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CASE REPORT

Community-onset *Clostridium difficile*-associated diarrhoea not associated with antibiotic usage

Two case reports with review of the changing epidemiology of *Clostridium difficile*-associated diarrhoea

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ABSTRACT

The emergence of hypervirulent strains of *Clostridium difficile* causing outbreaks in hospitals and nursing homes may result in a greater than before spread of the bacterium in the community. By consequence, the incidence of community-onset cases of *Clostridium difficile*–associated diarrhoea (CDAD) may increase outside known risk groups that are currently characterised by prior hospitalisation, prior antibiotic usage, older age and significant comorbidity. Here, we describe two case histories of community-onset CDAD. The first concerns a previously healthy young female with community-acquired CDAD without recent hospitalisation or antibiotic usage. The second patient developed diarrhoea in the community after discharge from a hospital where – in retrospect – an outbreak of CDAD occurred. The cases illustrate that CDAD should be included in the differential diagnosis of patients seeking care for community-onset diarrhoea, even in those without characteristic risk factors for CDAD.

KEYWORDS

Clostridium difficile, community-acquired diarrhoea, passive immunotherapy

INTRODUCTION

Recently, outbreaks of diarrhoea due to *Clostridium difficile* PCR-ribotype 027 have been reported in Canada, the United States and Europe, including the Netherlands.[1-5] Typically, outbreaks occur in a hospital or nursing home and primarily affect elderly individuals who suffer significant underlying conditions that make them susceptible to acquiring *C. difficile*-associated diarrhoea (CDAD).[6] Among the predisposing conditions, exposure to antibiotics during an extended period of hospitalisation is regarded as most significant.[7] The severity of CDAD can range from transient, mild diarrhoea to fulminant colitis. A recently circulating strain of *C. difficile* characterised as toxinotype III, North American pulsed field type 1, restriction endonuclease analysis group BI and PCR ribotype 027 has been associated with enhanced virulence, apparently due to the production of higher amounts of toxins.[1,4,8]

As can be expected from the known risk factors of CDAD (i.e., hospitalisation, old age, antibiotic usage, underlying medical conditions, gastrointestinal surgery, nasogastric tubes, etc), most of the outbreak reports have dealt with nosocomial CDAD. There is an overall lack of information on community-onset CDAD. Here, we describe two such cases: a case of truly community-acquired CDAD without any known risk factors and a case of community-onset CDAD caused by an epidemic strain likely acquired during

a recent stay in hospital. The two cases illustrate various aspects of community-onset CDAD and indicate that physicians should be aware of the possibility of CDAD cases in the community, also in those who do not have known risk factors for CDAD. Moreover, such cases suggest that the epidemiology of CDAD may be changing, with a greater than before circulation of the bacterium in the community due to increased introduction of the bacterium from hospitals and institutions with outbreaks.

CASE REPORT

A 28-year-old female presented to the emergency department because of syncope and severe diarrhoea. Her prior medical history was unremarkable with the exception of two caesarean sections, performed years before the present admission. On presentation, she complained of cramping abdominal pain, nausea and vomiting of one day's duration, and passing of profuse watery stools mixed with blood. Soon after her symptoms began she noticed light-headedness and briefly lost consciousness during passage of stools. Because of the peracute nature and severity of her symptoms, her general practitioner referred her to the emergency room of the nearby hospital.

The patient was not taking any medication, nor had she recently used any. On physical examination, she did not appear severely ill. A blood pressure of 80/40 mmHg and a pulse of 60 beats/min were noted. Examination of heart and lungs was unremarkable, her abdomen was tender. Rectal examination revealed pink stools with some mucus.

Laboratory investigation revealed a leucocytosis of 12.9 x 10^9/l, with a neutrophil count of 11.2 x 10^9/l. The haemoglobin level was 8.5 mmol/l and the erythrocyte sedimentation rate (ESR) was 5 mm/h. Urea, creatinine, glucose, electrolyte and liver enzyme levels were within normal limits.

