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Investigation on Food Poisoning Outbreak Caused by *Staphylococcus aureus* Enterotoxin A and Incidence of Staphylococcal Food Poisoning in the Kingdom of Bahrain

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Abstract

Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases resulting from the ingestion of food contaminated with *Staphylococcus aureus* enterotoxins. The aim of the study is to provide an overview of the incidence of staphylococcal food poisoning in Bahrain and to describe the epidemiological and microbiological investigations of food poisoning outbreak among school members in 2018. A single-center, retrospective study was conducted at Public Health Laboratories (PHL) in Bahrain. All food poisoning samples received at PHL from 2013 to 2021 were included in this study. The epidemiological and laboratory investigations of an outbreak in 2018 among school members were included. Overall, staphylococcal enterotoxin food poisoning accounted for 28.1% of confirmed food poisoning cases; Staphylococcal enterotoxin A (SEA) was the most prevalent toxin (60%). In the school outbreak, the laboratory investigation showed the presence of SEA in chicken shawarma. SEA has also been isolated from patients' stool samples and food handlers' hands, nose and throat swabs. Outbreak investigations revealed that poor hygiene practices during food processing and improper food handling practices accounted for this outbreak. SEA was the cause of food poisoning outbreak among members of the school due to poor personal hygiene of the food handlers and improper food handling. Training workers to follow all hygienic protocols and practices for preparing, handling, and cooking food should be considered to avoid future outbreaks.

Keywords

Staphylococcus aureus, Staphylococcal Food Poisoning, Foodborne Outbreak, Enterotoxins, Food Handlers, Detection, Analysis

1. Introduction

Staphylococcus aureus is a major cause of nosocomial and community-acquired infections [1]. The pathogenicity of *S. aureus* is due to toxins and invasive enzymes [2]. Staphylococcal food poisoning is one of the most prevalent foodborne illnesses worldwide. According to the Centers for Disease Control, 240,000 cases occur annually in the USA, leading to 1000 hospitalizations and six deaths. The European Food Safety Authority has documented an increasing incidence of SFP outbreaks in Europe; in 2015, there were 434 SFP outbreaks, which equals 10% of all outbreaks reported [3]. Staphylococcal food poisoning results from eating foods contaminated with enterotoxins produced by *S. aureus*. It is characterized by sudden onset of nausea, vomiting, stomach cramps, diarrhea, and fever. Symptoms usually develop within thirty minutes to eight hours after eating food contaminated with enterotoxins [4].

Twenty-eight different staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like toxins (SEls) have been identified along with many variants [5]. The true SEs, are toxins that show emetic activity and SEls, are non-emetic toxins or toxins that have not been tested. Staphylococcal enterotoxin A (SEA), Staphylococcal enterotoxin B (SEB), Staphylococcal enterotoxin C (SEC), Staphylococcal enterotoxin D (SED) and Staphylococcal enterotoxin E (SEE) are well defined and classified as classic enterotoxins [6]. These five classical SEs (SEA, SEB, SEC, SED, SEE) contribute to 95% of SFP outbreaks, SEA is the most common comprising > 50% of SFP outbreaks [7]. Staphylococcal enterotoxins are non-glycosylated, antigenically distinct, low molecular weight (25 - 30 kDa) proteins consisting of approximately 220 - 240 amino acids that all fold into homologous globular structures [8] [9]. The genes encoding the different enterotoxins are carried and distributed by different mobile genetic elements (prophages, plasmids, pathogenicity islands, enterotoxin gene cluster (egc) and the staphylococcal cassette chromosome (SCC)) [10], Sea gene composed of 771 base pairs encodes for SEA precursor of 257 amino acid residues [11].

Staphylococcus aureus is a gram-positive coccus, facultative anaerobic, non-motile, non-spore forming bacterium [12]. This bacterium produces a carotenoid pigment that appears golden, leading to the appearance of golden colonies [6]. *Staphylococcus aureus* is found as a part of normal flora on the skin and mucous membranes [10]. It can be found in the air, water, dust, and excrement of humans and animals [2]. *Staphylococcus aureus* can survive for prolonged periods after initial contact on hands and inanimate and environmental surfaces [13]. Staphylococcal enterotoxins are short proteins that are soluble in water and saline solutions, heat-stable, and resistant to several environmental conditions such as freezing, drying and degradation from proteolytic enzymes such as pepsin or trypsin, enabling them to function in the gastrointestinal tract after ingestion [14]. The nuc gene, which is responsible for heat resistance, is highly related to enterotoxin production and can be considered a marker of *S. aureus* enterotoxin infection [1].

