EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Saccharomyces cerevisiae* CBS 5926

Final

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Saccharomyces cerevisiae* CBS 5926
Herbal preparation(s)	Not applicable
Pharmaceutical form(s)	Not applicable
Rapporteur(s)	J. Wiesner
Assessor(s)	F. Stolte B. Merz F. Deget
Peer-reviewer	A. Assisi

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

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1. Introduction

Disclaimer

Due to the initial decision of the HMPC to conduct a scientific assessment in order to possibly establish a European Union (EU) herbal monograph on Saccharomyces cerevisiae, a draft assessment report and draft monograph supporting a traditional and well-established use of herbal medicinal products containing Saccharomyces cerevisiae CBS 5926 were prepared, in line with EMA/HMPC standard procedures and the provisions of Article 10a of Directive 2001/83/EC (well-established use) and of Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC (traditional use).

However, no absolute majority required for adoption of the monograph was achieved. HMPC is of the opinion that a monograph on Saccharomyces cerevisiae CBS 5926 cannot be established due to different opinions on the classification of this yeast. On one hand, yeasts are considered 'fungi' in a broad sense and, as such, could be considered falling under the 'herbal substance' definition of Directive 2001/83/EC. On the other hand, living yeast could also be considered covered by the Live Biotherapeutic Products general monograph of the European Pharmacopoeia.

Therefore, this assessment report is published for information and transparency purposes only. This document is without prejudice to any classification of Saccharomyces cerevisiae as either a herbal substance or a live biotherapeutic substance, which is ultimately within the remit of individual EU/EEA Member States. The present HMPC assessment report shall in no way be regarded as a HMPC position on the classification of Saccharomyces cerevisiae as an herbal or biological active substance of a medicinal product.

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

The HMPC has discussed whether living yeast cells (fungi) such as *Saccharomyces cerevisiae* (strain CBS 5962) may be considered herbal substances/preparations, based on Directive 2001/83/EC which defines herbal substances as "All mainly whole, fragmented or cut plants, plant parts, algae, fungi, lichen in an unprocessed, usually dried, form, but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal substances. Herbal substances are precisely defined by the plant part used and the botanical name according to the binomial system (genus, species, variety and author)."

For the purposes of this assessment report, a specific yeast strain of *Saccharomyces cerevisiae* was considered as a herbal substance. However, the proposal for adoption of a monograph on this herbal substance did not reach the required absolute majority in the HMPC, in view of divergent views on the classification of *Saccharomyces cerevisia* CBS 5927 (see section 6 "Overall conclusions (benefit-risk assessment)").

- Herbal substance(s)

Yeasts are eukaryotic single-cell microorganisms classified in the kingdom Fungi, but they do not form a single taxonomic or phylogenetic group. As it is known from fungi, yeasts can have asexual and sexual reproductive cycles. Mainly, the vegetative growth in yeasts is asexual reproduction by budding or fission.

Yeast species either require oxygen for aerobic cellular respiration (obligate aerobes) or are anaerobic, but also have aerobic methods of energy production (facultative anaerobes). They use different kinds

of carbohydrates to produce carbon dioxide and water or in the absence of oxygen a fermentation process is used to produce ethanol and carbon dioxide.

The use of different carbohydrates is a characteristic property to differentiate between various yeast species.

Yeasts grow best in a neutral or slightly acidic pH environment.

At present, a correct definition and classification for yeast species are still a matter of debate. The common classification is listed below:

According to the common scientific classification system, there are two separate phyla: Ascomycota and Basidiomycota. Within the phyla of the Ascomycota, two different subphyla are known: the Saccharomycotina and the Taphrinomycotina. The only class in the subphylum Saccharomycotina is Saccharomycetes (Eriksson and Winka, 1997). The class of Saccharomycetes contains the order Saccharomycetales, a budding yeast. Twelve families are included in the order Saccharomycetales. One of these families is the so-called Saccharomycetaceae, which is present in a wide variety of habitats. This family contains, among others, the species *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae is one of the most important and useful yeasts. A strain of *Saccharomyces cerevisiae* was the first eukaryotic genome to be completely sequenced (Sankoff, 2009). Because of their useful physiological properties, many yeast species are considered of importance in food production. Strains of *Saccharomyces cerevisiae* have a long tradition of use in baking and brewing. It is assumed that *Saccharomyces cerevisiae* strains are isolated from fruits. This is also known for the *Saccharomyces cerevisiae* strain described in the current monograph (McFarland, 2010).

The correct taxonomy of the *Saccharomyces cerevisiae* strain has been discussed over years. The strain has received considerable discussion about its valid nomenclature (McFarland, 1996; McCullough *et al*., 1998). Before the actual era of yeast taxonomy by means of gene sequences and other molecular criteria, identifications were based on phenotypic tests and assimilation profiles.

In 1996, McFarland postulated that *Saccharomyces boulardii* has been shown to be a separate species of *Saccharomyces* on the basis of several taxonomic, metabolic and molecular parameters. *Saccharomyces boulardii* is a wild *Saccharomyces* strain that does not produce ascospores or uses galactose as a carbon source (as do wild *Saccharomyces cerevisiae* strains). This strain is also given a separate designation by the American Type Culture Collection (ATCC 74012). *Saccharomyces boulardii* has different oxidative utilisation and fermentation patterns that can distinguish it from *Saccharomyces cerevisiae* (McFarland, 1996).

Although the phenotype sometimes can be used to correctly identify species, in the following years molecular comparisons have shown that many earlier identifications based on phenotype have been incorrect (Kurtzman and Fell, 2006). Rapid detection and accurate identification of yeasts are now possible by use of a variety of molecular methods. Many phylogenetic relationships among the yeasts and other fungi have been clarified by analysis of gene sequence divergence (Kurtzman and Fell, 2006). Based on various molecular methods, it is consistently shown that *Saccharomyces boulardii* is similar to *Saccharomyces cerevisiae* and cannot be differentiated as a separate species (Vaughan-Martini and Martini, 1987; McCullough *et al*., 1998; Blaschek *et al*., 2013; Vaughan-Martini, 2003). Therefore, the yeast previously named *Saccharomyces boulardii* is considered as a special strain within the heterogeneous species *Saccharomyces cerevisiae* (Bassetti *et al.,* 1998; Piarroux *et al.,* 1999; Perapoch *et al*., 2000; Lherm *et al.,* 2002; Mitterdorfer, 2002a; Mitterdorfer, 2002b; Cassone *et al*., 2003; Riquelme *et al.,* 2003).

However, the term *Saccharomyces boulardii* has been used more frequently as medicinal term in the therapeutic usage than the taxonomically correct term *Saccharomyces cerevisiae* strain Boulard.

Instead of labelling the strain, it is more common to use the name of the culture collection indicating the respective number of the deposit: "*Saccharomyces cerevisiae* CBS 5926", which is the primarily used reference for this strain. The strain is also deposited in the American Type Culture Collection with the number ATCC 74012 and additionally in the Institut Pasteur de Paris as I-745.

Differences and similarities of *Saccharomyces cerevisiae* CBS 5926 and laboratory strains of *S. cerevisae* – without any characterisation – are observed (table 1) (Edwards-Ingram *et al*., 2007; Lukaszewicz, 2012). The majority of the studies show that especially the ability for pseudohyphal switching, the survival at low acid pH and higher optimal growth temperature are features having a direct influence on the use in medicinal products.

Table 1: Differences and similarities of *Saccharomyces cerevisiae* CBS 5926 and laboratory strains of *Saccharomyces cerevisae* (taken from McFarland, 1996; Edwards-Ingram *et al*., 2004; Malgoire *et al*., 2005; Edwards-Ingram *et al*., 2007; Lukaszewicz, 2012)

***Saccharomyces cerevisiae* CBS 5926**	***Saccharomyces cerevisiae* (laboratory strain)**
Higher optimal growth temperature (ca. 37°C)	Lower optimal growth temperature (ca. 30°C)
Higher resistance to low pH	Lower resistance to low pH
Does not use galactose	Uses galactose
Asporogenous in contrast to *Saccharomyces cerevisiae* but may produce fertile hybrids with *Saccharomyces cerevisiae* strains	Sporogenous
Lost all intact Ty1/2 elements	Contains several Ty1/1* elements
Microsattelite typing shows genotypic differences	
Trisomic for chromosome IX	Stable strains with various ploidy

*Ty 1/1 is a specific transposable element for yeast

- Herbal preparation(s)

Saccharomyces cerevisiae CBS 5926 is being commercialised as a freeze-dried powder obtained from an aqueous suspension using a fermentative culture procedure referring to a Cell-Bank-System. For this reason, the manufacture is inspected according to GMP Annex 2 "Manufacture of Biological active substances and Medicinal Products for Human Use" and authorised by Regulatory Authorities as a biological active substance. Usually, the freeze-dried yeast is a preparation containing lactose as excipient during the freeze-drying process. However, there are also some preparations, which are produced by fluid bed drying. These two possible manufacturing routes can be considered as comparable taking into account the specification characteristics of the yeast preparation, as described below.

As main characteristic of the yeast, the number of viable cells is determined, in addition to the strain identity. The respective specification for the content of viable cells is set according to the yeast used in the clinical studies and is assayed by dilution series, smear and count of the colonies grown. Regarding the specification of the yeast used in the clinical studies, the acceptance criterion for the parameter "content of viable yeast cells" is set to "$\geq 2 \times 10^{10}$ CFU (colony forming units)/g dried (native) yeast". As it is common for microbiological results, the limit of the result must be interpreted in such a way that a deviation of ± ½ power of ten is still considered "within the specification".

The identity is proven based on microscopic and macroscopic tests, a visual test based on the form and colour of the cell colonies grown on Sabouraud agar. A DNA-fingerprint is used to compare the yeast with reference to *Saccharomyces cerevisiae* CBS 5926. Furthermore, a biochemical identification test should be performed by means of the different carbohydrate assimilation profiles of yeast strains resulting in a specific code by metabolising different substrates.

In the majority of the clinical studies included in this assessment report, hard capsules containing the dried yeast powder are used as pharmaceutical dosage form. In the release specification, the acceptance criterion for the parameter assay the content of viable cells is defined as "$\geq 2 \times 10^{10}$ CFU/g dried yeast powder". During stability, at least "4×10^{9} CFU/g dried yeast powder" should be ensured. As it is mentioned before, the limit of the result must be interpreted so that a deviation of ± ½ power of ten is still considered "within the specification". These acceptance criteria are set according to the specification of the finished product used in the clinical studies. On the market, there is also the dried yeast powder in sachets, which is considered a comparable dosage form.

For an application of other strains than the above-mentioned *Saccharomyces cerevisiae* CBS 5926, on which this assessment report is based, the genetic identity should be documented. Referring also to the respective decisions of the European Food Safety Authority (EFSA) via its Scientific Opinions on the substantiation of health claims to Article 13(1) of Regulation EC No 1924/2006, restriction fragment length polymorphism analysis (RFLP) or sequencing analysis of DNA taxonomic markers should be used for identification of a yeast species. The strain identification must be supported by chromosome length polymorphism analysis by Pulsed-field Gel Electrophoresis (PFGE), Random Amplified Polymorphic DNA (RAPDs), microsatellite DNA polymorphism analysis or other internationally accepted genetic typing molecular techniques (EFSA, 2010; 2012a; 2012b). Furthermore, characteristics reported from literature in Table 1 should also be considered concerning properties of different strains.

In literature, different terms have been used for *Saccharomyces cerevisiae* CBS 5926, *Saccharomyces cerevisiae* as well as *Saccharomyces boulardii*. For the investigations cited in this assessment report, the terms used by the authors are maintained.

- Herbal medicinal products

In the clinical studies assessed in the present document, different medicinal products have been administered to the patients. According to the homepage of Biocodex (http://www.biocodex.com/en/therapeutic-areas-products) *Saccharomyces boulardii* CNCM I-745 (*Saccharomyces cerevisiae* CBS 5926) is the active principle of the several medicinal products. Hence, it is concluded that all these medicinal products are identical with regard to *Saccharomyces cerevisiae* strain and number of living cells. Recent package leaflets mention amounts of living cells as follows: e.g. $\geq 1.8 \times 10^{10}$ viable cells/g lyophilisate or 5×10^{9} viable cells/250 mg sachet (corresponding to 2×10^{10} viable cells/g).

For a specific product called Floratil®, contradictory information is available. On the Biocodex homepage, Floratil® is characterised as a 'natural yeast-based probiotic' and thus differs from the medicinal products mentioned before. Based on the patent description and on package leaflets available, Floratil® does not differ from medicinal products containing *Saccharomyces boulardii*.
A recent package leaflet mentions not less than 5×10^{9} cells *Saccharomyces boulardii*/g lyophilisate whereas the reference Corrêa *et al.* (2011) mentions 4×10^{9} viable cells in one 200 mg capsule which correlates to 2×10^{10} viable cells/g.

1.2. Search and assessment methodology

This assessment report was based on the literature on *Saccharomyces boulardii/cerevisiae* available at the "Federal Institute for Drugs and Medicinal Devices" in Germany and the publications provided by the Association of the European Self-Care Industry (AESGP), Biocodex and Pierre-Fabre in response to the EMA/HMPC call for data on *Saccharomyces boulardii/cerevisiae* of 14 February 2014. Furthermore, a literature search in the German Institute for Medical Documentation and Information (DIMDI) database was performed in July 2015 using the following terms: *Saccharomyces boulardii/cerevisiae*, humans, clinical, preclinical, safety. A separate literature research was performed for the therapeutic use of *Saccharomyces boulardii/cerevisiae* in the indication irritable bowel syndrome in the specific DIMDI database 'XMEDALL' (terms used: irritable bowel syndrome, *Saccharomyces*).

To update the list of references, in March and April 2020 a second literature search was performed in PubMed database for articles published in the last 5 years using the following terms: "*Saccharomyces boulardii/cerevisiae,* humans, diarrhea" and "*Saccharomyces boulardii/cerevisiae*, humans, irritable bowel syndrome" respectively and using the EBSCO Discovery Service with the following terms: (*Saccharomyces boulardii* OR *Saccharomyces cerevisiae*) AND humans AND (clinical OR preclinical OR safety) AND (diarrhea OR diarrhoea) from July 2015 to April 2020. Literature was also included from the single Periodic Safety Update Report (PSUR) assessment PSUSA/00009284/201702 (CMDh, 2017).

As diarrhoeal diseases remain a major threat to global health, in the recent years the number of publications on *Saccharomyces boulardii/cerevisiae* has increased continuously (Lukaszewicz, 2012). Only the articles considered as relevant for the establishment of this assessment report on a well-established and traditional use of *Saccharomyces cerevisiae* CBS 5926 within the EU were included in the reference list. In view of the large quantity of articles on *Saccharomyces boulardii/cerevisiae* published, it was not attempted here to give a complete overview of all studies and medical indications having been investigated with this yeast preparation.

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information obtained from the request on 17 April 2014 concerning drug preparations containing *Saccharomyces cerevisiae/boulardii* on the market in the EU showed the following results:

No products have been authorised in Bulgaria, Croatia, Ireland, Poland, Serbia, United Kingdom and Slovenia.

Table 2: Overview of data obtained from marketed medicinal products

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
1) *Saccharomyces boulardii*	a) Prevention of diarrhoea caused by ingestion of broad-spectrum antibiotics in individuals who are	Hard capsules 125 mg/ 250 mg (2×10^{10} viable cells/g)	WEU; 2012; Belgium

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
	predisposed to diarrhoea by *Clostridium difficile* b) Prevention of a relapse (recurrence) of diarrhoea caused by *Clostridium difficile* c) For the treatment of acute diarrhoea in children under 12 years, in addition to oral hydration	a) and b) 4 times 125 mg per day, respectively 2 times 250 mg per day for 4 weeks corresponding to 500 mg per day c) 2-4 times 125 mg per day, respectively 1-2 times 250 mg per day for 4-7 days corresponding to 250-500 mg per day	
2) *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926)	See 1)	Hard capsules, powder for oral suspension (sachet) 250 mg (≥2.4 x 10^{10} viable cells/g) Adults: 2 times 1-2 doses (250 mg) per day Children: 2 times 1 dose (250 mg) per day	WEU; 2004; Belgium
3) *Saccharomyces boulardii*	(Not available)	Containing 50 mg	1968-2005; Belgium
4) *Saccharomyces boulardii* (dried) Institut Pasteur: I-745 (*Saccharomyces cerevisiae* CBS 5926)	Adjuvant therapy of acute infectious diarrhoea Prevention and therapy of colitis and diarrhoea caused by antibiotics In combination with vancomycin and metronidazol for therapy of colitis caused by *Clostridium difficile*	Powder for oral suspensions in sachets/ hard gelatine capsules 250 mg (containing not less than 1 x 10^9 total viable cells count)/sachet or hard capsule Adolescents and children over 4 years of age: 1-2 bags/hard capsules once to twice per day (250-1,000 mg per day).	Full MA; 2002; Czech Republic

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
	Adjuvant therapy of irritable bowel syndrome	Children 3-4 years of age: 1 to 2 bags/hard capsules per day (250-500 mg per day)	
5) *Saccharomyces boulardii* (Lyophilised) Institut Pasteur: I-745 ATCC (American Type Culture Collection): ATCC 74012 CBS (Centraalbureau voor Schimmelcultures): CBS 5926	Disorders of the intestine caused by disturbances of the intestinal microflora Prophylaxis and treatment of diarrhoea caused by use of antibiotics In adjunct to vancomycin or metronidazole treatment to prevent the recurrence of *Clostridium difficile* induced diarrhoea	Powder for oral suspension 250 mg (≥18 x 10^9 cells/g; ≥2.5 x 10^9 cells/sachet) 1 or 2 sachets, given once or twice per day (250-1,000 mg per day)	Full MA; 1997; Estonia
6) *Saccharomyces boulardii* (Lyophilised) (Details see 5)	Disorders of the intestine caused by disturbances of the intestinal microflora Prophylaxis and treatment of diarrhoea caused by use of antibiotics In adjunct to vancomycin or metronidazole treatment to prevent the recurrence of *Clostridium difficile* induced diarrhoea	Hard capsule. 250 mg (≥18 x 10^9 cells/g; ≥2.5 x 10^9 cells/capsule) 1 or 2 capsules, given once or twice per day (250-1,000 mg per day)	Full MA; 2003; Estonia
7) *Saccharomyces boulardii*	Acute diarrhoea Antibiotic-associated diarrhoea Traveller's diarrhoea	Hard capsule/sachet for oral suspension 250 mg lyophilised powder (≥31.8 x 10^9 CPU/g, >5 x 10^9 CPU/g	Full MA, bibliographic (national) application; 1996; Finland

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
	Recurrent *Clostridium difficile* infection	in the finished product in the release specification, respectively) Children and adolescents: 250 mg twice per day	
8) *Saccharomyces boulardii*	Symptomatic treatment of diarrhoea in addition to rehydration	Hard capsule 50 mg of *Saccharomyces boulardii* Children over 6 years of age and adults: 100 mg twice per day	WEU; 1961; France
9) *Saccharomyces boulardii*	Symptomatic treatment of diarrhoea in addition to rehydration	Hard capsule 200 mg of *Saccharomyces boulardii* Children over 6 years of age and adults: 200 mg per day	WEU; 1970; France
10) *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926)	Symptomatic treatment of diarrhoea in addition to rehydration	Powder for oral solution in sachet (1 sachet = 100 mg of *Saccharomyces boulardii*) Children over 2 years of age and adults: 1 sachet twice per day (= 200 mg per day)	WEU; 1997; France
11) *Saccharomyces boulardii*	Prophylaxis and treatment of antibiotic-associated adverse reactions of the intestine Complementary treatment in acute diarrhoeas of adults and children Preventive treatment of recurrence of	Capsule 250 mg Adults: 2-4 capsules per day (500-1,000 mg per day) Children: 1-2 capsules (250-500 mg per day)	WEU; 2010; Greece (Capsules containing 50 mg *Saccharomyces boulardii* were approved 1968)

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
	Clostridium difficile disease in addition to vancomycin/ metronidazole Preventive treatment of broad-spectrum antibiotic-associated diarrhoea		
12) *Saccharomyces boulardii* (lyophilised cells)	Acute diarrhoea of bacterial and viral aetiology in children and adults Treatment and prevention of antibiotic-related colitis Prevention of recurrence of *Clostridium difficile* diseases Irritable bowel syndrome Prevention of diarrhoea associated with tube-feeding	Capsule 250 mg and powder for oral suspension 250 mg per sachet 250-500 mg 1-2 times per day (250-1,000 mg per day)	Full MA; 1997; Latvia
13) *Saccharomyces boulardii* (freeze-dried)	Adults: Adjuvant to antibiotics to prevent relapse of *Clostridium difficile* diarrhoea (CDD) Prophylaxis of antibiotic-associated diarrhoea (ADD) Children: Prophylaxis of antibiotic-associated diarrhoea (ADD) in vulnerable patients	Capsule, hard 250 mg Adults: 2 capsules 2 times per day (1,000 mg per day) Children: ≥3 years of age: 1 capsule 2 times per day (500 mg per day) 2-3 years of age: 1 capsule per day (250 mg per day) Duration of use:	Full MA; 1995; Sweden

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
	Immunosuppressed patients have been excluded in the studies	Not more than 4 weeks Treatment should be initiated 48-72 hours from the start of the antibiotic treatment and continue until at least 3 days after completed antibiotic treatment	
14) *Saccharomyces cerevisiae* CBS 5926	a) Symptomatic treatment of acute diarrhoea b) Prevention and symptomatic treatment of traveller's diarrhoea c) Diarrhoea associated with tube feeding d) Adjuvant treatment of chronic acne	Capsule 250 mg Powder 250 mg/sachet (>1.8 x 10^{10} viable cells/g lyophilisate) Children>2 years of age and adults: a) Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg b) Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg Starting 5 days before departure c) 750 mg/1.5 l nutrient solution d) Single dose: 250 mg 3 times per day Daily dose: 750 mg	WEU; 1995; Germany
15) *Saccharomyces cerevisiae* CBS 5926	See 14)	Hard capsule 50 mg (>1.8 x 10^{10} viable cells/g lyophilisate) Children >2 years of age and adults: a) Single dose: 100–150 mg 3 times per day Daily dose: 300-450 mg	WEU; 1995; Germany

