HHS Public Access

Author manuscript

J Food Prot. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

J Food Prot. 2018 August ; 81(8): 1283–1292. doi:10.4315/0362-028X.JFP-17-494.

Salmonella enterica Serotype Javiana Infections Linked to a Seafood Restaurant in Maricopa County, Arizona, 2016

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Abstract

On 10 August 2016, the Maricopa County Department of Public Health identified culture-confirmed *Salmonella enterica* serotype Javiana isolates from two persons who reported eating at a seafood restaurant; seven additional cases were reported by 15 August. We investigated to identify a source and prevent further illness. We interviewed persons with laboratory-reported *Salmonella* Javiana infection. Pulsed-field gel electrophoresis (PFGE) and whole genome sequencing of isolates were performed. A case was defined as diarrheal illness in a person during July to September 2016; confirmed cases had *Salmonella* Javiana isolate yielding outbreak-related PFGE patterns; probable cases had diarrheal illness and an epidemiologic link to a confirmed case. Case finding was performed (passive surveillance and identification of ill meal companions). A case-control study assessed risk factors for *Salmonella* Javiana infection among restaurant diners; control subjects were chosen among meal companions. No restaurant workers reported illness. Foods were reportedly cooked according to the Food Code. Food and environmental samples were

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SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-17-494.s1.

collected and cultured; *Salmonella* Javiana with an indistinguishable PFGE pattern was isolated from portioned repackaged raw shrimp, halibut, and a freezer door handle. We identified 50 *Salmonella* Javiana cases (40 confirmed and 10 probable); illness onset range was from 22 July to 17 September 2016. Isolates from 40 patients had highly related PFGE patterns. Thirty-three (73%) of 45 patients interviewed reported eating at the restaurant. Among 21 case patients and 31 control subjects, unfried cooked shrimp was associated with illness (odds ratio, 6.7; 95% confidence interval, 1.8 to 24.9; $P = 0.004$). Among restaurant diners, laboratory and case-control evidence indicated shrimp as the possible outbreak source; poor thermal inactivation of *Salmonella* on shrimp is theorized as a possible cause. Cross-contamination might have prolonged this outbreak; however, the source was not identified and highlights limitations that can arise during these types of investigations.

Keywords

Case-control study; Outbreak; Restaurant; *Salmonella*; Shrimp; Thermal inactivation

Salmonellosis is estimated to cause 1.2 million illnesses each year in the United States, with approximately 23,000 hospitalizations and 450 deaths (20). *Salmonella* infections most often cause gastroenteritis that can range from mild to severe, and they sometimes develop into potentially serious infections, including bacteremia or endovascular infections (8). More than 2,500 serotypes of *Salmonella* have been described (24). *Salmonella enterica* serotype Javiana is the sixth most common *Salmonella* strain identified in the United States, accounting for approximately 3.5% of nontyphoidal *Salmonella* isolates (24). Substantial seasonality has been reported, with 72% of cases reported during July to October (26). Since 1980, several southeastern U.S. states have had the highest reported age-standardized rates of *Salmonella* Javiana infections, although increasing rates have been observed in the southwest, and infections have been reported throughout the United States (24, 26).

Outbreaks of *Salmonella* Javiana have previously been associated with various sources, including contaminated tomatoes, watermelon, cheese, and other foods and with amphibians (5, 6, 14, 15, 21, 31). *Salmonella* Javiana has previously been detected in fruit and vegetables (e.g., cantaloupe, green onions, papaya, collard greens, and cucumbers), meat products (e.g., chicken, frozen shrimp, and ground beef), spices (e.g., coriander and pepper powder), and other foods (e.g., candy and corn bread) (23). In addition to food sources, ill food handlers infected with *Salmonella* Javiana who work while symptomatic have been known to propagate an outbreak, as reported at a children's hospital in 2003 (10). According to the National Antimicrobial Resistance Monitoring Systems for Enteric Bacteria, 97% of *Salmonella* Javiana isolates have antimicrobial pan-susceptibility (i.e., not resistant to any antibiotics) (7).

During 2011 to 2015, *Salmonella* Javiana cases were reported sporadically in Arizona, with zero to three cases reported per month. All *Salmonella* isolates from private or commercial laboratories in Arizona are required to be submitted to the Arizona State Public Health Laboratory (ASPHL) for serotyping and pulsed-field gel electrophoresis (PFGE). On 10 August 2016, the Maricopa County Department of Public Health (MCDPH) identified two

unique culture-confirmed *Salmonella* infections in persons who reported eating at the same restaurant (restaurant A) in Phoenix during the week preceding symptom onset. By 15 August, the Arizona Department of Health Services notified the MCDPH of seven additional cases or suspect cases: a PFGE-matched cluster of five *Salmonella* Javiana cases in Maricopa County (one illness was in a person who had reported eating at restaurant A) and two additional persons infected with *Salmonella* reporting eating at restaurant A within the exposure time frame. An investigation was initiated to identify cases, characterize the outbreak, and determine initial outbreak source among restaurant A diners and to prevent additional cases and make recommendations for control and prevention of salmonellosis.

MATERIALS AND METHODS

Case definition and laboratory testing.

