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Isolation of *Clostridium difficile* from the Environment and Contacts of Patients with Antibiotic-Associated Colitis

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Clostridium difficile is the most important cause of antibiotic-associated colitis, but its epidemiology remains unknown. Using a selective medium for the isolation of *C. difficile*, cultures were obtained from the environment and contacts of hospitalized patients carrying *C. difficile* in their stools. In areas where carriers had diarrhea, 85 (9.3%) of 910 cultures of floors and other surfaces, especially those subject to fecal contamination, were positive. In areas where there were no known carriers, only 13 (2.6%) of 497 cultures of similar sites were positive ($P < 0.005$). *C. difficile* was isolated from hands and stools of asymptomatic hospital personnel, from sewage and soil, and from the home of a patient. Environmental isolates were toxigenic. *C. difficile* inoculated onto a floor persisted there for five months. Further studies are needed to document how often *C. difficile* shed by patients with antibiotic-associated colitis is acquired by other persons and whether isolation precautions are capable of limiting the organism's spread.

Although *Clostridium difficile* is the most important cause of antibiotic-associated colitis [1–17], the sources of the organism and the mechanisms of its spread are still incompletely defined. Intestinal carriage rates of up to 3% have been reported for healthy adults [6]. Uncertainty exists as to whether antibiotic-associated colitis results from overgrowth of *C. difficile* in an asymptomatic carrier or whether in some cases the organism may have been newly acquired from patients, hospital personnel, fomites, or other reservoirs. The possibility of exogenous infection is suggested by recognized hospital outbreaks of pseudomembranous colitis [8–12].

We observed several patients with antibiotic-associated colitis in the University of Michigan Medical Center Hospital, Ann Arbor, whose organisms may have been transmitted to other persons. In each case one or more additional patients on the same ward were later found to carry *C. difficile*. The present studies were done to determine whether environmental or human sources of *C. difficile* could be found on these wards.

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Materials and Methods

A prereduced selective agar medium, cycloserine, cefoxitin, fructose, and egg yolk agar (CCFA), developed by George et al. [13] was used to culture environmental items, stools, and other specimens. CCFA is capable of detecting as few as 100 cfu of *C. difficile*/g of stool. Plastic 15- × 65-mm Rodac plates (Falcon Plastics, Oxnard, Calif.) containing 17.5 ml of prereduced CCFA were used for environmental sampling by pressing the raised agar surface onto the surface to be cultured. Cultures of air (30 cu ft per sample) were obtained on CCFA plates using a single-stage, slit-impactor air sampler (Mattson-Garvin Co., Maitland, Fla.). Cultures of nose, throat, urethra, stools, and skin were obtained using a prereduced Dacron® swab (Scientific Products, Evanston, Ill.) which was then inoculated onto CCFA. Soil, stool, and sewage samples cultured quantitatively (~1 g or 1 ml) were suspended in prereduced phosphate-buffered saline, and serial 10-fold dilutions were inoculated onto CCFA. Hands were cultured by pressing the fingers onto CCFA. Foods were inoculated onto CCFA using Rodac or conventional culture plates.

All cultures were incubated for 48–72 hr at 37 C in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.). Colonies morphologically resembling *C. difficile* [13] were identified using biochemical reactions and gas chromatography of volatile organic acids, as detailed in the laboratory manual of the Virginia Polytechnic Institute and State University Anaerobe Laboratory, Blacksburg [14].

Fibroblast cell monolayer cultures were used to detect the cytopathic toxin of *C. difficile* in stools or supernatants of brain-heart infusion broth cultures, as described by Rifkin et al. [15, 16]. Specificity of the toxin was determined by demonstrating neutralization of CPEs of positive specimens using five units of *Clostridium sordellii* antitoxin (lot no. 40067-3647; Bureau of Biologics, Food and Drug Administration, Bethesda, Md.).

In this study, symptomatic patients with *C. difficile* and its toxin in their stools were categorized as having colitis if sigmoidoscopy revealed definite gross or microscopic evidence of inflammation. If sigmoidoscopy was not performed or revealed no inflammation, patients were categorized as having *C. difficile*-associated diarrhea.

