity when analyzed directly, but strains isolated from

vomit samples of three children (strains 7.1, 8.1, and 8.2) produced enterotoxins (Table 3). A total of 20 out of 21 stool samples of children admitted to the hospitals contained coagulase-positive *S. aureus* and five out of six stool samples of kitchen workers were positive for *S. aureus*. On the other hand, the six swabs from hands of kitchen workers did not contain any coagulase-positive *S. aureus*, *Salmonella* spp. or *E. coli*.

Table 3. Characterization of coagulase-positive *S. aureus* isolates from food poisoning outbreaks among school children in Ninh Binh Province, Vietnam.

Isolate	Origin	MLST	<i>spa</i> Type	Classical	Virulence Genes	Antimicrobial
				Toxins ⁱ		Resistance ⁱⁱ
4.1	Chicken floss	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN
7.1	Vomit	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN
8.1	Vomit	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN
8.2	Vomit	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN
11.1	Frozen chicken	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN
11.4	Frozen chicken	ST6	t701	+	<i>coa, sea, sec, femA</i>	TET, PEN

ⁱ Phenotypic test by 3MTMTECRATM Staph Enterotoxin kit; ⁱⁱ PEN, penicillin; TET, tetracycline; “+” means presence of toxins.

S. aureus enterotoxins were detected directly from chicken floss but not from any of the other lunch food items served (deep-fried shrimp, tomato soup, and cooked rice). *S. aureus* was isolated from deep-fried shrimp (5.0×10^2 CFU/g) and chicken floss (1.5×10^3 CFU/g), but not in tomato soup or cooked rice. Two samples of frozen chicken from the canteen food storage used by the school canteen to prepare the lunch and the abattoir providing the chicken contained coagulase-positive *S. aureus* (3.5×10^5 CFU/g and 9.3×10^4 CFU/g). Enterotoxin was found in both frozen chicken samples. Three *S. aureus* isolated from chicken floss (isolate 4.1) and frozen chicken (isolates 11.1 and 11.4) produced enterotoxins (Table 3), whereas none of the strains isolated from deep-fried shrimp produced toxins.

The six *S. aureus* isolated and characterized from vomit and food samples all contained the *coa, sea, sec, and femA* virulence genes and produced enterotoxins (Table 3). Among all antimicrobials tested, the strains only showed resistance to penicillin and tetracycline (Table 3).

MLST profiling revealed that all six isolates were sequence type (ST) 6 and *spa* type t701 (Table 3). eBURST analysis revealed that ST6 is closely related to ST5 through ST2750 (Figure 1).

