

An Outbreak of Foodborne Illness Among Attendees of a Wedding Reception in Wisconsin Likely Caused by *Arcobacter butzleri*

Victoria Lappi,¹ John R. Archer,² Elizabeth Cebelinski,¹ Fe Leano,¹ John M. Besser,¹
Rachel F. Klos,² Carlota Medus,³ Kirk E. Smith,³
Collette Fitzgerald,⁴ and Jeffrey P. Davis²

Abstract

Background: *Arcobacter* species, primarily *Arcobacter butzleri*, are widely distributed among animals, infrequently isolated from humans, and previously not associated with outbreaks of foodborne illness. We report results of an investigation of a foodborne outbreak that occurred among attendees of a wedding reception in Wisconsin, United States, and was likely caused by *A. butzleri*.

Methods: We conducted a case-control study among reception attendees and a laboratory investigation to determine the extent, source, and cause of the outbreak. A clinical case was defined as diarrhea in an attendee with illness onset ≤ 7 days following the wedding reception.

Results: The case-control study included 47 of 51 case patients and 43 non-ill attendees. Results demonstrated that consuming broasted chicken was the only factor significantly associated with illness (odds ratio 10.51; 95% confidence interval 1.28, 476.4). Five patients provided stool specimens. Comprehensive culture and polymerase chain reaction (PCR) testing did not detect common bacterial or viral pathogens. Subsequent testing with PCRs targeting 16S/23S rDNA of the three most clinically relevant *Arcobacter* spp. and the *rpoB/C* gene of *A. butzleri* provided products confirmed as *A. butzleri* (four patients) and *A. cryaerophilus* (one patient) by sequence analysis.

Conclusions: The results of this investigation suggest that *A. butzleri* should be considered an agent that can cause outbreaks of foodborne illness. Rigorous investigation of outbreaks of undetermined etiology is valuable for incrementally increasing our understanding of emerging agents causing foodborne illnesses.

Introduction

THE GENUS *ARCOBACTER*, formerly known as aerotolerant *Campylobacter*, includes 12 validly named species, and several new potential species that are found among a wide diversity of habitats and hosts, and are considered emerging enteric pathogens and potential zoonotic agents (Collado and Figueras, 2011; Ho *et al.*, 2006; Snelling *et al.*, 2006; Wesley and Miller, 2010). *Arcobacter butzleri*, *A. cryaerophilus*, *A. skirrowii*, and *A. cibarius* are associated with human or nonhuman disease (Jiang *et al.*, 2010).

A. butzleri is most frequently associated with human infections (Kiehlbauch *et al.*, 1991; Vandenberg *et al.*, 2004) and has been isolated from numerous sources including aborted

bovine and porcine fetuses (Ellis *et al.*, 1977; Ellis *et al.*, 1978), healthy dairy cattle (Wesley *et al.*, 2000), retail poultry, beef, lamb, and pork (Atabay and Corry, 1997; Atabay *et al.*, 1998; Rivas *et al.*, 2004), shellfish (Collado *et al.*, 2009), raw milk (Scullion *et al.*, 2006), primates (Anderson *et al.*, 1993), lettuce (Gonzalez and Ferrús, 2011), surface water (Collado and Figueras, 2011), ground water (Rice *et al.*, 1999), and drinking water treatment plants and a reservoir (Jacob *et al.*, 1993; Jacob *et al.*, 1998). Investigation of an outbreak of gastrointestinal illness among visitors to an island in Lake Erie revealed that *A. butzleri* was isolated from 7 of 16 groundwater samples, but was not isolated from ill patients; however, neither *Arcobacter*-specific culture methods nor polymerase chain reaction (PCR) were used to examine stool specimens (Fong *et al.*, 2007).

¹Public Health Laboratory, Minnesota Department of Health, St. Paul, Minnesota.

²Bureau of Communicable Diseases and Emergency Response, Wisconsin Division of Public Health, Madison, Wisconsin.

³Acute Disease Investigation and Control Section, Minnesota Department of Health, St. Paul, Minnesota.

⁴Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, Georgia.