Results

The first environmental survey was performed when we detected two patients colonized with *C. difficile* in a surgical intensive care unit (ICU). The index case was a 61-year-old man who developed colitis due to *C. difficile* after receiving prophylactic cephalosporins perioperatively. Two weeks later, a second patient who then occupied the bed previously used by the index case developed severe *C. difficile*-associated diarrhea (he had experienced transient mild diarrhea of unknown cause before his admission to the surgical ICU). Stool cultures were obtained over the next two weeks from the other 14 patients in the unit. One additional patient was found to carry *C. difficile*, but he had no gastrointestinal symptoms. Nose, throat, and urethral cultures from all patients were negative for *C. difficile*.

Thus, at least three patients in the surgical ICU harbored *C. difficile* within a period of about one month. A total of 432 environmental samples was collected from objects and surfaces by the end of that period, and 48 (11.1%) cultures were positive for *C. difficile* (table 1). Positive cultures were obtained most often from used bedpans, bedpan hoppers, floors of utility rooms, toilet seats, and floors at the bedsides of culture-positive patients. Usually <10 colonies were found on each positive Rodac plate. Clean bedpans, walls, and windows were negative for *C. difficile*, as was the air. Only six (2.8%) of 218 environmental samples were positive in another surgical ICU that was selected for study because we had not detected any *C. difficile* infections in patients there. The difference in rates of positive environmental cultures in the two units was statistically significant ($P < 0.005$). Positive cultures in the second surgical ICU were most often from bedpan hoppers and floors. Cultures of 25 hospital food items were negative.

A second survey was conducted a few months later in our pediatric hospital. The index case was an infant who had been treated with oral amoxicillin and parenteral ampicillin and gentamicin because of otitis media. She was admitted to the

Table 1. Distribution of *Clostridium difficile* isolates taken from the environments of two adult surgical intensive care units (ICUs).

	No. positive/no. sampled (%)	
Sites cultured	Case-associated ICU	Control ICU
Surfaces		
Bedpans (clean)	0/20	0/20
Bedpan hoppers, used bedpans	15/58 (25.9)	2/25 (8)
Counter tops	0/40	0/20
Hospital garments	0/20	0/15
Medical devices*	0/20	0/15
Plants	0/5	ND
Floors of patients' rooms	8/55 (14.5)	1/25 (4)
Floors of soiled utility room	10/50 (20)	3/25 (12)
Toilet seats	15/45 (33.3)	ND
Walls	0/40	0/20
Washbasins, tubs, sinks	0/40	0/20
Windows	0/20	0/20
Air conditioner filters	0/5	0/5
Food products	0/5	0/3
Air†	0/9	0/5
Total	48/432 (11.1)‡	6/218 (2.8)‡

NOTE. ND = not done.
* For example, stethoscopes.
† Thirty cu ft per sample.
‡ $P < 0.005$.

Table 2. Isolation of *Clostridium difficile* from the stools of patients on two pediatric units.

Type of unit	No. of patients studied	Mean age of patients (months)	No. of patients with culture-positive stools (%)	No. of patients with toxin (%)*	No. of patients who later developed diarrhea
Pediatric ward (case-associated)	21	8.9	15 (71)†	9 (43)	5
Newborn intensive care unit (control)	14	1.1	3 (21)†	2 (14)	0

* *C. difficile* toxin detected in stool cultures.
† $P < 0.005$.

hospital because of severe watery diarrhea. Rectal biopsy revealed cryptitis; her stool yielded *C. difficile* (10^5 cfu/ml) and contained *C. difficile* toxin at a titer of 1:10. Stool cultures were negative for other bacterial pathogens, ova, and parasites. There was no response to conservative therapy, but there was a prompt response to treatment with oral vancomycin. The ward environment was studied when an antibiotic-treated roommate of the index case developed diarrhea and had stools containing *C. difficile* (10^5 cfu/g) and its toxin (at a titer of 1:10). Skin cultures (legs and hands) from this infant were positive. A newborn ICU was selected for comparison because we had never isolated *C. difficile* there.

There were 21 infants on the case-associated pediatric ward, and 15 (71%) of them had stool cultures positive for *C. difficile* (10^2–10^7 cfu/g) at least once during the next two weeks (table 2); nine of these 15 also had *C. difficile* toxin in their stools in titers as high as 1:1,000. The mean age of these patients was about nine months. Only two infants (the two mentioned above) had diarrhea when first studied. However, five of the colonized infants subsequently developed diarrhea, and two developed colitis due to *C. difficile* requiring treatment with vancomycin after they were given antibiotics.

Two of 12 cultures obtained from the hands of personnel working on the pediatric ward were found to be positive. One was from a nurse and the other was from a technician who performed venipunctures. No more than three colonies were detected on each positive plate. Stools from two of 11 asymptomatic nurses working on the ward were positive for *C. difficile*.