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
		b) Single dose: 100-150 mg 3 times per day Daily dose: 300-450 mg Starting 5 days before departure c) 750 mg/ 1.5 l nutrient solution d) Single dose: 250 mg 3 times per day Daily dose: 750 mg	
16) *Saccharomyces cerevisiae* CBS 5926	See 14)	125 mg/hard capsule (≥2.5 x 10^9 viable cells/ capsule fluid bed drying) Posology see 13)	WEU; 2001; Germany
17) *Saccharomyces cerevisiae* CBS 5926	See 14)	250 mg/capsule (≥5.0 x 10^9 viable cells/ capsule fluid bed drying) Posology see 13)	WEU; 2001; Germany
18) *Saccharomyces cerevisiae* CBS 5926	See 14)	Hard capsule 375 mg (≥7.5 x 10^9 viable cells/ capsule fluid bed drying) a) 375 mg per day b) 375 mg per day starting 5 days before departure c) 750 mg/1.5 l nutritive solution per day d) 750 mg per day	WEU; 2001; Germany
19) *Saccharomyces cerevisiae* CBS 5926	a) Symptomatic treatment of acute diarrhoea b) Prevention and symptomatic treatment of traveller's diarrhoea and diarrhoea	Hard capsule 250 mg (≥5.0 x 10^9 viable cells/capsule, fluid bed drying) a) Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg	WEU; 2001; Germany

Active substance	**Indication**	**Pharmaceutical form** **Strength** **Posology** **Duration of use**	**Regulatory Status (date, Member State)**
	associated with tube feeding	b) Traveller's diarrhoea: Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg Starting 5 days before departure Tube feeding: 750 mg/ 1.5 l nutrient solution	
20) *Saccharomyces cerevisiae* CBS 5926	See 19)	Hard capsule 250 mg ($\geq 1 \times 10^{10}$ viable cells/g lyophilisate) Posology see 18)	WEU; 2005; Germany
21) *Saccharomyces cerevisiae* CBS 5926	Symptomatic treatment of acute diarrhoea Prevention and symptomatic treatment of traveller's diarrhoea	Hard capsule 250 mg ($\geq 5.0 \times 10^{9}$ viable cells/capsule, fluid bed drying) 1 hard capsule once to twice per day (250-500 mg per day)	WEU; 2013; Germany
22) *Saccharomyces cerevisiae* CBS 5926	Diarrhoea: enteritis, colitis Prevention and treatment of traveller's/summer diarrhoea Enteral dysbiosis Diarrhoea associated with antibiotics and chemotherapeutics Acne	Capsule 50 mg Daily dosage in babies, children and adults: Acute diarrhoea: 3 times 100 mg; max. 3 times 200 mg Chronic diarrhoea, prophylaxis, during antibiotic treatment: 3 times 50 mg Acne: 3 times 100 mg for 14 days; thereafter 3 times 50 mg; for a treatment period of 3 months at least	WEU; 1975 to 2003; Germany

Active substance	Indication	Pharmaceutical form Strength Posology Duration of use	Regulatory Status (date, Member State)
23) *Saccharomyces cerevisiae* CBS 5926	See 14) (without acne)	Capsule 250 mg Posology see 14)	WEU; 1978 to 2003; Germany
24) *Saccharomyces boulardii*	Antimycotic	Ointment (44 mg/g)	1962-2008; Belgium
25) *Saccharomyces cerevisiae* CBS 5926	a) Symptomatic treatment of acute diarrhoea b) Symptomatic treatment of traveller's diarrhoea and diarrhoea associated with tube feeding c) Adjuvant treatment of chronic acne	Capsule 250 mg ($\geq 2.5 \times 10^9$ viable cells) Children from 2 years of age and adults: a) 250-500 mg per day b) Traveller's diarrhoea: 250-500 mg per day Tube feeding diarrhoea: 500 mg/l nutritive solution c) 750 mg per day	Full MA; 2000; Austria

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Further data on the administration of *Saccharomyces boulardii/cerevisiae* were provided by the different countries:

Undesirable effects:

AT: Flatulence, hypersensitivity reactions (itching, urticaria, localised or generalised exanthema, Quincke edema).

BE: Very rare (< 1/10.000): fever in case of fungemia, mycosis, anaphylaxia, dyspnea, itching, exanthema, Quincke edema, epigastric pain and abdominal meteorism (these symptoms were observed in clinical studies and do not require to interrupt the course of treatment), thirst.

CZ: Gastrointestinal disorders (stomach pain, bloating, constipation), allergic reactions (itching, redness, urticaria), systemic mycotic infection.

DE: Flatulence, allergic reactions (itching, urticaria, localised or generalised exanthema, Quincke edema, dyspnea, anaphylaxia), fungemia in patients with central venous catheter, life-threatening diseases, severe underlying disease, reduced immune defense.

EE: Rare cases of digestive tract disturbances have been reported, not requiring that treatment to be discontinued.

EL: Rare: flatulence; very rare: rash, cutaneous allergy, urticaria, pruritus, anaphylactic reaction, angiooedema, exanthema.

FR: Very rarely: allergic reactions (Quincke oedema), flushes, itches; rarely: urticaria.

SE: Uncommon (≥1/1,000 to <1/100): urticarial, allergic reactions, constipation, thirst; rare (≥1/10,000 to <1/1,000): angioedema, exanthema. Cases of sepsis caused by *Saccharomyces boulardii* have been reported for patients with seriously impaired general condition.

Use in children and adolescents:

AT: Due to missing data, the medicinal product may be used in children < 2 years on doctor's orders only.

DE: The content of the capsule may be mixed into food or beverages. The food may not be too hot (>50° C) or ice cold. Due to missing data on posology, the medical product should not be used in children <2 years of age.

EE: The contents of the sachet should be mixed with water or another liquid or food and the contents should be poured in a baby's feeding-bottle. In young children under 6 years of age, it is recommended not to swallow capsules (risk of false passage) but to open them and tip the contents into a beverage or food.

EL: In order to be administered to children, the capsule should be opened, and the content should be mixed with milk (feeding-bottle) or food.

LV: For children less than 6 years of age capsules should be opened and powder mixed with liquid.

Duration of treatment:

AT and DE: No restriction of treatment duration: Treatment of diarrhoea should be continued for several days following a cessation of complaints. For the treatment of chronic acne administration for several weeks is recommended.

BE: adults: Prevention of relapses of diarrhoea caused by *C. difficile*: 4 weeks; children: Treatment of diarrhoea in addition to rehydration therapy: 1 week. The treatment should not be interrupted too early, because diarrhoea can recur.

CZ: If the symptoms persist for more than 2 days during the use of the medicinal product, the treatment method should be reconsidered.

EL: If diarrhoea persists after 2 days or if blood in faeces appears or if fever appears, treatment should be reconsidered and the necessity to introduce oral or parenteral rehydratation should be considered. After diarrhoea is stopped, the treatment may be continued for some days.

FI: 1-4 weeks depending on the indication.

Contraindications:

AT: Hypersensitivity to ingredients of the medicinal product.

BE: Hypersensitivity to one of the ingredients or other yeast, patient with central venous catheter.

CZ: Patients with central venous catheter.

DE: Hypersensitivity to *Saccharomyces boulardii*. Due to the risk of fungemia treatment is contraindicated in patients with life-threatening diseases, reduced immune defense, and in patients with central venous catheter. Children <2 years are excluded from self-medication and have to be treated only after consultation of a medical doctor.

EE: Hypersensitivity to *Saccharomyces boulardii*. Patient with central venous catheter.

EL: Known hypersensitivity to one of the components. Patients having a Central Venous Catheter-CVC, allergy to yeast, especially *Saccharomyces boulardii*.

FI: Hypersensitivity to the active substance

LV: Hypersensitivity to one of the ingredients. Patient with central venous catheter and with immune deficiency disorders.

SE: Hypersensitivity to the active substance. Patients with central venous catheter.

Special warnings/precautions:

AT: Especially in children the most important therapeutic measure is a substitution of water and electrolytes.
If diarrhoea lasts longer than 2 days or is associated with blood or elevated body temperature, a doctor should be consulted.
In case of microbiological stool examination during or shortly after treatment with this medicinal product the laboratory should be informed on its administration, as false-positive results may be possible.
Due to missing data, the medicinal product may be used in children < 2 years on doctor's orders only.
Because of unratable risk of fungemia patients with impaired immune status (e.g. HIV-infection, chemotherapy, or radiotherapy) should not use this medicinal product without consulting a doctor.

BE: As diarrhoea causes serious loss of water and electrolytes, substitution of water and electrolytes is important.
If freezing liquids or food, or liquids or food, which can be heated above 50°C, are used to prepare a suspension for oral use, the acticity of the medicinal product can decrease.
Precautions in fever above 38°C, severe abdominal pain, blood in stool, vomiting associated with diarrhoea, diarrhoea for more than 3 days.
In case of microbiological stool examination *Saccharomyces boulardii* can cause false-positive results.
The medicinal product is contraindicated in patients with severe immune deficiency (e.g. HIV-infection, organ transplantation, leukaemia, progredient malignant tumour, radiotherapy, chemotherapy, large-dose long-term glucocorticoids treatment) should not use this medicinal product without consulting a doctor.

CZ: If the symptoms persist for more than 2 days during the use of the medicinal product, the treatment method should be reconsidered. Not to be mixed with hot (more than 50°C) or iced beverages or meal. Not to be combined with alcohol.

DE: Risk of fungemia in patients with life-threatening diseases, reduced immune defense, and in patients with central venous catheter.
If diarrhoea lasts longer than 2 days, aggravates or is associated with blood or elevated body temperature, a doctor should be consulted.
Especially in children the most important therapeutic measure is a substitution of water and electrolytes.
In case acne deteriorates or does not improve a doctor should be consulted.
In case of microbiological stool examination during or shortly after treatment with this medicinal product the laboratory should be informed on its administration, as false-positive results may be possible.
Due to missing data on posology, the medicinal product should not be used in children <2 years of age.

EE: Contains living cells. This drug should therefore not be mixed with very hot (over 50°C), iced or alcoholic drinks or food.

The treatment does not replace rehydration when this is necessary. The rehydration dose and its route of administration (oral) should be adapted to the severity of the diarrhoea and to the age and state of health of the patient.
Saccharomyces boulardii is a living organism associated with risks of sytemic fungus infection by gastrointestinal translocation or hand-borne contamination. Rare cases of fungemia have been reported in hospitalised severely ill patients, most often because of gastrointestinal disease, with a central venous catheter.

EL: In children below 2 years of age, a medical advice is recommended due to risk of dehydration. The treatment does not replace rehydration when this is necessary. The rehydration dose and its route of administration [oral - i.v.] should be adapted to the severity of the diarrhoea and to the age and state of health of the patient.
Contains living cells. Therefore, it must not be mixed into very hot (above 50°C), icy drinks or foods (e.g. drinks stored in the refrigerator, ice creams, very hot meals) and alcohol-containing drinks.
It is advisable not to open the capsules in the surroundings of patients with a central venous catheter-CVC-, to avoid any colonisation, especially hand-borne of the catheter. There have been reports of patients with a central venous catheter-CVC-, even not treated with *Saccharomyces boulardii*, of very rare cases of fungemia (penetration of blood by yeast), most often resulting in pyrexia and blood cultures positive for *Saccharomyces*. The outcome in all these cases has been satisfactory after administration of antifungal treatment and, when necessary, removal of the catheter.
It is not possible to draw safe safety data on the content of 250 mg for patients, who are more prone to side effects or systemic fungal infections, such as immunosuppressed patients, patients with AIDS, patients in Intensive Care Units and patients receiving enteral feeding in the hospital environment.

SE: There have been reports in patients with a central venous catheter of very rare cases of fungemia (blood cultures positive for *Saccharomyces*). It is advisable not to open capsules in the surroundings of patients with a central venous catheter, to avoid any colonisation, especially hand-borne, of the catheter.

Drug interactions:

AT and DE: The co-administration of antimycotics may impair the treatment results of *Saccharomyces boulardii*.
The concomitant use of monoamine oxidase inhibitors may increase blood pressure.

BE: The intake of antimycotics neutralises the effect of the medicinal product.

CZ: Not to be used concomitantly with systemic oral and parenteral antimycotics.

EE: Because of its fungal nature, *Saccharomyces boulardii* must not be administered with systemic or oral antifungal drugs.

EL: Because of its fungal nature, it must not be administered with parenteral or oral antifungal drugs.

SE: Should not be used concomitantly with antimycotic drugs.

Fertility, pregnancy and lactation:

AT: The widespread use of yeast as food indicates no risk for pregnancy and lactation.

BE: Due to the lack of data, the use in pregnancy and lactation is not recommended.

CZ: Safety during pregnancy and lactation has not been established. In absence of sufficient data, the use during pregnancy and lactation is not recommended.

DE: Due to the lack of data, *Saccharomyces boulardii* should not be used in pregnancy.

EE: *Saccharomyces boulardii* does not absorb through the gastrointestinal tract. *Saccharomyces boulardii* does not transfer into breast milk. No data about fertility.

EL: There are no reliable animal teratogenesis data. Clinically, no malformed nor foetotoxic effect has been reported to date. However, monitoring of pregnancies exposed to this medicine is insufficient to rule out any risk. Therefore, although *Saccharomyces boulardii* is not absorbed, as a precautionary measure, it is preferable to weight benefit/risk before using this medicine during pregnancy. *Saccharomyces boulardii* is not absorbed. In the absence of data, it is preferable to weight benefit/risk for the breastfed infant before using it during lactation.

SE: Pregnancy and breast-feeding: *Saccharomyces boulardii* is not absorbed.

Overdose:

AT: Flatulence can increase. A specific antidote is not known.

CZ and PT: Not known.

DE: No cases of overdose have been reported. It is possible that the symptoms described in "Undesirable effects" may be increased.

EE: None.

SE: No case of overdose has been reported.

Effects on ability to drive or operate machinery or impairment of mental ability:

AT and EL: Not known.

BE and CZ: The product does not influence the ability to drive or operate machinery.

DE: No limitations.

SE: No effects have been observed.

Information on relevant combination medicinal products marketed in the EU/EEA

Combination products (ointment, suppositories) containing alcoholic extracts of *Saccharomyces cerevisiae* (unknown DER) and oil from shark liver for the symptomatic treatment of haemorrhoids have been reported by Czech Republic. These products are not regarded as relevant for the assessment of the medicinal use of *Saccharomyces cerevisiae* CBS 5926.

In France, several combination products containing *Saccharomyces cerevisiae* are on the market. They refer to various indications and differ with regard to their composition and pharmaceutical form. These products are not regarded as relevant for the assessment of the medicinal use of *Saccharomyces cerevisiae* CBS 5926.

Information on other products marketed in the EU/EEA (where relevant)

Several food supplements containing *Saccharomyces cerevisiae/boulardii* are included in the database of the Latvian Food Centre (mono and combination products). No further information is given.

2.1.2. Information on products on the market outside the EU/EEA

In the United States, *Saccharomyces boulardii* is regarded as a probiotic, which as a dietary supplement does not require approval by the Food and Drug Administration (FDA). Due to its regulation as a dietary supplement, it is intended for use by the general healthy population, not as a drug to prevent, treat or mitigate disease (Venugopalan *et al*., 2010).

According to CFR-Code of Federal Regulations Title 21 (FDA), dried yeast (*Saccharomyces cerevisiae* and *Saccharomyces fragilis*) may be safely used in food provided the total folic acid content of the yeast does not exceed 0.04 mg/g yeast (approximately 0.008 mg of pteroyglutamic acid per gram yeast) (21CFR172.896).

2.2. Information on documented medicinal use and historical data from literature

During his visit to Indochina in 1920, the French biologist Henri Boulard discovered a closely related strain of *Saccharomyces cerevisiae* which was named *Saccharomyces boulardii*. Its discovery was based on the observation that, during an outbreak of cholera, some people, who either had chewed on the skins of lychee and mangosteen fruits or drunken a special tea prepared from the lychee fruit, were not affected by the disease. In 1947, Laboratories Biocodex bought the patent for this yeast strain and started the process of research and manufacturing (McFarland, 2010). Since then, this *Saccharomyces* strain (initially named *"boulardii"*) has been used in a variety of indications, which mainly refer to the gastrointestinal tract.

In 1988, Kommission E (BAnz, 1988) published a first monograph on *Faex medicinalis*, which consists of fresh and dried cells *of Saccharomyces cerevisiae* MEYER and/or *Candida utilis* and contains vitamins, glucanes and mannans. As indications, lack of appetite and adjuvant treatment of acne and furunculosis are mentioned. The dosage recommended is 6 g per day.

According to the monograph of the Kommission E, which was published thereafter in 1994 (BAnz, 1994) and Blaschek *et al*. (2013), there is a well-established use (WEU) of *Saccharomyces boulardii* (*Saccharomyces cerevisiae* HANSEN CBS 5926) in the following indications:

- Symptomatic treatment of acute diarrhoea
- Prophylaxis and symptomatic treatment of traveller's diarrhoea
- Diarrhoea associated with tube feeding
- Adjuvant treatment of acne

The corresponding posologies are listed in Table 3.

Table 3: Overview of historical data

Country	**Herbal preparation**	**Documented Use/Traditional Use**	**Pharmaceutical form Strength (where relevant) Posology Duration of use**	**Reference**
Germany	*Saccharomyces cerevisiae* HANSEN CBS 5926 (1 g of the lyophilisate contains ≥1.8 x 10^{10} viable cells)	WEU: a) Symptomatic treatment of acute diarrhoea b) Prophylaxis and symptomatic treatment of traveller's diarrhoea c) Diarrhoea associated with tube feeding d) Adjuvant treatment of acne	Adults and children >2 years of age: a) 250-500 mg per day b) 250-500 mg per day; 5 days before departure c) 500 mg/l nutrient solution d) 750 mg per day	Monograph of Kommission E (15 April 1994) Blaschek *et al*. (2013)

2.3. Overall conclusions on medicinal use

According to the results of the market overview, preparations from *Saccharomyces cerevisiae* are used in many European countries, with the first medicinal products having been marketed in 1968 (Belgium). The following preparations have 30 years of medicinal use in the EU: symptomatic treatment of acute diarrhoea, prevention and symptomatic treatment of traveller's diarrhoea and adjuvant treatment of chronic acne (for assessment of the clinical data, see chapter 4).

Table 4: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	**Indication**	**Posology, Strength**	**Period of medicinal use**
Saccharomyces cerevisiae CBS 5926 (>1.8 x 10^{10} viable cells/g lyophilisate)	Treatment of acute diarrhoea	Adolescents, adults and elderly: Single dose: 250 mg 2 times per day Daily dose: 250-500 mg Children (6 months to 11 years): Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg	Since 1978

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
		Infants from 6 months to 2 years should be treated under the care of a doctor, only	
Saccharomyces cerevisiae CBS 5926 (>1.8 x 10^{10} viable cells/g lyophilisate)	Prevention of traveller's diarrhoea	Adolescents, adults and elderly: Single dose: 250 mg 1-2 times per day Daily dose: 250-500 mg Starting 5 days before departure	Since 1978
Saccharomyces cerevisiae CBS 5926 (>1.8 x 10^{10} viable cells/g lyophilisate)	Adjuvant treatment of acne	Adolescents and adults: Single dose: 250 mg 3 times per day Daily dose: 750 mg	Monograph of Kommission E (15 April 1994) Since 1995

3. Non-Clinical Data

The pharmacological properties of *Saccharomyces cerevisiae* have been investigated extensively both by *in vitro* and *in vivo* methods and the results have been published in numerous publications. *Saccharomyces cerevisiae* is considered as a probiotic according to the definition of the World Health Organisation (2002) i.e. "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." A comprehensive review on the various mechanisms of action of *Saccharomyces cerevisiae* is given by Kelesidis and Pothoulakis (2012), Im and Pothoulakis (2010), McFarland (2010) and Moslehi-Jenabian *et al.* (2010).

Considering the multitude of studies with preclinical data on *Saccharomyces cerevisiae*, a systematic review is not given here. This section is rather the result of an attempt to select illustrative studies relevant for the medicinal use.

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

3.1.1. Primary pharmacodynamics

As summarised by Kelesidis and Pothoulakis (2012) and McFarland (2010), the pharmacodynamic effects of *Saccharomyces boulardii* can be classified into three main domains of action:

- Luminal action, including effects against several bacterial toxins, antimicrobial activity, modulation of intestinal flora, metabolic activity;
- Trophic action on the intestinal mucosa, including enzymatic activity;
- Regulation of the immune response by acting as immune stimulant, by reducing pro-inflammatory responses and promoting mucosal anti-inflammatory signaling effects.

While some of the effects were only investigated in humans and therefore are not further mentioned here, others were also seen in non-clinical tests.

For at least some of the effects, it was claimed that the substances responsible for these effects are released by the cells of *Saccharomyces cerevisiae* during their intestinal catabolism, rather than being secreted by viable cells (Kelesidis and Pothoulakis, 2012; McFarland, 2010):

Luminal action:

- Antitoxin effects

In several studies inhibition of toxin receptor binding sites, stimulation of antibody production against *Clostridium difficile* toxin A and direct proteolysis of the pathogenic toxins/secretion of enzymatic proteins (production of a serine protease that cleaves *C. difficile* toxin A, production of a 63 kDa phosphatase that destroys the endotoxin of pathogenic *Escherichia coli* and production of a 120 kDa protein that reduces the effects of cholera toxin) was shown.

- Antimicrobial activity

Saccharomyces boulardii is capable of directly or indirectly interfering with intestinal pathogens. Inhibition of growth of bacteria and parasites, reduction of gut translocation of pathogens, neutralisation of bacterial virulence factors and suppression of host cell adherence that interferes with bacterial colonisation were reported.

- Modulation of intestinal flora

Rapid re-establishment of normal microbiota could be shown in antibiotic-exposed mice after administration of *Saccharomyces boulardii*.

According to a review by Moré and Swidsinski (2015), *Saccharomyces boulardii* is able to create a favorable growth environment for the beneficial intestinal microbiota, while constituting extra protection to the host mucus layer and mucosa.

Trophic action:

Saccharomyces boulardii can reduce the number of infected cells and stimulate the growth and differentiation of intestinal cells in response to trophic factors, prevent apoptosis and synthesis of TNF-α. It reduces mucositis, restores fluid transport pathways, stimulates protein and energy production and restores metabolic activities in colonic epithelial cells, secretes mitogenic factors that enhance cell restitution, enhances release of brush border membrane enzymes, stimulates the production of glycoproteins in the brush border and the production of intestinal polyamines, restores normal levels of colonic short chain fatty acids (SCFA), stabilises gastrointestinal barrier function and strengthens enterocyte tight junctions and can reduce crypt hyperplasia and cell damage in colitis models.

