Clinical evaluation of the synbiotic Prodefen Plus® in the prevention of the antibiotic-associated diarrhoea in subjects requiring antibiotic treatment

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Abstract

Prodefen® is a dietary food supplement formulated as a synbiotic that has shown additional benefit to the standard supportive therapy in the management of acute viral diarrhoea in children. There is scarce evidence of this synbiotic in adults. The objective of this randomised double blind placebo-controlled clinical trial was to evaluate the efficacy and safety of Prodefen Plus® in the prevention of antibiotic-associated diarrhoea (AAD) in an adult population requiring either antibiotic treatment for an oral infection or antibiotic prophylaxis for a dental surgical procedure in a dental consultation. 151 subjects were randomised to the active (synbiotic) or control arm (placebo) for 14 days. There was a significantly higher reduction in the AAD incidence, and an improvement in the stool consistency in the active group. A higher reduction in both the frequency and duration of the diarrhoea episodes in the active group was also observed, as it was an improved perception of the diarrhoea severity. Overall, the study medication was well tolerated. In conclusion, results from this study confirm the beneficial effect of the synbiotic administered as adjuvant therapy in preventing the antibiotic-associated diarrhoea.

Keywords: antibiotic-associated diarrhoea, synbiotic, Prodefen Plus®, adult, prevention

1. Introduction

Antibiotic treatment has widely proved to cause intestinal dysbiosis, a qualitative and quantitative imbalance and alteration of the gut microbiota, with a significant reduction of the host microbial diversity and a proliferation of pathogenic bacteria, resulting in several diseases and dysfunctions, such as antibiotic-associated diarrhoea (AAD) (McFarland, 1998; Modi *et al.*, 2014).

Prevalence of AAD ranges from 5 to 39% in patients taking antibiotics, and it depends upon the specific type of antibiotic, the population characteristics (age, health status, etc.), and the pathogenic microorganism (McFarland, 1998). Broad-spectrum antibiotics and those that are

poorly absorbed in the colon or are excreted in the bile are associated with higher rates of AAD. Amoxicillin/clavulanate, a widely used antibiotic, is associated with a high risk of AAD, and more frequently than with amoxicillin alone, possibly due to an increased intestinal motility effect (Gomez *et al.*, 2012). AAD appears in individuals of any age, however it shows its highest frequency in patients over 65 years old (MacFarland, 2008).

According to the WHO, diarrhoea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of organisms (Anonymous, 2017). Likewise, diarrhoea can be assessed by means of the Bristol Stool Form Scale

(BSFS), using the stool form grading as a guide to the whole intestinal transit time (Figure 1). In this scale, diarrhoea would be graded from 5 to 7 according to the shape and consistency of the stool (Lewis and Heaton, 1997).

In 2014, an expert panel, convened by the International Scientific Association for Probiotics and Prebiotics (ISAPP) to discuss the field of probiotics, reiterated with one small change the definition of probiotic issued by the FAO/WHO in 2001 (Hill *et al.*, 2014). While a probiotic were described as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host', including those microbial species that have been shown in properly controlled studies to confer benefits to health (Hill *et al.*, 2014), the term prebiotic would be used to define 'a substrate that is selectively utilised by host microorganisms conferring a health benefit' (Gibson *et al.*, 2017), and the term synbiotic would be chosen instead to refer to a product containing both probiotics and prebiotics (De Vrese and Schrezenmeir, 2008; Markowiak and Slizewska, 2017; Pandey *et al.*, 2015; Schrezenmeir and De Vrese, 2001). The synergistic stimulation of probiotics by prebiotics would not only affect the gut microbiota, by immune-modulating the metabolic activity in the intestine (De Vrese and Marteau, 2007; Markowiak and Slizewska, 2017), but they would also confer health benefit to extraintestinal sites (Bubnov *et al.*, 2019; Gibson *et al.*, 2017). Presently, the most widely accepted prebiotics are the fructants or fructooligosaccharides (FOS) and the galactans or galactooligosaccharides (Gibson *et al.*, 2017).

