Laboratory Review of Foodborne Disease Investigations in Washington State 2007–2017

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Abstract

The Washington State Department of Health Public Health Laboratories (WAPHL) has tested 11,501 samples between 2007 and 2017 for a foodborne disease using a combination of identification, serotyping, and subtyping tools. During this period there were 8037 total clinical and environmental samples tested by pulsed-field gel electrophoresis (PFGE), including 512 foodborne disease clusters and 2176 PFGE patterns of Salmonella enterica subsp. enterica. There were 2446 Shiga toxin–producing Escherichia coli samples tested by PFGE, which included 158 foodborne disease clusters and 1174 PFGE patterns. There were 332 samples of Listeria monocytogenes tested by PFGE, including 35 foodborne disease clusters and 104 PFGE patterns. Sources linked to outbreaks included raw chicken, unpasteurized dairy products, various produce types, and undercooked beef among others. As next-generation sequencing (NGS) replaces PFGE, the impact of this transition is expected to be significant given the enhanced cluster detection power NGS brings. The measures presented here will be a reference baseline in future years.

Keywords: Washington State, PFGE, Salmonella, Listeria, foodborne illness and disease, PulseNet

Introduction

Approximately 3000 notifiable enteric foodborne illnesses are reported annually in Washington (WA) State, with 1–10 associated deaths (CDC, 2015a). The foodborne disease category is a leading cause of infectious illnesses in WA. Clinical laboratories in WA are required to submit specimens or isolates from patients diagnosed with listeriosis, salmonellosis, shigellosis, vibriosis, or infection with Shiga toxin Department of Health–producing Escherichia coli (STEC) to the Washington State Public Health Laboratories (WAPHL). Submissions are characterized to confirm the initial identification and some isolates are further serotyped and subtyped.

The PulseNet program is a national laboratory network that allows participating laboratories to link molecular characteristics of bacterial isolates from foodborne illness cases to detect outbreaks (Swaminathan et al., 2001). WAPHL was among the first four state PHLs to join the Centers for Disease Control and Prevention (CDC)-sponsored PulseNet program in 1996 (Stephenson, 1997; CDC, 2016b) and has continued its key role as the Western PulseNet Region Area Laboratory for >20 years. PulseNet relies on the use of standardized pulsed-field gel electrophoresis (PFGE) equipment, methodology, and analysis tools that link data across participating laboratories to detect clusters.

The primary source of infection with Listeria monocytogenes, STEC, Salmonella enterica, Campylobacter jejuni, Yersinia spp., Vibrio cholerae, or Vibrio parahaemolyticus is undercooked or adulterated food. Although listeriosis and STEC infections represent a small proportion of all foodborne illnesses, outcomes can be severe so each case is carefully investigated. Listeriosis occurs primarily in individuals with immunosuppression, pregnant women, neonates, and the elderly as invasive infection that can carry a mortality rate of at least 16% (Farber and Peterkin, 1991; Barton Behravesh et al., 2011; CDC, 2016a). STEC infections can also be severe because of the risk of developing hemolytic uremic syndrome that carries a high mortality rate particularly, for children

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younger than 4 years (Barton Behravesh et al., 2011). Along with listeriosis, salmonellosis causes the most deaths because of a foodborne disease in WA, despite a lower case fatality rate. This is because salmonellosis is among the most common bacterial foodborne infections, second only to campylobacteriosis (CDC, 2015b; Laufer et al., 2015).

The aim of this publication was to summarize the work that WAPHL has carried out over the past 11 years (2007–2017) in the area of foodborne disease investigations. The transition as next-generation sequencing (NGS) replaces PFGE is expected to have a significant impact given the enhanced cluster detection power because of the increase in resolution of NGS. In addition, the use of culture independent diagnostic testing (CIDT) and its impacts on the need for isolates are briefly addressed. The measures presented here will be a baseline for reference in future years. Although WAPHL has applied PFGE to organisms other than those already mentioned, this summary will focus on only these organisms and the work WAPHL has performed for WA residents.

Materials and Methods

**Bacteria isolation, identification, and subtyping**

STEC were isolated and identified using MacConkey with sorbitol (SMAC), tellurite, and cefixime (CT-SMAC), and Rainbow agar with novobiocin and tellurite. Specimens not already in Gram Negative (GN) broth were enriched by inoculating GN broth. All specimens were initially screened for functional Shiga toxin utilizing a lateral flow enzyme immunoassay (EIA) test (Meridian ImmunoCard STAT™) or Alere SHIGA TOXIN QUIK CHEK™) which detects and differentiates Shiga toxin 1 and Shiga toxin 2 (Staples et al., 2017). Isolates were tested for Shiga toxin production and were confirmed biochemically. If the isolate was Shiga toxin positive and biochemically resembling *E. coli*, the isolate was serotyped using *E. coli* OK antisera or antibody-coated latex beads. Turnaround time for STEC isolation and confirmation was 4–7 business days. These isolates were routinely tested by PFGE.

