

The administration of probiotics and synbiotics in immune compromised adults: is it safe?

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Abstract

This study aimed to systematically evaluate safety of probiotics and synbiotics in immune compromised adults (≥ 18 years). Safety was analysed using the Common Terminology Clinical Adverse Events (CTCAE version 4.0) classification, thereby providing an update on previous reports using the most recent available clinical data (2008–2013). Safety aspects are represented and related to number of participants per probiotic strain/culture, study duration, dosage, clinical condition and selected afflictions. Analysis of 57 clinical studies indicates that probiotic and/or synbiotic administration in immune compromised adults is safe with regard to the current evaluated probiotic strains, dosages and duration. Individuals were considered immune compromised if HIV-infected, critically ill, underwent surgery or had an organ- or an autoimmune disease. There were no major safety concerns in the study, as none of the serious adverse events (AE)s were related, or suspected to be related, to the probiotic or synbiotic product and the study products were well tolerated. Overall, AEs occurred less frequent in immune compromised subjects receiving probiotics and/or synbiotics compared to the control group. In addition, the results demonstrated a flaw in precise reporting and classification of AE in most studies. Furthermore, generalisability of conclusions are greatly limited by the inconsistent, imprecise and potentially incomplete reporting as well as the variation in probiotic strains, dosages, administration regimes, study populations and reported outcomes. We argue that standardised reporting on adverse events (CTCAE) in ‘food’ studies should be obligatory, thereby improving reliability of data and re-enforcing the safety profile of probiotics.

Keywords: food safety legislation, immunocompromised people, prebiotics, probiotics, synbiotics

1. Introduction

Advances in care and surgical techniques have considerably improved the prognosis and outcome of hospitalised and critical ill patients (Liu *et al.*, 2013); however, complications such as comorbidity and infections remain a major concern and prolong the hospitalisation (Vincent *et al.*, 2009). These complications increase the healthcare expenditures and lead to higher recurrences and death rates (Liu *et al.*, 2013). Prescribing antibiotics prophylactically in order to prevent infectious complications is a less favourable option after decades of extensive antibiotic use (Barraud *et al.*, 2010). Due to a high prevalence of bacterial antibiotic resistance

and a relatively exhausted antibiotic pipeline, there is a need for alternative strategies (Saavedra, 2001). Many complications in hospitalised patients are associated with a dysbiosis of the intestinal microbiota (Watkinson *et al.*, 2007). There is increasing evidence that probiotics have the potential to restore this aberrant composition of the intestinal ecosystem, thereby preventing these common complications (Sanders *et al.*, 2013). Probiotics are defined as ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ (FAO, 2001). Probiotics can be supported by prebiotics, which are ‘non-viable food components that confer a health benefit on the host associated with modulation of the microbiota’ (Pineiro

et al., 2008). A combination of probiotics and prebiotics is referred to as a synbiotic (Guarner *et al.*, 2012). Probiotics are proposed to enhance intestinal integrity, regulate the immune system, prevent pathogenic colonisation and play a key role in metabolic pathways (Alonso and Guarner, 2013; Kamada *et al.*, 2013; Vyas and Ranganathan, 2012). A high number of randomised trials have been conducted to prove probiotic efficacy with contradicting results (Sanders *et al.*, 2013). Nevertheless, probiotics demonstrated to be promising in several areas, for instance in reducing the incidence of infection, antibiotic associated diarrhoea and intensive care unit stay (Barraud *et al.*, 2013; Hempel *et al.*, 2012; Morrow *et al.*, 2012).

Despite the potential benefits of probiotic administration, there are some concerns regarding the safety of probiotics. Hospitalised individuals are often more susceptible to infections due to an impaired intestinal barrier function, an imbalance of the immune system and defective microbial clearance (Clayburgh *et al.*, 2004; Schuijt *et al.*, 2011). In these individuals probiotic species might become opportunistic and also translocate through the gastrointestinal barrier, causing bacteraemia and other complications. Several case-reports indicate that probiotic species are indeed able to cause bacteraemia (Salminen *et al.*, 2004). A randomised controlled trial was even terminated when there appeared a significant higher mortality rate after administration of probiotics in patients with acute pancreatitis (Besselink *et al.*, 2008). Hence, a safety profile of probiotics and synbiotics is necessary to determine the potential risks, in particular, in a susceptible population comprising immune compromised subjects. A previous report indicated that probiotics and synbiotics are safe in the setting of controlled trials in infants less than two years of age (Van den Nieuwboer *et al.*, 2014). In specific, in comprehensive meta-analyses probiotics demonstrated to be effective and safe in highly vulnerable immune compromised preterm neonates, which have a very immature intestinal barrier and are at risk of developing necrotic enterocolitis (Alfaleh and Bassler, 2008; Wang *et al.*, 2012). This study will focus on probiotic and synbiotic safety in immune compromised adults (≥ 18 years). The aim of this report is to provide an update with the most recent interventional studies based on the previous safety analysis by Hempel *et al.* (2011) and provide a detailed overview of safety concerns for high-dosage and chronic probiotic use in immune compromised adults, by taking health conditions, probiotic intake and study duration into account.

2. Methods

A comprehensive literature study was conducted to analyse the safety data of probiotics and synbiotics in immune compromised adults according to previously published methodology (Van den Nieuwboer *et al.*, 2014). All clinical studies were retrieved from the online database PubMed

(National Library of Medicine, includes MEDLINE). The search strategy was confined to human interventional studies using a single probiotic, a probiotic mix or synbiotics within the last five years, covering the recent clinical studies. By only including studies published between 2008 and 2013 an update is provided on previous extensive safety analyses that did not indicate any associated health risks (Claassen *et al.*, 2010; Hempel *et al.*, 2011). An in-depth literature search using the search terms 'probiotics' and 'synbiotics' in combination with immune compromised conditions was performed to retrieve all relevant studies. Examples of immune compromised search terms are 'HIV', 'organ transplantation', 'cancer', 'critical ill', 'hospitalised' and 'immune deficient'. In addition, interventional studies in individuals with disorders such as inflammatory bowel disease (IBD) or rheumatoid arthritis (RA) were also included if participants were allowed to continue their treatment with immunosuppressant drugs or biologicals (e.g. corticosteroids, azathioprine, tumour necrosis factor- α inhibitors). All animal, *in vitro* and sub-studies were excluded from analysis and by applying a filter, only clinical trials were included. All original and follow-up studies with immune compromised adults (≥ 18 year) were considered eligible; there was no restriction on probiotic or synbiotic species or study design (open label pilot to double-blinded, randomised, placebo-controlled studies). Both mechanistic studies as well as studies attempting to cure, treat, alleviate or prevent an illness were incorporated into this analysis.