Staphylococcal food poisoning can occur with any food that provides sufficient

carbon and amino acid sources for *S. aureus* growth [15]. Several types of food act as optimal media for the growth of *S. aureus* like meat, poultry, egg, dairy products, salad, bakery products, and sandwich fillings [13]. Certain strains of *S. aureus* are able to resist high lactic acid concentrations, which promotes their proliferation in a variety of foods, such as cheese, meat, salads, and milk chocolate [16]. *Staphylococcus aureus* can grow and produce SEs over a wide range of temperatures (7˚C - 48.5˚C; optimal 30˚C - 37˚C), pH (4.2 - 9.3; optimal 7 - 7.5), and sodium chloride concentrations up to 15% NaCl [13].

Staphylococcal enterotoxins are transmitted to human from food contaminated with *S. aureus* through food surfaces, food handlers, contaminated raw materials, unsuitable handling of processed food and insufficient cooling of food [5] [12]. Food handlers contaminate food through manual contact with their noses or hands or through respiratory tract secretions [10]. Approximately one-third of healthy human populations carry *S. aureus* in their noses, which often contaminate the face, hands and fingers. Therefore, nasal carriers can act as a source of cross-contamination in food [17].

The severity of food poisoning symptoms depends on the quantity of toxin ingested and the health status of the affected person [14] and on the toxin type [10]. When the number of *S. aureus* organisms/g in the food exceed 100,000, the intoxication dose of SE is less than 1.0 ug. However, in more sensitive individuals, ingesting 100 - 200 ng of enterotoxin can already result in symptoms [18]. Symptoms appear rapidly within 30 minutes after ingesting the SE contaminated food. The incubation period is from two to seven hours, and symptoms disappear within 12 hours [10]. Symptoms are commonly characterized by abdominal pain, diarrhea, nausea and vomiting [8]. Staphylococcal food poisoning is often self-limiting with recovery occurring one to three days after the onset of symptoms. However, symptoms may be more severe in young, elderly, and immunocompromised patients [9]. Treatment is generally aimed at restoring fluid and electrolyte losses as a consequence of severe vomiting [14].

Staphylococcal enterotoxins can penetrate the small intestine via epithelial or mucus-producing goblet cells. Staphylococcal enterotoxin A stimulates the release of serotonin and histamine from mast cells. Serotonin then stimulates the vagus nerve in the vomiting center of the brain by triggering an emetic response [15]. The release of inflammatory mediators is accountable for the local damage of the gastrointestinal tract, mostly appearing in the stomach and the upper part of the small intestine [19]. Despite strong induction of emesis, the symptoms of diarrhea are often less apparent in SFP, which may be due to the inability of some SEs, such as SEA and SEC, to cause the fluid exudation and dilation of the small intestine [9]. Diarrhea may happen as a result of water inhibition and electrolyte reabsorption in the small intestine [19].

This study aimed to provide an overview of staphylococcal food poisoning, identify the association of *S. aureus* enterotoxin with food poisoning outbreaks and describe the epidemiological and microbiological investigations of the food

poisoning outbreak in 2018 among school members in Bahrain.

2. Materials and Methods

2.1. Study Design and Study Setting

A single-center, retrospective study was conducted at PHL in Bahrain on all food poisoning samples received from 2013 to 2021. This includes the epidemiological and laboratory investigations of food poisoning outbreak in 2018 among 90 school members (87 students and three teachers). The data of all samples received from 2013 to 2021 was extracted from local database at PHL. The sampling strategy used in this study was based on routine inspections in the Food Control Department as part of the inspectors' regular duties, targeting food establishments and their products for evaluation, rather than on a random or blinded approach.