The beneficial effects of probiotics have been demonstrated in many diseases, including the prevention of AAD in both children and adults, through the stabilisation or reconstitution of the physiological balance between the intestinal microbiota and its host. Therefore, measures to prevent AAD include the use of probiotics. Several randomised clinical trials (RCTs) and meta-analysis of RCTs and systematic reviews have shown in both children and adults a reduction in the risk of developing AAD with the administration of certain probiotic microorganisms (Blaabjerg *et al.*, 2017; Cai *et al.*, 2018; Evans *et al.*, 2016; Goldenberg *et al.*, 2015; Guo *et al.*, 2019; Hempel *et al.*, 2012; Hickson, 2011; Johnston *et al.*, 2016; Wright *et al.*, 2015).

The beneficial effects of probiotics seem to be strain-specific. It is well-known that different bacterial strains of the same genus and species may exert completely different effects on the host. Antibiotic resistance and transferability of antibiotic resistance genes from probiotic strains to commensal microbiota in the gut are important components of the safety of bacteria used as probiotics (Campana *et al.*, 2017). In addition, meta-analyses on the effect of probiotics on AAD suggest a dose-response effect (Ouwehand, 2017). It should be noted that the probiotic use decreased the risk of AAD by more than 50% when probiotics were started within 72 h of the onset of antibiotic treatment (Boyanova and Mitov, 2012).

Figure 1. Patient study flow. ITT = intention to treat; AT = as treated; PP = per protocol. * Patients who were incorrectly assigned to the other treatment group, but the blinding was preserved until the end.

Prodefen Plus® (Italfarmaco, Alcobendas, Madrid) is a dietary food supplement formulated as a synbiotic, with a combination of prebiotics and 7 probiotic strains, that has shown additional benefit to the standard supportive therapy in the management of acute viral diarrhoea in both hospitalised (Allahverdi *et al.*, 2010) and outpatient paediatric populations (Garcia-Menor *et al.*, 2016). Prodefen Plus contains, among its probiotic strains, *Lactobacillus rhamnosus* GG (LGG), one of the most effective strains in preventing the AAD in adults and children treated with antibiotics for any reason, as confirmed in a systematic review and meta-analysis of 12 RCTs (Szajewska and Kolodziej, 2015), and as endorsed in several International Guidelines (Agamennone *et al.*, 2018; Floch, 2014; Goldenberg *et al.*, 2017; A. Guarino *et al.*, 2014; Szajewska *et al.*, 2016). Furthermore, LGG has shown to improve traveller's diarrhoea, relapsing *Clostridium difficile* colitis, and to reduce the severity and duration of the attacks in infantile diarrhoea. It has been demonstrated that LGG colonises the gastrointestinal tract for 1 to 3 days in most individuals and up to 7 days following its administration (Gorbach, 2000). In addition, both the prebiotic component of the synbiotic Prodefen Plus (FOS) and the rest of the contained probiotic strains (*Lactobacillus casei*, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium infantis* and *Bifidobacterium bulgaricus*), either alone or in combination, have been demonstrated to be safe and effective in preventing AAD in adults and children (Canani *et al.*, 2007; Dietrich *et al.*, 2014; Gao *et al.*, 2010; Garcia Trallero *et al.*, 2019; Guarino *et al.*, 2020; Guo *et al.*, 2019; Hickson *et al.*, 2007; Lai *et al.*, 2019; Thibault *et al.*, 2004; Vandenplas *et al.*, 2011), through multi-factorial and strain-dependent mechanisms (Mekonnen *et al.*, 2020). Furthermore, the probiotic strains contained in the synbiotic Prodefen Plus, specially *L. rhamnosus*, *L. casei* and *L. acidophilus*, meet all the strain-dependent biological properties (in terms of adhesive ability, resistance to antibiotics and gut biological fluids) required by the probiotic guidelines to be considered a safe and an effective treatment for the gut microbiome modulation (Bubnov *et al.*, 2018).

