A Foodborne Outbreak of *Staphylococcus aureus* Associated with Fried Chicken in Republic of Korea

Hyeon, Ji-Yeon¹, Gyung-Tae Chung¹, Sun-Hye Bing², Kyung-Sook Kwon², Hyeon-Hee Lee³, Soo-Jin Kim¹, Se-Eun Jeon¹, Yeon-Ho Kang¹, and Junyoung Kim¹*  

¹Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, 187, Osong-eup, Cheongwon-gun, Chungbuk-do, 363-951, Korea  
²Division of Infectious Disease, Research Institute of Public Health and Environment, Daejon 302-789, Korea  
³Seo-gu Daejon Health Center, Daejon, 302-150, Korea  

Received: October 10, 2012 / Revised: October 17, 2012 / Accepted: October 18, 2012

**Abstract**  
An outbreak of *Staphylococcus aureus* infections occurred in a university with an enrollment of 80 students in the city of Daejon, Republic of Korea. All nine *S. aureus* isolates from patients (n = 7), staff members (n = 1), and the fried chicken served as the lunch (n = 1) harbored the enterotoxin A gene and showed an identical antibiotic-resistant profile, PFGE banding pattern (STAS16.001), and sequence type, ST 6. These results suggested that the outbreak was associated with eating the fried chicken that had been handled by an infected staff member. This case report demonstrated a practical approach to identifying the source and transmission of an infection.

**Key words:** *Staphylococcus aureus*, outbreak, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST)

**Introduction**  
Staphylococcal infection is a frequent cause of foodborne gastroenteritis in the world, following the ingestion of staphylococcal enterotoxins (SEs) [4, 10]. Over the past 10 years, *Staphylococcus aureus* (*S. aureus*) was the fourth most frequent pathogen, after pathogenic *E. coli*, *Salmonella*, and *Vibrio* spp. (http://kfda.go.kr/e-stat/) in the Republic of Korea. From 2002 to 2012, a total of 2,357 cases were reported from 174 staphylococcal food poisoning outbreaks (7.38% of the total number of outbreaks) in the Republic of Korea. In 921 of the 2,357 food poisoning outbreaks (39.1%), the causes (pathogen, food, water, and other) of outbreak have not been identified in the past 10 years in the Republic of Korea.

When *S. aureus* outbreaks have occurred during large social events, many different phenotyping and genotyping methods have been used to distinguish *S. aureus* strains to understand the epidemiology, population biology, and genetic diversity [4, 6]. Pulsed-field gel electrophoresis (PFGE) is considered to be the gold standard for typing *S. aureus* isolates in epidemiological studies [1]. Multilocus sequence typing (MLST) based on sequencing has also been proposed for the assessment of evolutionary relationships among *S. aureus* strains [6]. Therefore, in this study, we describe a practical and effective approach to identify the source and transmission route of infection in foodborne *S. aureus* outbreaks, by combining epidemiological study and molecular subtyping methods, including PFGE and MLST.

**Results**  
The outbreak of foodborne *S. aureus* infections involved 80 students in a university in the city of Daejon, Republic of Korea. Among the 80 students, 10 students exhibited symptoms of *S. aureus* infection. The symptoms included diarrhea (23.5% of the patients), abdominal cramps (23.5%), nausea (17.6%), vomiting (14.7%), and fever and chills (8.8%). The incubation period ranged from four to six hours after lunch.

A cohort study was performed with symptomatic students and 49 controls to analyze the risk of illness. Odds ratios with 95% confidence interval (CI) values are reported as measures of association. A *p* value of < 0.05 was accepted as being statistically significant.

Stool specimens from nine symptomatic students and six staff members working in the packed-lunch store were examined, in addition to nine environmental specimens, which included seven lunch meals stored in the refrigerator. For the isolation of *S. aureus* from the stool specimens, the samples were plated onto MSA agar (Difco). A total of five screened colonies were picked from each plate and identified using VITEK II (bioMérieux). Enterotoxin typing of *S. aureus* isolates was performed by multiplex PCR using the PowerChek *S. aureus* toxin ID PCR kit.
(Kogenebiotech). The antimicrobial susceptibility profiles of the isolates were determined by the disk diffusion method, as described in a previous study [8].

The genetic relatedness of the S. aureus isolates was investigated by PFGE using the method described in a previous study, with some modifications [5]. Briefly, for the preparation of plugs, bacterial cells were suspended in TE buffer (10 mM Tris and 1 mM EDTA, pH 8), and 170 µl of the cell suspension was mixed with 20 µl of lysostaphin (20 mg/ml) and 10 µl of lysozyme (10 mg/ml) followed by incubation at 37°C for 10 min. Next, 5 µl of proteinase K (20 mg/ml) and 200 µl of 1.2% SeaKem Gold Agarose (FMC) were added. The plugs were reacted with ES buffer (0.5 M EDTA, pH 9.0, and 1% sodium lauryl sarcosine) to lyse the bacterial cells. For restriction endonuclease digestion, the lysed plugs were incubated at 37°C for 2 h with 30 U of SmaI (Roche), and PFGE was performed with 1% agarose gels in 0.5× Tris-borate-EDTA buffer at 14°C using a CHEF mapper apparatus (BioRad) at 6 V/cm with a linearly ramped switching time of 5.16–40.17 s for 18 h. Interpretation of DNA fingerprint patterns was accomplished using the Bionumerics 5.1 software (Applied Maths). The banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

For the PCR amplifications of MLST, 0.5 mM DNA template was added to 25 µl of a PCR mixture consisting of ExTaq Premix (Takara) and 1 µM of each primer from seven loci (arc, aro, glm, gmk, pta, tpi, and yqi) (http://saureus.mlst.net). The mixture was initially denatured at 98°C for 5 min, which was followed by 40 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 1 min, and finally elongated at 72°C for 5 min. After PCR product purification, the DNA sequences were analyzed by a company for genetic technology, Solgent (Daejeon, Republic of Korea). Allele numbers were assigned a sequence type (ST) after the distinct allele sequences were submitted to the dedicated database (http://saureus.mlst.net).