All samples were analyzed according to the microbiological criteria approved by the GCC Standardization Organization (GSO). The reference bacterial strain used in this study was *Staphylococcus aureus* ATCC 6538. This study was approved by Health Research Committee in Ministry of Health, Bahrain on January 2023.

2.2. Epidemiological Investigation

A notification was received from the hospital to the public health at 1:30 p.m. that two suspects of food poisoning reported eating breakfast in school at 9:00 a.m., and five more cases were reported from another hospital at 4:15 p.m. Epidemiological investigation to collect information and samples from patient, food and food handlers was done. The Food Control Department was notified to take the necessary actions.

Five samples were collected and sent to PHL for microbiological analysis. A sample from a chicken shawarma leftover and four swabs from kitchen surfaces and cutting boards (meat table, vegetable table, meat cutting board and vegetable cutting boards) were collected. Swabs were collected from the hands, nose, and throat of the four food handlers. Nine stool samples from the students were also collected.

2.3. Laboratory Investigation

Microbiological analysis of the samples and detection of SEA were performed. Food samples were evaluated according to the Microbiological Criteria for Foodstuffs (GSO 1016/2015) under the ready-to-eat foods category. The limit of *S. aureus* in the ready-to-eat foods category is equal to 20 CFU/g ("m" value), below which is considered satisfactory, and 10^2 CFU/g ("M" value), above which is considered unsatisfactory. Samples were analyzed according to ISO 6888-1:1999 "Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species)".

A 10^{-1} dilution of chicken shawarma was prepared in buffered peptone water (CM1049), and aliquots of the food dilution were spread on Baird-Parker agar plates (CM1127) (Oxoid, Thermo Fisher Scientific, UK). The chicken shawarma leftover was tested for the detection of SE using VIDAS SET2, an automated qualitative test carried out on the miniVIDAS instruments for the detection of staphylococcal enterotoxins (SEA-SEE) using an enzyme linked fluorescent assay (bioMérieux, Marcy-L'Etoile, France). Reconstituted extraction buffer (25 ml) was added to 25 g of food in a blender bag and centrifuged for 15 minutes at 3000 - 5000 g at 18˚C - 25˚C. The supernatant was filtered and 500 µL of the filtrate was added to the VIDAS strip well. Environmental swabs, food handlers' hand, nose, and throat swabs, and patient stool samples were inoculated on Baird-Parker agar plates. Plates were incubated at 35˚C or 37˚C for 48 h ± 2 h.

Suspected colonies of *S. aureus* (black/grey, shiny colonies with white and clear zones) were counted and confirmed using the Staphaurex latex agglutination test (Remel, Thermo Fisher Scientific, UK) and identified using Maldi-Tof MS Microflex LT system (Bruker Daltonik, Germany). *Staphylococcus aureus* isolates from chicken shawarma, food handler swabs, and patient stool samples were tested for the production of SEA-SED by SET-RPLA Kit (Oxoid, Thermo Fisher Scientific, UK). The isolates were inoculated into tryptone soya broth (CM129) and incubated at 37˚C for 18 - 24 h (Oxoid, Thermo Fisher Scientific, UK). The broth was then filtrated using a 0.22 mm low protein-binding membrane filter. A Summary of the workflow for the laboratory investigations performed at PHL is shown in Figure 1.