*Salmonella* were isolated and identified using MacConkey (MAC), Hektoen Enteric (HE) agar, Salmonella-Snigella (SS) agar, and brilliant green agar. Stool were inoculated into selenite broth and tetrahionate broth as a selective enrichment broth for better recovery of *Salmonella* spp. Isolates resembling *Salmonella* were confirmed using biochemicals. From 2007 to 2012, *Salmonella* isolates were serotyped utilizing *Salmonella* antisera to determine O and H antigens. From 2012 to 2017, molecular techniques (Illumina xMAP *Salmonella* serotyping assay) were used to serotype *Salmonella* isolates, supplemented with *Salmonella* antisera (Dunbar et al., 2015). Turnaround time for *Salmonella* isolation and confirmation was 4–7 business days. All *Salmonella* isolates were routinely tested by PFGE.

*Listeria* from clinical specimens were identified using blood agar plates (BAP), brain–heart infusion (BHI) broth agar slant or a heart infusion agar (HIA) slant, and MAC to look for purity, hemolysis (BAP), and inhibited growth (MAC). A single colony was picked from the BAP to inoculate a set of biochemicals to confirm *L. monocytogenes*. If the results were not typical for *L. monocytogenes*, then hippurate and CAMP tests were performed to help with the identification. Turnaround time for *Listeria* identification was 3–5 business days.

*Listeria* isolates were routinely tested by PFGE for subtyping and a BHI/HIA was referred to the CDC for further studies.

*Listeria* from food samples and environmental samples were isolated and identified using a modified Food and Drug Administration Bacteriological Analytical Manual procedure for detecting *Listeria* in food (FDA, 2017).

Media and test reagents for *Salmonella, E. coli, and Listeria* isolation and identification were purchased commercially with a few exceptions. Antisera were purchased from Difco, Denka Seiken, orSSI Diagnostica. Media and most biochemicals were purchased from Remel and Hardy Diagnostics. The antibody-coated latex beads were purchased from Pro-Lab for *E. coli* Non-O157 (*E. coli* Non-O157 Latex Test Reagent Kit) and from Remel for *E. coli O157* (Remel RIM *E. coli* O157:H7 Latex test). Carbohydrate biochemicals and nutrient broths were made in-house at the WAPHL. All manufactured media were used following the manufacturer guidelines. All WAPHL in-house media use followed the Enterics and Special Bacteriology Reference Units laboratory procedure manuals and microbiology reference books (Holt, 1994; Weyant, 1996; MacFaddin, 2000; de la Maza, 2004; Garcia and Isenberg, 2010; Jorgensen, 2015).

**PFGE subtyping**

PFGE subtyping was carried out using PulseNet protocols for running and analyzing PFGE gels (Graves and Swaminathan, 2001; Ribot et al., 2001, 2006; Swaminathan et al., 2001; Parsons et al., 2007). Turnaround time for PFGE was 4 business days. PFGE patterns were compared with other patterns both in the WA database and in the national CDC PulseNet database using BioNumerics software. Any pattern matches were further assessed to determine if they should be considered a cluster and clusters were reported to an epidemiologist.

**Cluster definition**

For this publication a cluster identified by WAPHL is defined as two or more cases with matching PFGE patterns and similar illness onset date (within 60 d). Other supportive information for defining a cluster is similar geographic distribution or similar demographics, especially for a common PFGE pattern (Bender et al., 2001; Barrett et al., 2006; Tauxe, 2006). A foodborne disease outbreak is defined as two or more people with the same illness from a shared identified food or drink. Outbreaks vary in size and are classified depending on the spread of disease as local, multicounty, or multistate (CDC, 2015b). Ill people from the same household are not counted as a cluster.

**Results**

Between 2007 and 2017 WA received a total of 33,079 notifiable bacterial disease case reports for foodborne illnesses. During this period WAPHL received a total of 12,885 human enteric isolates of which 11,134 received PFGE characterization (Fig. 1). Of human enteric reports (confirmed, probable, and suspect cases), 51% were attributed to campylobacteriosis, 27% to salmonellosis, 9% to STEC, and 10% to other enteric illnesses including listeriosis, vibriosis, cholera, and shigellosis.
There were 8759 salmonellosis and typhoid fever cases (confirmed, probable, and suspect) reported during the period and 7829 Salmonella isolates were subtyped at WAPHL (Table 1). Among the S. enterica subsp. enterica isolates tested, the most frequent serotypes identified, in order, were Enteritidis, Typhimurium, I 4,[5],12:i:-, Heidelberg, and Newport. Table 2 presents the most common serotypes reported in WA. Less common serotypes detected in WA are reported elsewhere (Washington State Department of Health). Serotypes Enteritidis and Typhimurium topped all serotypes for each year during 2007–2017, except for 2015 when a large outbreak of serotype I 4,5,12:i:- associated with roasted whole hogs occurred (Kawakami et al., 2016).

Within serotypes Enteritidis, Typhimurium, and I 4,5,12:i:- there were 110, 287, and 97 distinct PFGE patterns, respectively (Table 1). For all Salmonella serotypes there was an average of 45 Salmonella PFGE clusters per year (Table 1). Salmonella Enteritidis was responsible for multiple confirmed outbreaks linked to travel to Mexico, dining at local restaurants, or consuming poultry (Table 3). One outbreak linked to alfalfa sprouts and spicy sprouts sickened 25 people, 10 residing in WA. Three people were hospitalized and the investigation was closed on July 6, 2011, after the company voluntarily recalled the product (CDC, 2011). Salmonella Typhimurium outbreak vehicles included chicks, peanut butter, alfalfa sprouts, hedgehogs, a teaching laboratory exposure, and restaurants. An outbreak as a result of rotisserie chicken salad contaminated with Salmonella Typhimurium was identified in 2016.

