Staphylococcal Food Poisoning Caused by Imported Canned Mushrooms

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From February through April 1989, four outbreaks of staphylococcal food poisoning in the United States were associated with eating mushrooms canned in the People's Republic of China (PRC). In the four outbreaks, 99 persons who ate at a suspect facility developed gastrointestinal symptoms within 24 h, including 18 who were hospitalized. Illness was associated with eating mushrooms at a university cafeteria (relative risk [RR] = 53.0), a hospital cafeteria (RR = 13.8), a pizzeria (odds ratio [OR] = ∞), and a restaurant (OR = ∞) (all $P < .0001$). Staphylococcal enterotoxin A was found by ELISA in mushrooms at the sites of two outbreaks and in unopened cans from the three plants thought to have produced mushrooms implicated in outbreaks. These investigations led to multistate recalls and a US Food and Drug Administration order to restrict entry into the United States of all mushrooms produced in the PRC; until this action, the United States imported ~50 million pounds yearly.

In February 1989, we investigated an outbreak of what clinically appeared to be staphylococcal food poisoning among 11 students and faculty members at Mississippi State University (MSU). The outbreak was identified by a health service physician and reported to the Mississippi State Department of Health, which in turn contacted the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA). Following confirmation of the initial outbreak in February 1989 and evidence that linked this outbreak to canned mushrooms, CDC contacted all state health departments to request that they report any possible outbreaks linked to this commercial product.

Materials and Methods

Epidemiologic investigations. In each of four outbreaks that were intensively investigated, a case-patient was defined as a person who ate at a suspect facility and within 24 h developed two or more of the following symptoms: nausea, vomiting, abdominal cramps, and diarrhea. For outbreaks 1 and 2, questionnaires were distributed in the suspect cafeterias. For outbreak 3, patients were identified through self-reporting, credit card receipts, and lists of take-out food patrons; controls were dining partners or others without illness identified through these methods. For outbreak 4, cases were identified through self-reporting before and after a press release about the outbreak that named the restaurant but that did not discuss possible vehicles of transmission; controls were their dining partners who had not become ill. Information was gathered on illness and on foods consumed on the day of the outbreak using standard questionnaires self-administered for respondents for outbreaks 1 and 2 and by telephone conversations with a trained interviewer for outbreaks 3 and 4.

In each investigation, all food handlers were interviewed to determine the details of mushroom handling. Hands were inspected for lesions. Refrigerator temperatures were measured, and kitchen hygiene was reviewed. Remaining cans that appeared to be from the same box or shipment were forwarded for analysis to an FDA laboratory.

Statistical tests on proportions were done using Yates's corrected χ^2 and Fisher's two-tailed exact test.

Laboratory methods. Extracts of mushrooms and other suspect foods were prepared and analyzed for staphylococcal enterotoxin by ELISA [1]. To detect the presence of partially denatured enterotoxin, extracts were urea-treated before testing [2]. An ELISA (Staphylococcal Enterotoxin Visual Immunoassay; Tecra Diagnostics, Roseville, Australia) was used, using polyvalent antisera to toxin types A–E in a double polyclonal antibody sandwich configuration [3]. The absorbance of the test portions was determined in a microtiter tray reader (V Max Kinetics Microplate Reader; Molecular Devices, Menlo Park, CA), using a dual wavelength (405–490 *A*). Test portions with an optical density (OD) of >0.200 were considered to contain enterotoxin. When specimens were positive by the polyvalent ELISA, tests for specific entero-

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toxin type and confirmation were done using the microslide gel diffusion or reverse passive latex agglutination (RPLA) methods [3].

To estimate the concentrations of staphylococcal enterotoxin in mushrooms corresponding to ELISA OD measurements of mushroom extracts, dilutions of pure enterotoxin were added to 100 g of canned mushrooms from the People's Republic of China (PRC). Mushrooms were processed, and OD was measured as described above.

Staphylococci were isolated from cultures of foods and stool specimens, and isolates were tested for enterotoxin production using methods previously described [3–5].

