Investigation of Consecutive Families' Food Poisoning at A Restaurant

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Abstract

In the evening of May 22, 2007, Family A dined in Restaurant A which is located in the Zuoying District. That evening, 2 of the 4 family members who dined at the restaurant showed symptoms of fever, vomiting, and diarrhea. In addition, on May 27, 2007, Family B and Family C also dined at Restaurant A. 3 of the 5 family members in Family B showed symptoms of vomiting, stomachache, and diarrhea. All of the 4 family members in Family C showed symptoms of vomiting, abdomen pains, and diarrhea after dining at the restaurant. After showing symptoms, all three families sought out medical assistance at Kaohsiung Veterans General Hospital and E-Da Hospital. 2 anal swab samples were collected from Family A; 2 anal swab samples from Family B; 3 anal swabs and 1 vomitus sample was collected from Family C. The results showed that 5

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anal swab samples and the 1 vomitus sample from Family A and C were positive *Staphylococcus aureus* with A type enterotoxin, whereas the 2 anal swab samples collected from Family B turned up negative for the bacterium. Due to the fact that Family C's case was reported by the E-Da Hospital as an epidemic through the food poisoning report system, the Food Safety Division of Health Department of Kaohsiung County Government also collected 3 environmental samples and 1 sample from the cooking personnel's hands which were later shown to be negative. Because the sample results for Family A and C were positive for *Staphylococcus aureus* with A type enterotoxin, the bacterium isolates were sent to the CDC Laboratory Research and Development Center for PFGE matching. The results confirmed the same source of food poisoning contamination. However, the samples from the restaurant showed negative in the results. In the future, additional pharynx/larynx and nasal samples can also be taken from the cooking staff in a suspected food poisoning incident in order to elevate the chances of determining the transmission source. In addition, due to the increased numbers of community *Methicillin resistant Staphylococcus aureus* (MRSA), a further antibiotics sensitivity test was conducted. The results confirmed that the bacterium involved in this incident is MSSA.

Key Words: Food poisoning, Staphylococcus aureus with A type enterotoxin, pulsed-field gel electrophoresis (PFGE)

Introduction

The Fifth Branch, Centers for Disease Control (CDC) discovered three collective food poisoning incidents in May of 2007. These three cases involved three families which had dined in the same restaurant yet on different times. The symptoms were mainly vomiting and diarrhea with a latent period of 2-4

hours. After the screening the cases' anal swab and vomitus specimens, two of the cases showed the *Staphylococcus aureus* with A type enterotoxin. Further antibiotics sensitivity testing resulted in the determination of *Methicillin susceptible Staphylococcus aureus* (MSSA). To determine whether the transmission sources were related each other or not, *Staphylococcus aureus* isolates were sent to the CDC Laboratory Research and Development Center for pulsed-field gel electrophoresis (PFGE) matching. The results showed that the 2 cases of *Staphylococcus aureus* isolates had the same PFGE spectrum; therefore, we assume that the transmission source is likely to have been the same restaurant in which the cases dined.

Investigation of the Epidemic

In the evening of May 22, 2007, Family A dined in Restaurant A which is located in the Zuoying District. That evening, 2 of the 4 family members who dined at the restaurant showed symptoms of fever, vomiting, and diarrhea 3 hours later. After seeking medical assistance at Kaohsiung Veterans General Hospital, the patients' symptoms were slightly relieved. The hospital reported the collective diarrhea incident through the symptomatic reporting system. In addition, on May 27, 2007, Family B and Family C also dined at Restaurant A. 3 of the 5 family members in Family B showed symptoms of vomiting, stomachache, and diarrhea. After seeking medical assistance at Kaohsiung Veterans General Hospital, the incident was reported. All of the 4 family members in Family C showed symptoms of vomiting, abdomen pains, and diarrhea 2-3 hours after dining at the restaurant A. E-Da Hospital reported the food-poisoning incident after Family C arrived seeking medical assistance.