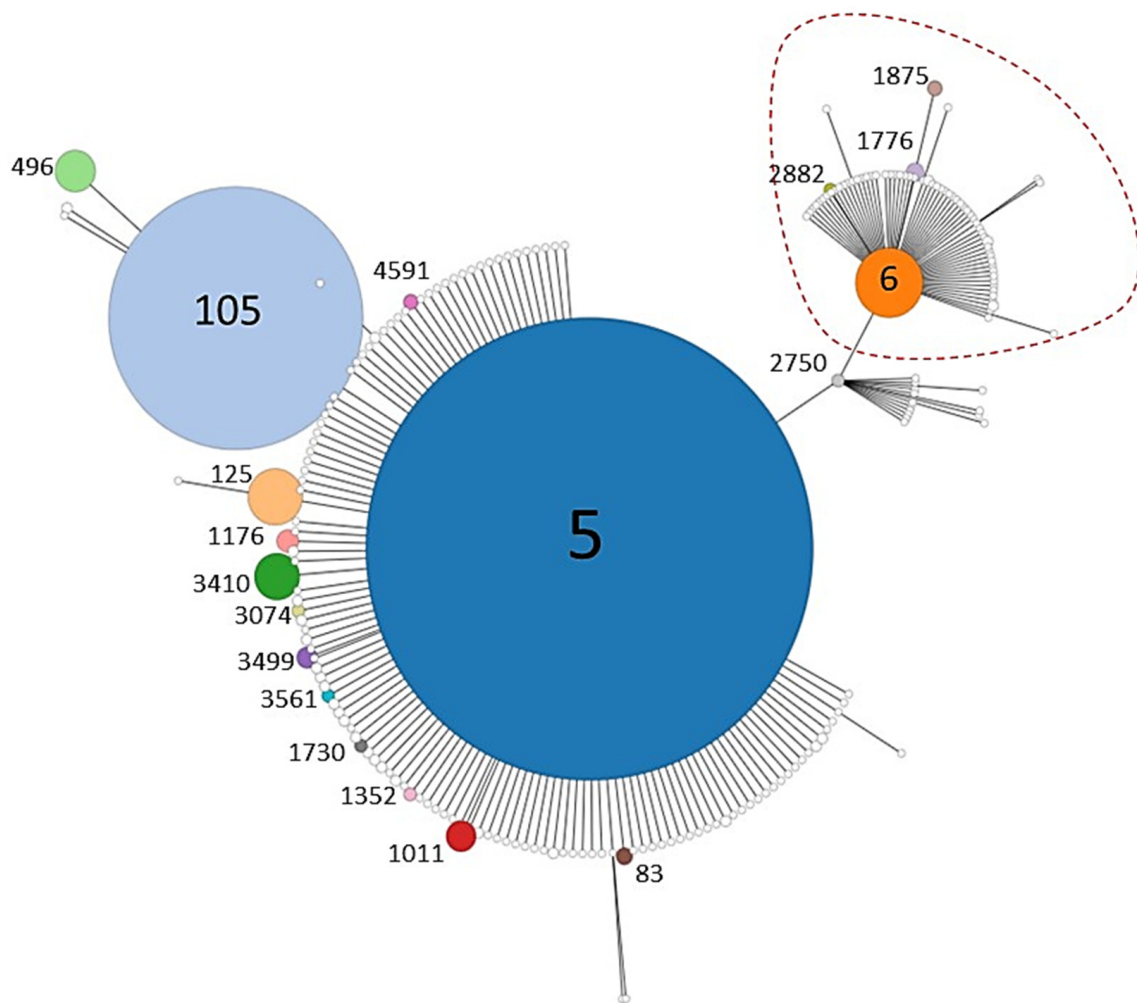


Figure 1. Clonal complex (CC) analysis of the six *S. aureus* isolates from this outbreak based on MLST profile and eBURST analysis. Each sequence type (ST) is represented by a node (circle), with the node size correlating with the number of isolates in each ST. Closely related STs form a clonal complex group, with the most prevalent ST located in the center. Each black line connecting two nodes indicates one locus difference. All *S. aureus* isolates in this study belonged to ST6.

4. Discussion

We were notified of a large-scale food poisoning outbreak at a primary school in Ninh Binh Province on 5 October 2018 after more than 300 children became ill with symptoms including nausea, vomiting, abdominal pain, and headache only hours after having lunch at the school canteen. Based on case symptoms, *S. aureus* was suspected to be the cause of the outbreak. Laboratory tests identified up to 10^3 CFU *S. aureus* per g in two food items served at lunch (chicken floss and deep-fried shrimp) and 10^5 CFU/g in two frozen chicken samples from the storage facility of the school canteen and the slaughter house delivering the chicken. Staphylococcal enterotoxins were directly detected from chicken floss and both frozen chicken samples. *S. aureus* was also isolated in all vomit samples and 25/33 stool samples collected, though no enterotoxin was detected. Since the presence of enterotoxin-producing *S. aureus* in food sources at concentrations of 10^5 to 10^8 CFU/g is typically considered as an intoxication dose for human [19], and enterotoxins were directly detected in chicken floss and frozen chicken samples, we believe that *S. aureus* was the cause of this food poisoning outbreak with chicken floss as the likely vehicle of infection. The attack rate found in our study was 38.0%, with common symptoms including vomiting (72; 80.9%), abdominal pain (64; 71.9%), nausea (38; 42.7%), fever (36; 40.4%), and diarrhea

(4; 4.5%). Our attack rate is lower than attack rates of 71% to 74% reported for previous outbreaks [20,21].