The primary objective of this study was to evaluate the effectiveness of the synbiotic Prodefen Plus in the prevention of AAD in an adult population requiring antibiotics, either for treatment of an oral infection or as prophylaxis for a dental surgical procedure in a dental consultation.

2. Materials and methods

Study design

This was a multicentre, double-blind, placebo-controlled, parallel-group, clinical trial conducted in 20 Spanish dental care clinics from January 2018 to November 2018, whose objective was to evaluate the efficacy and safety of a

synbiotic, in the prevention of AAD in an adult population. The study was conducted following the principles of ICH Good Clinical Practice, and according to the criteria set by the Declaration of Helsinki. The study was approved by the Ethics Committee of the Hospital Puerta de Hierro (Madrid).

Participants

Eligible study subjects were all patients presented to a dental consultation who required either antibiotic treatment for an oral infection or prophylactic treatment for a surgical procedure with Augmentin® (875 mg of amoxicillin and 125 mg of clavulanic acid) three times a day for 7 days, as per routine clinical practice. In addition, to be finally included in the study, participants should be subjects between the ages of 18 and 65 years (inclusive), without any serious health condition, and must have provided their written informed consent.

The following patients were excluded from the study: if they presented an intrinsic non-AAD; suffered from any other diseases that might cause diarrhoea (such as alimentary allergies and intolerances, chronic gastrointestinal diseases, immunosuppression, pneumonia, etc.); had a heart valve implant and/or had a history of infective endocarditis; had allergies to probiotics, Augmentin, or any of their ingredients; had received probiotics and/or antibiotic treatment within the previous 3 and 4 weeks, respectively; were participating in another study; women who were either pregnant, breast-feeding or planning to become pregnant during the course of the trial; individuals who referred an excessive alcohol consumption; patients who were diagnosed with *C. difficile* infection within the previous 3 months; and those subjects who would not need to return to the dental consultation after antibiotic therapy.

Study procedures and outcomes

On the same day of the dental consultation, all eligible patients, who met the inclusion criteria and signed the informed consent form, were randomly allocated (1:1) to receive either 1 sachet of the synbiotic Prodefen Plus or a matching placebo daily for 14 days, added to the standard antibiotic treatment of Augmentin 875/125 mg every 8 h daily for 7 days. Subjects were assigned into one of the study groups by means of a simple randomisation procedure, using computer-generated random numbers with a sequential allocation order. Treatment allocation sequence was blinded, and concealed from patients, researchers, the Clinical Research Organization (CRO), and the study sponsor.

Patients were instructed to take Prodefen Plus 2 h after taking the morning antibiotic dose. The synbiotic Prodefen Plus contains a combination of prebiotics (FOS 990 mg)

and seven probiotic strains (1×10^{10} cfu LGG; 1×10^9 cfu each *L. casei*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. infantis* and *B. bulgaricus*) per sachet. Patients who required antibiotic treatment for an oral infection were seen at the dentist's office on the first day of the study (baseline visit) and subsequently revisional visits on the 7th day and 14th day (final visit) of the study. Those patients who underwent a surgical procedure had an additional visit within 15 days prior to the study treatment initiation (day 1 or baseline visit).

The primary objective of this study was to evaluate the effectiveness of the synbiotic Prodefen Plus in the prevention of AAD. Secondary study objectives were:

1. to assess the incidence and/or duration of any other antibiotic-associated symptomatology;
2. to evaluate the tolerability and safety profile of the synbiotic; and
3. to evaluate treatment acceptance and impact on daily activities.

