Implication of *Clostridium difficile* Strain Typing in Recurrent *C. difficile* Infection

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Abstract

Introduction: Recurrent *Clostridium difficile* infection (CDI) is a debilitating problem. We sought to determine if patients in our healthcare system had relapsing disease or reinfection by analyzing the strain types of the bacteria causing CDI. We hypothesize that typing results could imply relapse (when the patient strains are repeatedly the same), or reinfection and the need for increased infection control measures (when infection is with a new strain). To test this we did PCR ribotyping of *C. difficile* cultures from CDI patients who recurred at ≥60 days.

Methods: Stool was archived beginning in February 2015. Any patient with a second CDI episode ≥60 days from the original sample had both stools cultured. Toxigenic *C. difficile* recovered from both samples was prepared for PCR ribotyping. Isolates were grown for 24 hours and DNA extracted/ amplified following the method of Fawley et al. (PLoS One. 2015). PCR products were separated using a 3500*XL* genetic analyzer using GeneScan 600 LIZ as size standards. Ribotype was determined using the WEBRIBO database (https://webribo.ages.at/). Ribotype pairs were compared to each other and also to baseline data gathered from a previous study.

Results: 51 *C. difficile* isolates from 25 patients were typed. Twenty one patients (84%) had a second infection with the same strain and 4 (16%) had a new strain type. For patients whose second positive test was at 60-90 days, 1/13 (7.6%) acquired a new strain, and for those whose second positive test was at >90 days, 3/13 (23%) had a new strain. The number of patients with different strains vs the same was not significant (p= 0.59). In all, nineteen strain types were recovered from the 25 patients. Comparing recurrence with the same strain type to our baseline data found only 5 (20%) patients having the major clones circulating in our population. This suggests this was relapse. For the patients with a new clone, 3 of 4 were strains we had not seen previously, suggesting new CDI acquisition outside of our healthcare system or the introduction of a new clone

Conclusion: Majority of the patients tested in this timeframe had relapsing disease and not new infection from exposure to our healthcare environment. Periodic typing of *C. difficile* isolates can help focus Infection Control efforts.

Background

Multiple bouts of *Clostridium difficile* infection is a debilitating problem for patients. In order to help address the issue, we sought to determine if patients in our healthcare system had recurring disease or reinfection by analyzing the strain types of the bacteria causing the disease. Typing results could help determine patient management when the patient strains are repeatedly the same, or the need for increased infection prevention measures when infection with new strains is common. We examined *C. difficile* isolates from stool samples of patients who had positive tests >60 days apart. If the number of patients with different strain types was significantly greater than the number of patients with the same strain type, infection prevention measures for *C. difficile* would be reexamined.

Methods

Growth of Isolates: Stool sample aliquots from patients testing positive with the Cepheid Xpert ® *C. difficile* /Epi stool PCR test were frozen beginning February 2015. When a patient had a second positive stool test >60 days from the original sample, both stools were cultured. Samples were cultured onto CCFA-HT agar (Anaerobe Systems) and incubated for 5 days at 35ºC anaerobically. Colonies resembling *C.difficile* were confirmed as such if the isolates were large Gram positive or variable bacilli, non-aerotolerant and Pro Disk positive (Remel, Inc).

PCR Ribotyping: When *C. difficile* was recovered from both samples, the pair of isolates were prepared for PCR ribotyping. Isolates were grown for 24 hours under anaerobic conditions and the DNA was extracted and amplified following the method described by Fawley et al (1). PCR products were separated using a 3500*XL* genetic analyzer using GeneScan 600 LIZ as size standards. Fragment sizing was achieved by using the GeneMapper 4.1 software and ribotype was determined using the WEBRIBO database accessed at https://webribo.ages.at/. (last accessed 04/2016). Additionally, following PCR, the products were separated on an Agilent 2100 Bioanalyzer (Agilent Technologies) for visualization (Figure 1).

Circulating Strains: In 2013, 217 stool samples were sent to TechLab, Inc. (Blacksburg, VA) as part of an Infection Control initiative and tested for a variety of parameters, including strain typing by PCR ribotyping (2). These strain types are considered baseline data for this analysis. The most common strains found are listed in Table 2.

Results

Fifty-one *C. difficile* isolates from 25 patients with recurrent disease were typed. Of the 25 patients, 21 (84%) had a second infection with the same strain and 4 (16%) had a different strain type (Table 1).