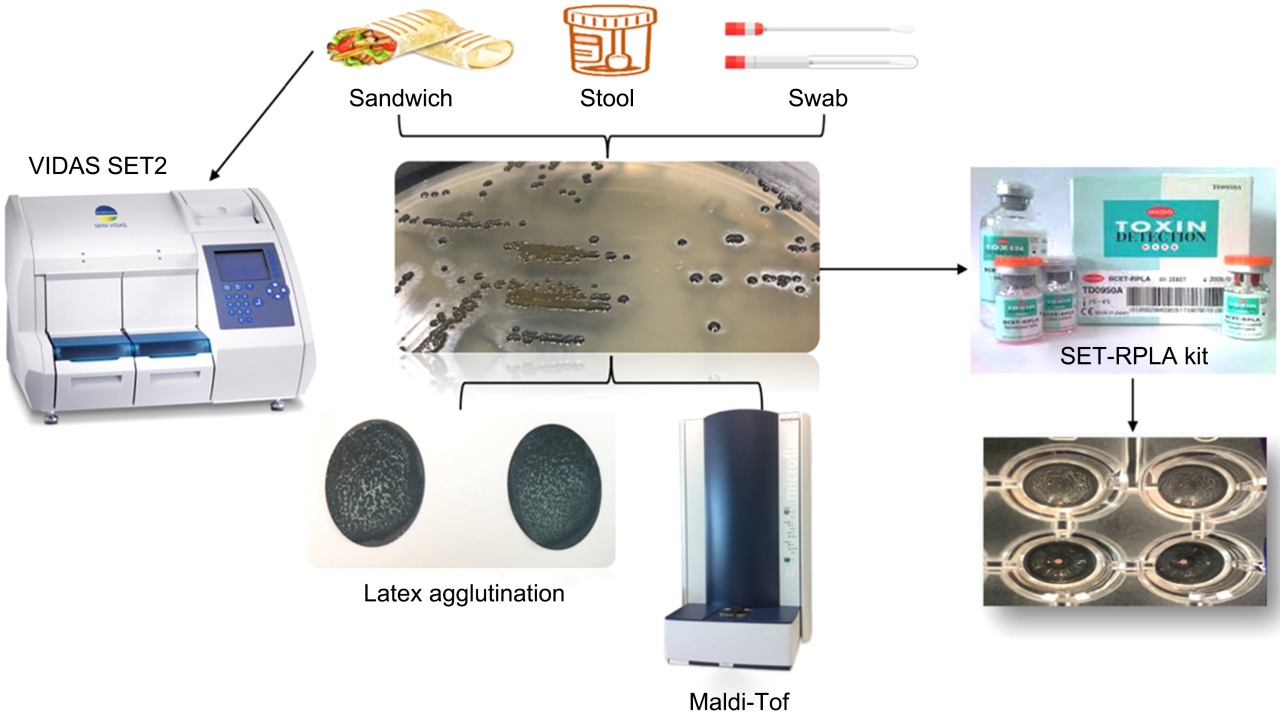

Figure 1. General overview of analytical methods used for detection of SE.

2.4. Statistical Analysis

The analysis in this study was performed using Microsoft Office Excel (2016), which was utilized primarily for graphical representation of the data and included the following information: number and type of samples tested, food category, number of food poisoning cases and microbiological parameters with test result. The data was analyzed as number and percentage and presented in tables and graphs.

3. Results and Discussion

3.1. Incidence of Staphylococcal Food Poisoning

In Bahrain, approximately 680 food poisoning cases were reported to the Food Control Department from 2013 to 2021 (Figure 2), 57 cases were confirmed and the causative agents involved in these food poisoning cases were isolated. *Salmonella* bacteria were the most common cause of food poisoning accounting for 27 (47.4%) confirmed cases, followed by *Staphylococcal aureus* enterotoxins 16 (28.1%) and *Bacillus cereus* enterotoxin 14 (24.6%). Among the 16 cases of SFP, 20 types of SEs were identified and SEA was the most prevalent toxin detected in 12 (60%) cases followed by SEB 3 (15%), SED 3 (15%), and SEC 2 (10%) (Figure 3). The main factors contributing to these SFP outbreaks were poor personal hygiene, improper food handling practices and inadequate refrigeration of foods. Hot and humid weather at Bahrain increases the risk of SFP as it contributes to increase microbial activity and spoilage of food. The most commonly reported food categories involved in SFP are ready-to-eat food (61%), followed by salad and appetizers (22%), cakes and desserts (11%), and dairy products (6%) (Figure 4). The most commonly reported food category involved in SFP is ready-to-eat food. The disease is frequently associated with protein-rich food such as meat and meat-based products [12]. Traditional Bahraini cuisine includes meat, dairy, and egg-based dishes, which are common carriers of SEs. Buffet-style dining and communal food sharing increase contamination risks. Improper handling and storage of these foods increase contamination risks. Bahrain's hot and humid climate creates an ideal environment for bacterial growth. *Staphylococcus aureus* thrives between 7˚C - 48˚C, with an optimum of 37˚C, making improper food storage a major risk.