The following study outcomes were evaluated: (1) percentage of patients who developed AAD, defined as the passage of 3 or more loose or liquid stools per day (or more frequently than what is normal for the individual) and a stool consistency of 5 to 7 types according to the BSFS, for at least one day (or two or more consecutive days) along the study period; (2) number of episodes of diarrhoea (an episode defined as one or more days with diarrhoea, and same episode considered if only one day without diarrhoea occurred in between two consecutive days with diarrhoea); (3) duration of AAD episodes; stool frequency per day and stool consistency (according to the BSFS). In addition, the following variables were also assessed: incidence and/or duration of all the associated symptoms, such as nausea, vomits, bloating, abdominal pain, abdominal cramps, blood in the stool, etc.; study treatments tolerability profile (measured as incidence and duration of adverse events); study treatment acceptance; study treatment impact on daily activities; grade of treatment compliance; and the need for medical intervention or further concomitant treatment of AAD.

At baseline visit, a first diary card was provided to all study participants in order to collect the necessary information to analyse the study variables: timing of administration for the synbiotic/placebo and Augmentin, number and consistency of daily stools, associated symptomatology, other medications for treating the AAD, adverse events, impact of treatment on daily activities, treatment acceptance, etc. A second diary card was given to the participants at the follow-up visit on the 7th day.

Statistical analysis

Statistical methods

Descriptive statistics were used to describe the characteristics of the study population, both at baseline and along the study (at days 3, 7, 10 and 14). Continuous variables were described by measures of central tendency (mean, median) and dispersion (standard deviation (SD), interquartile range (IQR), maximum and minimum), including the total number of valid values and the confidence interval (CI). Categorical variables were described by means of absolute and relative frequencies.

For the inferential analysis, two study samples were selected, one for the efficacy analysis and another for the safety analysis. For efficacy, the as treated (AT) population was analysed. AT population was defined as all study subjects who received at least 1 dose of the study medication, independently of the initially assigned treatment group. For the safety analysis, all the subjects who received at least one dose of the study medication were included.

Statistical tests for comparison between groups included Student's t test for continuous variables (parametric) or Wilcoxon and Mann-Whitney U tests (non-parametric). Categorical variables were analysed by either the McNemar or the chi-squared (χ^2) tests. All tests were two-tailed, with a significance level of 0.05. In addition, the AAD associated relative risk (RR) with its CI 95% was calculated. All statistical analyses were performed with the Statistical Analysis Software (SAS, Cary, NC, USA) version 9.4.

Furthermore, the evolution of the cumulative proportions of patients who developed AAD along the study period in both active and placebo groups was adjusted by non-linear regression to a one-phase association equation, and both curves were compared by the ANOVA test. These calculations were made using the GraphPad Prism 6.0 (La Jolla, CA, USA).

Sample size

Based on the McFarland meta-analysis (2006) (McFarland, 2006), we estimated a sample size of 75 subjects for each study arm to detect a minimum odds ratio (OR) of 0.27 for AAD reduction, assuming an exposure prevalence in the control group of 29%, a two-sided power of 80%, a significance level of 5%, and an estimated patient dropout rate of 15%.

3. Results

A total of 152 subjects were recruited for the study, and 151 were randomised to active treatment or placebo. Eventually, 147 patients were considered for the AT analysis and for

the safety evaluation. 74 patients were finally assigned to the active group and treated with Prodefen Plus (of whom, 12 had been originally allocated to the placebo arm, but incorrectly assigned to the active group), whereas 74 subjects were assigned to the control group and treated with Placebo (with 13 patients who had been initially allocated to the active arm, and incorrectly assigned to the placebo group). Nevertheless, the study blinding and concealment was preserved until the study completion. The per protocol (PP) study sample consisted of 93 study subjects (Figure 1).

Regarding the population baseline clinical and sociodemographic characteristics, 60 subjects (40.8%) were males, and mean (SD) age was 45.77 (12.71) years old. Median (IQR) values for body mass index (BMI) were 24.90 (23.99-29.38) for males, and 24.63 (20.80-26.90) for women, as per the AT population. Reasons for consultation for the AT population were oral infection in 87 (59.2%) and surgical procedure in 60 (40.8%) study patients. Additionally, just

6 patients (4.1%) of the entire AT population referred a relevant personal or family medical history, and only 15 subjects (10.2%) regarded themselves as occasional alcohol consumers. Finally, 17 patients (11.6%) were receiving any kind of concomitant therapy prior to their inclusion in this study. Baseline characteristics are listed in Table 1. Except for the age, where there was a significantly higher mean value in the active compared with the placebo group ($P=0.0310$), in the AT and PP populations, both groups presented similar clinical and sociodemographic profiles.