Results

Epidemiologic investigations. Outbreak 1 was identified on 13 February 1989, when 11 MSU students and faculty presented to the health service with nausea, vomiting, abdominal cramps, and diarrhea within hours after eating at the cafeteria. Nine were hospitalized, including a 22-year-old man treated in intensive care for hypovolemic shock. Of 280 persons who responded to questionnaires administered in the cafeteria on 20 February and who reported eating in the cafeteria on the day of the outbreak, 12 had illness fitting the case definition; 2 were among the 11 who had presented previously. Nine (60%) of 15 who ate mushrooms served in an omelet or with grilled hamburgers or chicken became ill compared with 3 (1%) of 265 who did not (relative risk [RR] = 53.0, $P < .00001$). All 11 students who presented to the student health service on the day of the outbreak and 1 other person who later reported becoming ill fit the case definition and had eaten mushrooms either with omelets or with foods served from the char-grill in the cafeteria. A total of 22 patients were identified in this outbreak.

Cafeteria employees reported that a 68-oz can of mushrooms was opened at 6:15 A.M. on the morning of the outbreak and split between two half-gallon trays. One tray was placed in a refrigerator and the other in the refrigerated area at the omelet bar. The mushrooms in the first tray were later heated with butter, placed on the char-grill, and served with hamburgers or chicken when the char-grill opened at 11 A.M. The mushrooms had been grown and canned in the PRC.

Outbreak 2 was identified when employees at a hospital in New York City developed an acute gastrointestinal illness on 28 February 1989, hours after eating at the hospital cafeteria. Forty-five (27%) of 169 questionnaire respondents who ate lunch in the cafeteria on 28 February became ill compared with 1 (1%) of 112 who did not eat this meal (RR = 29.8, $P < .001$). Forty (53%) of 76 persons who recalled eating at the salad bar became ill compared with 5 (7%) of 72 who did not (RR = 7.6, $P < .001$). Among those who ate at the salad bar, 38 (86%) of 44 who ate mushrooms became ill compared with 2 (6%) of 32 who did not (RR = 13.8, $P < .0001$). At 11 A.M. on the day of the outbreak, a 68-oz can of mushrooms had been opened, and mushrooms had been placed in a 48-oz crock on ice at the salad bar.

Outbreak 3 was identified when the Allegheny County Health Department in Pennsylvania was notified that people developed vomiting and diarrhea on 17 April 1989, several hours after eating at a restaurant. An investigation identified 12 ill persons, 2 of whom were hospitalized. All 12 had consumed mushrooms served on pizza or with a parmigiana sauce compared with only 2 of 21 controls who ate at the restaurant on the same day (odds ratio [OR] = ∞, $P < .0001$). All 12 persons ate meals with mushrooms that came from the same can; the mushrooms were served within several hours after the can was opened.

Outbreak 4 was identified on 23 April 1989, when 6 persons presented to hospitals in Clearfield County and Center County, Pennsylvania, with nausea, vomiting, abdominal cramps, and diarrhea several hours after eating take-out pizza with mushrooms from a local pizzeria. Fourteen other ill persons were identified through responses to a press release. All 20 ill persons, but only 6 of 18 controls, who ate pizza from the pizzeria the same evening had eaten mushrooms with their pizza (OR = ∞, $P < .001$). No other food item was associated with illness. Four 68-oz cans of mushrooms were opened between 6:00 and 6:30 P.M. and served the evening of the outbreak.

Inspection of each of the facilities where outbreaks occurred revealed no deficiencies in storage temperatures, food handling, or sanitation.

Of the 99 patients identified in the four outbreaks, 33% sought medical attention, and 18% were hospitalized. In each outbreak, >80% of patients had nausea, >75% had diarrhea, >65% had abdominal cramps, and >60% had vomiting. The peak onset time was 2 h after eating mushrooms for outbreaks 1 and 3 and 3 h for outbreaks 2 and 4.