Salmonella was isolated using Hektoen enteric selective agar plating and then identified using matrix-assisted laser desorption–ionization time of flight mass spectrometry at the ASPHL. Isolates were subtyped by using the xMAP *Salmonella* serotyping assay (Luminex Corporation, Austin, TX). Isolates serotyped as *Salmonella* Javiana were further characterized by PFGE by using the CDC PulseNet protocol for XbaI restriction digest and electrophoresis. A subset of *Salmonella* Javiana isolates with XbaI PFGE pattern JGGX01.1855 or highly related patterns were forwarded to the Centers for Disease Control and Prevention (CDC) for whole genome sequencing by using high-quality single-nucleotide polymorphism analysis to determine the genetic relatedness of the isolates. Isolates were also tested for antimicrobial susceptibility by using MIC testing. Food and environmental samples were collected and cultured for *Salmonella* Javiana at the ASPHL by using methods specified in the Bacteriological Analytical Manual (U.S. Food and Drug Administration [FDA], Washington, DC) or by methods specified by the CDC. A confirmed case was defined as onset of diarrhea (three or more loose stools in 24 h) during 28 June to 30 September 2016 in a Maricopa County resident or visitor during the 2 weeks before illness and infection with *Salmonella* Javiana outbreak strain (i.e., PFGE pattern JGGX01.1855 or a highly related pattern). A probable case was diarrheal illness in a person with an epidemiologic link (e.g., person lived in the same household or ate at the same restaurant) to a confirmed case.

Case finding and interviews.

All ASPHL isolates were reviewed for outbreak strains. While the outbreak was ongoing, the Arizona Department of Health Services and the ASPHL notified the MCDPH immediately (<1 h after identification) when a new *Salmonella* Javiana case was identified and gave updates as PFGE pattern results were available to determine whether the case definition was met. The *Salmonella* Javiana outbreak strain PFGE gel images were uploaded using BioNumerics (Applied Maths Inc., Austin, TX) to PulseNet, the national molecular subtyping network for foodborne disease surveillance, to identify indistinguishable isolates from other locations. The outbreak strain and highly related strains had not previously been identified in Arizona or elsewhere. Epidemiologists investigating the outbreak used the System for Enteric Disease Response, Investigation, and Coordination, a software platform

used to access PulseNet data, and communicated with the CDC and other states to identify cases in other locations.

The MCDPH used an expanded standard *Salmonella* questionnaire (see supplemental material) to interview patients, including many possible exposures during the week before illness, such as exposures via restaurants, grocery stores, and consumption of seafood or other food items. Binomial probabilities were calculated to compare foods consumed by ill persons with expected Arizona consumption rates in the 2016 Arizona Food, Animal, and Water Exposure Survey, a survey of exposures and foods consumed by healthy persons living in Arizona. The MCDPH also used a supplemental menu-specific questionnaire if the ill person reported eating at restaurant A during the 2 weeks preceding illness. The MCDPH contacted ill persons to gather additional information by using these questionnaires. Detailed information about foods consumed at restaurant A, at other restaurants, at home, and at locations outside of the home was gathered as cases were identified. The MCDPH and Maricopa County Environmental Services (MCES) requested additional information from restaurant A regarding specific ingredients in entrées and other foods consumed.

Environmental sampling and traceback.

MCES staff visited restaurant A to collect samples based on ingredients with a high frequency of consumption from patient reports on 26 August, 1 September, and 7 September; these visits were all after the last outbreak-associated cases ate at the restaurant. Food samples from 26 August included parsley, repackaged halibut, frozen shrimp in the original packaging (unknown size), whipped butter, unsalted butter, and lemon juice. Food samples from 1 September included frozen halibut in the original packaging, various marinated and plain skewered shrimp, shrimp stuffing, bagged shrimp, bacon-wrapped shrimp, and frozen shrimp in the original packaging (shrimp ranged in type and size [large, extralarge, jumbo, extracolossal]). Food samples from 7 September consisted of frozen shrimp in the original packaging (extracolossal lot 36011V). Twenty food items and four environmental surface swabs (three nonfood contact surfaces and one food contact surface) were collected and tested at the ASPHL for enteric pathogens, including *Salmonella*.

Case-control study.

The MCDPH conducted an unmatched case-control study. Case patients were defined as persons with a confirmed infection that met the case definition who had eaten at restaurant A in the week before illness onset during July to August 2016. Control subjects were defined as nonill meal companions (same meal date) of case patients. Case patients and control subjects were interviewed using the same menu-specific questionnaire through a telephone interview or online, and they received a $5.00 gift card for their time for questionnaire completion. Restaurant A is a chain seafood restaurant, and considerations were made during the investigation regarding how different seafood was prepared or cooked. Because fried seafood is coated in a batter before cooking and unfried (but still cooked) seafood is not, we grouped seafood, such as shrimp, by cooking methods as follows: breaded and fried, or unfried but still cooked by being grilled, sautéed, or boiled. During hypothesis-generating interviews preceding the case-control study, case patients frequently reported consuming shrimp. Shrimp consumption was analyzed in three categories: fried, unfried, and both fried

and unfried shrimp (i.e., all cooked types). Incomplete responses were excluded from the analysis for all food-related question types (responses were included even if information was missing for demographic characteristics, treatment, and hospital information). Cross-tabulations based on illness status were created for each food item. Fisher's exact test was used to calculate odds ratios and 95% confidence intervals (CIs). A significant association was one with a CI that did not include 1.0. CDC human subjects' protection officers reviewed this study and deemed the work to be nonresearch. Identifiable data were anonymized using numerical identifications generated from Arizona's Medical Electronic Disease Surveillance Intelligence System, and all results were reported in aggregate.

RESULTS

Case information.

