

2427. A Laboratory Simulation and Field Study of Simplified Qualitative and Quantitative Environmental Cultures for Clostridioides difficile

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Session: 253. HAI: *C. difficile* - Surveillance
Saturday, October 5, 2019: 12:15 PM

Background. Reliable, simple methods to assess environmental contamination with *Clostridioides difficile* are needed for studies of terminal disinfection effectiveness and transmission of *C. difficile* from fomites. We compared two novel sampling methods for assessment of *C. difficile* contamination: (1) isopropanol wipe sampling with broth enrichment (IW), where alcohol wipes are used to sample surfaces followed by immersion in CDBB-TC broth and (2) ethanol-shock hand imprint sampling (HI), where surfaces are sampled by gloved hands moistened with ethanol hand sanitizer followed by imprinting hands on selective agar (CDBA-TC). Both methods allow for sampling of complex surfaces common in hospital environments.

Methods. We performed a laboratory simulation study using six 10-fold dilutions (10¹-10⁶) of *C. difficile* spores from two strains sampled from fiberglass surfaces by IW and HI. We then performed a field study of 10 sites in 11 hospital rooms of patients with *C. difficile* infection (CDI) diagnosed < 48 hours prior to sampling. Results for the HI and IW method were quantitative and qualitative, respectively. We compared room contamination prevalence (total CFU per room by HI and sites positive/total sites per room) to the cycle crossing threshold (C_c) from the diagnostic *C. difficile* PCR test (Cepheid) from the room occupant.

Results. The IW laboratory limit of detection was 10¹ spores compared with 10⁴ spores for HI. In the field study, IW and HI detected *C. difficile* contamination in 9/11 (82%) rooms at 22/110 (20%) sites and 5/11 (45%) rooms at 22/110 (20%) sites, respectively (Table 1). Six rooms with no detection by the HI method had a lower median number of sites positive per room compared with the 5 rooms with ≥1 cfu (1 vs 6, P < 0.05) (Table 1). The most commonly contaminated sites were linens (53%), call bells (43%), toilets (40%), blood pressure cuffs (40%), over-bed tables (38%), and bedrails (30%) (Table 2). Higher tcdB C_c was associated with a lower total room contamination prevalence (Figures 1 and 2).

Conclusion. Our data suggest that the IW method is more sensitive for detection of *C. difficile* in the hospital environment, but the HI method offers quantitation. The prevalence of hospital room contamination for CDI patients appears to correspond with their toxin burden as estimated by PCR.

Table 1. Field study of two methods for *C. difficile* recovery from 11 rooms occupied by recently-diagnosed patients with *C. difficile* infection. For each room, 10 sites per room were cultured.

Room	Unit type	Hours from diagnosis to sampling	tcdB - Ct	sites positive by HI	sites positive by IW	Total positive sites	Total CFU	Sites positive by HI	Sites positive by IW
1	ICU	26	29.6	0	1	1	0	-	P
2	ICU	23	26.6	1	1	2	1	A	S
3	ICU	24	20.6	5	4	6	70	CRTSA	CPST
4	ICU	25	32.8	0	1	1	0	-	X
5	floor	5	23.3	7	7	9	38	CSTRPOX	CSPRBDX
6	ICU	31	34.2	0	0	0	0	-	-
7	floor	13	23.5	7	4	7	177	RBPSTOC	RBPS
8	floor	16	29.4	2	0	2	4	OV	-
9	floor	29	36.3	0	1	1	0	-	V
10	floor	28	35.2	0	1	1	0	-	T
11	floor	23	29.0	0	2	2	0	-	PS

ICU= intensive care unit, tcdB-C_c= cycle crossing threshold for tcdB target on *C. difficile* PCR from the stool sample from the recently-diagnosed room occupant, HI=hand imprint method, IW= isopropanol wipe method, CFU=colony forming units, P=pillowcases, S=sheets, C= call bell, T=toilet, B=blood pressure cuff, X=sequential compression device, R=bed rail, A=patient abdomen, O=over-bed table, D= portable electronic vital signs monitor, V=visitor chairs

Table 2. Field study of two methods for *C. difficile* recovery from 112 sites in 11 rooms occupied by recently-diagnosed patients with *C. difficile* infection stratified by site sampled in descending frequency of sampling.

