

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



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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 symptomatic 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. PCR ribotyping was conducted using the Bidet primers and agarose gel electrophoresis. A dendrogram was constructed in Bionumerics by the un-weighted 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 suggestive of direct transmission were evenly split between hospital- (18 of 35, 51%) and community-acquired (17 of 35, 49%) cases. The median time between clustered cases was 50 days (range: 7 to 331 days). 35 of 70 (50%) of all isolates exhibited entirely unique strain types.

Conclusions: *C. difficile* isolates in our community hospital exhibited tremendous genetic diversity. The high 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*'s diverse 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, and for acquisition of *C. difficile* within the community, remain poorly understood.

Methods

- 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 performed using the Bidet primers and agarose gel electrophoresis.¹
- Ribotypes were identified and separated by the unweighted pair-group method with a threshold for identical strains set at 98% similarity.

Results

Table 1: Clinical and demographic characteristics.

	CO-CDI (n=32)	HCFA-CDI (n=66)
Age [IQR]	63 [53-72]	67 [55-74]
Gender, female	13 (41)	36 (55)
Race, black	7 (22)	33 (50)
CCMI	4.5 [2.3-7.3]	7.5 [4.3-9.7]
Co-morbidities:		
Diabetes	10 (55)	30 (55)
CKD	4 (22)	28 (51)
Cancer	3 (17)	13 (24)
CHF	8 (44)	24 (44)
Pulmonary	7 (39)	22 (40)
Antibiotics	22 (69)	56 (85)
PPI	8 (26)	26 (39)
Immune suppress	8 (25)	12 (18)
Prior surgery	13 (93)	36 (84)

Results (continued)

