Comparison of Bacterial Isolates from a Suspected Food Poisoning Outbreak at a Post-partum Care Center

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Abstract

*Staphylococcus aureu*s is one of the most common pathogens causing food poisoning in Taiwan. Secreted by the bacteria, the enterotoxin is heat-stable, and resistant to degradation by digestive enzymes. Symptoms of staphylococcal food poisoning are nausea, vomiting, and diarrhea. The Research and Diagnostic Center of the Centers of Disease Control received post-mortem samples of a male infant and a female child, suspected to be part of a larger food poisoning outbreak at a post-partum care center, on August 30 and September 5, 2007, respectively. No clinical significant bacteria were isolated from the male infant. *Staphylococcus aureus* was isolated from swabs of the throat, intestine, liver, kidney, and spleen from the female child. In addition, on October 17, 2007, the Research and Diagnostic Center also received *Staphylococcus aureus* isolates from the hand of a food handler taken on August 27. Comparison of the five isolates from both persons was performed to understand the association between the two persons. The results showed that the two groups of *Staphylococcus aureus* produced enterotoxin A, but their antibiotic sensitivity, pulsed-field gel electrophoresis and

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multilocus sequence typing patterns were different. Therefore, we conclude that the *Staphylococcus aureus* isolates from the child and the food handler were different.

Keywords: *Staphylococcus aureus*, staphylococcal enterotoxins, antimicrobial susceptibility pattern, pulsed-field gel electrophoresis, multilocus sequence typing

Background

According to the Food and Drug Administration of Taiwan, the most common cause of food poisoning is bacterial food poisoning. *Staphylococcus aureus* is the third most common cause of bacterial food poisoning. *Staphylococcus aureus* is a common pathogen for humans. It is ubiquitous in air, soil, water, food, eating utensils, and skin, hands, and nasal passages of healthy people. It is resistant to drying, heat, and salt. Therefore, the bacteria can cause a variety of diseases and symptoms. Pathogenicity of the bacteria depends on the cell wall structure, enzyme produced, and secretion of exotoxin [1]. *Staphylococcus aureus can contaminate food and secrete* Staphylococcal enterotoxin (SE), which causes food poisoning. This enterotoxin is resistant to digestive enzymes and heat-stable, capable of resisting 30 minutes of boiling. Therefore, food that has been cooked may still cause food poisoning. Symptoms of staphylococcal food poisoning include nausea, vomiting, and diarrhea. Incubation period is 1-6 hours [2]. To date, 15 different enterotoxins have been discovered. Most common types are SE-A and SE-E. Of these, SE-A is the most commonly found in food poisoning [2, 3].

In August 2007, a post-partum care center had cluster of suspected food poisoning. A total of 10 people became ill with nausea, vomiting, abdominal pain, diarrhea, dizziness, and fever. One girl died. At the same time, a male infant also died, after having purpura and shortness of breath. On August 30 and September 5,

2007, the Research and Diagnostic Center of the Centers of Disease Control received post-mortem samples taken from the two patients who died. On September 7, *Staphylococcus aureus* producing enterotoxin A was isolated from throat swab, intestine, liver, kidney, and spleen of the girl. On September 14, the laboratory reported that no clinically significant bacteria were isolated from tissues of the male infant. In addition, the local health department reported that the swabs, taken on August 27, of the food handler's hand yielded *Staphylococcus aureus*. *Staphylococcus aureus* was isolated from swabs of the throat, intestine, liver, kidney, and spleen from the female child. In addition, on October 17, 2007, the Research and Diagnostic Center also received *Staphylococcus aureus* isolates from the food handler taken on August 27.

The laboratory tested samples from the two autopsies and re-tested the isolate from the food handler provided by the local health department. To clarify the possible association between the *Staphylococcus aureus* isolated from the two different sources, the laboratory compared enterotoxin, antibiotic sensitivity, pulsed-field gel electrophoresis and multilocus sequence typing patterns of the bacterial isolates.

Material and Methods

A. Samples from autopsies and hand of the food handler

There were 15 samples from the autopsy of the girl, including swabs of the pericardium, throat, and ascites, brain, brain stem, basal ganglia, left lung, right liver, thymus, spleen, right kidney, colon, intestine, aerobic blood culture, and anaerobic blood culture. There were 17 samples from the autopsy of the male infant, including swabs of basal ganglia, brain stem, meninges, dura, oral cavity, trachea, lung, myocardium, stomach, pleural effusion, peritonium, bile,

intestine, colon, anus, aerobic blood culture, and anaerobic blood culture. There was one swab of the food handler's hand. After receiving these samples, the laboratory began processing and testing.

