Polyphasic Characterization of Five Different Serotypes of Salmonella enterica from a Food-Borne Outbreak that Occurred in Pennsylvania and Near-by States, July 2004

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Abstract

Background: Salmonella is a common food-borne pathogen, causing an estimated 1.4 million cases of salmonellosis each year in the United States. Five Salmonella enterica serotypes: Anatum, Javiana, Muenchen, Thompson, and Typhimurium, were isolated by the Pennsylvania Department of Health (PDEH) from a food-borne outbreak that occurred in Pennsylvania and near-by states in 2004, which was associated with the consumption of tomatoes. The PDEH reported that pulsed-field gel electrophoresis (PFGE) played a key role in identifying the outbreak-associated isolates. In this study, we characterized forty-one Salmonella enterica isolates from the outbreak using a diversity of methods.

Methods: Isolates were characterized using biochemical (API), serotyping (Kaufman-White), and molecular methods including rep-PCR (Bacterial Barcodes), Real-Time PCR, 16S Ribosomal RNA gene, and ribotyping (Riboprinter®).

Results: By employing the polyphasic approach, we verified that all 41 Salmonella strains from the outbreak were enterica serovars. Serotyping, Rep-PCR and ribotyping profiles further confirmed the serological typing results as previously reported. Moreover, diversity among the isolates was observed by molecular typing and serotyping. Among the molecular authentication methods, rep-PCR was less accurate than ribotyping in identifying the serotypes. This may be due to the limited number of serovars representative in the Bacterial Barcodes library. There was high concordance between serological testing and ribotyping for serotypes Anatum (group E), Javiana (group D), and Typhimurium (group B) and one sample each of Thompson and Muenchen. On the other hand, the concordance between serological testing and ribotyping for the serovars Muenchen (group C1 or C1 and C2) and Thompson (group C1 and C2) was less stringent.

Conclusions: Ribotyping was found to be a straightforward technique for molecular confirmation of enterica serovars, and it has advantages over other molecular methods such as PFGE which is more labor-intensive.

Introduction

BEI Resources strives to serve the scientific community by providing high quality products and is constantly searching for new methodologies to characterize and authenticate microorganisms. BEI Resources also maintains a impeccable Quality Assurance program to ensure that the products meet customer expectations as well as ISO guidelines. With this goal in mind, we performed a polyphasic analysis. Salmonella enterica associated with a food-borne outbreak.

Salmonella infection is one of the most frequently reported causes of food-borne disease in the United States (Mead et al., 1999). Diagnosis of the disease is often characterized by bacterial culture from stool samples or more by molecular techniques. In this study, we evaluated 41 Salmonella isolates deposited in the BEI Resources collection maintained by ATCC. The entire collection is comprised of five serotopes and was analyzed using biochemical, serotyping and molecular techniques. All of these methods are based on diverse analytical techniques and may have limitations due to the methodology and/or available database. The results of this polyphasic approach are reported here.

Methods

Bacterial Cultures:
The Salmonella isolates used in this study are from a recent outbreak described by Sandt et al., 2006. All have been deposited in BEI Resources (NR-4291 to NR-5430). Each strain was grown on Trypticase Soy Agar (TSA) plates for 18-24 hours at 37°C.

Biochemicals:
API 20E: Assay was performed following the manufacturer instructions.

Serological assay:
The Salmonella O and H antisera were purchased from Becton Dickinson (Poly group A1 Group A, B, C1, D2, D3, E, F, G, H, and V). The slide agglutination assay was performed in triplicate on each isolate per manufacturer instructions. Each of the 41 strains was tested against all the serotypes; a positive reaction (3+ and 4+) did not exclude that sample from further testing. Seria salina solution (0.8%) was used as a negative control. Agglutination reactions were scored from negative (no agglutination) to 4+ (100% of cells agglutinate). All reactions were read within one minute or less.

Genomic DNA:
BEI Resources provided 33 of the 41(NR-4572 to NR-4618) genomic DNA. The remaining eight samples were extracted using the Ultra Clean Microbial DNA Isolation kit (MO BIO Laboratories, Inc.).

16S sequencing: 16S sequencing was performed by the ATCC Molecular Authentication Resource Center (MAR) per standard ATCC practice.

Real-time PCR:
Real-time PCR was performed with the TaqMan® Salmonella enterica kit on an ABI Fast 7500 (Applied Biosystems). All assays were performed in optical 96-well plates. The DNA was diluted to a concentration of 10 pg/μL DNA from S. enterica (ATCC® 700631-5) used as a positive control and was serially diluted from 1.000 to 0.001 pg.