Safety of the evaluated probiotics or synbiotics products was assessed by analysis of the quantity and nature of the reported adverse events (AEs). An AE is defined as the occurrence of a complication or illness, or worsening of the condition throughout the study. An AE can be further classified according to its relationship with the study product and its severity, however this report did not further subdivided the AEs because this also depends on the judgement of the investigator and causal relationship should be determined in large meta-analyses. The Common Terminology Criteria for Adverse Events (CTCAE version 4.0, NIH, 2009) classification system was used to categorise the AEs which divides the AEs into 26 disorder areas (Table 1). This study did not grade the severity of the AEs as necessary information was missing or difficult to interpret. As not all AEs were properly reported, or were reported using alternative classification systems, an extra category 'unspecified' was added. All AEs that were unspecific were placed in this category.

Other relevant data, such as probiotic strains, dosage and treatment duration were taken into account for analysis of the intervention. Since properties of probiotics are strain specific, it is essential to determine what strains have been investigated at high-dosage and in chronic use with regard to their respective safety profile. It should be noted

Table 1. Common terminology clinical adverse events v. 4.0.

Category	Designation
Blood and lymphatic system disorders	I
Cardiac disorders	II
Congenital, familial and genetic disorders	III
Ear and labyrinth disorders	IV
Endocrine disorders	V
Eye disorders	VI
Gastrointestinal disorders	VII
General disorders and administration site conditions	VIII
Hepatobiliary disorders	IX
Immune system disorders	X
Infections and infestations	XI
Injury, poisoning and procedural complications	XII
Investigations	XIII
Metabolism and nutrition disorders	XIV
Musculoskeletal and connective tissue disorders	XV
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	XVI
Nervous system disorders	XVII
Pregnancy, puerperium and perinatal conditions	XVIII
Psychiatric disorders	XIX
Renal and urinary disorders	XX
Reproductive system and breast disorders	XXI
Respiratory, thoracic and mediastinal disorders	XXII
Skin and subcutaneous tissue disorders	XXIII
Social circumstances	XXIV
Surgical and medical procedures	XXV
Vascular disorders	XXVI

that this review used the terms probiotics and synbiotics interchangeably, as synbiotics contain probiotic strains.

A total of 64 relevant abstracts were identified using the above mentioned search strategy. After full text analysis, seven studies were not eligible for final analysis. Four studies did not meet all the inclusion criteria, two studies appeared to be sub-analyses from randomised controlled trials (RCT) published before 2008, and one study did not include immune compromised subjects. A total of 57 clinical studies were included into the final analysis (Table 2). All of the studies were original studies; no long-term follow-up studies were identified or retrieved.

3. Results

A total number of 4,914 participants were enrolled to the treatment and the control group in the 57 eligible studies of which 2,563 participants were allocated to a probiotic treatment. In the treatment arm, a number of 2,289 participants were analysed per-protocol from the 2,563 participants, resulting in a drop-out rate of 10.69%. The allocated and analysed per-protocol participants for the control group were 2,351 and 2,122 respectively,

with a drop-out rate of 9.74%. The data of the allocated population was used for the safety analysis, as the per-protocol population is lacking possible drop-outs due to experienced AEs. The majority of the studies were published between 2010 and 2011, whereas only one eligible study was published in 2013. The median duration of the interventional studies was 28 days (range: 3-365) (Figure 1). 88% of the interventions were shorter or equal to three months (50 studies). Only two studies evaluated long-term administration of one year, four studies determined the effects of 6-month administration and one study of 4 months. The identified immune compromised conditions were patients with HIV, critically ill patients, hospitalised patients, severely injured individuals, perioperative patients (tumour removal or normal surgery), organ disease (liver cirrhose, kidney disease), autoimmune disorders (IBD, multiple sclerosis (MS), RA) and 'other' encompassed radiotherapy and UV-therapy.

The allocated and per-protocol analysed participants for each subgroup is depicted in Figure 2. The majority of immune compromised adults subjected to an intervention were critically ill and hospitalised, had an organ disease or were HIV-infected. The HIV-infected subgroup included 9

Table 2. The clinical studies used for the analysis.

Agrawal <i>et al.</i> , 2012	Irvine <i>et al.</i> , 2010, 2011	Ranganathan <i>et al.</i> , 2009
Anukam <i>et al.</i> , 2010	Ishikawa <i>et al.</i> , 2011	Schunter <i>et al.</i> , 2012
Bouilly-Gauthier <i>et al.</i> , 2010	Jenks <i>et al.</i> , 2008	Sharma <i>et al.</i> , 2011
Chitapanarux <i>et al.</i> , 2010	Klarin <i>et al.</i> , 2008	Shimizu <i>et al.</i> , 2009
Cimperman <i>et al.</i> , 2011	Knight <i>et al.</i> , 2009	Song <i>et al.</i> , 2010
De los Angeles Pineda <i>et al.</i> , 2011	Lee <i>et al.</i> , 2010	Souza and Jorge, 2012
Diepenhorst <i>et al.</i> , 2011	Liu <i>et al.</i> , 2010, 2011, 2013	Stadlbauer <i>et al.</i> , 2008
Eguchiet <i>et al.</i> , 2011	Malaguarnera <i>et al.</i> , 2010	Steed <i>et al.</i> , 2010
Fleming <i>et al.</i> , 2011	Mandel <i>et al.</i> , 2010	Stephens and Hewett, 2012
Frohmadet <i>et al.</i> , 2010	Mangell <i>et al.</i> , 2012	Tan <i>et al.</i> , 2011
Fujimori <i>et al.</i> , 2009	Matthes <i>et al.</i> , 2010	Tanaka <i>et al.</i> , 2012
Gao <i>et al.</i> , 2010	Mittal <i>et al.</i> , 2011	Tandon <i>et al.</i> , 2009
Gianotti <i>et al.</i> , 2010	Morrow <i>et al.</i> , 2010	Tursi <i>et al.</i> , 2010
González-Hernández <i>et al.</i> , 2012	Peguet-Navarro <i>et al.</i> , 2008	Usami <i>et al.</i> , 2011
Hemsworth <i>et al.</i> , 2012	Peral <i>et al.</i> , 2009	Zhang <i>et al.</i> , 2012
Horvatet <i>et al.</i> , 2010	Pereg <i>et al.</i> , 2011	
Hummelen <i>et al.</i> , 2010, 2011a,b	Pozzoni <i>et al.</i> , 2012	