B. Isolation and identification of bacteria

Samples were inoculated on blood agar plat (BAP), chocolate agar, MacConky sorbitol agar, Salmonella-Shigella agar (SS), thiosulfate citrate bile salts sucrose agar (TCBS), and Baird-Parker agar (BP) that were placed in 37℃ for 18-24 hours of incubation. BP agar and chocolate agar were incubated for at least 48 hours.

Isolates were selected from the plates then cultured using TSA. Gram staining and biochemical testing for bacterial identification were performed after one day of incubation. Biochemical testing was conducted using oxidase, catalase and coagulase tests, as well as biochemical test kits API 20 E and 20 NE (bioMerieux, Marcy-I'Etoile, France), Vitek (bioMerieux) and Poenix (BD, MJ, USA). For the minority of isolates which had atypical biochemical profiles, 16S rDNA sequencing was performed to confirm identification.

C. Gene detection of *Staphylococcus aureus* and enterotoxin

DNA was extracted from samples, including culture media, tissue mixture and extract from blood culture bottle, using QIAamp DNA mini kit (Qiagen, Hilden, Germany). This was followed by real-time PCR to detect the *femB* gene, unique to *Staphylococcus aureus,* and the staphylococcal enterotoxin gene, *sea* [3]. Total volume of the mixture was 25 μL, including 200nM of Taqman probe 200, 600 nM of probe, 12.5 μL of master mix (Applied Biosystems, NJ, USA), 10 μL of DNA template, and sterile water. ABI 7000 Sequence Detection System was used to perform the following reactions: 50℃ for 2 minutes, 95℃ for 10 minutes, and 45 cycles of 15 seconds at 95℃ followed

by one minute at 60℃.

D. Testing of enterotoxin

Reversed passive latex agglutination, RPLA, was used to test *Staphylococcus aureus* isolates for their ability to produce enterotoxins, and to identify the type of enterotoxin produced (SEA-E). Enteroroxin-F kit (SEIKEN, Tokyo, Japan) was used for analysis. After isolates were incubated overnight in BHI media, the mixture was centrifuged at 8,000 rpm for 10 minutes and the supernatant was diluted to two times the volume using diluents from the kit. The mixture (25μL) was placed into six wells of a 96-hole well plate. In each of the six wells, one drop of each enterotoxin type testing gel and a control testing gel were added in. The 96-hole well plate was placed in a moisture box at room temperature. The result was read after incubation overnight.

E. antibiotic sensitivity tests

Testing for antibiotic sensitivity was performed using Phoenix analyzer and its companion testing kit. After finding the minimal inhibitory concentration (MIC), the result was reported based on the standards set by the Clinical and Laboratory Standards Institute (CLSI). The method is as follows: add one drop of indicator in AST broth and mix thoroughly; add 25 mL of fresh bacteria broth that has already been adjusted to McFarland 0.5-0.6 into the AST broth and mix thoroughly; place the mixture into the testing kit's testing well; put the sample on the Phoenix analyzer for incubation and testing; key in the name of the sample; examine the results printed out the next day [4].

F. Pulsed-field gel electrophoresis (PFGE) molecular typing

After adjusting the bacteria broth to suitable turbidity, a mixture of 300 μL of buffer, which contained 3 μL of lysostaphin (1 mg/mL) and 300 μL of 1.8% agarose, was added to the broth before it was placed into a mold. The gel was

then placed into 5 mL of EC solution (6 mM Tris-HCl, 100 mM EDTA, 0.5% sarcosine, 1 M NaCl, 0.5% Brij058, 0.2% sodium deoxycholate, pH 8.0) for reaction at 25℃ for four hours. Following this, it was rinsed with TE buffer four times, each time being shaken in 25℃ water bath for 30 minutes. The gel was then removed and cut into strips, and 30 U of *Sma* I restriction enzyme (New England Biolabs, MA, USA) was applied and allowed for reaction at 25℃ for four hours. After this reaction, the gel strips were placed into a mold, and Bio-Rad CHEF MAPPER electrophoresis unit (Bio-Rad Laboratories, CA, USA) was used for electrophoresis. Next, the gel was run using voltage at 6 V/cm, angle at 120°, pulse time between 5 to 40 seconds, and total run time of 21 hours. After the completion of electrophoresis, ethidium bromide was used for staining and the images were captured. Finally, BioNumerics 4.0 software (Applied Maths, Kortrijk, Belgium) was used for analysis [5].