In the newborn ICU, three (21%) of 14 neonates were found to have *C. difficile* in their stools. None of the three had an abnormal stool frequency or consistency. Two of them had stools that were toxin-positive (one at a 1:2 and the other at a 1:1,000 dilution). The numbers of *C. difficile* in stools ranged from 10^3 to 10^7 cfu/g (wet weight). The mean age of infants in this ICU was about one month.

The culture survey on the pediatric ward show-

Table 3. Distribution of *Clostridium difficile* isolates taken from the environment of two pediatric units.

Sites cultured	No. positive/no. sampled (%)	
	Pediatric ward (case-associated)	Newborn intensive care unit (control)
Surfaces		
Bedpan hoppers	0/25	0/15
Chart covers	1/30 (3.3)	0/20
Cribs (occupied)	2/45 (4.4)	1/20 (5)
Dust mops, dust pans on cleaning carts	2/6 (33.3)	0/4
Floors		
Bathroom	4/40 (10)	NA
Clean storage room	2/30 (6.7)	0/20
Patient's room	6/50 (12)	1/30 (3.3)
Soiled room	2/30 (6.7)	0/20
Hospital garments	0/20	0/11
Linens, blankets (clean)	0/20	0/8
Linens, blankets (in use)	5/50 (10)	3/37 (8.1)
Medical devices*	0/25	0/20
Mobiles, toys	1/20 (5)	0/12
Scales	8/40 (20)	1/30 (3.3)
Washbasins, sinks, tubs	4/40 (10)	1/30 (3.3)
Air†	0/7	0/2
Total	37/478 (7.7)‡	7/279 (2.5)‡

NOTE. NA = not applicable.
* For example, stethoscopes.
† Thirty cu ft per sample.
‡ $P < 0.005$.

ed that 37 (7.7%) of 478 environmental cultures were positive for *C. difficile*. Most isolates were taken from floors in bathrooms or rooms of colonized patients, but the organism was recovered from a wide variety of sites, including scales, washbasins, dust mops, dust pans, used linens and blankets, cribs, hospital charts, and toys (table 3). In the newborn ICU, seven (2.5%) of 279 environmental samples were positive, a rate which was significantly lower than in the other unit ($P < 0.005$). *C. difficile* was obtained from linens in use, cribs, floors, scales, and washbasins. Cultures from the hands of five persons working in the newborn ICU were negative. Nine air samples were negative in both units.

When the infant described as the index case developed a recurrence of *C. difficile*-associated diarrhea at home and had to be rehospitalized, we decided to study her family members and home environment. One of two asymptomatic brothers carried *C. difficile* in his stool. Three other family members were negative. In the home, 15 (12.2%) of 123 environmental items were positive (table 4),

Table 4. Distribution of *Clostridium difficile* isolates taken from two home environments.

Sites cultured	No. positive/no. sampled (%)	
	Case-associated home	Control home
Bathroom		
Floors	2/15 (13)	0/15
Sink cabinet	6/15 (40)	0/15
Inside toilet cover	1/10 (10)	0/10
Bedrooms		
Floors	1/15 (7)	0/10
Bookcase	1/4 (25)	0/5
Linens	0/10	0/5
Toys	0/15	0/7
Living room		
Crib	0/10	NA
Utility room		
Floor	1/10 (10)	0/5
Freezer door	1/5 (20)	0/5
Soiled clothing	0/10	0/5
Yard		
Soil	2/2 (100)	0/1
Tap water*	0/2	0/1
Total	15/123 (12.2)†	0/84†

NOTE. NA = not applicable.
* Samples taken from kitchen (100 ml per sample).
† $P < 0.001$.

including the bathroom sink cabinet, floors, toilet, bookcase, freezer door, and soil from beneath a swing in a play yard. None of 84 cultures was positive in another home where five family members did not carry *C. difficile*. Cultures were obtained of 20 soil samples from randomly chosen sites in Ann Arbor, Mich., and *C. difficile* was not isolated from any of them.