The trophic effects of *Saccharomyces boulardii* are the focus of a review by Moré and Vandenplas (2018). They report that *Saccharomyces boulardii* CNCM I-745 synthesises and secretes polyamines, which have a role in cell proliferation and differentiation. The administration of polyamines or *Saccharomyces boulardii* CNCM I-745 enhances the expression of intestinal digestive enzymes as well as nutrient uptake transporters. The signalling mechanisms leading to enzyme activation are not fully understood. However, polyamines have direct nucleic acid–binding capacity with regulatory impact. *Saccharomyces boulardii* CNCM I-745 induces signalling via the mitogen-activated protein kinase pathway. In addition, effects on the phosphatidylinositol-3 kinase (PI3K) pathway have been reported. As an additional direct effect, *Saccharomyces boulardii* CNCM I-745 secretes certain enzymes, which enhance nutrient acquisition for the yeast and the host. The increased availability of digestive enzymes

seems to be one of the mechanisms by which *Saccharomyces boulardii* CNCM I-745 counteracts diarrhoea; however, also people with certain enzyme deficiencies may profit from its administration. More studies are needed to fully understand the mechanisms of trophic activation by the probiotic yeast.

Regulation of the immune response:

- Acting as immune stimulant

It could be shown that *Saccharomyces boulardii* triggers activation of complement and migration of monocytes and granulocytes and enhances the number of Kupffer cells in germfree mice. Furthermore, experiments revealed that it enhances the mucosal immune response and secretory IgA intestinal levels, enhances systemic immune response and levels of serum IgG to *C. difficile* toxins A and B, contributes to earlier production of IFN-γ and IL-12, inhibits dendritic cell-induced activation of T cells, modifies migration of lymphocytes in a chronic inflammatory bowel disease model, modifies lymphocyte adherence to endothelial cells and improves cell rolling and adhesion.

- Reducing pro-inflammatory responses and promoting mucosal anti-inflammatory signaling effects

In several studies *Saccharomyces boulardii* decreased expression of pro-inflammatory cytokines (IL-8, IL-6, IL-1β, TNF-α and IFN-γ), increased expression of the anti-inflammatory cytokine IL-10, interfered with NF-κB-mediated signal transduction pathways, in immune and colonic epithelial cells, blocked activation of ERK1/2 and MAP kinases, decreased nitric oxid and inhibited production of inducible nitric oxid synthase, modulated T-cell migratory behavior and increased trapping of T helper cells into mesenteric lymph nodes and stimulated production of anti-inflammatory molecules in human colonocytes such as peroxisome proliferator-activated receptor-gamma.

To be an effective probiotic agent, *Saccharomyces cerevisiae* must fulfill several conditions, such as survival of the passage to its target organ (most commonly the colon) or resistance to stomach acids and bile acids.

Although much of the oral dose seems to be destroyed (usually stool levels are 100-1,000 times lower than the oral dose), surviving oral doses have been found to be effective (usually at levels over 10^8 organisms/g stool).

Table 5: Properties of *Saccharomyces cerevisiae* which should be considered to determine the efficacy [taken from Kelesidis and Pothoulakis, 2012]

Properties of *Saccharomyces boulardii*	**References**
Survives passage to its target organ (most commonly the colon): although much of the oral dose is destroyed (usually stool levels are 100-1000 times lower than the oral dose), surviving oral doses have been found to be effective (usually at levels over 108 organisms/gram stool)	Gorbach, 2000
Survives at body temperature (37°C): unique advantage of being one of the few yeasts that do best at human body temperatures	Graff *et al*., 2008
In lyophilised form, *Saccharomyces boulardii* survives gastric acid and bile	Graff *et al*., 2008
As is the case with all yeasts, *Saccharomyces boulardii* is naturally resistant to antibiotics	Graff *et al*., 2008
Saccharomyces boulardii is resistant to proteolysis	Buts, 2009

Properties of *Saccharomyces boulardii*	**References**
Saccharomyces boulardii exists in the competitive milieu of the intestinal tract	Buts, 2009
Saccharomyces boulardii levels are higher in patients with disturbed intestinal microbiota (due to antibiotic exposure) compared to patients without antibiotic exposure	Klein *et al*., 1993
When given orally, achieves steady-state concentrations within three days and is cleared within 3-5 days after it is discontinued	Blehaut *et al*., 1989; Elmer *et al*., 1999b
Some types of fiber (psyllium) increased *Saccharomyces boulardii* levels by 22%, while other types of fiber (pectin) showed no effect	Elmer *et al*., 1999a

Czerucka and Rampal (2019) described the diversity of *Saccharomyces boulardii* CNCM I-745 mechanisms of action against intestinal infections as follows:

"Saccharomyces boulardii strain CNCM I-745 is a probiotic yeast that by virtue of being a eukaryote differs from other probiotic strains, which are of bacterial origin (prokaryote). The research shows a great diversity in its mode of action and types of targets: pathogens, pathogenic toxins, gut microbiota and intestinal epithelium. Two main mechanisms were demonstrated: the first one is a large capacity of the wall to fix bacteria and toxins, which facilitates their elimination during intestinal transit and the second one is the synthesis by this yeast of several active factors. These factors include high molecular weight proteins, some of which have antisecretory effects, others act as proteases that degrade toxins or their receptors. Factors of small size and protein or non-protein nature that exhibit anti-secretory or anti-inflammatory activities are also involved in its action. Finally, Saccharomyces boulardii CNCM I-745 acts on different components that maintain the intestinal barrier: Tight junctions that regulate permeability; reconstitution of the microbiota after antibiotic therapy; and, activation of innate immunity, which stimulates innate defenses of the host during infection. The optimization of the use of this probiotic in infections requires a better knowledge of the different mechanisms of action."

3.1.2. Secondary pharmacodynamics

Kim *et al*. (2004) described the purification and characterisation of a novel antihypertensive angiotensin I-converting enzyme (ACE) inhibitory peptide from *Saccharomyces cerevisiae*. The purified inhibitor competitively inhibited ACE and showed a clear antihypertensive effect in spontaneously hypertensive rats at a dosage of 1 mg/kg body weight (b.w.). These results were similar to that of the antihypertensive drug captopril.

Investigations of Karen *et al*. (2010) in rats suggest that *Saccharomyces boulardii* may reduce lung injury by reducing bacterial translocation, which results in reduced infection, inflammation, and generation of proinflammatory cytokines in an experimental model of acute necrotising pancreatitis.

3.1.3. Safety pharmacology

Not available.

3.1.4. Pharmacodynamic interactions

Blackwell and Marley (1966) tested yeast extracts in rats, cats and guinea pigs concerning interactions with monoamine oxidase inhibitors because attacks of hypertension and headache have occurred in patients taking monoamine oxidase inhibitors and Marmite® (a food product). The explored yeast

extracts were obtained locally, except for some samples of Marmite® (Salt Marmite®) and Salt-free Marmite® given by Bovril Ltd.
The product injected i.v. into untreated rat and cat produced a fall followed by a rise in blood pressure and contracted the nictitating membrane. These sympathomimetic effects were prolonged by previous treatment with an amine oxidase inhibitor. The effects of yeast extract after intraduodenal injection were only obtained in the rat or cat after treatment with an amine oxidase inhibitor. The inhibitors used were of the amine, hydrazine and hydrazide variety. Furthermore, some histamine-like effects of the yeast extracts both after i.v. injection and intraduodenal injection were observed. The histamine-like effects could be attributed to the absorption of a histamine-like substance from the yeast extract in the intestine which was facilitated by inhibition of monoamine oxidase. In the guinea pig, the i.v. or intraduodenal injection of yeast extract increased resistance on inflation of the lungs. The authors summarised that yeast extracts had sympathomimetic and histamine-like properties.

Assessor's comment:

Since the product tested consists not only of yeast extract but also of high amounts of salt, vegetable extract and various vitamins, the results should be regarded as not appropriate for the assessment of the medicinal use of Saccharomyces cerevisiae.

Izquierdo-Pulido *et al*. (1995) examined the influence of *Saccharomyces cerevisiae* var. *uvarum* on histamine and tyramine formation during beer fermentation. They found that the yeast did not produce histamine or tyramine during fermentation. Yeast recycling did not influence biogenic amines formation.

3.1.5. Conclusions

In many review articles, the pharmacodynamic effects of *Saccharomyces cerevisiae* were classified into three main domains of action: a luminal and a trophic action as well as an influence on the immune response. Several mechanisms of actions are described within these 3 categories. Some of the knowledge was obtained using *in vitro* data; therefore, the biological relevance of these results cannot be evaluated. Also, *in vivo* data obtaind in animals with different physiology concerning the digestive system might only be supportive for the human situation. Nevertheless, since some of the effects could also be seen in patients, it seems that at least basically the non-clinical results support the usage of *Saccharomyces cerevisiae* in the conditions linked to diarrohea.

Concerning possible interactions with monoamine oxidase inhibitors, the available preclinical data do not refer to the specific *Saccharomyces* strain covered by this assessment report. Furthermore, these data are inconsistent as regards histamine and tyramine findings.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

In vivo

In 1989, Blehaut *et al*. investigated the kinetics of *Saccharomyces boulardii* in humans, rats and mice (see chapter 4.1.2). They conducted a multiple dose study and a single dose study in rats and presented the results of continuous administration of *Saccharomyces boulardii* in mice (lyophilised *Saccharomyces cerevisiae* CBS 5926 with a total number of yeast cells (±SD) of $10^{10.98\pm0.02}$ per gram corresponding to $10^{10.55\pm0.08}$ viable yeast cells per gram).

Single dose in rat:

A total of 54 rats weighing 200±20 g on a yeast free regimen received 0.4 g/kg of *Saccharomyces boulardii* suspension as a single dose (lyophilised *Saccharomyces cerevisiae* CBS 5926 with a total number of yeast cells (±SD) of $10^{10.98\pm0.02}$ per gram corresponding to $10^{10.55\pm0.08}$ viable yeast cells per gram) (oesophageal tube). A group of 6 rats were sacrificed at each of the following times: control, 0 (immediately post-dosing), 0.08, 0.25, 2, 3, 4, 8, and 24 hours.

Measurements made in three levels of the gastrointestinal tract (oesophagus and stomach, small intestine and large intestine) show the progression of live and dead cells through the length of the gastrointestinal tract. Live cells appeared to move faster than dead cells.

The recovery of live cells decreased from 87% at 15 min to a value of 13% at 24 hours. An apparent disappearance half-life of 9 hours was calculated from the last four time points (3, 4, 8 and 24 hours).

The time course of dead cells was much slower. Initially, there was a plateau with a slight increase in recovery to 108% at 2 hours. This phenomenon was explained with the addition of dead cells arising from the rapid disappearance of live cells. The apparent disappearance half-life calculated from the last four points was 44 hours. This value is only an estimate since measurements were available for less than one half-life.

Multidose study in rat:

Six male rats weighing 200±20 g received 0.8 g/kg of *Saccharomyces boulardii* suspension (lyophilised *Saccharomyces boulardii* with a total number of yeast cells (±SD) of $10^{10.88\pm0.04}$ per gram corresponding to $10^{10.28\pm0.04}$ viable yeast cells per gram) as a single daily dose (oesophageal tubes) from day 1 to 14 and were fed with a yeast-free regimen.

Twenty-four hours after the beginning of *Saccharomyces boulardii* treatment, faecal concentration of live cells reached a steady state in all animals. Less than 1% of the administered live cells were recovered in the faeces. In the period following *Saccharomyces boulardii* treatment (days 15-18), the concentrations of live cells decreased by three orders of magnitude within 24 hours. Consequently, a precise measure of half-life could not be obtained. However, the authors estimated that its value is shorter than 3 hours. Dead cells reached a steady state within 24 hours, too.

The recovery of dead cells based on total dose of *Saccharomyces boulardii* ranged from 9.3% to 15.8% with an overall mean of 12.4±2.4%. The recovery based on the dose of dead cells ranged from 12.4% to 21.2% with an overall mean (±SD) of 16.6±3.2%. The half-life time is estimated on 36 hours.

Continuous administration in mice:

Ten nude and ten control mice (haired, thymic) received *Saccharomyces boulardii* (lyophilised *Saccharomyces boulardii* with a total number of yeast cells (±SD) of $10^{10.98\pm0.02}$ per gram corresponding to $10^{10.55\pm0.08}$ viable yeast cells per gram) in a 5% (w/V) suspension as the source of drinking water. In addition, 10 nude and 10 control mice received only saline.

After 70 days of oral administration, the caecum only contained detectable levels of yeast. No living cells could be detected in the mesenteric lymph nodes, in the liver, lungs, heart or kidney.

In vitro

Graff *et al*. (2008) investigated to what extent *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926) is sensitive to gastrointestinal pH conditions. The survival of different concentrations of *Saccharomyces boulardii* applied as freeze-dried yeast or as aqueous suspension was examined in conditions mimicking the stomach pH (pH 1.1; 0.1 N HCl) and intestinal pH (pH 6.8; phosphate buffer). The viability of both forms of *Saccharomyces boulardii* remained stable for 6 hours in

phosphate buffer, whereas under acidic conditions for both forms of *Saccharomyces boulardii* from 5 min a significant decrease of viability was observed. At the highest concentration of 200 g/l the initial pH value of 1.1 increased to 3.2 demonstrating a protective effect. This investigation shows that in order to improve oral availability of viable *Saccharomyces boulardii*, it has to be protected from gastric destruction.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

The monograph of the German Kommission E (1994) states that a single oral administration of 3 g/kg b.w. produced no toxic reactions in mice and rats.

Acute toxicity tests were performed in rats (Sudha, 2011). *Saccharomyces boulardii* (corresponding to 5 x 10^9 CFU/g) administered at single oral doses of 6,500 mg per kg of b.w. produced no treatment-related changes in the test animals. The animals were observed over a period of 14 days.

3.3.2. Repeat dose toxicity

The administration of 330 mg/kg b.w. over a period of 6 weeks on 6 days per week to dogs and the oral administration of 100 mg/kg b.w./day to rats and rabbits showed no drug induced changes (Kommission E, 1994). For sub-acute toxicity studies, Sprague-Dawley rats were fed with oral doses of *Saccharomyces boulardii* (corresponding to 5 x 10^9 CFU/g) up to 1,300 mg/kg b.w. for 14 consecutive days (Sudha, 2011). During the observation period of 28 days, *Saccharomyces boulardii* was well tolerated and there was no morbidity nor any toxic clinical symptom displayed either in male or female rats.

3.3.3. Genotoxicity

According to the Kommission E (1994) monograph, no mutagenic effects of *Saccharomyces cerevisiae* HANSEN CBS 5926 have been detected in an AMES-test with *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without S9 activation.

3.3.4. Carcinogenicity

No studies on cancerogenicity for the yeast *Saccharomyces boulardii* are available.

3.3.5. Reproductive and developmental toxicity

No studies are available either on reproduction toxicology and the fertility-influencing, or on embryo-foetal and peri-/postnatal toxicity effect for the yeast *Saccharomyces boulardii*.

3.3.6. Local tolerance

Not applicable.

3.3.7. Other special studies

<u>Pathogenicity</u>

Maejima *et al*. (1980) examined *Saccharomyces cerevisiae* for its pathogenicity and colonisation in mice and cynomolgus monkeys as the models of the biological containment level. Adult mice given perorally 5.5 or 2.4 x 10^7 cells of *Saccharomyces cerevisiae* strain MC16 excreted them rapidly and no colonisation of the cells in the abdominal organs, lymph nodes or gastrointestinal wall was demonstrated. No change in the faecal flora was observed. After peroral administration of 4.9 x 10^7 or 7.8 x 10^8 cells, cynomolgus monkeys showed a similar tendency of rapid excretion and lack of colonisation. Cortisone acetate treatment had no significant effect. Intravenous administration of 3.9 x 10^7 yeast cells had no pathogenic effect and no viable yeast was detected in the blood. The biological containment level of *Saccharomyces cerevisiae* was suggested not to be lower than that of *E. coli* K12 biosafety level 1. The possibility of achieving the biosafety level 2 was suggested. [Biosafety level 1: suitable for work involving well-characterised agents not known to consistently cause disease in healthy adult humans, and or minimal potential hazard to laboratory personnel and the environment. Biosafety level 2 is similar to Biosafety level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. These agents cause only mild disease to humans or are difficult to contract via aerosol in a laboratory setting.] (Chosewood and Wilson, 2009).

To assess the pathogenic potential, Clemons *et al*. (1994) analysed 13 clinical isolates (e.g. lung, blood, peritoneal fluid), 10 non-clinical isolates (e.g. bread yeast, wine yeast) and 5 constructed strains of *Saccharomyces cerevisiae*. All were *Saccharomyces cerevisiae* by biochemical profiles, sporulation or genetic evidence. To initiate the model of infection, 4-week-old male CD-1 mice from the virus antibody-free colony were inoculated intravenously with 2 x 10^7 viable CFU of *Saccharomyces cerevisiae*. At various times after infection, 4-10 mice were killed, and brains, spleens, livers, kidneys, and lungs were removed aseptically and residual burden of *Saccharomyces cerevisiae* in each organ was determined. Prolonged persistence of *Saccharomyces cerevisiae* especially in the brains was found. Furthermore, some of the clinical isolates tested demonstrated a modest proliferation in the brain over the first 7 days of infection (5-fold); infection due to non-clinical isolates declined. By authors' definition of virulence, the results indicate that the comparative pathogenicity of *Saccharomyces cerevisiae* isolates is represented by a continuum rather than a clear virulent or avirulent result. The majority of the clinical isolates were better able to persist *in vivo* than isolates from non-clinical sources. 12 of the 13 clinical isolates were assigned to groups considered as being virulent or intermediate in virulence. This is in contrast to the non-clinical isolates where only 4 of 10 were assigned to the intermediate virulence group and the rest were considered avirulent. However, even within the latter group, there were degrees of resistance to eradication by host mechanism. The authors concluded that these data indicate that recovery of *Saccharomyces cerevisiae* in a clinical setting, especially from severely compromised patients, should not be ignored.

To further characterise *Saccharomyces cerevisiae* pathogenesis, Byron *et al*. (1995) studied a virulent clinical isolate and an avirulent non-clinical isolate in C5-deficient mice. Complement deficiency (in particular, of the fifth component [C5]) has been implicated in increasing susceptibility of humans and mice to fungal infections with *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* as well as infections caused by gram-negative bacteria. The mice were infected intravenously with 10^7 CFU and temporal burdens of yeast cells in various organs were determined. After infection, the virulent clinical isolate increased by 20-fold in the brain from day 0 to 3 and by 4-fold in the kidneys. The avirulent non-clinical isolate increased by 13-fold in the brain from day 0 to 3 and decreased by 16-fold in the kidneys. Both isolates declined in number in other organs (spleen, liver, lungs). In all studies, 90% of mice infected with 10^7 CFU of the virulent clinical isolate died between days 2 and 7, whereas no mice infected with equivalent numbers of the avirulent non-clinical isolate died. No mice died after infection with 10^6 CFU of the virulent clinical or the avirulent non-clinical isolate. The importance of C5 was confirmed by studies using C5-deficient mice (C5-) and their congenic

C5-sufficient (C5+) counterparts. Again, the C5- mice were most susceptible to infection with *Saccharomyces cerevisiae*, with 63% infected with the virulent clinical isolate dying by day 7; no C5+ mice died. No mice infected with the avirulent non-clinical isolate died, and mean burdens in the brain at day 14 were sevenfold lower in C5+ mice than in C5- mice. The authors concluded that C5 is important in the early innate host response to infection with some isolates of *Saccharomyces cerevisiae* able to cause mortality. However, in comparing the relative degrees of virulence of the various isolates in C5-sufficient and C5-deficient mice, it is apparent that different isolates have developed differing genetic strategies for the manifestation of virulence, regardless of the status of host response. *Saccharomyces cerevisiae* has been added to the list of emerging pathogens to which an immunocompromised host is susceptible and must now be looked at in the same light as other more common fungal pathogens.

3.3.8. Conclusions

There are only very limited non-clinical toxicological data available.

As some clinical case reports describe, immunocompromised patients run the risk of a *Saccharomyces cerevisiae* sepsis and therefore the use of *Saccharomyces cerevisiae* is contraindicated in these patients. This contraindication is supported by the non-clinical data concerning pathogenicity mentioned above.

3.4. Overall conclusions on non-clinical data

In many review articles, the pharmacodynamic effects of *Saccharomyces cerevisiae* were classified into three main domains of action: a luminal and a trophic action as well as an influence on the immune response. Since many results reflect on *in vitro* data or were obtained in animals with different physiology concerning the digestive system, the biological relevance of these results cannot be evaluated. Nevertheless, since some of the effects could also be seen in patients, it seems that at least principally the non-clinical results on pharmacodynamics support the usage of *Saccharomyces cerevisiae* in conditions linked to diarrhoea.

Immediately after introduction in the oesophagus, live and dead cells are moved through the gastrointestinal tract simultaneously with an irreversible degradation. Disappearance from the stomach and oesophagus was followed by appearance in the small intestine. Ultimately, *Saccharomyces cerevisiae* appears in the bowel and faeces thereby were less than 1% of the administered live cells recovered in faeces. The recovery of dead cells is incomplete, because there is an irreversible loss in the gastrointestinal tract by digestion of the cell wall. The majority of dead cells (83% in rat) introduced in the gastrointestinal tract are destroyed in a 24 hours period. Cells of irreversible loss in the gastrointestinal tract by digestion of the cell wall do not cross the gastrointestinal wall.

There are only very limited toxicological data available. Studies on reproductive and developmental toxicity are not available. In the case of i.v. administration, the non-clinical data suggest that virulence of *Saccharomyces boulardii* cannot be excluded, particularly in immunocompromised patients.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

4. Clinical Data

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal

substance(s)/preparation(s) including data on relevant constituents

As already presented in 3.1.1, according to Kelesidis and Pothoulakis (2012) and McFarland (2010), the pharmacodynamic effects of *Saccharomyces boulardii* can be classified into three main domains of action: a luminal action, a trophic action as well as an influence on the immune response. The following effects have been confirmed in humans:

Luminal action:

Within the gastrointestinal lumen both anti-toxin and antimicrobial effects have been described. Anti-toxin effects are directed against *C. difficile* toxin A and B (54 kDa protease), endotoxins of pathogenic *E. coli* (63 kDa protein phosphatase), and cholera toxins (120 kDa protein). Anti-toxin effects are due to different mechanisms: inhibition of pathogen receptor sites or direct proteolysis of pathogenic toxins via a secretion of enzymatic proteins.