Figure 1: PCR ribotyping of isolates

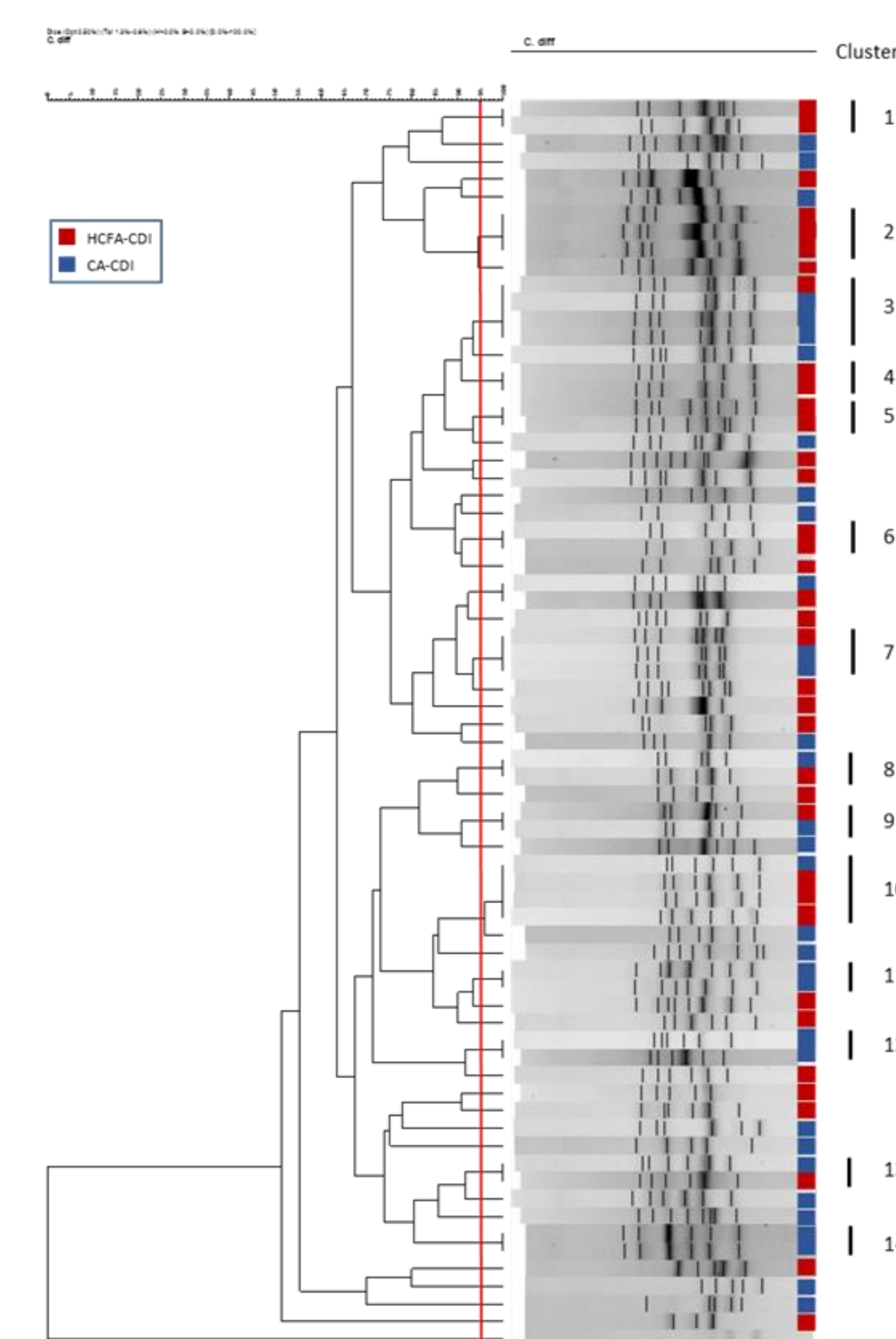


Figure 2: Histogram of ribotypes according to source.

