

Efficacy of the probiotic, Bio-K+® CL1285®, in the control of a nosocomial outbreak of severe *Clostridium difficile*-associated diarrhea at the Centre Hospitalier Pierre-Le Gardeur: a case report.

Pierre-Jean Maziade MD¹, Doris Gagné RN¹, Gilles Murray MD¹, Christine Jacob RN¹, Pascale Pereira dt.p¹, Line Méthot, B.Pharma, M.Sc. adm².

¹ Service de prévention des infections et ²Département de pharmacie,
Centre Hospitalier Pierre-Le Gardeur

Short running title: Primary prevention of *Clostridium difficile* colitis with a probiotic.

Note: Centre hospitalier Pierre-Le Gardeur is the principal institution from which the study originates.

Correspondence: Dr. Pierre-Jean Maziade
Département de microbiologie
Centre hospitalier Pierre-Le-Gardeur
911 Montée des Pionniers
Lachenaie (Québec) J6V 2H2
Tel: 450-654-7525
Fax: 450-585-8297
E-mail: pierre-jean.maziade@ssss.gouv.qc.ca

Summary:

During the year 2003, many care centers in the province of Quebec experienced an increase in the morbidity and mortality rates due to *Clostridium difficile*-associated diarrhea. The Centre hospitalier Pierre-Le Gardeur was one of them. We had established a good program in prevention of infections in 1998 and since 2001 we also had a pharmacovigilance committee restricting the prescription of antibiotics. In spite of these measures, we were not able to control the outbreak. On February 1st 2004, we decided to administer Bio-K+® CL1285®, a probiotic, to all inpatients on antibiotics. After one month of utilisation, the outbreak was under control.

Abstract:

Introduction: Despite all our efforts to control a nosocomial outbreak of *Clostridium difficile*-associated diarrhea in 2003, we were unable to decrease the mortality and the morbidity due to this disease.

Methods: The study was conducted from February 1st 2004 to August 31st 2005. Every adult inpatient (≥ 18 years old) receiving antibiotics at our Center was automatically administered the probiotic Bio-K+[®]CL1285[®] (a patented fermented milk, containing 50×10^9 *Lb acidophilus*, CL1285[®] and *Lb casei*). This product was used in February and March 2004 and administered for a period of one month in daily doses of 98 ml. There were no exclusion criteria. We switched to two capsules per day of the same product in May 2004. The incidence of new nosocomial cases per 1000 admissions of *Clostridium difficile* associated diarrhea (mild and severe) was calculated monthly. We compared the total and the severe cases incidence with the inpatients of the outbreak period (from August 1st to January 31st). Isolates of *C.difficile* were typed by pulsed-field gel electrophoresis in both the study and the outbreak period. We analysed the compliance to the fermented milk product only. We checked blood and pus cultures for *Lactobacillus* during the study.

Results: There was a 94% decrease in the incidence of severe cases during the study period (from a mean of 5.1 cases/1000 admissions during the outbreak compared to 0.3 cases/ 1000 admissions during the study). A 73% decrease in the total incidence of *Clostridium difficile*-associated colitis (from a mean of 18.4 cases/1000 admissions compared to 5.0 cases/1000 admissions during the study period) was observed. Also, there was a 39% decrease in recurrences. The two groups were similar in term of co morbidities (APACHE 2 score). All but one isolates of *C.difficile* were from a single predominant strain(pulsovar A). This strain was highly resistant to fluoroquinolones. The compliance to the fermented milk with a high concentration of *Lb acidophilus*, CL1285[®] was 66% (77% when we counted those who took it partially). We have not found any infection due to the *Lactobacillus* in the probiotic used during the study.

Discussion: Considering the rapid control of the outbreak of *Clostridium difficile*-associated colitis at our center and the safety of Bio-K+[®]CL1285[®], it is reasonable to use it as primary preventive measure in patients receiving antibiotics.