Food vehicles leading to recurrent outbreaks associated with other Salmonella serotypes included pot pie and pig roast linked to Salmonella I 4,[5],12:i:- (Kawakami et al., 2016) and frozen raw chicken linked to Salmonella Heidelberg (Green et al., 2018). Sources linked to multiple Salmonella serotypes included live chicks, pet reptiles, and multiple restaurants. Produce vehicles linked to salmonellosis outbreaks included mangoes, green onions, peppers, and pistachios. In 2015 there were two outbreaks resulting from exposure to peanut butter (Salmonella Newport) and spicy tuna rolls [Salmonella Paratyphi B L(+) Tartrate(+)]. One Salmonella Saintpaul outbreak in 43 U.S. states and Canada linked to jalapeno and serrano peppers, and possibly to raw tomatoes, affected 1442 people with 2 deaths (CDC, 2008b) (Table 3). In 2007 a WA outbreak involving 12 illnesses was linked to the use of an improperly cleaned food slicer contaminated with Salmonella Seftenberg. During the 2007–2017 period there were a total of 23 deaths associated with salmonellosis in WA.

The total number of confirmed, probable, and suspect cases as a result of STEC reported between 2007 and 2017 was 2525, of which 1373 cases were attributed to E. coli O157, 293 cases were attributed to E. coli O26, and 691 were attributed to other E. coli serotypes (not shown). Among E. coli O157 isolates there were 1398 PFGE patterns and 129 PFGE clusters (Table 1). Outbreaks were linked to consuming undercooked beef (2007, 2009), cookie dough (2009), or unpasteurized milk (Table 3); in addition, outbreaks occurred at day care centers, at petting zoos, or owing of contact with grazing animals. There were 10 STEC-related fatalities reported during this period (Table 1). For E. coli non-O157 there were 776 PFGE patterns and 29 PFGE clusters (Table 1), which included outbreaks because of raw sprouts and uncooked flour. In addition, lettuce, leafy greens, kale, and spinach were also found to be STEC vehicles (Table 3) as well as produce (lettuce, kale, cantaloupe, and onions).

**Discussion**

Salmonellosis has several characteristics that make control difficult (Ailes et al., 2008). It occurs naturally in cattle, poultry, and eggs and is not considered an adulterant in raw
| Criteria | STEC (all serotypes) | STEC (O157) | Salmonella Enteritidis | Salmonella Typhimurium | Salmonella I 4,5,12:i- | Salmonella Newport | Salmonella Heidelberg | Salmonella Typhi | All other Salmonella serotypes (non-Typhi) | Listeria monocytogenes | Shigella sonnei and Shigella flexneri | Vibrio | 11-Year totals |
|----------|---------------------|-------------|-----------------------|------------------------|------------------------|-------------------|---------------------|----------------|------------------------------------------|-------------------------|--------------------------|--------|----------------|
| 2007–2017 |                     |             |                       |                        |                        |                   |                     |                |                                       |                         |                          |        |                |
| No. of unique PFGE patterns | 776 | 398 | 110 | 287 | 97 | 168 | 75 | 2 | 1439 | 104 | 313 | 6 | 3773 |
| No. of clusters | 29 | 129 | 66 | 89 | 45 | 31 | 22 | 2 | 259 | 35 | 18 | 1 | 726 |
| No. of local clusters | 12 | 54 | 15 | 30 | 8 | 4 | 6 | 55 | 8 | 13 | 0 | 205 |
| No. of multistate clusters | 17 | 75 | 51 | 59 | 37 | 27 | 16 | 204 | 27 | 5 | 0 | 518 |
| Total WA food/environmental isolates pulsed | 24 | 30 | 208 | 114 | 1 | 2 | 379 | | | | | | |
| Total WA clinical case isolates pulsed | 1078 | 1314 | 1788 | 1086 | 619 | 392 | 444 | 131 | 3369 | 218 | 631 | 42 | 11,134 |
| Confirmed/suspect/probable cases | 1152 | 1373 | 1842 | 1107 | 565 | 385 | 433 | 117 | 4310 | 249 | 1733 | 682 | 13,948 |
| Deaths | 10 | 3 | 3 | 4 | 1 | 2 | | 10 | 18 | 0 | 1 | 52 |
| 2007 |                     |             |                       |                        |                        |                   |                     |                |                                       |                         |                          |        |                |
| No. of local clusters | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | |
| No. of multistate clusters | 0 | 5 | 1 | 3 | 2 | 3 | 0 | 13 | 4 | 0 | 0 | |
| Confirmed/suspect/probable cases | 13 | 119 | 120 | 121 | 45 | 58 | 39 | 375 | 25 | 159 | 25 | |
| Deaths | 0 | NA | NA | NA | NA | NA | NA | NA | 2 | 0 | 0 | |
| Total local and multistate clusters | 0 | 8 | 1 | 3 | 2 | 3 | 0 | 15 | 4 | 1 | 0 | |
| Total WA clinical case isolates pulsed | 12 | 126 | 112 | 127 | 48 | 56 | 36 | 332 | 16 | 133 | 0 | |
| 2008 |                     |             |                       |                        |                        |                   |                     |                |                                       |                         |                          |        |                |
| No. of local clusters | 0 | 4 | 2 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | |
| No. of multistate clusters | 0 | 1 | 5 | 5 | 2 | 1 | 0 | 5 | 7 | 1 | 0 | |
| Confirmed/suspect/probable cases | 24 | 151 | 199 | 133 | 15 | 39 | 31 | 429 | 29 | 116 | 29 | |
| Deaths | 1 | NA | NA | NA | NA | NA | NA | NA | 3 | 0 | 0 | |
| Total local and multistate clusters | 0 | 5 | 7 | 5 | 2 | 2 | 0 | 9 | 2 | 2 | 0 | |
| Total WA clinical case isolates pulsed | 12 | 144 | 197 | 129 | 22 | 37 | 31 | 304 | 22 | 96 | 0 | |
| 2009 |                     |             |                       |                        |                        |                   |                     |                |                                       |                         |                          |        |                |
| No. of local clusters | 0 | 5 | 2 | 3 | 0 | 0 | 1 | 3 | 1 | 4 | 0 | |
| No. of multistate clusters | 0 | 12 | 4 | 14 | 3 | 1 | 4 | 26 | 1 | 0 | 0 | |
| Confirmed/suspect/probable cases | 32 | 159 | 147 | 148 | 17 | 29 | 63 | 416 | 24 | 153 | 48 | |
| Deaths | 0 | NA | NA | NA | NA | NA | NA | NA | 4 | 0 | 0 | |
| Total local and multistate clusters | 0 | 0 | 6 | 17 | 3 | 1 | 5 | 29 | 2 | 4 | 0 | |
| Total WA clinical case isolates pulsed | 28 | 156 | 146 | 157 | 26 | 29 | 72 | 318 | 25 | 125 | 0 | |