Demography Data:

13 people were involved in the investigation of this case with 9 people who portrayed symptoms of food poisoning. Among these 9 people, male subjects took up 55.6% of the ratio whereas female subjects had a 44.4% ratio. The age span of the cases is 7-58 years of age with an average age of 45 years.

Investigated Number of Subjects:

The cases are defined by any of the following symptoms: vomiting, abdomen pains, and diarrhea. Therefore, Families A, B, and C all fit the case profile with a total of 9 cases.

Symptoms:

The 9 cases' symptoms are distributed as following: vomiting—8 (88.9%); diarrhea—8 (88.9%); abdomen pains—4 (44.4%); fever—1 (11.1%); and stomachache—1 (11.1%).

Specimen Collecting and Laboratory Examination

2 anal swab samples were collected from Family A (results showed the *Staphylococcus aureus* with A type enterotoxin); 2 anal swab samples were collected from Family B (both results were negative); 3 anal swab samples and 1 vomitus sample were collected from Family C due to the fact that the hospital reported the incident as food poisoning(both results were *Staphylococcus aureus* with A type enterotoxin). In addition, the Food Safety Division of Health Department of Kaohsiung City Government has conducted an investigation of the restaurant and collected samples from the environment including those from the raw food cutting board, cooked food cutting board, knives, and a sample from the hands of a cook (all results showed negative). However, because Family A and Family B were reported as collective diarrhea incidents and not food poisoning incidents, no food hygiene personnel were involved in the investigation; thus only

human samples were collected.

Due to the fact that all three cases dined at Restaurant A in the Zuoying District and two of the families (Family A and Family C) displayed the *Staphylococcus aureus* with A type enterotoxin in the human samples, the isolates of the two cases were sent to CDC Laboratory Research and Development Center for PFGE matching. Results showed that both *Staphylococcus aureus* isolates from the two cases had the same PFGE spectrum. Due to the increased appearance of the community acquired *Methicillin resistant Staphylococcus aureus* (MRSA) in the past few years, the *Staphylococcus aureus* isolates were sent for further drug sensitivity testing.However,the results were all positive for MSSA .Although the test results for the samples from the restaurant were negative, the chances that the transmission source came from the restaurant are still quite high.

Materials and Methods:

1. The food poisoning bacillus culture used CBS, DHL, HE, BP, MYP, Peptone water, plus 1 % NaCl and Selenite broth and were set overnight for culturing.
2. The bio-testing for Staphylococcus uses API Staph in calculating the biochemistry reaction grade of the overnight cultured bacteria. Further matching with the API system was conducted.
3. Testing for Staphylococcus blood serum used the Staphylase test.
4. After cultured overnight in the BHI broth, SET-RPLA was conducted to determine the presence of Staphylococcus.
5. Gene types of the coli isolates from the two cases were matched using Pulsed-field gel electrophoresis (PFGE). The chromosomes were truncation cut and compared with the gene results.
6. The antibiotics sensitivity tests for the *Staphylococcus aureus* isolates used the

agar disc diffusion test.

Results:

1. Suspected *Staphylococcus aureus* bacteria were found in the blood BP culture.
2. According to the API Staph system matching, 97% were positive in reaction as *Staphylococcus aureus*.
3. The Staphylase test showed positive results.
4. The SET-RPLA test results turned up positive (see Table 1).
5. According to the match results of the PFGE, No. 04-901-225061 and 04-901-225062 from Family A and 04-901-194544 from Family C displayed the same PFGE spectrum (see Figure 1)
6. Results of antibiotics sensitivity test results are shown in Table 2.

Precautionary Measures

According to 4th section of the 11th article and the 31st article of the Food Sanitation Act, the Food Safety Division of Health Department of Kaohsiung City Government has issued a deadline for the restaurant to improve their environment conditions and a fine. Following inspections have shown the restaurant to meet the standards.

