Abstract

Background: Clostridium difficile infection (CDI) incidence has increased dramatically in the past decade, making CDI one of the most common causes of infectious diarrhea and an urgent public health threat (1). Understanding the biological features and spread of C. difficile strains can help target control efforts. PCR-ribotyping, the current method of choice for C. difficile typing, remains subjective and challenging for inter-laboratory comparisons. Multilocus sequence typing (MLST), based on the allelic variation of seven housekeeping genes, represents a more robust tool that would enhance inter-laboratory reproducibility. However, a comprehensive translation system to ribotyping is a prerequisite. Here we describe the concordance between MLST and PCR-ribotyping.

Methods: The Centers for Disease Control and Prevention’s (CDC) Emerging Infections Program (EIP) conducts CDC surveillance in 10 U.S. sites. C. difficile isolates cultured from a subset of cases underwent capillary electrophoresis PCR-ribotyping at CDC. A representative sample, selected from the top 30 ribotypes (RTs), underwent whole genome sequencing (WGS) at Minnesota Department of Health. An additional subset of isolates, representing the top 10 RTs, underwent WGS at CDC. At both laboratories, the Illumina MiSeq platform was used to obtain 250 bp paired-end sequencing reads. MLST analyses were done using the pubMLST C. difficile scheme.

Results: A total of 479 C. difficile isolates, including at least 10 isolates for each RT, were analyzed by WGS. Among the 30 RTs represented, 35 different MLST sequence types (STs) were identified. Twenty-two of the RTs (including 077) were each associated with a single/1:2 unique ST, while 8 RTs (020, 014, 015, 076, 046, 153-251, 027, and 075) presented more genetic diversity with single-locus or double-locus variants, resulting in multiple STs within one ribotype. There were two instances of two different RTs sharing the same ST.

Conclusion: Multilocus sequence typing and PCR-ribotyping showed comparable discriminatory abilities. However, the ST is not always predictive of the RT and vice versa. This represents the first step towards a transition to using WGS for standard C. difficile typing.

Prevalence of C. difficile Ribotypes in the US, 10 EIP sites, 2012 – 2016

Majority of Ribotypes Showed Good Correlation with Sequence Types

• 22 (73%) of 30 ribotypes had a single sequence type

Reference


MLST results concord with Whole genome hg-SNP analyses and epidemiological background

Conclusion/Future directions

Δ MLST showed promising results as a replacement for traditional ribotyping as the primary subtyping tool.

Δ Additional crosswalk efforts are needed, including comparing more samples for each ribotype and performing a crosswalk between MLST and less frequently identified C. difficile ribotypes.

Δ The actual genetic relatedness of C. difficile isolates within the same ribotype and MLST need further investigation via whole genome analyses approach.

CDC disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.