(continued)
| Criteria                      | STEC (all serotypes) | STEC (O157) | Salmonella Enteriditis | Salmonella Typhimurium | Salmonella I 4,5,12:i:- | Salmonella Newport | Salmonella Hiedenberg | Salmonella Typhi | All other Salmonella serotypes (non-Typhi) | Listeria monocytogenes | Shigella sonnei and Shigella flexneri | Vibrio | 11-Year totals |
|-------------------------------|----------------------|-------------|------------------------|------------------------|-------------------------|--------------------|-----------------------|-----------------|------------------------------------------|-------------------------|---------------------------------------|--------|---------------|
| 2010                          | 2                    | 6           | 3                      | 7                      | 1                       | 0                  | 0                     | 6               | 3                                        | 2                       | 0                                    |        |               |
| No. of local clusters         | 2                    | 6           | 3                      | 7                      | 1                       | 0                  | 0                     | 6               | 3                                        | 2                       | 0                                    |        |               |
| No. of multistate clusters    | 0                    | 8           | 11                     | 8                      | 2                       | 2                  | 2                     | 2               | 19                                       | 0                       | 1                                    | 0      |               |
| Confirmed/suspect/probable cases | 77                  | 110         | 173                    | 127                    | 10                      | 50                 | 52                    | 368                          | 24                                       | 112                                   | 59      |               |
| Deaths                        | 1                    | NA          | NA                     | NA                     | NA                      | NA                 | NA                    | NA              | 1                                        | 0                       | 0                                    |        |               |
| Total local and multistate clusters | 2                | 14          | 14                     | 15                     | 3                       | 2                  | 2                     | 25                           | 3                                        | 3                                    | 0      |               |
| Total WA clinical case isolates pulsed     | 78                  | 103         | 166                    | 133                    | 18                      | 50                 | 53                    | 234                          | 20                                       | 102                                   | 0      |               |
| 2011                          | 0                    | 8           | 1                      | 3                      | 2                       | 1                  | 1                     | 6               | 1                                        | 2                       | 0                                    |        |               |
| No. of local clusters         | 0                    | 8           | 1                      | 3                      | 2                       | 1                  | 1                     | 6               | 1                                        | 2                       | 0                                    |        |               |
| No. of multistate clusters    | 0                    | 5           | 4                      | 2                      | 2                       | 1                  | 1                     | 10                           | 2                                        | 0                                    | 0      |               |
| Confirmed/suspect/probable cases | 88                 | 104         | 137                    | 88                     | 16                      | 20                 | 27                    | 301                          | 24                                       | 153                                   | 45      |               |
| Deaths                        | 1                    | NA          | NA                     | NA                     | NA                      | NA                 | NA                    | NA                           | 0                                        | 0                                    | 0      |               |
| Total local and multistate clusters | 0               | 13          | 5                      | 5                      | 4                       | 2                  | 2                     | 16                           | 3                                        | 0                                    | 0      |               |
| Total WA clinical case isolates pulsed     | 80                  | 98          | 132                    | 82                     | 15                      | 27                 | 29                    | 271                          | 17                                       | 91                                   | 26      |               |
| 2012                          | 1                    | 4           | 0                      | 3                      | 0                       | 0                  | 0                     | 7               | 0                                        | 1                       | 0                                    |        |               |
| No. of local clusters         | 1                    | 4           | 0                      | 3                      | 0                       | 0                  | 0                     | 7               | 0                                        | 1                       | 0                                    |        |               |
| No. of multistate clusters    | 3                    | 8           | 0                      | 4                      | 1                       | 2                  | 1                     | 31                           | 2                                        | 0                                    | 0      |               |
| Confirmed/suspect/probable cases | 100                | 118         | 151                    | 93                     | 19                      | 39                 | 87                    | 453                          | 26                                       | 133                                   | 67      |               |
| Deaths                        | 0                    | NA          | NA                     | NA                     | NA                      | NA                 | NA                    | NA                           | NA                                       | 0                                    | 0      |               |
| Total local and multistate clusters | 4               | 12          | 0                      | 7                      | 1                       | 2                  | 1                     | 38                           | 2                                        | 1                                    | 0      |               |
| Total WA clinical case isolates pulsed     | 94                  | 111         | 150                    | 87                     | 27                      | 36                 | 87                    | 350                          | 20                                       | 23                                   | 0       |               |
| 2013                          | 0                    | 9           | 0                      | 3                      | 0                       | 0                  | 0                     | 3               | 1                                        | 0                       | 0                                    |        |               |
| No. of local clusters         | 0                    | 9           | 0                      | 3                      | 0                       | 0                  | 0                     | 3               | 1                                        | 0                       | 0                                    |        |               |
| No. of multistate clusters    | 3                    | 12          | 2                      | 7                      | 7                       | 2                  | 1                     | 19                           | 4                                        | 0                                    | 0      |               |
| Confirmed/suspect/probable cases | 137                | 165         | 141                    | 98                     | 24                      | 21                 | 35                    | 330                          | 21                                       | 122                                   | 90      |               |
| Deaths                        | 3                    | NA          | NA                     | NA                     | NA                      | NA                 | NA                    | NA                           | 0                                        | 0                                    | 0      |               |
| Total local and multistate clusters | 3               | 21          | 2                      | 10                     | 7                       | 2                  | 1                     | 22                           | 5                                        | 0                                    | 0      |               |
| Total WA clinical case isolates pulsed     | 130                 | 157         | 146                    | 94                     | 38                      | 21                 | 34                    | 305                          | 25                                       | 13                                   | 0       |               |
| 2014                          | 1                    | 0           | 2                      | 0                      | 0                       | 0                  | 0                     | 1               | 0                                        | 1                       | 0                                    |        |               |
| No. of local clusters         | 1                    | 0           | 2                      | 0                      | 0                       | 0                  | 0                     | 1               | 0                                        | 1                       | 0                                    |        |               |
| No. of multistate clusters    | 4                    | 4           | 7                      | 10                     | 4                       | 2                  | 2                     | 19                           | 5                                        | 0                                    | 0      |               |
| Confirmed/suspect/probable cases | 159                | 103         | 217                    | 67                     | 67                      | 21                 | 31                    | 336                          | 24                                       | 157                                   | 92      |               |
| Deaths                        | 2                    | NA          | NA                     | NA                     | NA                      | NA                 | NA                    | NA                           | NA                                       | 0                                    | 0      |               |
| Total local and multistate clusters | 5               | 4           | 9                      | 12                     | 4                       | 2                  | 2                     | 20                           | 5                                        | 1                                    | 0      |               |
| Total WA clinical case isolates pulsed     | 153                 | 94          | 206                    | 60                     | 70                      | 22                 | 31                    | 291                          | 20                                       | 8                                    | 0      |               |