Key words: *Clostridium difficile*, colitis, probiotic, antibiotic, Bio-K+[®]CL1285[®]

Introduction:

Clostridium difficile-associated colitis was a frequently observed nosocomial infection in our institution. During fiscal year 2002-2003, the reported incidence was 9.5 cases per 1000 admissions. Nevertheless, the infections, even when recurring, were not severe and responded to the standard treatment of metronidazole or oral vancomycin.

Between August and October 2003, there was an increase of almost 50% in the incidence of nosocomial cases, with an increased severity and mortality rates rarely seen with this type of pathology. Furthermore, response to the standard treatment was somewhat slow or ineffective. In March 2003, several hospital in Montreal and Eastern Township noted a marked increase in the incidence of *Clostridium difficile*-associated diarrhea. The mean incidence was 28,2 nosocomial cases/1000 admissions, nearly five times that was observed 2 years ago (5,7 cases/1000 admissions)¹.

In November 2003, a specific action plan was put into effect to overcome this situation. First, infected patients were isolated in designated areas with dedicated hospital staff assigned to them. Second, a rigorous cleaning program of the hospital was implemented, including disinfection of bathrooms, toilets, floors and walls with special attention to the rooms previously occupied by patients with diarrhea. In addition, disinfection of medical equipment (cuffs, bed pans, etc ...) was performed after each use to reduce inter-patient contamination. Third, monitoring of antibiotic utilization, mainly 2nd and 3rd generation cephalosporins was continued (the pharmacovigilance program exist since 2001) ; in addition, moxifloxacin, which had been on hospital formulary since spring 2003, was withdrawn as a safety measure. In fact, this latter antibiotic was involved in 35% of the cases identified between

August and October 2003 (15% when used in monotherapy). Moreover, up to 5.6% of patients treated with moxifloxacin developed *C. difficile*-associated colitis (2.2% when administered in monotherapy). In comparison, 4.6% patients treated with 2nd and 3rd generation cephalosporins suffered from colitis (1.2% when used in monotherapy) and only 0.9% patients treated with clindamycin were affected (0.4% when used in monotherapy). Several recent publications have reported such an association between quinolone use and the onset of pseudo membranous colitis^{2,3,4,5}. Fourth, since April 2003 we have a team that washing the hands of inpatients twice a day seven days a week. We use a waterless antibacterial hand sanitizer made with alcohol and chlorexidine.

Despite the protective measures aforementioned, the incidence of *C. difficile* cases continued to progress among the hospitalized patient population. Consequently, on February 1st, 2004, we implemented the addition of Bio-K+[®] CL1285[®], a probiotic prepared by Bio-K+ International, to the treatment regimen of all our patients receiving antibiotherapy. Our main goal was to decrease the incidence of severe *C. difficile*-associated colitis.

Methods:

Centre hospitalier Pierre-Le Gardeur is a 250 beds community hospital. However, since April 16th 2004, the hospital was relocated in a new building with 284 beds. The study was conducted from February 1st to August 31st 2005. Due to the temporary shutdown of many units during the hospital move, the study did not include the incidence of *C. difficile* for the month of April.

C. difficile-associated diarrhea was defined by the presence of diarrhea and a positive assay for *C. difficile* toxins A and B; by the sudden onset of diarrhea with no alternative explanation and a diagnosis of pseudomembranous colitis on the basis of endoscopy; or by histologic evidence of the condition. A case was considered nosocomial if symptoms started 72 hours or more after a patient was admitted or if *C. difficile*-associated diarrhea was diagnosed within one month after a previous admission⁶. A recurrent episode was considered if it occurred less than eight weeks after a previous diagnosis of *C. difficile*-associated diarrhea. Recurrent cases were included only once. Pediatric inpatients were excluded. Furthermore, nosocomial cases were classified according to their degree of severity measured 30 days after the first diagnosis. Severity was defined based on at least one of the following criteria: attributable or contributive cause of death within 30 days after the diagnosis, a need for intensive care or colectomy, septic choc or toxic mega colon. For each death, two physicians judged the cause independently whether is attributable, contributive or unrelated. In the case of a disagreement, the two physicians reached a consensus.