The source of transmission seemed to be the chicken bought from the abattoir, because *S. aureus* was found in frozen chicken samples originating from there. Though *S. aureus* was also found in stool samples of kitchen workers, no *S. aureus* was identified in their hand swab samples, thus it is unlikely that there were additional sources of contamination. Molecular analysis revealed that all strains found in chicken floss, vomit samples, and frozen chicken samples were identical and of clonal origin, confirming that the chicken from the abattoir was the sole contamination source of this outbreak. It is likely that there was a cross-contamination from raw meat to the cooked meat during food preparation process. However, it was not possible to obtain swab samples of kitchenware such as chopping board and knives to confirm such transmission during food preparation.

The single *S. aureus* strain responsible for this outbreak belonged to sequence type 6 (ST6), had *spa* type t701, was *coa*-positive, and carried two classical enterotoxin genes, *sea* and *sec*. This strain was tetracycline- and penicillin-resistant but oxacillin-, erythromycin-, gentamycin-, methicillin-, and vancomycin-sensitive. PCR tests detected the presence of *femA*, a risk factor known to influence the level of methicillin resistance in *S. aureus* [22]. However, *mecA*, the most well-known methicillin-resistant gene of *S. aureus*, was not detected, consistent with the result of our antimicrobial susceptibility test. ST6 is a clone commonly associated with foodborne *S. aureus* infections, e.g., in China [23–26], whereas other clones predominated in such outbreaks in South Korea (ST1, ST59, and ST30 [19]), Japan (ST45 and ST81 [20–22]) and Europe (ST45 and ST5 [23,24]). In China, both ST6-t701 [25] and ST6-t304 [23] have been described as being highly prevalent. The resistance profiles of the ST6 isolates in China are highly diverse, ranging from being fully susceptible to resistant to several antimicrobials with our outbreak ST6-t701 clone, showing penicillin and tetracycline resistance only. As in the present outbreak, practically all ST6 outbreak-associated isolates contain at least the *sea* gene and often more virulence genes as is the case for the current clone [23–27].

S. aureus enterotoxins (SEs) are classified based on their emetic activities with the gastrointestinal toxins being potent at just minute quantities at concentration ranges of nanograms to micrograms [27]. Due to their heat stability, most classical SEs cannot be destroyed by common food preparation processes [28]. Among staphylococcal classical enterotoxins, SEA is most frequently associated with staphylococcal food poisoning, with some reports suggesting that a dose as little as 20–100 ng of enterotoxin A can cause symptoms in exposed adults [28]. A recent study on three staphylococcal food poisoning cases in Europe found the levels of enterotoxins A and C in food leftovers were 0.015–0.019 and 0.132 ng/g, respectively [29]. In our study, we were able to detect ≥ 0.211 ng/g of enterotoxin A in both frozen raw and cooked chicken meats. This level of enterotoxin A is well within the widely recognized dose required to cause foodborne illnesses. It was pointed out in a recent study by Grispoldi and colleagues that during the production process of canned meat, enterotoxin was only detectable in bacteria-spiked products after 10 hrs at 37 °C or 48 hrs at 20 °C, and no enterotoxin was detected in products kept at 10 °C [30]. Thus, the conditions in and duration for which cooked food was kept in the school canteen before being served to the children would also significantly affect the enterotoxin level in food items. We do not disregard the hypothesis that, in addition to the possibility of *S. aureus* transmission between raw and cooked meats, intact heat-stable enterotoxin A left in cooked meat after the cooking process could also be the cause of this large-scale outbreak.

Overall, the results of the laboratory tests and epidemiological investigation revealed that *S. aureus* and staphylococcal enterotoxins were the cause of this outbreak, and frozen raw chicken meat as well as chicken floss were the source and vehicle of contamination. Several lines of prevention can be proposed to prevent future outbreaks, including (1) monitoring the hygiene of the slaughtering and raw food preparation process; (2) controlling the microbial quality of raw meat before cooking according to standard regulations (e.g.,

Salmonella spp., *S. aureus*, and *E. coli*); (3) conduct training on the principles of hazard analysis and critical control points (HACCP) for kitchen workers to avoid cross-contamination between fresh and cooked foods, ensuring hand hygiene by hand wash with soap and water as well as disinfection with alcohol 70% or other antimicrobial reagents, and wearing of gloves, a mask, and a hair net during food preparation; (4) conducting periodic inspection of cooked foods at school canteens to assess the quality during processing.