Antimicrobial effects include a direct or indirect inhibition of growth of pathogens, preservation of the tight junctions between enterocytes, reduction of gut translocation of pathogens, and suppression of host cell adherence that interferes with bacterial colonization. Investigations both in mice (Barc *et al*., 2008) and patients with chronic idiopathic diarrhoea (Swidsinski *et al*., 2008) showed that after the discontinuation of antibiotic treatment the restoration of normal intestinal microbiota is achieved faster under the administration of *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926).

Garcia Vilela *et al*. (2008) investigated the effect of *Saccharomyces boulardii* on intestinal permeability in 34 patients with Crohn's disease in remission. As compared to placebo, the administration of 200 mg lyophilised *Saccharomyces boulardii* (about 4 x 10^8 cells; *Saccharomyces cerevisiae* CBS 5926) every 8 hours over a period of 3 months in addition to baseline treatment improved intestinal barrier function as measured by lactulose/mannitol ratio.

Akil *et al*. (2006) evaluated how the oral intake of *Saccharomyces boulardii* affects the number of *E. coli* colonies in the colon. 24 healthy children (age 3-16 years) received 5 billion CFU of *Saccharomyces boulardii* once daily for 5 days. Following treatment with *Saccharomyces boulardii*, the mean number of *E. coli* colonies in g/ml stool significantly decreased from 384,625±445,744 to 6,283±20,283. During the same interval, the number of *Saccharomyces boulardii* colonies increased from 0 to 11,047±26,754 in g/ml stool.

Furthermore, within the gut lumen *Saccharomyces boulardii* shows metabolic activity by increasing SCFA which favour normal colonic function. Following the oral administration of *Saccharomyces boulardii* (1 g per day) on 6 consecutive days, Girard-Pipau *et al*. (2002) observed an increase in the SCFA acetic, propionic and butyric acid in patients with enteral nutrition.

Schneider *et al*. (2005) assessed the effects of *Saccharomyces boulardii* on faecal flora and SCFA in 10 patients on long-term total enteral nutrition (TEN). Treatment with *Saccharomyces boulardii* (500 mg b.i.d. (two times daily) as a lyophilised powder) significantly increased total faecal SCFA as compared to 15 healthy controls. At the end of treatment, faecal butyrate was significantly higher whereby faecal flora remained unchanged. According to the authors, the preventive effects of *Saccharomyces boulardii* on TEN-induced diarrhoea may be due to the increase of faecal SCFA.

Swidsinski *et al.* (2016) investigated the impact of antibiotics and *Saccharomyces boulardii* (Sb) on bacterial composition in human faeces. Samples were collected from three groups of women (n=20 each) treated for bacterial vaginosis with ciprofloxacin + metronidazole. Group A received the combined antibiotic regimen, whereas the A/Sb group received concomitant *Saccharomyces boulardii*, and the A_Sb group received *Saccharomyces boulardii* prophylaxis following the 14-day antibiotic course. One 250 mg capsule *Saccharomyces boulardii* (1.8 x 10^{10} CFUs/g lyophilisate) three times

daily was administered. The decrease in the concentrations of the fermenting biomass and inconsistency of the bacterial diversity caused by antibiotics were effectively averted by *Saccharomyces boulardii* prophylaxis. A total of 88% of patients receiving *Saccharomyces boulardii* quickly restored their initial individual microbial profiles. The total microbial concentrations recovered completely in the two *Saccharomyces boulardii* groups within 3 months post-antibiotic treatment. However, although they were different shortly after antibiotic treatment, both concomitant and subsequent *Saccharomyces boulardii*-treated groups were similar at the end of the observational period.

Kabbani *et al.* (2017) compared and contrasted the effects of *Saccharomyces boulardii* (SB), an antibiotic (Amoxicillin-Clavulanate, AC) and the combination on the intestinal microbiota of healthy humans in a single-centre, open-label, randomised controlled trial. In addition, they examined the effects of these interventions on gastrointestinal symptoms with a particular focus on antibiotic-associated diarrhoea (AAD). 53 subjects were enrolled, and 49 were considered for statistical analyses. They were randomised to one of 4 study groups: SB for 14 days, AC for 7 days, SB plus AC, control (no treatment). Participants gave stool samples and completed gastrointestinal symptom questionnaires. Microbiota changes in the stool specimen were analysed using 16s rRNA gene pyrosequencing (bTEFAP). Control subjects had a stable microbiota throughout the study period. Significant microbiota changes were noted in the AC alone group during treatment, which reverted toward baseline, but were not yet completely restored 2 weeks after antibiotic therapy. No significant shifts in bacterial genera were noted in the SB alone group. Adding SB to AC led to less pronounced microbiota shifts including less overgrowth of *Escherichia* and to reduction in AAD scores.

Trophic action:

Buts *et al*. (1986) investigated the response of the small intestinal mucosa to *Saccharomyces boulardii* in 7 healthy adults. Following the administration of high doses of lyophilised *Saccharomyces boulardii* (250 mg four times per day; 250 mg with a biological activity of 9.4×10^9 viable cells) over a period of 2 weeks, a peroral suction biopsy was performed. As compared to the initial biopsy, the histological examination of the post-trial biopsy showed no morphological alterations with regard to villus height or crypt depth. After treatment, a statistically significant increase of the specific enzymatic activity of disaccharidases (sucrase, lactase, maltase) was observed, whereas mucosal protein content remained unchanged.

The effects of *Saccharomyces boulardii* on duodenal mucosa were also investigated by Jahn *et al*. (1996) by means of morphometry and determination of brush border enzyme activity. Twelve healthy volunteers received lyophilised *Saccharomyces boulardii* 5 capsules three times a day (t.i.d.) (one capsule containing 50 mg (10^9 viable cells)) over a period of 21 days. A comparison of intra-individual histochemical results pre- and post-administration of *Saccharomyces boulardii* revealed a statistically significant increase of enzymatic acitivity of lactase, α-glucosidase and alkaline phosphatase in the brush border of enterocytes. According to the authors, this effect as well as a tendency of an increased villous surface possibly may be caused by an accelerated maturation of enterocytes.

Influence on the immune response:

Both locally in the gut and systemically, *Saccharomyces boulardii* modulates the immune response either by its action as an immune stimulant or by reducing pro-inflammatory responses. Based on their studies in growing rats, Buts *et al*. (1990) assume that glucan and mannans, which are complements of the yeast external capside, could play an immunogenic role.

Ozkan *et al*. (2007) described an enhancement of immune response for *Saccharomyces boulardii*. Following the oral administration of 250 mg *Saccharomyces boulardii* b.i.d. for 7 days to 16 patients (age 6 months to 10 years) with acute diarrhoea as compared to 11 children on placebo, a significant

increase in serum IgA and decrease in C-reactive protein was observed. The percentage of CD8 lymphocytes at the end of treatment was significantly higher, too. Secretory IgA is important for the maintenance of intestinal barrier function.

Machado Caetano *et al*. (1986) investigated the effects of *Saccharomyces boulardii* on specific and non-specific defense in 96 immunocompetent human volunteers. Following the oral administration of 4 times 250 mg per day for 7 days (250 mg corresponding to 3.5 x 10^9 yeast cells), a significant increase of erythrocytes, leucocytes, neutrophils, complement components, serum anti-complementary activity and leukocyte chemokinesis, especially when autologous serum and antigen have been added to the culture medium, and decrease of complement haemolytic activity were observed. The overall changes in serum proteins suggested changes of acute phase proteins typical of an inflammatory process. *Saccharomyces boulardii* had no mitogenic response of lymphocyte populations. These results demonstrate that *Saccharomyces boulardii* oral ingestion induces cellular and humoral changes in humans, which result from reticulo-endothelial system stimulation and activation of the complement system. *Saccharomyces boulardii* did not affect the specific immune response, as it lacks mitogenic effects of its own and did not modify lymphocyte populations nor their response to both phytohaemaglutinin and Cowan I mitogens.

Stickl (1987) performed a double-blind study and investigated the effects of *Saccharomyces cerevisiae* Hansen (either 500 mg per day or 200 mg per day *Saccharomyces cerevisiae* Hansen versus placebo) on IgA in saliva. Within the 3 weeks of treatment, no effects on IgA in saliva were observed. While *Saccharomyces* had no influence on T4-lymphocytes, T8-lymphocytes slightly increased.
As T8-lymphocytes are known to possess cytotoxic and suppressor properties, the author concluded from the study results that *Saccharomyces cerevisiae* might have positive effects on the treatment of diseases such as atopia or autoimmune diseases.

Apart from the trophic effects on intestinal mucosa, Jahn *et al*. (1996) also investigated the effects of *Saccharomyces boulardii* on lymphocytes. Only minimal effects on the immune system of the peripheral blood and the intestine were observed. The percentage of activated $CD25^+$ T-helper/inducer cells increased only slightly, while the concentration of IgA was not affected.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

An overview on pharmacokinetics of *Saccharomyces boulardii* and other biotherapeutic agents is given by Martin *et al*. (1999). According to them, in healthy humans, *Saccharomyces boulardii* is not a natural coloniser of the gut and is not absorbed following oral administration.

Pharmacokinetics of *Saccharomyces boulardii* and *Saccharomyces cerevisiae* were investigated in healthy volunteers or patients with *Clostridium difficile* disease (CDD) or HIV-infections. Furthermore, the concomitant use of antibiotics and antifungals was studied.

In 1989, Blehaut *et al*. investigated the kinetics of *Saccharomyces boulardii* in humans. Eight healthy volunteers received 500 mg *Saccharomyces boulardii* each in the morning and in the evening for a period of 14 days. The capsules contained lyophilised *Saccharomyces boulardii* with a total number of yeast cells (±SD) of $10^{10.98\pm0.02}$ per gram corresponding to $10^{10.55\pm0.08}$ viable yeast cells per gram. The examination of stool samples showed that the concentration of *Saccharomyces boulardii* increased rapidly over the first 2 days of administration and achieved a steady state on day 3. Less than 1% (mean 0.36±0.31%) of the dose of live cells administered was recovered in faeces and a half-life of 6 hours was reported. The rate of recovery from dead cells ranged from 17% to 58% with an overall mean of 31.5±12.1%. Within 7 days following the termination of administration, yeast cells were not

detected in faeces anymore. There was no evidence that cells of *Saccharomyces boulardii* cross the gastrointestinal wall in this study.

Another pharmacokinetic trial was performed by Pecquet *et al*. (1991) in 6 healthy volunteers, who received a daily dosage of 3 x 10^8 life-dehydrated *Saccharomyces cerevisiae* cells for 5 days. Counts of *Saccharomyces cerevisiae* faecal excretion gradually increased to a maximum of 10^5 CFU/g faeces. Within 5 days after the end of treatment, yeast cells disappeared from faeces.

Klein *et al*. (1993) studied the recovery and elimination of *Saccharomyces boulardii* in healthy human volunteers. For the investigation of *Saccharomyces boulardii* dose on recovery of stool, daily doses of 0.2, 1.0 and 3.0 g *Saccharomyces boulardii* were administered sequentially to 8 healthy volunteers over a period of one week each. For each dose, steady-state levels occurred by 72 hours. With increasing *Saccharomyces boulardii* doses, the main steady-state concentration of *Saccharomyces boulardii* increased significantly, whereas the percentage of recovery was independent from dose. The effect of ampicillin on single-dose elimination kinetics was investigated in 10 volunteers. Following the single oral administration of 1 g *Saccharomyces boulardii*, total 24 hours stool was collected for 7 days. After a wash-out period of 7 days, ampicillin was given for another 7 days at a dose of 250 mg four times a day. 24 hours after the first dose of ampicillin, the test persons received a single dose of 1.0 g *Saccharomyces boulardii*. For the following 7 days, total 24 hours stool was collected. Ampicillin significantly increased ($p<0.01$) the area under the concentration versus time curve and maximum faecal concentration. In order to investigate the effect of ampicillin on *Saccharomyces boulardii* elimination at steady-state in 6 volunteers the same procedure was used as for the study of single-dose elimination kinetics, with the exception that 0.5 g *Saccharomyces boulardii* was taken b.i.d. for 7 days. Mean faecal output of *Saccharomyces boulardii* was measured between 72 and 120 hours, when steady-state levels were reached. With ampicillin steady-state recovery of the drug increased about two-fold ($p<0.05$) and steady-state levels were about 2.4 times higher ($p<0.01$).

Elmer *et al*. (1999b) reported a rapid clearance of *Saccharomyces boulardii* in 97 patients with recurrent *Clostridium difficile* disease (CDD). *Saccharomyces boulardii* was given in addition to standard antibiotic treatment (either vancomycin 2 g per day or 500 mg per day or metronidazole 1 g per day) for 10 days. On day 7, according to randomisation, 47 patients received placebo, 50 patients oral treatment with 1 g of *Saccharomyces boulardii* (500 mg b.i.d.; 1 g containing about 10^{10} viable organisms) for 4 weeks. In 48 patients, the clearance of *Saccharomyces boulardii* was investigated. In 94% of the patients, *Saccharomyces boulardii* was cleared by the third day after the discontinuation of treatment. As compared to studies in healthy volunteers having received the same dosage of *Saccharomyces boulardii*, faecal concentrations were 1-2 log lower. Patients, who were asymptomatic at the time of stool collection in this study had significantly higher concentrations than patients with disease symptoms. This clinical study also showed that patients with low faecal concentrations of *Saccharomyces boulardii* had a higher risk of recurrence of CDD.

Results, which are controversial to those by Graff *et al*. (2008) (see non-clinical pharmacokinetics), were achieved by Scevola *et al*. (2003). They, too, evaluated acid tolerance *in vitro* and faecal recovery *in vivo* of a *Saccharomyces cerevisiae* strain (*Saccharomyces* strain unknown) after oral administration to 16 healthy volunteers. From pH 1.0 to pH 7.0 the release of *Saccharomyces cerevisiae* in buffer solutions increased. The selected yeast strain showed good tolerance to low pH, which mimics the gastric environment. After one month of treatment at a dose of 100 million cells per day, *Saccharomyces cerevisiae* grew from the faeces of 6 (37.5%) of the 16 healthy, treated volunteers. These findings, however, are of limited validity, since the dose of *Saccharomyces* administered was low (4.7 x 10^7 CFU/ml) and the drug product also contained vitamins (B1, B2, B6, B8, B12 and folic acid).

4.2. Clinical efficacy

4.2.1. Dose response studies

Not available.

4.2.2. Clinical studies (case studies and clinical trials)

At the beginning of this section, it is emphasised that in the clinical studies assessed different medicinal products containing *Saccharomyces boulardii* have been administered to the patients. Unfortunately, only in a few studies, information on the *Saccharomyces boulardii* strain and number of living cells was given. In these cases, the information was included in the assessment report. If no information is given, the authors did not include these data in their publication.

Treatment of acute diarrhoea

Höchter *et al.* (1990) assessed safety and efficacy of a *Saccharomyces boulardii* treatment for patients with acute diarrhoea (more than 3 watery stools during the last 24 hours) in a randomised double-blind, placebo-controlled, multicentre clinical trial for the reduction of stool frequency. 107 ambulatory patients were randomised. The data of 15 patients were not included in the statistical evaluation because of violation of the inclusion and exclusion criteria (to one patient additionally one antibiotic was applied which could cause diarrhoea; 14 patients had less than 3 watery stools). A total of 92 patients were included in the statistical evaluation (*Saccharomyces boulardii*: n=43; placebo: n=49). Treatment lasted 7 days. Control visits were performed on day 1, 3 and 8. Frequency and consistency of stool were the main efficacy variables, which were assessed by a score for consistency (1=formed, 2=soft, 3=liquid), multiplied by the number of stools per day. Efficacy and tolerability were evaluated on basis of a score ranging from very good to bad separately by patient and investigator. Under *Saccharomyces boulardii* the reduction of score derived from stool frequency and consistency was significantly higher than under placebo (-17.2 and –13.6, respectively; p=0.035) after 2 days of therapy. Concerning accompanying variables, there were significant advantages of the *Saccharomyces boulardii* treatment in comparison to placebo: improvement of nausea (day 3: 78.4% and 51.3% respectively (p=0.014); day 8: 100% and 81.1%, respectively (p=0.022)) and positive judgement of therapy on day 3 by patients (very good/good) 95.1% and 76.1%, respectively (p=0.013). Positive judgement of therapy on day 3 by investigator was 88.4% for *Saccharomyces boulardii* and 78.8% for placebo. On day 3 treatment with *Saccharomyces boulardii* resulted in 21%±40% liquid stools and with placebo in 22%±40%, at day 8 under treatment with *Saccharomyces boulardii* 3%±16% and under placebo 12%±33% (p=0.026).
Two adverse reactions (slight constipation and vomiting) were reported during the study drug treatment period by two patients receiving *Saccharomyces boulardii* and by two patients in the placebo group.

Mayr *et al.* (1996) treated 222 patients with acute diarrhoea with 500 mg *Saccharomyces boulardii* daily for 7 days (either 2 capsules A (n=110) or 2 capsules B (*Saccharomyces cerevisiae* CBS 5926) (n=112)) in a controlled, randomised multicentre double-blind study. Both products differed only in their excipients: lactose was contained only in B. According to the publication both products contain identical strains with, at least 10^{10} live micro-organisms/g. The average number of stools per day decreased from 7.0 (A) and/or 6.8 (B) on day 0 to 1.94 and 2.25 on days 3-6. The statistical analysis showed at least an equivalent efficacy of both products. In total, 13 patients reported adverse events. The symptoms mentioned were dizziness, recurrence of diarrhoea, nausea, vomiting, abdominal pain, dyspnoea and weakness. In one patient myocardial infarction was suspected. In the publication, the

causal evaluation was missing. However, the authors concluded that the safety of treatment with *Saccharomyces* was good.

Lacarrière and Rieckhoff (1986) assessed *Saccharomyces cerevisiae* Hansen for its efficacy in acute diarrhoea with at least three loose stools per day in a post-marketing uncontrolled study with 3,026 patients from 12 European and African countries. The patients should be older than one year; diet and concomitant oral treatment e.g. for rehydration should be applied as stated by the investigator. *Saccharomyces cerevisiae* was taken alone as a single therapy. The time of the medication was limited to 4 days. The efficacy was assessed on basis of the duration of diarrhoea. A total of 92.3% out of 2,911 cases analysed responded well to a monotherapy with *Saccharomyces cerevisiae* in acute diarrhoea. *Saccharomyces cerevisiae* was also effective in children aged between one and five years (469; 19 excluded) and the efficacy was as good as in the age group of ≥6 years (92% and 92.4%, respectively). Efficacy was also assessed in consideration of the geographic origin. In Europe 93% of patients ≤5 years and 92% of patients >5 years responded well, in Africa 86% and 94% respectively. In 77% of the patients, diarrhoea stopped within the first 3 days or less of treatment.
The tolerability of the treatment was very good. A total of eight patients reported side effects, such as abdominal pain or meteorism, which did not lead to a discontinuation of treatment.

Cottrell *et al.* (2015) conducted a prospective, randomised, single (investigator)-blind, three-arm, parallel group, non-inferiority clinical trial in adults with acute diarrhoea at clinics in Mexico and India. The patients were international travellers, expatriates and local residents and aged 18 years or over. They had symptoms of diarrhoeal illness with onset during the prior 48 hours, a minimum of three unformed stools in the 24 hours before study entry with the most recent stool unformed, and with abdominal discomfort within the prior 4 hours. 415 subjects (ITT) were randomly assigned to loperamide–simeticone 2/125 mg capsule-shaped tablet (caplet) (n=139), loperamide–simeticone 2/125 mg chewable tablets (n=139) or *Saccharomyces boulardii* 250 mg capsules (≥1.8 x 10^{10} viable cells/g) (n=137). Two dosage units of loperamide were taken initially at the investigator site, followed subsequently by one dosage unit after each unformed stool, with a maximum of 4 dosage units in a 24-hour period, for up to 48 hours. *Saccharomyces boulardii* was administered twice daily for 5 days. The primary endpoint was the number of unformed stools passed between 0 and 24 hours following the initial dose of study medication (NUS 0-24). Both loperamide-simeticone groups had a significantly lower mean NUS 0-24 than the *Saccharomyces boulardii* group (both p<0.001). Mean NUS 0-24 values were 3.3 in the loperamide–simeticone caplet group, 3.2 in the chewable tablet group and 4.3 in the S*accharomyces boulardii* group. The (upper) limit of the one-sided 97.5 % CI for the difference (caplets-tablets) was 0.48, within the predetermined non-inferiority margin. Median time to last unformed stool, to complete relief of diarrhoea and of abdominal discomfort were also significantly lower in the loperamide-groups compared to *Saccharomyces boulardii*. At 7-day follow-up most subjects reported passing stool at least once since the final study visit (loperamide–simeticone caplet 94.1%, loperamide–simeticone chewable tablet 94.8%, *Saccharomyces boulardii* 97.0%), did not experience continued or recurrent diarrhoea [loperamide–simeticone caplet 3.7% (p<0.03 vs. *Saccharomyces boulardii*), loperamide–simeticone chewable tablet 3.7%, *Saccharomyces boulardii* 5.7%] and felt completely well [loperamide–simeticone caplet 96.3% (p<0.02 vs. *Saccharomyces boulardii*), loperamide–simeticone chewable tablet 96.3% (p<0.02 vs. *Saccharomyces boulardii*), *Saccharomyces boulardii* 88.6%].
A total of 17 (4.1%) subjects experienced at least one adverse event; three (2.2%) subjects in the loperamide–simeticone caplet group, seven (5.0%) subjects in the loperamide–simeticone chewable tablet group and seven (5.1%) subjects in the *Saccharomyces boulardii* capsule group. The most commonly reported adverse events were nausea, asthenia and anorexia [in three (0.7%) subjects each, overall]. Constipation was reported by two (1.4%) subjects in the loperamide–simeticone chewable tablet group, with no reports in the other two groups. No subject experienced a serious

adverse event or was withdrawn from the study due to an adverse event.
This study was single-blinded and performed in Mexico and India without a placebo-controlled group. Therefore, this study did not assess the efficacy of *Saccharomyces boulardii* compared to placebo.