Fifty persons had an illness that met the case definition; 40 cases were confirmed and 10 were probable. Confirmed cases of *Salmonella* Javiana that met the outbreak case definition, by illness onset date, are shown in Figure 1. Illness onset dates were during 28 June to 7 September. The interval from meal-to-illness onset among 22 confirmed cases with known restaurant A meal dates was 0 to 8 days, with a median of 2 days and a mean of 2.7 days. Thirty-three (73%) of 45 interviewed persons reported eating at restaurant A, with exposure meal dates occurring during 16 July to 18 August (shown in Fig. 2, in addition to a timeline of investigation events); in total, 25 illnesses met the confirmed case definition and 8 met the probable case definition. We were unable to obtain an onset date for four of five persons with unknown restaurant A exposure; the laboratory specimen collection date was used as the onset date. Restaurant A exposure meal dates were unavailable for 3 of 25 confirmed cases that had known restaurant A exposure. Twelve persons with illnesses that met the confirmed and probable case definition reported no exposure to restaurant A; they reported exposure to 15 other restaurants or fast food establishments. Only one common fast food establishment was reported by two confirmed case patients who reported consuming a meal at this establishment. Four (33%) of these 12 ill persons reported eating shrimp from other restaurants or stores; 2 of the 12 ill persons reported eating other types of seafood (halibut and Icelandic cod). No other common food exposures were identified among patients who did not eat at restaurant A. The first case with onset on 28 June was a 71-year-old female whose exposures included frozen shrimp, frozen cod, carrots, celery, cauliflower, bell pepper, salads, soup, beef, watermelon, peaches, blueberries, peanut butter, tomatoes, and eggs. She did not report eating at restaurant A or any other restaurants. We obtained specific ingredient information based on food exposure history reported by all patients; many dishes shared similar ingredients. The reported shrimp exposure (63%) among all patients was significantly ($P < 0.001$) higher than the expected shrimp exposure among Arizona residents by using the Arizona Food, Animal, and Water Exposure survey data (24%), but we do not have information on the frequency of shrimp exposures among seafood-restaurant patrons.

Demographic characteristics and outcomes were available for 25 (76%) confirmed case patients with restaurant A exposure; patients' ages were 12 to 71 years, with a median age of 46 years (Table 1). Among these 25 case patients, 15 (60%) were female, 19 (86%) of 22 were white, and 17 (68%) were Maricopa county residents; 10 (43%) of 23 visited an

emergency department for their illness, 7 (29%) of 24 were hospitalized, and no deaths were reported. Not all case patients reported complete demographics, treatment, or hospital information. Five of 25 confirmed cases of *Salmonella* Javiana with restaurant A exposure were residents of other states (Colorado, Indiana, Nevada, Texas, and Wisconsin); out-of-state patients were identified from PFGE isolates uploaded to PulseNet.

Laboratory testing.

Forty clinical isolates were available for PFGE testing; 35 (87.5%) isolates matched the PFGE pattern JGGX01.1855. Five clinical isolates were sent to the CDC for whole genome sequencing. These five clinical isolates represented four *Salmonella* Javiana PFGE patterns —JGGX01.2890, JGGX01.2891, JGGX01.2892, and JGGX01.1855—and were highly related within one to four alleles (median, 2.5) by whole genome sequencing; all four PFGE patterns were included in this outbreak case definition. Two of the five isolates were from persons who had restaurant A exposure (JGGX01.2891 and JGGX01.2892), and three of five isolates were from persons who did not have restaurant A exposure (JGGX01.2890, JGGX01.2892, and JGGX01.1855). The CDC completed antimicrobial susceptibility testing and reported that all five isolates were pan-susceptible on the CDC National Antimicrobial Resistance Monitoring Systems for Enteric Bacteria panel (8, 22).

Environmental assessment of equipment and food handling.

After notification of the initial two unique culture-confirmed *Salmonella* cases reporting eating at restaurant A, the MCDPH contacted the MCES to inquire about recent public complaints of illness mentioning restaurant A. The MCES had received one complaint on 4 August by a person who became ill with a gastrointestinal illness 1 day after eating at restaurant A; this person was later identified as a case patient. The MCES conducted an environmental assessment of restaurant A on 11 August at the request of the MCDPH. The MCES did note that tongs used to handle raw chicken or seafood were in a container with equipment such as scoops and spatulas used to handle ready-to-eat food items. The container water was kept at 170°F (77°C), above the 135°F (57°C) hot-holding minimum temperature requirement in the Arizona Food Code (1); the water level allowed for submersion of the utensil surfaces that touched raw protein items. It was not observed whether a particular utensil used to handle raw proteins was submerged into the container for at least 15 s before handling ready-to-eat proteins. Hence, the person in charge was advised by the MCES to separate equipment used for raw and ready-to-eat food items out of an abundance of caution to prevent cross-contamination if bacteria were still present. At the retail level, the Food Code does not discriminate in cooking temperatures between different types of seafood. Non–ready-to-eat halibut and non–ready-to-eat shrimp all fall under the same category. This also means that during preparation, it would not be considered a violation if an employee works with non–ready-to-eat halibut and then continues with non–ready-to-eat shrimp (or vice versa) without a proper hand wash and continued use of equipment used for the previous protein. No employees had been reported ill since 1 July, and the restaurant requires documentation of medical clearance before returning to work.

Environmental assessment of food products.

Raw seafood and raw chicken were observed being prepared side by side; one employee handled each type of food and each type had its own scale. A separate preparation table for produce and starches (e.g., bread) was used. Cooking and holding temperatures of suspect food items were found to be in compliance; however, Maricopa County code does not require logs of cooking oil temperatures or the final cooking temperature of food products. In addition, the restaurant did not keep calibration records for their thermocouples or thermometers; no cooking logs were observed along the main cook line. During the investigation, it was recommended to start using cooking logs. During hypothesis generation, we calculated the reported exposure to shrimp as significantly higher than the expected shrimp exposure among Arizona residents, which helped inform the MCES of what additional information needed to be gathered during the environmental assessments. Much of restaurant A's fresh seafood arrives daily; no time frame was established for the arrival of frozen seafood. Shrimp was delivered frozen, thawed for different preparations such as skewered, portioned, and then repackaged and refrozen. Different types and sizes of shrimp (e.g., large or jumbo) were imported from several areas such as wild-caught from Texas or Mexico and farm raised from Vietnam, and some shrimp were deveined in-house. During the assessment, the MCES observed the preparation and portioning of all of the suspect seafood, and no violations were noted. The MCES reinforced the recommendation for hand washing by all food handlers and exclusion of ill persons from work. The restaurant performed an overnight deep cleaning of the kitchen and dining areas as a precautionary measure after the environmental assessment on 11 August; specific cleaning practices were not reported. The findings of the environmental assessment helped to inform the case-control study.

