Epidemiology and Molecular Characterization of *Clostridium difficile* Strains from Patients with Diarrhea: Low Disease Incidence and Evidence of Limited Cross-Infection in a Swedish Teaching Hospital

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We prospectively studied the epidemiology of *Clostridium difficile*-associated diarrhea (CDAD) in a 900-bed hospital over the course of 12 months by PCR-ribotyping of *C. difficile* isolates. A total of 304 cases were diagnosed, corresponding to an overall incidence of 7/1,000 admissions, with higher rates in nephrology, hematology, and organ transplantation wards (37, 30, and 21/1,000), and 72% were classified as hospital associated (onset in hospital or onset at home but after a hospital stay within 2 months). All 382 isolates from 227 of 304 (75%) patients available for PCR-ribotyping were typeable, yielding 70 PCR-ribotypes. The three most common types comprised 30% of hospital-associated and 34% of community-associated cases, indicating import via admitted patients as a major source of *C. difficile* strains occurring in the hospital. Of the 227 patients studied, 38% each contributed 2 to 13 fecal samples positive for *C. difficile* over the course of the study period. Repeat isolates of the same PCR-ribotype as the first isolate were found in 79% of these patients and in 95% of specimens delivered within 30 days, compared to 63% of those obtained at 31 to 204 days. Nosocomial acquisition of CDAD, defined as the proportion of cases sharing *C. difficile* type and admitted to the same ward within 2 or 12 months, was 20% and 32% of hospital-associated cases and 14% and 23% of all cases, respectively. Thus, most CDAD cases diagnosed over the course of the study period, including those associated with hospitalization, appeared to be caused by endogenous *C. difficile* strains rather than by strains truly being acquired in the hospital.

Clostridium difficile-associated diarrhea (CDAD) has become an increasing clinical problem as a nosocomial disease affecting mainly the elderly, patients with serious underlying diseases, and surgical patients (4, 5, 9, 15, 17, 23, 28). *C. difficile* probably represents the most common current cause of bacterial diarrhea in developed countries and, besides caliciviruses, the most common nosocomial diarrheal pathogen (9, 18). Based on laboratory reports, at least 5,000 cases of CDAD occur every year in Sweden, corresponding to 60 cases per 100,000 inhabitants per year, and more than 70% of the cases are associated with a hospital stay (9).

Clusters of nosocomial cases of CDAD have been attributed to transmission of *C. difficile* between patients but also indirectly through the hands of health care workers or via contaminated surfaces or fomites (10, 15, 19). Furthermore, some strains may be more transmissible and also more virulent than others and thus be associated with higher attack rates and a high local incidence of CDAD (12, 13, 21). Also, antibiotic treatment policies and infection control efforts such as hand hygiene and isolation practices may differ not only between

hospitals and wards but also between time periods. These factors influence the rate of selection and nosocomial transmission of *C. difficile* and thus the local incidence of CDAD. Such factors might also explain reported outbreaks of CDAD even though molecular typing of isolates did not support nosocomial transmission of a particular *C. difficile* strain (11, 14, 20, 30).

The aim of the present study was to prospectively investigate the molecular epidemiology of CDAD at a university hospital during a stable endemic situation by PCR-ribotyping of *C. difficile* isolates from consecutive patients with *C. difficile* infection.

MATERIALS AND METHODS

Patients. From 10 February 2000 through 10 February 2001, consecutive patients with *C. difficile* infection at Huddinge University Hospital, Stockholm, Sweden, were included in the study. The hospital serves a population of approximately 900,000 residents in the southern and western parts of Stockholm and provides 908 beds, comprising 20 clinical departments and 60 wards. Many wards were sharing staff and linked two by two and are referred to as linked wards.

Patients were selected for the study by laboratory criteria (see below). Generally, a fecal sample for *C. difficile* diagnosis was sent to the laboratory within 3 days of onset of diarrhea. Date and duration of hospitalizations, date of samplings, and transfers of patients between wards were registered. The study was approved by the ethics committee of the Karolinska Institute (Huddinge, Sweden).