Table 6: Clinical studies (controlled and uncontrolled) on the treatment of acute diarrhoea

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute diarrhoea Höchter *et al*., 1990	Placebo-controlled, double-blind, randomised, multicentre Duration: 8 days	Per capsule:50 mg *S. cerevisiae* CBS 5926 (≥1.8 x 10^{10} viable cells/g), 6.5 mg lactose, 93.5 mg saccharose 3 times 4 capsules at day 1 and 2, 3 times 2 capsules from day 3 to day 7 Oral use	92 out of 107 randomised patients (41 female, 51 male) Verum: 43 Placebo: 49 Age: 18-65 years, mean age: 38 years	Acute adult diarrhoea with more than 3 loose stools during the last 24 hours before consultation of a doctor	Primary endpoint: score derived from stool frequency and quality (number of stools x consistency 1=formed, 2=soft, 3=loose) at day 3 Day 1 Verum 22.7±12.5 Placebo 20.3±9.7 Day 3 Verum 5.5±6.8 Placebo 6.7±8.7 P=0.035 Subgroup of patients with diarrhoea ≤2 days at time of study inclusion	Mann-Whitney-U-test for the primary endpoint and separate evaluation of stool frequency and quality Chi-square test for the other secondary endpoints	Under *S. boulardii* the reduction of score derived from stool frequency and consistency was significantly higher than under placebo (-17.2 and -13.6, respectively; p=0.035) after 2 days of therapy

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					Day 1 Verum 22.0±11.7 Placebo 19.3±7.1 Day 3 Verum 4.5±4.3 Placebo 6.6±9.2 Without significance calculation Secondary endpoints day 3 and day 8: stool frequency, stool quality, nausea, abdominal pain, vomiting, temperature, global efficacy and global tolerability		
Treatment of acute diarrhoea	Controlled, randomised multicentre double-blind	2 capsules A(n=110) or 2 capsules B(n=112) according to 500 mg *S. cerevisiae* CBS 5926	222 patients ≥18 years (128 male, 94 female)	Acute diarrhoea (not longer than 24 hours) with more than 3	Primary endpoint: stool frequency on day 2; cumulated stool frequency day 3 to day 6	Non-inferiority: confidence-interval-	Missing placebo control group, therefore, efficacy

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Mayr *et al*., 1996		(at least 10^{10} live micro-organisms/g) Duration: 7 days	ITT-population 219, because of 3 patients with missing data at day 2 (108 A, 111 B) PP-population 198: 6 patients not conform with inclusion criteria, 12 with medicine intake too low, 3 with incomplete documentation	loose stools during the last 24 hours before inclusion	Secondary endpoints: stool consistency, nausea, vomiting, overall assessment by physician and patient statistical analysis showed at least an equivalent efficacy of both products Additional explorative analysis showed a significant superiority of A concerning the cumulated stool frequency and consistency day 3 to day 6	inclusion-method, α=0.025 Superiority (explorative): covariance analysis, one-sided U-test, one-sided exact Fisher-test	cannot be assessed objectively, equivalent result of both products
Treatment of acute diarrhoea Cottrell *et al.,* 2015	Prospective, randomised, single (investigator)-blind, three-arm,	Loperamide–simeticone 2/125 mg capsule-shaped tablet (caplet) (n=139): Loperamide–	415 (ITT) international travellers, expatriates and local residents and aged 18	Onset of diarrhoea during the prior 48 hours, a minimum of	Primary endpoint: number of unformed stools passed between 0 and 24 hours following the initial dose of study medication (NUS 0–24):	The test for non-inferiority (NUS 0–24) of loperamide–simeticone caplets versus	Single blinded and performed without a placebo-controlled group

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	parallel group, non-inferiority clinical trial in Mexico and India Duration: 7 days	simeticone 2/125 mg chewable tablets (n=139): 2 dosage units initially, followed subsequently by one dosage unit after each unformed stool, with a maximum of 4 dosage units in a 24-hour period, for up to 48 hours *S. boulardii* 250 mg capsules (≥1.8 x 10^{10} viable cells/g) (n=137): twice per day for 5 days	years or over with mean age of 36.4 years (range 18-79) Male 64% Female 36% Origin: Mexico 27%, USA/Canada 23%, India 20%, Europe 17%, Russia 7%, South America 2%, Other 3%	three unformed stools in the 24 hours before study entry with the most recent stool unformed, and with abdominal discomfort within the prior 4 hours	Both loperamide-simeticone groups had a significantly lower mean NUS 0-24 than the *S. boulardii* group (both p<0.001) Some secondary endpoints: median time to last unformed stool, to complete relief of diarrhoea and of abdominal discomfort were also significantly lower in the loperamide-groups compared to *S. boulardii*	tablets was one-sided with α equal to 0.025; all other tests were two-sided at α 0.05 level Chi-square test or Fisher's exact test	
Treatment of acute diarrhoea	Open post marketing uncontrolled study	Capsules containing *S. cerevisiae* CBS 5926: ≥1.8 x 10^{10} viable cells/g (the amount of *S.*	3,026 subjects ≥1 year; 115 excluded resulted in 2911 patients >5	Acute diarrhoea with more than 3 loose stools during the last	Duration of diarrhoea Cured: 1 to 2 formed stools during 24 hours	Total population: 92.3% cured	Limited relevance for efficacy

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Lacarrière and Rieckhoff, 1986		*cerevisiae* /capsule is not given) Children 1-5 years: 3 times 3 capsules Patients >5 years: 3 times 4 capsules Treatment duration: 4 days	years and 450 ≤5 years	24 hours before consultation of a doctor	Not cured: diarrhoea lasts more than 4 days; need for another antidiarrhoeal after 2 days	Patients ≤5 years: 92% cured Patients >5 years: 92.4% cured	because uncontrolled No differences in consideration of age and geographical origin Good tolerability

Special kinds of diarrhoea and gastrointestinal disorders

Mansour-Ghanaei *et al.* (2003) enrolled 57 adult patients with acute amebiasis in a double-blind, randomised clinical trial in Iran. Three patients were excluded because of non-compliance. The patients were randomised either to regimen 1 (metronidazole (750 mg t.i.d.) and iodoquinol (630 mg t.i.d.) for 10 days, n=27) or regimen 2 (capsules of lyophilised *Saccharomyces cerevisiae* CBS 5926, 250 mg t.i.d.) orally in addition to regimen 1, n=27). Patients were re-examined at 2 and 4 weeks after the treatment, and stool was examined at the end of week 4. Student's t-test, chi-square and McNemar's tests were used for statistical analysis. In group 1, diarrhoea lasted 48.0±18.5 hours and in group 2, 12.0±3.7 hours ($p<0.0001$). In group 1, the durations of fever and abdominal pain were 24.0±8.8 and 24.0±7.3 and in group 2 they were 12.0±5.3 and 12.0±3.2 hours, respectively ($p<0.001$). Duration of headache was similar in both groups. At week 4, amebic cysts were detected in 5 cases (18.5%) of group 1 but in none of group 2 ($p<0.02$).

Besirbellioglu *et al.* (2006) evaluated the efficacy of *Saccharomyces boulardii* against *Giardia lamblia* infections in a double-blind, placebo-controlled study in Turkey. Group 1 (30 adult patients with giardiasis) included metronidazole 750 mg 3 times daily along with *Saccharomyces boulardii* capsules (250 mg b.i.d. orally, *Saccharomyces cerevisiae* CBS 5926) while group 2 (35 patients) was treated with metronidazole 750 mg 3 times daily and placebo for 10 days. In group 1, 17 patients were symptomatic (diarrhoea, abdominal pain etc.) and the other 13 patients were asymptomatic. In group 2, 15 patients were symptomatic and 20 asymptomatic. Patients were re-examined at 2 and 4 weeks after treatment, and stool examinations were performed. Statistical analysis was performed by the Mann-Whitney U-test and Fisher's exact test. At week 2, *G. lamblia* cysts were detected in 6 cases (17.1%) of group 2 and none in group 1. The proportion of patients with clearance of microscopical findings after 2 weeks was 100% (group 1) and 82.8% (group 2) ($p=0.027$). At the end of the fourth week, presence of the cysts continued in the same 6 cases in group 2. These findings indicated that *Saccharomyces boulardii* may be effective in treating giardiasis when combined with metronidazole therapy.

Saint-Marc *et al.* (1995) conducted a double-blind, placebo controlled, parallel group trial in 35 patients with stage IV AIDS to evaluate the efficacy of *Saccharomyces boulardii* in AIDS-related diarrhoea unresponsive to standard therapy. Mean age was 34.9 years. Most patients were male. The cause of diarrhoea was identified in 54.3% of cases (cryptosporidiosis in 20%). Eighteen patients were assigned to *Saccharomyces boulardii* therapy (3 times 1,000 mg per day) and 17 to placebo. Resolution of diarrhoea was recorded in 61% of *Saccharomyces boulardii* patients versus 12% of placebo patients after one week ($p<0.002$). Significant improvements were also noted in the *Saccharomyces boulardii* group regarding the daily diarrhoea score based on stool number, weight and volume ($p<0.002$), abdominal pain, abdominal distension, asthenia, weight gain and Karnofsky index. Tolerability was very good. These data show that over a one-week period *Saccharomyces boulardii* is an effective treatment for persistent AIDS-related diarrhoea.

Prevention of acute antibiotic-associated diarrhoea (AAD)

The definition of antibiotic-associated diarrhoea (AAD) is "otherwise unexplained diarrhoea that occurs in association with the administration of antibiotics." (Bartlett 2002). According to McFarland (2006) the primary outcome for AAD is diarrhoea (≥3 loose stools per day for at least 2 days or ≥5 loose stools/48 hour) within 2 months of antibiotic exposure. Its incidence depends on the kind of antibiotic administered, patient's characteristics such as age and general health status, severity of disease requiring intensive care, inpatient or outpatient treatment. Rates of up to 60% have been reported during hospital outbreaks (McFarland 2006). Symptoms vary in intensity from mild to life-threatening and may occur soon after the initiation of antibiotic treatment or up to 2 weeks after the end of antibiotic therapy (Micklefield 2014). The antidiarrhoeal effects of *Saccharomyces boulardii* may be

explained pharmacologically by its trophical (increase of short chain fatty acids in the colon) and antitoxin effects (Micklefield 2014).

In a review by McFarland (2010) on the use of *Saccharomyces boulardii* in adults, 10 randomised controlled clinical studies (see Table 7, no. 1-7, Cremonini *et al.*, 2002a, Duman *et al.*, 2005, Cindoruk *et al.*, 2007, see Table 8) have been included. According to the meta-analysis of these studies, *Saccharomyces boulardii* was assessed as significantly protective of AAD.

This effect has also been described in another meta-analysis by McFarland (2006) who investigated the efficacy of probiotics for the prevention of AAD and treatment of CDD (*Clostridium difficile* disease) on the basis of published randomised, controlled clinical trials (no. 1; 3; 4; 5; Kotowska *et al*., 2005; Cremonini *et al.*, 2002a). From their meta-analysis on the effect of probiotic administration on AAD which included the clinical trials no. 3-5, Cremonini *et al*. (2002b) concluded that the results obtained suggest a strong benefit of probiotic administration on AAD, but that the evidence for beneficial effects is still not definitive. According to the authors, published studies are flawed by the lack of a placebo design and by peculiar population features.

D'Souza *et al*. (2002) included 9 randomised, double-blind, placebo-controlled clinical trials in their meta-analysis on probiotics in the prevention of AAD. In 4 of these trials (no. 1; 3; 4; 5), *Saccharomyces boulardii* was administered. The authors concluded that probiotics can be used to prevent AAD and that *Saccharomyces boulardii* has the potential to be used in this situation. The efficacy of probiotics in this indication still remains to be proved.

Another meta-analysis published by Szajewska and Mrukowicz (2005) evaluated the effectiveness of *Saccharomyces boulardii* in the prevention of AAD in children and adults. Five randomised clinical studies (no. 1; 3; 4; 5; Kotowska *et al.*, 2005, n=1,076 patients) have been included in the review and *Saccharomyces boulardii* was assessed as moderately effective in preventing AAD in children and adults treated with antibiotics. According to them, for every 10 patients receiving daily *Saccharomyces boulardii* with antibiotics, one fewer will develop AAD.

In 2012, another systemic review and meta-analysis on probiotics for the prevention and treatment of AAD was published by Hempel *et al*. They assessed parallel randomised controlled trials with different probiotics for the prevention of AAD and found that the pooled evidence suggests that probiotics are associated with a reduction in AAD. Nevertheless, more research is needed to determine whether this association varies systematically by population, antibiotic characteristic or probiotic preparation.

Xie *et al*. (2015) published a systematic review concerning probiotics for the prevention of antibiotic-associated diarrhoea (AAD) and *Clostridium difficile* diarrhoea (CDD) in older patients. Six trials with a total of 3,562 patients were included. Only one of them investigated *Saccharomyces cerevisiae (boulardii)* in 69 patients; however, a benefit from *Saccharomyces boulardii* in preventing AAD and CDD in this patient group was not identified (Lewis *et al*. (1998)). In their review, the authors concluded that probiotics may not reduce the risk of AAD and CDD in older patients. More robust studies were demanded, which should include large samples sizes, multicentre and double-blind designs and should isolate and examine factors such as probiotic strains and the types of antibiotic.

Szajewska and Kolodziej (2015b) published an update of their 2005 meta-analysis. In addition to the previously identified five randomised clinical trials, 16 new trials were included. A total of 4,780 participants (2,441 in the experimental group and 2,339 in the control group) were included. 12 studies were placebo-controlled; in the remaining studies, there was no intervention in the control group. 15 trials (Chu *et al.* 2012, Table 7 except Ehrhardt *et al.* (2016) and Table 8 except Lee *et al.* (2011)) were performed in adults, and six trials in children (see below). Only two trials (Kotowska *et al.* 2005 and Pozzoni *et al.* 2012) were at low risk of bias. The daily dose of *Saccharomyces boulardii* ranged from 50 mg to 1,000 mg. In adults, compared with placebo or no treatment, *Saccharomyces*

boulardii reduced the risk of diarrhoea from 17.4% to 8.2% (15 RCTs, n=3,114; relative risk (RR): 0.49, 95% CI: 0.38-0.63; number needed to treat (NNT): 11, 95% CI: 9-15). Subgroup analysis based on age showed that the administration of *Saccharomyces boulardii* did not reduce the risk of *C. difficile*-associated diarrhoea in adults. One major limitation is that the methodological quality of included trials varied. Definition of AAD and/or diarrhoea differed. There were wide differences in the duration of follow-up, which varied from 2 weeks to 1 year after cessation of antibiotic treatment, or it was not specified. The optimal dose of probiotics, including *Saccharomyces boulardii*, and the duration of treatment have not been established. The only study with low bias in adults (Pozzoni *et a*l. 2012) showed that *Saccharomyces boulardii* was not effective in preventing the development of AAD. Data regarding therapy-related adverse effects were available from 16 of the included trials. In these trials, *Saccharomyces boulardii* was well tolerated. Adverse events rate was similar in experimental and control groups.
One important question remains according to the authors whether the use of *Saccharomyces boulardii* shall be considered 'in all subjects receiving antibiotics or only in select populations'. This will require clinical judgement.

Apart from the studies listed in the Table 7, another clinical study has been identified in literature (Table 7: no. 9) which has not been included in the meta-analyses mentioned above.

Ehrhardt *et al.* (2016) (Table 7: no. 9) performed a multicentre, randomised, double-blind, placebo-controlled phase III study in hospitalised patients, who received systemic antibiotic treatment in 15 hospitals in Germany between July 2010 and October 2012. Participants received 250 mg capsules *Saccharomyces boulardii* (at least 1.8×10^{10} live cells/g lyophilisate) or a matching placebo orally twice per day within 24 hours of initiating antibiotic treatment, continued treatment for 7 days after antibiotic discontinuation, and were then observed for 6 weeks. 2,444 patients $\geq$ 18 years were screened. The trial was stopped early for futility after inclusion of 477 participants. 246 patients aged 60.1 ± 16.5 years and 231 patients aged 56.5 ± 17.8 years were randomised to the *Saccharomyces boulardii* group and the placebo group, respectively. 21 AADs occurred in the *Saccharomyces boulardii* group and 19 AADs in the placebo group (p=0.87). Two cases of *Clostridium difficile*-associated diarrhoea (CDAD) were observed in each group. None of the prespecified factors (centre, age, sex, duration of antibiotic treatment, and readministration of antibiotics) were associated with risk of AAD. Nine serious adverse events were recorded in the *Saccharomyces boulardii* group, and 3 serious adverse events in the placebo group. None were related to study participation. The authors found no evidence for an effect of *Saccharomyces boulardii* in preventing AAD or CDAD.

Table 7: Clinical studies on the prevention of acute antibiotic-associated diarrhoea (AAD) with *Saccharomyces boulardii* in adults

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Prevention of AAD Adam *et al*., 1977	Placebo-controlled, randomised, multicentre	*S. cerevisiae* CBS 5926: 4 cps per day (i.e. 200 mg per day) Duration: 7 days	Verum: n=199 (96 male/103 female) Placebo: n=189 (96 male/93 female) Mean age: Verum: 39.3 years Placebo: 37.6 years	Administration of oral antibiotic treatment (tetracycline or beta-lactam) for ≥5 days because of broncho-pulmonary or ENT infection Age: >15 years	Occurrence of diarrhoea or candida infection Diarrhoea: Verum: 4.5% Placebo: 17.5% Candidiasis: Verum: 2.0% Placebo:12.2%	Diarrhoea/ candidiasis: Chi-square test Both tests were highly significant	Older investigation, publication in French only, follow-up too short
Prevention of AAD Monteiro *et al*., 1981	Placebo-controlled, double-blind	*S. cerevisiae* CBS 5926: 4 cps per day Duration: 6 days	Verum: n=121 Placebo: n=119	Administration of oral antibiotic treatment (tetracycline or beta-lactam)	Occurrence of diarrhoea (>2 bowel motions per day) or candida infection diarrhoea Verum: 15.7% Placebo: 27.7% Candidiasis:	Diarrhoea/ candidiasis: student's t-test	Older investigation, publication in Portuguese only

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					Verum: 1.7% Placebo: 10.7%		
Prevention of AAD Surawicz *et al*., 1989a	Placebo-controlled, double-blind, 2:1 randomisation	*S. boulardii*: 1,000 mg per day within 48 hours after start of antibiotic treatment Duration: 2 weeks after last antibiotic therapy	318 patients enrolled. 138 patients not included into statistical evaluation n=180 patients for statistical evaluation Verum: n=116 Placebo: n=64 Mean age: 47.8 years Male: 68.9%	In-patients receiving new antibiotic treatment for ≥3 days	Incidence of diarrhoea (≥3 loose or watery stools per day for at least 2 days): Verum: 9.5% Placebo: 21.8%	Incidence of diarrhoea: Chi-square test (statistically significant difference)	Antibiotic therapy not specified
Prevention of AAD McFarland *et al*., 1995	Placebo-controlled, double-blind, multicentre, 1:1 randomisation	Lyophilised *S. boulardii* 1 g per day (3 x 10^{10} CFU) within 72 hours of the start of antibiotic	n=193 patients Verum: n=97 Mean age: 40.7 years	In-patients receiving new prescriptions for at least one beta-	Incidence of diarrhoea (≥3 loose or watery stools per day for at least 2 days):	Incidence of diarrhoea Binominal exact test (statistically significant)	Efficacy investigated in the prevention of beta-lactam associated diarrhoea only

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	USA	treatment; administration until 3 days after the discontinuation of antibiotic treatment Follow-up for 7 weeks	Male: 63.9% Placebo: n=96 Mean age: 42.3 years Male: 65.6%	lactam antibiotic for ≥48 hours	ITT-population verum: 7.2% placebo: 14.6%		
Prevention of AAD Lewis *et al*., 1998	Placebo-controlled, randomised UK	*S. boulardii* (*S. cerevisiae* CBS 5926) 113 mg b.i.d. throughout antibiotic therapy	n=69 patients Verum: n=33 Mean age: 75 years Placebo: n=36 Mean age: 77 years	Patient older than 65 years with antibiotic treatment within the preceding 24 hours	Incidence of diarrhoea (≥3 loose stools per day) Verum: 21% Placebo: 13.9%	2-tailed Mann-Whitney or chi-square test No statistically significant difference	Daily dose of *S. boulardii* low No follow-up after the end of antibiotic therapy Only elderly patients included
Prevention of AAD Can *et al*., 2006	Randomised, antibiotic + placebo vs antibiotic + *S. boulardii*,	*S. boulardii* 48 hours after initiation of antibiotic therapy	n=151 Verum + antibiotic: n=73	In-patients (age: 25-50 years) with chemotherapy not	Incidence of diarrhoea Verum: 1.4% Placebo: 9.0%	Student's t-test: statistical significance	Age was found to be a risk factor of AAD. Age was significantly higher in the AAD

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	Turkish in-patients	at a dosage of 500 mg per day 250 mg contains 5 x 10^9 CFU Observation period: 4 weeks after the end of antibiotic treatment	Placebo + antibiotic: n=78	requiring intensive care			than in the non-AAD group No information given, if the treatment groups were comparable initially
Prevention of AAD Bravo *et al*., 2008	Randomised, double-blind, Chile	*S. boulardii* 500 mg per day for 12 days. (≥1.2 x 10^{10} viable cells)	n= 86 Age: 15-81 years Verum: n=41 Placebo: n=45	Adult patients with acute infectious diseases receiving treatment with amoxicillin for 5-10 days	Incidence of diarrhoea Verum: 9.8% Placebo:11.1%	No statistically significant differences between both treatments	Only abstract in English
Prevention of AAD Pozzoni *et al*., 2012	Placebo-controlled, randomised, double-blind, single-centre	*S. boulardii* (*S. cerevisiae* CBS 5926). 5 x 10^9 CFU b.i.d. within 48 hours	n=275 Verum: n=141 mean age: 79.9 years	Hospitalised patients >50 years of age with antibiotic therapy for<48 hours	Incidence of diarrhoea (>3 passages of liquid stool per day for at least 2 days or	No statistically significant difference Chi-square test, Fisher's exact test	Only elderly hospitalised patients included *S. boulardii* was not effective in preventing

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		after the start of antibiotic therapy Treatment duration: 7 days Follow-up: 12 weeks after the end of antibiotic treatment	placebo: n=134 mean age: 78.5 years		≥5 passages within 48 hours) Verum: 15.1%, Placebo: 13.3%		the development of AAD
Prevention of AAD Ehrhardt *et al*. 2016	Placebo-controlled, randomised, double-blind, multicentre	250 mg capsules *S. boulardii* (at least 1.8 x 10^{10} live cells/g lyophilisate) or a matching placebo orally b.i.d. within 24 hours of initiating antibiotic treatment, continued treatment for 7 days after	n=477 ITT analysis. n=292 (with complete observations) PP analysis Verum: n=246 Mean age: 60.1 ± 16.5 years. Placebo: n=231 Mean age: 56.5 ± 17.8 years	Hospitalized patients ≥18 years of age with systemic antibiotic therapy	Primary endpoint (ITT): Risk of AAD (diarrhoea (passage of 3 or more loose or liquid stools (mostly in larger amounts) within 24 hours for at least 2 days) with onset not before the 3rd day of antibiotic treatment)	No statistically significant differences between both treatments Primary endpoint: Prentice, Williams and Peterson (PWP) model analyses Secondary endpoints:	No evidence for an effect of *S. boulardii* in preventing AAD or CDAD limitations e.g.: Based on a 15% incidence of AAD, 686 patients would be needed in each group for 80% power to detect 5% difference in the cumulative incidence of AAD

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		antibiotic discontinuation Follow-up: 6 weeks after the end of antibiotic treatment	2,444 patients were screened. The trial was stopped early for futility after inclusion of 477 participants.		Verum=21 episodes of AAD Placebo: 19 episodes of AAD p=0.87, hazard ratio of AAD in the verum group compared with the placebo group: 1.02 (95% CI, 0.55-1.90; p=0.94) Secondary endpoints: Risk of CDAD: 2 cases in each group Mean duration of AAD: Verum=4.48 days Placebo=4.26 days p=0.79	Cox proportional hazar model Cochran-Mantel-Haenszel χ^2 test The findings were robust in the PP as well as several sensitivity analyses	between treatment group. Unplanned, masked interim analysis according to the Müller and Schäfer procedure due to slow recruitement and unexpectedly few events. The authors concluded that based on the lower observed incidence of AAD in the 2 groups, they still would not have found a difference between the groups, if they had reached the target sample size. 1,967 of 2,444 screened patients

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					Mean time to onset: Verum=18.4 days Placebo=18.9 days		were ineligibile for participation, one third due to contraindications. 100 cases (verum) and 85 cases (placebo) with missing or incomplete data concerning stool frequencies. However, ITT analysis used all available data.