Table 1. (Continued)
| Criteria | STEC (all serotypes) | STEC (O157) | Salmonella Enteritidis | Salmonella Typhimurium | Salmonella I 4,5,12:i:- | Salmonella Newport | Salmonella Heidelberg | Salmonella Typhi | All other Salmonella serotypes (non-Typhi) | Listeria monocytogenes | Shigella sonnet and flexneri | Vibrio | 11-Year totals |
|----------|----------------------|-------------|-----------------------|-----------------------|------------------------|------------------|---------------------|----------------|---------------------------------|-------------------------|------------------------|---------|----------------|
| 2015     |                      |             |                       |                       |                        |                  |                     |                |                                 |                         |                        |         |                |
| No. of local clusters | 7  | 4  | 3  | 3  | 4  | 0  | 3                | 4             | 1                | 0                   | 0                      |                        |         |                |
| No. of multistate clusters | 2  | 9  | 7  | 3  | 5  | 4  | 3                | 21          | 4                | 1                   | 0                      |                        |         |                |
| Confirmed/suspect/probable cases | 181 | 157 | 208 | 74 | 224 | 31 | 36              | 461 | 21 | 152 | 68 |                        |         |                |
| Deaths | 1  | NA | NA | NA | NA | NA | NA                | NA          | 0                | 0                   | 0                      |                        |         |                |
| Total local and multistate clusters | 9  | 13 | 10 | 6  | 9  | 4  | 6                | 25           | 5                | 1                   | 0                      |                        |         |                |
| Total WA clinical case isolates pulsed | 165 | 149 | 198 | 69 | 234 | 35 | 38              | 350 | 21 | 8 | 0 |                        |         |                |
| 2016     |                      |             |                       |                       |                        |                  |                     |                |                                 |                         |                        |         |                |
| No. of local clusters | 0  | 8  | 2  | 4  | 0  | 0  | 1                | 11           | 0                | 1                   | 0                      |                        |         |                |
| No. of multistate clusters | 2  | 6  | 8  | 1  | 6  | 6  | 2                | 22           | 2                | 2                   | 0                      |                        |         |                |
| Confirmed/suspect/probable cases | 154 | 97 | 195 | 79 | 70 | 16 | 18              | 376 | 14 | 191 | 63 |                        |         |                |
| Deaths | 0  | NA | NA | NA | NA | NA | NA                | NA          | 0                | 0                   | 1                      |                        |         |                |
| Total local and multistate clusters | 2  | 14 | 10 | 5  | 6  | 6  | 3                | 33           | 2                | 3                   | 0                      |                        |         |                |
| Total WA clinical case isolates pulsed | 153 | 91 | 188 | 74 | 71 | 18 | 18              | 239 | 16 | 27 | 0 |                        |         |                |
| 2017     |                      |             |                       |                       |                        |                  |                     |                |                                 |                         |                        |         |                |
| No. of local clusters | 1  | 3  | 0  | 2  | 1  | 2  | 0                | 10           | 0                | 1                   | 0                      |                        |         |                |
| No. of multistate clusters | 3  | 5  | 2  | 2  | 3  | 3  | 0                | 17           | 2                | 0                   | 0                      |                        |         |                |
| Confirmed/suspect/probable cases | 187 | 90 | 147 | 79 | 44 | 61 | 14              | 465 | 17 | 285 | 96 |                        |         |                |
| Deaths | 1  | NA | NA | NA | NA | NA | NA                | NA          | 3                | 0                   | 0                      |                        |         |                |
| Total local and multistate clusters | 4  | 8  | 2  | 4  | 5  | 0  | 27               | 27           | 2                | 0                   | 1                      |                        |         |                |
| Total WA clinical case isolates pulsed | 173 | 85 | 147 | 74 | 50 | 61 | 15              | 375 | 16 | 5 | 16 |                        |         |                |