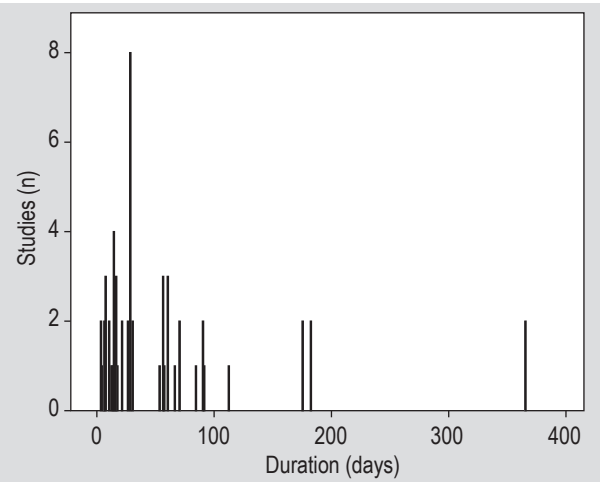


Figure 1. Duration of the interventional studies in days.

(15.8%) studies with 375 and 369 participants allocated to the treatment and control group respectively. Within this subgroup, participants were highly active antiretroviral therapy (HAART)-treated as well as anti-retroviral (ARV) therapy naïve. The median duration of the intervention was 30 days (range: 28-175).

The critically ill, severely injured and hospitalised patients were combined into the subgroup denoted ‘critically ill’, consisting of 15 (26.3%) studies. In the treatment and control group were 938 and 852 subjects allocated, respectively. Subjects allocated to this subgroup were considered critically ill if they required enteral nutrition, mechanical ventilation, suffered from Systemic Inflammatory Response Syndrome (SIRS), acute pancreatitis or were hospitalised due to a need of antibiotic treatment. The severely injured subjects that were included in this subgroup suffered from

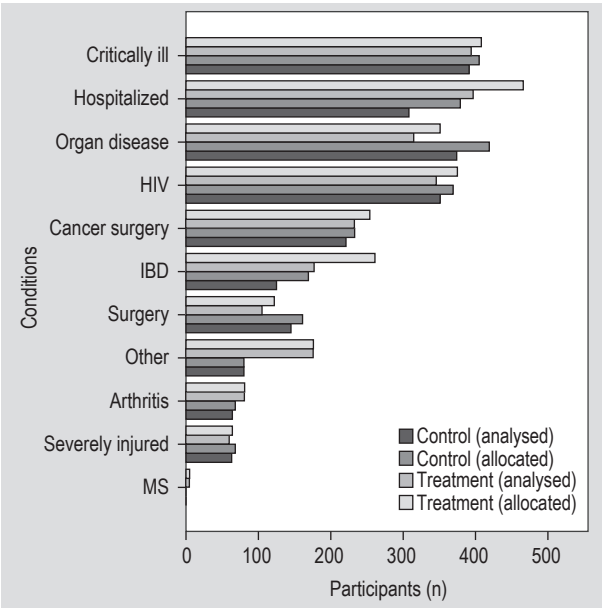


Figure 2. Allocated and analysed (per-protocol) participants for each conditions for both the treatment and control group.

traumatic brain injury or burn injury. The median duration of the intervention in the studies of the ‘critically ill’ patient subgroup was 14 days (range: 7-28).

The perioperative subgroup consisted of patients with colonic, hepatic or oesophageal cancer requiring radical surgery as well as patients undergoing colonic resection or pancreaticoduodenectomy. In addition, this subgroup included one study involving patients undergoing liver transplantation. In 11 (19.2%) studies a total of 376 and 394 participants were allocated to the treatment and control group, respectively. The participants received probiotics

preoperative for a median of 3 days (range: 0-8) and perioperative for a median of 16 days (range: 3-28).

In the organ disease subgroup, 351 participants were allocated to the treatment group and 419 participants to the control group, respectively. A total of 9 (15.8%) studies included patients with compensated and alcoholic liver cirrhosis, chronic hepatitis and chronic kidney disease. The participants underwent probiotic or synbiotic treatment for a median of 60 days (range: 5-365).

Participants suffering from an autoimmune disorder including RA, spondyloarthritis, MS and the IBDs, ulcerative colitis (UC) and Crohn's disease, were exposed to the intervention for a median of 56 days (range: 7-365). In 10 studies (17.5%) a total of 347 and 237 participants were allocated to the treatment and control group, respectively. The participants were allowed to continue their standard treatment with or without immunosuppressant drugs. The remaining subgroup 'other' consisted of cancer patients receiving radiotherapy or patients receiving UV-induced immune suppression. A total of 176 and 80 participants were allocated in 3 (5.2%) studies in the treatment and control group, respectively. The median duration of the intervention was 57 days (range: 53-70).

Interventional product

In 50.8% of the trials a commercially available probiotic or synbiotic product was administered to the participants. Eighteen (31%) of the administered study products was a single strain probiotic product, 28 (48.2%) was a multi strain probiotic product consisting of a combination of two or more probiotic strains. A single synbiotic supplementation was the least frequently used formulation, 4 (6.9%) times, whereas a synbiotic mixture containing two or more probiotic species was administered in 8 (13.7%) interventions. The strain designation was not reported for 68 (43.6%) probiotic species used in the interventions. In one case the probiotic species was not provided at all (Malaguenera *et al.*, 2010) and Mittal and colleagues (2011) did not provide the probiotic genus, species or strain.