Prevention of acute antibiotic-associated diarrhoea (AAD) caused by triple therapy of *Helicobacter pylori* infection

Helicobacter pylori, which was first discovered by Marshall and Warren in 1984, is an important factor in the pathogenesis of gastroduodenal ulcers and gastritis. In addition, chronic infections with *H. pylori* are associated with an increased risk for gastric carcinoma and gastric MALT (Mucosa-associated lymphoid tissue)-lymphoma (Fischbach *et al.*, 2009). According to the guideline on *Helicobacter pylori* and gastroduodenal ulcer disease (Fischbach *et al.*, 2009) in Germany the prevalence of *H. pylori* infections ranges between 5% (children) and 24% (adults) and is higher in immigrants (36-86%). Triple therapy (combination of proton pump inhibitor (PPI) plus amoxicillin plus clarithromycin) is indicated for the eradication of *H. pylori* infections. In order to evaluate if the administration of *Saccharomyces boulardii* is effective in preventing side-effects (e.g. occurrence of diarrhoea) of triple therapy for *Helicobacter pylori* infection, several clinical studies have been performed (Table 8).

Cremonini *et al*. (2002a) included 85 *H. pylori* positive, asymptomatic patients in their clinical study who were randomised in four groups (*Lactobacillus casei* subsp. *rhamnosus* GG, *Saccharomyces boulardii*, *Lactobacillus* spp+biphidobacteria, placebo) to receive probiotic or placebo treatment during and for 7 days after a 1-week triple therapy (20 mg rabeprazole b.i.d., clarithromycin 500 mg b.i.d., tinidazole 500 mg b.i.d). 21 patients were randomised to receive *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926, 5 x 10^9 CFU/sachet, b.i.d.), 21 patients to placebo. For the assessment of side-effects, a questionnaire developed by de Boer *et al*. (1996) was used. This questionnaire was proposed as a standard side-effect scoring instrument for exploring *H. pylori* treatment regimens. The questionnaire included the following items: loss of appetite, nausea, vomiting, taste disturbance, dizziness, stomach pain, diarrhoea during treatment, diarrhoea after treatment, headache, rash, others). During the first week of treatment, the incidence of diarrhoea was 5% in the *Saccharomyces boulardii* group and 30% in the placebo group (p=0.018). As compared to the placebo group, during week 2 incidence of diarrhoea remained lower in the *Saccharomyces boulardii* group, however, with borderline significance. Taste disturbance, too, was observed significantly less frequently in the *Saccharomyces boulardii* group (5% vs. 40%, p=0.0027). No major adverse events were reported.

In a multicentre open clinical trial, Duman *et al.* (2005) enrolled 389 patients with peptic ulcer disease or non-ulcer dyspepsia for *H. pylori* eradication therapy with clarithromycin (500 mg b.i.d.), amoxicillin (1,000 mg b.i.d.), and omeprazole (20 mg b.i.d.) for 14 days. These patients were then randomised to *Saccharomyces boulardii* (500 mg b.i.d.) (n=204) or no treatment (n=185). The aim of the study was to assess the efficacy and safety of *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea due to *H. pylori* eradication. Diarrhoea was defined as a change in bowel habits with at least 3 semi-solid or watery bowel movements per day for at least two consecutive days. The patients were observed during the entire treatment period and the following 4 weeks. The incidence of diarrhoea during the overall study period was 6.9% in the treatment and 15.6% in the control group (p=0.007). In one patient, treatment with *Saccharomyces boulardii* was discontinued because of a skin reaction. Although the validity of this study is limited due to its open design, it indicates that *Saccharomyces boulardii* may be effective in preventing antibiotic-associated diarrhoea in patients receiving triple treatment for eradication of *H. pylori* infection.

In a double-blind clinical study, Cindoruk *et al.* (2007) randomised 124 patients with *H. pylori* infection receiving 2 weeks of triple therapy (clarithromycin 500 mg b.i.d., amoxicillin 1,000 mg b.i.d., lansoprazole 30 mg b.i.d.) to *Saccharomyces boulardii* (n=62) at a dosage of 500 mg b.i.d. (*Saccharomyces cerevisiae* CBS 5926) or placebo (n=62). Side-effects of treatment were recorded by the questionnaire according to de Boer *et al.* (1996). This questionnaire was filled out by the patients during the 2 weeks of treatment and the following 2 weeks. During the overall study period, 9 patients

(14.5%) in the treatment group and 19 patients (30.6%) of the control group experienced diarrhoea ($p<0.05$). Statistically significant differences between the groups were also detected with regard to epigastric discomfort: 9 (14.5%) patients in the *Saccharomyces boulardii* versus 27 (43.5%) patients in the control group experienced epigastric discomfort ($p<0.01$). The incidence of diarrhoea during the treatment phase was 11.2% in the *Saccharomyces boulardii* group and 25.8% in the control group, during follow-up the respective incidences were 3.2% and 4.8%. The overall assessment of tolerability on a 5-point scale was significantly superior in the treatment group ($p<0.001$). No major side-effects were observed.

Song *et al.* (2010) evaluated the additive effects of *Saccharomyces boulardii* and *Saccharomyces boulardii*+mucoprotective agent to PPI-based triple therapy alone. 991 patients with *H. pylori* infections were randomised to one of the 3 groups. The first group received triple therapy alone, which consisted of 20 mg omeprazole, 1,000 mg amoxicillin and 500 mg clarithromycin, twice a day for 7 weeks (n=331). In addition to triple therapy, in the second group, one capsule with *Saccharomyces boulardii* (3×10^{10} CFU/g, 250 mg *Saccharomyces cerevisiae* CBS 5926) was given three times a day for 4 weeks (n=330). The third group receiving also a mucoprotective agent is not considered here. During the study period, patients had to note the side-effects into a diary. Diarrhoea occurred more frequently in the group with triple therapy alone (6%) than in the group with the addition of *Saccharomyces boulardii* (3.3%). The frequency of overall side-effects in the *Saccharomyces boulardii* group was statistically significantly lower than that in the group with triple therapy alone (14.5% vs. 19%; $p<0.05$).

Another randomised clinical trial to investigate the efficacy and safety of adding the probiotic *Saccharomyces boulardii* to standard triple therapy for eradication of *H. pylori* was performed by Zojaji *et al.* (2013). 80 patients were randomised to treatment with amoxicillin (1,000 mg, b.i.d.), clarithromycin (500 mg, b.i.d.), omeprazole (20 mg, b.i.d.) and *Saccharomyces boulardii* (250 mg, b.i.d., *Saccharomyces cerevisiae* CBS 5926) for 14 days (groupe A). 80 patients received triple therapy alone (group B). Patients were asked to report any side effects of therapy during the treatment period (end of first, second, third and fourth weeks of treatment) and were given a possible side effect list, such as epigastric pain, diarrhoea, taste disturbance, constipation and stomatitis. The frequency of side effects such as nausea, diarrhoea, abdominal discomfort and bloating in the *Saccharomyces boulardii* group A was significantly lower than that in the group B in first and second weeks ($p<0.05$). No remarkable side-effects of treatment with *Saccharomyces boulardii* were noted.

Kyriakos *et al.* (2013) investigated if *Saccharomyces boulardii* enhances the efficacy of classic triple therapy in eradicating *H. pylori*. 70 patients with peptic ulcer or functional dyspepsia according to Rome III criteria and *H. pylori* infection were treated with omeprazole 20 mg b.i.d., clarithromycin 500 mg b.i.d. and amoxicillin 1 g b.i.d. for 14 days. A total of 36 out of 70 (51%) patients were randomised to *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926), two capsules t.i.d. for 14 days (group A) and 34 (49%) on no intervention (group B). Seven patients in group B (20.6%) and 1 patient in group A (2.8%) stopped treatment because of diarrhoea (95% CI 3.3% to 32.7%, $p=0.026$).

In another study by Chu *et al*. (2012) on the efficacy and safety of *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926 250 mg b.i.d. for 14 days) in combination with PPI-based triple therapy (omeprazole 20 mg b.i.d., amoxicillin 1 g b.i.d., clarithromycin 0.5 g b.i.d. for 14 days) for *H. pylori* related peptic ulcer, a significant difference in the rate of adverse effects was observed. 100 patients were randomly assigned to treatment with standard triple therapy alone (n=50) or standard triple therapy plus *Saccharomyces boulardii* (n=50). The most common side-effects were mild to moderate and self-limiting (e.g. nausea, vomiting, diarrhoea, melena, dizziness). The incidence of side-effects in the group receiving *Saccharomyces boulardii* was statistically significantly lower than in the

control group (15.6% vs. 57.8%; p<0.01). As in this clinical study the incidence of the diarrhoea was not stated explicitly, the study was not included in Table 8.

The effect of *Saccharomyces boulardii* as an adjuvant to a 14-day triple therapy (clarithromycin 500 mg b.i.d., amoxicillin 1,000 mg b.i.d., lansoprazole 30 mg b.i.d.) for eradication of *H. pylori* has been investigated by Lee *et al.* (2011) and published as an abstract. 223 patients infected by *H. pylori* were randomised to 14 days of triple therapy alone (n=116, control group) or triple therapy in combination with *Saccharomyces boulardii* (n=107, treatment group). Side-effects of treatment were assessed by means of a validated questionnaire for 2 weeks from the start of therapy. The incidence of diarrhoea was 29.9% in the treatment group and 43.1% in the control group. This difference was statistically significant (p=0.036). Two patients from the treatment goup and 5 patients from the control group stopped treatment because of adverse events. The validity of this clinical study is also limited due to its open design. The dosage of *Saccharomyces boulardii* is not mentioned in the abstract.

In 2010, a meta-analysis on randomised controlled trials was performed by Szajewska *et al*. to investigate the effects of *Saccharomyces boulardii* as supplementation to standard triple therapy on *H. pylori* eradication rates and therapy-associated side effects. Five clinical studies with 1,307 patients in total were included: 4 studies were performed in adults (Cindoruk *et al.,* 2007, Cremonini *et al.,* 2002a, Duman *et al.,* 2005, Song *et al*., 2010), one study included children (Hurduc *et al*., 2009; see: prevention of AAD in children). Szajewska *et al*. (2010) observed a statistically lower risk of therapy-related diarrhoea in patients receiving concomitant treatment with *Saccharomyces boulardii*. Treatment with *Saccharomyces boulardii* compared with placebo reduced the risk of AAD from 17.2% to 6.7% (RR: 0.47, 95% CI: 0.32-0.69, NNT: 16, 95% CI: 11-30). Due to the majority of patients being adults, the authors stated that their results may be applicable only to this population. The daily doses of *Saccharomyces boulardii* ranged from 500 mg to 1,000 mg. The authors concluded that, in patients with *H. pylori* infection, there is evidence to recommend the use of *Saccharomyces boulardii* along with standard triple therapy as an option for decreasing overall therapy-related side effects, particularly diarrhoea.

In 2015, Szajewska *et al.* updated their meta-analysis on supplementation of triple therapy with *Saccharomyces boulardii*, as since then more randomised clinical studies had been published in this indication: Zhao *et al.* (2014), Zojaji *et al.* (2013), Kyriakos *et al.* (2013), Chu *et al.* (2012), Gao *et al.* (2012), Lee *et al.* (2011). The studies by Chu *et al*. (2012) and Gao *et al.* (2012) had been published in Chinese and had been translated in order to allow an assessment by the authors.

In this assessment report, these two studies are not included, since a translation is not available. In the meta-analysis of Szajewska *et al.* (2015), a total of 2,000 patients were included, of them 330 children with an age range from 3-18 years. As compared to triple treatment alone, the concomitant administration of *Saccharomyces boulardii* reduced the risk of overall adverse effects, especially diarrrhoea (RR: 0.51, 95% CI: 0.42-0.62; high quality evidence). None of the trials reported on adverse effects other than those related to eradication therapy. As the majority of patients included in this meta-analysis were adults, more studies are needed in children.

Table 8: Clinical studies on the prevention of acute antibiotic-associated diarrhoea (AAD) with *Saccharomyces boulardii* in adults caused by triple therapy of *Helicobacter pylori* infection

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Prevention of AAD due to triple therapy of *H. pylori* infection Cremonini *et al*., 2002a	Placebo-controlled, triple-blind, randomised	*S. cerevisiae* CBS 5926; 5 x 10^9 CFU b.i.d. during the antibiotic week and 1 week thereafter Observation period: 4 weeks	n=97 Verum: n=22 Placebo: n=21 Age: 18-61 years Male: n=43 Female: n=54	Symptom-free, *H. pylori* positive patients receiving triple antibiotic treatment (rabeprazole/clarithromycin/tinidazole) for 7 days	Incidence of diarrhoea during the first week Verum: 5% Placebo: 30%	Chi-square test: statistical significance (p=0.018)	Small study groups
Prevention of AAD due to triple therapy of *H. pylori* infection Duman *et al*., 2005	Open, randomised, *S. boulardii* vs. no treatment, multicentre	*S. cerevisiae* CBS 5926; 2 times 500 mg per day for 2 weeks Follow-up: 4 weeks	n=389 Verum: n= 204 Control: n= 185 Verum: Male: 50% Mean age: 45.7 years. Control: Male: 47.5%	Patients with peptic ulcer or non-ulcer dyspepsia receiving triple antibiotic therapy (clarithromycin/amoxicillin/omeprazole) for *H. pylori* for 2 weeks	Incidence of diarrhoea (≥3 semi-solid or watery stools per day on 2 consecutive days) Verum: 6.9% Control: 15.6%	Chi-square test: statistical significance p=0.007	Due to open study design, only limited validity

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
			Mean age: 44.7 years				
Prevention of AAD due to triple therapy of *H. pylori* infection Cindoruk *et al*., 2007	Placebo-controlled, randomised, double-blind	*S. boulardii* 2 x 500 mg per day for 2 weeks Follow-up: 6 weeks after the end of treatment	n=124 Verum: n=62 Male: 41.9% Mean age: 45.8 years Placebo: n=62 Male: 29% Mean age: 47.6 years	Patients receiving 2 weeks of triple therapy (clarithromycin, amoxicillin, lansoprazole) for *H. pylori* eradication	Prevention of side-effects related to eradication therapy; frequency of diarrhoea Verum: 11.2% (treatment phase) 3.2% (follow-up) 14.5% (overall) Placebo: 25.8% (treatment phase) 4.8% (follow-up) 30.6% (overall)	Chi-square test: statistical significance	*S. boulardii* improved treatment tolerability
Prevention of AAD due to triple therapy	Randomised, controlled	*S. cerevisiae* CBS 5926; 3 x 10^9 CFU/250 mg?	Group + *S. boulardii*: n=330	Patients with *H. pylori* infection receiving proton pump inhibitor based triple treatment (omeprazole/amoxicilli	Incidence of diarrhoea group + *S. boulardii*: 3.3%	Statistically significant difference ($p<0.05$)	No double-blind study design

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
of *H. pylori* infection Song *et al*., 2010		750 mg per day for 4 weeks Follow-up: 5-8 weeks	Mean age: 49.8 years Control group: n=331 Mean age: 49.8 years	n/clarithromycin) for 7 days	Control group: 6.0%		
Prevention of AAD due to triple therapy of *H. pylori infection* Zojaji *et al*., 2013	Randomised, controlled	*S. boulardii* (250 mg b.i.d.) for 14 days Group A: triple therapy + *S. boulardii*. Group B: triple therapy alone Observation period: 4 weeks	n=160 Mean age: 47.1 years Female: 58.7% n=80 in each group	Adult patients (>15 years of age) with *H. pylori* infection receiving triple treatment with amoxicillin/clarithromycin/omeprazole for 14 days	Occurrence of diarrhoea Group A: Week 1/2/3/4 13%/12.5%/6.3%/1.3 % Group B: 30%/26.3%/11.3%/5%	Non-parametric t-test, chi-square test Statistically significant difference in favour of *S. boulardii* in week 1 and 2	No double-blind study design

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Prevention of AAD due to triple therapy of *H. pylori* infection Kyriakos *et al*., 2013	Prospective, randomised, controlled, single-centre	Group A: triple therapy plus *S. boulardii* for 14 days Group B: triple therapy alone Triple therapy: Clarithromycin/ amoxicillin/ omeprazole	n=70 Group A: n=36 Mean age: 47 years. Group B: n=34 Mean age: 45 years	Adult patients (18-75 years) with *H. pylori* related peptic ulcer disease or functional dyspepsia during a 3-year period	Withdrawals because of the occurrence of diarrhoea: Group A: 2.8% Group B: 20.6%	Statistical significance p=0.026	No double-blind clinical trial
Prevention of AAD due to triple therapy of *H. pylori* infection. Lee *et al*., 2011	Randomised, controlled	Control group: triple therapy for 14 days Treatment group: triple therapy for 14 days + *S. boulardii*	Control group: n=116 Treatment group: n=107	223 patients infected with *H. pylori*	Side-effects of treatment, tolerability by means of a validated questionnaire for 2 weeks from the start of the study Frequency of diarrhoea: Treatment group: 29.9% Control group: 43.1%	Statistical significance p= 0.041	Dosage of *S. boulardii* not mentioned; open study design; abstract only

Prevention of recurrence of *Clostridium difficile* disease (CDD)

According to Bartlett (2002) about 10 to 20% of the cases of AAD are caused by infections with *Clostridium difficile*. Most of the cases of colitis due to antibiotic therapy, however, are associated with *C. difficile*. Antibiotics, which are most commonly implicated with CDD, are clindamycin, cephalosporins and penicillins. Clinical symptoms of CDD may range from uncomplicated diarrhoea to pseudomembranous colitis (PMC). For the standard treatment of CDD, the antibiotics vancomycin and metronidazole are used. Although this medication is effective in 80% of the patients with CDD, in 20% further episodes of diarrhoea or colitis occur within 3 to 28 days after the end of antibiotic treatment (Mc Farland *et al*., 1994).

Several clinical studies have been performed in this indication with *Saccharomyces boulardii* given as an adjunct to standard treatment. In 1989b Surawicz *et al*. published the results of an open trial, which investigated the efficacy of *Saccharomyces boulardii* for the treatment of recurrences of *C. difficile*-associated colitis in humans. Thirteen patients with recurring *C. difficile* cytotoxin-positive diarrhoea were treated with 10 days of vancomycin and a 30-day course of *Saccharomyces boulardii* (500 mg b.i.d.). Eleven (85%) had no further recurrences. The authors concluded that *Saccharomyces boulardii* may have a role in treating recurrent *C. difficile* diarrhoea and colitis. Due to its open design, the study is not included in the following table (Table 9).

According to McFarland (2010) two randomised, double-blind clinical studies have been performed with *Saccharomyces boulardii* for the treatment of CDD. Both trials included patients, who suffered from diarrhoea and had a positive culture for *C. difficile*. While McFarland *et al.* (1994) observed a statistically significant reduction of relapses with *Saccharomyces boulardii* only in the subgroup of patients with recurrent disease (34.6% vs. 64.7%; p=0.04), Surawicz *et al*. (2000) reported that, only in the subgroup of patients receiving treatment with high dose vancomycin and *Saccharomyces boulardii*, relapses of CDD tended to decrease. Statistically significant differences were not demonstrated in this study.

Both clinical trials have also been assessed in a review by Tung *et al*. (2009). The authors concluded that *Saccharomyces boulardii* may be effective for secondary prevention of CDD in some specific patient populations with particular concurrent antibiotic treatment.

Dendukuri *et al*. (2005) included the randomised clinical studies listed here in their review on probiotic therapy for the prevention and treatment of *C. difficile*-associated diarrhoea.

They calculated the following risk difference (95% CI) (placebo-probiotic):

McFarland *et al*., 1994:		20.5 (2.2 to 37.0)
	First time CDD:	4.9 (-17.6 to 26.4)
	Recurrent CDD:	30.0 (2.3 to 50.6)
Surawicz *et al*., 2000:		9.8 (-6.7 to 25.6)
	High dose vancomycin:	33.0 (-0.3 to 62.0)
	Low dose vancomycin:	-6.4 (-16.2 to 28.1)
	Metronidazole:	-1.9 (-25.8 to 29.2)

Only McFarland *et al.* (1994) reported a significant beneficial effect overall. In a post hoc analysis, the authors found that this effect was almost entirely limited to the subgroup with recurrent CDD.

According to Dendukuri *et al*. (2005), the studies conducted to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat CDD in adults. Better designed and larger studies are needed.

In their guidelines for *Clostridium difficile* infection (CDI) in adults, Cohen *et al*. (2010) assess the use of probiotics for the primary prevention of CDI as follows: "*Administration of currently available probiotics is not recommended to prevent primary CDI, as there are limited data to support this approach and there is a potential risk of bloodstream infection*."