G. Multilocus sequence typing (MLST)

Seven genes of *Staphylococcus aureus* were amplified using PCR. After confirming that the amplified sequence was the correct gene, the product was purified before being submitted to the laboratory for sequencing. After the seven genes were sequenced, the sequences were entered into the MLST database (http://www.mlst.net) to compare with the sequences of the *Staphylococcus aureus* ST gene [6].

Results

A. Samples from autopsies and hand of the food handler

There were 15 samples from the autopsy of the girl, including swabs of the pericardium, throat, and ascites, brain, brain stem, basal ganglia, left lung, right liver, thymus, spleen, right kidney, colon, intestine, aerobic blood culture,

and anaerobic blood culture. There were 17 samples from the autopsy of the male infant, including swabs of basal ganglia, brain stem, meninges, dura, oral cavity, trachea, lung, myocardium, stomach, pleural effusion, peritonium, bile, intestine, colon, anus, aerobic blood culture, and anaerobic blood culture. There was one swab of the food handler's hand. After receiving the samples, the laboratory began processing and testing.

Staphylococcus aureus was isolated from the throat swab, intestine, liver, kidney, and spleen of the girl. *Staphylococcus aureus* was the predominant isolate on culture media. *Staphylococcus aureus* was also isolated from the brain stem, basal ganglia, and colon, but it was not the predominant isolate on culture media. No clinically significant isolates were cultured from the tissues of the male infant. Bacteria isolated from both the aerobic and anaerobic blood culture bottles were normal flora for humans. The specimen collected on August 27 from the hand of the food handler was placed in Cary-Blair media. After receiving the specimen on October 17, the culture of the specimen at the CDC laboratory yielded *Staphylococcus aureus*.

Because no *Staphylococcus aureus* was isolated by direct culture of specimens obtained from the male infant, real-time PCR for *femB* and *sea* genes unique to *Staphylococcus aureus* was performed. Of the 17 specimens, no *femB* or sea genes were found using PCR. In contrast, throat swab and intestine tissue from the girl were both positive for these two genes. It was concluded that all of the samples from the male infant were negative for *Staphylococcus aureus*.

Five isolates of *Staphylococcus aureus* from the girl's throat swab, intestine, kidney, spleen, and liver (S-throat, S-intestine, S-kidney, S-spleen, S-liver) and five isolates of *Staphylococcus aureus* from the food handler's hand (H3, H5, H8, H81, and H9) were selected for comparison.

B. Testing of enterotoxin

As shown in figure 1, all the isolates from the girl produced SEA; four of the five isolates from the food handler's hand produced SEA. Only H5 showed no enterotoxin.

Table 1. Comparison of *Staphylococcus aureus* isolates from index case and hand swab of food handler.

SE[a]	Isolates[b]									
	S-throat	S-intestine	S-kidney	S-spleen	S-liver	H3	H5	H8	H81	H9
SEA	+	+	+	+	+	+	-	+	+	+
SEB	-	-	-	-	-	-	-	-	-	-
SEC	-	-	-	-	-	-	-	-	-	-
SED	-	-	-	-	-	-	-	-	-	-
SEE	-	-	-	-	-	-	-	-	-	-

[a] SE: Staphylococcal enterotoxins; SEA: Staphylococcal enterotoxin A; SEB: Staphylococcal enterotoxin B; SEC: Staphylococcal enterotoxin C; SED: Staphylococcal enterotoxin D; SEE: Staphylococcal enterotoxin E.

[b] S-throat, S-intestine, S-kidney, S-spleen, and S-liver indicate Staphylococcus isolated from throat, intestine, kidney, spleen, and liver of the index case; H3, H5, H8, H81, and H9 indicate isolates isolated from the hand of the food handler.

C. Comparison of antibiotic sensitivity pattern

The same strain of bacteria usually has the same antibiotic sensitivity pattern. Therefore, antibiotic sensitivity pattern might help determine whether the strains have the same origin. Results of the 21 antibiotic sensitivity tests are shown in table 2. Most notable is the difference in resistance to erythromycin. The girl's strain is resistant to erythromycin, while the isolate from the food handler's hand is sensitive to erythromycin. This indicates that the two strains of *Staphylococcus aureus* have different antibiotic patterns.

Table 2. Comparison of *Staphylococcus aureus* antibiotic resistance between isolates from the girl and the food handler's hand.