As illustrated in Figure 3, *Lactobacillus acidophilus* (unspecified) and *Bifidobacterium longum* (unspecified) were administered most frequently to the participants. *L. acidophilus*, *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* GR-1 were all administered to more than 300 subjects. Between 200 and 300 subjects received *Streptococcus thermophilus* (unspecified), *Lactobacillus bulgaricus* (unspecified), *Bifidobacterium breve* (unspecified), *B. bifidum* (unspecified), *Bifidobacterium infantis* (unspecified) and *Lactobacillus plantarum*. The probiotic strains *Lactobacillus casei* (unspecified), *L. rhamnosus* GG, *L. casei* LBC80R, *L. acidophilus* CL1285, *Pediococcus pentosaceus* 5-33:3, *Leuconostoc mesenteroides*

32-77:1, *L. plantarum* 2362, *Lactobacillus paracasei* 19, *Lactobacillus johnsonii* La1, *Saccharomyces boulardii*, *L. plantarum* (GMCC no. 1258), *L. acidophilus*-11, *B. longum*-88, *B. breve* Yakult, *L. paracasei* (unspecified), *L. casei* Shirota, *L. rhamnosus* R0011 and *L. acidophilus* R0052 were each administered to 100-200 subjects. The safety data of the remaining 27 evaluated strains proved less reliable as these strains were administered to less than 100 subjects. Figure 3 illustrates the administered daily cfu for each evaluated strain. The median supplemented daily dosage was 2.0×10^9 cfu, ranging from the lowest administered dosage of the helminth *Trichurius suis* of 2.5×10^4 ova once each two weeks to the highest dosage of 4.0×10^{11} cfu/day with *B. longum* (unspecified). The probiotic strains *B. longum* (unspecified), *L. rhamnosus* GR-1, *L. plantarum* 299v, *L. rhamnosus* GG, *S. thermophilus* (unspecified), *L. bulgaricus* (unspecified), *L. acidophilus* (unspecified), *B. bifidum* (unspecified), *L. casei* Shirota and *L. johnsonii* La1 were evaluated according to a large range of dosages, whereas the other strains were solely evaluated for a single dose, lower than 5.0×10^9 cfu or the applied dosage was not provided in the report. The clinical studies did not provide a clear daily cfu for the strains *B. bifidum* W23, *B. infantis* (unspecified), *B. infantis* W52, *B. lactis* Bi-07, *B. longum* KB35, *L. acidophilus* KB31, *L. acidophilus* W70, *L. casei* W56, *L. paracasei* (unspecified), *L. plantarum* (unspecified), *L. plantarum* (ATCC 10241), *L. rhamnosus* CAN-1, *L. rhamnosus* HN001, *L. salivarius* W24, *Lactococcus lactis* W58 and *S. thermophilus* KB27.

The various underlying disorders required different probiotic properties for alleviation, mitigation, prevention or treatment. The majority of the HIV-patients received *L. rhamnosus* GR-1 (n=299), whereas most critical ill patients received *L. rhamnosus* GG (n=178). The largest proportion of perioperative participants received the probiotic strains *L. plantarum* (CGMCC no.1258), *L. acidophilus*-11 and *B. longum*-88 (n=139). The most common administered probiotics strains in participants with an organ disease were *L. acidophilus* (unspecified), *L. bulgaricus* (unspecified) and *S. thermophilus* (unspecified; n=148), whereas participants with an autoimmune disorder and 'others' received most frequently *B. longum* (unspecified; n=170) and *L. johnsonii* La1 (n=144), respectively. With regard to the evaluated dosage per type of underlying condition, perioperative participants received the largest daily cfu, whereas the group 'other' received the lowest daily cfu. Figure 4 demonstrates that for each condition a wide range of cfu is evaluated, with autoimmune participants receiving the most diverse dosages of administration.

Safety

The eligible studies were, regardless of the self-reported adverse events, analysed for the non-specific overall safety statement of the study product (e.g. 'No significant