Among the efficacy outcomes of the study, the cumulative incidence of AAD was significantly lower in the active arm compared with the placebo group ($P<0.001$), especially along the first seven days of the study. The co-administration of the synbiotic and Augmentin reduced the number of subjects experiencing AAD. On the third day the cumulative percentage of subjects experiencing AAD was 6.8% in the active and 16.4% in the placebo group, and

Table1. Baseline characteristics (as treated population).¹

Characteristic	Synbiotic ²	Placebo	P-value ³
Age, years, mean (SD)	47.9(12.5)	43.6(12.4)	0.031
Sex (females), n (%)	41 (55.4)	46 (63.0)	0.348
BMI (kg/m ²), median (IQR)			
Males	24.8 (23.8-28.7)	25.0 (24.0-29.41)	0.715
Females	25.5 (20.8-26.8)	24.3 (20.8-27.3)	0.705
Reasons for dental consultation, n (%)			0.789
Dental infection	43 (58.1)	44 (60.3)	
Surgical procedure	31 (41.9)	29 (39.7)	
Alcohol consumption, n (%)			0.545
Occasional drinker	9 (12.2)	6 (8.2)	
Abusive drinkers ⁴	0 (0.0)	0 (0.0)	
Ex-drinkers	0 (0.0)	1 (1.4)	
Never-drinkers	59 (79.7)	57 (78.1)	
Unknown	6 (8.1)	9 (12.3)	
Relevant medical history, n (%)			1.000
IBD	0 (0.0)	0 (0.0)	
Respiratory diseases	0 (0.0)	0 (0.0)	
Cardiovascular diseases	0 (0.0)	0 (0.0)	
Immunodeficiency disorders	0 (0.0)	0 (0.0)	
Allergies to probiotics	0 (0.0)	0 (0.0)	
Allergies to antibiotics	0 (0.0)	0 (0.0)	
Other (not specified)	3 (4.1)	3 (4.1)	
Concomitant therapies prior to study inclusion, n (%)	9 (12.2)	8 (11.0)	0.819

¹ SD = standard deviation; BMI = body mass index; IQR = interquartile range; IBD = inflammatory bowel disease.

² Prodefen Plus[®] is a synbiotic that contains a combination of prebiotics (fructooligosaccharides 990 mg) and seven probiotic strains (1×10^{10} cfu *Lactobacillus rhamnosus* GG and 1×10^9 cfu each *Lactobacillus casei*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Bifidobacterium infantis* and *Bifidobacterium bulgaricus*) per sachet.

³ Fisher exact test.

⁴ Alcohol abuse was defined as consumption of more than 14 alcoholic drinks per week, or more than 4 drinks in a day, for a healthy male (<65 years); or more than 7 alcoholic drinks per week, or more than 3 drinks in a day, for a healthy non-pregnant woman (<65 years).

raised to 10.8% in the active and 20.5% in the placebo group on the seventh day (5 vs 12 and 8 vs 15, on days 3 and 7, respectively). Statistically significant differences in AAD incidence between the study groups were maintained once the antibiotic treatment was finished (Figure 2).

Significant differences ($P<0.05$) were also found in the stool consistency throughout the study, with a significantly

greater number of loose and watery stools (type 5 to 6 of the BSFS) and completely watery stools (type 7 of the BSFS) within the placebo group compared to the group that received the synbiotic (Table 2). The greatest frequency of watery stools were observed on days 3 and 4 of treatment, with a total number of 12 stools (12%) in the active group and 26 (22.2%) in the placebo group on day 3, and total number of 4 stools (3.9%) in the active group and 29 (23%) in the placebo group on day 4.