A diagnosis of vasovagal syncope due to a severe bout of gastroenteritis and mild dehydration was made. The patient was admitted for fluid resuscitation (3 litres in the first 24 h). Stool cultures were negative for *Salmonella, Shigella, Yersinia* and *Campylobacter* and stool examination did not reveal any parasites, such as *Gardia lamblia*. An enzyme-linked immunosorbent assay (ELFA, Biomerieux) for *C. difficile* toxin A on stool was positive. Her stools were not cultured for *C. difficile*. Treatment with oral metronidazole 500 mg three times daily for ten days was initiated, a regimen to which she responded favourably. After three days she was discharged and she completed her ten-day course of antibiotics at home. After completion of the antimicrobial regimen, she participated in an experimental protocol aimed at reducing the occurrence of relapses of CDAD and received a bovine immune milk preparation (anti-CD WPC) for two weeks. During a follow-up up to 60 days after start of the immune milk, the patient remained asymptomatic, and contact over one year later indicated that CDAD had not recurred.

Regarding the possible source of her *C. difficile* infection, an extensive history was taken. This revealed that three months previously the patient's two infant sons had been admitted to another hospital for two days because of a respiratory tract infection. The patient had spent one night in the hospital with her children during their admission. There had been no *C. difficile* outbreak in this hospital. Of note, neither the sons nor other family members had experienced diarrhoea. Moreover, after the diagnosis of CDAD had been made in the patient, stool cultures of her husband and two sons were taken but were negative for *C. difficile*. In conclusion, no plausible source of exposure could be established. It seems highly unlikely that the patient's episode of CDAD is related to her one-day stay in hospital three months earlier.

The second case concerns a 71-year-old male who was admitted with progressive diarrhoea. He had suffered a stroke in the past and had vascular dementia with secondary parkinsonism, chronic obstructive pulmonary disease, hypertension and chronic renal failure due to nephrosclerosis. One week before admission, he had been discharged from hospital where he was being examined for chronic watery stools with concomitant loss of an already compromised kidney function. Diarrhoea had been present for six months. At that admission, peripheral eosinophilia (0.7 x 10^9/l) was noted. A computed tomography scan of the abdomen showed extensive arterial wall abnormalities compatible with atherosclerosis and thickening of the sigmoid wall. Colonoscopy had not revealed abnormalities, whereas biopsies of the sigmoid showed mild inflammation with infiltration of eosinophils. Microbiological examination of stools including multiple tests for *C. difficile* toxin and extensive parasitological examination were negative. On the basis of these findings and especially the fact that repeatedly, no infectious agent could be demonstrated, a differential diagnosis of eosinophilic colitis or cholesterol embolism was made. Symptomatic treatment with loperamide and haemodialysis was started. Upon readmission the patient had a fever up to 39.1°C. His medication consisted of loperamide, aspirin, clopidogrel, atorvastatin, perindopril, metoprolol, temazepam, levodopa/carbidopa, alfacalcidol, epoetin beta iv during haemodialysis and ipratropium albuterol inhalations; he had not received antibiotics recently. The patient lived in a nursing home. Physical examination, including the abdomen, did not reveal abnormalities except for a mild tachycardia of 110 beats/min and increased bowel sounds. Laboratory investigation showed a leucocytosis of 15.6 x 10^9/l and an ESR of 48 mm/h. The eosinophilia had decreased. His C-reactive protein level was 322 mg/l. A rapid immunoassay for *C. difficile* toxin A (ELFA, BioMérieux) was positive. *C. difficile* was cultured from the stool as well; the strain was typed as ribotype 027. This case later on proved to be part of an epidemic due to ribotype 027

in this hospital. Treatment was started with oral vancomycin 250 mg four times a day for 14 days. After the antimicrobial regimen, the patient also participated in the experimental protocol and received a bovine immune milk preparation (anti-CD WPC) for two weeks. His condition improved and he was discharged from hospital; rapid stool tests for *C. difficile* toxins were repeatedly negative.

DISCUSSION

The cases presented here concern community-onset CDAD. The first case illustrates that CDAD can be acquired in the community in the absence of any of the known risk factors for this disease. The second case illustrates how exposure to *C. difficile* in a hospital in which CDAD is endemic can cause CDAD to spread into the community. It underlines once more that prior use of antibiotics is not a necessary factor for CDAD to develop.