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Recently, *A. butzleri* was established as an etiology of travelers' diarrhea in 8% of 201 U.S. and European travelers with acute diarrhea acquired in India, Mexico, or Guatemala (Jiang *et al.*, 2010).

While consumption of *Arcobacter*-contaminated food is considered a mode of transmission (Collado and Figueras, 2011), foodborne outbreaks of *A. butzleri* infections have not been reported. We describe the investigation of an outbreak of foodborne illness associated with consumption of broasted chicken served during a wedding reception in Wisconsin, United States. Intensive laboratory testing indicated that *A. butzleri* likely was the etiologic agent that caused this outbreak.

Materials and Methods

Background

On October 21, 2008 (all dates in 2008), a Wisconsin local health department (LHD) was informed of multiple cases of diarrhea among 280 attendees of a wedding reception held on October 17 at a rural banquet and catering facility. The LHD staff immediately contacted Wisconsin Division of Public Health staff and an investigation was initiated. The wedding began at 4:00 p.m. on October 17; dinner was served at 5:30 p.m. There was no rehearsal dinner and no other wedding-related events involving >20 people. The LHD staff obtained a guest list from the bride's family and a menu of reception food and drink items from the caterer.

Definitions

A clinical case was defined as diarrhea occurring in an individual who attended the wedding reception and dinner (attendee) on October 17, with illness onset occurring within 7 days of the reception. Criteria for a laboratory-confirmed case could not be applied during the investigation because of the long interval needed to establish an etiologic agent.

Case-control study

The Wisconsin Division of Public Health staff developed a questionnaire to obtain data regarding demographic and clinical features, and consumption of 16 food and 4 beverage items. The questionnaire was mailed with a self-addressed, prepaid return envelope to all wedding attendees. Ill and well attendees were identified based on data from returned questionnaires. A case-control study was conducted to identify food or beverage items and other factors associated with illness. Case patients included attendees with illnesses meeting the clinical case definition. Controls included attendees who did not develop symptoms. Completed questionnaires were returned during October 23–November 14.

Statistical methods

Statistical analyses were conducted using Epi Info software version 3.4.3 (CDC, Atlanta, GA; <http://wwwn.cdc.gov/epiinfo/>) to calculate odds ratios and Taylor 95% confidence intervals and conduct a multivariate logistic regression analysis.

Environmental investigation

On October 21, an inspection was conducted of the banquet and catering facility. The food manager was interviewed regarding employee health and food-handling practices.

Laboratory investigation

During October 22–24, LHD staff arranged for collection of stool specimens in modified Cary-Blair transport media (Meridian Bioscience, Cincinnati, OH) from five symptomatic attendees and sent the specimens to the Wisconsin State Laboratory of Hygiene (WSLH) for standard enteric bacterial culture (*Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli* O157:H7) and real time-PCR testing for norovirus. Specimens were archived at 4°C.

Because expanded testing was required for identification of the etiologic agent, the five patient samples were sent overnight on cold packs on November 3, from the WSLH to the Minnesota Department of Health, Public Health Laboratory (MDH-PHL). The only remaining food available for testing was one piece of chicken retrieved from an attendee's refrigerator by LHD staff and stored frozen in the LHD laboratory. The chicken was shipped overnight on cold packs from the LHD to MDH-PHL on December 16. Patient specimens were tested using PCR for adenovirus, astrovirus, norovirus, rotavirus, sapovirus, *Yersinia pseudotuberculosis*, and diarrhea-genic *E. coli* virulence factors. Standard bacteriological methods were used for isolation of *Vibrio* and *Yersinia* species, *Aeromonas* and *Plesiomonas*. Patient specimens and the food sample were further tested by culture for *Campylobacter* and *Arcobacter* species by MDH-PHL in collaboration with the *Campylobacter* and *Helicobacter* Reference Laboratory, Centers for Disease Control and Prevention, which included use of *Arcobacter*-specific culture media (Atabay *et al.*, 1998; Atabay and Corry, 1998; Johnson and Murano, 1999; Fitzgerald and Nachamkin, 2007). Stool specimens were extracted for DNA using modifications of a published method (Zhu *et al.*, 1998). These modifications included adding preprocessing wash steps and further purifying the ethanol-precipitated DNA using a QIAquick PCR Purification kit (Qiagen, Valencia, CA). The PCR analysis targeted the 16S rDNA gene of *A. butzleri* and *A. skirrowii* and 23S rDNA gene of *A. cryaerophilus* in a multiplex PCR (Houf *et al.*, 2000) and the *rpoB/C* gene of *A. butzleri* (Brightwell *et al.*, 2007).