Definitions. Diarrhea was defined as ≥ 3 loose stools per day for at least 2 days. CDAD was defined as diarrhea in a patient with a stool culture positive for *C. difficile* and/or fecal cytotoxin B (15). Hospital-associated CDAD was defined as

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FIG. 1. Section of polyacrylamide gel showing PCR products stained with silver. PCR-ribotyping of *C. difficile* isolates was carried out as described in Materials and Methods. Note the differences between the patterns of type s21 (identical to that of reference strain of serotype H) and s21b (identical to that of reference strain of serotype A8). The positions of molecular size markers (in base pairs) are shown to the right.

episodes with onset after more than 72 h of hospitalization or present on admission in patients who had been hospitalized within the previous 2 months. Community-associated cases were defined as episodes occurring in patients within 72 h after admission but without a history of recent hospitalization, essentially as defined also by others (15, 19, 20). Patients who were hospitalized on the same ward within 2 months and from whom *C. difficile* of identical PCR ribotypes were isolated were presumed to reflect nosocomial acquisition of the organism (30). The first patient in each cluster was considered the index case, and the remaining ones were considered nosocomially acquired cases. Contact cases were defined as those hospitalized concomitantly in the same or an adjacent room as an index case.

Cytotoxin B detection and isolation of *C. difficile*. CDAD was diagnosed by examining feces for the presence of cytotoxin B by a McCoy cell assay in conjunction with a toxin/antitoxin kit (TechLab, Blacksburg, Va.). *C. difficile* was isolated as colonies of typical appearance on cycloserine-cefoxitin fructose agar supplemented with sodium taurocholate (6). The colonies were tested for toxin B production by the same McCoy assay. A fecal sample positive for *C. difficile* by culture and/or positive for cytotoxin B was considered positive.

PCR-ribotyping. All *C. difficile* isolates were subject to PCR-ribotyping. Cell pellets from overnight cultures of *C. difficile* were boiled in 5% Chelex, and PCR amplification of DNA between the genes encoding the 16S and 23S rRNAs was performed with Ready-to-Go PCR tubes (Amersham Biosciences) with the primer pairs and conditions described by Stubbs et al. (21) with minor modifications. The primers used were 5'-CTGGGGTGAAGTCGTAACAAGG-3' and 5'-GCGCCCTTTGTAGCTTGACC-3'. After an initial denaturation at 94°C for 5 min, the PCR conditions were 94°C for 60 s, 55°C for 60 s, and 72°C for 60 s, repeated for 30 cycles on a PTC-200 (MJ Research Inc.). After the final cycle, samples were heated for 72°C for 7 min and cooled to 4°C.

PCR products were separated on precast polyacrylamide gels (GeneGel Excel 12.5/24; Amersham Biosciences) and visualized by silver staining. In addition to the clinical isolates, the serogroup reference strains CCUG 37766 to 37787, corresponding to serogroups C, A2, A3, A4, A5, A6, A7, A8, A9, A10, S1, S3, S4, A, B, D, F, G, H, I, K, and X, respectively, were analyzed. Two reference strains (CCUG 37766 [serogroup C] and CCUG 37779 [serogroup A]) were included on each gel, and a 100-bp DNA ladder was run in every fifth lane. The use of polyacrylamide gels and silver staining resulted in higher resolution (Fig. 1) and thus higher discrimination than the standard agarose gels and ethidium bromide staining used by Stubbs et al. (21) (data not shown). For this reason, we used our own PCR ribotype numbers and prefixes (Sweden).

Analysis of banding patterns. Digitized gels were analyzed in Molecular Analyst Fingerprinting Plus 1.6 software (Bio-Rad, Applied Maths) by cluster correlation and the unweighted pair group method for arithmetic averages. The clustering of the banding patterns was double checked manually, and each unique pattern was given a number and, when necessary, a suffix to indicate a closely related but distinct ribotype.

Statistical methods. The Mann-Whitney *U* test was used to compare ages in subsets of study patients, and the chi square test was used to evaluate differences in characteristics among patient subgroups. JMP software (SAS Institute, Cary, N.C.) was used for all statistical calculations.

RESULTS

Epidemiological data and patient characteristics. During the study year, 304 patients were positive for *C. difficile* by culture and/or fecal detection of cytotoxin B, corresponding to 7 cases per 1,000 admissions. There was a slight predominance of cases during February through June, 8 cases per 1,000 admissions, compared to the remaining 7 months, 5 cases per 1,000 admissions. In total, 382 isolates from 227 of 304 patients (75%) were available for PCR-ribotyping and included in the analysis. The 77 toxin-positive patients missing yielded no *C. difficile* isolate for typing.