Three factors are thought to explain the classical risk profile for CDAD. First, the patient must be exposed to the pathogen. Although the bacterium is ubiquitous and can be isolated from many sources both inside and outside hospitals, CDAD is most frequently acquired in hospitals and care institutions where the bacterial load is likely high because host factors predispose the population admitted to these institutions to develop clinical disease.[6] Second, prior administration of antibiotics and consequent disruption of the resident bowel flora has always been considered important, if not necessary, for colonisation by *C. difficile*. In particular clindamycin, cephalosporins, fluoroquinolones (especially of the later generations) and less so macrolides and intravenous β-lactams with β-lactamase inhibitors have been associated with CDAD.[2,7,9-19] Lastly, a host factor appears to determine, at least in part, whether or not colonisation is followed by clinical manifestations of CDAD. Older age cohorts admitted for extended periods because of severe underlying disease are at highest risk for CDAD.[7,19-22] Presumably, in these individuals a lack of effective antitoxin humoral immunity is a decisive factor in developing CDAD, since long duration of disease and relapse has been associated with lower concentrations of circulating and faecal antibodies against *C. difficile* toxins A and B.[23-25]

Since early 2003, an increase in the incidence of CDAD has been reported in Canada and subsequently in the upper part of the United States of America and Europe. The CDAD cases in this outbreak were remarkable because they ran a more severe course.[19,26-28] The greater morbidity was associated with the emergence of PCR ribotype 027.[1,4,8] In just a few years, outbreaks of CDAD due to PCR ribotype 027 occurred in the Netherlands as well.[3,4,29-31] Of note, the outbreaks concerned hospitalised or institutionalised patients. One report already noted an increase in community-acquired cases of CDAD in a population not considered at risk but unfortunately only a few strains of *C. difficile* were available for typing and type 027 was not found.[32]

The rate of community-acquired (CA) CDAD, formerly a very rare entity, appears to be increasing.[19,33-35] *Table 1* summarises findings in the studies that have been published on this subject. Some of these cases may actually be hospital acquired, since definitions of CA-CDAD vary. However, some clearly do not fit the classical risk profile.[19,32,36] A systematic surveillance of CA-CDAD has not been performed until recently. Stool samples of 703 patients with diarrhoea submitted by general practitioners in an area of 3.6 million inhabitants in Germany were investigated for pathogens including *C. difficile* by culture and enzyme immunoassay for *C. difficile* toxin A/B. The *C. difficile*-toxin A/B assay was positive in 66 (9.3%) of the stool samples. Thirty-one (47%) of 66 patients had healthcare-associated diarrhoea (i.e., defined as an onset of symptoms within four weeks after hospital discharge) whereas 35 (53%) were truly community-acquired. Recent usage of antibiotics was reported by 34/66 (52%) patients, most frequently cephalosporins (33%) and fluoroquinolones (33%).[37]

If the incidence of CA-CDAD is indeed increasing, what could be the cause? The emergence of CDAD in hospital outbreaks undoubtedly leads to the spread of the pathogen among admitted patients, not all of whom will develop symptoms of CDAD during hospitalisation. As illustrated by the second case, some cases of CDAD can be expected to occur in the weeks or even months following discharge. In addition, the increased circulation of *C. difficile* within hospitals will increase the rate of asymptomatic *C. difficile* carriership within the population, due to temporary excretion of the pathogen by discharged patients and/or healthcare workers. Contact with such cases will in the end lead to some cases of community-acquired CDAD. Furthermore, it has been suggested that an animal reservoir may play a role in the emergence of community-acquired CDAD.[38] *C. difficile*-associated disease and carriage have been reported in pets and farm animals. In 1993, the role of pets as a reservoir was investigated comparing restriction endonuclease analysis types of *C. difficile* isolates from pets, veterinary clinics, humans and hospitals.[39] In that study, there was no correlation between isolates from pets and humans and therefore it was concluded that animals do not form an important reservoir for strains that cause human disease. However, *C. difficile* seems to have become more important as an animal pathogen[40] and a number of recent studies have found overlap between animal and human ribotypes, suggesting that there is interchange of strains between animals and humans.[41,42] Of note, *C. difficile* could be cultured from 20% of retail meat samples in a Canadian study, with a majority of the toxigenic isolates being *C. difficile* type 027.[43] Incidentally, neither of the patients we describe had had contact with any possible animal source.

