



# Prevalence of *Clostridium difficile* Ribotypes in Minnesota, 2012-2014

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### Abstract

**Background:** *Clostridium difficile* infections (CDI) are the leading cause of healthcare-associated diarrhea and colitis. PCR ribotyping has been used to describe the molecular epidemiology of CDI. Ribotype (RT) 027 has historically predominated nationally and RT 078 has been associated with animal exposure. We describe the RTs circulating in Minnesota (MN) from 2012-2014. **Methods:** The MN Department of Health performs active, population and laboratory based surveillance for CDI as part of the U.S. Centers for Disease Control and Prevention's (CDC) Emerging Infections Program. *C. difficile* positive stool specimens were sent to the MN Public Health Laboratory for culture. Recovered isolates were randomly forwarded to CDC for molecular characterization and polymerase chain reaction (PCR) ribotyping. Cases were defined as community-associated (CA) if stool was collected as an outpatient, or as an inpatient  $\leq 3$  days of admission with no overnight stay in a healthcare facility 12 weeks prior; remaining cases were defined as healthcare-associated (HA). Medical records were reviewed and CA cases were interviewed to assess healthcare exposures, medications and animal exposures. **Results:** Of 1474 *C. difficile* isolates, 754 (51%) were characterized; 103 unique RTs were recovered. The most prevalent RTs were 106 (79, 10%), 002 (70, 9%), 020 (69, 9%), 014 (52, 7%), and 027 (50, 7%). Of 1,120 CA cases, 37% were ribotyped; predominant strains were 020 (44, 11%), 106 (41, 10%), 002 (36, 9%), 014 (30, 7%), and 027 (22, 5%). Of 855 HA cases, 40% were ribotyped; predominant strains were 106 (38, 11%), 002 (34, 10%), 027 (28, 8%), 020 (25, 7%), and 014 (22, 7%). The predominant RTs did not change significantly over the study time period and no difference in predominant RTs was detected when comparing healthcare exposure in the 12 weeks prior to infection. Of the 19 RT 078 isolates identified from CA cases, 2 (11%) reported exposure to agricultural animals. **Conclusion:** A diverse population of RTs were found in MN with no single RT accounting for >10% of circulating RTs in total and over time. RT 106 rather than 027 predominated and few 078 were detected limiting the ability to identify potential risk factors. Continued characterization of RTs and linkage with epidemiologic data is needed to monitor changes in circulating strains and determine RT specific risk factors.

### Background

- Clostridium difficile* infections (CDI) are the leading cause of healthcare-associated diarrhea and colitis resulting in 453,000<sup>1</sup> number of cases per year. Ribotype (RT) 027 has historically predominated nationally and RT 078 has been associated with animal exposure.
- C. difficile* isolates have been traditionally characterized using pulsed-field gel electrophoresis (PFGE) subtyping; however, advances in laboratory technology have gradually transitioned from PFGE characterization to PCR RT characterization
- We describe the RTs circulating in Minnesota from January 1, 2012-December 31, 2014