Of the 227 cases, 164 (72%) were classified as hospital and 63 (28%) as community associated, although 122 patients (54%) had onset of diarrhea during their hospital stay, and 105 (46%) fell ill at home (Fig. 2). The median age of these 100 males and 127 females was 63 years (range, 1 to 95 years), and 203 patients (89%) had received antibiotics within 2 months prior to the onset of diarrhea. The subset of study patients did not differ from the total patient population with regard to age (median, 61.5 years; range, 1 to 95 years, $P = 0.7$), gender (135 males and 169 females, $P = 0.9$), or proportion of hospital-associated cases (214, 70%) versus community-associated cases (90, 30%) ($P = 0.7$).

The median number of days between admission and the date of microbiological diagnosis of *C. difficile* infection was 10 (range, 2 to 73 days) for hospital-associated cases and 3 (range, 2 to 22 days) for community-associated cases. Of the 227 study patients, 60 (26%) died within 2 months after their episode of *C. difficile* infection. These patients had higher median age, 75.5 years (range, 1 to 95 years), than the survivors, 57 years (range, 1 to 93 years) ($P = 0.0001$), and 54 of 60 (90%) had severe underlying diseases: cardiac failure, cerebrovascular lesion, chronic obstructive pulmonary disease, diabetes mellitus, malignancy, renal insufficiency, and rheumatoid arthritis. In none of these patients was *C. difficile* infection considered the primary cause of death.

Department-specific incidence of *C. difficile* infection. The patients were distributed among 17 of the 20 hospital departments (85%). Seven of these (nephrology, hematology, organ transplantation, infectious diseases, pediatrics, geriatrics, and gastroenterology) contributed 80% of the cases. These departments accounted for approximately 40% of the hospital beds. Their incidences of CDAD were 37.1, 30.2, 21.1, 18.3, 14.6, 13.9, and 4.6 cases/1,000 admissions, respectively, during the study period.

Patient contacts. Fecal samples from 59 patient contacts to 21 arbitrarily selected index patients were analyzed for *C. difficile*. The median age of the contacts, 24 males and 35 females, was 74 years (range, 30 to 92 years). Of the 59 patient contacts studied, seven (12%) ward mates to 5 of 21 index patients (24%) were positive for *C. difficile* by culture and/or fecal detection of cytotoxin B. Three of these seven patient contacts subsequently developed diarrhea.

FIG. 2. Distribution of patients in the study population. Hospital-associated CDAD was epidemiologically defined as an episode diagnosed during hospitalization or on admission in patients who had been hospitalized within the previous 2 months. Community-associated cases had no history of hospitalization. Patients who were hospitalized on the same ward within 2 months and from whom *C. difficile* of identical PCR-ribotype was isolated were presumed to reflect nosocomial acquisition of the organism (see text for details).

PCR-ribotypes of *C. difficile* and their epidemiology. Of the 382 fecal samples culture positive for *C. difficile*, 198 (52%) were also positive for cytotoxin B, 119 (31%) only by isolation of toxigenic strains, and 65 (17%) yielded isolates negative for toxin B. Of the initial samples from each patient, 45 (20%) yielded *C. difficile* strains that did not produce toxin B in vitro.

All 382 isolates available were typeable by PCR-ribotyping. Among the 227 initial patient isolates, 70 different PCR-ribotypes were distinguished. Three of these, s20, s21, and s21b, whose ribotype was identical to that of the serogroup G, H, and A8 type strains accounted for 31% of the isolates (Table 1). Ten PCR-ribotypes were represented by more than five patients each, and 22 types were represented by two to five patients each, whereas 39 PCR-ribotypes were sporadic, i.e., found in only one patient each. Eight of 10 PCR-ribotype s3 strains (80%), five of five ribotype s32 strains (100%), and

three of three ribotype s5 strains (100%) did not produce toxin B in vitro.