The first patient recovered quickly after treatment with metronidazole. In the recent outbreaks, however, the relapse

Table 1. *Results of published studies concerning community-acquired CDAD*

Study	Country, year, setting	Overall incidence of CDAD (/100,000 py)*	Number of patients with CDAD	Definition of CDAD	Proportion of CA-CDAD (%)	Definition of CA-CDAD	Proportion of COHA-CDAD (%)	Proportion of noso-comial CDAD (%)	Proportion of CDAD with unknown location of onset
Karlström[35]	Sweden, 1995, GP/hospital	58	1888	Stool positive in any test for CD and CT+	28	CO, no hospitalisation preceding 4 weeks	15	52	5
Kyne[25]	Ireland, 1995, hospital	?	73	Diarrhoea and CT+	11	CO or onset in first 72 hours of admission without hospitalisation preceding 60 days	9.6	79.4	0
Wheeler[33]	UK, 1993-1996, community	160	6	Diarrhoea and CT+	100†	CO	?	0	0
Wheeler[33]	UK, 1993-1996, GP	20	17	Diarrhoea and CT+	100†	CO	?	0	0
Dial[19]	UK, 1994-2004, GP	<1 in 1994 to 22 in 2004	1672	Clinical diagnosis and/or CT+	74	CO without hospitalisation preceding year	26	0	0
Paltansing[34]	Netherlands, 2005, hospital	16/10,000 admissions	81	Diarrhoea and CT+	6§	CO	30§	61	3
Riley[49]	Australia, 1988 GP	5.5% of stool samples	16	Diarrhoea, CD cultured or CT+	100†	CO	?	0	0

CDAD = *Clostridium difficile*-associated diarrhoea; py = person years; CA-CDAD = community-acquired CDAD, COHA-CDAD = community onset healthcare-associated; GP = general practice; CD = *Clostridium difficile*; CT+ = cytotoxin test positive in stool; CO = community onset. *Unless otherwise specified. †CA-CDAD and COHA-CDAD were not separated. §Data partially through personal communication.

rate of CDAD has increased from about 20% to as high as 47% in cases caused by PCR ribotype 027. Unfortunately, besides increasing the dose or extending the course of antibiotics, switching metronidazole into oral vancomycin and using alternating or pulsed regimens, there is little one can do to prevent cycles of relapses and even the measures mentioned have not been proven to be efficacious. Also, the efficacy of strategies including probiotics, bacteriotherapy, toxin-absorbent resins and intravenous immunoglobulins is currently uncertain and not supported by evidence from clinical trials.[44,45] Previously, we reported on the use of passive immunotherapy with anti-*C. difficile* whey protein concentrate (40%; anti-CD-WPC) made from milk from cows immunised with inactivated *C. difficile* toxins and killed bacterial cells. Anti-CD-WPC neutralises the action of toxins *in vitro* and protects against CDAD in an animal model.[46] As a milk product, it was found safe for use in humans with CDAD[47] and in a first, uncontrolled trial an about 50% reduction in relapse rate was observed.[48] However, the efficacy of this treatment modality still has to be submitted to a dose-finding and placebo-controlled randomised trial.

In conclusion, the emergence of new strains of *C. difficile* causing outbreaks in hospitals and nursing homes in recent years may also forward the circulation of such strains in the general population, and increase the incidence of community-acquired cases of CDAD outside the well-known risk groups. The present case histories illustrate that CDAD should be included in the differential diagnosis of both acute and chronic community-onset diarrhoea, even when the patient has not recently taken antibiotics, is young and has no comorbidity. It also underscores that strict hygienic measures should be taken in all patients with diarrhoea to prevent spread of the pathogen.

REFERENCES

1. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of Clostridium difficile. N Engl J Med 2005;353:2433-41.
2. Pépin J, Saheb N, Coulombe M-A, et al. Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile-associated diarrhea: a cohort study during an epidemic in Quebec. Clin Inf Dis 2005;41:1254-60.
3. Van Steenbergen J, Debast S, van Kregten E, van den Berg R, Notermans D, Kuijper E. Isolation of Clostridium difficile ribotype 027, toxinotype III in the Netherlands after increase in C. difficile-associated diarrhea. Eurosurveillance Wkly 2005;10.
4. Kuijper EJ, Coignard B, Tüll P; the ESCMID Study Group for Clostridium difficile. Emergence of Clostridium difficile-associated disease in North America and Europe. Clin Microbiol Infect 2006;12(Suppl 6):2-18.
5. Kato H, Ito Y, van den Berg RJ, Kuijper EJ, Arakawa Y. First isolation of Clostridium difficile in Japan. Eurosurveillance Wkly 2007;12(1).