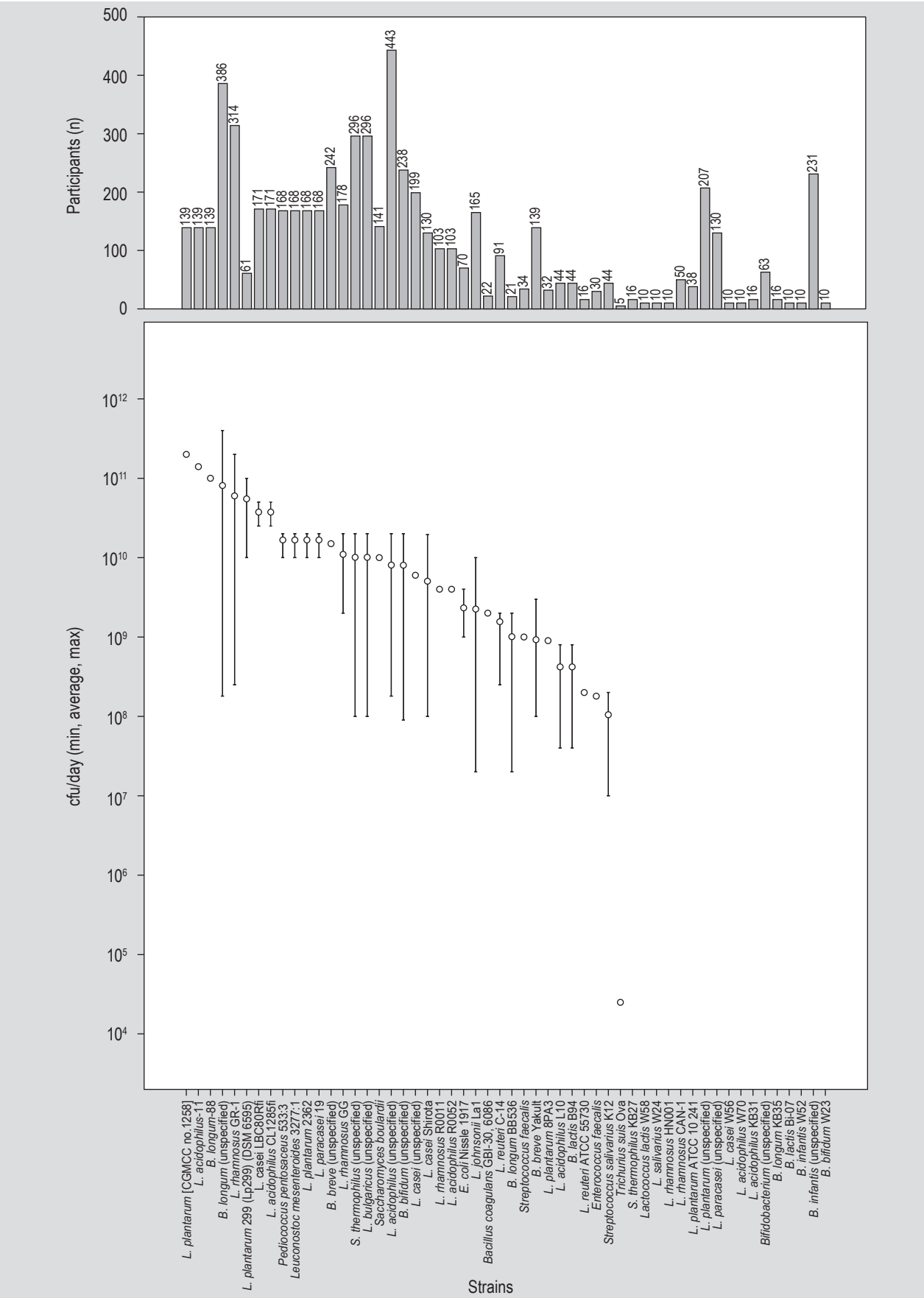


Figure 3. Range of daily administered CFU per probiotic strain and the respective number of participants receiving the probiotic strain.

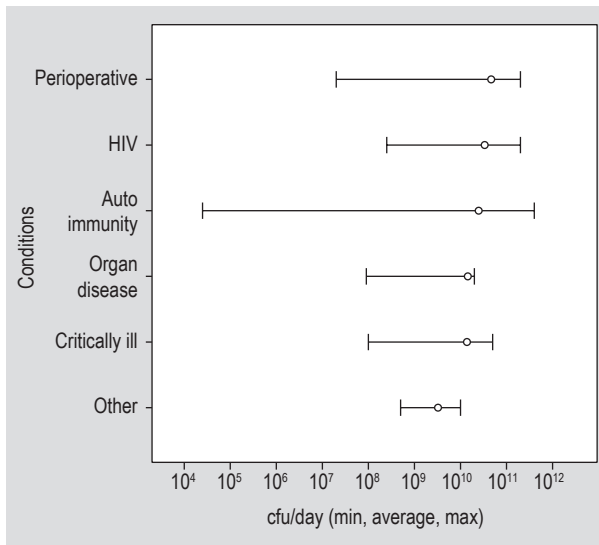


Figure 4. Daily administered cfu (minimal, average and maximal administered dosage) for each immune compromised conditions.

difference in AEs between the treatment and control group' and 'study product was well tolerated'). As illustrated in Figure 5, 27.1% of the analysed studies did not encounter any AEs or no AEs were reported by the participants. In 18.6% of the studies, the investigators did not observe a significant difference in AEs between the treatment group and the control group. In 10.1% of the studies the investigator only stated that no serious AEs occurred during the intervention period. The reported AEs were unrelated to the study product in 6 (10.1%) studies. The intervention was 'well tolerated,' 'safe' or even led to 'reduced complications' in 5, 1.7 and 5% of the studies, respectively. Strikingly, 22% of the analysed studies did not discuss safety or AEs of any kind. In addition these studies did not provide any overall safety statement regarding the administration of probiotic or synbiotic study product.

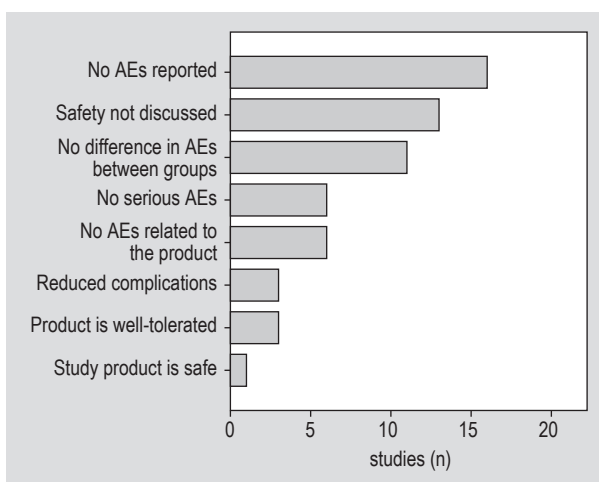


Figure 5. Reported overall safety statement of each analysed study.

In the eligible studies a total of 1,997 AEs were identified, 831 AEs in the treatment arms and 1,166 AEs in the control arms. Figure 6A illustrates the distribution of all the reported AEs in both the treatment arms as well as the control arms. The most common AEs could be categorised as gastrointestinal disorders (category VII) or infections and infestations (category XI). This included diarrhoea (VII), abdominal pain (VII), ventilator associated pneumonia (VAP; XI), abdominal discomfort or bloating (VII) and sepsis (XI). In both the treatment as well as in the control group, gastrointestinal disorders (category VII) occurred most frequently, 471 and 639 AEs respectively. Infections and infestations (XI) were the second most frequent reported AEs, 172 AEs in the treatment group compared to 318 in the control group. A total of 174 AEs could not be categorised properly according to the CTCAE, 97 and 77 in the treatment and control group respectively. The other CTCAE categories were not reported, or only observed in a low frequency.

With regard to the specific subgroups of immune compromised adults, the incidence of AEs in HIV-patients was 313, with the majority being of gastrointestinal nature. The incidence of gastrointestinal disorders (VII) was 102 and 181 in the treatment and control group, respectively. There was a lower or equal incidence rate of AEs in all CTCAE categories in the treatment group compared to the control group. Overall, there were less AEs reported (n=97) in the treatment arm (Figure 6B). Gastrointestinal disorders (VII) occurred most frequent in critical ill patients, followed by infections and infestations (XI). Despite of 8 more AEs in the treatment group within the category of skin disorders (XXIII), the overall incidence of AEs was lower compared to the control group (n=370 vs. 416 AEs respectively; Figure 6C). In perioperative participants, infections and infestations (XI) were most frequently observed, followed by gastrointestinal disorders (VII) and 'unspecified' AEs (XXVII). The occurrence of AEs in these categories were all higher in the control group compared to the treatment arm, although there were some single reported events in the treatment group concerning an endocrine disorder (V), nervous system disorder (XVII), renal and urinary disorder (XX), respiratory, thoracic and mediastinal disorder (XXII) and an AE in a surgical or medical procedure (XXV; Figure 6D). Overall the incidence of AEs was 197 and 341 in the treatment and the control group respectively. With regard to participants with an organ disease, hepatobiliary disorders (IX) and gastrointestinal disorders (VII) were the most common AEs. Of all the reported AEs, the incidence was lower in the treatment group (n=76) compared to the control group (n=163; Figure 6E). In the subgroup of participants suffering from an autoimmune disease, the frequency of AEs was relatively low. 'Unspecified' AEs (XXVII) and gastrointestinal disorders (VII) were reported most frequent. Both occurred more frequently in the treatment group compared to the control group (n=40