Flatley *et al.* (2015) performed a retrospective chart review to evaluate the effects of the removal of *Saccharomyces boulardii* from an automatic antibiotic order set and hospital formulary on hospital onset *C. difficile* infections (hCDI) rates. At an USA hospital, an increase in the incidence of CDI in 2006 had prompted the following changes: 1) more stringent cleaning procedures; 2) CDI positive patients were isolated and placed in contact precautions and 3) implementation of a protocol that linked an order to initiate *Saccharomyces boulardii* therapy (250 mg b.i.d.) to patients receiving antibiotics highly associated with causing CDI like i.v. formulation of clindamycin, cefepime, ceftazidime, ceftriaxone, cefuroxime and fluoroquinolones. In 2009, the hospital re-evaluated its CDI prevention strategies and *Saccharomyces boulardii* was removed. In their retrospective analysis, Flatley *et al*. compared a control group admitted from November 2008 through November 2009 to the study group admitted from January 2010 through January 2011. The primary outcome was the incidence rates of hCDI in all hospitalised patients during the control and study group. There were 167,157 hospital patient days with 167 hCDI cases in the control group and 183,867 hospital patient days with 191 hCDI cases in the study group, without statistically significant difference in the incidence of hCDI between the two groups. Receipt of linked antibiotics was similar in patients acquiring hCDI. The secondary outcome was the incidence of hCDI among patients, who received a linked antibiotic. In total, 8,708 patients in the control group and 8,411 patients in the study group were treated with linked antibiotics. 109 (1.25%) and 127 (1.51%) patients acquired hCDI in the control and study group, respectively (p=0.698). The authors concluded that administering *Saccharomyces boulardii* with i.v. broad-spectrum antibiotics as prophylaxis was not effective for preventing hCDI.

Table 9: Clinical studies on the prevention of recurrence of *Clostridium difficile* disease (CDD)

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Prevention of recurrent CDD McFarland *et al*., 1994	Placebo-controlled, double-blind, randomised, parallel-group	2 times 2 capsules with 250 mg *S. boulardii*; according to 3 x 10^{10} CFU per day or placebo in addition to a standard antibiotic therapy Duration of therapy: 4 weeks Follow-up: 4 weeks	124 adults 57 *S. boulardii* Mean age: 56.8 years ± 20.4 67 placebos Mean age: 59.2 ± 21.1 9 drop-outs during treatment, 5 drop-outs during follow up 5 patients died (1 pneumonia under *S. boulardii*, 4 under placebo) 1 patient refused	Active CDD (initial CDD and/or recurrent CDD) ranging from uncomplicated diarrhoea to pseudomembranous colitis (PMC) who were receiving one of two oral standard treatments (vancomycin or metronidazole) at the time of enrolment	Efficacy calculated from the formula ([IP – IT]/IP) x 100, IP=incidence of CDD recurrence in placebo group, IT=incidence of CDD recurrence in *S. boulardii* group Relative risks (RRs) calculated from cumulative incidence ratios, and two-tailed 95% test-based confidence interval (CIs) that excluded 1 were defined as significant Unadjusted RRs of *S. boulardii* compared to placebo: 0.47; 95% CI: 0.22 to 1.00	ITT-basis, Student's t-test, Mann-Whitney U test, chi-square test, Fisher's exact test, Kaplan-Meier method, mantel Log-Rank Test Jadad Score 5 (Dendukuri, 2005)	It is not clear if the use of multivariate logistic regression model to control for confounding factors was predefined, otherwise the primary outcome was not significant. In addition, efficacy could only be confirmed in the subgroup with recurrent CDD and not in the subgroup

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
			participation because of a side effect (rash due to vancomycin)		Using a multivariate logistic regression model to control for confounding factors the RRs of *S. boulardii* compared to placebo was significantly reduced: 0.43; 95% CI: 0.20 to 0.97 Overall: Recurrence rate: Placebo: 44.8 % *S. boulardii*: 26.3% Initial CDD: Recurrence rate: Placebo: 24.2% *S. boulardii*: 19.3% Recurrent CDD: Recurrence rate:		with initial CDD. Neither the dose nor the duration of antibiotic was controlled.

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					Placebo: 64.7% *S. boulardii*: 34.6%		
Prevention of recurrent CDD Surawicz *et al*., 2000	Placebo-controlled, double-blind, explorative study	2 times 2 capsules with 250 mg *S. boulardii* or placebo in addition to a 10-day course of a standard antibiotic therapy from day 7 Duration of therapy: 4 weeks	168 adults (32 patients on high-dose vancomycin, 83 patients on low-dose vancomycin, 53 patients on metronidazole)	Active diarrhoea (change in bowel habits with ≥3 loose or watery stools per day for ≥2 consecutive days, or >8 loose stools within 48 hours) before standard antibiotic treatment, positive C. difficile assay, ≥1 recent, prior episode of CDD within 1 year	Diarrhoea cessation was defined as a return to normal bowel frequency (<3 loose or watery stools per day) for at least 48 hours Neither the 10-day course of lower dose of vancomycin nor metronidazole given with either *S. boulardii* or placebo was significantly effective 3 (16.7%) of the 18 patients receiving high-dose vancomycin and *S. boulardii* had a recurrence of CDD, compared with 7 (50%) of 14 patients receiving	Student's t-test, Mann-Whitney ranked sum test, chi-square test, Fisher's exact test Two-tailed tests of significance were used for all tests at level of p≤0.05 Jadad Score 3 (Dendukuri, 2005)	Patients were treated for CDD according to the preference of their private physicians and not per randomisation and were then referred to the authors of the study. Only one subgroup is presented without indicating if this design was predefined in

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					high-dose vancomycin and placebo (p=0.05, by the Fisher's exact test)		the study protocol.

Prevention of diarrhoea associated with tube-feeding

The occurrence of diarrhoea during tube feeding is a common complication. Possible causes include alteration of bacterial flora, hypoalbuminemia, infusion rate or concomitant medication.

A list of randomised controlled clinical studies, which investigated the anti-diarrhoea efficacy of *Saccharomyces boulardii* in tube-fed patients, has been published by McFarland (2010).

One of the causes of diarrhoea in tube-fed patients may be the consequences on colonic trophicity of a deficiency in luminal short-chain fatty acids (SCFA). Schneider *et al*. (2005) investigated the effects of *Saccharomyces boulardii* (0.5 g b.i.d. for 6 days) on faecal flora and SCFA in 10 patients on long-term total enteral nutrition. As compared to 15 healthy volunteers, treatment with *Saccharomyces boulardii* increased total faecal SCFA levels – especially butyrate - which remained high even 9 days after the discontinuation of treatment. According to the authors, *Saccharomyces boulardii*-induced increase of faecal SCFA concentrations may explain its preventive effects on diarrhoea induced by total enteral nutrition (see also 4.1.1).

Clinical experience with the use of *Saccharomyces boulardii* in prevention of enteral nutrition-related diarrhoea is very limited. Only a low number of patients has been included in clinical studies. The dosages applied ranged from 1 g per day (Bleichner *et al*., 1997) to 2 g per day (Tempé *et al*., 1983, Schlotterer *et al*., 1987).

Table 10: Randomised controlled clinical studies for the prevention of diarrhoea in tube-fed patients

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Prevention of diarrhoea in tube-fed patients Tempé *et al*., 1983	Randomised, double-blind	*S. cerevisiae* CBS 5926 dosage: 1 x 10^{10} per day Treatment duration: 11-21 days	n=40 Placebo: n=20 Mean age: 65.5 years *S. boulardii*: n=20 Mean age: 67.8 years	Intensive care patients on enteral feeding for at least 10 days	Days of diarrhoea: *S. boulardii:* 34/389 day (8.7%) Placebo: 63/373 days (16.9%)	p<0.001	Small study population
Prevention of diarrhoea in tube-fed patients Schlotterer *et al*., 1987	Randomised, double-blind	*S. cerevisiae* CBS 5926; 2 g per day vs. placebo	n=18 *S. boulardii*: n=9 Mean age: 32.9 years Placebo: n=9 mean age: 43.4 years	Patients 18-70 years with burns (18-70% of body surface) with enteral nutrition for ≥8-28 days	Days of diarrhoea: *S. boulardii*: 3/204 day (1.5%) Placebo: 19/208 day (9.1%)	p<0.001	Small and special study population, higher posology than usually recommended for medicinal products in the European Union
Prevention of diarrhoea in tube-fed patients	Randomised, double-blind, placebo-controlled, multicentre	*S. boulardii* 4 x 500 mg per day (no further information) vs. placebo	n=128 *S. boulardii*: n=64 Mean age: 61.6 years Male: 45%	Critically ill patients with need for enteral nutrition for >6 days	Per cent of days with diarrhoea per feeding days: 1) Diarrhoea was defined according to a diarrhoea	p=0.0069	higher posology than usually recommended for medicinal products in the European Union

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Bleichner *et al*., 1997			Placebo: n=64 Mean age: 64.9 years Male: 46%		score based on volume and consistency of each stool: *S. boulardii*: 14.2% Placebo: 18.9% 2) Diarrhoea was defined as three or more non formed stools per day *S. boulardii*: 7.7% Placebo: 12.7%	p<0.01	

Treatment of irritable bowel syndrome (IBS)

Irritabel bowel syndrome (IBS) is a functional gastrointestinal disorder in which abdominal discomfort or pain is associated with changes in bowel habits, stool consistency and other features of disordered defectation. IBS is considered to be one of the most frequent clinical problems in gastroenterology with an estimated prevalence in the Western world of up to 20% (CHMP, 2014). In 2014, a guideline has been published by the CHMP on the evaluation of medicinal products for the treatment of IBS (CHMP, 2014). Currently, the Rome III criteria, which replaced the Rome II criteria, are regarded to be the standard diagnostic criteria:

"Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months (with symptoms being present for the last 3 months and onset at least 6 months prior to diagnosis) associated with 2 or more of the following

- Improvement with defaecation
- Onset associated with a change in frequency of stool
- Onset associated with a change in form (appearance of stool)

Sub-typing of IBS patients is performed by the predominant stool pattern present in a patient."

Short-term treatment intermittent use of compounds should be evaluated in repeated treatment courses shorter than 8 weeks. Long-term continuous treatment should be evaluated in studies with a duration of at least 6 months (CHMP, 2014).

In 1983, Maupas *et al*. investigated if *Saccharomyces boulardii* is effective in the treatment of IBS in a randomised, double-blind clinical study. 34 patients (*Saccharomyces boulardii*: n=16; placebo: n=18) were treated with *Saccharomyces boulardii* (3 times 1 capsule per day) or placebo over a period of one month. The statistical evaluation showed that *Saccharomyces boulardii* was statistically significantly superior to placebo with respect to the opinion of the physician and patient, number and consistency of stools. Regarding the clinical symptoms pain, distension and dyspepsia, however, no statistically significant differences were observed between the groups. As this clinical study had been performed in 1983, the current guidelines by the CHMP on the evaluation of medicinal products for the treatment of IBS have not been considered.

The clinical study performed by Maupas *et al*. (1983) was the only study performed with *Saccharomyces boulardii*, which McFarland and Dublin (2008) included in their meta-analysis of probiotics for the treatment of IBS. They concluded "*in summary, the present meta-analysis suggests that probiotics offer promise for the treatment of IBS. Results should be interpreted cautiously given the methodological limitations of published studies. Future studies are needed, in particular larger studies of longer duration with greater methodological rigor. In addition, more data are needed regarding which specific strains and doses are most likely to be effective. The use of probiotics for IBS warrants further study, particularly given the chronic nature of this condition, its major impact on patients' quality of life, and the dearth of other effective treatments.*"

Since then, more clinical studies investigating the effects of *Saccharomyces boulardii* in the treatment of IBS have been reported. Choi *et al*. (2011) evaluated the effects of *Saccharomyces boulardii* on quality of life and symptoms in 90 patients with diarrhoea-predominant or mixed-type IBS in a randomised, double-blind, placebo-controlled multicentre trial. Diagnosis of IBS was based on Rome II Criteria and in both groups about 72% of the patients suffered from diarrhoea-predominant IBS. Although the results of this study indicated a statistically significant higher improvement of quality of life (QOL) in the patients treated with *Saccharomyces boulardii* (15.4% vs. 7.0%; $p<0.05$), IBS

symptoms were reduced to a similar extent in both groups. Bowel frequency and stool consistency did not change in either group. The drop-out rate was high in both groups.

The studies performed by Maupas *et al*. (1983) and Choi *et al*. (2011) were included by Korpela and Niittynen (2012) in a review on the effects of probiotics on the gastrointestinal symptoms of IBS. The authors concluded that general recommendations on the use of probiotics in IBS cannot be given at that time. Further clinical trials and data on the mechanism of action are required.

In 2011, the results of another randomised, double-blind, placebo-controlled clinical study with *Saccharomyces boulardii* in 70 patients with diarrhoea-predominant IBS were published by Kabir *et al*. No significant difference between the two groups was found in any of the parameters evaluated (number of stools, consistency of stools, abdominal pain, abdominal distension, personal life and professional life) on any of the observation days (0, 30 and 60). *Saccharomyces boulardii* treatment for 30 days in diarrhoea-predominant IBS patients did not result in any improvement.

Bafutto *et al*. (2013) evaluated the effects of mesalazine and *Saccharomyces boulardii* given alone or in combination for the treatment of patients with diarrhoea-predominant IBS. A pilot study was performed in 53 patients, 12 patients received *Saccharomyces boulardii* alone. The results of the study showed a statistically significant improvement of symptom scores in all treatment groups. The improvement of the symptom score was greater with mesalazine alone or in combination with *Saccharomyces boulardii* as compared with *Saccharomyces boulardii* alone. Due to the small number of patients, the short study duration (30 days) and the pilot character of the study with lack of randomisation and blinding, the results obtained in this study do not support a WEU of *Saccharomyces boulardii* for the indication IBS.

Akhondi-Meybodi *et al.* (2014), too, studied the effect of *Saccharomyces boulardii* on the treatment of IBS in a randomised, double-blind clinical study in 60 patients. IBS was diagnosed by a gastroenterologist based on clinical symptoms, laboratory tests and medical examinations such as colonoscopy, colon biopsy, sonography and stool culture. 30 patients were treated with *Saccharomyces boulardii* (1 capsule per day, *Saccharomyces cerevisiae* CBS 5926, at least 1 x 10^{10} living cells/g), 30 patients received placebo for an interval of 3 weeks. The aim of the study was to investigate the effect of *Saccharomyces boulardii* on improving symptoms of IBS (abdominal pain, flatulence, urgent defecation, diarrhoea, obstipation, gurgling, eructation, gas release from the anus). Severity of symptoms was assessed by the patients by means of a Likert scale at the beginning and end of treatment. At the end of treatment, statistically significant differences between the groups favouring *Saccharomyces boulardii* were found for pain severity (p=0.008), diarrhoea (p=0.001) and gurgling (p=0.317). The validity of this study, however, is limited due to the small number of patients included and the short treatment period. Furthermore, there are doubts with regard to the blinding procedure, since according to the authors placebo capsules were only "similar" to verum capsules "except that they did not contain *Saccharomyces* powder and had no coating". Another important aspect is that, apart from abdominal pain, symptoms of IBS which are essential for making the IBS diagnosis (e.g. improvement with defecation, frequency and consistency of stool) have not been included as primary endpoints in the statistical evaluation.

Pineton de Chambrun *et al*. (2015) performed a randomised clinical trial of *Saccharomyces cerevisiae* (n=86) versus placebo (n=93) in 179 patients with IBS (Rome III criteria). The patients in the treatment group received *Saccharomyces cerevisiae* (CNCM I-3856) 500 mg once daily over a period of 8 weeks and, as result, a reduction of abdominal pain/discomfort scores without altering stool frequency and consistency was observed. Since genetic identity, however, was not proven for the strain of *Saccharomyces cerevisiae* administered in this clinical study, the results cannot be transferred to the *Saccharomyces cerevisiae* CBS 5926 assessed here. Therefore, the clinical study was not included in the table below.

Table 11: Clinical studies in the indication irritable bowel syndrome (IBS)

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of IBS Maupas *et al*., 1983	Double-blind, randomised, three centres	3 times 1 cps per day (*S. cerevisiae* CBS 5926) vs. placebo Duration: 1 month	Verum: n=16 Placebo: n=18 Sex: 20 male, 14 female Mean age: 42 years	Patients >18 years of age with IBS (abdominal pain, distension, episodes of diarrhoea)	Statistically significant differences ($p<0.05$) with regard to physician's and patient's subjective opinion Decrease in number of stools Improvement in stool consistency No statistically significant differences with regard to the symptoms pain, distension, dyspepsia	Student's t-test	Small number of patients, no AE mentioned
Treatment of IBS Choi *et al*., 2011	double-blind, randomised, placebo-controlled, multicentre	*S. cerevisiae* CBS 5926; 2 x 10^{11} live cells	*S. boulardii*: n=45 Mean age: 40.2 years	Patients (20-65 years of age) with diarrhoea-predominant IBS	Primary efficacy variable: IBS-QOL: statistically significant better improvement with *S.*	Student's t-test	IBS symptoms were secondary efficacy variables only

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		2 times 2 capsules per day for 4 weeks vs. placebo	Male: 51.4% Placebo: n=45 Mean age: 40.6% Male: 48.7% Drop-outs: *S. boulardii*: n=11 Placebo: n=12	or mixed type IBS (Rome II criteria)	*boulardii* (15.4% vs. 7.0%) ($p<0.05$) Secondary efficacy variable: scores for IBS symptoms (abdominal pain, discomfort, hard/lumpy stool, loose/watery/stool, straining, urgency, sense of incomplete evacuation, mucus in stool, bloating, passage of gas): total scores were reduced in both groups to a similar extent Secondary variable: frequency and consistency of stool (Bristol stool scale):		One AE in the placebo group: worsening of abdominal pain and flatulence. High rate of drop-outs

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					no significant change in either group		
Treatment of IBS Kabir *et al*., 2011	Double-blind, randomised, placebo-controlled	*S. boulardii* 250 mg sachet twice a day vs. placebo Duration: 30 days	*S. boulardii*: n=35 Mean age: 32.4 years. Male: n=32 Placebo: n=35 Mean age: 29 years. Male: n= 32	Patients with diarrhoea predominant IBS (Rome II criteria) Age: 18-50 years	Single scores for: Number of stools, consistency of stools, abdominal pain, abdominal distension, personal life, professional life No difference between the groups in any score at days 0, 30 and 60	Student's t-test	Study from Bangladesh
Treatment of IBS Bafutto *et al*., 2013	Pilot study.	Mesalazine alone (MG): 800 mg t.i.d. Mesalazine/ *S. boulardii* (MSbG): 800 mg t.i.d./200 mg t.i.d.	MG: n=20 Mean age: 46 years. MSbG: n=21	53 patients with diarrhoea-predominant IBS (>18 years of age) (Rome III criteria)	Symptom evaluation at baseline and after treatment (4-point likert scale): Stool frequency, form and consistency (Bristol Scale),	difference between baseline and end of treatment: Paired t-test	short observation period small number of patients pilot study

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		S. boulardii alone (SbG:): 200 mg t.i.d. duration: 30 days	Mean age: 50 years SbG: n=12 Mean age: 43 years		abdominal pain and distension Statistically significant improvement of symptom score (pre/post treatment comparison): MG: 10.7 (pre) vs. 5.5 (post) (p<0.0001) MSbG: 10.67 (pre) vs. 5.0 (post) (p<0.0001) SbG: 9.75 (pre) vs. 7.08 (post) (p<0.003) Significant differences (p<0.03) were seen when comparing MG, MSbG and SbG	Kruskal-Wallis test	not randomised or blinded

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of IBS Akhondi-Meybodi, 2014	Double-blind, randomised, placebo-controlled	*S. cerevisiae* CBS 5926 (at least 1 x 10^{10} living cells/g) 1 capsule per day vs. placebo Duration: 3 weeks	*S. boulardii* n=30 Male: 43.3% Mean age: 37.3 years Placebo: n=30 Male: 40% Mean age: 44.2 years	60 patients with IBS as diagnosed by a gastroenterologist	Severity score of symptoms (Likert scale) on day 0 and after 3 weeks: Abdominal pain, flatulence, urgent defecation, diarrhoea, obstipation, gurgling, eructation, gas release from anus Aim of the study: effect of *S. boulardii* on improvement of symptoms Statistically significant difference between the groups regarding: Pain severity (p=0.008) Diarrhoea (p=0.001) Gurgling (p=0.317)	T-test, paired samples t-test	Small number of patients Short treatment duration Doubtful blinding

Prevention of traveller's diarrhoea

In 2007, McFarland published a meta-analysis of randomised clinical studies with various probiotics for the prevention of traveller's diarrhoea. From 12 studies identified in literature, two were performed with *Saccharomyces boulardii*:

Kollaritsch *et al*. (1989) investigated the value of four different non-antibiotic preparations for prophylaxis or treatment of traveller's diarrhoea. All studies were placebo-controlled, double-blind field trials with Austrian tourists (n=2,271) visiting countries with warm climates. Apart from *Saccharomyces,* the following preparations were administered: *Lactobacillus acidophilus*, an oral vaccine consisting of heat inactivated Enterobacteriaceae, a combination product containing carbo medicinalis, Bolus alba, pectin, lactose, whey powder. With regard to *Saccharomyces*, healthy Austrian travellers were given either placebo (n=406; mean age: 43.2 years) or a daily dose of 125 mg b.i.d. (n=426; mean age: 42.4 years; group I) or 250 mg b.i.d. (n=399; mean age 41.5 years; group II) of *Saccharomyces cerevisiae* CBS 5926 containing 5 x 10^9 or 10^{10} revivable cells. Intake started 5 days prior to departure and continued during the whole stay abroad. In addition, the travellers received a questionnaire to record personal data, information of the holidays' conditions and information of diarrhoea.

Episodes of diarrhoea occurred in 42.6% of the placebo group and statistically significantly less frequent in the *Saccharomyces* group: 33.6% (group I; p<0.007) and 31.8% (group II; p<0.002). The incidence of diarrhoea as compared to placebo was reduced by 21.2% in group I and 25.4% in group II. Dose dependency was suggested but not significant in the study. Side-effects were not observed. 9 participants reported improvement of acne.

