Anaerobe 25 (2014) 1–4

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical microbiology

Bacterial counts from five over-the-counter probiotics: Are you getting what you paid for?

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ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form
24 October 2013
Accepted 24 October 2013
Available online 1 November 2013

Keywords:
Probiotics
Clostridium difficile
Lactobacillus
Saccharomyces

ABSTRACT

There is concern that the bacterial colony counts present at the time of manufacture and listed on the probiotic package may not be reflective of the numbers viable colonies at the time of purchase and patient consumption thereby diminishing efficacy. We performed a colony count study of three separate samples of five different probiotics purchased from three different stores: *Bifidobacterium infantis* (Align®); *Lactobacillus acidophilus* CL1285® and *Lactobacillus casei* LBC80R® (Bio-K+®); *Lactobacillus rhamnosus* GG (Culturelle®); *Saccharomyces boulardii* (Florastor®) and "*L. acidophilus*" and "*Lactobacillus helveticus*" (Lactinex®). Approximately 1 g of powder of each (Lactinex® tablets were crushed before testing) was reconstituted in sterile distilled water, serial 10-fold dilutions were prepared and plated in duplicate onto blood agar plates, with incubation for 48 h in an anaerobic chamber (except the *Saccharomyces* which was incubated aerobically) after which colony counts were performed. The Florastor® packaging did not state an expected concentration and was found to have 9.2×10^9 -1.3×10^{10} CFU/g. Lactinex®, Align®, Bio-K+®, and Culturelle® had viable colony counts that were similar to those stated on the package.

1. Introduction

Antibiotics disrupt the normal host colonic flora which is an important factor in thwarting the protective microbiota and one of the major risk factors for the development of both antibiotic associated diarrhea, which can occur in 30% of treated patients, and of *Clostridium difficile* associated diarrhea (*C. difficile* infection, CDI) [1,2]. For the past 30 years, therapy of CDI has relied on the use of antimicrobial agents such as metronidazole and vancomycin. Despite this approach, CDI has increased in prevalence and severity while the relapse rate for CDI has ranged from 20 to 30%, especially in elderly patients and those who require continued concomitant antimicrobial therapy to treat their primary infections during the course of CDI [3–5]. Because of this unacceptable relapse rate, there has been a search for new and improved therapies as well as adjunctive therapies for CDI [6].

The importance of maintaining the fecal microbiota is illustrated by the emerging therapy of bio-transplantation for patients with relapsing CDI [7]. Probiotics have been used as adjunctive therapy to mitigate the disruptive effect of the antibiotic on the normal fecal flora but their use, role and efficacy are controversial topics. The World Health Organization defines a probiotic as a preparation that consists of a living organism that, when given in adequate amounts, delivers a health benefit to the patient [8].

In 2009, the European Food Safety Agency did not substantiate more than 500 health claims for probiotics such as improving the immune system, treating diarrhea, lowering cholesterol levels, helping with lactose intolerance and others. Part of this controversy is related to varied and less stringent regulations on over-the-counter products such as probiotics that differ from FDA approved therapies, as well as the role of specific probiotic strains, combinations of probiotic strains, their purity and even the presence of an effective dose in the retail products. It has been noted [9] that "probiotics and probiotic products are different from each other" and that each probiotic should be individually scientifically evaluated and moreover, that one cannot extrapolate findings from one micro-organism to another even if they belong to the same species or genus in the plethora of available products. Often patients go to health food stores and self-medicate with probiotics, and some ask their physicians for guidance on selection without critical evaluation.

Grzeskowiac et al. [10] studied 15 *Lactobacillus rhamnosus* GG products produced by different manufacturers using amplified

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http://dx.doi.org/10.1016/j.anaerobe.2013.10.005

polymorphic DNA analysis, enterobacterial repetitive intergenic consensus analysis and pulsed-field electrophoresis profiles. They demonstrated that the manufacturing process (production and methods) and the food carrier used can influence the properties of various probiotics and that quality control should be considered for each strain, even within the same genus/species. Even beyond clinical efficacy, one of the questions about the quality control of manufacture of these various agents is whether the dosage advertised on the packages (colony forming units per gram, CFU/g) is consistent with what the patient is receiving in the purchased preparation. In other words, do patients get what they pay for? Many products may stay of the shelf for long periods, under variable temperature and climatic conditions, thus the stability and viability at the time of patient ingestion are of concern.

In order to answer this question, we purchased three different lot numbers of five different commercial preparations from different local pharmacies, online and health food stores, performed quantitative colony counts of these preparations and compared them to the package advertising.