Laboratory investigations. In outbreak 1, staphylococcal enterotoxin was identified by ELISA in a mushroom omelet (OD = 1.604), mushrooms from the omelet bar (OD = 1.649), and liquid from mushrooms at the char-grill (OD = 0.218). The presence of enterotoxin A was confirmed by the microslide test in the first two test portions and by RPLA in the third. *Staphylococcus aureus* did not grow on direct plating of any of these test portions, although typical gram-positive cocci in clusters were seen on a Gram's-stained smear of the mushrooms from the char-grill. In outbreak 3, staphylococcal enterotoxin type A was identified in a test portion of remaining pizza made with mushrooms (ELISA OD = 1.717, with RPLA confirmation) and in lower concentrations from five test portions of sauces made with mushrooms (OD = 0.229–0.432); no staphylococci grew in culture. Enterotoxin A was also found in mushrooms in the can from which the mushrooms for the pizza were taken (OD = 2.400, with RPLA confirmation). In outbreaks 2 and 4, no mushrooms from the portions served were available for testing.

Cans of mushrooms from the same shipment as those implicated in outbreak 1 had embossed codes indicating that they were produced by a plant in Fujian Province (plant A). Re-

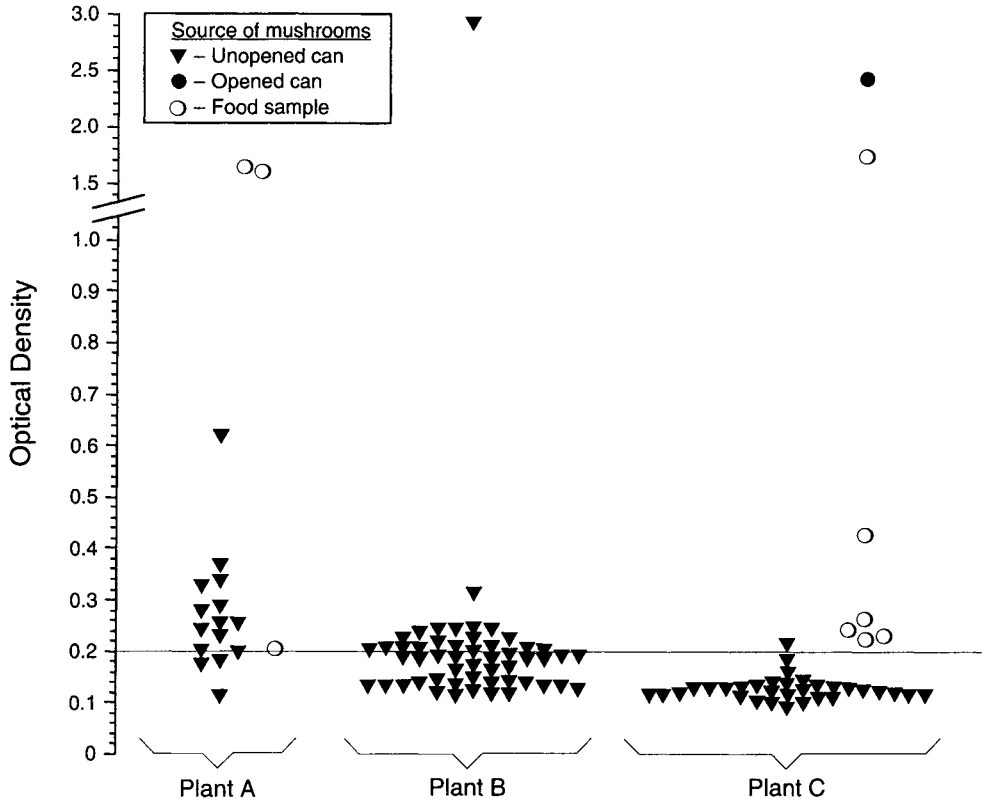

Figure 1. Results of ELISAs without urea for staphylococcal enterotoxin in mushrooms from unopened cans, opened can from which implicated mushrooms were served, and remaining implicated foods containing mushrooms. Plants identified by can codes as having processed mushrooms are indicated at bottom. Optical density >0.200 (horizontal bar) was considered positive.

maining cans found in the hospital cafeteria where outbreak 2 had occurred had codes indicating a facility in Sichuan Province (plant B). Cans found at the sites of outbreaks 3 and 4 had codes indicating a plant in Anhui Province (plant C). Ninety-nine test samples from 107 previously unopened cans from these three plants were tested for enterotoxin (for 8 test samples, aliquots from 2 cans from the same plant were combined). When tested without the addition of urea, 11 of 15 test samples from plant A had ODs >0.200, as did 20 of 52 test samples from plant B and 1 of 32 test samples from plant C (figure 1). Duplicates of 40 of the 67 test samples that initially had ODs ≤0.200 were treated with urea. The ODs were then >0.200 in 11 (28%) of these test samples: 1 of 4 from plant A, none of 7 from plant B, and 10 of 29 from plant C. All three of those that tested positive that were retested by the microslide method were confirmed as containing staphylococcal enterotoxin type A. The ELISA readings for the implicated food items are shown in figure 1, together with the results obtained without urea for the test samples from unopened cans.