All patients receiving antibiotic treatment, whether hospitalized or under observation in the emergency department, were given a probiotic for a period of 1 month. The probiotic used in the study was Bio-K+[®] CL1285[®]. The product contains a human strain of *Lactobacillus acidophilus*, CL1285[®] and *Lactobacillus casei* and was characterized at *Institut Pasteur*. The probiotic exists in 2 formats: fermented milk containing 50 billion live and active bacteria per 98 ml bottle and capsules, each containing 30 billion bacteria. In February and March 2004, the dosage used was 98 ml of the fermented milk format administered daily and from May 2004 and on, switched to 2 capsules (60 billion bacteria) per day. The pharmacy department was responsible for dispensing the probiotic. In order to ensure the rapid adherence to this new treatment protocol, a permanent directive was established to mandate the addition of the probiotic at the beginning of each antibiotic treatment.

We compared the patients in the study to those with *C. difficile*-associated diarrhea between August 1st and January 31st. We used a computerized medical chart (ChartMaxx) to consult demographic, laboratory and clinical data. We calculate the APACHE 2 score for every patients at admission in both group.

Pulsed-field gel electrophoresis and susceptibility testing of *C. difficile* isolates was performed by the Public Provincial Laboratory of Quebec to determine if it is a clonal outbreak⁷. The susceptibility testing to ceftriaxone, ciprofloxacin, clarythromycin, clindamycin, gatifloxacin, levofloxacin, meropenem, metronidazole, piperacillin/tazobactam and vancomycin was done according to the guidelines of the Clinical and Laboratory Standards Institute⁸.

Statistical analysis...

Ridascreen® Elisa test (R-Biopharm) was used for the detection of *C. difficile* toxins A and B. Tests were performed 5 days a week on stool samples with no preservatives.

Patient's compliance to the probiotic (fermented milk format only) was assessed during 3 days in March 2004 and reasons for non-compliance were identified. This analysis was not performed for capsules, as patients seemed to comply easily with a capsule format. Patient's compliance could not be verified after hospital discharge; however, a prescription for Bio-K+® CL1285® was handed out to all patients.

Infection due to *Lactobacillus* was checked through blood and pus cultures.

Results:

Between February 1st and August 31st 2005, 4968 patients received Bio-K+® CL1285®. Results are summarized in Figure 1 and Table 1.

The APACHE 2 score was similar in the two groups (table2).

During the outbreak, (August 2003 to January 2004) the mean incidence of severe cases was 5.1 per 1000 admissions compared to 0.3 per 1000 admissions during the study, a 94% reduction (table 2). In addition, we have observed a reduction in the total incidence of *C. difficile* cases. During the study period, the mean incidence was at 5.0 cases per 1000 admissions compared to 18.4 cases per 1000 admissions during the outbreak, a 73% reduction.

We had 38.5% of recurrence during the outbreak compared with 23.4% during the study. This is a 39% reduction.

Pulsed-field gel electrophoresis was done on 16 *C.difficile* isolates during the outbreak (18%) and on 9 isolates during the study (12%). All but one isolate were the pulsovar A (96%). The susceptibility testing demonstrate for the pulsovar A a high resistance to fluoroquinolones, to third generation cephalosporin and to macrolide. In the other end, the strain was relatively susceptible to clindamycin, to meropenem and to piperacillin/tazobactam. Also, it remains susceptible to metronidazole and vancomycin.