The three dominating PCR-ribotypes, s20, s21, and s21b, comprised 30% and 34% of hospital-associated and community-associated cases, respectively (Table 1). In contrast, three other types, s12, s16, and s25b, were isolated exclusively from hospital-associated CDAD cases, representing 11% of these, and three additional ribotypes (s3, s22, and s25) were on average twice as common among hospital as among community-associated cases (14% versus 6%).

The distribution of the 10 most frequent PCR-ribotypes among departments, wards/linked wards, and over time is shown in Tables 2, 3, and 4. There was only minor apparent clustering of cases due to the same ribotype in space (Tables 2 and 3) and in time (Table 4). Among the isolates from 164 CDAD cases classified as hospital associated, 27 PCR-ribotypes occurred in more than one patient. Of the 138 patients having shared PCR-ribotypes, 56 occurred in 24 clusters of only two to three patients each and cared for in the same ward or two linked wards within the same 2-month period. Regarding the first CDAD patient of each pair or triplet as the index case transmitting his/her *C. difficile* strain to the other patient(s), an estimated 20% (32 of 164) of hospital-associated CDAD cases or 14% (32 of 227) of all CDAD cases reflected nosocomial acquisition of the strain (Fig. 2). Assuming that a ward served as a potential reservoir of a *C. difficile* ribotype not only for 2 months but during the whole 12 months of the study period, due to asymptomatic carriage among patients plus spores present in the rooms, the rate of nosocomial acquisition of the strain was estimated to 32% in hospital-associated cases and 23% in all cases. Notably, 77 of 304 (25%) patients were excluded from the analysis due to missing bacterial isolates for PCR-ribotyping.

PCR-ribotypes of repeat isolates of *C. difficile*. Consecutive repeat isolates from 87 of the 227 patients (2 to 13 isolates per patient, total of 237 isolates) were available for typing. In as many as 69 (79%) of these patients, the same PCR-ribotype was found in the initial and the following samples, indicating a

TABLE 1. PCR-ribotypes of initial isolates of *C. difficile* from 227 patients with diarrhea

PCR-ribotype (n = 70)	No. of patients (%)		Presumed serogroup ^a
	Hospital associated (n = 164)	Community associated (n = 63)	
s21	24 (15)	9 (14)	H
s21b	17 (10)	6 (10)	A8
s20	8 (5)	6 (10)	G
s22	10 (6)	1 (2)	
s3	9 (5)	1 (2)	D ^b
s16	8 (5)	0	A
s29b	5 (3)	3 (5)	
s19	4 (2)	3 (5)	
s12	6 (4)	0	A2
s25	5 (3)	1 (2)	
s14	4 (2)	1 (2)	
s32	4 (2)	1 (2)	
s25b	4 (2)	0	
Other ^c	56 (34)	31 (49)	

^a PCR-ribotype identical to that of the respective serogroup reference strain (see text).

^b Toxin A/B negative.

^c Each type represented by fewer than four patients.

TABLE 2. Distribution of PCR-ribotypes of initial isolates of *C. difficile* from 227 patients with diarrhea, including hospital-associated and community-associated cases, by clinical department

Department	No. of patients with PCR-ribotype:											Total
	s3	s12	s16	s19	s20	s21	s21b	s22	s25	s29b	Other ^a	
Pediatrics	2	0	0	1	4	9	4	0	0	3	13 ^b	36
Gastroenterology	0	0	0	1	1	2	1	2	0	1	5	13
Hematology	3	1	1	0	1	2	0	2	0	0	14 ^c	24
Infectious diseases	1	1	2	3	2	9	3	2	1	1	21 ^d	46
Geriatrics	0	2	1	1	2	3	6	3	3	0	10 ^e	31
Nephrology	4	0	1	0	0	2	5	1	0	0	11 ^f	24
Organ transplantation	0	1	1	0	2	1	0	0	1	0	6 ^g	12
Urology	0	0	0	0	0	0	0	0	0	1	3	4
Rheumatology	0	0	0	0	0	0	0	0	0	1	1	2
Orthopedics	0	1	0	0	0	0	0	0	1	0	1	3
Oncology	0	0	0	0	1	1	0	0	0	0	1	3
Neurology	0	0	1	1	0	0	0	0	0	0	0	2
Rehabilitation	0	0	0	0	0	0	0	0	0	0	2	2
Lung medicine	0	0	0	0	0	1	0	0	0	0	2	3
Cardiology	0	0	0	0	0	1	0	0	0	0	1	2
Emergency	0	0	0	0	1	0	0	0	0	0	3	4
Endocrinology	0	0	0	0	0	0	1	1	0	1	3	6
Other departments	0	0	1	0	0	2	2	0	0	0	5	10
All departments	10	6	8	7	14	33	22	11	6	8	102	227