The association between clinically defined illness and the consumption of specific foods is presented in Table 1. The results of interviews with all of the patients (n = 10) and the asymptomatic control students (n = 49) suggested that there was no food with a statistically significant association with the illness (p > 0.05, odds ratio < 1). In addition, the odds ratios of the rice, eggs, and fried chicken could not be obtained, because these foods were ingested by 58 of the 59 individuals.

S. aureus isolates (n = 9) were isolated from seven of nine patients, one of six staff members, and the fried chicken tested. The environmental samples and other food samples were negative for pathogenic bacteria and viruses. The same toxin type, staphylococcal enterotoxin A (SEA), was detected in all of the S. aureus isolates (Fig. 1). This finding was consistent with reports that SEA is the most dominant enterotoxin type in foods and food poisoning outbreaks worldwide, including in the Republic of Korea [2, 3, 7]. In addition, all of the isolates showed an identical antibiotic resistance pattern, resistance to ampicillin and tetracycline (Fig. 1), which is consistent with the results of another previous study in the Republic of Korea and in other countries [8].

In the results of both PFGE and MLST, the S. aureus isolates from the fried chicken and the stool of a staff member were genetically indistinguishable from those isolated from the patients. All nine S. aureus outbreak-associated strains showed identical PFGE banding patterns (STAS16.001). The PFGE patterns generated with SmaI ranged in size from 20.5 to 600 kb and included 16 fragments (Fig. 1). MLST identified identical allelic profiles or sequence types (STs) among all nine S. aureus outbreak-associated strains, ST 6 (12-4-1-4-12-1-3) (Fig. 1). ST6 was the most common ST that was observed; this type was identified in 34 (63.5%) of the 133 isolates from seven outbreaks between 2006 and 2009 in South China [1]. However, ST1, ST59, and ST72 were the major STs of S. aureus strains in food-poisoning cases collected from 10

<table>
<thead>
<tr>
<th>Food</th>
<th>Cases (n = 10)</th>
<th>Controls (n = 49)</th>
<th>p Value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed</td>
<td>Unexposed</td>
<td>Exposed</td>
<td>Unexposed</td>
<td></td>
</tr>
<tr>
<td>Rice*</td>
<td>10</td>
<td>0</td>
<td>48</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Egg*</td>
<td>10</td>
<td>0</td>
<td>48</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Fried chicken*</td>
<td>10</td>
<td>0</td>
<td>48</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Pickled radish</td>
<td>9</td>
<td>1</td>
<td>40</td>
<td>9</td>
<td>1.00</td>
</tr>
<tr>
<td>Kimchi</td>
<td>9</td>
<td>1</td>
<td>44</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>Pizza</td>
<td>8</td>
<td>2</td>
<td>42</td>
<td>7</td>
<td>0.64</td>
</tr>
<tr>
<td>Mineral water</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>47</td>
<td>0.43</td>
</tr>
<tr>
<td>Water from purifier</td>
<td>4</td>
<td>6</td>
<td>21</td>
<td>28</td>
<td>1.00</td>
</tr>
<tr>
<td>Coke</td>
<td>8</td>
<td>2</td>
<td>42</td>
<td>7</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*The odds ratios of the rice, eggs, fried chicken, and other beverage could not be calculated.

*Odds ratio > 1, p < 0.05, CI > 1, there is statistically significant association between food and illness.
FOODBORNE OUTBREAK OF STAPHYLOCCUS AUREUS IN REPUBLIC OF KOREA

To our knowledge, this study is the first report on an S. aureus ST6 outbreak in the Republic of Korea, and active surveillance and effective controls are needed to prevent the further occurrence of S. aureus ST6.

The results of the molecular subtyping methods suggested that the most significant association with illness was the ingestion of fried chicken that had been handled by the infected staff member. The fried chicken was cooked using the following simple steps: frying of frozen chicken without a thawing process, chopping, and packing. We concluded that the S. aureus contamination moved from the hands of the staff member to the chicken at the chopping step and that the hygiene practices of the staff members in charge of cooking are critical in preventing foodborne outbreaks. This case report could be a practical study on identifying the source and transmission route of an infection, and a supportive study for epidemiological analysis in the case of similar foodborne outbreaks.

Acknowledgments

This study was supported by a grant from the Korea Centers for Disease Control and Prevention. The authors declare that they have no conflicting interests in relation to this work.

REFERENCES


Fig. 1. A PFGE dendrogram of all of the S. aureus isolates digested with Smal, using Bionumerics with percent similarity calculated using the Dice coefficient (tolerance 1.5%). Also shown is the corresponding sequence type (ST) by MLST for each PFGE pattern. AM, ampicillin; TET, tetracycline.