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SHORT REPORT
Calling all Campy – how routine investigation and molecular characterization impacts the understanding of campylobacteriosis epidemiology – Alaska, United States, 2004–2013

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SUMMARY

Unlike most jurisdictions in the United States, Alaska performs pulsed-field gel electrophoresis (PFGE) characterization of all *Campylobacter* sp. isolates at the state public health laboratory – a practice that started in 2002. Moreover, in order to ensure early detection and response to campylobacteriosis outbreaks, the Alaska Section of Epidemiology has investigated all incident *Campylobacter* sp. case reports since 2004. This report summarizes the public health impact of routine incident case investigations and molecular characterization of all *Campylobacter* sp. isolates. In sum, we found that these efforts have contributed to better characterization of the epidemiology of campylobacteriosis in Alaska, and facilitated more rapid outbreak detection, more public health investigations, and earlier public health interventions.

Key words: Bacterial infections, bacterial typing, *Campylobacter*, community outbreaks, foodborne infections.

In Alaska, campylobacteriosis has been legally mandated to be reported by healthcare providers and laboratories per directive 7 AAC 27·005 and .007 to the Alaska Section of Epidemiology (SOE) since 1984. *Campylobacter* sp. is the most frequently reported bacterial enteric pathogen in Alaska, with an average of 90 cases reported annually (approximately 10–16 infections/100 000 population). In the absence of county-level public health authorities, all cases are reported to the state-level SOE and each patient is interviewed to determine possible sources of exposure. Many other jurisdictions may not have the legal framework to compel reporting and/or the resources to conduct individual interviews. Additionally, these jurisdictions may also not routinely perform pulsed-field gel electrophoresis (PFGE) on *Campylobacter* sp. isolates [1]. However, the State of Alaska Public Health Laboratory (ASPHL) has performed routine PFGE of *Campylobacter* sp. isolates since 2004. We summarize here Alaska's experience of universally investigating *Campylobacter* sp. case reports and molecular typing of isolates to refute the commonly held notion that *Campylobacter* sp. infections are typically sporadic illnesses.

The Alaska SOE refers all laboratory-confirmed campylobacteriosis case reports to local public health nurses to conduct a telephone interview with each patient using a standardized pathogen-specific interview form. Patients are asked about exposures to certain foods, activities, and venues, among other risk factors. All patients are offered information on *Campylobacter* sp. infection, prevention, and hand hygiene. Completed interview forms are returned to SOE for review of data collected regarding possible sources of

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infection, others who may be ill, and high-risk activities and occupations. When warranted, additional interviews and clinical specimens may be pursued. If indicated, SOE collaborates with the Alaska Department of Environmental Conservation for on-site facility inspection and/or environmental sampling. Alaska data that meet the criteria for outbreaks are submitted to the United States National Outbreak Reporting System (NORS). During the 2004–2013 study period, data specifications of events to be recorded in NORS (previously called eFORS) evolved. Examples of changes included adding person-to-person transmitted norovirus outbreaks and refining the classification of multistate 'clusters' with pattern matches and unclear sources of infection.

During 2004–2013, ASPHL classified all *Campylobacter* sp. PFGE data by frequency of pattern matches. Clinical, environmental, and animal *Campylobacter* sp. isolates and/or *Campylobacter* sp.-positive stool samples were received at ASPHL. Isolates were identified by growth on CVA (cefoperazone, vancomycin, amphotericin B) agar plates (PML Microbiologicals, USA), and results from testing of motility, Gram stain, oxidase (BD, USA), and hippurate hydrolysis (PML Microbiologicals). All hippurate-negative isolates were sent for identification to the National *Campylobacter* and *Helicobacter* Reference Laboratory in Atlanta, GA, USA. Preparation of *C. jejuni* DNA, macrorestriction analysis using the restriction enzymes *Sma*I and *Kpn*I (Roche, New England Biolabs, USA) and PFGE of all isolates were performed according to the *C. jejuni* PulseNet protocol [2]. PFGE and cluster analysis was performed using BioNumerics v. 5·1 (Applied Maths, USA) and the Dice coefficient using the unweighted pair-group method with arithmetic averages (UPGMA). Isolates were assigned to the same PFGE pattern when they clustered at >95% similarity (for *Sma*I and *Kpn*I, 0·45% optimization and 0·85% position). Time and resource costs for processing isolates were estimated. Factors considered included reagents, consumables, equipment, personnel time, and indirect costs.

Initially, clusters were defined as two or more isolates that had indistinguishable primary restriction enzyme *Sma*I PFGE pattern numbers assigned by PulseNet, and isolated within a 60-day period. Following summer 2008 when ASPHL began performing both restriction digest PFGE patterns, a cluster was later defined as two or more isolates with indistinguishable *Sma*I and *Kpn*I pattern numbers assigned by PulseNet, and isolated within a 60-day period. An outbreak was defined as a cluster with an identified shared epidemiological exposure.