Case-control analysis.

In total, 21 laboratory-confirmed cases were included in the case-control analysis (four persons could not be contacted). The online survey for the case-control study was sent to 52 meal companions by e-mail or conducted by telephone; 38 (73%) meal companions completed the survey. Seven (18%) of 38 meal companions reported diarrheal illness within 14 days after their meal; these 7 meal companions were excluded as control subjects and counted as epidemiologic-linked probable cases (3 probable cases had previously been identified resulting in 10 probable cases in total). Of the seven persons reporting diarrheal illness within 14 days after their meal, the interval from meal-to-illness onset was 1 to 5 days, with the median and mean both being 2.5 days. Therefore, 31 well meal companions of ill persons completed the survey after not reporting illness and were included as control subjects.

Select foods eaten by case patients from restaurant A are shown in Table 2. Because the non–restaurant A consumption of shrimp (33%) was not different from the population exposure rate (24%), the percentage of nonill controls that consumed shrimp (20 [64%] of 31 controls consumed shrimp) might reflect the baseline rate of shrimp consumption in seafood restaurants. Among all foods evaluated, unfried (but still cooked such as boiled, sautéed, etc.) shrimp consumption was the only food significantly associated with illness (odds ratio, 6.7; 95% CI, 1.8 to 24.9; $P = 0.004$) (Table 2). All other associations were not

significant. Only 4 (24%) of 17 laboratory-confirmed cases with restaurant A exposure with information available reported eating shrimp cocktails (shrimp were cooked but served cold); among these four cases, two had only consumed shrimp in the form of a shrimp cocktail, and two had consumed both shrimp cocktail and cooked unfried shrimp in other dishes. Of note, two controls (not ill) had shrimp cocktails as their only shrimp exposure.

Environmental sample testing results.

A freezer door handle environmental sample and repackaged halibut sample collected on 26 August and five prepared but uncooked shrimp samples (two skewered, two bagged, and one bacon wrapped) of four different sizes (large, extralarge, jumbo, and extracolossal) collected on 1 September tested positive by PCR for *Salmonella*; isolates were identified as serotype Javiana PFGE pattern JGGX01.1855, matching the outbreak strain. One frozen unopened box of extracolossal shrimp (also known as U10; ≤10 shrimp weigh 1 lb [454 g]) collected on 1 September tested positive for *Salmonella* serotype Weltevreden PFGE pattern JQPX01.0385, which is different from the outbreak strain. All other items sampled tested negative for *Salmonella* by PCR. Environmental samples negative for *Salmonella* by PCR from 26 August included a hanging air curtain in the walk-in refrigerator, walk-in refrigerator handle, and protein preparation table surface.

Recommendations made.

Restaurant A removed all halibut from its menu as a precaution after being notified of the *Salmonella*-positive halibut sample. On 30 August, the MCES recommended that restaurant A perform another deep cleaning procedure, including cleaning freezer door handles. After positive test results from the prepared uncooked shrimp samples for *Salmonella* by PCR, the MCES and MCDPH provided recommendations to restaurant A to document that the internal temperature of the cooked shrimp and halibut reached 165°F (74°C) for 15 s, keep a log book of periodic or hourly quality temperature checks, and reeducate employees on hand hygiene and best practices. Restaurant A discarded all portioned and thawed U10 shrimp and set aside frozen U10 shrimp if any more sampling was required.

Traceback information.

Traceback (to determine where the product had been distributed and originated) was conducted by the MCES, Arizona Department of Health Services, and the FDA on the U10 frozen raw shrimp, after identification of *Salmonella* Weltevreden from product samples; the U10 frozen raw shrimp product originated from a farm in Vietnam and had been distributed to wholesale, retail, and restaurant locations in 10 states (Arizona, Connecticut, Illinois, Kentucky, Massachusetts, New Jersey, New York, Rhode Island, Texas, and Virginia). The FDA has a zero tolerance (nondetectable) for *Salmonella* in seafood, meaning the detection of *Salmonella* by using an FDA-recognized method causes the seafood to be adulterated (29, 30). The supplier recalled this product on 19 September, and PulseNet was queried in September 2016; no additional isolates matching this strain of *Salmonella* Weltevreden were identified.

The MCES performed a routine, unannounced inspection of restaurant A on 14 September. No violations were cited other than shellfish tags that were not retained for the proper length

of time. With no further reports of illness or complaints associated with restaurant A, this investigation was closed on 3 October 2016.