Item/Site sampled	Positive/Total (%)	Positive by IW	Positive by HI	HI CFU range**
Sink*	0/12 (0%)	0	0	-
Toilet	4/11 (36%)	2	3	9-138
Bed rail	3/10 (30%)	2	3	3-14
Over-bed table	3/10 (30%)	0	3	1-6
Pillowcases	5/10 (50%)	5	2	2-5
Call bell	3/9 (33%)	2	3	1
Sheets	5/9 (56%)	5	3	1-4
Computer keyboard	0/8 (0%)	0	0	-
Doorknobs	0/6 (0%)	0	0	-
Visitor chairs	2/6 (33%)	1	1	2
Blood pressure cuff	2/5 (40%)	2	1	9
Sequential compression device	2/4 (50%)	2	1	20
Monitor lead wires	0/3 (0%)	0	0	-
Patient abdomen	2/3 (66%)	0	2	1-21
Fecal management system	0/1 (0%)	0	0	-
Portable electronic vitals unit	1/1 (100%)	1	0	-
Room telephone	0/1 (0%)	0	0	-
Portable thermometer probe	0/1 (0%)	0	0	-
Ventilator	0/1 (0%)	0	0	-
Totals	32/111 (29%)	22	22	

IW = isopropanol wipe broth enrichment technique, HI= hand imprint ethanol shock technique, *1 additional portable hallway sink was sampled outside the 11th room sampled
**where only 1 site was positive the CFU at the site is listed

Figure 1. Total hand imprint CFU per room vs PCR crossing threshold

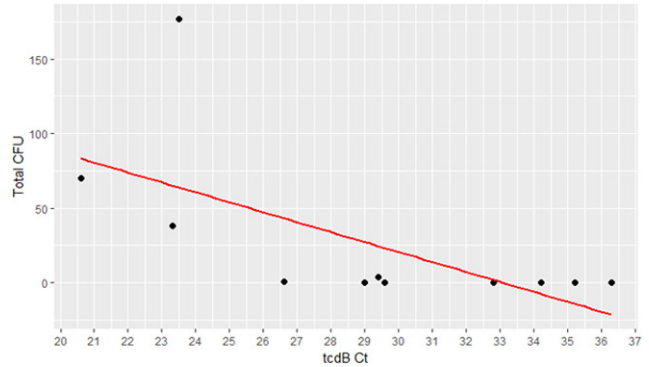
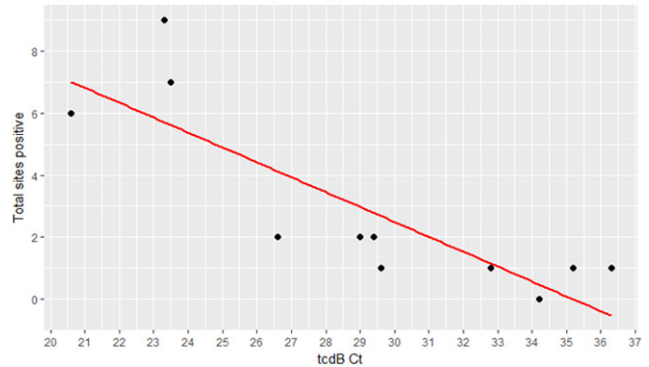


Figure 2. Total number of sites positive per room vs PCR crossing threshold



Disclosures. All authors: No reported disclosures.

2428. Whole-genome Sequencing to Determine Clostridium difficile Transmission

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Background. *Clostridium difficile* is a major problem in healthcare institutions due to its substantial attributable morbidity, mortality and costs. Although traditionally recognized as a nosocomial infection, there is increasing evidence that hospital-based transmission may not be as common as previously thought. Whole-genome sequencing (WGS) has superior discriminatory ability than other previously used techniques for *C. difficile* typing. This study aimed to investigate whether WGS could help to elucidate *C. difficile* transmission patterns at The Royal Melbourne Hospital (RMH).

Methods. All *C. difficile* isolates (N = 138) identified in patients admitted to RMH from November 1, 2015 to October 31, 2016 had molecular typing performed by WGS, multilocus sequence typing (MLST) and PCR ribotyping. Clinical epidemiological data were collected for each episode so that patient locations could be examined together with molecular typing information to determine putative transmissions in the hospital.

Results. After combining molecular and clinical epidemiology, a picture of diverse *C. difficile* emerged. Only 7 (6%) of isolates appeared to have been transmitted from other hospital patients, according to combined WGS and patient location data. However, both PCR ribotyping (33%) and MLST (44%) had significantly higher proportions of putative transmissions in this cohort.

Conclusion. This finding has significant implications for the Infection Prevention team as efforts toward prevention of *C. difficile* infection may need to be redirected away from the current focus on prevention of nosocomial transmission. Future studies are needed to broaden understanding of *C. difficile* transmission dynamics so that other sources can be identified and targeted for intervention.

Disclosures. All authors: No reported disclosures.

2429. Whole-genome Sequencing of Healthcare-Onset C. difficile Infection (HO-CDI) Cases Shows Widespread Presence of Antimicrobial Resistance Genes

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