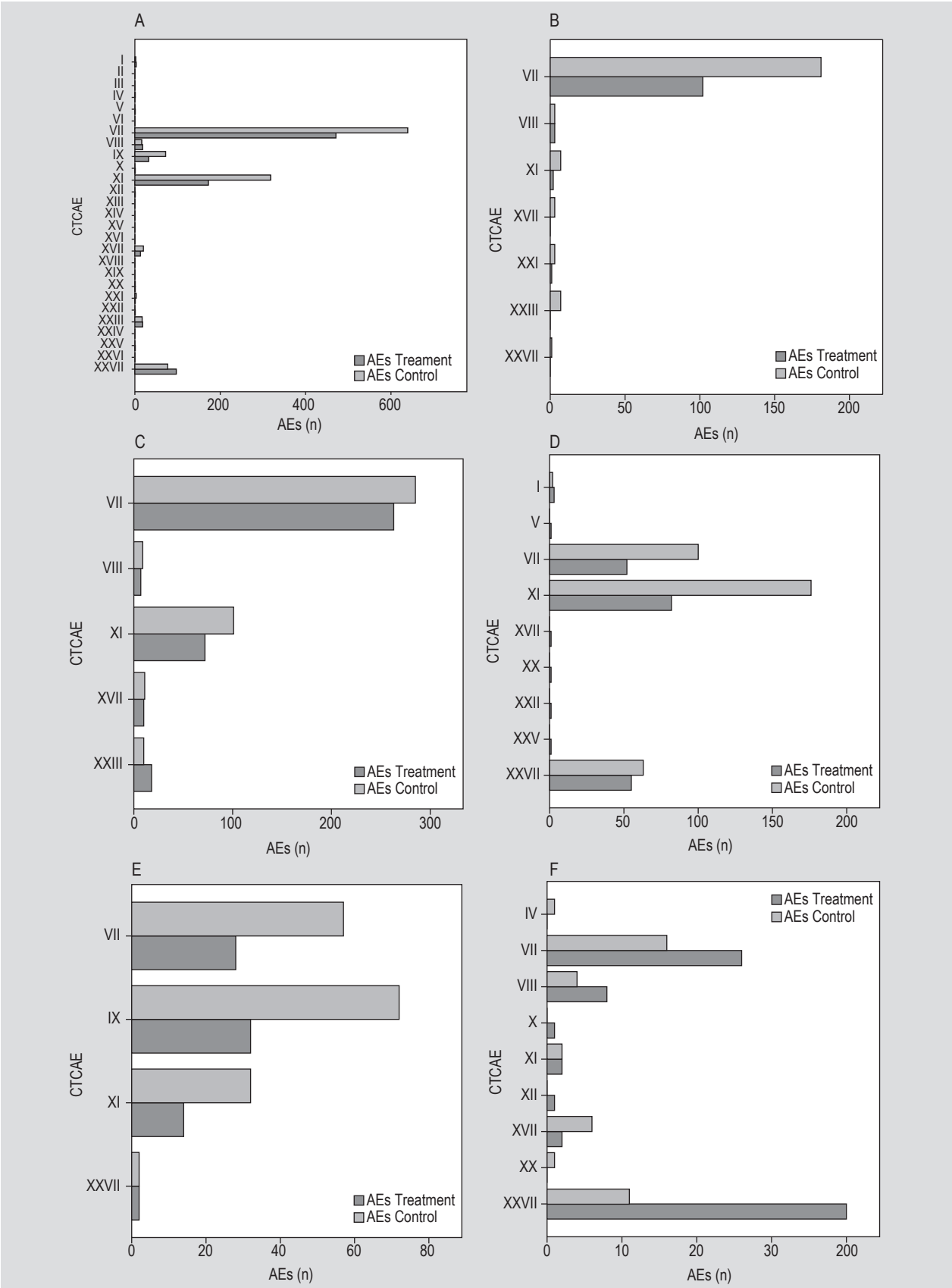


Figure 6. Total observed AEs (A) for all the CTCAE categories; (B) in HIV-infected participants; (C) in critical ill participants; (D) in perioperative participants; (E) in participants with an organ disease; and (F) in participants with an autoimmune disorder, in the allocated treatment arm and the control arm.

vs. $n=11$ in category XXVII and $n=26$ vs. $n=16$ in category VII for the treatment and control group respectively). Other CTCAE categories that were higher (but not significant) in the treatment group were immune system disorders (X), injury, poisoning and procedural complications (XII) and general disorders and administration site conditions (VIII). Although of concern, these complications occurred in relatively low numbers (Figure 6F). In the 'unspecified' category (XXVII) there were 20 more AEs reported in the treatment group compared to the control arm. The study by Matthes *et al.* (2010), investigating the effect of probiotic enemas on participants with UC, mainly contributed to this difference. The treatment group in this study comprised of three arms with dosages of *Escherichia coli* Nissle 1917 ranging from 1×10^9 to 4×10^9 cfu/day. Hence, 50 more subjects were allocated to the treatment group compared to the control group ($n=70$ and $n=20$, respectively). Due to a larger population it is expected that the incidence of AEs in the treatment group is higher. The incidence of AEs was not significantly different between the treatment and control group.

In the group 'other', only severe diarrhoea (grade II/III) was reported after radiotherapy (Chitapanarux *et al.*, 2010). In the treatment and control group an AE was reported three and 14 times respectively ($P=0.002$). The AEs were not attributable to the study products (data not shown).