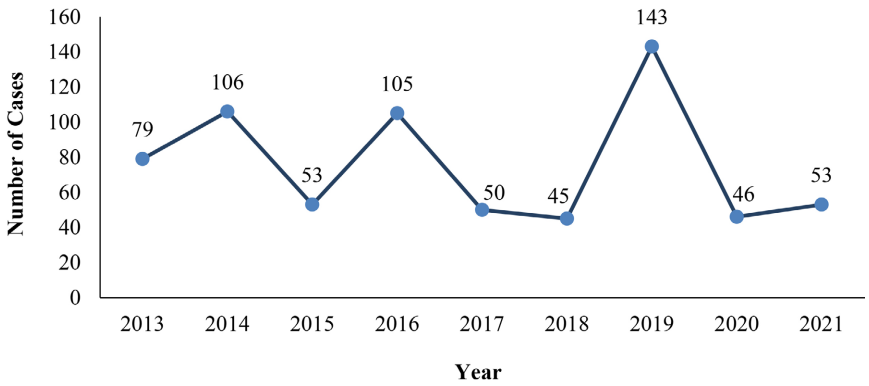

Figure 2. Food poisoning cases reported from 2013-2021.

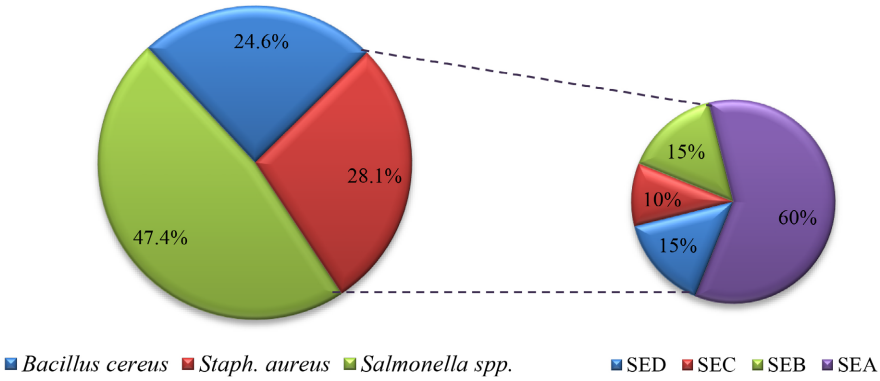

Figure 3. Causative agents involved in food poisoning with % of cases from 2013-2021.

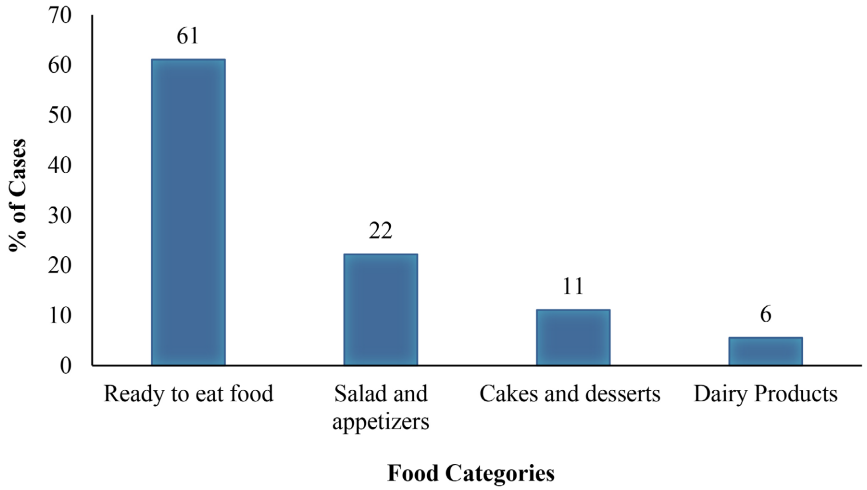

Figure 4. Food categories involved in staphylococcal food poisoning.