Figure 2 shows the relationship between enterotoxin A concentration and ELISA OD, obtained when known quantities of enterotoxin A were added to mushrooms and the mushrooms were extracted and processed by the same procedures as those for sampled mushrooms.

Investigation of other outbreak reports. Three other outbreaks reported to CDC in 1989 in response to CDC and FDA alerts were possibly due to canned mushrooms, but these investigations were incomplete. The suspect cans in these three outbreaks, however, were from two of the same plants implicated in the four outbreaks described above (plants A and B).

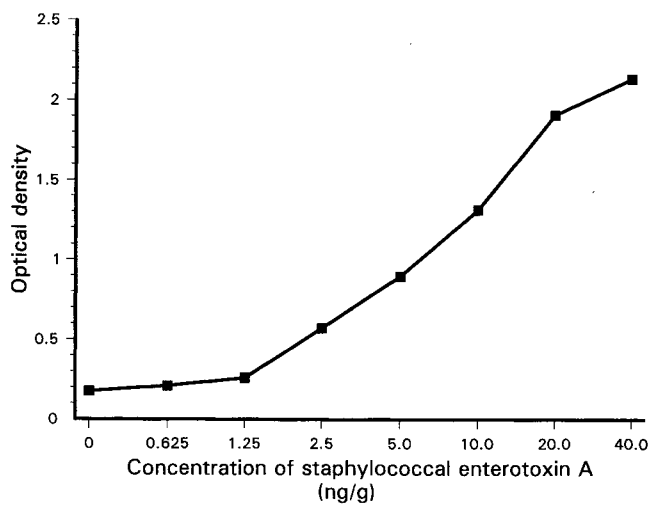

Figure 2. Results of ELISAs for staphylococcal enterotoxin in mushrooms, using mushroom test samples to which known concentrations of purified enterotoxin were added.

Discussion

We conclude that multiple outbreaks of staphylococcal foodborne disease were caused by canned mushrooms contaminated with staphylococcal enterotoxin type A. First, investigations of four outbreaks demonstrated that persons who ate canned mushrooms had elevated risks for illness characteristic of staphylococcal foodborne disease. Second, two of the outbreaks were microbiologically confirmed, with staphylococcal enterotoxin identified in implicated foods made with mushrooms and in leftover mushrooms. Third, in no outbreak was there evidence of improper mushroom storage or handling after cans were opened. Finally, staphylococcal enterotoxin type A was found in unopened cans taken from each of the sites where outbreaks occurred.

Although ELISA ODs of implicated foods and several tested mushrooms from cans were high, ODs of many tested cans were 0.200–0.300, appearing borderline positive (figure 1). The curve correlating enterotoxin A concentration with the ELISA OD of extracted mushrooms showed that an OD of 0.209 corresponded to an enterotoxin concentration of 0.625 ng/g. A 4-oz serving of these mushrooms (113 g) would contain 71 ng of enterotoxin. One human volunteer study has shown that a dose of 50 ng/kg of body weight (3500 ng for a person weighing 70 kg) is necessary to cause vomiting or diarrhea, and 10 ng/kg (700 ng for a 70-kg person) to cause milder symptoms [6]. Therefore, cans with low-level contamination may have had insufficient enterotoxin to cause illness in most exposed persons.

Staphylococcal enterotoxin type A was subsequently found in cans produced by seven other factories in the PRC (FDA, unpublished data). Although the large institutional-sized cans were implicated in the confirmed outbreaks, enterotoxin was also found in small retail-sized cans (FDA, unpublished data).