In Vietnam, the local provincial Departments of Food Safety and Hygiene are responsible for initiating investigations of foodborne disease outbreaks and collect samples, sending samples to be analyzed to the provincial Preventive Medicine Centers or National Institute for Food Control. In case of serious outbreaks, samples are sent to the National Institute for Food Control or the National Institute for Food Control will collect samples directly. The National Institute for Food Control often contacts and engages in questionnaire interviews of patients, hygiene inspections, and collection and laboratory analysis of food samples. Underreporting of foodborne diseases is well-known in developed, but less known in less developed countries. This is the case in Vietnam where it is difficult to trace the source of infection of people affected, and even more difficult to trace the source of contamination of the foods involved, partly due to limited capacity at district and provincial levels and the vast geographical areas that national food safety institutions have to cover. In such cases, studies such as the present one will provide the much-needed qualitative and quantitative data to aid strain monitoring and guiding preventative measures for future outbreaks.

5. Conclusions

In this study, we investigated the epidemiology and molecular characteristics of a large-scale food poisoning outbreak at a school canteen in Ninh Binh Province, Vietnam, that led to 352 children being hospitalized. A single clone of SE-producing *S. aureus* strain was identified as the causative pathogen of the outbreak. Both enterotoxins and the producing strain were found in prepared chicken floss and frozen chicken. The strain was also found in vomit samples of patients and stool samples of both patients and kitchen workers. The outbreak strain was identified as sequence type 6 (ST6), *spa* type t701, was *coa*-positive, and carried two classical enterotoxin genes, *sea* and *sec*. This strain was tetracycline- and penicillin-resistant but oxacillin-, erythromycin-, gentamycin-, methicillin-, and vancomycin-sensitive. These data not only provide necessary information to better understand and monitor *S. aureus* strains associated with food poisoning outbreaks but also aid surveillance of antimicrobial resistance in *S. aureus* in Vietnam.

Author Contributions: T.T.N. conceptualized the study, designed experiments, and edited the final manuscript. H.H.T.L. performed interviews, collected samples, performed experiments, analyzed data, and draft the manuscript. A.D. and P.S.A. contributed to the study's conceptualization, experiment design, and data analysis, as well as drafted and revised the manuscript. H.M.N. and Y.T.T. performed laboratory investigations, analyzed experiment data, and drafted and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by the Specific Task Program 2019 (Vietnam Ministry of Health, numbered 149/QD-BYT).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Please contact author for data requests.

Acknowledgments: We thank members of our laboratories for meaningful discussion and technical assistance. This work made use of the PubMLST website MLST (<https://pubmlst.org/saureus/> (accessed on 27 November 2018)) [13] and the SpaServer website (<http://spaserver2.ridom.de> (accessed on 27 November 2018)).

Conflicts of Interest: The authors declare no competing interests.