6. McFarland LV, Mulligan ME, Kwok, RY, Stamm WE. Nosocomial acquisition of Clostridium difficile infection. N Engl J Med 1989;320:204-10.

7. Bignardi GE. Risk factors for Clostridium difficile infection. J Hosp Infect 1998;40:1-15.

8. Pépin J, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile-associated disease during an epidemic caused by a hypervirulent strain in Quebec. Can Med Assoc J 2005;173:1037-42.

9. Yip C, Loeb M, Salama S, Moss L, Olde J. Quinolone use as a risk factor for nosocomial Clostridium difficile-associated diarrhea. Infect Control Hosp Epidemiol 2001;22:572-5.

10. McKusker ME, Harris AD, Perencevich E, Roghmann M-C. Fluoroquinolone use and Clostridium difficile-associated diarrhea. Emerg Infect Dis 2003;9:730-3.

11. Gaynes R, Rimland D, Killum E, et al. Outbreak of Clostridium difficile infection in a long-term care facility: association with gatifloxacin use. Clin Inf Dis 2004;38:640-5.

12. Palmore TN, Sohn S, Malak SF, Eagan J, Sepkowitz KA. Risk factors for acquisition of Clostridium difficile-associated diarrhea among outpatients at a cancer hospital. Infect Control Hosp Epidemiol 2005;26:680-4.

13. Modena S, Bearelly D, Swartz K, Friedenberg FK. Clostridium difficile among hospitalized patients receiving antibiotics: a case-control study. Infect Control Hosp Epidemiol 2005;26:685-90.

14. Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of Clostridium difficile-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol 2005;26:273-80.

15. 15.Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to Clostridium difficile, Clostridium perfringens, and Staphylococcus aureus. J Clin Microbiol 2006;44:2785-91.

16. Raveh D, Rabinowitz B, Breuer GS, Rudensky B, Yinnon AM. Risk factors for Clostridium difficile toxin-positive nosocomial diarrhea. Int J Antimicrob Ag 2006;28:231-7.

17. Gifford AH, Kirkland KB. Risk factors for Clostridium difficile-associated diarrhea on an adult hematology-oncology ward. Eur J Clin Microbiol Infect Dis 2006;25:751-5.

18. Biller P, Shank B, Lind L, et al. Moxifloxacin therapy as a risk factor for Clostridium difficile–associated disease during an outbreak: attempts to control a new epidemic strain. Infect Control Hosp Epidemiol 2007;28:198-201.

19. Dial S, Delaney JAC, Barkun AN, Suissa S. Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. JAMA 2005;294(23):2989-95.

20. McFarland LV, Surawitz CM, Stamm WE. Risk factors for Clostridium difficile carriage and Clostridium difficile-associated diarrhea in a cohort of hospitalized patients. J Infect Dis 1990;162:678-84.

21. Sheck FW, Stacey BSF, Rendell J, Hellier MD, Hanson PJV. The rise of Clostridium difficile: the effect of length of stay, patient age and antibiotic use. J Hosp Infect 2000;45:235-7.

22. Kyne L, Sougioultzis S, McFarland LV, Kelly CP. Underlying disease severity as a major risk factor for nosocomial Clostridium difficile diarrhea. Infect Cont Hosp Epidemiol 2002;23:653-9.

23. Warny M, Vaerman J-P, Avesani V, Delmée M. Human antibody response to Clostridium difficile toxin A in relation to clinical course of infection. Infect Immun 1994;62:384-9.

24. Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhea. Lancet 2001;357:189-93.

25. Aronsson B, Granström M, Möllby R, Nord CE. Serum antibody response to Clostridium difficile toxins in patients with Clostridium difficile diarrhoea. Infection 1985;13:97-101.

26. Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications. Ann Surg 2002;235:363-72.

27. Pépin J, Valiquette L, Alary ME, et al. Clostridium difficile-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ 2004;171:466-72.