4. Discussion and conclusions

Administration of probiotic strains to immune compromised adults indicated no increased adverse health risk in the setting of the 57 analysed probiotic and synbiotic intervention studies, published between 2008 and 2013. More specifically, the evaluated strains appeared to be safe with the respective administered daily dosage. The general safety statement in the majority of the studies indicated that there were no AEs reported by the participants, or there was no significant difference in incidence of AEs between the treatment and control arm. In general, none of the serious AEs were related, or suspected to be related, to the probiotic or synbiotic product and the study products were well tolerated. The incidence of the reported AEs in each study was either similar or significantly lower in the treatment arm compared to the control group; the incidence of AEs never appeared to be significantly lower in the control group. In addition, the incidence rate in the treatment arm is 0.32 vs. 0.49 in the control group, further reinforcing the alleged safety. Indeed, three studies stated that probiotics and synbiotics reduced complications during the probiotic intervention (Liu *et al.*, 2010, 2013; Zhang *et al.*, 2012). Both the studies of Zhang *et al.* (2012) and Liu *et al.* (2013) performed a perioperative probiotic intervention on participants undergoing elective radical surgery for colon cancer. The reduced complications encompassed a significant reduction in the incidence of sepsis, bacteraemia

and postoperative infections. Liu *et al.* (2010) reported reduced complications but did not further specify this.

Unfortunately, a high proportion (22%) of studies did not report or discuss safety aspects of the probiotic/synbiotic intervention. Especially in the case of immune compromised adults this is of major concern. Perhaps, as more than half (50.8%) of the studies evaluated the efficacy of commercially available products, the investigators depended on previous safety data or on a safety profile provided by the manufacturer. The majority of the eligible clinical studies focussed on efficacy rather than safety. These studies were not designed for proper AE evaluation. Although probiotic and synbiotic administration did not indicate an increased risk of any complications, future studies should take potential harms into account and be beware of the potential risks. Overall, the incidence of reported AEs was only (not significantly) higher in the treatment group for the CTCAE categories I, V, VIII, X, XII, XXII, XXIII, XXV and XXVII, and often reflected one or two more AEs reported in the treatment arm.

In none of the reported cases of infection, a probiotic strain was shown to have caused the bacteraemia. In each case the causal microbe was another organism than the administered probiotic strains. This is in line with our previous report in infants (Van den Nieuwboer *et al.*, 2014) and the safety analysis by Hempel *et al.* (2011). In addition, literature did not indicate an increase of *Lactobacillus* bacteraemia despite a substantial increase in probiotic use (Salminen *et al.*, 2002). Furthermore, clinical infections are rarely associated with probiotic intake and many probiotic strains are designated as GRAS (generally considered safe by qualified experts) by the FDA (Von Wright, 2005). In one case however, there was a concern regarding the isolated strain. The RCT by Knight *et al.* (2009) was able to culture a *Leuconostoc* species from a single tracheal aspirate after administration of a probiotic mixture of *P. pentosaceus*, *L. mesenteroides*, *L. paracasei* and *L. plantarum*. Repeated attempts to isolate the organism failed, and *Leuconostoc* is a common oropharyngeal commensal, which made this finding probably insignificant.

The rationale for using probiotics in HIV is that the gut is the most afflicted site in this disorder. The CD4⁺ T-cells and dendritic cells in the gut associated lymphoid tissue are depleted by the virus (Dandekar, 2007; Mehandru *et al.*, 2004). These alterations lead to ongoing immune activation and level of inflammation, and results in epithelial barrier disruption, dysbiosis and microbial translocation into the bloodstream (Marchetti *et al.*, 2013). It is even hypothesised that this chronic increased level of inflammation aggravates the disease progression (Brenchley *et al.*, 2006). Despite the possibility of ARV therapy such as HAART, treatment is not accessible for everyone or the CD4⁺ counts are too high to commence therapy. These individuals are still

afflicted by micronutrient deficiencies, diarrhoea and other HIV-associated complications (Dandekar, 2007). By administration of probiotics, the gut permeability could be decreased and intestinal inflammation could be reduced, thereby improving the disease progression. The analysis of interventional studies with HIV-infected participants resulted however in poor efficacy. The most observed effect was an increase in CD4⁺ T cells and a decrease in febrile episodes (Anukam *et al.*, 2008; González-Hernández *et al.*, 2012; Irvine *et al.*, 2011). The most commonly evaluated probiotic strain was *L. rhamnosus* GR-1, and overall the strains were administered in a wide range of dosages. Even at high dosages of 2.0×10^{11} cfu/day the occurrence of AEs in the treatment group were relatively low compared to the control group. The number of AEs for each category was lower in the treatment group, although more participants were allocated to this arm. This further reinforces safety of probiotics in adult HIV-patients. It should be noted that the study population consisted of both HAART treated as well as ARV therapy naïve individuals. None of the allocated participants had a CD4⁺ T cell count below 200 cells/mm³, a marker for clinical progression to AIDS and heavily increased risk of developing infections (Leserman *et al.*, 2002). Future studies are needed to the risks/benefit situation in individuals with AIDS.

The subgroup comprising critically ill, hospitalised and severely injured subjects is more vulnerable, due to on-going systemic inflammation, which facilitates infections and other complications. The level of inflammation determines the risk of infection and outcome of the patient. On the other hand, severe trauma, such as brain injury, induces a state of severe immune depression by e.g. production of glucocorticoids (Tan *et al.*, 2011). In these cases one is more prone to bacterial translocation and sepsis (Alverdy *et al.*, 2003). Critically ill patients often require parenteral nutrition and or mechanical ventilation, which predisposes to infections due to their immune compromised state. For instance, VAP occurs in 9-27% of the intubated patients, leading to prolonged hospital stay, morbidity, mortality and healthcare cost (Kollef, 2005). Despite the increased risk of developing bacteraemia, the incidence of AEs for each CTCAE category, except for skin and subcutaneous tissue disorders (XXIII), was lower in the treatment group. A higher incidence of skin AEs (n=8) in the treatment group was observed in the study of Pozzoni *et al.* (2012). The hospitalised, antibiotic treated subjects received 1×10^{10} cfu of *S. boulardii*, of whom 12 reported pruritus and five a cutaneous rash compared to eight respectively two cases in the control group. Both incidences of AEs did not significantly differ between the intervention arms. Due to the modest difference in number of reported AEs in category XXIII and the higher number of patients allocated to the treatment arm, this is of no major concern. Safety data thus indicated that the current investigated probiotic strains are safe at their evaluated dosages. The probiotics

were only administered during a relative short time (less than one month). Hence, safety is not proved in chronic administration to the critically ill. In addition, the range of tested dosages was insufficient and did not exceeded 5.0×10^{10} cfu/day. In conclusion, the administration of probiotics and synbiotics do not appear to pose a risk to critically ill patients, which allows future studies to identify the optimal choice of probiotic strain(s).

