Molecular Epidemiology of *C. difficile* Within a Community Hospital: A Pilot for a Regional Survey

**Abstract**

**Background**: *Clostridium difficile* is the leading cause of healthcare-associated infection. As incidence rises, its epidemiology is also evolving. 20-50% of cases are now community-acquired. *C. difficile* cases arise from more diverse sources than previously thought. In this study, we investigated the diversity of *C. difficile* within a community hospital.

**Methods**: Stool samples were collected from symptom-positive adults with a positive *C. difficile* PCR admitted to Duke Regional Hospital from 7/2016 to 7/2017. Healthcare-associated CDI was defined by any admission to a hospital, nursing, or dialysis facility in the preceding 30 days. *C. difficile* was isolated by ethanol shock followed by plating on CDSA media. DNA was extracted using a Chelex-based protocol and PCR ribotyping was conducted using the Biodet primers and agarose gel electrophoresis. A dendrogram was constructed in Bionumerics using the unweighted pair-group method with the threshold for identical strains set at 95% similarity.

**Results**: *C. difficile* was successfully isolated from 85% of submitted specimens. For this pilot study, PCR ribotyping was performed on a convenience sample of 70 isolates. *C. difficile* exhibited substantial diversity: 47 distinct ribotypes were observed among 70 isolates (figure 1). 14 clusters involving identical strain types were observed. totaling 35 isolates. Identical strain types were also observed. The median time between clustered cases was 50 days (range: 7 to 371 days). 35 of 50 (70%) of all isolates exhibited entirely unique strain types.

**Conclusions**: *C. difficile* isolates in our community hospital exhibited tremendous genetic diversity. The proportion of strains with entirely unique ribotypes suggests diverse sources of acquisition. These results are consistent with a growing body of literature in which 30-50% of *C. difficile* isolates are genetically distinct, even when direct transmission was suspected. We are currently expanding our survey to include a network of regional hospitals and clinics, with the goal of better characterizing *C. difficile* diversity and still poorly understood sources.

**Background**

- *C. difficile* is a leading cause of healthcare-associated infection, however its epidemiology is evolving. 20-50% of US cases are now community-acquired.
- Factors contributing to the shift in *C. difficile* from hospitals to the community hospital, and for acquisition of *C. difficile* within the community, remain poorly understood.

**Methods**

- Stool samples were collected from adults with a positive *C. difficile* PCR admitted to Duke Regional Hospital from 7/2016 to 7/2017. Healthcare-associated CDI was defined by any admission to a hospital, nursing, or dialysis facility in the preceding 30 days.
- *C. difficile* was isolated by ethanol shock followed by plating on CDSA media. DNA was extracted using a Chelex-based protocol and PCR ribotyping was conducted using the Biodet primers and agarose gel electrophoresis. A dendrogram was constructed in Bionumerics using the unweighted pair-group method with the threshold for identical strains set at 95% similarity.

**Results (continued)**

**Figure 1**: PCR ribotyping of isolates.

**Table 1**: Clinical and demographic characteristics.

**Table 2**: Epidemiologic characteristics of cases caused by identical ribotypes (presumed clusters).

**Figure 2**: Histogram of ribotypes according to source.

**Results**

- Stool samples were collected from adults with *C. difficile* infection admitted to Duke Regional Hospital from 7/2016 to 7/2017.
- Healthcare-associated CDI was defined by any admission to a hospital, nursing, or dialysis facility in the preceding 30 days.
- *C. difficile* was isolated by ethanol shock and plating on CDSA media. DNA was extracted using a Chelex-based protocol and PCR ribotyping was performed using the Biodet primers and agarose gel electrophoresis.
- Ribotypes were identified and separated by the unweighted pair-group method with a threshold for identical strains set at 98% similarity.

**Conclusions**

- *C. difficile* shows substantial genetic diversity, suggestive of diverse sources of acquisition and consistent with results from Eyre et al in the Oxford Hospital system.
- The epidemic strain 027 was conspicuously absent from Duke Regional Hospital in 2017.
- In a small sample size to date, HA and CA-CID showed relatively little intermixing of strain types.