PFGE, pulsed-field gel electrophoresis; STEC, Shiga toxin-producing Escherichia coli; WA, Washington.
meat products; so producers can attempt but are not required to control it. *Salmonella* spp. can grow as biofilms on common surfaces used to process food, including stainless steel. Cross-contamination may be one of the main obstacles in reducing the prevalence of these bacteria in restaurants and other food-processing establishments as sources of recurrent outbreaks in WA (CDC, 2008a, 2013; Paz-Mendez et al., 2017; Green et al., 2018). Several reports have highlighted the potential for various serotypes of *S. enterica* to grow within the phyllosphere of several food-producing plants when exposure to this pathogen occurs through the soil or irrigation water (Barak et al., 2008; Gu et al., 2011; Zheng et al., 2013; Haendiges et al., 2018). These characteristics make *Salmonella* outbreaks linked to produce categories likely to occur in the future. Travel abroad is another well-recognized risk factor for salmonellosis (Ekdahl et al., 2005) as noted in this report. Contact with live poultry and amphibians was another common outbreak source in Washington that is well-recognized as a risk factor (Woodward et al., 1997; Behravesh et al., 2014; Basler et al., 2016; Bosch et al., 2016; Ribas and Poonlaphdecha, 2017).

Several large outbreaks in WA have been linked to *Salmonella* contamination of foods. An outbreak in 2014 linked to eating a raw beef “kitfo” dish sickened over 40 people. Starting in 2007, peanut butter was recognized as a new vehicle for salmonellosis (Sheth et al., 2011). WA reported 27 ill from 2 nut butter outbreaks since 2007. In 2015 there was the largest pork-associated salmonellosis outbreak in WA history (CDC, 2015a; Kawakami et al., 2016). This multiclonal *Salmonella* outbreak was linked to whole hogs from a slaughter facility and resulted in a large pork recall. Slaughter facilities in the past have been recognized as the most important source of *Salmonella* contamination for *Salmonella*-free hogs (Swanenburg et al., 2001a, 2001b).