Antibiotics	S-throat [b]	S-intestine	S-kidney	S-spleen	S-liver	H3	H5	H8	H81	H9
Amoxicillin/ Clavulanate	S (≤1/0.5) [a]	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)	S (≤1/0.5)
Ampicillin	R *	R *	R *	R *	R *	R *	R *	R *	R *	R *
Chloramphenicol	S (4)	S (8)	S (4)	S (4)	I (16)	S (8)	S (4)	I (16)	I (16)	S (8)
Ciprofloxacin	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)
Clindamycin	X (≤0.25)	X (≤0.25)	X (≤0.25)	X (≤0.25)	X (≤0.25)	S (≤0.25)	S (≤0.25)	S (≤0.25)	S (≤0.25)	S (≤0.25)
Erythromycin	R (>4)	R (>4)	R (>4)	R (>4)	R (>4)	S (≤0.25)	S (≤0.25)	S (≤0.25)	S (≤0.25)	S (≤0.25)
Gentamicin	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)	S (≤2)
Gentamicin -Syn	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)	- (≤500)
Levofloxacin	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)
Linezolid	S (2)	S (2)	S (2)	S (2)	S (2)	S (1)	S (2)	S (2)	S (2)	S (2)
Moxifloxacin	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)	S (≤1)
Nitrofurantoin	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)	S (≤16)
Oxacillin	S (0.25)	S (0.5)	S (0.5)	S (0.5)	S (0.5)	S (0.5)	S (0.5)	S (1)	S (1)	S (0.5)
Penicillin G	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)	R (>1)
Quinupristin-dalfopristin	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)
Rifampin	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)
Streptomycin -Syn	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)	- (≤1000)
Teicoplanin	S (1)	S (1)	S (1)	S (1)	S (1)	S (≤0.5)	S (≤0.5)	S (1)	S (≤0.5)	S (1)
Tetracycline	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)	S (≤0.5)
Trimethoprim/ Sulfamethoxazole	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)	S (≤0.5/9.5)
Vancomycin	S (2)	S (2)	S (2)	S (2)	S (2)	S (1)	S (2)	S (2)	S (2)	S (2)

[a] MIC, Minimal Inhibitory Concentration (μg/mL).
[b] All isolates are confirmed positive for the resistance marker of beta-lactamase (nitrocefin based).
* When beta-lactamase is detected in staphylococci, an interpretation of susceptibility for penicillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, and piperacillin is reported as resistance.
X Macrolide-resistant staphylococci with an interpretation of susceptibility or intermediary for clindamycin may have inducible resistance to clindamycin or may be resistant to macrolides only.
\- High-dosage antibiotics, used for clinical drug choice only, do not come along with an interpretation of Susceptibility (S), Intermediary (I), and Resistance (R).

D. PFGE molecular typing

PFGE molecular typing is a standard method for clarifying whether the bacteria are related. The result is shown in figure 1. Of the five strains isolated from the girl, PFGE patterns are the same. Strains from the food handler's hand have two different PFGE patterns, four strains that produced toxin, and H5, which did not produce toxin. All three PFGE patterns have similarity in about 10 segments. Using BioNumerics software for analysis, the similarity in PFGE patterns between strains from the girl and the non-toxin producing strain from the food handler's hand was 59.3%, while the similarity between the strains from the girl and the toxin producing strains from the food handler's hand was 39.2%. This indicates that the differences between the strains are vast, and that they are not associated.

