Staphylococcal Food Poisoning in a Restaurant in Taichung City, Taiwan

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Abstract

Public Health Bureau in Taichung City, was informed of a suspected food poisoning in a restaurant on September 8th, 2008. 2 patients were sent to the emergency room. 2 rectal swab samples and 2 vomiting samples from these 2 patients were sent to the Bureau. 3 rectal swab samples from 3 kitchen workers of this establishment were sent to Taiwan CDC. Meanwhile, 2 food samples (from a goose meat lunch box) and 3 environmental samples (chopping board swab, knife swab and water) were sent to Bureau of Food and Drug Analysis for further examination. Staphylococcus aureus enteral toxic type A was isolated from 2 vomiting samples, 1 rectal swab from a patient, and 2 food samples. The isolated pathogens were analyzed by pulsed-field gel electrophoresis (PFGE) and the result revealed the same strain map suggesting the isolated strains and

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the food-contaminated strains come from the same source, goose meat lunch box. Similar bacterial strains isolated from patient vomit and rectal swab samples reveal that rectal swab examinations are an effective tool for detecting food poisoning caused by *Staphyloccocus aureus*.

Keywords: food poisoning, *Staphyloccocus aureus*, enteral toxin, pulsed-field gel electrophoresis.

Introduction

Staphylococcus aureus is a Gram positive bacteria and is also a common pathogen responsible for food poisoning. The clinical signs are based on the enterotoxin produced by this pathogen. There are 14 types of enterotoxins, including A, B, C, D, E and G~O [1]. Type A~E are the most commonly seen enterotoxins in the event of food poisoning, especially exterotoxin type A which is also the most virulent [2]. The activity of enterotoxins and superantigens produced by *Staphylococcus aureus* may induce proliferation of macrophages and T lymphocytes, which release non-specific cytokines and induce hypersensitivity reactions [3].

The clinical signs of staphylococcal toxicity include nausea, vomiting and diarrhea. The incubation period is from 30 minutes to 8 hours. The clinical signs are related to the inflammatory reaction induced by enterotoxins. The enterotoxins bind to the receptors on the surface of macrophages and reactivate the macrophages [4], which may lead to inflammatory mediators such as histamine or serotonin releasing [5]. Nausea is the earliest symptom. It is believed that the releasing of serotonin may irritate the vagal nerve and reactivate vomiting center in the medulla, and thus, nausea is induced [6,7]. The inflammatory mediators irritate the nerve system in the gastrointestinal tract and reactivate adenylyl

cyclase. This reaction inhibits the electrolyte absorption and induces releasing electrolytes from crypt enterocytes which leads to fluid losing and diarrhea [8,9]. The incubation period is related to individual tolerance, amount of enterotoxin, and ingested amount, however, lethal cases were still recorded [3,10].

Staphylococcus aureus lives in the environment, mammals and birds. It is also possible to discover this pathogen from the skin, hair, nasal cavity, throat and GI tract in a healthy person, and nasal cavity is believed to be the natural habitat of this pathogen [11,12]. A contaminated wound may also contain large amount of *Staphylococcus aureus* [13]. The natural temperature for *Staphylococcus aureus* is between 7-48℃, and fast proliferation and release of enterotoxin occurs between 20-37℃. *Staphylococcus aureus* is resistant to high-salt or high-sugar environment and thus meat products may contain high level of enterotoxin. Furthermore, enterotoxin is very stable during heating. It may only be destroyed by 100℃ heating for 2 hours. Thus, sterile food but containing enterotoxins may also cause enterotoxification effect [14].

Contaminated food manipulators are also the common source for staphylococcal food toxification [15]. Nasal cavity is the natural habitat for this pathogen and staphylococcal carriers, including sustained carriers and opportunistic carries, are the main source of this disease [16]. This pathogen may also be found from throat [17]. The standard sampling procedure and monitoring for suspected food manipulators are usually taking rectal sample and neglect throat, nasal cavity and wound, which may not correctly following the pathogen sources.

Public Health Bureau, Taichung City, was informed of a suspected

food poisoning in a restaurant on September 8th, 2008. 2 patients were sent to the emergency room. Samples from the patients, restaurant workers, food and environment were collected and sent to Taiwan CDC and Bureau of Food and Drug Analysis for further examination. The result of these examinations revealed *Staphylococcus aureus* from the patients and food. The isolated bacteria were then examined by pulsed-field gel electrophoresis (PFGE) and the relation between food and patients was approved.