Discussion

Food poisoning is defined as "an incident in which two or over two people display similar symptoms after consuming the same foods. In addition, samples collected from patient's fecal matter, vomitus, blood, or other related environment samples (such as air, water, soil, etc.) that are further separated into the same types (such as blood serum or bacteriophage types) and determined as the poisoning source" [1,2]. In this investigation report, Families A, B, and C have 2, 3, and 4 cases of food poisoning. All their symptoms showed vomiting and

diarrhea. The three families not only consumed food from the same source, two of the families were tested positive for *Staphylococcus aureus* with A type enterotoxin. Therefore, this case is defined as a food poisoning epidemic. In a food poisoning epidemic, the first issued to be addressed is the source of the epidemic. This can be conducted in four main areas; symptoms displayed, latent period, symptom continuity period, and attack rate (the number of people infected) [3]. Furthermore, understanding the conditions of the food (raw or improperly stored food) and water consumed by the cases can also aid in determining the transmission source of the poisonings. In addition, the traveling history of the cases is also relevant.

According to the Bureau of Food Safety material categorization chart accumulated from past food poisoning incidents, bacterial type food poisonings are the most commonly seen. The most commonly seen bacteria are *Vibrio parahaemolyticus, salmonella enteric, Staphylococcus aureus,* and *Bacillus cereus.* Apart from the latent period and patient symptoms, in determing the source as *Staphylococcus aureus,* laboratory results are the main indicators. Cases fitting any one of the following descriptions can be categorized as *Staphylococcus aureus* food poisoning: 1. *Staphylococcus aureus* is found in two or more case samples from fecal matter and vomitus; 2. *Staphylococcus aureus* is found in the suspected food contamination source; 3. 10^5 organisms/gram of *Staphylococcus aureus* is found in the suspected food contamination source ,provided specimen is properly handled [5]. In this report, the results for the 2 anal swab samples all showed the *Staphylococcus aureus* with A type enterotoxin. The 3 anal swab samples and 1 vomitus sample from Family C also showed positive results for the *Staphylococcus aureus* with A type enterotoxin. Both cases fit the first description in determining *Staphylococcus aureus* food

poisoning. Although the samples taken from Family B did not show the presence of this bacterium, 3 of the 5 the family members still experienced symptoms of vomiting and diarrhea after dining at the restaurant. Therefore, it is highly suspicious that this incident of food poisoning was caused by *Staphylococcus aureus*.

Staphylococcus aureus will cause food poisoning due to the enterotoxin it creates. Categorized according to immunology, enterotoxin can be categorized as A, B, C, D, E, and G-O types. Among this, types A-E are the main types that cause food poisoning. The production of *Staphylococcus aureus* enterotoxin is influenced by temperature (best temperature at 21-37℃), PH (best PH at 5.0), and other specific materials (such as Amino Acids carbohydrates, minerals). Thus,

Staphylococcus aureus can live quite well in foods but without producing enterotoxin [6]. In addition the enterotoxin of *Staphylococcus aureus* can resist high temperatures; thus it is not easily broken down by high temperatures. On the other hand, it is hard to detect the bacteria in the foods due to the fact that the contaminated foods do not give off a different flavor. Therefore, it can easily become the cause of food poisoning. Once *Staphylococcus aureus* food poisoning occurs, the main symptoms are vomiting and diarrhea with a 2-4 hour latent period.

The main cause of *Staphylococcus aureus* food poisoning origins from the lack of care put into personal hygiene by food processing personnel. Usually, the bacterium is carried in the personnel's throat, nasal area, and skin surface and normally without any syndromes [8, 9]. This means that the carrier is in a healthy condition. Therefore, it is extremely easy for the carrier to neglect related hygienic measures and bring the bacterium onto the food. In addition, the right storage environment temperatures will result in the production of