^a PCR-ribotypes represented by fewer than six patients each.^b Including two patients with PCR-ribotype s32.^c Including two patients with PCR-ribotype s25b and two patients with PCR-ribotype s30.^d Including two patients with PCR-ribotype s17 and two patients with PCR-ribotype s2.^e Including two patients with PCR-ribotype s21d and two patients with PCR-ribotype s34.^f Including two patients with PCR-ribotype s25b.^g Including two patients with PCR-ribotype s5.

low overall rate of reinfection. Of 109 repeat isolates sampled within 30 days after the initial isolate, 104 (95%) were of the same PCR-ribotype as the index isolate, compared to 26 of 41 (63%) for repeat isolates obtained at 31 to 204 days. In one

patient contributing isolates from 13 sampling occasions over a period of 1 to 204 days after the first one, the first and seventh isolate were of the rare PCR-ribotype s5, whereas all other isolates were of the most common type, s21. In another patient

TABLE 3. Distribution of PCR-ribotypes of initial isolates of *C. difficile* from 227 patients^a with diarrhea, including hospital-associated and community-associated cases, by different wards^b

PCR-ribotype	No. of hospitalizations per ward or linked ward																
	Pediatri- rics		Infectious diseases				Geriatrics			Emergency unit (1/2)	Gastro- enter- ology		Nephrology (1/2)	Transplantation (1/2)	Hematology (1/2)	Cardiology (1/2)	ICU ^d (1)
	1/2 ^c	3	1	2	3	4	1/2	3/4	5/6		1/2	3/4					
s2c	0	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	0
s3	1	0	0	1	1	1	0	0	0	0	0	0	5	1	3	0	0
s5	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
s12	0	0	0	0	1	0	0	2	0	2	0	0	0	1	2	0	0
s16	0	0	2	0	0	0	0	0	1	1	0	0	1	1	1	0	1
s17	1	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0
s19	1	0	2	0	0	0	1	0	0	2	1	0	0	0	0	0	0
s20	6	0	1	0	1	0	0	0	2	0	1	1	0	2	1	0	0
s21	8	2	3	4	2	3	0	0	3	3	3	1	3	1	3	4	2
s21b	4	1	2	1	0	0	2	3	2	2	0	1	5	1	1	1	1
s22	0	0	3	0	1	0	1	1	1	3	1	2	2	0	2	0	0
s22d	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0
s25	0	0	0	1	0	0	2	0	2	1	0	0	0	1	0	0	0
s25b	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0	0
s29b	3	1	0	1	0	0	0	0	0	1	0	2	0	0	0	0	0
s30	1	0	0	1	0	0	1	0	0	0	0	0	0	0	2	0	0
s32	2	0	0	0	0	0	1	0	0	0	0	0	2	0	0	1	0
s34	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0

^a One patient may have more than one hospitalization.^b Only wards with at least two patients with identical PCR-ribotypes are included.^c Wards within each department are indicated by number. Two numbers combined by a slash (e.g. 1/2), indicate linked wards.^d ICU, intensive care unit.

TABLE 4. Distribution of PCR-ribotypes of initial isolates of *C. difficile* from 227 patients with diarrhea, including hospital-associated and community-associated cases, by month

Month	No. of patients with PCR-ribotype:											All
	s3	s12	s16	s19	s20	s21	s21b	s22	s25	s29b	Other ^a	
January	0	1	0	1	1	1	0	0	1	1	8	14
February	1	0	1	0	2	3	3	3	0	0	13	26
March	1	0	0	1	1	6	1	0	1	2	9	22
April	3	0	1	1	2	3	2	1	0	1	14	28
May	0	1	1	1	1	4	1	1	0	1	13	24
June	3	1	1	0	2	3	1	2	1	0	7	21
July	0	0	2	0	1	3	2	0	0	0	5	13
August	0	0	0	1	1	4	1	1	2	0	6	16
September	1	3	0	0	1	4	5	1	0	0	2	17
October	1	0	0	1	0	2	3	1	0	0	8	16
November	0	0	2	0	1	0	2	0	1	2	6	14
December	0	0	0	1	1	0	1	1	0	1	11	16
Total	10	6	8	7	14	33	22	11	6	8	102	227

^a Each type represented by fewer than six patients.

who contributed repeat isolates from 10 samplings at 1 to 157 days after the first one, all isolates were PCR-ribotype s21 except the ninth one, which was of type s3.