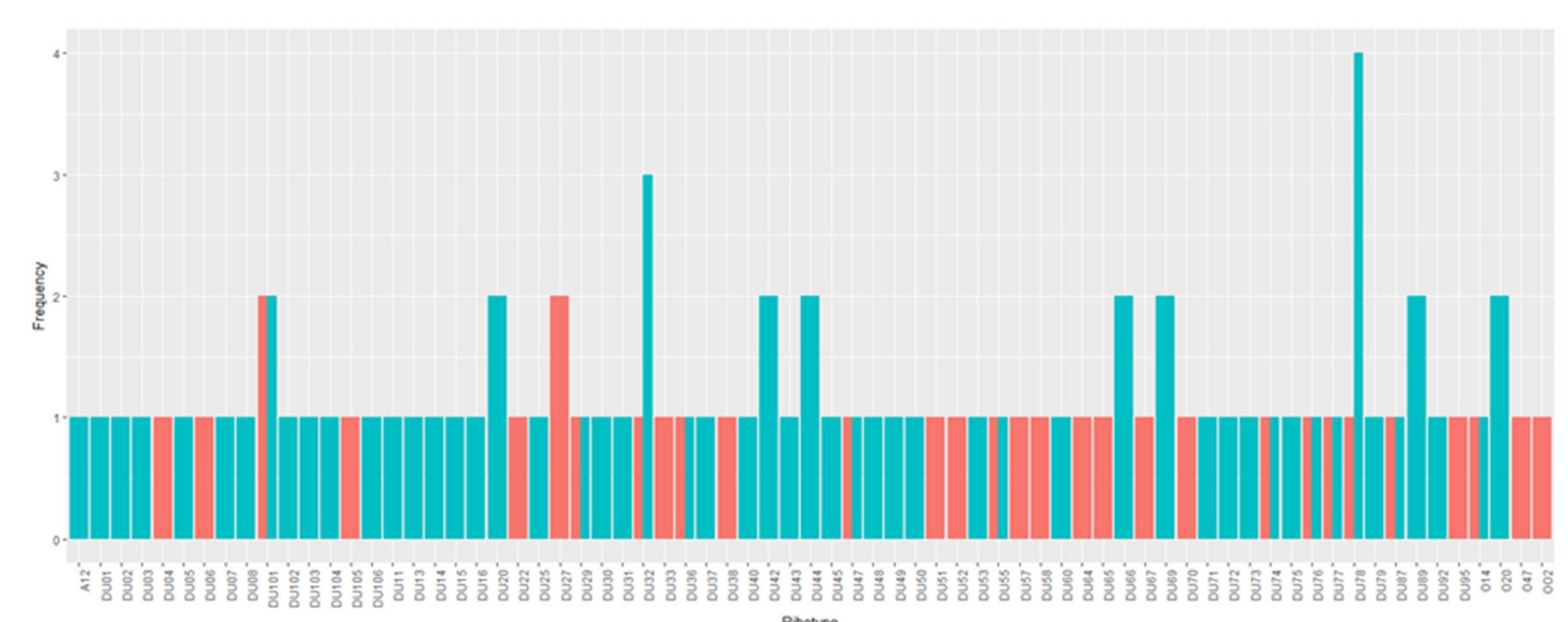


Table 2: Epidemiologic characteristics of cases caused by identical ribotypes (presumed clusters).

Ribotype	Service	Age	Date Collected	Dialysis	Prior CDI	Prior Surgery	LabID	Strict
DU101	Internal Medicine	67	07/08/16	Yes		02/17/16	HCFA	HCFA
DU101	Internal Medicine	61	07/14/16	Yes	02/23/16	02/22/16	HCFA	HCFA
DU101	Internal Medicine	91	08/03/16	No	03/15/15		CO	CO
DU101	Internal Medicine	40	08/12/16	No			CO	CO
DU32	Internal Medicine	51	08/27/16	No	06/25/16		CO	CO
DU32	Intensive Care	55	09/19/16	No	08/22/16		HCFA	HCFA
DU32	Internal Medicine	43	09/26/16	No	08/14/16		HCFA	HCFA
DU32	Internal Medicine	83	11/17/16	No			CO	HCFA
DU78	Internal Medicine	67	07/07/16	Yes		08/21/15	HCFA	HCFA
DU78	Internal Medicine	62	07/12/16	No		12/24/14	CO	CO
DU78	Internal Medicine	78	07/24/16	Yes	11/16/15		HCFA	HCFA
DU78	Internal Medicine	85	01/06/17	No		01/04/17	HCFA	HCFA
DU78	Internal Medicine	68	02/06/17	Yes			CO	HCFA

Figure 3: Tables of top ten observed ribotypes from national (CDC) data and Duke Regional Hospital.

A) CDC HA-CDI² B) Duke Regional HA-CDI

Ribotype	No. of isolates (n=504)	Percent isolates	Ribotype	No. of isolates (n=66)	Percent isolates
027	102	19	DU78	4	6
106	48	9	020	2	5
002	40	7	DU20	2	3
014	36	7	DU31	2	3
020	30	6	DU42	2	3
015	18	3	DU44	2	3
001_072	15	3	DU66	2	3
056	17	3	DU89	2	3
017	15	3	DU101	2	3
005	14	3	A12	1	2
Others	201	37	Others	45	68

C) CDC CA-CDI² D) Duke Regional CA-CDI

Ribotype	No. of isolates (n=619)	Percent isolates	Ribotype	No. of isolates (n=32)	Percent isolates
106	58	9	002	2	6
027	52	8	DU101	2	6
014	46	7	047	1	3
020	40	7	014	1	3
002	35	6	DU04	1	3
015	21	3	DU06	1	3
054	20	3	DU22	1	3
005	19	3	DU23	1	3
056	18	3	DU27	1	3
046	17	3	DU105	1	3
Others	288	47	Others	20	63

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.³
- The epidemic strain 027 was conspicuously absent from Duke Regional Hospital in 2017.
- In a small sample size to date, HA and CA-CDI showed relatively little intermixing of strain types.



1. Bidet et al. *FEMS Microbiol* 1999.
2. CDC Annual Report on Emerging Infections, 2015.
3. Eyre et al. *NEJM*. 369(13). 2013.