Thirty AAD episodes were recorded along the study period; 12 in 12 subjects in the active group, and 18 in 16 subjects in the placebo group. The median (interquartile range, IQR) duration for all the AAD episodes in the study was 1.0 (1.0, 3.0) for the active group and 2.0 (1.0, 3.0) for the placebo group. The maximum duration was 6 and 8 days for the active and placebo groups, respectively.

A significant association ($P<0.001$) between the presence of AAD and its impact on the daily living activities along the whole study was observed (Supplementary Table S1). Overall, there was a small percentage of participants (10–25%) who referred any kind of impact on their quality of life. They were more distressed in the initial days. The biggest differences were found on day 3: the percentage

Figure 2. Incidence of antibiotic-associated diarrhoea (AAD) in the as treated population. AAD for at least 1 day (i.e. passage of 3 or more loose stools per day and a stool consistency of 5 to 7 according to the Bristol Stool Form Scale).

Table 2. Consistency of stools: total number of stools and number (percentage) of loose and watery stools.¹

	Total n (100%)	Type 5 n (%)	Type 6 n (%)	Type 7 n (%)	P-value
Day 1					0.002
Active	59	6 (10.2%)	1 (1.7%)	0 (0.0%)	
Placebo	74	8 (10.8%)	14 (18.9%)	4 (5.4%)	
Day 2					0.002
Active	95	13 (13.7%)	6 (6.3%)	3 (3.2%)	
Placebo	103	11 (10.7%)	10 (9.7%)	20 (19.4%)	
Day 3					0.054
Active	100	16 (16.0%)	11 (11.0%)	12 (12.0%)	
Placebo	117	10 (8.5%)	18 (15.4%)	26 (22.2%)	
Day 4					0.0005
Active	103	15 (14.6%)	12 (11.7%)	4 (3.9%)	
Placebo	126	18 (14.3%)	10 (7.9%)	29 (23.0%)	
Day 5					0.001
Active	112	17 (15.2%)	15 (13.4%)	5 (4.5%)	
Placebo	103	8 (7.8%)	10 (9.7%)	18 (17.5%)	
Day 6					0.135
Active	98	16 (16.3%)	9 (9.2%)	4 (4.1%)	
Placebo	103	14 (13.6%)	13 (12.6%)	9 (8.7%)	
Day 7					0.032
Active	104	13 (12.5%)	12 (11.5%)	2 (1.9%)	
Placebo	101	16 (15.8%)	6 (5.9%)	11 (10.9%)	

¹ Bristol Stool Form Scale: loose and watery stools (type 5 to 6); completely watery stools (type 7).

of subjects who had activities of daily living affected was reduced if taking the synbiotic (13.5%) compared to those taking the placebo (23.5%), although these differences did not reach the statistical significance. Furthermore, the degree of impact in affected subjects was lower if they received the synbiotic compared to if they received the placebo (Figure 3 and 4).

As to the associated symptomatology recorded by the study patients in their diary card, and analysed in the AT population, the most frequently reported were flatulence (in 38 subjects, with a mean (SD) duration of 3.76 (3.54) days), abdominal pain (in 27 subjects, with a mean (SD) duration of 2.96 (2.10) days), and nausea (in 12 subjects, with a mean (SD) duration of 1.42 (0.67) days). Overall, the incidence of the associated symptoms throughout the whole study was low (less than 15%), and there were not statistically significant differences between groups.

Treatment tolerability was regarded by most of the participants along the study (74 to 80%) as good or very good (Supplementary Figure S1). No significant differences were observed in type or frequency of adverse events between the study groups. The most frequently reported adverse events (AE) were gastrointestinal problems: flatulence (25.9%), abdominal pain (18.4%) and nausea

(8.2%). Finally, 70% of the study subjects reported a good/very good study treatments acceptance and satisfaction (Supplementary Figure S2), with no observed significant differences between both study groups.