To verify the multiplex results and increase detection sensitivity, the *A. butzleri* specific 16S rDNA primers were also used as a singleplex. Because of weak product amplification, secondary amplification was used to generate sufficient PCR product to confirm band identity by DNA sequencing. Sequencing was completed on a Beckman Coulter CEQ™ 8800 using the methods of Beckman Coulter Genome Lab™. All obtained sequences were screened against the GenBank sequence database using Basic Local Alignment Search Tool analysis.

Results

Case finding and description

Among the 280 attendees, 109 (39%) returned questionnaires. Among respondents, 51 (47%) had illnesses meeting the clinical case definition, 43 reported no symptoms, and 15 had illnesses not meeting the clinical case definition. Signs and symptoms of illness reported by ≥50% of the 51 case patients included diarrhea, abdominal cramps, fatigue, nausea, chills, body/muscle aches, and headache (Table 1). Among case patients, 67% were female and the median age was 44 (range 19–90) years. The mean incubation period was 31.6 hours

TABLE 1. COMPARISON OF REPORTED SIGNS AND SYMPTOMS AMONG ATTENDEES WITH DIARRHEAL ILLNESSES MEETING THE CLINICAL CASE DEFINITION (CLINICAL CASES) TO REPORTED SIGNS AND SYMPTOMS AMONG ATTENDEES WITH NONDIARRHEAL ILLNESSES NOT MEETING THE CLINICAL CASE DEFINITION

Sign or symptom	Clinical cases (N = 51)		Nondiarrheal illnesses not meeting case definition (N = 12)	
	Number	%	Number	%
Diarrhea	51	100	0	0
Abdominal cramps	42	82	9	75
Fatigue	38	75	9	75
Nausea	35	69	6	50
Chills	34	67	7	58
Body aches	32	63	7	58
Headache	29	57	7	58
Muscle aches	24	47	4	33
Sweats	20	39	1	8
Vomiting	14	27	0	0
Fever	6	12	2	17
Bloody diarrhea	3	6	0	0

(range 5.5–82.5 hours, five had incubation periods <12 hours) among 46 case patients with specific known onset dates and times (Fig. 1); mean illness duration was 3.0 days (range 1–6 days). No case patients sought medical attention or were hospitalized. Illnesses experienced by 12 attendees who reported nondiarrheal illnesses and illness onset dates and times were also characterized by abdominal cramps, fatigue, nausea, chills, body aches, and headache. Each symptom was reported by $\geq 50\%$ in this illness category (Table 1). Their illness incubation periods were similar (mean 32.6 hours) to those of case patients (Fig. 1), but illness durations (mean 2.2 days) were shorter. One attendee with fever, abdominal cramps, body aches, and fatigue received outpatient care. Among control individuals, 57% were female and the median age was 51 (range 20–79) years.

Case-control study

Forty-seven case patients and 43 non-ill (control) individuals provided completed food and beverage data and were included in the analysis. Of 16 foods and 4 beverages served during the reception and dinner, the only item significantly associated with illness was broasted chicken; 46 (98%) of 47 case patients reported eating chicken, compared to 35 (81%) of 43 control individuals (odds ratio [OR] 10.51; 95% confidence interval [CI] 1.28–476.4; Fisher's Exact $p=0.010$). The only other item with $p<0.15$ was dressing; 42 (89%) of 47 case patients reported eating dressing, compared to 33 (77%) of 43 controls (OR 2.55; 95% CI 0.79, 8.17; Mantel Haentzel $p=0.11$). In a logistic regression analysis that included only these two variables, broasted chicken and dressing consumption, broasted chicken was the only food independently associated with illness (adjusted OR 9.57; 95% CI 1.13, 80.96; $p=0.038$). Eleven attendees with nondiarrheal illness provided food and beverage data; all 11 reported eating chicken and 10 (91%) reported eating dressing. Their inclusion as case patients would not have affected these results.