Investigations of PRC mushroom plants by FDA investigators and consultants to a mushroom importer found several reasons why mushrooms may have been contaminated at multiple facilities. Mushrooms were extensively handled, since they were hand-picked at >10,000 small farms for each plant and manually separated by size. The mushrooms were stored without refrigeration, often anaerobically, in plastic bags or in brine—conditions that promote the selective survival and growth of staphylococci [7]. Sanitation deficiencies were widespread. In the one plant (not outbreak-associated) where investigators cultured samples, *S. aureus* that produced enterotoxin A was identified on the hands of 7 workers and in recently sliced mushrooms (FDA, unpublished data).

If environmental contamination with staphylococci was widespread at the canneries, contamination may have occurred both before and after thermal processing. Since staphylococcal enterotoxin is heat stable, findings of high enterotoxin concentrations but few staphylococcal colonies may be explained either by thermal processing following heavy staphylococcal contamination or by nutrient depletion and death of organisms following postprocessing contamination through functional microleaks during cooling. In some sampled cans, particularly from plant C, enterotoxin was found only after treatment with urea, suggesting that enterotoxin had been partially heat denatured during canning [2]; yet many unopened cans from plants A and B and the mushrooms implicated in outbreaks had detectable enterotoxin even before urea treatment. Postprocessing contamination has previously been the main explanation for staphylococcal outbreaks caused by canned foods [8, 9].

In Canada in 1981, two staphylococcal-like outbreaks occurred in association with retail-size cans of mushrooms from the PRC [10]. Although these outbreaks could not be microbiologically confirmed, all PRC mushrooms were detained for inspection for several months. In 1988, the year before these outbreaks, the United States imported >50 million pounds of PRC canned mushrooms [11]. Considering the extent of contamination discovered during our investigations, as well as the other unconfirmed reports of outbreaks in 1989, the number of cases of illness caused by this product probably far exceeded the number reported here.

These investigations led to multistate recalls of PRC-canned mushrooms and an order to prohibit entry of this product into the United States until deficiencies could be corrected [12–14]. Through March 1996, strict import restrictions and intensive product inspections remain in effect; periodic testing by FDA has shown continuing evidence of product contamination (FDA, unpublished data).

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T Lymphocytic and Immature Macrophage Alveolitis in Active Pulmonary Tuberculosis

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The phenotype of bronchoalveolar cells from 11 healthy subjects and from affected and unaffected lungs of 15 patients with pulmonary tuberculosis (PTB) was determined. An immature macrophage alveolitis was found in the affected lung and the unaffected lung versus controls as determined by morphology and peroxidase activity. T lymphocytic alveolitis also was found in the affected but not the unaffected tuberculous lung compared with healthy controls. The majority of alveolar lymphocytes in unaffected and affected PTB lungs were T cells expressing the $\alpha\beta$ T cell receptor. Alveolar T cells from both unaffected and affected lungs were activated, as determined by increased expression of CD69 and HLA-DR. Interleukin-2 receptor (IL-2Rα) expression was, however, unchanged on alveolar lymphocytes from affected lung and was decreased in the unaffected lung. Thus, activated T lymphocytes and immature macrophages in the tuberculous lung are basic to the local immunopathogenesis of PTB.

The most common organ affected in tuberculosis (TB) is the lungs, yet very little is known about the cell types or the immunologic events occurring at this site of disease activity. Several studies show an increase in alveolar lymphocytes (AL) [1–6], and one study reports an increase in neutrophils within the bronchoalveolar cells (BAC) of patients with pulmonary TB (PTB) obtained by bronchoalveolar lavage (BAL) [6]. HLA-DR also is increased on AL from patients with PTB [3].

Most available data on characterization of AL in PTB patients, however, fail to control for the site of actual tuberculous infection in the lungs, antituberculous therapy before BAL, or smoking habits. Further, thorough analysis of the phenotype of AL and their state of activation have not been reported. Therefore, the purpose of the current study was to characterize the cellular pattern of BAC and to determine the phenotype and state of activation of autologous AL and blood lymphocytes obtained from the radiographically affected and unaffected lung during active PTB.

Materials and Methods

Study subjects. Study subjects with new-onset PTB (n = 15) were recruited at the National Institute for Respiratory Diseases

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