References

1. Kim, Y.B.; Seo, K.W.; Jeon, H.Y.; Lim, S.K.; Lee, Y.J. Characteristics of the antimicrobial resistance of *Staphylococcus aureus* isolated from chicken meat produced by different integrated broiler operations in Korea. *Poult. Sci.* **2018**, *97*, 962–969. [[CrossRef](#)]
2. Le Loir, Y.; Baron, F.; Gautier, M. *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* **2003**, *2*, 63–76.
3. Wieneke, A.A.; Roberts, D.; Gilbert, R.J. Staphylococcal food poisoning in the United Kingdom, 1969–1990. *Epidemiol. Infect.* **1993**, *110*, 519–531. [[CrossRef](#)]
4. Wu, S.; Huang, J.; Wu, Q.; Zhang, J.; Zhang, F.; Yang, X.; Wu, H.; Zeng, H.; Chen, M.; Ding, Y.; et al. *Staphylococcus aureus* Isolated From Retail Meat and Meat Products in China: Incidence, Antibiotic Resistance and Genetic Diversity. *Front. Microbiol.* **2018**, *9*, 2767. [[CrossRef](#)]
5. Balaban, N.; Rasooly, A. Staphylococcal enterotoxins. *Int. J. Food Microbiol.* **2000**, *61*, 1–10. [[CrossRef](#)]
6. Wang, X.; Meng, J.; Zhang, J.; Zhou, T.; Zhang, Y.; Yang, B.; Xi, M.; Xia, X. Characterization of *Staphylococcus aureus* isolated from powdered infant formula milk and infant rice cereal in China. *Int. J. Food Microbiol.* **2012**, *153*, 142–147. [[CrossRef](#)] [[PubMed](#)]
7. Bui, T.M.H.; Zahid, H.M.; Sucharit, B.N.; Afework, K.; Nguyen, V.N.; Alizadeh, M.; Masayuki, Y.; Fusao, O.; Nguyen, T.L.; Ha, T.A.D.; et al. Toxigenicity and genetic diversity of *Staphylococcus aureus* isolated from Vietnamese ready-to-eat foods. *Food Control* **2010**, *21*, 166–171.
8. Veras, J.F.; do Carmo, L.S.; Tong, L.C.; Shupp, J.W.; Cummings, C.; Dos Santos, D.A.; Cerqueira, M.M.; Cantini, A.; Nicoli, J.R.; Jett, M. A study of the enterotoxigenicity of coagulase-negative and coagulase-positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais, Brazil. *Int. J. Infect. Dis.* **2008**, *12*, 410–415. [[CrossRef](#)] [[PubMed](#)]
9. Yoshida, R.; Kuwahara-Arai, K.; Baba, T.; Cui, L.; Richardson, J.F.; Hiramatsu, K. Physiological and molecular analysis of a *mecA*-negative *Staphylococcus aureus* clinical strain that expresses heterogeneous methicillin resistance. *J. Antimicrob. Chemother.* **2003**, *51*, 247–255. [[CrossRef](#)] [[PubMed](#)]
10. Strommenger, B.; Kettlitz, C.; Weniger, T.; Harmsen, D.; Friedrich, A.W.; Witte, W. Assignment of *Staphylococcus* isolates to groups by *spa* typing, Smal macrorestriction analysis, and multilocus sequence typing. *J. Clin. Microbiol.* **2006**, *44*, 2533–2540. [[CrossRef](#)] [[PubMed](#)]
11. Urwin, R.; Maiden, M.C. Multi-locus sequence typing: A tool for global epidemiology. *Trends Microbiol.* **2003**, *11*, 479–487. [[CrossRef](#)] [[PubMed](#)]
12. Maiden, M.C.; Bygraves, J.A.; Feil, E.; Morelli, G.; Russell, J.E.; Urwin, R.; Zhang, Q.; Zhou, J.; Zurth, K.; Caugant, D.A.; et al. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 3140–3145. [[CrossRef](#)] [[PubMed](#)]
13. Jolley, K.A.; Maiden, M.C. BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinf.* **2010**, *11*, 595. [[CrossRef](#)] [[PubMed](#)]
14. Spratt, B.G.; Hanage, W.P.; Li, B.; Aanensen, D.M.; Feil, E.J. Displaying the relatedness among isolates of bacterial species—The eBURST approach. *FEMS Microbiol. Lett.* **2004**, *241*, 129–134. [[CrossRef](#)]
15. Feil, E.J.; Li, B.C.; Aanensen, D.M.; Hanage, W.P.; Spratt, B.G. eBURST: Inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J. Bacteriol.* **2004**, *186*, 1518–1530. [[CrossRef](#)] [[PubMed](#)]
16. Mellmann, A.; Weniger, T.; Bessenbrugge, C.; Rothganger, J.; Sammeth, M.; Stoye, J.; Harmsen, D. Based Upon Repeat Pattern (BURP): An algorithm to characterize the long-term evolution of *Staphylococcus aureus* populations based on *spa* polymorphisms. *BMC Microbiol.* **2007**, *7*, 98. [[CrossRef](#)]
17. Argimon, S.; Abudahab, K.; Goater, R.J.E.; Fedosejev, A.; Bhai, J.; Glasner, C.; Feil, E.J.; Holden, M.T.G.; Yeats, C.A.; Grundmann, H.; et al. Microreact: Visualizing and sharing data for genomic epidemiology and phylogeography. *Microb. Genom.* **2016**, *2*, e000093. [[CrossRef](#)]
18. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [[CrossRef](#)]
19. Doyle, M.P.; Beuchat, L.R. (Eds.) *Staphylococcus aureus*. In *Food Microbiology: Fundamentals and Frontiers*, 3rd ed.; ASM Press: Washington, DC, USA, 2007; pp. 493–518.
20. Gumbo, A.; Bangure, D.; Gombe, N.T.; Mungati, M.; Tshimanga, M.; Hwalima, Z.; Dube, I. *Staphylococcus aureus* food poisoning among Bulawayo City Council employees, Zimbabwe, 2014. *BMC Res. Notes* **2015**, *8*, 485. [[CrossRef](#)]
21. Pillsbury, A.; Chiew, M.; Bates, J.; Sheppard, V. An outbreak of staphylococcal food poisoning in a commercially catered buffet. *Commun. Dis. Intell.* **2013**, *37*, E144–E148.
22. Berger-Bachi, B.; Barberis-Maino, L.; Strassle, A.; Kayser, F.H. FemA, a host-mediated factor essential for methicillin resistance in *Staphylococcus aureus*: Molecular cloning and characterization. *Mol. Gen. Genet.* **1989**, *219*, 263–269. [[CrossRef](#)]
23. Chen, Q.; Xie, S. Genotypes, Enterotoxin Gene Profiles, and Antimicrobial Resistance of *Staphylococcus aureus* Associated with Foodborne Outbreaks in Hangzhou, China. *Toxins* **2019**, *11*, 307. [[CrossRef](#)]
24. Li, G.; Wu, S.; Luo, W.; Su, Y.; Luan, Y.; Wang, X. *Staphylococcus aureus* ST6-t701 isolates from food-poisoning outbreaks (2006–2013) in Xi'an, China. *Foodborne Pathog. Dis.* **2015**, *12*, 203–206. [[CrossRef](#)] [[PubMed](#)]
25. Liao, F.; Gu, W.; Yang, Z.; Mo, Z.; Fan, L.; Guo, Y.; Fu, X.; Xu, W.; Li, C.; Dai, J. Molecular characteristics of *Staphylococcus aureus* isolates from food surveillance in southwest China. *BMC Microbiol.* **2018**, *18*, 91. [[CrossRef](#)]

