ORIGINAL ARTICLE

Randomised Placebo-controlled Double Blind Multicentric Trial on Efficacy and Safety of *Lactobacillus acidophilus* LA-5® and *Bifidobacterium* BB-12® for Prevention of Antibiotic-Associated Diarrhoea

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Abstract

Aims: To evaluate the effectiveness, safety and tolerability of a probiotic formulation containing ***Lactobacillus acidophilus*** LA-5® and ***Bifidobacterium*** BB-12® in the prevention of antibiotic associated diarrhoea (AAD).

Methods and Material: A double-blind randomised placebo controlled multicentric trial was conducted in adults who were prescribed a seven-day course of oral antibiotic (either cefadroxil or amoxycillin) for a documented indication. The effectiveness of a 14-day therapy (concomitant with antibiotic course and seven days thereafter) of the probiotic formulation in preventing AAD was evaluated. Safety profile was assessed by monitoring of all treatment emergent adverse events and tolerability on a global well being scale.

Results: The incidence of AAD in the probiotic group was 10.8% compared to 15.6 % in the placebo group, the difference being statistically non-significant ($p = 0.19$). The relative risk for AAD was 0.7 with the 95% CI being 0.4 to 1.2. The diarrhoea duration in the probiotic group was two days with an interquartile range of 1- 3 days and was significantly less ($p = 0.01$) than the placebo group which was four days with an interquartile range of 3 - 5.5 days. Subgroup analysis of subjects with AAD showed that the incidence of severe diarrhoea (watery stools) was 96% in the placebo group (25 out of 26) compared to 31.6% (6 out of 19) in the probiotic group and this difference was significant statistically ($p < 0.001$). Four mild, non-serious, adverse events were detected (2.0%) in the probiotic group but there were none in the placebo group.

Conclusion: This randomised controlled trial shows that prophylactic administration of the probiotic formulation containing *Lactobacillus acidophilus* LA-5® and *Bifidobacterium* BB-12®, did not effectively lower the incidence of AAD in adults. However, compared to placebo the duration of diarrhoea in the probiotic group was significantly reduced. Its tolerability and safety profile were good.

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Received: 04.07.2012; Revised: 16.08.2012; Accepted: 05.09.2012

Introduction

Antibiotic associated diarrhoea (AAD) is unexplained diarrhoea that occurs in association with the administration of antibiotics. The incidence is 5 - 30% and the rates are higher if the antibiotic spectrum gets

broader and in elderly patients. AAD possibly results from an alteration of the normal gut microflora which occurs due to inadvertent killing of the protective gut microflora by the inciting antibiotic. In 20 - 30% of hospitalised cases it could be due to infections caused by opportunistic bacteria like *Clostridium difficile* and *Klebsiella oxytoca* which colonise the gut and play a role in the pathogenesis of colonic lesions.[1-3]

Probiotics are live beneficial microbes either bacteria or yeasts, which confer beneficial effects to the host. Published reports have suggested that probiotics stabilise the gut microbial environment by enhancing the immunologic barrier of gut mediated mainly through mucosal IgA responses.

Due to its wide availability, there is growing interest in evaluating its beneficial role in prevention and treatment of various types of diarrhoea mainly AAD, *Clostridium difficile* infections, inflammatory bowel disease, irritable bowel syndrome and other gastro-intestinal disorders. There are conflicting reports regarding their effectiveness in these indications.

Several published trials[3-7] in adults and children have shown that probiotics may be beneficial in preventing AAD while some have failed to document its effectiveness. Many of these trials were conducted with single agent probiotic like *Lactobacillus* strains or *Saccharomyces boulardii* and some were combination products. A Cochrane database systematic review of 2007[4] on the role of probiotics on paediatric AAD has suggested that future studies in this area should be conducted to provide detailed information about the efficacy of probiotics on outcome measures like duration of diarrhoea, duration of antibiotic use and severity of diarrhoea to assist clinicians to decide whether probiotics should routinely be recommended for the prevention of AAD. The rationale for selecting the marketed product of Lactobacillus acidophilus LA-5® and Bifidobacterium BB-12® was based on the observation that at the time of study inception there were no published studies that had evaluated the efectiveness and safety of this combination probiotic preparation for prevention of preventing AAD in Indian population. Secondly, this probiotic product is available as a techsule which has better product stability than the capsule dosage form. Therefore, this phase IV, randomised, multicentric placebo-controlled double blind trial was conducted to evaluate the comparative efficacy and safety/tolerability of a formulation containing Lactobacillus acidophilus LA-5® and Bifidobacterium BB-12® in preventing AAD and its effects on duration and severity of AAD in outpatient department requiring oral antibiotic.