28. McDonald LC, Owings M, Jernigan DB. Clostridium difficile infection in US short-stay hospitals, 1996-2003. Emerg Infect Dis 2006;12:409-15.

29. Long S, Fenelon L, Fitzgerald S, et al. First isolation and report of clusters of Clostridium difficile PCR 027 cases in Ireland. Eurosurveillance Wkly 2007;12(4).

30. Indra A, Huhulescu S, Hasenberger P, et al. First isolation of Clostridium difficile PCR ribotype 027 in Austria. Eurosurveillance Wkly 2006;11(9).

31. Kuijper EJ, Coignard B, Brazier J, et al. Update of Clostridium difficile-associated disease due to PCR ribotype 027 in Europe. Euro Surveill 2007;12:3-5.

32. Centers for Disease Control and Prevention (CDC). Severe Clostridium difficile-associated disease in populations previously at low risk: four states, 2005. MMWR Morb Mortal Wkly Rep 2005;54(23):1201-5.

33. Kyne L, Merry C, OÇonnell B, Keane C, O'Neill D. Community-acquired Clostridium difficile infection. J Infect 1998;36:287-8.

34. Wheeler JG, Sethi D, Cowden JM, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. BMJ 1999;318:1046-50.

35. Paltansing S, van den Berg RJ, Guseinova RA, Visser CE, van der Vorm ER, Kuijper EJ. Characteristics and incidence of Clostridium difficile-associated disease, The Netherlands, 2005. Clin Microbiol 2007;13:1058-64.

36. Karlström O, Fryklund B, Tullus K, Burman LG. A prospective nationwide study of Clostridium difficile-associated diarrhea in Sweden. The Swedish C. difficile Study Group. Clin Infect Dis 1998;26:141-5.

37. Weil H-P, Fischer-Brügge U,.Harmanus C, Mattner F, Gastmeier P, Kuijper EJ. High incidence of Clostridium difficile-associated diarrhea with a community onset in a hyperendemic region in Germany [Oral presentation]. ECCMID, Munich, 2007.

38. Rupnik M. Is Clostridium difficile-associated infection a potentially zoonotic and foodborne disease? Clin Microbiol Infect 2007;13:457-9.

39. O'Neill G, Adams JE, Bowman RA, Riley TV. A molecular characterization of Clostridium diffic1e isolates from humans, animals and their environments. Epidemiol Infect 1993;111:257-64.

40. Songer JG, Anderson MA. Clostridium difficile: An important pathogen of food animals. Anaerobe 2005;12:1-4.

41. Arroyo LG, Kruth SA, Willey BM, Stämpfli HR, Low DE, Weese JS. PCR ribotyping of Clostridium difficile isolates from human and animal sources. J Med Microbiol 2005;54:163-6.

42. Rodriguez-Palacios A, Stämpfli HR, Duffield T, et al. Clostridium difficile PCR ribotypes in calves, Canada. Emerg Infect Dis 2006;12:1730-6.

43. Rodriguez-Palacios A,Stämpfli HR, Duffield T, Weese JS. Clostridium difficile in Retail Ground Meat. Canada. Emerg Infect Dis 2007;13:485-7.

44. McFarland L. Alternative treatments for Clostridium difficile disease: what really works? J Med Microbiol 2005;54:101-11.

45. http://www.genzyme.com/corp/media/GENZ%20PR-070607.asp

46. Van Dissel JT, de Groot N, Hensgens CMH, et al. Bovine antibody-enriched whey to aid in the prevention of a relapse of Clostridium difficile associated diarrhoea: preclinical and preliminary clinical data. J Med Microbiol 2005;54:197-205.

47. Young KWH, Munro IC, Taylor SL, Veldkamp P, van Dissel JT. The safety of whey protein concentrate derived from the milk of cows immunized against Clostridium difficile. Regul Toxicol Pharmacol 2007;47:317-26.

48. Numan S, Veldkamp P, Kuijper EJ, van den Berg RJ, van Dissel JT. Clostridium difficile-associated diarrhea: bovine anti-Clostridium difficile whey protein to help aid the prevention of relapses. Gut 2007;56:888-9.

49. Riley TV, Wetherall F, Bowman J, Mogyorosy J, Colledge CL. Diarrheal disease due to Clostridium difficile in general practice. Pathology 19991;23:346-9.