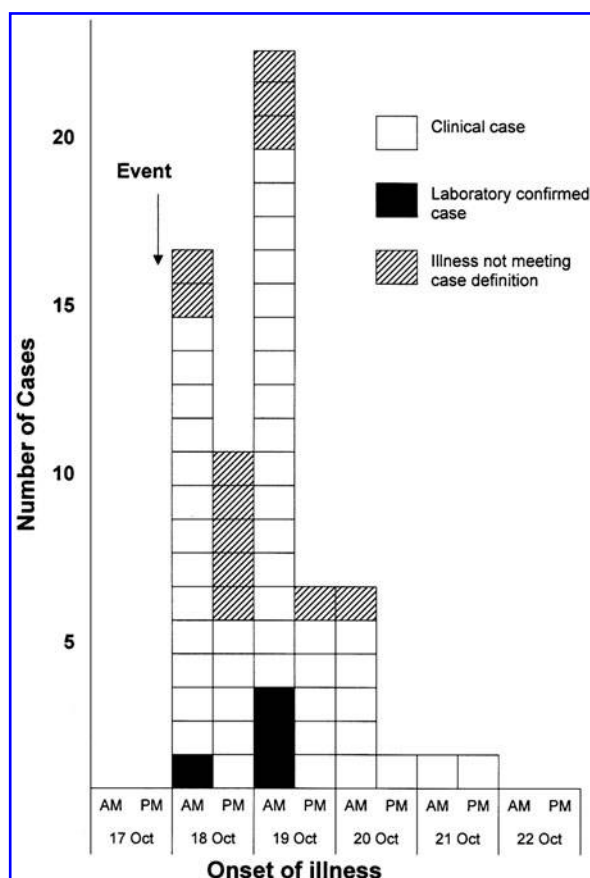


FIG. 1. The number of cases of reported diarrhea ($n=51$) and nondiarrheal ($n=12$) illnesses among attendees of a wedding reception and dinner by 12-hour interval of onset, Wisconsin, October 17–21, 2008. Among five attendees with reported diarrhea who submitted stool specimens for testing, four had test results positive for *Arcobacter butzleri*, including one attendee who also had test results positive for *Arcobacter cryaerophilus*.

Environmental investigation

Inspection of the banquet and catering facility did not identify any major violations or ill food workers; employees were observed wearing gloves while preparing food. No employees were ill or had recent gastrointestinal illness. The chicken was pre-cut, packed in 40-pound cases, and procured from a single source. The chicken was prepared on the day of the wedding by one individual and was marinated using a commercial marinade in multiple large, covered plastic containers held in a closely monitored walk-in cooler at 36–37° F. After marinating, the chicken was broasted. Broasting is a trademark applied to a method of cooking chicken and other foods using a pressure fryer and condiments (<http://en.wikipedia.org/wiki/Broasting>). The only deficiency noted during food preparation was that cooking was based on length of time; internal temperatures were not measured after cooking the chicken. One attendee reported that the chicken was lukewarm.

Laboratory investigation

Cultures of all five stool specimens and the chicken were negative for *Salmonella*, *Shigella*, *E. coli* O157:H7, *Campylobacter*, and *Arcobacter* spp. All five stool specimens were PCR-negative

for adenovirus, astrovirus, norovirus, rotavirus, sapovirus, and *Yersinia pseudotuberculosis*, and culture-negative for *Vibrio*, *Yersinia*, *Aeromonas*, and *Plesiomonas* spp. Except for a single specimen positive for the diffusely adherent *E. coli* *afaC* gene marker, all *E. coli* virulence factor PCR tests of stool specimens were negative; the chicken was not tested.

Of the five stool specimens, three had positive *Arcobacter* multiplex PCR results for *A. butzleri* and four had positive singleplex results because of improved detection sensitivity. The 16S rDNA PCR product was confirmed as *A. butzleri* using sequence analysis. One specimen further yielded a 23S rDNA PCR product sequence-confirmed as *A. cryaerophilus*. The additional *rpoB/C* PCR assay generated PCR product from the five specimens only when secondary amplification was performed. The sensitivity of this assay for human stool specimens was not verified; variable amplification could be associated with being at or near the assay's limit of detection. Sequence analysis of the *rpoB/C* gene PCR products supported the identity of *A. butzleri*. *Arcobacter* PCR testing of the chicken sample was negative.