After observing the relatively high prevalence of *C. difficile* in the environments of patients with active illness due to *C. difficile*, we studied other parts of the hospital where cases had been seen earlier. On an oncology ward where antibiotic use was intensive, 17 (40%) of 43 patients had *C. difficile* in their stools at least once during a four-month period; four of the 17 patients were also positive for *C. difficile* toxin. Three of the four patients developed colitis due to *C. difficile* during the study; two patients responded to treatment with vancomycin, and colitis contributed to the death of the third. Thus, 7% of the 43 patients on this ward developed colitis during our study. No colonized patient lost the organism spontaneously. We restudied 30 patients whose stools were initially negative. Four (13%) of them developed positive stools after one to three negative cultures, and one of them also developed colitis due to *C. difficile*.

Only five (2.1%) of 239 cultures obtained from environmental sites before any of the patients developed colitis were positive. Six weeks later, when one of the patients developed colitis, environmental sampling was repeated. In that patient's room, 19 (19.6%) of 97 samples were positive (table 5). In contrast, five (6.8%) of 74 samples were positive in the room of a patient who was an asymptomatic carrier of *C. difficile*. Only two (2.6%) of 78 samples were positive in the room of a patient whose stools were negative for *C. difficile*; this rate was about the same as that observed in the first survey of the ward and control areas (tables 1 and 3).

The hands of two of four persons who changed the bed clothing of a patient on the oncology ward having *C. difficile*-associated diarrhea were positive; both persons had negative stool cultures. Two patients with *C. difficile* in their stools on that ward also had positive cultures of hands, and only one had diarrhea.

Positive environmental cultures were uncom-

Table 5. Distribution of *Clostridium difficile* isolates taken from the room environments of three patients in an onocology unit.

Sites cultured	No. positive/no. sampled (%)		
	Patient with *C. difficile* colitis and diarrhea	Asymptomatic stool carrier of *C. difficile*	Patient with diarrhea, culture-negative for *C. difficile*
Bedpans (clean)	0/3	ND	ND
Bedpans (used)	ND	ND	0/3
Counter tops	1/10 (10)	0/5	0/5
Floors of patients' rooms	5/20 (25)	2/15 (13.3)	0/15
Floors of bathrooms	4/10 (40)	1/5 (20)	0/10
Food trays	0/5	0/4	0/4
Hospital garments	1/6 (16.7)	0/5	0/4
Linens, blankets (in use)	4/16 (25)	0/9	0/8
Slippers	ND	1/4 (25)	1/4 (25)
Toilet seats	1/5 (20)	1/5 (20)	1/5 (20)
Washbasins, sinks, tubs	1/10 (10)	0/10	0/10
Sink cabinets	2/5 (40)	0/5	0/4
Doorknobs of bathrooms	0/3	0/3	0/3
Windows	0/4	0/4	0/3
Total	19/97 (19.6)	5/74 (6.8)	2/78 (2.6)

NOTE. ND = not done.

mon in other parts of the hospital. The barium enema room in the radiology department and the sigmoidoscopy room were of special interest. All of 76 samples from the barium enema room were negative, but one of 125 samples from the sigmoidoscopy room was positive. The positive culture was taken from a container used for soiled instruments. Disposable plastic sigmoidoscopes were used in this room, and cultures from the non-disposable handles were negative for *C. difficile.*

Since soiled linens had yielded *C. difficile* in our survey, cultures were obtained from the hospital laundry. In the area where soiled linens were sorted, seven (8%) of 84 surface cultures were positive.

Ten (77%) of 13 samples of sludge from the outflow trap of the main hospital sewer yielded *C. difficile*, with a mean of 4×10^2 cfu/g. Raw influent at the municipal wastewater treatment plant about 1.5 miles away yielded 6×10^2 cfu/100 ml; samples from all final stages of water processing were negative.

A sample of 20 environmental isolates of *C. difficile* was tested for toxigenicity. Supernatants of brain-heart infusion broth cultures of all 20 isolates were positive for the cytotoxin of *C. difficile* after incubation for 72 hr, with titers of toxin ranging from 1:10 to 1:10,000.

The floor of an unused hospital room was intentionally contaminated with a 72-hr brain-heart infusion broth culture of an environmental isolate, and the surface was cultured at regular intervals thereafter (table 6). *C. difficile* could be isolated from the floor for five months, although the numbers of recoverable organisms declined markedly (99%) within the first day or two after inoculation.