Material and methods

Disease investigation

In September, 2008, Public Health Bureau, Taichung City, was notified of a suspected food poisoning by Lin Shin Hospital. The hospital indicated that 2 patients were sent to the emergency room due to vomiting, nausea, diarrhea and tenesmus. Both patients mentioned having goose meat lunch box from a restaurant. 1 patient had this meal at 9:30pm in the evening and revealed clinical signs at the midnight. The other patient had this food at 9 o'clock in the evening and clinical signs of vomiting and diarrhea occurred at 10:30pm. 3 other patients consumed the contaminated food and all of them revealed similar clinical signs (vomiting, diarrhea). The incubation period was1.5-2.5 hours. Lin Shin Hospital reported this event to Public Health Bureau, Taichung City through communicable disease reporting system and related investigation was proceeded.

Sample collection

Public Health Bureau, Taichung City, collected rectal and vomiting samples from the 2 patients, and 3 rectal swab samples from 3 suspected restaurant workers. These samples were sent to the Central Regional Laboratory,

Center for Research and Diagnostics, Taiwan CDC for further examination. 2 food samples (rice, goose meat, and bamboo shoots) were examined in the Central Regional Laboratory, Bureau of Food and Drug Analysis.

Bacterial isolation, culture and identification

Samples from patients and workers were cultured by Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS Agar), Salmonella Shigella agar (SS agar), Baird-Parker agar (BP agar) and Mannitol-Egg yolk-Polymyxin agar (MYP agar) for bacterial isolation for common food toxification pathogens, such as Salmonella spp., Staphylococcus aureus, Vibrio spp., and Bacillus spp [18]. The agars were incubated in a 37℃ environment for 16 hours. Dark, raised, and round bacterial colonies with metal reflection and non-transparent loop were found on BP agar, which was suspected as *Staphylococcus aureus*. The suspected bacterial colonies were then transferred to TSA agar in a 37℃ environment for 16 hours and examined by staphylase agglutination test (OXOID, Hampshire, UK). Staphylase-positive colonies were inoculated with Brain Heart Infusion broth (BHI broth) and incubated in a 37℃ environment for 16 hours. The broth was centrifuged and upper layered fluid was collected for reverse passive latex agglutination test (RPLA test) for enterotoxin typing. The commercial examination kit (DENKA SEIKEN Co., Tokyo, Japan) was able to identify enterotoxin type A, B, C and D.

Food and environmental samples were cultured for *Staphylococcus aureus*, *Salmonella* spp., *Bacillus* spp., pathogenic *E. coli*, *Vibrio* spp. and *Clostridium perfringens* and the bacterial amount per food gram was calculated. The isolated *Staphylococcus aureus* was then proceeded RPLA test for enterotoxin typing.

PFGE analysis

Based on the RPLA test procedure published by Dr. McDougal et.al. [19], the isolated bacterial strains were buried, decomposed and gel washed, *SmaI* restricted enzyme incision, and PFGE examined. The DNA was stained by ethidium bromide, photographed and transferred as TIFF document. These photo documents were then analyzed by BioNumerics software (Applied Maths, Kortrijk, Belgium) for standardizing, comparing and analyzing.

Result and discussion

Staphylococcus aureus enterotoxin type A was isolated from the samples collected from the patients, including 2 vomiting samples and 1 rectal swab sample, by the Central Regional Laboratory, Center for Research and Diagnostics, Taiwan CDC. The rectal samples from 3 restaurant workers were negative in bacterial isolation (Table 1). 2 food samples (goose meat lunch box, including rice, goose meat and bamboo shoot) were examined and 10^2-10^6 cfu/g *Staphylococcus aureus* enterotoxin type A were calculated by the Central Regional Laboratory, Bureau of Food and Drug Analysis. No *Staphylococcus aureus* was isolated from the environmental samples (swabs from cutting board, knives and water).

In order to investigate the relationship between bacterial strains isolated from vomiting, rectal and food samples, we selected 13 strains, 5 strains and 11 strains from 2 vomiting samples, rectal samples and food samples (Table 1), respectively, for PFGE analysis. The result revealed the same PFGE map from those samples (Figure 1).