2. Methods

We purchased five probiotic products from store-shelf or online stock at eight different commercial sources in the Los Angeles area and one online provider (Table 1) in December of 2011. Storage conditions (shelf at ambient temperature or refrigeration), package marked expiration date and stated colony counts from the packaging were recorded. Products were stored as instructed on the packaging at ambient temperature, except BioK+, which was refrigerated. The agents and their labeled per unit (capsule, tablet or sachet) composition were as follows: Align® (Procter & Gamble, Inc., Ohio, USA), *Bifidobacterium infantis* 35624, 1×10^9 CFU; Bio-K+® (Bio-K Plus International, Inc., Quebec, Canada), *Lactobacillus acidophilus* CL1285® and *Lactobacillus casei* LBC80R®, 5×10^{10} CFU; Culturelle® (Amerfit, Connecticutt, USA), *L. rhamnosus* GG, 1×10^{10} CFU and Florastor® (Biocodex, France), *Saccharomyces boulardii* (250 mg) with active ingredients lactose (33 mg). Lactinex® (Becton Dickenson and Co., Maryland, USA) was labeled to contain "*L. acidophilus*" and "*Lactobacillus helveticus* (*bulgaricus*)", the latter being uncertain nomenclature. Query to BD clarified that the strains are *Lactobacillus gasseri* (ATCC 4962, originally deposited as *Lactobacillus bifidus*) and *L. helveticus* (ATCC 33409, originally deposited as *Lactobacillus bulgaricus*). Lactinex® has no unit concentration claimed on the package; however, 1×10^6 CFU are claimed on its website (http://www.bd.com/ds/technicalCenter/productFaqs/FaqLactinex.asp). None of the products had duplicate lot numbers, except Florastor® whose packages all had the same lot number.

To determine the quantitative counts at the time of purchase, approximately 1 g of each powder was weighed out into a test tube. Lactinex® tablets were crushed before weighing. Each specimen was reconstituted with a measured amount of sterile water to prepare a 1:10 dilution. Seven additional serial 10-fold dilutions ranging to 10^{-8} were prepared and 0.1 ml from each tube was transferred onto Brucella blood agar (Hardy Media, Santa Maria, CA) in duplicate. The plates were incubated in an anaerobic chamber, except the *Saccharomyces*, which was incubated aerobically, for 48 h at 37 °C. Colonies were counted on each dilution and plate to determine the CFU/g. For preparations containing more than one probiotic, the count reflected the total number of colonies that grew on the plate and not by individual genera or species.

3. Results

Results are shown in Table 1. All probiotic preparations were noted to be before their stated expiration date (4–30 months). While there was some lot-to-lot variability, of those products that indicated an expected concentration per unit (capsule or tablet), three of four contained within the log CFU/unit of viable organisms stated on the packaging. Lactinex® had the lowest concentration with $1.1–1.4 \times 10^7$ CFU/g but exceeded the per tablet claim. The Florastor® packaging claimed a weight rather than a unit concentration and was found to have $9.2 \times 10^9–1.3 \times 10^{10}$ CFU/g. Culturelle® and Bio-K+® contained somewhat more than the claimed concentrations per unit. Align® lot numbers had the most variability among which did not seem to correlate with time to expiration. Only Florastor® packages had the same lot numbers and concentrations among the three packages were within approximately one log CFU/g of each other. Florastor® had the longest expiration dates and Align® and Lactinex® expiration dates had the greatest variability. In all cases, the colonies appeared as typical of Lactobacillus or Bifidobacterium colonies and no contamination by other organisms was seen.

Table 1
Active ingredients of six randomly purchased probiotic brands and culture results of three different packages of each product.

Probiotic product	Active ingredients	Unit	Recommended daily dosage	Stated count (CFU/unit)	Months to expiration date	Lab assay (CFU/g)	Average (CFU/g)	Avg. unit weight (g)	Assay count (CFU/unit)
Align®	Bifantis® *(Bifidobacterium infantis* 35624)	Capsule	1	1×10^9	4	1.2×10^9	3.0×10^9	0.2	6.1×10^8
					12	8.4×10^8			
					18	7.1×10^9			
Bio-K+®	*Lactobacillus acidophilus* CL1285®, *L. casei* LBC80R®	Capsule	1–2	5×10^{10}	18	1.1×10^{11}	1.3×10^{11}	0.5	6.5×10^{10}
					18	1.5×10^{11}			
			1–2	2.5×10^{10}	18	8.7×10^{10}		0.5	4.4×10^{10}
Culturelle®	*Lactobacillus rhamnosus* GG	Capsule	1	1×10^{10}	17	1.3×10^{11}	8.1×10^{10}	0.3	2.4×10^{10}
					18	5.6×10^{10}			
					18	5.8×10^{10}			
Florastor®[c]	*Saccharomyces boulardii*, 250 mg 33 mg lactose	Capsule	2	NS[a]	29	9.2×10^9	1.1×10^{10}	0.3	3.15×10^9
					29	1.3×10^{10}			
					30	9.3×10^9			
Lactinex®	"*Lactobacillus acidophilus*", "*L. helveticus*", 240 mg lactose, 12 mg glucose, 125 mg sucrose	Tablet	12–16	1×10^6	5	1.1×10^7	1.2×10^7	0.5	6×10^6
					13	1.4×10^7			
					13	1.2×10^7			

[b] Unit = one capsule, tablet or sachet.
[a] NS, not stated.
[c] None of the products had duplicate lot numbers, except Florastor® whose packages all had the same lot number.