Bacterial Barcodes (BBC): Each sample was assayed by Rep-PCR in duplicate and each assay was repeated at least three times. The DiversityLab® Salmonella kit (BioMérieux Clinical Diagnostics) was used to perform all Rep-PCR reactions as described in the manufacturer instructions with AmpliTag® DNA Polymerase (Applied Biosystems). The PCR reactions were loaded into DiversiTaq and run on a Caliper biocycler (Caliper Technology Corp.). Results were analyzed by two methods: Pearson Correlation (PC) or modified Kullback-Leibler (KL), to calculate the percent similarities. Each set of samples, comprising one or more serovars, was submitted to the appropriate Salmonella Bacterial Barcode Library. Each strain of the serovar was then directly compared to each of the Libraries Top Five Matches (five highest similarity values) using the interactive overlay program. Based on the number of commonly shared peaks and the associated similarity value, the serovar was chosen from one of the Libraries Top Five Match.

Riboprinter® (Riboprinter®): DNA samples were run on the Riboprinter®. All 41 strains were run in duplicate on the Dupont Qualicon Riboprinter®. Cells were prepared as described in the Quaiction manual. DNA was restricted with PvuII. The Riboprinter® electrophoresis gel was scanned and compared each sample to the appropriate library and assigns a Dupont identification (Dup-ID) for each sample with a similarity value of 0.85 or greater. When the Dup-ID did not achieve the expected serotype, the sample’s Riboprinter® pattern was compared to the pattern of its nearest neighbor. The nearest neighbor is a list of potential Dup-IDs taken from the Dup-Library.

Results and Discussion

API 20E:
The API 20E® is a commonly used approach for the biochemical characterization of Salmonella strains. API identified all 41 strains as Salmonella spp. with a confidence level ranging from 87 to 99%.

Real-Time PCR and DNA Sequencing of the 16S Ribosomal RNA genes:
Real-Time PCR and 16S DNA Sequencing confirmed that all were Salmonella enterica.

Serotyping - Slides agglutination assay:
The slide agglutination assay is one of the most accurate methods for serotyping S. enterica. All 41 strains exhibited the expected serotype (Table 1); however, a few strains from three different serotypes – Muenchen, Thompson, and Typhimurium – exhibited cross reactively with different serotypes (Table 1). This cross reactivity could lead to some confusion, stressing the need to use a polyphasic approach for serotyping S. enterica.

Table 1: Serological Identification of Salmonella enterica

<table>
<thead>
<tr>
<th>Number of strains</th>
<th>Anatum</th>
<th>Javiana</th>
<th>Muenchen</th>
<th>Thompson</th>
<th>Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>8</td>
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<tr>
<td>0.8</td>
<td>5</td>
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<td>10</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>0.6</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>0.4</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2: BBC Classification report vs. Riboprinter® Dupont Identification

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Number of Times</th>
<th>PC-BBC</th>
<th>KL-BBC</th>
<th>Riboprinter®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatum</td>
<td>5</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Javiana</td>
<td>10</td>
<td>60%</td>
<td>80%</td>
<td>90%</td>
</tr>
<tr>
<td>Muenchen</td>
<td>10</td>
<td>62.5%</td>
<td>25%</td>
<td>62.5%</td>
</tr>
<tr>
<td>Thompson</td>
<td>10</td>
<td>60%</td>
<td>80%</td>
<td>60%</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>10</td>
<td>87.5%</td>
<td>87.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Molecular serotyping – Ribosomal analysis (Dupont Identification):
Four strains, (Anatum and three Thompson), were assigned a Dup-ID other than the expected serotype. The percentage of samples assigned to the expected serotype is shown in Table 2. When any sample was assigned a Dup-ID other than the expected serotype, the sample was then compared to its nearest neighbours. The assigned Dup-ID was consistently the highest and closest match.

Conclusions

A polyphasic approach should be employed in any study in which S. enterica is being characterized and serotyped. Any unknown isolates should be assigned to the appropriate genus and species by biochemical and sequencing (16S) techniques. This investigation shows that ribotyping is a useful tool for screening S. enterica serotypes during a food-borne outbreak.

Despite assigning only 76% of the strains to their expected serotype the riboprinter's software gave consistently high similarity values (0.92 or greater). Ribotyping offers a distinct advantage over other molecular biological techniques including Pulse-field gel electrophoresis (PFGE) because it does not require previous DNA extraction, it is very user friendly, and highly automated.

References


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