Patient's compliance was based on 201 observations in 3 days. A compliance of 66% was estimated for the fermented milk format. Twenty-three percent of the patients refused the probiotic while 11% took only part of the recommended dose. The main reason for this non-compliance proved to be the taste (including the fruity flavoured product). In addition, difficulty in managing the fermented milk product in the pharmacy, (need for refrigeration, and lack of recycling facility in the hospital for disposal of empty containers) prompted the switch to the capsule format.

There was no infection related to the probiotic used. In fact, no blood or pus cultures were positive for *Lactobacillus acidophilus* or *Lactobacillus casei* in patients receiving Bio-K+®CL1285®.

Discussion:

In a 1997 survey of 18 Canadian institutions, the mean incidence of *C.difficile*-associated diarrhea was 6 per 1000 admissions, and 1.5 percent of affected patients died as a direct or indirect result of this disease^{9,10}. During our outbreak, we have three times the incidence described in the Canadian survey and almost fourteen times the rate of direct or indirect death¹⁰. In a recent retrospective chart review done in a region of Quebec from 1991 to 2003, showed an increase in incidence of four to five times and a mortality rate three times higher of that seen in 1991¹¹.

Probiotics have proved their effectiveness in the primary prevention of antibiotic-associated diarrhea as reported in two recent meta-analysis^{12,13}. The proposed mechanism of action is thought to be through restoration of the gastrointestinal flora partly destroyed by antibiotherapy. Another possible mechanism is the modification by the probiotics of immune processes to destroy the invading

organism¹⁴. In fact, *Lactobacillus* increases the numbers of cells that secrete immunoglobulins in the intestinal mucosa and it stimulates the local release of interferon¹⁵. In addition, a recent randomised study that utilised BioKplus in primary prevention of *C.difficile*-associated diarrhea was presented at the American College of Gastroenterology¹⁶. Only one patient out of 44 who take BioKplus developed *C.difficile*-associated diarrhea compared to seven out of 45 in the placebo group (p=0,058).

Rapid results arose from the massive use of probiotics at our institution, where better hygiene practice alone was insufficient to stop the outbreak. There are two main reasons to explain the limited effect of the improved hygiene practices implemented in November 2003. First, the old building was more difficult to clean and maintain, it had limited bathroom availability and a considerable number of patients were placed in the hallways of the emergency departments as well as in hallways of medical wards due to lack of bed availability. Second, the number of patients infected or colonised by the *C. difficile* strain had exceeded the critical mass.

Better hygiene and cleaner environment are clear advantages of the new facility. In addition, 70% of the individual rooms are equipped with private bathrooms; the remaining rooms are semi-private and also have their own bathrooms. The maximum number of patients per room is 2 and the emergency ward has no hallways. Regardless of the improvements of the new building, it is important to note that *C. difficile*-associated nosocomial cases had already significantly decreased two months before the relocation. In addition, during autumn and winter 2004-2005 we have a mean of fifty patients in overload units due to a lack of beds in regular units. Each of these overload units consist of a group of five to ten inpatients in a room with a single bathroom. The patients are very close at each other (less than three feet). In these units, there are one or two inexperienced nurses. Not unusually, we find some inpatients with diarrhea in these units that are not isolated. Even with this promiscuity, we continue to have a very low incidence of *C.difficile* colitis. In comparison, in a recent study in 12 Quebec hospitals between January 11 to June 26 2004, the incidence was 22.5 per 1000 admissions and 22.8 % of complications⁵. At the same time, in our institution, we have an incidence of 14.8 per 1000 admissions and 10% of complications, and the majority of these happened before the use of the probiotic BioK+. Also, we can noted the loss of seasonal variation in the incidence of *C.difficile*-associated diarrhea during the winter 2004-2005. Effectively, we supposed to have a higher incidence during the winter due to the increasing use of antibiotics for respiratory infections, but we continued to have a very low incidence with no complications.

