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Letters to the Editor

Recovery of *Clostridium difficile* from Hospital Environments

In the paper entitled "Use of a Selective Enrichment Broth to Recover *Clostridium difficile* from Stool Swabs Stored under Different Conditions" by Arroyo et al., published in the October 2005 issue of the *Journal of Clinical Microbiology* (1), the authors concluded that the use of selective enriched broth (0.1% sodium taurocholate and cycloserine-cefoxitin fructose broth [TCCFB]) was superior to the use of cycloserine-cefoxitin fructose agar (CCFA) and to the use of alcohol shock followed by inoculation onto blood agar and that neither the storage time nor the storage temperature affected the recovery of *C. difficile* from stool swabs. This conclusion was based on a comparison of three methods using nine standard culture swabs from each stool sample received in a research laboratory within 1 week of collection. One swab was used immediately, while the remaining swabs were randomly assigned to two storage groups: one at room temperature and the other at 4°C. After 1, 2, 4, and 8 weeks, an appropriate swab from each storage group was inoculated on CCFA and then placed in 9 ml of TCCFB. CCFA was incubated in an anaerobic chamber at 37°C for up to 5 days, and suspicious colonies were subcultured and identified. TCCFB was incubated up to 7 days, and 2 ml was mixed with an equal amount of absolute ethanol and left at room temperature; this procedure was followed by centrifugation and inoculation of the pellet onto blood agar and incubation under an anaerobic condition, as described above.

We used the same 0.1% sodium taurocholate-enriched cycloserine-cefoxitin-amphotericin B mixture (CCA) (bioMerieux, Marcy L'Etoile, France) (TCCA) broth for recovery of *C. difficile* strains from hospital environmental swabs and from the palms/fingernails of the medical personnel. One hundred sixty-four environmental swabs were collected from hospital environments of maternity (94 swabs) and surgical (70 swabs) wards (5, 6). Environmental samples from a 76-bed maternity ward were collected from neonates' bedrails, baby sinks, baskets for diapers, surfaces of baby-changing tables, baby scales, and walls behind radiators. From a 60-bed surgical ward, environmental samples were collected from patients' beds, stretchers, push-chairs, sinks, and toilets (Table 1). In both hospital wards, *C. difficile* strains were at the same time isolated from 17% fecal samples of neonates and 9% fecal samples of surgical patients (4). Out of 164 collected environmental swabs inoculated on TCCA Columbia blood agar and subsequently inoculated in TCCA broth, *C. difficile* strains were isolated in 8 and 12 (13%) cases, respectively, in the maternity hospital and in 3 and 5 (7.4%) cases, respectively, in the surgical ward. Ribotyping classified the majority of *C. difficile* strains isolated from the maternity environment (type A) and from the surgical environment (type B) in the same fashion. Moreover, ribotyping classified strains from the maternity ward and neonatal fecal samples and from the surgical ward environment and patients' fecal samples as the same types (data not shown). One hundred fifty swab samples taken from the palms/fingernails of medical personnel were *C. difficile* negative.

Interestingly, the majority of nontoxigenic *C. difficile* strains did not grow on TCCA Columbia blood agar and were recovered only in TCCA broth.

Because stool samples usually contain a high number of bacterial spores (2), Columbia blood agar with an antibiotic mixture is a good choice when stool samples are cultured within the first 24 to 48 h after collection. When environmental swab samples are cultured for *C. difficile*, the use of broth enriched with 0.1% sodium taurocholate and an antibiotic mixture is recommended because in the hospital environment, the number of spores is smaller than that in fecal samples. The contamination of the hospital environment may play an important role in transmission, since spores of *C. difficile* can survive for up to 6 months (3, 8). It is also important from an epidemiological point of view to recognize the source of *C. difficile* strains and to compare strains isolated from different sources in terms of clonality (7).

TABLE 1. Characteristics of environmental strains of *Clostridium difficile* isolated from maternity and surgical wards

Strain no.[a]	Source	Growth on:		Toxigenicity TechLab A/B result[c]	Ribotyping result (spacer type)[d]
		TCCA	TCCB[b]		
1	Bedrail 1	+	+	+	A
2	Basket 1	−	+	−	A
3	Sink	+	+	+	A
4	Surface of table 1	+	+	+	A
5	Basket 2	−	+	−	A
6	Wall behind radiator	+	+	+	A
7	Bedrail 2	−	+	−	D
8	Basket 3	+	+	+	A
9	Scale 1	−	+	−	A
10	Bedrail 3	+	+	−	A
11	Scale 2	+	+	+	A
12	Surface of table 2	+	+	+	A
13*	Patient's bed	−	+	+	B
14*	Stretcher	−	+	+	B
15*	Push-chair	+	+	+	B
16*	Sink	+	+	+	B
17*	Toilet	+	+	+	B
Total	17	12	17	12	A (D)/B

[a] Strains 1 to 12, *C. difficile* strains isolated from maternity ward environment; strains 13 to 17, *C. difficile* strains isolated from surgical environment (marked by asterisks).

[b] TCCB, 0.1% sodium taurocholate and cycloserine-cefoxitin-amphotericin B-enriched broth.

[c] TechLab A/B, *Clostridium difficile* toxins A/B ELISA (TechLab, Blacksburg, VA).

[d] Analysis of the variations in lengths of the ribosomal intergenic spacer regions was performed by using SP1/SP2 primers and a PCR program according to the method described in reference 5.

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