The efficacy profile was assessed by evaluating the incidence of antibiotic associated diarrhoea in comparison to the placebo during the study period of 14 days. Comparative evaluation of the duration of diarrhoea, daily stool frequency and severity of diarrhoea was also done. The safety and tolerability was evaluated by monitoring of all treatment emergent adverse events (serious and non-serious) and the global well being score assessment.

Methods and Materials

This study was conducted at an outpatient setting in four tertiary care city hospitals in India during the period 2009-2010. The study was initiated in each trial site after receiving approval from the respective Institutional Ethics Committee. All the subjects were enrolled after getting their informed consent signed.

Subject selection criteria: Subjects were enrolled in the age group of 18 to 70 years of either sex who were prescribed a systemic oral antibiotic (cefadroxil or amoxycillin) for seven days for a documented indication. Subjects, who had a course of systemic antibiotic in the last one month prior to screening, with severe infections requiring hospitalisation and parenteral antibiotic therapy, were excluded. Subjects with underlying GI disorder like ulcerative colitis, Crohn's disease, malabsorption, GI bleeding etc. were not included in the study.

Subjects were enrolled in each group after they fulfilled the selection criteria and given written informed consent was obtained. Randomisation was done using computer generated random number sequence with equal allocation ratio. Allocation concealment was done by using sequentially numbered opaque sealed envelopes. The test group received 2 techsules twice daily for 14 days plus the prescribed antibiotic (amoxycillin 500 mg thrice daily or cefadroxil 500 mg twice daily) for the first 7 days (Day 0 onwards). The control group received identical looking placebo capsules for 14 days (Day 0 onwards) plus the prescribed antibiotic (amoxycillin or cefadroxil) for the first 7 days (Day 0 onwards). Complete haemogram was done at baseline and study end visit. Assay for *C. difficile* was not done in the AAD subjects but routine stool examination was done in subjects who developed diarrhoea.

All subjects were provided with a subject symptom diary in which they recorded all symptoms (daily stool frequency, consistency and colour of stool, abdominal pain, bloating and other symptoms, if any). Antibiotic associated diarrhoea (AAD) was defined as passage of at least three or more watery or loose stools per day for at least two consecutive days.The diary was reviewed at the day 7 follow-up visit and at study end visit (day 14) for diarrhoeal incidence assessment. Adverse events if any were recorded and clinical examination was also performed at each

visit. Subjects were withdrawn from the study, if they experienced any suspected serious adverse event (AE) related to the study medication, protocol violation, or they withdrew consent.

The sample size for the study was calculated on the assumption that this study would be able to detect a difference of 10% in the incidence of AAD (based on previous published report[8] of about 25% incidence rates in placebo arm), power of 80% and α (level of significance) at 0.05%. The target number of subjects required in each arm would be, 199 considering a drop out of 15%. Therefore a total of 400 for even distribution of patients were to be recruited across the 4 study centres in India.

The efficacy endpoint was the incidence of AAD in each arm (test versus placebo) at study end. Comparative evaluation of the duration of diarrhoea, daily stool frequency and percentage of subjects who had severe diarrhoea was also done to detect statistically significant differences.

Summary statistics of the baseline demographic and disease profile was done and for comparison of differences between groups, appropriate parametric (unpaired t-test) and non-parametric tests (Mann Whitney U test) were applied. The efficacy data analysis was done as a modified intention to treat analysis (i.e. all subjects who had attended at least one post-baseline visit were considered evaluable). The incidence of antibiotic associated diarrhoea in the two treatment arms was compared for detecting any statistically significant differences using Chi-square test/Fisher's Exact Test. The relative risk (RR) with its 95% CI was also calculated. The level of significance for the analysis was considered as $p < 0.05$.

Table 1 : Comparative Profile of subjects with AAD

Parameters		Probiotic Group n=19	Placebo Group n=26
Age (yr)	18-40	14	17
	>40	5	9
Sex	Male	17	17
Antibiotic intake	Amoxycillin	15	23
	Cephadroxil	4	3
Indication for which antibiotic was used	URTI	8	9
	LRTI	8	10
	Others	3	7

All statistical analysis was done using *GraphPad Prism* software version 5.