Discussion

The most important finding in this study was that *C. difficile* could be detected with ease in the environment of patients with antibiotic-associated diarrheal syndromes. The prevalence of *C. difficile* was significantly higher there than in areas where there were no known cases of colitis due to *C. difficile* ($P < 0.005$). Overall, we isolated the organism from 85 (9.3%) of 910 environmental cultures in the case-associated wards, but from only 13 (2.6%) of 497 cultures from wards where there were no known cases. There seemed to be a higher rate of positive cultures when carriers had diarrhea than when they did not. Objects most likely to yield the organism were those most likely to be contaminated with feces (bedpan hoppers, toilet seats, sinks, and scales), but floors, dust

Table 6. Persistence of *Clostridium difficile* inoculated onto the floor of an unused hospital room.

Time of sample	No. of organisms recovered from floor inoculated with		
	BHI control broth	10^4 cfu of *C. difficile**	10^6 cfu of *C. difficile**
Before inoculation	0	0	0
After inoculation			
1 hr	0	TNTC	TNTC
18 hr	0	3	TNTC
2 days	0	0	8
3 days	0	0	5
7 days	0	1	8
2 weeks	0	1	6
4 weeks	0	2	8
8 weeks	0	0	2
12 weeks	0	0	1
16 weeks	0	0	2
20 weeks	0	0	1

NOTE. The floor of an unused hospital room was intentionally contaminated with 72-hr BHI (brain-heart infusion) broth cultures of an environmental isolate of *C. difficile*. Data are expressed as cfu per Rodac plate (Falcon Plastics, Oxnard, Calif.). The surface area of the Rodac plates was ~0.03 sq ft. TNTC = too numerous to count.

* Inoculations are expressed as cfu per sq ft, in 1.0 ml of BHI broth.

mops, and bed clothing also were often positive. All of 20 environmental isolates studied produced a cytotoxin.

When we intentionally contaminated the floor of an unused hospital room with *C. difficile*, <1% of the inoculum was detectable 48 hr later, but small numbers of the organisms survived for five months. Prior contamination from unrecognized cases might explain the fact that 2.6% of cultures from our control areas were positive.

Our results suggest but do not prove that in some cases *C. difficile* may have spread from one patient to another, particularly on the oncology ward where 40% of the patients were colonized. The environment was not necessarily the source of organisms carried by patients. Bacteriophage typing and other epidemiologic techniques for distinguishing different strains of *C. difficile* and tracing their spread are not yet available. Further studies are indicated when these techniques are developed.

C. difficile was isolated from the hands or stools of several persons working on the contaminated wards, who thus might have been responsible for transmitting the organism. We were not able to isolate *C. difficile* from the air during our studies, but we cannot exclude its possible presence there in small numbers. Food items supplied to our patients were found to be negative. Keighley et al. [17] were unable to find any staff carriers of the organism in a hospital where there appeared to be an outbreak of *C. difficile*-associated disease, but five patients who previously had pseudomembranous colitis were found to be stool carriers of *C. difficile*. These investigators were able to isolate the organism from an inanimate object (the shelf on which bedpans were stored) on the wards only once. The sensitivity of their cultural methods is not known [17, 18], and it is also possible that different strains vary in their persistence in the environment. Mulligan et al. [19] have recently recovered *C. difficile* from environmental sites in three hospital rooms occupied by a patient with colitis. As in the present study, floors and bathrooms were most often positive and the degree of contamination seemed to decline after the patient became asymptomatic.

There are few other recognized sources of the organism that might be responsible for infecting patients. *C. difficile* appears to be an infrequent component of the colonic flora of healthy adults. Using nonselective media, George et al. [6] reported the detection of *C. difficile* in the stools of four (2.9%) of 137 healthy adults. *C. difficile* was first identified by Hall and O'Toole [20], who isolated it in 1935 from the feces of four of 10 healthy infants aged two weeks to one year. Snyder [21] recovered *C. difficile* from the feces of 10 of 22 healthy infants in the same age group. On our pediatric ward, 15 of 21 infants carried the organism. Nine infants had toxin-positive stools, but only two had diarrhea when first studied. We cannot explain why infants with toxin-positive stools often are asymptomatic. They may have passively acquired immunity or undeveloped binding sites for the toxin or the organism. It is also conceivable that the *C. difficile* cytotoxin by itself is not responsible for the disease.

George et al. [22] detected *C. difficile* cytotoxin in the stools of asymptomatic patients receiving cefoxitin. Hafiz et al. isolated *C. difficile* from the urogenital tracts of 72% of 108 women attending a venereal disease clinic and from 100% of 42 men

with nonspecific urethritis [23]. These rates are surprisingly high, but the identity of some of the isolates has been confirmed [24]. It is attractive to speculate that infants may be colonized with *C. difficile* during delivery, but the rate of carriage in the urogenital tracts of healthy or pregnant women is not known.

