Selective Enterotoxin Production by a *Staphylococcus aureus* Strain Implicated in a Foodborne Outbreak

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ABSTRACT

More than 80 of 230 participants (>34.7%) at a literary conference became ill with acute gastroenteritis 3 to 14 h after a catered meal. Attack rate data implicated cheese tortellini as the suspect food (p=0.0087). Selective plating of partially prepared and finished tortellini produced *Staphylococcus aureus* counts of 6.0 x 10⁷ and 1.0 x 10⁶ CFU per gram, respectively. Enterotoxin, phage typing, antibiotic sensitivity testing, and other biotyping studies were applied to *S. aureus* isolates from the suspect food and the single food-handler involved. All isolates reacted identically by all criteria, and each isolate produced both type A and C staphylococcal enterotoxins. Type A enterotoxin (0.90 ug/100 g) alone was detected in samples of the suspect food. The production of type C enterotoxin by the outbreak strain was delayed approximately 4 h relative to production of enterotoxin A when grown in Heart Infusion broth (pH 5.5). This study serves as an example of selective enterotoxin production by *S. aureus* in suspect foods which can be misleading to outbreak investigators.

The role of enterotoxin-producing strains of *Staphylococcus aureus* in foodborne gastroenteritis outbreaks has been well documented for years. Strains producing multiple enterotoxins have been implicated in a number of outbreaks (3,7,9). In many cases, *S. aureus* isolates recovered from food-handlers have been shown to be identical to strains responsible for enterotoxin production in suspect foods. Confusion can result, however, if an *S. aureus* strain elaborating different enterotoxins is isolated from food-handlers and the implicated food, but only one performed enterotoxin is demonstrated in the food samples. The link between the responsible enterotoxin and the food-handler may be questionable. The kinetics of enterotoxin production by *S. aureus* strains producing distinct enterotoxins is a potential regulating factor in such cases according to Noleto and Bergdoll (6). This outbreak, with its laboratory investigation, is an example of such enterotoxin production implicated in an outbreak of gastroenteritis.

THE OUTBREAK

On April 12, 1986, a well-publicized outbreak of gastroenteritis occurred in Sacramento, California. More than 80 of 230 participants (>34.7%) at a literary conference became ill with vomiting and diarrhea 3 to 14 h after the noon meal. A total of 28 individuals were treated at local hospital emergency rooms. Analysis of food-specific, attack-rate data implicated cheese tortellini as the suspect food (p=0.0087, Fishers Exact Test).

Preparation of the tortellini was accomplished primarily by a single food-handler and had begun the previous day. Fifty pounds of dried tortellini were boiled and cooled within a 3- to 4-h period, layered in plastic bags, and refrigerated overnight in a small refrigerator. The following morning a cheese sauce was prepared and mixed with the tortellini by hand. This procedure took approximately 45 min. The completed mixture was then placed in shallow pans with foil covers and transported to the conference site for final reheating in large ovens. Difficulty was experienced in setting the desired oven temperature, and the oven controls were reset 2 or 3 times during the 1-h-and-15-min heating period. The final temperature of the ovens was 350°F.

MATERIALS AND METHODS

Bacterial strains and food samples

The *S. aureus* isolates from both the suspect food and the primary food-handler (hands and nares) were obtained from the Sacramento County Health Department Laboratory. Frozen samples of cheese tortellini salvaged from the suspect meal were also provided by the county laboratory. Isolation and enumeration of *S. aureus* from the food were accomplished by the county laboratory on Tellurite-Polymyxin-Egg-Yolk Agar (Difco).

Phage typing, biotyping, and antibiotic sensitivity testing

Phage typing of all *S. aureus* isolates was performed by the Centers for Disease Control Laboratories in Atlanta, Georgia. Biochemical identification and antibiotic sensitivity tests on the *S. aureus* isolates were done by standard laboratory procedures (4). Biochemical tests included fermentation of glucose and mannitol.
Selective enterotoxin production by S. aureus

Results

A sample of the cheese tortellini saved by the food-handler before reheating gave an S. aureus count of 6.0 x 10^7 CFU per gram. The finished (reheated) tortellini had an S. aureus count of 1.0 x 10^8 CFU per gram. The concentration of S. aureus may be underestimated in this sample since direct plating may not enumerate heat-stressed or damaged organisms with high efficiency. All staphylococcal isolates were identified as S. aureus by accepted biochemical criteria. All isolates, including those from the tortellini and the food-handler, belonged to the same phage type (53/83A+) and gave the identical antibiotic sensitivity pattern (sensitive to clindamycin, methicillin, penicillin, vancomycin, ampicillin, cephalothin, neomycin, trimethoprim-sulfamethoxazole, and nitrofurantoin; resistant to tetracycline and erythromycin). All isolates produced "type A" (ring-shaped zone) reactions on Buffered Caseinate Agar after a 48-h incubation at 35°C. All isolates produced both type A (SEA) and type C (SEC) staphylococcal enterotoxins on semi-solid Brain Heart Infusion Agar as measured by the microslide gel double diffusion test. Samples of finished cheese tortellini had a pH of 5.5 and yielded only SEA (0.90 ug per 100 g) in test runs with a sensitivity of 0.10 ug per 100 g of sample. Enterotoxin was not detected in a sample of tortellini obtained before reheating.

The epidemic strain was used to prepare a 36-h rotary shake culture at 35°C in BHI Broth adjusted to the pH of the suspect tortellini. Table 1 gives the rate of growth and the results of micro-slide gel-diffusion enterotoxin assays on test portions on the rotary shake culture obtained at various times during the incubation. The enterotoxin amounts were determined by comparison of precipitin lines formed by the test portions with those produced by known enterotoxin solutions in the microslide gel double-diffusion test. The production of SEC lagged some 4 h relative to the production of SEA in this experiment.

Discussion

Several deviations from accepted food-handling practices were noted in this study that presumably contributed to the outbreak. Unacceptable practices included excessive hand contact with the food, prolonged time at holding temperatures between 10 to 60°C during the first cooling step, and inadequate reheating that may have resulted in multiplication of contaminating organisms. Total time at the holding temperatures was estimated to be between 4 and 5.5 h for the suspect food. The results of phage typing, biotyping, antibiotic sensitivity testing, and enterotoxin assays definitively identified each isolate recovered from the food-handler as the epidemic strain. The absence of detectable amounts of SEC in the remaining reheated tortellini is most likely explained by a low rate of SEC production in the tortellini during temperature abuse. If a significant amount of SEC had been produced, it should have survived the final heating of the tortellini, as shown by the recent work of Tibana et al. (8). While the enterotoxin assays made on the timed shaken broth cultures adjusted to pH 5.5 do not constitute an exhaustive study, the lag in SEC production by the epidemic strain was clearly demonstrated. Selective enterotoxin production in suspect foods should be considered when establishing a link between S. aureus isolates from food-handlers and the enterotoxin types produced in food samples.

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References


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