For patients whose second positive test was at 60-90 days, 1/13 (7.6%) had a new strain the second time, and for those whose second positive test was at >90 days, 3/13 (23%) had a new strain (Table 2). The number of patients with different strain types was not significantly greater than the number of patients with the same strain type (p= 0.59).

In all, nineteen strain types were recovered from the 25 patients. The most common strain type was 027 in 6 patients, followed by 203 with 3 patients and 016, 56 and 37 with 2 patients.

Table 1. *C. difficile* Strain Typing Results for Paired Isolates Feb 2015 to Jan 2016

Entries in red had a different strain type with the second illness

Patient No.	First Test	Ribotype	Second Test	Ribotype	No. Days between Positive Tests
20	05/28/15	027	08/21/15	014	60-90
2	08/04/15	508	10/15/15	508	60-90
7	11/06/15	203	08/13/15	203	60-90
8	10/30/15	053	01/23/16	053	60-90
9	11/16/15	037	01/08/16	037	60-90
11	06/15/15	076	09/24/15	076	60-90
16	10/09/15	106	12/15/15	106	60-90
17	04/11/15	106	08/01/15	106	60-90
17	04/11/15	106	06/05/15	106	60-90
21	05/18/15	027	07/31/15	027	60-90
25	08/13/15	027	10/30/15	027	60-90
23	10/10/15	037	01/16/16	037	60-90
22	04/03/15	027	06/06/15	027	60-90
1	08/21/15	203	01/04/16	39/2	>90
4	04/10/15	056	11/20/15	083	>90
12	03/24/15	003	07/30/15	446	>90
3	08/09/15	153	01/04/16	153	>90
5	07/30/15	049	12/14/15	049	>90
6	04/03/15	621	10/19/15	621	>90
10	09/17/15	203	01/07/16	203	>90
13	02/16/15	82/1	06/29/15	82/1	>90
14	04/17/15	027	07/18/15	027	>90
15	04/02/15	592	11/23/15	592	>90
18	03/21/15	020/14	08/25/15	020/14	>90
19	02/20/15	056	06/18/15	056	>90
24	05/19/15	027	10/12/15	027	>90

Table 2. Circulating Strains NorthShore University HealthSystem Oct 2012 to Sept 2013

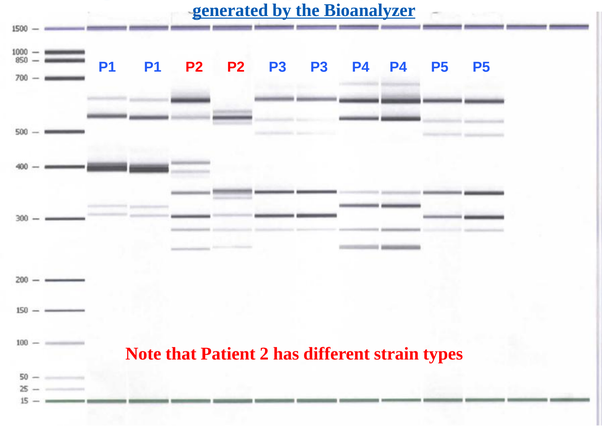
Ribotype	No. patients
027	61
014	35
106	17
002	15
056	15
054	7
015	5
057	5
103	5
126	5
009	4
012	4
001	3
039	3
005	2
043	2
046	2
053	2
075	2
232	2
251*	2

*** 22 additional strains were identified from 22 patients**

Discussion

Majority of patients had the same strain type during the second infection, suggesting relapse with the original strain. However, since strain type 027 is our most common clone, it is possible that the 5 patients with recurrent 027 disease could have picked up a new 027 strain, but it is impossible for us to tell. These results suggest that during this timeframe, strategies to prevent re-infection would have been most beneficial to patients as opposed to increased infection control measures in the hospital.

Figure 1. Virtual Gel Image of *C. difficile* PCR Products generated by the Bioanalyzer



Conclusion

- Majority of the patients tested in this timeframe had recurring disease
- Periodic strain typing of *C. difficile* isolates can help focus patient management efforts

References

1. Fawley et al. (PLoS One. 2015 Feb 13;10(2):e0118150 doi: 10.1371/journal.pone.0118150)
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