Research Note

Enterotoxin H in Staphylococcal Food Poisoning

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ABSTRACT

Seven members of one family became ill with vomiting and diarrhea 4 h after eating a type of cheese produced in the state of Minas Gerais, Brazil. Staphylococcus aureus (2.9 × 10⁸ CFU/g) that produced enterotoxin H (SEH) was isolated from the cheese. A low level of this enterotoxin was detected in the cheese extract before and after concentration 20-fold by copper chelate chromatography. The amount of SEH produced by the staphylococcal strain was 180 ng/ml of culture supernatant with production by the sac culture method. If only the ELISA ball kit had been used, it would have been concluded that enterotoxin D was the cause of the food poisoning.

Key words: Enterotoxin H, staphylococcal food poisoning

Staphylococcal food poisoning is one of the most common types of foodborne disease worldwide. It is not commonly investigated in developing countries because of many other disease problems in these countries. The isolation of at least 10⁶ CFU of staphylococci per g of food is circumstantial evidence for this type of food poisoning, along with the symptoms of vomiting and diarrhea developing in 1 to 6 h after consuming the food. Additional information, such as the production of enterotoxin by the isolated staphylococci, is reasonable proof that the food poisoning was due to staphylococci. However, the best proof is the detection of enterotoxin in the implicated food. In order to detect enterotoxin in food it is necessary to use one of the sensitive methods for detection of the enterotoxin, such as an enzyme-linked immunoassay (ELISA) or reversed passive latex agglutination (RPLA). Only in recent years has investigation of staphylococcal food poisoning been undertaken in Brazil (1, 2, 3, 4, 10).

This report presents the results of the investigation of a food-poisoning outbreak in a family after they consumed a common type of cheese produced in the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

Isolation of staphylococci

Twenty-five grams of the cheese was suspended in 225 ml of buffered peptone water; 0.1 ml was placed on Baird-Parker agar plates and incubated for 48 h at 37°C. The colonies were jet black, but were not typical, as they were small without halos and were mucoid. Five colonies were selected for further testing. Each colony was transferred to two test tubes containing 1 ml of BHI broth and incubated for 24 h at 37°C. Tests were made for coagulase and thermonuclease (TNase) production, anaerobic fermentation of glucose and mannitol, and production of hemolysin using sheep blood. Any colonies that were positive for these characteristics were considered Staphylococcus aureus and were tested for enterotoxin production. For staphylococcal counts, additional Baird-Parker plates were prepared with 10-fold dilutions if necessary (12).

Enterotoxin production

For enterotoxin production, inocula were prepared by combining the five isolates and incubating them in brain heart infusion (BHI) broth overnight at 37°C. Enterotoxin production was assayed by the membrane-over-agar plate method described by Robbins et al. (9).

Detection of enterotoxin in culture supernatant fluid

The optimum-sensitivity-plate (OSP) gel diffusion method was used as described by Robbins et al. (9) for enterotoxin detection.

The more sensitive methods, RPLA and ELISA, also were used for testing the culture supernatant fluid and the cheese extract for enterotoxin. The Oxoid RPLA kit (Denka Seiken Co. Ltd., Tokyo, Japan) employs latex particles coated with the specific antisera to the enterotoxins, each on separate latex particles. In the presence of enterotoxin the latex particles agglutinate. The limit of sensitivity is about 0.5 ng of enterotoxin per g of food (8). The ELISA ball kit (Dr. Bomelli AG, Lübbeufeld-Bern, Switzerland) was used according to the directions of the manufacturer (6). If any color develops, the sample is judged to contain the enterotoxins for which a color develops. Also, the RIDASCREEN ELISA kit (R-Biopharm GmbH, Darmstadt, Germany), which employs monoclonal antibodies, was used for testing the culture supernatant fluid. Microtiter strips coated with antibodies to each of the enterotoxins, plus a positive and negative control are provided.
The detection of enterotoxin H (SEH) was performed by Su and Wong (11) with an ELISA method they developed with the antibodies to SEH.

