Since 2013, Western Australia (WA) has experienced an emerging of predominantly community-associated C. difficile infection (CA-CDI) caused by PCR ribotype (RT) 012. C. difficile RT 012 was first identified in WA in September 2012. This previously uncommon RT quickly rose to the equal seventh most prevalent RT in 2013 and the equal second most prevalent RT by the end of 2014 (unpublished data). The increased numbers of C. difficile RT 012 cases in WA are shown in Figure 1.

Following the isolation of C. difficile RT 012 from WA-born organic beetroot in April 2015 [1], foodborne transmission of RT 012 was suspected. In response, a retrospective case-control study was carried out to determine whether consumption of certain vegetables or exposure to certain environmental factors increased the risk of CDI caused by RT 012. While this case-control study was taking place, a prevalence study in the Asia-Pacific region performed by our research group identified RT 012 as one of the most common RTs causing human CDI in Asia (16.7% in Hong Kong, 13.6% in Singapore, 11.4% in China, 6.8% in Taiwan and 5.3% in Thailand) [2]. Subsequently, the relatedness of Australian food and clinical RT 012 isolates to Asian clinical RT 012 was investigated using whole-genome sequencing (WGS) and high-resolution core genome phylogenetic analysis.

Materials and methods

A retrospective case-control study on emerging C. difficile RT 012 infection was conducted in WA to determine whether consumption of certain vegetables or exposure to certain environmental factors increased the risk of CDI caused by RT 012. Cases were defined as hospital patients who previously had RT 012 cultured from their stool samples and confirmed by molecular typing as previously described [1], between January 2014 and March 2015. Each case was matched with two controls for age (± 2 y) and gender. Controls were hospital patients identified in the same period of time, who had not had CDI caused by a RT other than RT 012. Statistical analysis was performed using SPSS. Characteristics of cases and controls were compared using Fisher’s exact test. Univariate odd ratios (ORs) were calculated and used to identify risk factors for C. difficile RT 012 infection.

WGS and core genome single-nucleotide variant (SNV) analysis were performed on 11 C. difficile RT 012 strains (Table 2). Genomic DNA was extracted using lysis matrix B (MP Biomedicals) and the QuickGene DNA tissue kit 5 (Kurabo). Multiplex paired-end (PE) genome libraries were constructed using standard Nextera XT protocols (Illumina Inc.) and sequencing was performed on a MiSeq platform (Illumina) that generated 250 reads. WGS data was assembled and annotated, and in silico multilocus sequence typing (MLST), phylogenetic analysis and comparison of SNVs were performed as described [3].

Results

Table 2. C. difficile RT 012 collection for WGS.

<table>
<thead>
<tr>
<th>Lab ID</th>
<th>Host</th>
<th>Sample type</th>
<th>Country</th>
<th>Source</th>
<th>CDI exposure</th>
<th>Sample date</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI 032</td>
<td>Food</td>
<td>Beetroot</td>
<td>Australia</td>
<td>WA</td>
<td>-</td>
<td>April-2015</td>
</tr>
<tr>
<td>WA2952</td>
<td>Adult-CIDI</td>
<td>WA</td>
<td>CIDI</td>
<td>CAI</td>
<td>Feb-2014</td>
<td></td>
</tr>
<tr>
<td>WA3410</td>
<td>Adult-CIDI</td>
<td>WA</td>
<td>CIDI</td>
<td>CAI</td>
<td>Jul-2014</td>
<td></td>
</tr>
<tr>
<td>WA3835</td>
<td>Adult-CIDI</td>
<td>WA</td>
<td>CIDI</td>
<td>HAI-HCFO</td>
<td>Nov-2014</td>
<td></td>
</tr>
<tr>
<td>SQ477</td>
<td>Adult-CIDI</td>
<td>Australia</td>
<td>SA</td>
<td>UNK</td>
<td>May-2013</td>
<td></td>
</tr>
<tr>
<td>HT0066</td>
<td>Adult-CIDI</td>
<td>Hong Kong</td>
<td>Hong Kong</td>
<td>UNK</td>
<td>Jul-2014</td>
<td></td>
</tr>
<tr>
<td>HT0088</td>
<td>Adult-CIDI</td>
<td>Taiwan</td>
<td>Kaohsiung</td>
<td>UNK</td>
<td>Jul-2014</td>
<td></td>
</tr>
<tr>
<td>HT0104</td>
<td>Adult-CIDI</td>
<td>Singapore</td>
<td>Changi</td>
<td>UNK</td>
<td>Aug-2014</td>
<td></td>
</tr>
<tr>
<td>HT0171</td>
<td>Adult-CIDI</td>
<td>Thailand</td>
<td>Bangkok</td>
<td>UNK</td>
<td>Sep-2014</td>
<td></td>
</tr>
<tr>
<td>HT0184</td>
<td>Adult-CIDI</td>
<td>South Korea</td>
<td>Daejeon</td>
<td>UNK</td>
<td>Sep-2014</td>
<td></td>
</tr>
<tr>
<td>HT0405</td>
<td>Adult-CIDI</td>
<td>China</td>
<td>Shanghai</td>
<td>UNK</td>
<td>Nov-2014</td>
<td></td>
</tr>
</tbody>
</table>

WA, Western Australia; SA, South Australia; CAI, community-associated infection; HAI-HCFO, hospital-associated healthcare facility outbreak; UNK, unknown.

Figure 2. Genomic core SNV distance between 11 C. difficile RT 012.

WGS and comparative genomics

MST

All 11 C. difficile RT 012 belong to sequence type 54 within clade 1.

In silico antimicrobial resistance (AMR) profile

Of the 11 sequenced isolates, AMR genes were identified in 72.7% (n = 8) of the isolates.

AMR genes encoding resistance to tetracycline (tet(M)), aminoglycosides (aac(6’)-Ia, AacC4-Aph, S4A4 and Aph A3-I), and macrolides-lincosamides-streptogramins (ermB) were detected in a food isolate from WA (WA325).

Of the 4 human isolates from Australia, only WA3835 carried AMR genes (ermB, tetM, AacC4-Aph).

The human isolates from China, Hong Kong, Korea and Thailand (HT0405, HT0066, HT0184 and HT0171, respectively) carried genes encoding resistance to aminoglycosides (aac(6’)-Ia-Aph) and macrolides-lincosamides-streptogramins (ermB).

The Singapore (OT0104) and Taiwan (OT0088) isolates were carrying ermB, tetM and AacC4-Aph genes.

SNVs analysis

A heatmap of pairwise SNV differences between the 11 genomes shown in Figure 2.

Two clonal groups were observed. First, a human isolate from Shanghai, China (OT0405) had zero SNV differences with a human isolate from Hong Kong (OT0066). These were isolated 5 months apart and separated by 1,26 km. Second, a human isolate from WA (WA3835) had a clonal relationship of ≤ 2 SNVs difference in their core genome with a human isolate from Thailand (HT0171).

The food isolate from WA was genetically very distant from all other sequenced isolates, with an average SNV difference of BR4.

References

2. Coles, C. The University of Western Australia, 2006.