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## Pulsed-Field Gel Electrophoresis in Alaska: A Tool to Assist Epidemiologic Investigations

### Epidemiologic Investigation

On December 15, 2006, Quest Laboratories (Seattle) isolated *Salmonella* serotype Tennessee (ST) from a urine specimen of an Alaska child with a urinary tract infection that first became symptomatic on December 10. The child was treated as an out-patient and symptoms resolved. Quest faxed a report to the Alaska Section of Epidemiology (SOE) on December 18 and subsequently forwarded the isolate to the Alaska State Public Health Laboratory (ASPHL). On December 29, ASPHL confirmed the isolate as ST and on January 3, 2007, pulsed-field gel electrophoresis (PFGE) was performed. Results were posted to PulseNet—an international network of shared molecular data on various bacterial species.

Once in the PulseNet system, the patient's PFGE pattern was compared to other ST patterns posted on PulseNet in the prior 30 days; it matched a PFGE pattern seen in six other states. On February 5, SOE was notified by the Centers for Disease Control and Prevention (CDC) that the Alaska case's pattern matched isolates from numerous other ST cases for which illness was suspected to be associated with consumption of certain brands of peanut butter.

On February 14, a nationwide CDC alert revealed that isolates from 288 ST cases in 39 states had a matching PFGE pattern. A multi-state case-control study confirmed that illness was strongly associated with consumption of peanut butter brands produced by ConAgra, which subsequently ceased production. Also on February 14, the Food and Drug Administration issued an initial recall for peanut butter product dating back to December 2005. A secondary recall, issued March 14, included product dating back to October 2004.

The outbreak strain of ST was isolated from the product and environmental samples from the plant. Further investigation by ConAgra found contamination originated inside its own plant. Moisture from a roof leak and a faulty sprinkler system allowed the growth of *Salmonella* that was likely present in raw peanuts or peanut dust.

Prior to being notified about the matching pattern, SOE had not identified a likely source of ST for the child. Follow-up questioning revealed that the child had indeed consumed an implicated brand and lot number of peanut butter. To date, there are >700 cases of ST illness in the United States that have been associated with ConAgra-produced peanut butter.

### Pulsed-Field Gel Electrophoresis (PFGE)

PFGE is a method of bacterial subtyping that can detect disease clusters or outbreaks. Agarose plugs are made containing a bacterial suspension. Restriction enzymes then cut the bacterial DNA into large fragments, which are separated by gel electrophoresis. By alternating the direction of the electrical field, the DNA fragments show up in the gel as a pattern of discrete bands. These DNA "fingerprints" are compared to a fingerprint database to determine if strains are related. By matching PFGE patterns, epidemiologists can link cases of illness that were not previously known to be associated. PFGE can also be used to link human illness to environmental isolates, such as those cultured from food products or animals.

### PFGE at the Alaska State Public Health Laboratory

ASPHL began performing PFGE in 1998. Isolates for PFGE are obtained from cultures either performed at ASPHL or submitted by local hospital laboratories. Current isolate DNA

fingerprints are compared to those in the Alaska fingerprint database to help identify clusters of gastroenteritis. The Table below summarizes some characteristics of *Salmonella* spp. isolates in the Alaska database.

**Table. *Salmonella* spp. Isolates Analyzed by ASPHL, 2000–2007.**

Number run by PFGE	652
Number of serotypes	80
Unique Alaska patterns	366
Clusters*	4
Outbreaks*	2

\*Data only available for 2007

Clusters are isolates with matching patterns seen in higher numbers than expected, but without a common exposure. Outbreaks are clusters that share an exposure. One 2007 cluster involved six cases of *S. Enteritidis* identified during the spring in the Norton Sound region, but a common exposure was never identified. In contrast, the ST case presented above was considered part of an outbreak.

ASPHL and other public health laboratories in the United States and Canada are part of the Molecular Subtyping Network for Foodborne Disease Surveillance, also known as PulseNet, run by CDC. Laboratorians must receive certification from PulseNet to perform PFGE assays. After DNA fingerprints are compared to the local database, they are uploaded to PulseNet to assist with the detection of outbreaks.

### Discussion

Outbreaks of acute gastroenteritis are easily identified when large numbers of ill persons present to health care providers soon after having a common exposure. More commonly, sporadic cases of acute gastroenteritis are reported to SOE without an obvious source of exposure. The example described above demonstrates the usefulness of this epidemiologic tool, especially in characterizing such sporadic cases of illness. Associations made using PFGE links can lead to important public health consequences such as a nationwide recall of a food product.

One noteworthy limitation to PFGE analysis is that the molecular variability of some bacterial species may not yet be fully understood such that when subsequent, more detailed molecular analyses are performed, some "matches" are later determined to be distinctly different isolates.

### Recommendations

1. Health care providers who have patients with acute gastroenteritis that they suspect may be part of a larger cluster of illness should contact the Section of Epidemiology at 907-269-8000 during business hours, or 1-800-478-0084 after hours.
2. Laboratorians should submit subcultures of enteric bacterial pathogens isolated in clinical laboratories to ASPHL for confirmation. All *Salmonella*, *Shigella*, *Campylobacter*, STEC positive *E. coli* including O157:H7, and *Vibrio* species isolates are routinely evaluated by PFGE. These services are free of charge.
3. Contact ASPHL for information on specimen collection and current shipping regulations at 907-334-2100 or refer to the ASPHL Laboratory Service Manual, available at: [http://www.hss.state.ak.us/dph/labs/publications/image/Lab\\_Svcs\\_Manual.pdf](http://www.hss.state.ak.us/dph/labs/publications/image/Lab_Svcs_Manual.pdf).