Enterotoxin detection in the cheese
One hundred grams of the cheese was homogenized in a Waring blender with 1.5 ml of 0.02 M NaHPO4 in saline, pH 7.4, per gram of food (7). The slurry was adjusted to pH 4.5 and centrifuged for 20 min at 20,000 × g, and 4°C. The pH of the supernatant fluid was readjusted to pH 7.4 and tested for enterotoxin with the RPLA and ELISA ball kits.

Concentration of the food extract
The food extract was concentrated by the copper chelate Sepharose method as described by Dickie and Akhtar (5).

RESULTS

Staphylococcal isolates
Each of the five isolates was coagulase and TNase positive, fermented glucose and mannitol anaerobically, and was hemolytic and pigmented. It was concluded that the isolates were Staphylococcus aureus.

Staphylococcal count in the cheese
The staphylococcal count was 2.9 × 10^8 CFU/g of cheese.

Enterotoxin production by the staphylococci
Examination by the OSP method showed the cheese isolates to be negative for any of the identified enterotoxins. The culture supernatant fluid was negative in RPLA and by the RIDASCREEN ELISA method for any of the enterotoxins, but was positive for SED by the ELISA ball kit.

Enterotoxin detection in the cheese
The cheese extract was positive for SED by the ELISA ball method, both before and after concentration of the extract, but was negative by the RPLA method. To show that the positive reaction by the ELISA ball method was due to SED and not SEH, the positive reaction by the SED reagents with the culture supernatant fluid (16 ml), treated with 4 ml of antibodies specific to SED, was not neutralized. However, treatment of 4 ml of culture supernatant fluid from the standard SED strain (FRI-472, 0.5 to 1 J.lg/ml), with 4 ml of SED antiserum, did neutralize the SED reaction in the ELISA ball method. This strain does not produce SEH. The difference in the amount of culture supernatant fluid used was due to the amount of enterotoxin present in the two fluids.

Due to the fact that the original results for SED were questionable, the strain was tested for the production of enterotoxin H (SEH), the enterotoxin recently isolated by Su and Wong (11). These investigators showed that the staphylococcal strain produced 180 ng/ml of SEH.

DISCUSSION

This outbreak was a typical staphylococcal food poisoning outbreak in a family resulting from the eating of a type of semicured cheese, common to the state of Minas Gerais, Brazil. All those who ate the cheese became ill. The symptoms of vomiting and diarrhea developing in 4 h were typical of this type of food poisoning.

The staphylococcal count of 10^8 CFU/g of cheese was adequate for production of enterotoxin; this count is not unusual in staphylococcal food poisoning (13). Although the organisms were not typical of S. aureus on Baird-Parker plates, yet their biochemical characteristics were typical of this species. Normally, enterotoxin production by staphylococci isolated from implicated foods can be detected by gel diffusion methods such as the OSP method. However, occasionally production can be determined only by the use of a more sensitive detection method.

Initially the positive results with the ELISA ball kit for SED were accepted as positive for SED even though negative results were obtained with the RPLA method. Again the positive reaction in the food extract by the ELISA method appeared to confirm that SED was the enterotoxin involved even though the food extract was negative by RPLA. It has been reported that the RPLA method is not as sensitive as the ELISA ball method (13).

The testing with the RIDASCREEN ELISA method was done after the cheese strain tested positive for SEH. This method is highly specific because monoclonal antibodies are used in preparing the kit.

Fortunately at the time this investigation was being done Su and Wong (11) reported the identification of a new enterotoxin, SEH. They agreed to test the cheese strain for the production of SEH and found that it produced 180 ng of SEH/ml of culture supernatant fluid produced by the sac culture method. They also found that the mutant SED strain 1151m generally used for the purification of SED produced a small amount of SEH. This could account for the presence of antibodies to SEH in the SED antiserum used in the ELISA ball kit because the 1151m strain was used for purification of the SED used for antibody preparation. The antiserum used in the RPLA kit is purified by affinity chromatography, which would tend to eliminate any contaminating antibodies. The use of monoclonal antibodies provides the most highly specific reactions.

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REFERENCES