Use of PCR methods without culture confirmation raised concern regarding potential for false-positive results. To address this concern, laboratory contamination was investigated for two potential scenarios: laboratory contamination with *Arcobacter* amplicons from previous testing, and cross-contamination during testing of the outbreak samples. The absence of a positive signal in the no-template (water) controls run throughout the testing period and in 20 additional specimens (non-outbreak-related) tested after the investigation was evidence that the *A. butzleri* outbreak results were not related to laboratory contamination. Cross-contamination during testing was unlikely because clinical isolates and positive controls were not extracted concurrently with outbreak specimens. Also, the secondary PCR amplification step used for sequencing yielded no additional positive specimens. Notably, the MDH-PHL molecular testing workflow compartmentalizes the PCR assay steps (set-up, extraction, amplification) into three different rooms with dedicated equipment, further reducing risks of cross-contamination.

Discussion

Our finding that *A. butzleri* was the likely etiology of this foodborne outbreak is supported by multiple observations. First, four of five outbreak-related patient stool specimens were positive for *A. butzleri* using a specific *Arcobacter* PCR method. The presence of *A. butzleri* was confirmed using amplification of the second gene target, *rpoB/C*. Additionally, the signs and symptoms of illness and the epidemiologic profile were consistent with other published reports regarding *A. butzleri* infection (Vandamme *et al.*, 1992; Lerner *et al.*, 1994). Despite rigorous testing, no other recognized pathogen was identified in the case patients' specimens. We documented a strong epidemiologic association of illness with consumption of broasted chicken. This finding is highly plausible, as numerous studies have identified *A. butzleri* in retail chicken specimens, and *Arcobacter* prevalence on chicken is greater than among other meats and foods (Rivas *et al.*, 2004; Kabeya *et al.*, 2004; Collado *et al.*, 2009).

During this investigation, one patient specimen positive for *A. butzleri* also tested positive for *Arcobacter cryaerophilus* and one patient specimen negative for *A. butzleri* tested positive

for diffusely adherent *Escherichia coli* (DAEC). *Arcobacter cryaerophilus* has been detected in individuals with diarrhea and in asymptomatic individuals (Vandenberg *et al.*, 2004; Enberg *et al.*, 2000). It is the second most prevalent *Arcobacter* spp. isolated from chicken. During a study of 42 retail samples of chicken, *Arcobacter* spp. were detected in 86% of samples: *A. butzleri* only in 50% and both *A. butzleri* and *A. cryaerophilus* in 36% (Pentimalli *et al.*, 2009). Thus, while isolation of *A. cryaerophilus* may be an incidental finding, it is plausible that the patient acquired *A. cryaerophilus* from chicken.

Investigators have identified DAEC with similar frequency in specimens from patients with diarrhea and from asymptomatic control individuals. Intestinal carriage of DAEC strains has been reported to be widespread among older children and adults, but the consequences of this carriage are unknown (Le Bouguenec *et al.*, 2006). Genetic characterization of multiple gene markers and phenotypic examination of DAEC isolates might differentiate illness-causing strains from strains detected in healthy individuals. Because no other outbreak-related stool was DAEC-positive, we believe this organism is unlikely to be the cause of this outbreak.

The final outbreak case classification included 51 cases: 4 clinically defined and laboratory-confirmed cases of *A. butzleri* infection and 47 clinically defined cases epidemiologically linked to laboratory-confirmed cases. Additionally, 12 illnesses reported among attendees were temporally related to wedding events but did not fulfill clinical case criteria. The survey response rate was only 39%; additional cases likely occurred among attendees. Among respondents, 61% were ill, suggesting that illness was a possible motive for response. Because of the low response rate and potential for overrepresentation of ill subjects, data were analyzed using a case-control rather than cohort study design.