Table 1. Samples and the isolation results

Collecting date	Source of the samples	Sample type	Result of bacterial [a] culturea	Staphylase coagulative test	Enterotoxin type	Selected bacterial colony no.
09/08/2008	Patient A	Rectal swab	+	+	A	C08.1182-1 C08.1182-2 C08.1182-3 C08.1182-4 C08.1182-5
		Vomit content	+	+	A	C08.1183-1 C08.1183-2 C08.1183-3 C08.1183-4 C08.1183-5 C08.1183-6 C08.1183-7
09/08/2008	Patient B	Rectal swab	−			
		Vomit content	+	+	A	C08.1188-1 C08.1188-2 C08.1188-3 C08.1188-4 C08.1188-5 C08.1188-6
09/08/2008	Worker A	Rectal swab	−			
	Worker B	Rectal swab	−			
	Worker C	Rectal swab	−			
09/08/2008	Food box A	Rice	2.3×104 cfu/g	+	A	A1 A2 A3 A4 A5
		Meat	4.3×105 cfu/g	+	A	B1
		Bamboo shoot	7.0×102 cfu/g	+	A	C1 C2 C3
09/08/2008	Food box B	Rice	1.4×106 cfu/g	+	A	D1
		Meat	1.3×106 cfu/g	+	A	
		Bamboo shoot	8.8×105 cfu/g	+	A	
09/09/2008	Environment	Cutting board	−			
		Knife	−			
		Water	−			

a The examination for samples from human and environment was a qualitative test; +: positive bacterial isolation; - : negative bacterial isolation. The examination for food samples was quantitative test to calculate bacterial colonies per gram (cfu/g) of the sample.

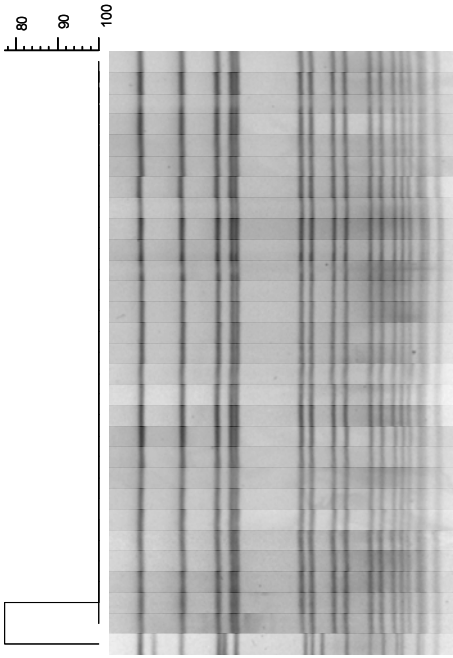

Figure 1. The comparison of PFGE maps of *Staphylococcus aureus* isolated from the patients and from the food.

The comparison of PFGE maps of bacterial culture and isolated strains revealed that the bacteria isolated from the patients and from the food were from the same source. Thus, the patients were infected by *Staphylococcus aureus* enterotoxin type A from the contaminated food.

Similar bacteria isolated from the vomiting samples and rectal swab samples indicated that *Staphylococcus aureus* may be isolated from the rectal samples in the case of staphylococcal food poisoning. The upper GI clinical sign of vomiting is due to enterotoxin reaction and the signs may occur shortly after infected. Thus, the vomiting content is the most direct samples for isolating pathogens and enterotoxins to approve staphylococcal

food toxification. Both contaminated food samples and vomiting samples were isolated high levels of bacterial pathogens. In our experience, the rate of positive bacterial isolation in staphylococcal food poisoing cases are low. Furthermore, the clinical sign of this disease is mainly vomiting. Thus, it is questionable whether rectal samples are the proper samples for bacterial isolation for *Staphylococcus aureus.* In the present study we used PFGE analysis to approve that the bacteria from vomiting content and rectal swab were the same and, thus, rectal swab samples may be suitable for the examination for this disease. The low bacterial isolation rate from the rectal samples may be due to short incubation time.

The rectal samples from 3 restaurant workers were negative for bacterial isolation. It is not correct to collect rectal samples from food manipulators to trace the source of bacterial origin. Many studies indicated that nasal cavity was the natural habitat for *Staphylococcus aureus* and wounds may usually be contaminated by this pathogen. *Staphylococcus aureus* contamination usually occurs when bad hygiene habit of the contaminated food manipulators, contaminated wounds on the hands and bad food manipulation procedures [15,20]. Improper sample collecting may be due to inexperienced disease prevention staffs. Regular education and training are highly recommended.

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