Even in a new facility, our inpatients continue to be infected by the pulsovar A of *C.difficile*. This genotype was recently characterised in outbreaks of *C.difficile* colitis in United states and Quebec^{4,5}. This strain was associated with a high morbidity and mortality *Clostridium difficile*-associated diarrhea and an unusually high level of resistance to fluoroquinolones. Even in the presence of this virulent strain, we managed to reduce the incidence and severity of this disease. It is important to say that even with a pharmacovigilance program since 2001, which consist in everyday surveillance of 2nd and 3rd generation cephalosporins and quinolones prescriptions, it is impossible to completely avoid these classes of antibiotics.

Because the two groups had no statistical difference in term of co morbidities (APACHE 2 score is similar in the two groups), it can not explain the wide difference in incidence and complications due to *C.difficile*-associated diarrhea between the two populations.

The *Clostridium difficile*-associated diarrhea had a recurrence rate of 5 to 35% depending of the study^{17,18}. A recent study in Quebec demonstrate a recurrence rate of 47% during the year 2003-2004 compared with a rate of 21% between 1991-2002¹⁹. We described a reduction of the recurrence rate of 39% (38,5% vs 23,4%) during the study period. The efficacy of probiotics to reduce the recurrence of *C.difficile*-associated diarrhea was not clearly demonstrated in other studies^{20,21,22}.

We found no infection due to *Lactobacillus acidophilus* or *CL1285* or *Lactobacillus casei* during the study period. *Lactobacillus* bacteremia is a rare entity. Other kinds of infection due to these bacteria are even rarer. A group of researchers in Finland found no increase in *Lactobacillus* bacteremia despite the increased use of probiotics^{23,24}. *Lactobacillus rhamnosus* is the most frequent species associated with bacteremia. This strain is not included in Bio-K+@CL1285®. Despite those facts, it is important to maintain a level of vigilance regarding the detection of possible rare cases of infection due to Bio-K+@CL1285®. If there is a possible infection, isolate should be sent to a reference laboratory for molecular characterization and confirmation²⁵.

We found that about one fourth of patients could not take the fermented milk product. It is well known that in North America, there are a lot of people who don't like the taste of yoghurt or fermented milk products. In addition, capsules are easier to distribute in hospital settings and the compliance is almost 100%. We thus decided to switch to Bio-Kaps[®] capsules for the remainder of the study. A recent meta-analysis stated that the form of probiotics is equally efficacious¹³.

Our study has a number of limitations. It was not a placebo-controlled trial making difficult to evaluate the full efficacy of the probiotic BioK+. With time, we began treatment more rapidly and more aggressively (for instance use vancomycin sooner) which can contribute to a better outcome. We moved to a new facility which can help in the control of the outbreak. However, we already have a good nosocomial infection program since 1998 and a pharmacovigilance program since 2001, and it didn't prevent the outbreak. After the patient release from the hospital, we don't have any control in regard of his compliance to take BioK+. Even with these limitations, the rapid decrease in the incidence and the complications cause by this highly virulent strain of *C.difficile*, can only be explain by the massive primary prevention with BioK+. The probiotic BioK+ can be a safe mean to prevent *C.difficile*-associated diarrhea and a multicentric placebo-controlled trial is needed.

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Figure Legends

Figure 1: Evolution of *Clostridium difficile* outbreak among hospitalized patients