### Table 2. Predominant Salmonella Serovars Detected in Washington State During 2007–2017

| Serotype       | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | Total |
|----------------|------|------|------|------|------|------|------|------|------|------|------|-------|
| Agona          | 13   | 25   | 9    | 15   | 18   | 9    | 9    | 6    | 11   | 4    | 7    | 126   |
| Anatum         | 3    | 9    | 7    | 7    | 8    | 3    | 8    | 2    | 3    | 5    | 62   |
| Bareilly       | 1    | 3    | 2    | 2    | 9    | 2    | 1    | 0    | 1    | 6    | 29   |
| Berta          | 0    | 0    | 0    | 3    | 3    | 4    | 3    | 2    | 6    | 6    | 2    |
| Braenderup      | 9    | 14   | 11   | 17   | 22   | 9    | 8    | 20   | 19   | 12   | 155  |
| Brandenburg     | 4    | 1    | 0    | 5    | 8    | 4    | 11   | 2    | 5    | 11   | 3    |
| Chester         | 2    | 3    | 1    | 10   | 0    | 1    | 2    | 1    | 2    | 3    | 27   |
| Dublin          | 6    | 2    | 4    | 8    | 5    | 2    | 3    | 8    | 6    | 8    | 5    |
| Enteritidis     | 120  | 199  | 147  | 173  | 137  | 151  | 148  | 217  | 208  | 195  | 147  |
| Hadar           | 7    | 9    | 15   | 6    | 12   | 13   | 6    | 8    | 14   | 3    | 7    |
| Havana         | 2    | 1    | 2    | 3    | 1    | 1    | 1    | 3    | 6    | 0    | 1    |
| Heidelberg      | 39   | 31   | 63   | 52   | 27   | 87   | 35   | 31   | 36   | 18   | 14   |
| I 4,12:i:-      | 8    | 6    | 8    | 0    | 0    | 1    | 0    | 2    | 10   | 0    | 5    |
| I 4,5,12:b:-    | 0    | 0    | 0    | 0    | 0    | 2    | 2    | 13   | 15   | 8    | 11   |
| I 4,5,12:i:-    | 46   | 17   | 19   | 10   | 13   | 28   | 38   | 67   | 224  | 70   | 44   |
| Infantis        | 10   | 11   | 15   | 18   | 11   | 22   | 13   | 19   | 18   | 24   | 28   |
| Javiana         | 10   | 10   | 9    | 11   | 11   | 8    | 7    | 7    | 13   | 17   |
| Kentucky        | 1    | 3    | 3    | 3    | 2    | 2    | 7    | 2    | 2    | 3    |
| Litchfield      | 1    | 16   | 4    | 4    | 2    | 1    | 2    | 3    |
| Mbandaka        | 7    | 6    | 5    | 10   | 6    | 6    | 4    |
| Montevideo      | 32   | 34   | 44   | 29   | 13   |
| Muenchen         | 12   | 6    | 12   | 12   |
| Newport          | 58   | 39   | 29   | 29   | 39   | 20   |
| Oranienburg      | 12   | 10   | 21   | 14   | 10   |
| Panama           | 3    | 3    | 5    |
| Paratyp A        | 3    | 2    | 3    | 1    | 3    |
| Paratyp B        | 2    | 1    | 1    | 1    |
| Paratyp B var. L(+) tartrate(+) | 17 | 19 | 18 | 14 | 11 | 8 | 14 | 5 | 8 | 10 | 28 | 152 |
| Poona            | 5    | 19   | 2    | 9    | 1    | 11   | 7    | 6    | 26   | 4    | 5    |
| Potsdam         | 1    | 1    | 6    | 0    | 2    | 0    | 0    | 1    | 1    |
| Saintpaul        | 31   | 27   | 22   | 12   | 5    | 8    |
| Sandiego         | 5    | 3    | 1    | 3    | 1    |
| Seftenberg       | 29   | 20   | 6    | 7    |
| Stanley          | 21   | 9    | 10   | 7    |
| Thompson         | 11   | 9    | 19   | 16   |
| Typhi            | 24   | 25   | 61   | 61   |
| Typhimurium      | 121  | 133  | 148  | 127  |
| Virchow          | 1    | 4    | 5    | 3    |
| Weltevreden      | 1    | 6    | 1    |

Additional serotypes reported every year can be found in the annual WA communicable disease surveillance reports (Department of Health).

Source: Washington State Department of Health.
Many of the STEC outbreaks (2007–2017) were associated with previously reported high-risk food vehicles particularly undercooked beef, raw sprouts, and unpasteurized milk (Erickson and Doyle, 2007; Neil et al., 2012; Luna-Gierke et al., 2014; Morton et al., 2017) in addition to flour, which has emerged as a risk factor for STEC infections in recent years (Morton et al., 2017). Animal exposures at petting zoos and state fairs are also a significant source of STEC infections. In 2015, WA reported an E. O157:H7 outbreak linked to attendance at a dairy education event. Environmental samples collected at the event site yielded PFGE patterns indistinguishable from the outbreak strain (Dunbar et al., 2015).

With the release of Shiga toxin EIA that allow clinical laboratories to better identify non-O157, there was a concomitant reduction in STEC culture submissions to WAPHL. In addition, with the emergence of polymerase chain reaction-based enteric testing, an increase in stool specimen submissions was noted (as opposed to isolate submissions). CIDT has impacted the workflow at WAPHL as specimen submissions have increased and isolate submissions have decreased. This trend is predicted to continue in future years. It will be important for the WAPHL to facilitate isolate recovery in future years as these new technologies expand and replace current testing workflows at clinical laboratories.

Listeriosis associated with ice cream, raw milk, and Mexican style soft cheeses was identified as a problem as early as 1985 and continues to this day (Linnan et al., 1988; Jackson et al., 2018). The ubiquity of L. monocytogenes in the environment and its potential to grow in biofilms mean that a previously unrecognized food vehicle could cause a foodborne outbreak (Ferreira et al., 2014). WA had two notable recurring listeriosis outbreaks from dairy products.

