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Recovery of *C. difficile* from the hospital environment – results depend on the media

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Introduction and Purpose

- *Clostridium difficile* is the leading cause of nosocomial infections resulting in substantial morbidity and mortality, as well as economic burden.
- The role of the hospital environment as an important source for ongoing *C. difficile* transmission in healthcare settings remains elusive and requires further investigation to provide an evidence base for infection control guidelines.
- Standardized protocols outlining the methodology for recovery of *C. difficile* from the environment are, however, lacking.
- The use of different media for culturing *C. difficile* from environmental swabs hampers comparability between and generalizability to other institutions.
- With the aim to inform future protocols serving as a reference for research and surveillance, we compared two different selective media and two different commercially available swabs regarding their yield of *C. difficile* from environmental samples.

Methods

- From September 2015 to February 2016, environmental samples were taken from 12 rooms of consecutive patients diagnosed with *C. difficile* infection.
- Environmental samples of the immediate patient-surroundings were performed by swabbing surfaces with a liquid-based collection and transport system (ESwab™, Copan, Brescia, Italy).
- The following 8 places were examined in each room: the bed grip, the patient's bell, the floor in front of the patient's bed, the side table, the door handle, the faucet knobs, the handle of the toilet flush, and the toilet seat.
- Swabs were taken from each spot and submitted to culture without any delay using two different commercially available culture media: cycloserine cefoxitin mannitol broth with taurocholate and lysozyme (CCMB-TAL, Anaerobe Systems, Morgan Hill, CA, USA) and selective cycloserine-cefoxitin-blood agar plates (CLO agar; bioMérieux, Marcy l'Etoile, France).
- In addition, the following two swabs were compared regarding the yield of *C. difficile* stratified by the two culture media:
  - the liquid-based collection and transport system (eSwab™,Copan, Brescia, Italy).
  - the agar gel-based collection and transport system (M40™ Transystem,Copan, Brescia, Italy).
- Cultures were incubated in an anaerobic chamber for eight days and *C. difficile* was identified according to standard laboratory methods.

Results

- Overall, 186 environmental swabs were taken from the eight predefined places in 12 patient rooms.
- *C. difficile* was recovered in 23.7% (22/93) of all samples using CCMB-TAL for culture and in 11.8% (11/93) of all samples plated onto CLO agar (p=0.035).
- Differences regarding the yield of *C. difficile* according to the different areas sampled are shown in **Table 1**.

	Anaerobic culture with CCMB-TAL			Anaerobic culture with CLO		
	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)
Bed grip	12	2	16.7	12	0	0
Patient's bell	12	3	25	12	1	8.3
Floor in front of the patient's bed	12	6	50	12	3	25
Side table	12	2	16.7	12	1	8.3
Door handle	12	0	0	12	0	0
Faucet knobs	12	2	16.7	12	2	16.7
Handle of the toilet flush	9	2	22	9	1	11
Toilet seat	12	5	41.6	12	3	25

Table 1: Differences between the two different culture media regarding the yield of *C. difficile* according to the different areas sampled

- Differences between the two swabs (eSwab™ and M40™ Transystem) regarding the yield of *C. difficile* using CCMB-TAL and CLO agar as culture media are shown in **Tables 2** and **3**, respectively. Our sample size is, however, too small to draw conclusions at this point and further sampling is ongoing.

	eSwab™			M40™ Transystem		
	Anaerobic culture with CCMB-TAL			Anaerobic culture with CCMB-TAL		
	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)
Bed grip	4	0	0	4	0	0
Patient's bell	4	0	0	4	0	0
Floor in front of the patient's bed	4	2	50	4	0	0
Side table	4	0	0	4	0	0
Door handle	4	0	0	4	0	0
Faucet knobs	4	0	0	4	0	0
Handle of the toilet flush	2	0	0	2	0	0
Toilet seat	4	1	25	4	1	25

Table 2: Differences between the two different swabs regarding the yield of *C. difficile* according to the different areas sampled using CCMB-TAL for culture

	eSwab™			M40™ Transystem		
	Anaerobic culture with CLO			Anaerobic culture with CLO		
	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)	Total (n)	*C. difficile* positive (n)	*C. difficile* positive (%)
Bed grip	4	0	0	4	0	0
Patient's bell	4	0	0	4	0	0
Floor in front of the patient's bed	4	0	0	4	0	0
Side table	4	0	0	4	0	0
Door handle	4	0	0	4	0	0
Faucet knobs	4	0	0	4	0	0
Handle of the toilet flush	2	0	0	2	0	0
Toilet seat	4	0	0	4	0	0

Table 3: Differences between the two different swabs regarding the yield of *C. difficile* according to the different areas sampled using CLO agar for culture

Conclusions

- Recovery of *C. difficile* was increased with the use of CCMB-TAL broth enrichment following plating to solid medium pointing to the important role of taurocholate to increase spore recovery by supporting germination.
- When investigating environmental contamination with *C. difficile*, selective culture media used for recovery of vegetative *C. difficile* in routine diagnostics may not be as sensitive as selective culture media containing taurocholate.