4. Discussion

Antibiotics are commonly prescribed in the primary care setting in Spain. The association of amoxicillin and clavulanic acid (A/C) is among the most frequently used ones, mainly indicated for ear nose and throat infections, chronic obstructive pulmonary disease exacerbations, complicated cellulitis or pneumonia, with a significant increase in prescription over the last years (Llor *et al.*, 2009). Likewise, A/C is one of the preferred prescribed antibiotic for endodontic and other oral infections in dental practice (Segura-Egea *et al.*, 2017), and the first-line prophylactic drug in both the pre- and post-operative periods for any dental surgical procedure (Camps-Font *et al.*, 2018).

Although the combination of amoxicillin and clavulanic acid has proven to be fairly safe, a high incidence (19.8%) of AAD has been reported, and much higher than with the use of amoxicillin alone (8.1%) (Kuehn *et al.*, 2015), or with fluoroquinolones, macrolides, tetracyclines and cephalosporins (Silverman *et al.*, 2017). Although the exact mechanism for this adverse effect is not well understood, apart from its direct effect on the gut microflora, it is believed that A/C does increase the small bowel motility in healthy individuals (Gomez *et al.*, 2012), and could also induce microbial dysbiosis leading to altered metabolism of key intestinal nutrients, and an osmotic diarrhoea (Silverman *et al.*, 2017). The consequence of this high AAD incidence is its impact on the treatment compliance, and the higher rates of antibiotic discontinuation, as shown in the A/C treated patients (Kuehn *et al.*, 2015).

Probiotics have demonstrated efficacy in preventing and/or delaying the onset of AAD, and specifically some studies have proven to show a beneficial effect of probiotics in A/C associated diarrhoea (Evans *et al.*, 2016; Garcia Trallero *et al.*, 2019). The synbiotic Prodefen Plus has previously shown additional benefit to the standard supportive therapy in the management of acute viral diarrhoea in paediatric populations (Allahverdi *et al.*, 2010; Garcia-Menor *et al.*, 2016). In this study, the primary objective was to evaluate the effectiveness of the synbiotic in the prevention of AAD in a healthy adult population who were treated with Augmentin (875 mg of amoxicillin and 125 mg of clavulanate) three times a day for 7 days, either for an oral infection or as prophylaxis for a surgical procedure in a dental consultation. Additionally, the incidence and/or duration of any other antibiotic-associated symptomatology and the tolerability and treatment acceptance of the synbiotic were evaluated. Therefore, the novelty of this trial lies in the use of this synbiotic for the first time in the prevention of AAD in an

Figure 3. Subjects with impact on activities of daily living. ns = not significant.

Figure 4. Grade of impact of the affected subjects on activities of daily living. ns = not significant.

adult outpatient population, and in a clinical setting for which neither health care professionals (Agamennone *et al.*, 2018) nor current guidelines specifically recommend prescribing probiotics (Aragon-Martinez *et al.*, 2019).

The study met its primary endpoint by achieving a significant reduction in the AAD incidence, especially during the first seven days of treatment, when the antibiotic, Augmentin, was administered, and a significant improvement in the stool consistency, in the active group compared with the placebo group. The AAD incidence reduction in the active group during the very first days of antibiotic treatment, lowering by half the number of subjects presenting with diarrhoea, has a significant clinical relevance, since this is the most critical period for antibiotic discontinuation that generally occur as a consequence of this inconvenient symptom.

These results are in accordance with findings previously reported in both individual published studies or systematic revisions and meta-analysis of the preventive effect of probiotics as adjunct treatment in AAD in adult populations (Beaugerie *et al.*, 2003; Blaabjerg *et al.*, 2017; Cai *et al.*, 2018; Cimperman *et al.*, 2011; Evans *et al.*, 2016; Goldenberg *et al.*, 2015; Hickson, 2011; Jafarnejad *et al.*, 2016; Pozzoni *et al.*, 2012; Song *et al.*, 2010; Szajewska *et al.*, 2015). Overall, most probiotic therapies have shown to be effective and safe in reducing the incidence of AAD, and the mean duration of diarrhoea, the stool consistency and the number of diarrhoea episodes at some extent. In this sense, published evidence suggest both dose-response and a specific-strain relationship, being *L. rhamnosus* the strain to present the highest effectiveness and tolerability for reducing the incidence of diarrhoea in prevention of AAD. However, these results should be interpreted with caution, since there is a reported evidence of some publication bias as well as statistically significant heterogeneity.