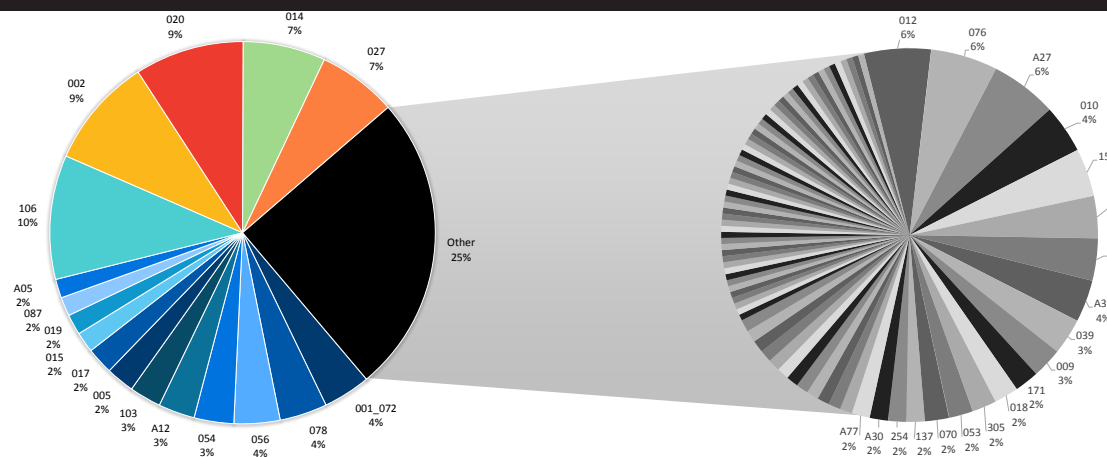
### Methods

- The Minnesota Department of Health (MDH) performs active, population and laboratory-based surveillance in five counties as part of the U.S. Centers for Disease Control and Prevention (CDC) Emerging Infections Program
- An incident case was defined as a positive *C. difficile* toxin or molecular assay on a stool specimen from a person without a positive assay in the prior 8 weeks. Medical chart abstractions were conducted for each incident CDI
- Cases were classified as healthcare-associated (HA) if stool was collected  $\geq 4$  days after hospital admission, if the case resided in a long-term care facility in the prior 12 weeks, or if the case had an overnight hospital stay in the 12 weeks prior to stool collection
- Cases were classified as community-associated (CA) if stool was collected  $\leq 3$  days after hospital admission, or as an outpatient with no prior healthcare stays in the previous 12 weeks. CA cases were interviewed to assess symptoms and additional exposures related to their CDI
- C. difficile* positive specimens from participating clinical laboratories were sent to the MDH Public Health Laboratory for culture
- Each quarter, 10 random HA isolates and every other CA isolate were sent to CDC for molecular characterization, including PCR ribotyping

### Results

- 1,975 incident cases were documented from January 1, 2012 to December 31, 2014
  - Isolates were recovered from 1,474 (75%) incident stool specimens
- 754 (51%) isolates were selected for molecular characterization at CDC and assigned a RT (Figure 1)
- 103 unique ribotypes were identified. The most prevalent RTs were 106 (79, 10%), 002 (70, 9%), 020 (69, 9%), 014 (52, 7%), and 027 (50, 7%) (Figure 1)
- Isolates from 37% of 1120 CA cases were characterized by PCR ribotyping
  - Predominant RTs were 020, 106, 002, 014, and 027 (Figure 2)
  - CA cases accounted for >50% of isolates among RTs 106, 002, 020, and 014 (Figure 2)
- Isolates from 40% of 855 HA cases were characterized by PCR ribotyping
  - Predominant RTs were 106, 002, 027, 020, and 014 (Figure 2)
  - HA cases accounted for >50% of isolates only for RT 027 (Figure 2)
- The proportion of CA cases was significantly higher than HA cases in RT 020 (64% vs. 36%,  $p=0.002$ ); however, the proportion of CA and HA cases were not statistically different when compared between all RTs ( $p=0.26$ ) (Figure 2)
- Isolates from 452 females and 302 males were characterized. Females accounted for >50% of isolates within each of the top five RTs (Figure 3)
  - The proportion of females was significantly higher than males in RT 106 (62% vs. 38%,  $p=0.004$ ), RT 020 (71% vs. 29%,  $p<0.0001$ ), and RT 027 (64% vs. 36%,  $p=0.009$ ), however the proportion of females and males were not statistically different when compared between all ribotypes ( $p=0.22$ ) (Figure 3)

Figure 1. Percent of *Clostridium difficile* Ribotypes among all Submitted Isolates, Minnesota, 2012-2014 (N=754)



- Cases aged  $\geq 65$  years were the most prevalent among RTs 106, 002, 014, and 027. Cases between 45-64 years of age were more prevalent in RT 020 (Figure 4)
- The predominant RTs did not significantly change over this time period, although RTs 020 and 106 appear to have become more prevalent (Figure 5)
- A total of 306 CA cases that were interviewed had corresponding isolates that were ribotyped

Figure 2. Percentage of Five Most Common *Clostridium difficile* Ribotypes Originating from Cases with Healthcare-associated or Community-associated Infections, Minnesota, 2012-2014

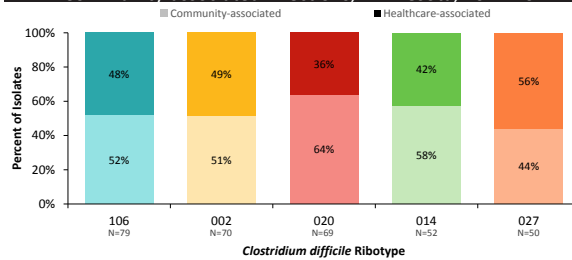
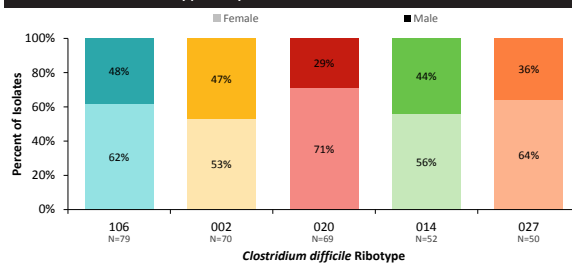


Figure 3. Percentage of Five Most Common *Clostridium difficile* Ribotypes by Sex, Minnesota, 2012-2014



- In CA cases that were interviewed, RT 002 and RT 014 were significantly associated with mild-moderate diarrhea (3-10 stools/day) when compared to severe diarrhea (>10 stools/day) in all RTs (RT 002: 82% vs. 15%,  $p=0.006$ ; RT 014: 85% vs. 11%,  $p=0.007$ ) (Table 1)
- RT 106 was significantly associated with less nausea when compared to all other RTs (32% vs. 53%,  $p=0.03$ ) (Table 1)
- No cases with RT 002 reported vomiting, significantly lower when compared to all other RTs (0% vs. 21%,  $p=0.006$ ) (Table 1)