DISCUSSION

Although the cause of this *Salmonella* Javiana outbreak is unknown, cross-contamination of *Salmonella* likely occurred in restaurant A, as evidenced by the *Salmonella* outbreak strain identified on multiple food types from different dates and environmental surfaces. Additional evidence that supports this conclusion is the positive freezer door handle matching the outbreak strain and the possibility that other food contact surfaces not swabbed and tested were contaminated, confirmed illnesses in persons who did not eat shrimp, and potentially the length of time between meal exposure dates of confirmed cases (4 weeks). It is possible that the positive food and environmental samples might have been contaminated after the illnesses occurred due to a different source. Unfried but still cooked shrimp was implicated as the most likely outbreak vehicle among restaurant A diners based on results of environmental sampling and case-control study. Because our case-control study focused only on restaurant A, these findings cannot be extrapolated beyond this location; in total, 15 other restaurants or fast food establishments were reported by 12 cases who did not have exposure to restaurant A; one common fast food establishment had more than one person reporting eating there within the week before illness onset with only two cases. Therefore, because the majority of cases ate at restaurant A, and due to limited resources and staffing to investigate the other restaurants, the investigators focused on the convenient sample of cases who ate at restaurant A. Restaurant A was likely involved in the transmission of the outbreak strain, because two-thirds of ill patients reported eating at restaurant A; however, other sources might be possible. The first case with onset on 28 June was possibly connected to the rest of the outbreak if the source of the outbreak was a contaminated food product, such as frozen shrimp, due to her known shrimp exposure; however, she did not report eating at restaurant A or any other restaurants.

Whole genome sequencing demonstrated that all isolates were genetically related, indicating that these illnesses, including the 12 illnesses not associated with restaurant A, were linked to a common source. There were several different but highly related PFGE patterns identified as the same outbreak strain among both ill restaurant A diners and ill persons who did not dine at restaurant A (all four PFGE patterns were from persons who had restaurant A exposure [JGGX01.2890, JGGX01.2891, JGGX01.2892, and JGGX01.1855], and three of four PFGE patterns were from persons who did not have restaurant A exposure [JGGX01.2890, JGGX01.2892, and JGGX01.1855]). These minor variations could be the result of natural variation among *Salmonella* organisms. Another possible explanation is that contamination with *Salmonella* occurred at the supplier, distributor, or other processing level, allowing sufficient time for slight genetic changes to occur before the contaminated food arrived at restaurants or grocery stores. Although laboratory testing demonstrated contamination of raw shrimp processed in restaurant A with the outbreak strain of *Salmonella*, no laboratory evidence indicated that shrimp was contaminated with *Salmonella* Javiana before processing at the restaurant. However, a negative result from testing items does not prove that intact shrimp were free of contamination. The absence of laboratory confirmation of bacterial contamination of a food implicated epidemiologically does not rule

out a role in transmission for the food. This situation is frequently encountered when the contaminated food is no longer available, and another batch or portion is tested. We do not know whether this was the case in this outbreak. The time that the cases ingested the contaminated food, resulting in subsequent illnesses, might have resulted from initial contamination of a food product (either from a source external or internal to the restaurant such as sick workers, contaminated food, or other means), with amplification of transmission in restaurant A through cross-contamination. *Salmonella* cross-contamination and recontamination episodes have been connected to poor sanitation practices, poor equipment design, and deficient control of ingredients (3); however, none of these problems were reported at restaurant A. Contamination might have occurred before the products arrived at the restaurant, at processing facilities where peeling, deveining, or removal of the head was performed. Conversely, contamination might have occurred at restaurant A during deveining and other processing. The deep cleaning by restaurant A on 11 August did not seem to have an impact on transmission; this could be attributed to the theory that food samples that had been previously prepped were later cooked and served and might have still resulted in transmission to restaurant patrons and in subsequent illnesses identified after 11 August. This aligns with our laboratory test results that multiple types of prepared but uncooked shrimp (two skewered, two bagged, and one bacon wrapped) tested positive for the outbreak strain; it is possible that illnesses continued because those food samples had been handled and prepared before the deep cleaning, and therefore were contaminated, and then refrozen for several days before being cooked and eaten (shrimp was delivered frozen, thawed for different preparations such as skewered, portioned, and then repackaged and refrozen). Therefore, the deep cleaning would not have prevented those additional cases; it is possible that only removal of those prepped foods would have prevented those additional cases.

The findings of restaurant A environmental assessments demonstrated that outbreaks can be propagated in a restaurant even when no violations are noted. The MCES reported that seafood items were cooked according to the Arizona Food Code that requires cooking to a temperature of 145°F (63°C) (1); however, we do not know whether the conditions observed in the restaurant were the same as those at the time of the outbreak, or whether the environmental assessment can capture all risk factors. We had several hypotheses to explain the absence of observed violations in the face of an outbreak. Unfried seafood might have been the vehicle of *Salmonella* infection, because the cooking temperature required by the Arizona Food Code might not be adequate to eliminate contamination of *Salmonella* on contaminated processed raw shrimp. Results from studies using volunteers to determine infectious dose showed high infective doses $>10^5$ organisms (17). However, investigations of outbreaks suggest that the infective dose was often low, $<10^3$ organisms (2). Specifically, an investigation of 1,000 cases of salmonellosis from a 1993 outbreak in Germany traced to paprika and paprika-powdered potato chips found (i) levels of 0.04 to 0.45 organisms per g of food, (ii) an estimated infectious dose of 4 to 45 organisms, and (iii) children younger than 14 years of age were primarily affected (of note, three serovars were implicated in this outbreak; however, the counts for *Salmonella* Javiana alone were not specified) (18). An analysis of *Salmonella* in outbreak-associated breaded and frozen comminuted raw chicken products identified that low levels of *Salmonella* may be associated with foodborne

outbreaks (one most-probable-number distribution of *Salmonella* per 0.33 to 556 g of product was revealed) (4). In addition, the FDA's Bad Bug Book (29) for nontyphoidal salmonellosis states the infective dose is as low as one cell, depending on age and health of host and strain differences among members of the genus (9).

