We investigated the use of whole-genome mapping and pulsed-field gel electrophoresis (PFGE) with isolates from an outbreak of Salmonella enterica serotype Saintpaul. PFGE and whole-genome mapping were concordant with 22 of 23 isolates. Whole-genome mapping is a viable alternative tool for the epidemiological analysis of Salmonella food-borne disease investigations.

Pulsed-field gel electrophoresis (PFGE), which relies on genetic polymorphisms within restriction enzyme recognition sites and large-scale insertions, deletions, and rearrangements that disrupt the restriction fragment profile (10), has been used for epidemiological analyses of Salmonella. In conjunction with the PulseNet tracking system, PFGE has been highly effective in assessing the epidemiological relationships of various Salmonella isolates associated with outbreaks of food-borne disease (1, 2). However, with some serotypes, such as Salmonella enterica serotype Enteritidis, it is difficult to interpret PFGE data because of genetic homogeneity (13). Therefore, other methods have been used to assess the epidemiological relatedness of Salmonella isolates, including determination of the variable number of tandem repeats and multilocus sequence typing (3).

An alternative molecular approach to global genomic analysis, termed whole-genome mapping, holds significant potential for the characterization of bacteria. Whole-genome maps have been used to assist in the closing of entire genomic sequences and, in addition, have been used to ascertain epidemiological relationships of Escherichia coli O157:H7 (4–7, 9, 11, 12, 14, 15).

A recent outbreak in 2009 involved infection with Salmonella serotype Saintpaul linked to the consumption of alfalfa sprouts (2). The investigation of that outbreak resulted in the detection of 228 cases of salmonellosis in 13 states occurring in two distinct waves of disease, as described by the Centers for Disease Control and Prevention. Twenty-three Salmonella Saintpaul isolates obtained from this well-defined outbreak provided the opportunity to evaluate whole-genome mapping as an epidemiological tool compared to PFGE.

Forty-three Salmonella strains were obtained from the Nebraska Public Health Laboratory, including 28 of Salmonella Saintpaul (Table 1), 6 of Salmonella serotype Typhimurium (10556, 10633, 10684, 10702, 10803, and 11022), 3 of Salmonella serotype Newport (10532, 10722, and 11023), 2 of Salmonella serotype Enteritidis (10454 and 10712), 2 of Salmonella serotype Heidelberg (10495 and 10773), and 1 each of Salmonella serotypes Stanley (100700) and Paratyphi B (11005).

Preparation of chromosomal DNA suitable for PFGE was performed essentially as described by Ribot et al. (10). DNA was digested with XbaI and analyzed using BioNumerics software (version 4.0; Applied Maths, Sint-Martens-Latem, Belgium). PFGE demonstrated that all of the S. Saintpaul isolates obtained from the first and second waves of illness (Table 1) were highly related (Fig. 1). Isolate 10600 contained one additional band of low molecular weight, which was determined to be of plasmid origin (data not shown), in comparison to the other S. Saintpaul isolates (Fig. 1). In contrast, the three S. Saintpaul isolates obtained prior to the first wave of illness (8995, 9495, and 10013) and the two isolates obtained after the second wave of illness (10946 and 11028) were distinguishable from the outbreak group as assessed by PFGE. Of these two S. Saintpaul isolates, 10013 and 11028 were indistinguishable from one another, although 10013 was isolated prior to the outbreak and 11028 was isolated following the second wave of illness. When other Salmonella serotypes from the repository were evaluated, all were found to be clearly distinguishable from the S. Saintpaul outbreak strains. Interestingly, each of four pairs of isolates (S. Typhimurium 10803 and 11022, S. Typhimurium 10633 and 10804, S. Heidelberg 10773 and 10495, and S. Enteritidis 10712 and 10454) had indistinguishable PFGE patterns and thus may have been epidemiologically related.

High-molecular-weight DNA of each Salmonella isolate was prepared directly from isolated colonies using the OpGen sample preparation kit (OpGen, Inc., Gaithersburg, MD) and the Agen-court Genfind v2 kit (Beckman Coulter). Whole-genome maps were produced with the Argus whole-genome mapping system. NcoI was chosen as the most appropriate restriction enzyme by using software (Enzyme Chooser; OpGen, Gaithersburg, MD) that identifies enzymes which result in a 6- to 12-kb average fragment size and no single restriction fragment larger than 80 kb. To determine the resolution of whole-genome mapping, a well-characterized and sequenced isolate of Francisella tularensis subsp. tularensis was used (GenBank accession number CP001633) (8). Two restriction enzyme digestions (NcoI, NdeI) of the isolate were performed and compared. Comparison of the control DNA sequences of F. tularensis demonstrated that whole-genome mapping is capable of correctly placing a 2-kb restriction fragment; therefore, the resolution was experimentally determined to be at least 2 kb; however, no fragments of less than 3 kb were used to characterize the Salmonella isolates.

Analysis of the 43 Salmonella isolates by whole-genome mapping was performed, and the results were compared to the PFGE results (Fig. 2). Using this approach, all S. Saintpaul
strains from the outbreak were determined to be indistinguishable by whole-genome mapping. The outbreak pattern included isolate 10600, in which an additional plasmid band had been seen using PFGE (Fig. 1). All five pre- and postoutbreak isolates were distinguishable from the outbreak isolates; 10013 and 11028 were considered indistinguishable, in concordance with the PFGE results. As expected, all non-S. Saintpaul whole-genome maps were distinct from those of the S. Saintpaul out-

TABLE 1  

<table>
<thead>
<tr>
<th>Time period</th>
<th>Dates</th>
<th>Salmonella Saintpaul isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to outbreak</td>
<td>Before 2009</td>
<td>8995, 9495, 10013</td>
</tr>
<tr>
<td>1st wave of illness</td>
<td>1 February–15 March 2009</td>
<td>10520, 10521, 10522, 10523, 10582, 10586, 10587, 10600, 10601, 10612, 10614, 10621, 10644, 10645, 10646, 10648, 10658, 10660</td>
</tr>
<tr>
<td>2nd wave of illness</td>
<td>16 March–18 April 2009</td>
<td>10680, 10690, 10695, 10701, 10741</td>
</tr>
<tr>
<td>Postoutbreak</td>
<td>After 18 April 2009</td>
<td>10946, 11028</td>
</tr>
</tbody>
</table>

a Dates are based on a CDC report defining the outbreak epidemic curve (2).

FIG 1 PFGE analysis of the Salmonella isolates used in this study.
break isolates. In concordance with PFGE results, isolate pairs 10803–11022 (S. Typhimurium), 10633–10684 (S. Typhimurium), and 10495–10773 (S. Heidelberg) were indistinguishable as assessed by whole-genome mapping. However, S. Enteritidis isolates 10454 and 10712 were divergent by whole-genome mapping, in contrast to the PFGE result.

Whole-genome mapping generates a finely detailed restriction enzyme map that has the added advantage of providing the order of the fragments in the genome. However, questions have arisen as to whether the increased resolution of whole-genome mapping would generate an excessively large number of fragments that would exceed the ability to link the variations to one serotype or outbreak strain. Results from this study demonstrate that molecular relationships determined by whole-genome mapping are equivalent to those from PFGE-generated data. Therefore, whole-genome mapping has potential value as an epidemiological analysis tool for the investigation of outbreaks of disease caused by Salmonella. Further work is required to determine whether whole-genome mapping can be used as an epidemiological tool with other bacteria of epidemiological importance such as Staphylococcus aureus and difficult-to-type bacteria such as Salmonella serotype Enteritidis and Clostridium difficile.

REFERENCES