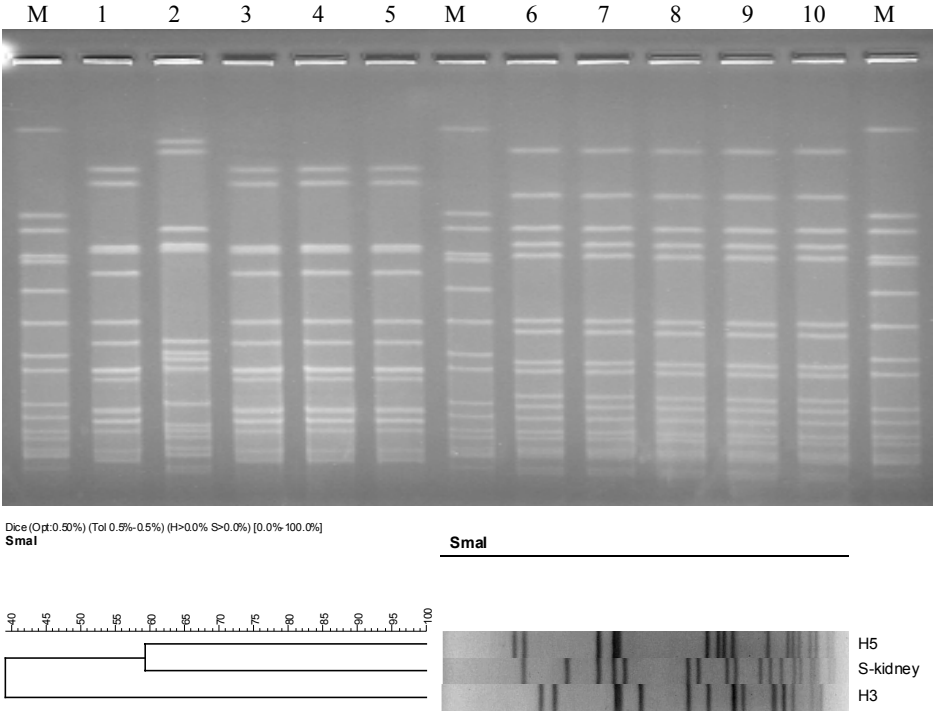

Figure 1. Comparison of PFGE patterns of *Staphylococcus aureus* isolates from the girl and the food handler (top figure). M: marker (NCTC8325); lanes 1-5 were isolates H3, H5, H8, H81, and H9 cultured from the food handler's hand; lanes 6-10 were isolates S-kidney, S-liver, S-spleen, S-throat, and S-intestine, taken from the girl's autopsy samples. Relatedness of the PFGE was analyzed using BioNumerics (bottom figure).

E. MLST molecular typing

MLST is another method frequently used in laboratories for molecular typing. Given that the five strains isolated from the girl had the same PFGE pattern and that the four strains of toxin producing strains from the food handler's hand had the same PFGE pattern, one strain from each patient was selected for

MLST analysis. S-intestine from the girl and H3 from the food handler's hand were selected. As shown in figure 3, these two strains were different when compared using MLST, indicating that the sources of these two strains were different.

Table 3. MLST comparison of *Staphylococcus aureus* isolates from the girl and the food handler.

Isolates	ST[a]	Allelic profile (allele no.)						
		arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
H3	-*	3	37	19	2	20	26	32
S-intestine	6	12	4	1	4	12	1	3

[a] ST, sequence type.
* nearest match, 813 (3, 37, 19, 2, 20, 26, 2) .

Discussion

During this incident, samples were collected from ill patients and the environment and tested in three different laboratories, but *Staphylococcus aureus* was only isolated from the autopsy specimens of the girl and the swab of the food handler's hand. The results from the enterotoxin testing, antibiotic sensitivity pattern, PFGE, and MLST molecular typing showed that although specimens from the girl and the food handler's hand both yielded SEA producing *Staphylococcus aureus*, the two strains had different antibiotic resistance patterns. The strains also had significant differences in PFGE and MLST [7]. Therefore, the conclusion is that the two strains do not have the same origin and have no meaningful association.

Adults suffering from staphylococcal food poisoning usually have self-limited mild symptoms. However, approximately 10% of the infected persons have severe symptoms which require hospitalization and treatment [9]. Persons with severe symptoms might be infants, children, or adults. Because each individual has a different level of sensitivity to enterotoxin, some patients might have severe

inflammatory response to enterotoxins secreted by *Staphylococcus aureus,* resulting in severe inflammation or even death [9-13]. In this incident, approximately four hours after eating lunch, the girl developed nausea and vomiting. *Staphylococcus aureus* producing enterotoxin A was isolated from the girl's throat swab, intestine, liver, kidney, and spleen. Based on the known pattern of Staphylococcal food poisoning and the result of laboratory testing, it was probable that the girl was infected by toxin-producing *Staphylococcus aureus* that resulted in severe reaction.

In most food poisoning incidents, pathogens usually do not survive in food, environment, and clinical specimens because of heating, changes in temperature or the lack of nutrients, leaving only traces of toxins in food or other specimens [8]. Another possibility is the failure to collect samples from the actual source of infection or the food that was actually, and as a result no pathogen could be identified in the specimens of the patient or the environment. In the present incident of suspected food poisoning, *Staphylococcus aureus* was isolated only in specimens from the girl and the food handler's hand. Staphylococcus was not found in any other specimens, including specimens from the male infant. In addition, isolated strains of the pathogen were shown to be different. Therefore, no infection source could be ascertained. Other than the reasons cited above, one could not rule out the possibility that other factors or pathogens might have played a role in this incident. Further investigation is needed to clarify the situation.

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