DISCUSSION

Continuous epidemiological surveillance of *C. difficile* infections is regarded to be of importance for detection of clustering of cases in space and time in order to identify and control potential outbreaks. Many studies of *C. difficile* infections in hospitals have described outbreaks due to identical strains, while others have shown a great diversity of *C. difficile* isolates, indicating various contributions from nosocomial transmission and reflecting factors related to patients and antibiotic treatment (10, 11, 15, 14, 19, 20, 30). Interestingly, a large multicenter survey in the United Kingdom showed a dramatic dominance of one PCR-ribotype among hospital but not among community isolates (21).

The incidence of CDAD at Huddinge University Hospital has remained rather stable during the last 5 years, approximately 300 cases annually, corresponding to 7 cases per 1,000 admissions. This rate is in the lower range compared to the incidences of 1 to 30 cases per 1,000 admissions reported from many hospitals (1, 15, 18, 19, 24). The incidence of *C. difficile* infection was highest in the nephrology and hematology units (37 and 30 cases/1,000 admissions, respectively), as found also by others (17, 23, 28). Factors such as infection control procedures and antibiotic treatment policies may explain the differences in CDAD incidence between hospitals and changes over time within individual hospitals and wards.

A wide range of phenotypic and genotypic methods have been used to analyze the relatedness of *C. difficile* isolates in nosocomial outbreaks (3, 5, 8, 21, 25, 30). PCR-ribotyping is a discriminatory, reproducible, and relatively rapid technique that provides many advantages over other methods (3, 5, 8, 21). It was recently reported that although serotype G strains are nontypeable by pulsed-field gel electrophoresis, the discriminatory power of that technique was somewhat higher than that of PCR-ribotyping, partly because serogroup H and A8 isolates had the same PCR-ribotype pattern (3). However, by using

polyacrylamide gels, we were able to clearly discriminate the serotype H and A8 type strains, obtaining the closely related PCR-ribotypes s21 and s21b (Fig. 1). In our study, polyacrylamide gels and silver staining revealed many weaker bands and thus provided additional PCR-ribotypes and improved discriminatory power compared to agarose gels and ethidium bromide staining of DNA.

In the present 1-year study, 70 different PCR-ribotypes of *C. difficile* were identified among 382 isolates from 227 patients. The cases and ribotypes were rather evenly distributed over the year, and the occurrence of PCR-ribotypes among cases defined as hospital associated and community associated was the same for several major PCR-ribotypes, indicating that these strains were often brought into the hospital by the patients. However, three PCR-ribotypes, s12, s16, and s25b, were unique to CDAD cases classified as hospital associated. No major clustering of any ribotype was found either by ward or in time, not even among the 10 most common ribotypes represented by more than five patients each. Instead, many apparent clusters comprising only two to three patients each were observed.

Seventy-two percent of the CDAD cases were primarily classified as hospital associated. Nosocomial acquisition of *C. difficile* was also assumed in cases sharing *C. difficile* of the same ribotype and admitted to the same ward within 2 months, suggesting hospital acquisition of the infecting strain for only 20% of nosocomial and 14% of all CDAD cases. Thus, a majority of the CDAD patients were apparently infected by endogenous rather than endemic ward strains, even among hospital-associated cases. It must be stressed, however, that 25% of the patients with *C. difficile* infection were excluded from the analysis because bacterial isolates were not available for PCR-ribotyping. The estimated rate of nosocomial acquisition could therefore be underestimated.