Arcobacter spp. are closely related to *Campylobacter* spp. Clinical data from investigations of ≥ 20 community outbreaks of *Campylobacter* enteritis (each involving ≥ 50 people) with varied outbreak case definitions demonstrated the following mean frequencies of signs and symptoms: diarrhea 84%, abdominal pain 79%, fever 50%, headache 41%, vomiting 20% and, in a smaller number of outbreaks, myalgia 42% and bloody stools 15% (Skirrow and Blaser, 2000). Abdominal pain associated with *Campylobacter* enteritis can be significant and is the most frequent reason for hospital admission (Skirrow and Blaser, 2000). In comparison, diarrhea, cramping, abdominal pain, nausea, and constitutional symptoms (chills, fatigue, headache, myalgia, and body aches) were frequently reported among ill individuals during this outbreak. Vomiting occurred in $>25\%$ of case patients, but fever was infrequently reported. Except for one attendee, no case patients sought medical care, although three reported bloody stools. Importantly, reception attendees were overwhelmingly adults and not representative of a community-wide population. In contrast, during the only other reported outbreak of *A. butzleri* infections, which involved person-to-person transmission in an Italian school, all 10 patients were children; all presented with abdominal cramps and none reported diarrhea, but three were sufficiently ill to be hospitalized (Vandamme *et al.*, 1992).

With availability of *Arcobacter*-specific culture media and directed molecular assays, frequencies of *Arcobacter* detection in stool specimens associated with human enteric illness have been considerable. During a 1995–2002 Belgian study that

involved 67,599 stool specimens and included a protocol to recover *Campylobacter* spp. and related organisms, *A. butzleri* was the fourth most common *Campylobacter*-like organism isolated (Vandenberg *et al.*, 2004). During a 2008–2009 Belgian study involving 2423 stool specimens from symptomatic outpatients and inpatients cultured for *Arcobacter* and common bacterial enteric pathogens, *Arcobacter*, detected in 1.24% of specimens, was the fourth most common genus isolated (Van den Abeele *et al.*, 2010). These results highlight the importance of testing for *Arcobacter* spp. when routine enteric pathogens are not found.

Arcobacter is a fastidious organism often requiring varied growth conditions for optimal detection and identification (Fitzgerald and Nachamkin, 2007). Although there are several published methods, the lack of a standard method for *Arcobacter* spp. isolation from fecal samples likely contributes to human cases remaining undetected, and it also poses a challenge to compare data between published studies (Merga *et al.*, 2011). Despite using appropriate selective media and providing recommended culture conditions, we were unable to detect *Arcobacter* using culture. The lack of *Arcobacter*-specific culture-positive specimens in this outbreak may be related to the time lag from collection to testing at MDH-PHL (> 12 days for stool, > 56 days for chicken), temperature abuse during transit or only nonviable organisms remaining in the specimens. *Campylobacter*, and other bacteria, can enter into a viable but nonculturable state upon exposure to starvation and physical stresses (Jones *et al.*, 1991; Barer and Harwood, 1999; Nogva *et al.*, 2000; Oliver, 2005). Recently, using *Campylobacter*-specific uniplex PCR and 16S rDNA sequencing, investigators (Bullman *et al.*, 2012) detected *Campylobacter* DNA in 93.6% of 204 culture-negative samples that were *Campylobacter*-positive using the EntericBio[®] system (SeroSep Ltd., Ireland). During our outbreak investigation, the specimens may not have been ideal for *A. butzleri* culture propagation but were suitable for identification using molecular techniques.

Conclusions

The results of our investigation suggest that *A. butzleri* can cause foodborne illnesses, and should be considered a potential cause of outbreaks of foodborne illness. Molecular techniques used in conjunction with standard culture methods to test for *Arcobacter* can improve detection sensitivity. Expanded suspicion beyond consideration of routine enteric pathogens provided direction in the identification of an organism previously not reported to cause foodborne illness outbreaks. Rigorous investigation of outbreaks of undetermined etiology is valuable for increasing our understanding of the burden of foodborne illness and the emerging agents of foodborne outbreaks.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Jeffrey P. Davis, MD

Bureau of Communicable Diseases and Emergency Response
Wisconsin Division of Public Health
1 West Wilson Street, Room 318
Madison, WI 53702

E-mail: jeffrey.davis@wisconsin.gov