Smith and King [25] isolated *C. difficile* from abdominal wounds in 1962. Gorbach and Thadepalli [26] isolated 152 strains of clostridia from 114 patients with various infections and identified four isolates as being *C. difficile* (one was from a blood culture, and three were from soft tissue infections).

McBee [27] isolated *C. difficile* (along with *C. sordellii*) from the contents of the large intestine of a seal in the Antarctic, and Hafiz and Oakley [28] found it in horse, donkey, and camel dung. We isolated *C. difficile* from the stools of ~5% of healthy hamsters in our laboratory, where studies on antibiotic-induced enterocolitis have been conducted for several years; these hamsters may have acquired the organism by contact with our animal quarters, where the organism is plentiful [29].

Although our data are not conclusive, they suggest that patients and ward personnel carrying the organism, and the ward environment as well, may be important sources of *C. difficile* contamination. Patients with diarrhea and colitis may be especially important sources, as they appear to shed more organisms than do asymptomatic carriers. We believe enteric isolation precautions should be used to prevent spread of the organism from symptomatic patients, but these measures have not been proven to be effective. Careful handwashing does seem worthwhile after contact with a patient with antibiotic-associated diarrhea, and inanimate articles exposed to their feces—such as sigmoidoscopes and bedpans—should be thoroughly cleaned and decontaminated. Much concern has been expressed about the possible role of sigmoidoscopes in transmitting *C. difficile*. In our hospital, they did not appear to be an important factor because disposable sigmoidoscopes were used. The sigmoidoscopy room had a low rate of contamination, and none of our patients underwent sigmoidoscopy before positive cultures were detected.

Since normal fecal flora may suppress *C. difficile*, patients being treated with antibiotics may be at greatest risk of acquiring the organism and developing colitis. Larson et al. [30] showed that hamsters pretreated with vancomycin developed enterocolitis after being given only a few cfu of *C. difficile* by mouth. When two asymptomatic infants who carried the organism during our survey were subsequently given antibiotics for intercurrent infections, both developed colitis due to *C. difficile*. A similar sequence of events was noted with adults on the oncology ward. To our knowledge, this is the first time asymptomatic patients known to carry the organism have been studied prospectively. Most cases of antibiotic-associated colitis during recent years have followed treatment with ampicillin, clindamycin, or cephalosporins, but many other antimicrobial agents have been implicated, although less frequently [9, 10]. The relative risk of developing colitis after treatment with different antibiotics in colonized patients is not known. The evidence suggests that treatment with antibiotics will not be followed by antibiotic-associated colitis unless the patient already carries or becomes colonized with *C. difficile* and, conversely, that the rate of colitis might be high when the organism is prevalent.

Suspected outbreaks of antibiotic-associated colitis due to cross-infection have been reported from Dallas, Tex., St. Louis, Mo., Chicago, Ill., and Birmingham, England [8–12]. Kabins and Spira [8] reported the occurrence in one institution of eight cases of clindamycin-associated colitis in a two-month period; six cases occurred in patients in an ICU. A retrospective survey detected only one case of antibiotic-associated colitis in the previous 20 months. In another hospital, there was a cluster of 28 cases among surgical patients [9, 10]. The incidence of diarrhea associated with the use of clindamycin in different geographic regions has ranged from 4% to 20% [12, 31], and the incidence of pseudomembranous colitis with clindamycin has ranged from 0.01% to 10% [11, 12]. The explanation for these variations is unknown, but cross-infection may be responsible in some cases.

Our results also may have implications concerning recurrences of colitis due to *C. difficile* after treatment with vancomycin [32, 33]. *C. difficile* isolates from such patients have remained susceptible to vancomycin. Recurrences may be a consequence of the failure to eradicate the spore

form of *C. difficile* from the intestines, but they may also result from reinfection with the organism from fomites or human sources such as those identified in this study. The enterocolitis that develops in hamsters after discontinuation of treatment with vancomycin also may be attributable to environmentally derived organisms [34]. We were able to isolate *C. difficile* from the home of one of our culture-positive patients, including the soil in her yard.

Much remains to be learned about the epidemiology and prevention of colitis due to *C. difficile*. The potential chain of transmission from healthy persons, animals, soil, plants, and food to hospital environment, patients, and personnel needs to be delineated better. It is apparent that prevention of this disease involves much more than just control of the antibiotic that precipitates the disease.

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