There was also a reduction albeit not statistically significant in both the number and duration of the diarrhoea episodes in the active group versus the placebo group in our study. Furthermore, the trial showed that the synbiotic Prodefen Plus was well tolerated and accepted by most of the study participants; the treatment compliance was graded as very good. Interestingly, there was a small percentage of study participants in either group who refer any type of study associated symptoms, and, likewise, a low reported impact on the daily living activities of the study patients, more favourable in those receiving the synbiotic. Similar results have been reported in published studies assessing the preventive effect of probiotics in AAD (Garcia Trallero *et al.*, 2019; Hungin *et al.*, 2013). The results on stool consistency, a key component of AAD severity perception (Shiro *et al.*, 2017), in addition to those observed on the impact on the daily life activities suggest the positive influence of the synbiotic in reducing the severity of the diarrhoea.

The study showed some strengths in its methodology and conduction. The study had statistical power enough to detect differences in several objectives between the groups, and its controlled design ensured the validity of the obtained results. Furthermore, this is the first study to prove the preventative efficacy of the synbiotic in reducing AAD incidence and its beneficial over the diarrhoea in an outpatient adult population.

Nevertheless, some study limitations must be acknowledged. An incorrect allocation of some study subjects to the other treatment group occurred (13 subjects from the active to the placebo arm, and 12 from the placebo to the active arm). Therefore, a bias in the final results due to a possible imbalance between both study arms cannot be completely ruled out, and this could be considered a study limitation. However, the study blinding was maintained throughout the whole study duration and the final numbers of the AT and PP study populations were comparable between the placebo and the active arms; therefore, it seems unlikely that this misallocation of some patients may have had any impact on the study results. Another potential bias might come from the heterogeneity of the two groups included in the study: patients with an oral infection requiring antibiotic therapy and patients requiring antibiotic prophylaxis prior to a dental surgery. However, it is well known that the type, dose and posology of the antibiotic has the greatest influence in the development and evolution of the diarrhoea (McFarland, 2008; Silverman *et al.*, 2017). In our study Augmentin 875/125 was prescribed to all the study subjects with the same dose and posology, and the synbiotic was always administered after the antibiotic intake. Hence, we considered that it would not influence the investigation of the effect of the probiotic in the diarrhoea.

A further study limitation could be the methodology used to record and collect the study outcomes; a patient diary card could be a potential source of bias. However, we reckon that diary cards are valid, objective and reliable assessment tools in the sense that they can reduce the risk of recall and the interviewer bias, when recording patients' symptoms, as well as permitting more accurately daily register. Larger trials would be necessary to confirm our results, and probably they should be conducted in other settings such as primary care practice.

In conclusion, results from this study confirm the clinical benefit of the synbiotic Prodefen Plus, administered as concomitant therapy in a healthy adult population treated with Augmentin, in preventing the antibiotic-associated diarrhoea. The synbiotic had a beneficial effect over the diarrhoea, improving the consistency of stools and decreasing the number and duration of the diarrhoea episodes. The effect of the synbiotic was clearly evident since the initial days of the antibiotic treatment. In addition, the intake of Prodefen Plus showed a positive influence on

activities of daily living. All these positive effects evidenced with the preventative action of the synbiotic are of great clinical relevance in assuring patient adherence and avoiding discontinuation of antibiotics as a consequence of the diarrhoea.

Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/BM2020.0062>.

Table S1. Severity of antibiotic associated diarrhoea.

Figure S1. Tolerability of administered treatment.

Figure S2. Acceptance and satisfaction with administered treatment.

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Conflicts of interest

CNM and JSA are full time employees of ITF Research Pharma S.L.U. (Spain); all the investigators received a research fee for their participation in the study.

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