The safety of a cooked food product depends on many variables, including cooking method, time and temperature combinations used, and initial level of contamination. The FDA Food Code recommends uncomminuted (e.g., not ground) fish is cooked to reach a minimum internal temperature of 145°F (63°C) for 15 s (27). When seafood is cooked and is not for immediate consumption, the FDA recommends seafood processors achieve a minimum 6-log reduction of *Listeria* monocytogenes (28). A 6-log reduction of *L. monocytogenes* is a higher thermal destruction recommendation than the Food Code because *L. monocytogenes* is considered the most heat-tolerant pathogen of concern in seafood and the recommendation is based on the "worst-case scenario" for potential contamination. For example, table A-3 of appendix 4 of the FDA Food Code states an internal product temperature of 145°F (63°C) should be held for at least 17 min, or at 165°F (74°C) the internal temperature should be held for at least 36 s. These recommendations are also based on an expectation of low level of contamination. If higher levels of initial contamination are anticipated, higher degrees of destruction might be needed; the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) notes that the cooking recommendation of 145°F (63°C) for 15 s is appropriate for consumers when a low level of bacterial contamination is reported, the seafood is properly handled before cooking, the cooked seafood will be consumed shortly after preparation (a specific time range is not specified), and leftovers will be handled properly and consumed within a short period (19). The NACMCF also indicates that the physical state of a product and thawing conditions of frozen seafood before cooking can substantially influence the degree of microbial inactivation achievable by heat, attributable to factors such as heat transfer characteristics or microbial thermal resistance. The MCES reported that restaurant A's practices were consistent with these recommendations, at least at the time of the environmental assessments. Frozen versus fresh, different preparations of shrimp (e.g., head on versus head off, stuffed versus unstuffed, and with sauce versus no sauce), season of harvest, and microbial load might affect efficacy of a particular cooking method (19). Although shrimp can be cooked at higher temperatures, these factors, in addition to changes in texture and taste of shrimp and other seafood cooked at higher temperatures, might affect the implementation of these practices on a larger scale.

Some data are available regarding thermal inactivation for seafood-associated pathogens, but results differ in part because of the variety of seafood products available and the range of cooking methods used, making generalization of a single recommended temperature for the broad category of seafood difficult (19). The FDA's Food Code (27) provides retailers with cooking guidance for uncomminuted fish based on *Salmonella* as the target organism for inactivation and an expectation of a low level of internal contamination. The decrease in illness that occurred after restaurant A was required to increase the cooking temperature of shrimp to 165°F (74°C) could indicate that previous cooking temperatures reached were insufficient to eliminate *Salmonella*. However, whether the reduction in the number of illnesses was attributable to the temporary increase in cooking temperature of the shrimp from 145°F (63°C) to 165°F (74°C) during the investigation period (and later reverted to

meet the minimum cooking temperature of 145°F [63°C]), attributable to the other control measures implemented around the same time (e.g., deep cleaning, education, and evaluation of food safety practices among employees, and temporarily removing halibut from the menu), or because the potentially contaminated lot(s) of product had already been used or served is unclear. Considering that cases unassociated with restaurant A ceased at the same time, the outbreak source could have been a contaminated product lot that had been distributed to other restaurants or grocery stores but was only available for a limited duration.

No human *Salmonella* Weltevreden isolates were identified during the time of the outbreak in Arizona. *Salmonella* Weltevreden, which was isolated from the recalled imported shrimp, is not a common cause of human illnesses; however, according to PulseNet, *Salmonella* Weltevreden has been isolated approximately 1,500 times from frozen shrimp, octopus, frozen lobster tails, chili powder, cilantro, and many more foods (23). Food commodities in the aquatic animal group were the most common sources for serotype Weltevreden (16).

This investigation has several limitations, many of which can serve as lessons learned for future investigations and of which could be avoided depending on the circumstances (resources, staffing, time, communication, or other unexpected barriers allow). Because of the number of menu items served at restaurant A, not all foods were tested. Although the exact product turnover rate is unknown, fresh fish arrived daily and frozen shrimp were reportedly cooked and served within a few days; the original source shipment might have been served to restaurant patrons before sampling and testing, or contamination within a batch might have been sporadic and not all cooked meals served would have had *Salmonella* present. In addition, a larger sample size would have been required to capture a significant association between rare events such as exposure to halibut or oysters. Ingredient lists for all entrees from the large menu, complete information for all ingredients possibly consumed by ill persons, and additional food samples for testing were not collected. We investigated reported cases and did not use bills or credit card information to contact patrons and identify additional cases. Another limitation is that we were unable to interview restaurant A workers directly to verify whether any of them were ill. During the initial visit by the MCES on 11 August, the environmental assessment was performed by the MCES and because there was not an official investigation initiated until the additional cases were identified linked to the restaurant on 15 August, public health had not accompanied the MCES. Public health was informed by the MCES that no food workers were ill based on face-to-face questioning; therefore, time and effort by public health were focused on interviewing cases, gathering ingredient lists, analyzing the data, and coordinating the environmental and food sampling and laboratory testing. No formal interviews were performed by public health to identify ill food workers because food workers were questioned by the MCES and they did review the sick log. On 26 August, the restaurant, which was part of a chain, sent its food safety compliance manager on site to perform an investigation that included interviewing employees; the manager reported that there had been no ill employees since 1 July. In addition, it is not standard public health practice to collect stool specimens on nonill people. It is possible that illness by a food worker was not reported and not severe enough to require sick time off, but this cannot be confirmed. Few restaurants provide paid time off for illness, and worker(s) might go to work without reporting illness because they cannot afford to miss

work and the reduction in pay (11, 25). The medical clearance requirement might be another impediment to reporting illness because of the cost and time involved. After this investigation, it was recommended that if an outbreak is suspected, public health and the MCES should go to the facility on site together, to help better coordinate during the investigation, especially regarding interviewing potentially ill or previously ill food workers. In addition, there is potential recall bias in respondents' responses. Depending on the number of food items for which statistical testing for association with illness was performed, association by chance alone might have been observed. Last, we did not have information about whether other restaurants at which patients ate used the same shrimp or other food suppliers; due to resource and staffing limitations, the investigation and the case-control study were limited to persons who ate at restaurant A. Food and environmental testing were also only performed at restaurant A and not at other restaurants reported by case patients, which limits broader implications to the actual outbreak vehicle.