The prevention of *C. difficile* has been a main concern for our hospital infection control team for many years. Control measures that are continuously taught among the staff include isolation of patients with suspected infectious diarrhea in a single room, strict general barrier nursing, and specific cleaning routines whenever *C. difficile* is diagnosed. Our results

support the notion that these efforts have been of value in minimizing the acquisition of *C. difficile* within the hospital. In recent years, a comprehensive infection control program has been launched to further reduce nosocomial infections due to *C. difficile* and antibiotic-resistant bacteria at Huddinge University Hospital.

Five of the 70 PCR-ribotypes, s3, s20, s21, s21b, and s22, accounted for as many as 40% of the *C. difficile* isolates. Ribotypes s3 and s22 were common at Huddinge University Hospital, whereas ribotypes s20, s21, and s21b were also common among hospitalized community-associated cases. Earlier studies indicated that certain serotypes of *C. difficile* might be more virulent than others (12). Whether the most common PCR-ribotypes are more transmissible or more virulent, contributing to their high overall incidence among our patients, has yet to be demonstrated. Studies are in progress to characterize the isolates from the dominant ribotypes found in our study more thoroughly with regard to toxin production, germination, and sporulation and also correlate such bacterial properties with the severity of clinical symptoms of the patients and with relapse risk.

Of more than 2,000 *C. difficile* isolates analyzed by PCR-ribotyping in the United Kingdom, one particular ribotype, denoted type 1, has shown a dramatic dominance in hospital settings, 55% of isolates compared to 7.5% among community isolates (21). No such dominant clone was observed in our material. The three most common PCR ribotypes s20, s21, and s21b, clustered together with the serogroup G, H, and A8 reference strains, respectively (Fig. 1). The banding pattern of s21b showed a pattern related to but different from that of s21 (Fig. 1), indicating a clonal relationship between these two. However, a preliminary genomic analysis of their surface layer proteins revealed significant differences between the respective type strains, CCUG 37784 (serogroup H) and CCUG 37773 (serogroup A8) (16). Thus, interpreting clonal relationships between bacterial strains on the results of only one typing method may not be sufficient. PCR ribotype s3 clustered together with CCUG 37781, belonging to serogroup D. This type strain is toxin A and B negative, and analysis of eight clinical isolates from this group showed that these were also toxin negative. Furthermore, all three strains from group s5 grouped together with type strain CCUG 37786 of serogroup K, a strain also noted to be toxin negative. The reason for the symptoms in these patients is unknown, but the presence of toxin-negative *C. difficile* in some patients may reflect a carrier state without a causal relationship to current diarrheal disease (22, 28) or that such strains carry unknown virulence factors.

In 79% of patients from whom two or more consecutive *C. difficile* isolates were available for PCR-ribotyping, the repeat isolate(s) was of the same ribotype as the initial one, indicating a low overall rate of reinfection. This is in agreement with several other reports (2, 27) and again apparently reflected successful infection control in our hospital, although the possibility of occasional reinfection with the same strain could not be ruled out. The fact that only 5% of the isolates were of a new ribotype in repeated fecal specimens collected within 30 days after the initial one also suggested that a single patient was rarely infected with multiple *C. difficile* strains, as reported also by others (2, 27).

Carriage of *C. difficile* in contacts to patients with CDAD is

not uncommon (7, 10, 15, 19). Among 59 such contacts in the present study, 12% were positive for *C. difficile* by culture and/or fecal detection of cytotoxin B. This carriage rate is relatively low, considering that CDAD patients were present in the same ward, again supporting only a low level of transmission of *C. difficile* in the hospital. Since none of these isolates were available for PCR-ribotyping, possible nosocomial acquisition could not be studied.

In conclusion, 70 distinct PCR-ribotypes of *C. difficile* were found at Huddinge University Hospital during this prospective 1-year study. Ten ribotypes were dominant, although not markedly clustered in specific wards or in time, and the dominant types were equally common in CDAD cases classified as community associated and hospital associated. These observations indicated that nosocomial transmission of *C. difficile* was not a major problem during the study period. Our results indicate that CDAD in most patients was due to endogenous rather than hospital strains of *C. difficile*, even in cases classified as hospital associated. The CDAD incidence in a hospital is an indicator of implementation of hygienic routines and antibiotic policy and should be monitored continuously. Well-conceived and functioning infection control programs and antibiotic guidelines are important to keep CDAD rates at a minimum.

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