Results

Out of 396 enrolled subjects (198 in probiotic arm, 198 placebo), 176 (89%) in the probiotic group and 167 (84%) in the placebo arm completed the study. Out of 22 non-evaluable subjects in probiotic arm - 18 were lost to follow up and 4 were protocol deviators while out of 31 non-evaluable subjects in placebo arm 22 were lost to follow up and rest were protocol deviators.

The baseline profile of the study subjects at recruitment were comparable in the two arms with regard to age, sex and the underlying disease for which antibiotic were prescribed and no statistically significant difference was detected therefore the subjects in both the test and control arm were comparable. The mean age of the enrolled subjects were comparable and there were 65.15% were males in probiotic group and 68.18 % in the placebo group. The indication for antibiotic use were mainly upper and lower respiratory tract infections URTI- 64.14% (probiotic group); 63.64% (placebo group); LRTI- 27.27% (probiotic group) 31.81% (placebo group) and other infections accounted for the rest.

There were 176 evaluable subjects for efficacy analysis in the probiotic group and 167 in the placebo group. The incidence of AAD in the probiotic group was 10.8% (19 out of 176) compared to 15.56% in the placebo group (26 out of 167). The incidence of AAD in the probiotic group though lower than the placebo group but it was not statistically significant ($p = 0.24$). The profile of the subjects with AAD are enlisted in Tables 1 and 2 . There was no statistically significant

Table 3 : Comparison of diarrhoeal duration, stool frequency and incidence of severe diarrhoea

	Probiotic group n=19	Placebo group n=26	p value
Duration of diarrhoea in days Median (IQR)	2 (1-3)	4 (3-5.5)	0.009*
Daily stool frequency	3.11 ± 1.41	4.08 ± 1.85	0.062
Number of subjects with watery stool	6 (31.6%)	25 (96%)	<0.0001*

IQR = interquartile rang; *statistically significant

Table 2 : Centre-wise Incidence of AAD in the two treatment groups

Center	Total number of evaluable subjects (both groups)	Probiotic		Placebo		p Value
		Evaluable subjects	Subjects with Diarrhea (%)	Evaluable subjects	Subjects with Diarrhea (%)	
Kolkata centre 1	82	42	10 (23.8)	40	19 (47.5)	0.021
Kolkata centre 2	105	54	7 (12.96)	51	5 (9.8)	0.609
Chennai	86	45	0	41	1 (2.4)	0.311
Delhi	70	35	2 (5.7)	35	1 (2.8)	0.554
Total	343	176	19 (10.79%)	167	26 (15.56%)	0.191

difference in the incidence of AAD between groups with respect to age, sex or antibiotic intake. The analysis of the duration and severity of diarrhoea in subjects who had AAD reveals that the median duration of diarrhoea in the probiotic group was 2 days with an interquartile (IQR) range of 1-3 days compared to 4 days (IQR of 3 to 5.5) in the placebo group. This difference was significant statistically (p = 0.009) (Table 3).

In the probiotic group the daily stool frequency was 3.1 ± 1.4 compared to 4.1 ± 1.8 in the placebo group. Though the stool frequency was lower in the probiotic group, it was not statistically significant (p=0.06). Subgroup analysis of subjects with AAD shows that the incidence of severe diarrhoea (watery stool) was 96% in the placebo group (25 out of 26) compared to 31.6% (6 out of 19) in the probiotic group and this difference was significant statistically (p < 0.001) (Table 3). The relative risk for AAD was 0.7 with the 95% CI of 0.4 to 1.2 which indicates that the difference in risk between groups was not statistically significant.

The Investigator's Global Well being score for tolerability showed that probiotic was very well tolerated with 98.9% reporting excellent or good tolerability in comparison to 96.4% in the placebo group. Fair or poor tolerability was reported only in 1.1% in probiotic compared to 3.6 % in the placebo group.

There were no serious adverse events in any of the treatment arms and the adverse events which were observed were all non-serious, mild and self-limiting ones, which did not require treatment withdrawal. The incidence of AEs in the probiotic group was 2.0% compared to none in the placebo group. This difference was however not statistically significant. The four adverse events reported in the probiotic group were epigastric discomfort, pain abdomen, belching and dyspepsia. Causality assessment was done for all the AE as per the WHO ADR causality assessment scores[9] and all were found to be of either probable or possible association.

The results of this study have demonstrated benefit of probiotic supplementation in the dose of 4 billion CFU/day including both the two strains in reducing the duration of AAD and its severity compared to placebo but it was not statistically significant between the two arms.