3.2. Epidemiological and Laboratory Investigation

Three of the educational staffs and 87 students were affected by the food poisoning, and 68.9% (62) were male. The majority of cases were between the age group of 3 - 5 years (51.1%), followed by 9 - 11 years (22.2%) and 6 - 8 years (20%) (Table 1). Symptoms appeared in the form of vomiting, abdominal pain, and diarrhea one to two hours after the students ate chicken shawarma in the school. Three cases had severe symptoms were admitted to the hospital. The school confirmed the distribution of 400 chicken shawarma sandwiches between students and some teachers: 300 shawarma were given to between kindergarten and first-grade students and 100 to other students and teachers.

Laboratory results indicated the presence of almost 2×10^3 CFU/g *S. aureus* in chicken shawarma, which was considered unsatisfactory according to the Microbiological Criteria for Foodstuffs (GSO 1016/2015). The VIDAS SET2 test result was positive for SE in the leftover sample. Moreover, S. aureus colonies were isolated from swabs of three food handlers (Table 2) and stool samples from eight students. The SEA was detected in chicken shawarma, food handler swabs and student stool samples.

Table 1. Details of cases according to age and gender.

Age group in years	Male	Female	Total
3 - 5	27	19	46
6 - 8	16	2	18
9 - 11	16	4	20
12 - 16	3	0	3
>16	0	3	3
Total	**62**	**28**	**90**

Table 2. The laboratory results of food handlers.

Food Handlers	Swabs			Staphylococcal Enterotoxin (SE)
	Nose	Throat	Hand	
1	Not Isolated	Not Isolated	Not Isolated	-
2	*S. aureus* Isolated	*S. aureus* Isolated	Not Isolated	SEA
3	*S. aureus* Isolated	*S. aureus* Isolated	*S. aureus* Isolated	SEA
4	*S. aureus* Isolated	*S. aureus* Isolated	Not Isolated	SEA

The laboratory results corresponded with the symptoms that appeared on the students quickly after eating chicken shawarma, as poisoning with the bacteria isolated in the laboratory usually appears within a period ranging from half an hour to seven hours after eating contaminated food [10]. Our investigation revealed that *S. aureus* enterotoxin A in chicken shawarma was the cause of this outbreak. Several studies have reported that *S. aureus* counts need to be $>10^5$ CFU/g to considered as toxic and produce SEs in food [5] [10] [12]. However, SEA was detected with *S. aureus* counts of up to 10^3 CFU/g in our study, this might be due to an insufficient amount of the leftover sample. This finding was similar to a study conducted by Le *et al.* [20] on food poisoning outbreak among children in primary school after eating lunch from a school canteen. *Staphylococcus aureus* was isolated from chicken floss at up to 10^3 CFU/g. *Staphylococcus aureus* enterotoxin A was detected directly from chicken floss. Denayer *et al.* [10] reported in their study on three food poisoning outbreaks (A, B, C) occurred in 2013 in Belgium that SEA was detected in outbreak A with *S. aureus* counting up to 200 CFU/g in mashed potato, and up to 10^3 CFU/g in chicken and sausage in outbreak B. Moreover, our investigation showed that SEA was detected from nasal/throat swabs of food handlers and patient stool samples which is similar to the findings of the Belgian study.

Staphylococcal food poisoning outbreak was likely caused by contamination of chicken shawarma with *S. aureus* from food handlers, either through their hands

or coughing and sneezing over food due to poor personal hygiene, improper handling of cooked food, improper cleaning of food preparation areas, and lack of sufficient tools for preservation and storage. Medical counseling was provided to the school canteen workers who were confirmed to be infected with *S. aureus*; appropriate treatment was also provided, and coordination was made for follow-up after treatment. Coordination was made with the school to follow all hygiene instructions and good practices in food preparation such as training workers on hygienic practices for preparing, handling and cooking food, as well as ensuring that no ill individuals could contaminate food or food contact surfaces.