Figure 4. Percentage of Five Most Common *Clostridium difficile* Ribotypes by Age Category, Minnesota, 2012-2014

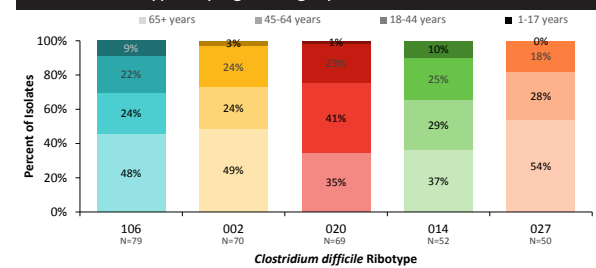
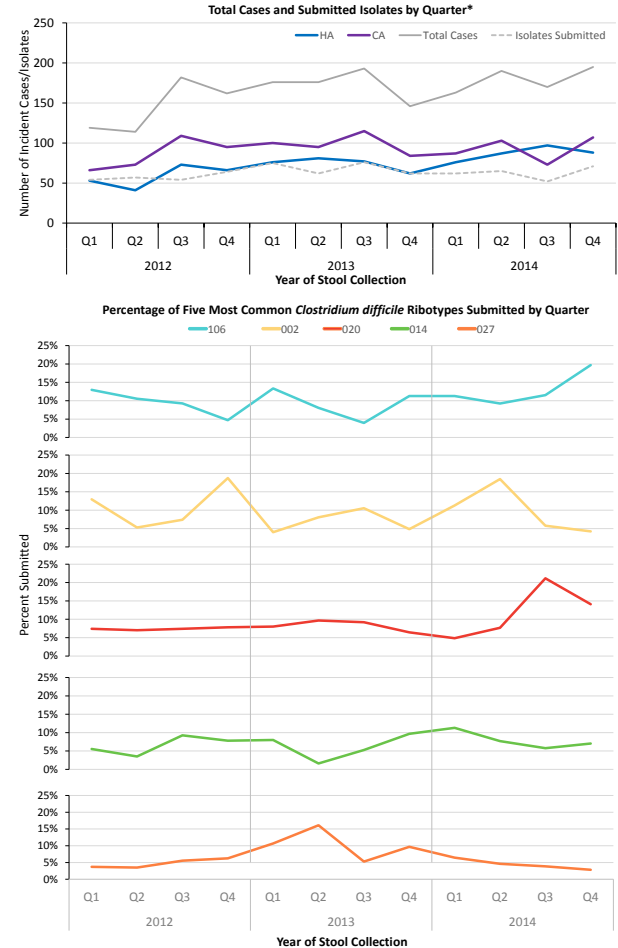


Table 1. Clinical Presentation of *Clostridium difficile* Infection in Community-associated Cases, Minnesota, 2012-2014

Symptoms	<i>Clostridium difficile</i> Ribotype, n(%)						P-value
	Total	106	002	020	014	027	
Diarrhea	272 (89)	27 (87)	27 (93)	22 (76)	19 (100)	13 (76)	0.07
<b>Diarrhea Frequency</b>							
3-10 stools/day (mild-moderate)	157 (58)	17 (63)	22 (82)	11 (50)	16 (85)	7 (54)	(referent)
10+ stools/day (severe)	108 (40)	10 (37)	4 (15)	10 (45)	2 (11)	6 (46)	0.03
Median Diarrhea Duration (Days, Range)*	12.5, 1-178	11.5, 3-60	12, 1-67	19, 3-50	10, 1-43	7, 3-22	0.45
Nausea	157 (51)	10 (32)	12 (41)	17 (59)	8 (42)	9 (53)	0.3
Vomiting	59 (19)	5 (16)	0	5 (17)	4 (21)	1 (6)	0.12

\*Only includes cases with resolved diarrhea at time of interview (Total, n=204; RT106, n=22; RT002, n=16; RT020, n=14; RT014, n=13; RT027, n=11)

Figure 5. Quarterly Comparison of Incident *Clostridium difficile* Infections, Isolates, and Ribotypes, Minnesota, 2012-2014



- A total of 19 RT 078 were isolated from CA cases, 2 (11%) reported exposure to agriculture animals on interview

### Conclusions

- A diverse population of ribotypes are currently circulating in Minnesota and no single ribotype accounted for >10% of circulating ribotypes in total and over time.
- Ribotype 106 rather than ribotype 027 predominated in Minnesota during this time period and ribotypes 106 and 020 appear to be increasing
- Continued characterization of ribotypes and linkage with epidemiologic data is needed to monitor circulating strains, and determine ribotype specific risk factors

### Acknowledgements

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### References

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