**Table 3. Foods Associated with Clusters and Outbreaks in Washington 2007–2017**

| IFSACa category | Etiology | Serotype(s) | No. of WA cases | No. of outbreaks |
|-----------------|----------|-------------|----------------|-----------------|
| Beef            | *Escherichia coli*, Shiga toxin–producing | O157:H7 | 9 | 2 |
| Beef            | *Salmonella enterica* | Senftenberg, Typhimurium, Braenderup | 20 | 3 |
| Chicken         | *S. enterica* | Heidelberg, I 4,[5],12:i:- | 104 | 5 |
| Dairy           | *E. coli*, Shiga toxin–producing | O157:H7; O121, O26:H11, O157:NM(H-), | 18 | 5 |
| Dairy           | *Listeria monocytogenes* | | | |
| Dairy           | *S. enterica* | Dublin | 3 | 1 |
| Eggs            | *S. enterica* | Enteritidis, Typhimurium | 69 | 2 |
| Fish            | *S. enterica* | Paratyphi B var. L(+) tartrate +, Weltevreden | 1 | 1 |
| Fruits          | *L. monocytogenes* | | | |
| Fruits          | *S. enterica* | I 4,[5],12:b:- var. L(+), Litchfield, Panama, Agona, Braenderup, Worthington, Enteritidis, Chayle, Infantis, Newport | 116 | 10 |
| Grains—beans    | *E. coli*, Shiga toxin–producing | O121, O26:NM | | |
| Herbs           | *S. enterica* | Wandsworth, Typhimurium | 33 | 4 |
| Nuts—seeds      | *E. coli*, Shiga toxin–producing | O157:H7 | 2 | 1 |
| Nuts—seeds      | *S. enterica* | Typhimurium, Newport, Hartford, Oranienburg, Gaminara, Montevideo, Seftenberg | 29 | 4 |
| Oils—sugars     | *S. enterica* | Virchow | 1 | 1 |
| Other           | *S. enterica* | Heidelberg, I 4,[5],12:b:- var. L(+), Javiana, Okatie, Thompson, Weltevreden | 16 | 1 |
| Pork            | *S. enterica* | Enteritidis, I 4,[5],12:i- | 215 | 5 |
| Seeded vegetables | *S. enterica* | Saintpaul, Newport, Paratyphi B, Poona | 66 | 5 |
| Sprouts         | *E. coli*, Shiga toxin–producing | O26, O121 | 12 | 2 |
| Sprouts         | *S. enterica* | Typhimurium, Newport, Enteritidis, Muenchen, Cubana, Kentucky | 34 | 4 |
| Turkey          | *S. enterica* | Subspecies IIIa, Hadar, I 4,[5],12:i:- | 12 | 3 |
| Vegetable row crops | *E. coli*, Shiga toxin–producing | O157:H7, O157:NM(H-), O26 | 28 | 9 |
| Vegetable row crops | *S. enterica* | Typhimurium, Javiana, Enteritidis | 30 | 3 |
| Multiple        | *E. coli*, Shiga toxin–producing | O157:H7, O121 | 79 | 6 |
| Multiple        | *L. monocytogenes* | | | |
| Multiple        | *S. enterica* | IV 50:z4,z23:-, Typhimurium, Sandiego, I 4,[5],12:i- | 279 | 18 |
|               |           | Enteritidis, Muenchen, Newport, Chester, Anatum, Heidelberg, Thompson, Paratyphi B var. L(+) tartrate + |

*a www.cdc.gov/foodsafety/ifsac/projects/food-categorization-scheme.html
Two patients hospitalized at the same facility in 2014–2015 and one a year later in 2016 developed listeriosis found to be linked to pasteurized ice cream served at the facility and produced by a local company (Rietberg et al., 2016). Pasteurized soft Mexican cheese produced by a local firm sickened several people in 2010 and again in 2015. Sushi and frozen vegetables have also been linked to listeriosis outbreaks in WA.

The implementation of policies or campaigns to encourage the use of specific interventions, in addition to the implementation of better identification tools (on-site rapid testing, whole-genome sequencing), may lead to the reduction in the incidence of enteric infections. There is strong evidence indicating that in areas of the country where these infections are investigated, such as FoodNet sites, there has been a reduction (by 30%) in illness incidence (Ailes et al., 2008). Better access to rapid test kits that can identify the presence of pathogens at food-processing facilities is also needed. Public health will, in the meantime, continue to rely on surveillance of notifiable conditions through the work of local health jurisdictions who conduct epidemiological and environment investigations. It is possible that the impact of the use of NGS tools may be overshadowed by the impact of CIDTs as fewer illnesses get characterized with an isolate culture that can then flow to get characterized by NGS. Nonetheless, NGS characterization offers unparalleled resolution in providing evidence to pathogen relatedness that will revolutionize the way foodborne disease investigations are conducted in the laboratory as PFGE is phased out.

To understand the impact of future laboratory testing as the use of NGS becomes more streamlined, it would be important for reference laboratories to track the amount of time it takes to detect clusters, number of outbreaks solved with food source identified, number of cases per cluster, and number of cases linked to a food source. In addition, there is work to be carried out to increase the proportion of stool samples submitted for laboratory testing for foodborne illnesses (Ailes et al., 2012) and in laboratory methodologies that ensure the recovery of an isolate. Characterization of isolates remains the key to a solved foodborne disease investigation (Hurd et al., 2012).

**Limitations**

Foodborne diseases attributed to botulism, norovirus, and yersiniosis were not evaluated. In addition, data for *Campylobacter* and *Shigella* are not complete as WAPHL did not test all the submitted isolates by PFGE. In WA the investigation of campylobacteriosis individual cases is considered optional (Washington State Department of Health, 2016).

Although the case counts were provided, most PHL data were missing vehicle source or cluster association data other than PFGE. All outbreaks and clusters reported herein were closed at the time of the writing of this article.

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