Food handlers have been implicated in a large number of foodborne diseases, where *S. aureus* is one of the important pathogens often transmitted to food through food handler's nasal and hand carriage [21]. Therefore, it is important to detect healthy and asymptomatic *S. aureus* carriers among food handlers to prevent possible food contamination. Due to the fact that nasal carriers frequently come into touch with hands, fingers, and the face, they can readily become skin carriers [22]. Iyevhobu and colleagues conducted a study to determine the prevalence of *S. aureus* from 300 nose and skin swab samples from healthy food handlers in restaurants in Nigeria. The results revealed that 30 (10%) of them were carriers of *S. aureus*, with the highest incidence of 24 (16%) from nasal swab and 6 (7.5%) from skin swab [21]. Another study conducted by Çakıcı *et al.* [22] on 300 food workers from different sectors in Türkiye demonstrated that *S. aureus* was isolated from 90 (30%) of individuals noses and 84 (28%) from hands. Forty-two (33.6%) *S. aureus* strains were positive for one or more SE genes out of 125 strains, and SEA was found at rates of 14.4%. Furthermore, A study conducted by Ahmed in Sudan on 186 food handlers who were working in different restaurants showed that SEA was the most common SE (19.4%) out of the total 93 isolated strains of *S. aureus* [23]. Alhashimi *et al.* [11] reported in their study on 332 food handlers related to SFP outbreaks in Iran that sea gene was detected in 16 (16%) out of 100 isolated strains of *S. aureus* from handlers.

Most cases of SFP can be prevented by adequate hygiene measures and maintained cooling chains [15]. Leaving food out for extended periods, especially in warm conditions, allows bacteria to multiply. Reheating does not destroy the toxin, so contaminated food must be discarded [16]. Maintaining the cold chain at temperatures below 7˚C - 10˚C is essential for reducing the risk of *S. aureus* growth and production of enterotoxin in food products [6]. The permissive temperature for growth and toxin production is between 6˚C and 46˚C. Therefore, the ideal temperature for cooked food should be either above 60˚C or below 5˚C. Other preventive measures should be considered, such as control of raw food, proper handling, cleaning, and disinfection of equipment and tools used in food processing and preparation [13], as stainless steel, which is widely used in the food business, is one of the inert surfaces on which *S. aureus* shows a good degree of adherence and facilitates the formation of biofilm that improve the ability to tolerate disinfectants [24].

Establishing mandatory health screening protocols, which include regular medical examinations for food handlers, is a critical measure to control food poisoning outbreaks by ensuring that individuals with skin infections or open wounds are identified before handling food. Additionally, conducting regular inspections can help ensure compliance with safety protocols and food safety standards. Including food safety training programs as a requirement for employment in the food sector would enhance preventive measures. Moreover, public awareness campaigns should focus on safe food handling and storage practices to reduce the risk of contamination and educate consumers about food safety and its risks.

4. Limitations

There were a number of limiting factors that influenced the epidemiological investigation process and thus the isolation of the causative agent. Some cases of food poisoning have been misdiagnosed as viral gastroenteritis at hospitals, and some patients refused to provide a stool sample for laboratory testing. Moreover, there was missing data that the patient did not provide during the investigation. Some patients, especially children, forget some information such as the time of onset of symptoms, type of symptoms, and food they ate. Furthermore, most of the samples received were not leftover due to delay in taking action either due to missing information or delay in reporting food poisoning cases, which reduces the chance of obtaining leftover samples, which explains the low number of confirmed cases.

Since the sampling process was neither random nor blinded, there may be selection bias. Despite these limitations, the methodology is consistent with practical food safety monitoring protocols, ensuring practical applicability and compliance with applicable regulations.

5. Conclusion

Staphylococcus aureus enterotoxin is the second common cause of confirmed foodborne outbreaks in Bahrain from 2013-2021, and SEA was the most prevalent toxin involved mostly in ready-to-eat food category. In this study, we investigated the epidemiological and microbiological investigations of food poisoning outbreaks among school members in 2018. The causative agent of the outbreak was SEA-producing *S. aureus* strain isolated from chicken shawarma, patient stool samples and food handlers' nose/throat swabs due to improper food handling and poor personal hygiene with the presence of nasal carriers among food handlers. Training food handlers to follow personal hygienic protocols and food safety practices for preparing, handling, and cooking should be considered to avoid future food poisoning outbreaks. Moreover, initiating the whole genome sequencing of *S. aureus* in food poisoning cases will create a qualitative shift in investigating and tracing the source of SFP outbreaks. Finally, initiating the whole genome sequencing for *S. aureus* isolates would create a qualitative shift in investigating and tracing the source of SFP outbreaks.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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