26. Yan, X.; Wang, B.; Tao, X.; Hu, Q.; Cui, Z.; Zhang, J.; Lin, Y.; You, Y.; Shi, X.; Grundmann, H. Characterization of *Staphylococcus aureus* strains associated with food poisoning in Shenzhen, China. *Appl. Environ. Microbiol.* **2012**, *78*, 6637–6642. [[CrossRef](#)]
27. Argudin, M.A.; Mendoza, M.C.; Rodicio, M.R. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* **2010**, *2*, 1751–1773. [[CrossRef](#)] [[PubMed](#)]
28. Hennekinne, J.A.; De Buyser, M.L.; Dragacci, S. *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiol. Rev.* **2012**, *36*, 815–836. [[CrossRef](#)] [[PubMed](#)]
29. Denayer, S.; Delbrassinne, L.; Nia, Y.; Botteldoorn, N. Food-Borne Outbreak Investigation and Molecular Typing: High Diversity of *Staphylococcus aureus* Strains and Importance of Toxin Detection. *Toxins* **2017**, *9*, 407. [[CrossRef](#)]
30. Grispoldi, L.; Popescu, P.A.; Karama, M.; Gullo, V.; Poerio, G.; Borgogni, E.; Torlai, P.; Chianese, G.; Fermani, A.G.; Sechi, P.; et al. Study on the Growth and Enterotoxin Production by *Staphylococcus aureus* in Canned Meat before Retorting. *Toxins* **2019**, *11*, 291. [[CrossRef](#)]