enterotoxin which will lead to later food poisoning. According to research documents, pharynx/larynx swab samples will show a higher ratio of *Staphylococcus aureus* than nasal swab samples [10]. Therefore it would be inadequate to only collect nasal swab samples in determining the source since most of the *Staphylococcus aureus* bacteria is located in the pharynx/larynx area [11]. Some of these carriers are continuous carriers whereas others are only coincidental carriers. There is a research that followed carriers for two-year period that discovered that the *Staphylococcus aureus* with the same gene structure was still present [10]. Thus, it is important in filtering out those cooking personnel that have recently visited hospitals or medical facilities. According to the investigation of the food poisonings, anal, hand, and wound swab samples were taken. However, nasal or pharynx/larynx swab samples were not collected. Although 2 of the human samples were positive in the *Staphylococcus aureus* with A type enterotoxin and the transmission source is extremely likely to be the restaurant, the samples collected from the environment and the hands of the cooking personnel displayed negative results in both *Vibrio parahaemolyticus* and *Staphylococcus aureus*. The possible reason may be that the hospital reported the incident as collective diarrhea and not food poisoning, resulting in no first-hand involvement of the Food Safety personnel in the investigation. Sanitation measures afterward may also have made it hard to detect the bacterium in the samples collected from the environment and cooking personnel after the report of the incident with Family C. In addition, the results from the samples taken from the cooking personnel showed to be negative, despite the fact that there were no wounds on his/her hands. However, only one hand sample was taken; thus we cannot completely eliminate the possibility of the cooking personnel being a carrier. Therefore, the accuracy of hospitals report

can be enhanced. At the same time, collecting nasal and pharynx/larynx swab samples not only increases the accuracy of determining *Staphylococcus aureus*, but also in pinpointing the transmission source. WHO has also pointed out that the samples to be taken in suspected food poisoning incidents include cases, suspected food, food processing cutlery, and the cooking personnel. Colon, pharynx/larynx, and pus or fluid from wounds (if existed) swab samples can also be taken [12]. It should be noted that when taking pharynx/larynx samples, if the location of which the sample is taken is incorrect (such as the surface of the tongue), it will lower the chances of distinguishing the bacterium [11]. Therefore, if we are to include collecting pharynx/larynx samples in food poisoning investigation standards, certified personnel should be there to collect the samples.

Testing displayed that the *Staphylococcus aureus* with A type enterotoxin found in the samples taken from Family A and Family C was MSSA. Research has proven that *Staphylococcus aureus* food poisoning may not only be caused by MSSA, but also the *methicillin-resistant Staphylococcus aureus* (MRSA) [13]. In addition, MRSA is currently a commonly seen infectious bacterium in hospitals and medical centers. Through visitation without adequate prevention in such facilities the bacteria can easily transmit into the community and result in a community epidemic, including food poisoning. However, due to the fact that there is no regular drug sensitivity testing conducted on the *Staphylococcus aureus* that causes food poisoning, we cannot assess the situation in Taiwan. We can only acknowledge the fact that the numbers of community MRSAs are rising. However, further research is needed to determine the exact ratio in food poisoning.

Suggestions

In reporting the epidemic, it is suggested that hospitals report suspected food poisonings through the epidemic report system and not collective diarrhea incidents through the symptom report system. This is due to the fact that food poisoning reports will notify Health Bureau Food Safety personnel; thus bringing the food sanitation element into the investigation. In accordance with the information the Centers for Disease Control on the investigated case, the origin for the epidemic can be more accurately pinpointed.

On the other hand, personnel who process food should pay more attention to personal hygiene. This refers to washing hands and wearing gloves and masks before processing foods. Food storage is also extremely important in preventing the growth of bacteria. In addition, it is also recommended that apart from the case, food, cooking environment, and cooking staff samples, pharynx/larynx and nasal samples can also be taken from the cooking staff in a suspected food poisoning incident. This not only assists in determining the transmission source but also in following precautionary measures.