Discussion

A multicentric, randomised, placebo-controlled, double-blind trial which was conducted in Korea,[9] evaluated the efficacy of a proprietary probiotic preparation containing Lactobacillus for the prevention of AAD in adults. Their study results showed that the incidence of AAD in the probiotic group was 3.9% (4 of 103 patients) and 7.2% (8 out of 111) in the placebo group (p = 0.44) which was not significant. However, the Lactobacillus group showed lower change in bowel frequency and consistency (50/103, 48.5%) than the placebo group (35/111, 31.5%) (P = 0.01). They concluded that although the probiotic preparation did not reduce the rate of occurrence of AAD in adult patients, the Lactobacillus group maintained their bowel habits to a greater extent than the placebo group.The results of our study are comparable. The incidence of AAD in the test group was 10.8% and 15.6% in the placebo group and the difference was not statistically significant (p = 0.24). The higher incidence of AAD in Indian population could be attributed to variations in nutritional, genetic or environmental factors but both the studies have shown that the difference in AAD rates amongst test and placebo groups were not statistically significant.

A meta-analysis report[3] of 2010, which included six adult and four paediatric trials and analysed the effects of a Lactobacillus single-agent regimen as a prophylactic agent during antibiotic treatment in reducing the risk of developing AAD compared to placebo. The overall combined risk ratio (RR) of developing AAD was significantly lower with Lactobacillus compared with placebo (RR 0.35, 95% CI 0.19 - 0.67). In a subgroup analysis, this held true for adults but not in pediatric patients (RR 0.24, 95% CI 0.08 - 0.75 and RR 0.44, 95% CI 0.18 - 1.08, respectively).

Another Cochrane database systematic review of 2007[4] on the role of probiotic (Lactobacilli spp., Bifidobacterium spp., Streptococcus spp., or *Saccharomyces boulardii* alone or in combination) in preventing AAD in children has shown that 9 out of 10 trials reported that the incidence of diarrhoea showed statistically significant results favouring probiotics over active/non active controls (RR 0.49; 95% CI 0.32 to 0.74) in a per protocol analysis, whereas intention to treat analysis non-significant results were noted overall (RR 0.90; 95% CI 0.50 to 1.63).

The possible mechanisms underlying its beneficial effects could be attributed to its actions on intestinal immunity. Some studies have suggested that these bacterial strains (Lactobacillus sp, Bifidobacterium sp. etc) may increase the number of IgA and other immunoglobulins secreting cells in the intestinal mucosa and stimulate local release of interferons. Probiotics could also function through enhancement of barrier function, immunomodulation, and competitive adherence to the intestinal mucosa thereby preventing or ameliorating various infective or inflammatory diseases.[11-13]

Another study from China[14] which was done on hospitalised patients has shown that the proprietary probiotic formulation containing *Lactobacillus acidophilus* CL + *Lactobacillus casei*) was effective in preventing AAD. The incidence of AAD in the probiotic and placebo treated groups were 15.5% and 44.1% respectively. The duration of diarrhoea was also shorter in the probiotic group (2.8 days) compared to the placebo group 6.4 days.

The strength of this study was that it was a randomised controlled double blind multicentric study, which was done in different cities of India. Secondly, the desired target sample size was achieved and finally, the manufacturer providing the study antibiotics minimises the chance of the antibiotic formulation confounding the study results.

The results of this randomised double blind placebo controlled multicentric study has provided evidence that although prophylactic treatment with probiotic entails additional costs but cost-benefit analysis may be favourable in terms of cost saving for investigation and treatment of an episode of severe AAD and reduction of morbidity (e.g. work loss days) related with the disease. Secondly prophylactic probiotic supplementation reduced the duration and stool frequency in those patients who developed AAD.

Some of the limitations of the present study include short follow up period and absence of microbiological examination of stool which could not be undertaken due to logistic and financial reasons. Secondly, the results of this study may lack generalisability since we evaluated the effects of this formulation on only two antibiotics.

Conclusion

The results of this trial have shown that the incidence of AAD was less with prophylactic administration of probiotic in adult subjects who were prescribed a seven day course of amoxycillin or cefadroxil but compared to placebo this difference was not statistically significant. However, it significantly reduced the duration of diarrhoea and daily stool frequency compared to placebo. The tolerability of the probiotic formulation was good and the incidence of adverse events was low and they were mostly mild and self-limiting.

Conflict of Interst

This was a sponsored study, the research grant was given by Zydus Cadila Healthcare Ltd. India and Chr. Hansen A/S, Netherlands.

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