During the 10-year time period 629 *Campylobacter* sp. isolates were processed at ASPHL; 45 were from environmental sources and 15 were not assigned a PulseNet number. Of the remaining 569 isolates, 184 (32%) were classified as a 'cluster' based on either primary enzyme or two-enzyme analysis, and further classified as an 'outbreak' if interviews revealed accompanying epidemiological links.

These 184 isolates comprised 34 incidents – either outbreaks or clusters. Of the 23 outbreaks reported to NORS: five (22%) were attributed to contaminated food, two to handling live poultry, and one each to unfiltered water, and person-to-person transmission; the source was undetermined for 14 (61%) of the outbreaks. Several of the more notable campylobacteriosis outbreaks above have been described in detail elsewhere [3, 4]. The combined PFGE and epidemiological data proved to be critical for the early identification of two smouldering *Campylobacter* sp. outbreaks which were not previously identified because the common exposure source was not recognized before the PFGE match was identified [3, 4].

The 11 other incidents not in NORS were either considered outbreaks but had not been entered into NORS/eFORS at the time of occurrence, or were classified only as 'clusters'. Some of these incidents like the outbreaks, had also prompted extra attention from public health authorities. For example, one cluster in a remote region without in-home piped water and without strong epidemiological links between cases, prompted additional community water testing. Another cluster in a dormitory-type housing unit gave rise to an environmental health inspection of a shared kitchen facility.

The remaining 385 isolates were characterized as 'sporadic' because they lacked molecular and/or epidemiological linkages to other isolates. Interestingly, there were several instances where isolates had matching patterns but the specimen collection dates were >60 days apart and thus not did not fit the study definition of clusters/outbreaks. Finally, some illnesses acquired in Alaska in non-residents, but identified outside the state, were not fully characterized because routine PFGE analysis was not being performed in those jurisdictions. These are not Alaska cases by counting convention based on residency, but also demonstrate additional national burden of campylobacteriosis because molecular data could allow for tabulation of more multistate outbreaks.

The cost of running a single isolate from receipt at ASPHL to PFGE processing was estimated at US$136 (2013 estimate). Costs were about 44% equipment, 21·5% each personnel time and overheads, and 13% for reagents, consumables, and other supplies. PFGE is performed concurrently with the phenotypic identification once the organism has been grown. Processing the isolate in the PFGE laboratory and running the gel electrophoresis takes 24 h. Analysis and uploading to PulseNet is performed once the electrophoresis is complete and the type pattern number is generally assigned 24 h after upload. *Sma*I and *Kpn*I digests have different PFGE running conditions and are placed on separate gels [2]. Once the decision was made in 2008 to perform both digests on all *Campylobacter* sp. isolates, the cost of running an isolate doubled to US$272. Therefore, an annual estimated cost for an average of 100 isolates would be about US$ 27 000. Whole genome sequencing (WGS) techniques are expected to replace PFGE analyses in the United States but will increase both the cost of evaluating isolates and the time needed to process/run samples. Additionally, interpreting results will require understanding of the significance of sequence differences from an epidemiological perspective as well as how laboratory analytical methods compare across nations.

Over 30% of the Alaska *Campylobacter* sp. cases were linked to an outbreak or cluster, leaving over 60% classified as truly sporadic. Although that estimate is vastly different than the >99% US national estimate [5], it is not surprising because links between uninvestigated cases are far less likely to be identified in the absence of epidemiological investigations. A recent case-case analysis in the UK comparing sporadic *vs.* outbreak-associated *Campylobacter* sp. infections similarly suggested under-appreciation of illness clusters/outbreaks and recommended enhanced surveillance to improve prevention and control of campylobacteriosis [6].

Routine reporting, investigation, and PFGE characterization of *Campylobacter* sp. isolates in Alaska has contributed to better characterization of the burden and epidemiology of campylobacteriosis, as well as more rapid and complete outbreak and cluster detection and response. The cost-effectiveness of these practices has not been formally assessed. Estimated costs for the PFGE analyses are noted above; the estimated costs for the epidemiological component were not calculated, nor were estimates of cost savings to overall public health based on interventions or health education and outreach. Of note, were Alaska to limit epidemiological and laboratory follow-up, we would expect documented outbreaks to decrease (regardless of changes in absolute case reports) due to the decrease in surveillance effectiveness.

Efforts taken nationally to improve the recognition and characterization of campylobacteriosis resulted in a successful proposal in 2014 from the U.S. Council of State and Territorial Epidemiologists to add *Campylobacter* sp. infections to the list of nationally notifiable conditions, which became effective in 2015 [7]. We anticipate that more systematic reporting at the national level will result in increased outbreak detection and concomitant opportunities for campylobacteriosis prevention. Conclusions about cost-effectiveness and feasibility may change as WGS techniques become more widely applied.

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DECLARATION OF INTEREST

None.

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