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Table 1. Test results for identifying Staphylococcus aureus and enterotoxin type

Date	Sample Source	Sample type	Sample No.	S. *aureus colony* (BP)	API	Staphylase test	SET-RPLA
960523	Family A	Anal swab	04-901-225061	(+)	S. *aureus* 97.8%	(+)	enterotoxin A
			04-901-225062	(+)		(+)	enterotoxin A
960528	Family C	Anal swab	04-901-194544	(+)	S. *aureus* 97.8%	(+)	enterotoxin A
			04-901-194547	(+)		(+)	enterotoxin A
			04-901-194549	(+)		(+)	enterotoxin A
			04-901-194548			(-)	
		vomit	04-901-194545	(+)	S. *aureus* 97.8%	(+)	enterotoxin A
			04-901-194546			(-)	

Table 2. Results of antibiotics sensitivity tests for Staphylococcus aureus

Tested coli isolates / Antibiotic Type	Family A 04-901-225061	Family A 04-901-225062	Family C 04-901-194544
Oxacillin 1 μ g	S	S	S
Vancomycin 30 μ g	S	S	S
Chloramphenicol 30 μ g	S	S	S
Erythromycin 15 μ g	I	I	S
Tetracyclin 30 μ g	R	R	R
Sulfamethoxazole/Trimethoprim 23.75/1.25 μ g	S	S	S

R: Resistant; I: Intermediate; S: Susceptible

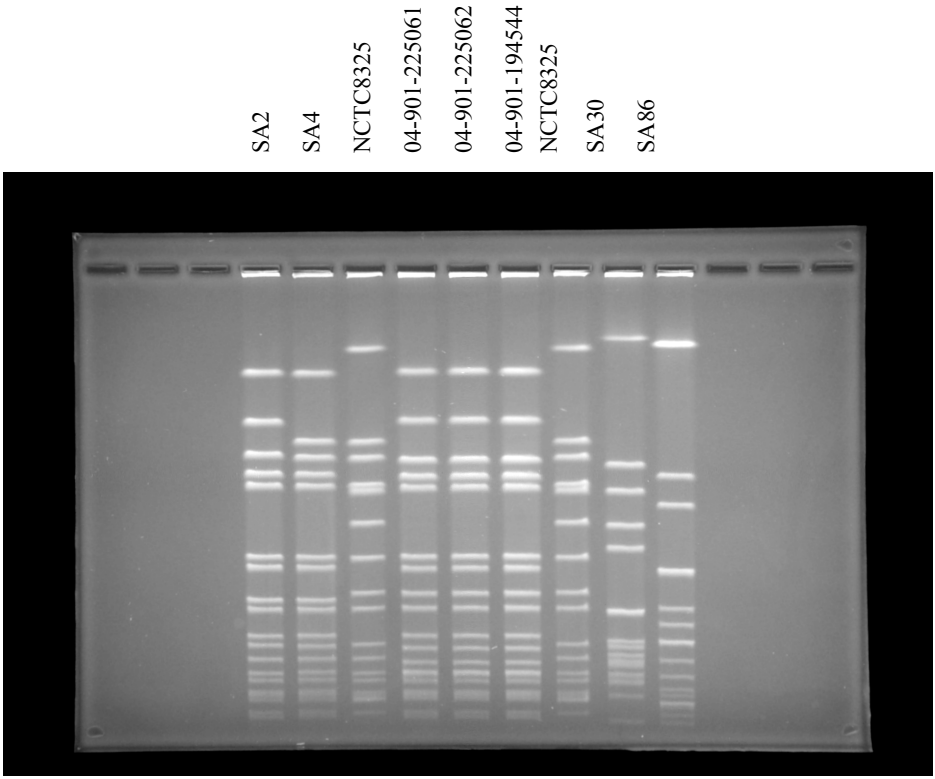

Figure 1. Staphylococus aureus PFGE Spectrum

04-901-225061, 04-901-225062, and 04-901-194544 are the isolated Staphylococcus aureus in this case. SA2, SA4, SA30, and SA86 are the laboratory isolated from other cases; NCTC 8325 is a reference isolate.