Figure 1

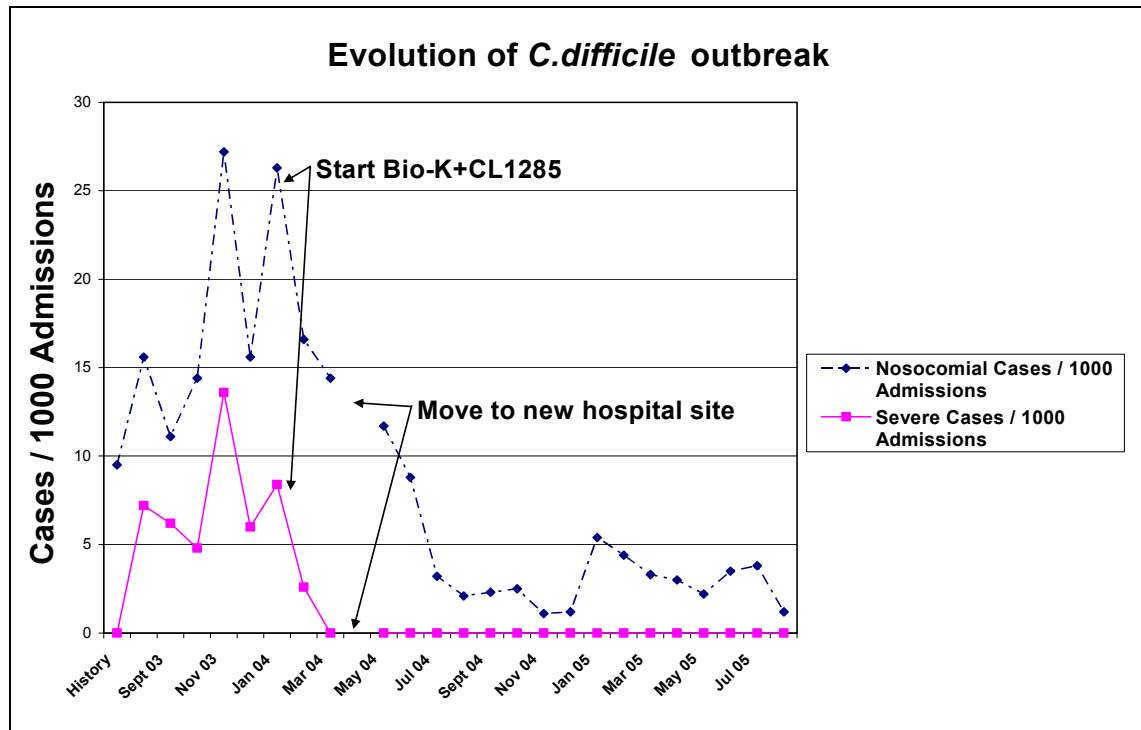


Table 1**Evolution of *C.difficile* outbreak among hospitalized patients**

Time Period	Nosocomial Cases / 1000 Admissions	Severe Cases / 1000 Admissions
Aug 03	15,6	6,4
Sept 03	11,1	3,7
Oct 03	14,4	4,4
Nov 03	27,2	7,6
Dec 03	15,6	3,9
Jan 04	26,3	4,8
Feb 04	15,3	2,6
Mar 04	13,2	0
Apr 04	Move to new hospital site	
May 04	10,5	0
Jun 04	8,8	0
Jul 04	3,2	0
Aug 04	2,1	0
Sept 04	2,3	0
Oct 04	2,5	0
Nov 04	1,1	0
Dec 04	2,4	0
Jan 05	5,4	0
Feb 05	4,4	0
Mar 05	4,5	2,3
Apr 05	3	0
May 05	2,2	0
Jun 05	3,5	1,2
Jul 05	3,8	0
Aug 05	1,2	0

Table 2**Characteristics of two Groups of Inpatients with *Clostridium difficile*-Associated Diarrhea.**

Description	Outbreak period	Study period	P value
Cases number	91	77	
Length of period (months)	6	18	
Mean total incidence (/1000 admissions)	18.4	5.0	
Mean severe cases incidence (/1000 admissions)	5.1	0.3	
Recurrent cases (%)	35 (38.5)	18 (23.4)	
Attributable death (%)	10 (11.0)	1 (1.3)	
Contributing death (%)	10 (11.0)	2 (2.6)	
Severe cases excluding death (%)	6 (6.6)	2 (2.6)	
APACHE 2 score (écart type)	11.1 (5.6)	11.1 (5.0)	
Pulsovar A (%)	16/16 (100)	8/9 (89)	