In conclusion, this outbreak of *Salmonella* Javiana might have been caused by *Salmonella* contamination of prepared, uncooked shrimp and *Salmonella* surviving the minimum cooking temperatures required for seafood in the Arizona Food Code, and by FDA Food Code, although it is not known whether the conditions observed in the restaurant were the same as those at the time of the outbreak. Because many factors affect the thermal inactivation for seafood-associated pathogens, restaurant workers, food preparers, and public health should be aware that complying with the Food Code of cooking to 145°F (63°C) for 15 s might be insufficient to eliminate *Salmonella* in seafood. There are currently no known plans to increase the minimum temperature requirement for seafood cooked by retailers as of the publication of this article. However, there is a need for studies considering the usefulness of increasing the minimum temperature requirement for seafood cooked by retailers and to which temperature the minimum requirement would be raised. The NACMCF more specifically suggested that "given the number of outbreaks and cases caused by *Salmonella*, there is a need to determine the thermal inactivation kinetics of this organism in seafood in order to determine whether the 2005 FDA Food Code times and temperatures are adequate" (19). Because food handlers have many opportunities to contaminate and cross-contaminate food during preparation, providing adequate food safety training before food handling occurs to ensure no one is sick before food handling, supervising food handlers who prepare or serve food, and providing adequate sick leave to employees are all important considerations (11–13, 25). Findings from the case-control study, environmental investigation, and laboratory testing during this outbreak were shared with restaurant A to guide future prevention efforts and enhance food handler training; although the information shared was not new to the public health literature, the lessons learned from the investigation and challenges in obtaining information could help inform better preparation for future similar complicated investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors thank the following persons for assistance during the outbreak or for writing the manuscript: Kristine Bisgard, Tammy Sylvester, Jigna Narang, Ronald Klein, Roumen Penev, Sherry Gower, Rob Tauxe, Heather Miller, Katherine Londen, Taylor Griswold, McKenzie Ricca, Jennifer Cripe, and the Student Aid for Field Epidemiology Response Team at the University of Arizona. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of Centers for Disease Control and Prevention.

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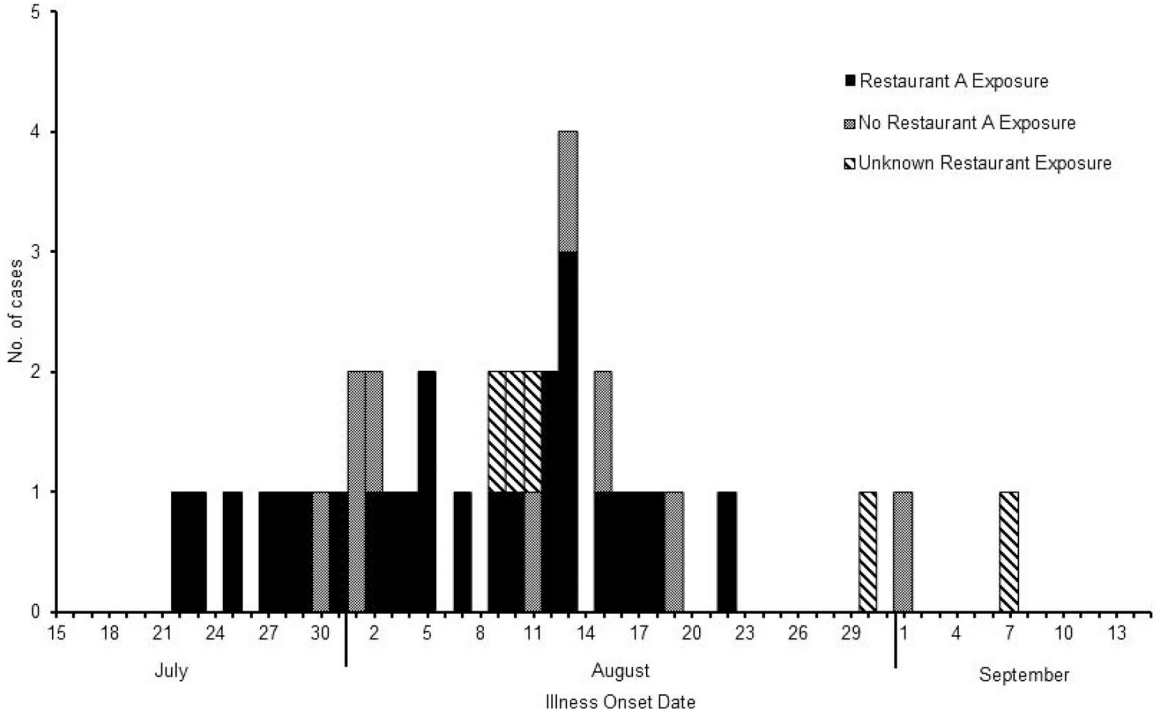

FIGURE 1.