The risk benefit analysis of probiotic administration in patients receiving elective surgery is highly relevant. Major gastrointestinal (cancer) surgery is associated with SIRS (Baigrie *et al.*, 1992). This state characterised by excessive levels of proinflammatory cytokine secretion is associated with morbidity and postoperative complication risk due to translocation of viable bacteria through the intestinal barrier (Besselink *et al.*, 2005). Some probiotic strains have the ability to improve the intestinal barrier (Mennigen and Bruewer, 2009). Abdominal surgery in general, and major surgery, such as liver transplantation and pancreaticoduodenectomy, is associated with increased risk of urinary tract infections, pneumonia and wound infections (Schroeder *et al.*, 2006; Tran *et al.*, 2004). Considering these available data, the safety of patients receiving perioperative probiotics and synbiotics is, however, excellent. In the control group overall 144 more AEs were reported. Even with a high dosage of 2.0×10^{11} cfu/day, there were no significant adverse health effects observed, but rather a protective effect (Liu *et al.*, 2013). Several single AEs were reported in the treatment group. However, these are likely to be unrelated to the study product as this concerned an adrenal insufficiency (V), delirium (XVII), urinary retention (XX), pleural effusion (XXII) and the necessity for nasogastric tubing (XXV). Although these safety data are promising, and there is a rationale for justifying perioperative administration of probiotic, the low numbers of enrolled patients, the various surgical conditions and procedures, the various strains administered and short intervention durations makes it yet impossible to claim general safety in major surgery.

Subjects with an organ disease, such as liver cirrhosis, own increased susceptibility to infections by opportunistic organisms due to defective microbial clearance (Fernández *et al.*, 2002; Mookerjee *et al.*, 2007). For instance, patients with liver disease have an aberrant innate immunity due to a dysfunction of neutrophils (Fiuza *et al.*, 2000). This commonly leads to bacterial infections and bacteraemia, often caused by intestinal bacteria (Riordan and Williams, 2006), which is of concern when ingesting high quantities of microorganisms. Nevertheless, safety data displayed a lower incidence of AEs in the treatment group for all CTCAE categories. The most commonly observed AEs were hepatobiliary disorders (IX) as expected in subjects with liver disease. It should be noted that less participants were allocated to the treatment group and the overall population

was too small to generalise a safety claim. In addition, very high dosages of probiotic were not administered during the trials (highest dosage of 2.0×10^{10} cfu/day).

In the 'autoimmune' subgroup, the lowest incidence of AEs was noted, of which, however, the majority occurred in the treatment group. This is of concern as inflammation of the gastrointestinal tract is associated with increased gut permeability as observed in both inflammatory bowel disease as well as rheumatoid arthritis (Antoni *et al.*, 2014; Ebert and Hagspiel, 2011). This might allow opportunistic bacteria to translocate and cause complications. In addition, many patients receive active treatment of immunosuppressive drugs such as corticosteroids, azathioprine and TNF- α blockers (Pithadia and Jain, 2011). Most AEs were observed in the categories VII (gastrointestinal disorder) and XXVII (unspecified). As previously discussed, this difference in number of AEs may be explained by the higher allocation of participants to the treatment group in the study of Matthes *et al.* (2010). The incidence of AEs in the treatment arm was also higher in some other CTCAE categories. Nevertheless, the studies did not report any serious AEs or any significant differences in incidence of AEs between the treatment and control groups. The strain *B. longum* was investigated at a very high dosage (4.0×10^{11} cfu/day), emphasising the safety of this strain in autoimmune patients. Not all participants received immunosuppressive drugs; those in remission continued maintenance treatment with 5-aminosalicylic acid (5-ASA) and, hence, were more immune competent. The data did not elucidate if patients who received probiotics in combination with their regular treatment of methotrexate/corticosteroids/azathioprine experienced a higher frequency of AEs. Future studies should focus more on patients receiving specific immunosuppressive drugs and treatment with biologicals. Noteworthy is the administration of *T. suis* ova in relapsing MS-patients (Fleming *et al.*, 2011). Only five subjects received these ova, and this is insufficient to provide a clear safety profile. Nevertheless, this pilot study identified no safety concerns, and justifies careful future research with helminths.

There were no safety concerns regarding the remaining subgroup 'other'. The immune suppression by radiotherapy and UV-therapy apparently did not induce adverse health effects as no AEs were reported.

It was expected that infections and gastrointestinal disorders were the most frequently observed AEs, as these were often the primary or secondary outcomes of the studies. Furthermore, many studies either did not quantify the common AEs (e.g. flatulence, bloating or constipation), did not report any AEs at all, or only reported major and serious AEs. Hence, there may be a substantial underrepresentation of AE reporting in some CTCAE categories. Taking these

AEs also into account, would provide a more comprehensive safety profile for the investigated probiotic strains.

In conclusion, the overall documentation of probiotic and synbiotic intervention studies is poor and do not properly address the safety aspects. Data on the specific strain administered and its dosage is often lacking. This is pivotal information, as probiotic properties are strain-specific and cannot be generalised to other probiotic strains of the same species. This means that new research generated from data as described in the current study should be cross-checked with the original paper and possibly reclassified. The dosage is important to know in order to determine the optimal dose-response relationship in order to achieve optimal efficacy and to enable a risk benefit analysis. A higher dosage also potentially increases the incidence of AEs, and safety should thus be evaluated on a strain-by-strain basis. Proper analyses of both efficacy as well as safety data are required for an adequate risk-benefit analysis. This review, however, did not focus on efficacy and future studies should incorporate these data. Whether single strain probiotics, a multi strain probiotic mixtures or synbiotics are most effective remains unclear and future studies as well as meta-analyses could provide more insight in this matter. In the controlled setting of clinical studies, the evaluated probiotic strains administered in their respective dosage in immune compromised adults seemed safe. Long-term follow-up studies were not available and, hence, a definite safety claim cannot be provided.

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