Confirmed cases of Salmonella Javiana that met the outbreak case definition, by illness onset date in Arizona, 2016 (n = 39), by restaurant A exposure (black bars), no restaurant A exposure (gray bars), and unknown restaurant exposure (cross-hatch bars). One person who had no restaurant A exposure reported illness onset 28 June (not shown here). Four of five persons with unknown restaurant A exposure were lost to follow-up and the date shown is based on the laboratory specimen collection date.

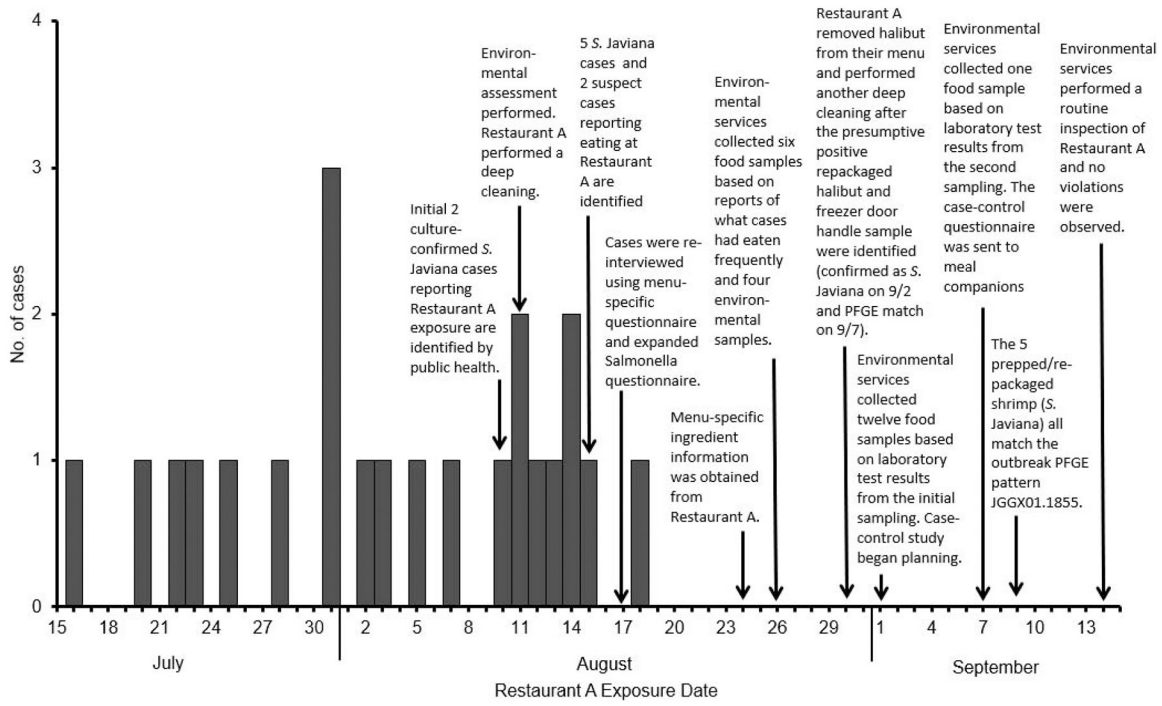

FIGURE 2.

Confirmed cases of Salmonella Javiana that met the outbreak case definition, by known restaurant A exposure date in Arizona, 2016 (n= 22), and by timeline of investigational events.

TABLE 1.

Characteristics and illness severity among patients with confirmed Salmonella Javiana cases with known information available, Arizona, 2016[a]

Demographics	All confirmed cases, no. (%) (n = 40)	Restaurant A exposure, no. (%) (n = 25)	No restaurant A exposure, no. (%) (n = 10)
Median age in yr (range)	42 (2–75)	46 (12–71)	41 (2–75)
Females	24 (60)	15 (60)	5 (50)
Race, ethnicity			
White, non-Hispanic	25 (76)	19 (86)	6 (75)
Black, non-Hispanic	4(12)	1 (5)	2 (25)
Asian	1 (3)	1 (5)	0 (0)
American Indian or Native Alaskan	1 (3)	1 (5)	0 (0)
White, Hispanic	1 (3)	0 (0)	0 (0)
State or state and county of residence			
Arizona, Maricopa County	32 (80)	17 (68)	10 (100)
Arizona, Yavapai County	2 (5)	2(8)	0(0)
Arizona, Pinal County	1 (3)	1 (4)	0 (0)
Other states[b]	5 (13)	5 (20)	0 (0)
Hospitalized	10 (29)	7 (29)	2 (20)
Emergency department visit	15 (44)	10 (43)	5 (50)

[a]Only *Salmonella* Javiana confirmed cases with isolates with outbreak-related PFGE patterns with known information are included.

[b]Other states include Colorado, Indiana, Nevada, Texas, and Wisconsin.

TABLE 2.

Case-control results of select foods eaten by case patients, restaurant A, in Arizona, 2016

Food item	Cases, no. (%) (n = 21)[a]	Control subjects, no. (%) (n = 31)[b]	Odds ratio[c]	95% CI	P value
Unfried shrimp	17 (81)	12 (39)	6.7	1.8–24.9	0.004
Oysters	4(19)	0 (0)	8.4	0.9–77.6	0.07
Halibut	3 (14)	0 (0)	6.7	0.7–64.8	0.15
Grilled octopus	2(10)	0 (0)	5.1	0.5–52.1	0.29
Grilled salmon	3 (14)	1 (3)	5.1	0.5–52.1	0.29
Crabmeat	11 (52)	9 (29)	2.7	0.8–8.5	0.15
Fried shrimp	1 (5)	8 (26)	0.1	0.1–1.3	0.07

[a]Confirmed or probable case of illness after *Salmonella enterica* serotype Javiana infection.

[b]Well meal companion.

[c]Using Fisher's exact test.