The clinical course in not preventable cases of diarrhoea was not influenced by this prophylaxis. Regional evaluation of efficacy, however, exhibited evident and statistically significant differences in protective capacity. The reduction of risk amounted to 58%, 59% and 40% respectively in Northern Africa, Western Africa and various tropical islands and thus was more pronounced than the overall risk reduction rate. Due to the differing protection rates according to the destination of travellers, according to the authors, a selective efficacy of *Saccharomyces cerevisiae* has to be taken into account. Only the application of *Saccharomyces cerevisiae* (strain Hansen CBS 5926) decreased the incidence of diarrhoea significantly.

Table 12: Incidence of traveller's diarrhoea according to region (from Kollaritsch *et al*., 1989)

Region	**Group**	**Frequency of diarrhoea**	**Reduction compared to placebo**	**Significant relating to overall reduction in group II**
North Africa (n=208)	placebo (n= 65) group I (n=73) group II (n=70)	50.7% 30.1% 21.4%	- 41% (p<0.01) 58% (p<0.01)	 p<0.0025
West Africa (n=51)	placebo (n= 19) group I (n=18) group II (n=14)	52.6% 33.3% 21.4%	- 37% 59%	 p<0.05

Region	Group	Frequency of diarrhoea	Reduction compared to placebo	Significant relating to overall reduction in group II
Middle East (islands) (n=123)	Placebo (n= 45)	40.0%	-	
	Group I (n=45)	28.9%	28% (p<0.1)	
	Group II (n=33)	24.2%	40% (p<0.05)	p<0.05
East Africa (n=251)	Placebo (n= 70)	48.6%	-	
	Group I (n=98)	35.7%	27% (p<0.05)	
	Group II (n=83)	36.1%	26% (p<0.1)	not significant
South America (n=97)	Placebo (n= 38)	50.0%	-	
	Group I (n=24)	33.3%	33%	
	Group II (n=35)	37.1%	26%	not significant
World tour	Placebo (n= 12)	50.0%	-	
	Group I (n=12)	25.0%	50% (p<0.1)	
	Group II (n=10)	40.0%	20%	not significant
Middle East (n=85)	Placebo (n= 21)	66.6%*	-	
	Group I (n=32)	68.6%	0	
	Group II (n=32)	65.6%	0	not significant
Far East (n=228)	Placebo (n= 86)	31.4%**	-	
	Group I (n=72)	25.9%	20%	
	Group II (n=70)	30.0%	5%	not significant
Central America (n=76)	Placebo (n= 32)	31.3%	-	
	Group I (n=18)	38.9%	0	
	Group II (n=26)	30.7%	0	not significant

* risk of diarrhoea significant higher (p<0.05) than the overall frequency in the placebo group;
** risk of diarrhoea significant smaller (p<0.05) than the overall frequency in the placebo group

The results of another placebo-controlled double-blind clinical study were published by Kollaritsch *et al*. in 1993. 3,000 Austrian travellers were randomly assigned to prophylactic treatment with *Saccharomyces boulardii* at daily dosages of 250 mg or 1,000 mg (*Saccharomyces cerevisiae* CBS 5926) or to placebo. Prophylactic administration started 5 days before departure and was continued for the entire stay abroad. The study participants were asked to fill in questionnaires and the data of 1,016 were available for the evaluation of efficacy (placebo: n=361; mean age 45.3 years; *Saccharomyces boulardii* 250 mg: n=352, mean age 43.9 years; *Saccharomyces boulardii* 1,000 mg: n=303, mean age 47.7 years). The incidence of diarrhoea was 39.1% in the placebo group and statistically significantly lower during the prophylactic administration of *Saccharomyces boulardii*: 34.4% in the low dose and 28.7% in the high dose group. The success of the prophylactic administration of *Saccharomyces boulardii* (especially for the dosage 1,000 mg),

however, was shown to depend directly on the rigorous use of the preparation. In this study, too, a varying regional and dose-dependent effect was noted for *Saccharomyces boulardii* which was particularly marked in North Africa and the Near-East.

According to McFarland (2007), *Saccharomyces boulardii* showed significant efficacy for the prevention of traveller's diarrhoea.

In his publication on the therapy for and prevention of traveller's diarrhoea, DuPont (2007) also mentions *Saccharomyces boulardii*, together with Lactobacillus GG [*Lactobacillus rhamnosus* GG], as one of the leading candidates for the prophylaxis in travel medicine. Although safe for use in immunocompetent subjects, the probiotic preparations have provided minimal protection against the development of traveller's diarrhoea.

In 2006, the Infectious Diseases Society of America (Hill *et al*., 2006) published guidelines for travel medicine. Regarding the prevention and management of traveller's diarrhoea, probiotics are not recommended for use, as they did not demonstrate sufficient efficacy. This assessment was based on investigations with Lactobacillus GG and the clinical trial with *Saccharomyces boulardii* performed by Kollaritsch *et al*. (1993).

Acne

According to the monograph of the German Kommission E, *Saccharomyces boulardii* is also indicated as an adjuvant in the treatment of chronic acne at a daily dosage of 750 mg. The effects of *Saccharomyces boulardii* in acne are ascribed to its antimicrobial mechanism of action (Reuter *et al*., 2010).

Bedi and Shonefelt (2002) mention *Saccharomyces boulardii* for the use in acne in their review on herbal therapy in dermatology.

In an evidence-based review on botanicals in dermatology, Reuter *et al*. (2010) concluded that *Saccharomyces* may have the potential to become standard treatment in acne. They searched published literature for the use of botanicals in dermatological indications with the focus on controlled clinical studies. The level of evidence as suggested by the UK National Health Service was assessed as level 'D' (i.e. expert opinions without explicit critical appraisal or based on physiology, bench research, or first principles).

The following clinical studies have been performed in acne:

In 1973, Mandrella reported the treatment results of 166 patients (121 female, 45 male) with acne. The majority of patients (56%) suffered from juvenile acne. Majority of the patients (67%) were ≤ 25 years old. The initial dose of *Saccharomyces boulardii* was 3 times 100 mg per day which was reduced to 3 times 50 mg per day after 2 weeks (*Saccharomyces cerevisiae* CBS 5926). The treatment period was longer than 6 months in about 80% of the patients. In almost 90% of the patients, very good/good results were achieved. There was no case of treatment failure.

Kujath and Sipp (1978) treated 41 soldiers (age 18-25 years) with *Saccharomyces cerevisiae* CBS 5926 for acne vulgaris. During the first 3 days of treatment, the dosage was 3 times 100 mg per day, which was gradually reduced over a period of 2 months to 50 mg per day. After 2-8 months of treatment, results in 80% of the patients were assessed as good/very good. In 7% of the cases, treatment was rated as ineffective. Side-effects were not observed.

In a randomised, controlled double-blind study involving 139 patients with various forms of acne, the effectiveness and tolerance of *Saccharomyces cerevisiae* Hansen CBS 5926 (3 times 250 mg per day) was studied in comparison with placebo over a maximum period of 5 months (Weber *et al*., 1989). The results of therapy were assessed by the physician as very good/good in 74.3% of

the patients receiving the preparation, as compared with 21.7% in the placebo group. In more than 80% of the former patients, the condition was assessed as healed/considerably improved, while the corresponding rate for the placebo group was only 26%. As the severity of acne and therapeutic efficacy were assessed mainly by subjective scores and statistical methods are only roughly described in the original publication, the study does not confirm the therapeutic efficacy of *Saccharomyces boulardii* in acne.

In another double-blind randomised clinical study by Stüttgen (1991), 94 patients with acne were treated with daily doses of 3 times 100 mg or 3 times 250 mg *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926). For the assessment of therapeutic efficacy, the number of papules within a defined area (3 times 5 cm), oiliness of the hair and frequency of hair washing were determined. After 5 months of treatment, the number of papules had decreased by 56.1% (300 mg per day) and 56.5% (750 mg per day). Therapeutic success was rated by the doctors as very good/good in 32.6% and 29.1% of the patients. In 37% (300 mg per day) and 37.5% (750 mg per day) of the patients, therapeutic efficacy was assessed as unsatisfactory. Statistically significant differences between the groups were not detected. As in this clinical study the rate of patients with unsatisfactory treatment effects was greater than the rate of patients with good treatment results, this study does not confirm a therapeutic efficacy of *Saccharomyces boulardii* in acne.

4.3. Clinical studies in special populations (e.g. elderly and children)

Acute unspecific diarrhoea in children

Controlled studies

Chapoy (1985, French publication) assessed lyophilised *Saccharomyces boulardii (Saccharomyces cerevisiae* Hansen CBS 5926-Chapoy 1986, German publication) (2 times 250 mg per day) in combination with standard oral rehydration therapy for the treatment of acute diarrhoea in 38 infants and toddlers with acute diarrhoea based on an acute gastroenteritis (age between two weeks and 30 months) in a controlled clinical trial in France. After admission to hospital, they received a special oral rehydration therapy. Normal nutrition was reintroduced in different episodes within about 72 hours. Each second child was allocated to the additional treatment of *Saccharomyces cerevisiae* for five days. Control visits were performed on day 1 and 4. A significant reduction in the number of stools ($p<0.01$), weight and consistency of stools ($p<0.05$), and transit time ($p<0.05$) could be confirmed by statistical evaluation of all 19 children in the *Saccharomyces cerevisiae* group. No adverse effect was reported.

Table 13: Results of Chapoy (1986, German publication)

Mean ± SD	***S. boulardii* group (n=19)**	**control group (n=19)**	**significance**
stool frequency			
Day 1	4.9±0.5	4.0±0.3	
Day 4	2.1±0.2	3.4±0.4	
Improvement	-2.8±0.5	-0.6±0.6	p<0.01

Mean ± SD	***S. boulardii* group (n=19)**	**control group (n=19)**	**significance**
stool weight (g)			
Day 1	283±42	192±25	
Day 4	144±28	180±37	
Improvement	140±43	-12±42	p<0.05
transit time via carmin red method (h)			
Day 1	7.2±1.0	10.4±1.6	
Day 4	16±1.6	12.3±1.6	
Improvement	8.8±1.6	3.0±1.9	p<0.05
stool consistency after 4 days			
Liquid	1	4	
Soft	3	8	p<0.05
Normal	15	7	

Cetina-Sauri and Sierra Basto (1989, 1991) evaluated the antidiarrhoeal effectiveness in acute diarrhoea and tolerance of *Saccharomyces boulardii* in a randomised double-blind placebo-controlled study, which included 130 Mexican infants from 3 months to 3 years of age. One group received 200 mg *Saccharomyces boulardii* every 8 hours for 4 days, the other placebo. All children were treated with a rehydration therapy additionally. Efficacy was defined less than 4 bowel movements per day and no liquid stools. The frequency of stools decreased and the consistency of the faeces improved at 24 hours of treatment in the *Saccharomyces boulardii* group as compared with the placebo group. The difference between the two groups became statistically significant at 48 and 96 hours, when clinical efficacy was assessed. Clinical cure rates were higher in the active treatment group than in the control group. The authors concluded that *Saccharomyces boulardii* may be used as an adjunct to oral rehydration for the treatment of acute diarrhoea in infants and very young children.

Table 14: Stool frequency: results of Centina-Sauri and Sierra Basto (1989, 1991)

Treatment day	***S. boulardii* group (n=65)**	**Placebo group (n=65)**	**Significance**
Start of treatment	7.50±2.15	6.66±2.26	
Day 1	5.15±1.93	5.47±2.40	
Day 2	3.76±2.31	4.38±2.73	p<0.05
Day 3	2.53±1.78	3.63±2.53	
Day 4	2.00±1.94	3.29±2.19	p<0.05

Urganci *et al.* 2001 evaluated the efficacy and tolerability of *Saccharomyces boulardii* (*Saccharomyces cerevisiae* Hansen CBS 5926) in a double-blind placebo-controlled study in 100 infants and small children (2–29 months; mean 10.8 months ± 0.9 months) with acute diarrhoea. 50 children got 250 mg *Saccharomyces boulardii* and rehydration solution and 50 children got placebo and rehydration solution. After 48 hours and 96 hours, children treated with *Saccharomyces boulardii* scored significantly better than controls.

Table 15: Number of stools: results of Urganci *et al*. (2001)

Treatment day	***S. boulardii* group (n=50)**	**Placebo group (n=50)**	**Significance**
	Mean±SD	Mean±SD	
Start of treatment	7.78±1.86	7.32±1.92	p>0.05
Day 2	3.78±0.71	4.24±0.99	p<0.01
Day 4	2.70±0.67	3.13±0.93	p<0.05

Kurugöl and Koturoglu (2005) evaluated the effect of *Saccharomyces boulardii* in Turkish children from 3 months to 7 years of age with acute diarrhoea. 232 children were enrolled, but 32 children were excluded. 23 children were prescribed antibiotics during the study period and 9 children were non-compliant to the protocol. 200 children received either *Saccharomyces boulardii* in a granulated form in a daily dose of 250 mg or placebo for 5 days.

Table 16: Results of Kurugöl and Koturoglu (2005)

	***S. boulardii* group (n=100)**	**Placebo group (n=100)**	**Significance**
Duration of diarrhoea (day)	4.7±2.5*	5.5±3.2	p=0.03
Duration of watery diarrhoea (day)	2.8±1.1	3.8±1.4	p<0.001
Duration of vomiting (day)	1.2±1.0	1.3±1.0	p=0.61
Duration of temperature >37.5°C (day)	1.0±0.8	1.1±0.9	p=0.28
Length of hospital stay	2.9±1.2	3.9±1.5	p<0.001

*Values are mean ± SD

In addition, the medians of the average stool frequency after the second day of treatment were significantly lower in the *Saccharomyces boulardii* group than in the placebo group (p=0.003). Four children from the placebo group versus only one child from the *Saccharomyces boulardii* group had persisting diarrhoea. One child in the *Saccharomyces boulardii* group had meteorism.

Billoo *et al.* (2006) assessed the efficacy and safety of *Saccharomyces boulardii* in acute watery diarrhoea and its role in reducing the frequency of episodes of diarrhoea in subsequent two months. Pakistani children from 2 months to 12 years of age with acute diarrhoea were randomised in *Saccharomyces boulardii* group (treated with oral rehydration salt, nutritional support and *Saccharomyces boulardii*, 250 mg b.i.d.) and in control group (treated with oral rehydration salt and nutritional support only). Active treatment phase was 5 days and each child was followed for two months afterwards. Frequency and consistency of stools as well as safety of drug were assessed on every visit. A comparison of the two groups was made in terms of number of diarrhoeal episodes in subsequent two months. There were 50 patients in each group. Baseline

characteristics such as mean age and the average frequency of stools were comparable in *Saccharomyces boulardii* and control group at the time of inclusion in the trial.

Table 17: Results of Billoo *et al*. (2006)

	***S. boulardii* group (n=50)**	**Control group (n=50)**	**Significance (t-test)**
Stool frequency day 0 (mean)	9.5	8.8	p=0.37
Stool frequency day 3 (mean)	2.8	4.4	p=0.01
Stool frequency day 6 (mean)	1.6	3.3	p=0.001
Duration of diarrhoea (day)	3.6	4.8	p=0.001

Mean numbers of episodes of diarrhoea by the end of two months were 0.56 in control group compared to 0.32 in *Saccharomyces boulardii* group (p=0.04). The drug was well accepted and tolerated. There were no reports of the side effects during treatment period. The authors concluded that *Saccharomyces boulardii* significantly reduced the frequency and duration of acute diarrhoea. The consistency of stool also improved. The drug was well tolerated.

Canani *et al.* (2007) compared the efficacy of five probiotic preparations recommended to parents in the treatment of acute diarrhoea in Italian children. The study was a prospective single-blind randomised controlled trial. 571 children aged between 3-36 months with acute diarrhoea (3 or more loose or liquid stools a day) were randomised to oral rehydration alone (n=91; 2 drop-outs; control group), or to oral rehydration in combination with a specific probiotic: *Lactobacillus rhamnosus* strain GG (n=98; drop-out 1); *Saccharomyces boulardii* (n=87; drop-out 2); *Bacillus clausii* (n=100; drop-out 1); mix of *L. delbrueckii* var *bulgaricus*, *Streptococcus thermophiles, L. acidophilus* and *Bifidobacterium bifidum* (n=94; drop-out 1); or *Enterococcus faecium* SF 68 (n=88; drop-out 1). The primary outcome measures were the total duration of diarrhoea and the number of stools a day and their consistency. In contrast to *Lactobacillus rhamnosus* and the combination preparation, *Saccharomyces boulardii* had no effect on duration of diarrhoea, stool outputs and consistency as compared to rehydration therapy alone. None of the preparations had a significant effect on the secondary outcomes fever, vomiting and hospital admissions. No adverse events were recorded.

Ozkan *et al.* (2007) assessed the efficacy of *Saccharomyces boulardii* and its immune response in 27 Turkish children aged between 6 months and 10 years with acute diarrhoea. The patients were randomised in two groups. 16 children received 250 mg *Saccharomyces boulardii* dissolved in 5 ml water twice daily for 7 days, and 11 children received placebo. The decrease of stool frequency was significantly greater in the *Saccharomyces boulardii* group on days 3 and 4 as compared to the placebo group.

Table 18: Results of Ozkan *et al*. (2007)

	***S. boulardii* group (n=16)**		**placebo group (n=11)**		**p value between the two groups**
	number of stools	**p value compared with baseline**	**number of stools**	**p value compared with baseline**	
Baseline	6.23±0.53		5.92±0.44		ns*
Day 1	4.50±0.36	<0.001	5.36±0.38	<0.001	ns*
Day 2	3.06±0.33	<0.001	4.27±0.38	<0.001	ns*
Day 3	1.68±0.23	<0.001	3.36±0.38	<0.001	<0.05
Day 4	0.43±0.22	<0.001	1.81±0.42	<0.001	<0.05

values are mean ± SD; *ns = not significant

Clinical symptoms such as fever and dehydration were resolved on the second day, with no significant difference between the two groups. The *Saccharomyces boulardii* group demonstrated a significant increase in serum immunoglobulin A and decrease in C-reactive protein levels on day 7. The percentage of CD8 lymphocytes on day 7 was significantly higher. No adverse reaction related to *Saccharomyces boulardii* therapy was observed during the study.

Villarruel *et al.* (2007) evaluated the efficacy of *Saccharomyces boulardii* as an adjuvant to oral rehydration solution (ORS) in shortening the duration of acute, mild to moderate diarrhoea in 100 children aged between 3 and 24 months in ambulatory care, in a randomised, double-blind, placebo-controlled study. 12 patients were excluded due to lack of compliance with protocol medication. Acute diarrhoea was defined as the presence of ≥3 liquid or loose stools in the preceding 24 hours but for less than 7 days. 16 breastfed patients were included (5 in the *Saccharomyces boulardii* group and 11 in the placebo group). Children under 1 year received 250 mg *Saccharomyces boulardii* (no further specification), and those over 1 year 2 times 250 mg per day or placebo for 6 days. 72 patients had a follow-up for one month (35 in the *Saccharomyces boulardii* group and 37 in the placebo group).

Table 19: Results of Villarruel *et al.* (2007)

	***S. boulardii* group**	**Placebo group**	**Significance**
Number of stools on day 4	2.5±1.4	3.5±1.8	p<0.001
Mean duration of diarrhoea	4.7 days (range 2-10 days)	6.16 days (range 2-13 days)	p<0.05
Risk of having diarrhoea lasting more than 7 days/number of patients	3/44	12/44	RR 0.25; 95% CI 0.1-0.8

A statistically significant difference was observed in the number of stools on the 4th and 7th day favouring the subgroup that received early treatment (within the first 48 hours of the onset of diarrhoea). However, no detailed information of stool frequency on day 7 is reported in the publication. No information of adverse events is given.

Vandenplas *et al.* (2007) evaluated the efficacy of *Saccharomyces boulardii* as an adjuvant to ORS in acute infectious gastroenteritis (GE) in children. 202 children less than 3 years old in 4 centres (3 in India, 1 in Indonesia) were included in the double-blind randomised placebo-controlled trial. All infants were treated according to WHO-recommendations (ORS for 6-7 hours, rapid re-alimentation

and ORS for every liquid stool) with 500 mg per day *Saccharomyces boulardii* or placebo as add-on treatment for 5 days. 188 (93%) children (93 *Saccharomyces boulardii*; 95 placebo) (age range 3-33 months) with diarrhoea (duration before inclusion 1-5 days) completed the study (drop-outs equal in both groups). Duration of diarrhoea was 66.57±52.52 h in the control group versus 53.65±38.74 hours (difference~13 hours or 20% of duration) in the verum group (p=0.05). 86% of the children in *Saccharomyces boulardii* group versus 74% in placebo group were cured on day 3 (p=0.04). The number of cured patients on day 5 did not differ (97% vs 90%, p=0.133). The groups did not differ in quality of stools, vomiting or use of other medication. Side effects were not reported.

Htwe *et al.* (2008) evaluated the efficacy of *Saccharomyces boulardii* in acute diarrhoea in 100 hospitalised children in Myanmar (age range= 3 months to 10 years; 89 children were aged between 3 and 24 months, 11 children were older than 2 years). 50 children were treated with *Saccharomyces boulardii* (2 times 250 mg, no further specification) for 5 days in addition to ORS and 50 were given ORS alone (control group). The mean duration of diarrhoea was 3.08 days in the *Saccharomyces boulardii* group and 4.68 days (p<0.05) in the control group. Stools had a normal consistency on day 3 in 38 (76%) of 50 patients in the *Saccharomyces boulardii* group as compared with only 12 (24%) of 50 in the control group (p=0.019). No severe side effects were observed.

Table 20: stool frequency in the study population: results of Htwe *et al.* (2008)

	***S. boulardii* (number of patients)**		**Control (number of patients)**		
Day	<3 stools per day	≥3 stools per day	<3 stools per day	≥3 stools per day	Chi-square test
1	0	50	0	50	ns*
2	27	23	15	35	0.019
3	39	11	28	22	0.019
4	48	2	39	11	ns*
5	50	0	48	2	ns*
6	50	0	50	0	ns*
7	50	0	50	0	ns*

*ns = not significant

Shen *et al.* (2008) evaluated the efficacy and safety of *Saccharomyces boulardii* for treatment of acute diarrhoea in 137 Chinese children aged from 1 month to 8 years in a multicentre, randomised and controlled trial. 75 children received *Saccharomyces boulardii* and 62 children dioctahedral smectite as control. The daily dose is not mentioned in the abstract. Frequency and consistency of stools as well as safety were assessed 48 hours and 72 hours after treatment. The improvement rate of diarrhoea after 48 hours was 84% in the *Saccharomyces boulardii* group and 69.35% in control group (p=0.041), the improvement rates were 60% and 53.22%, respectively, after 72 hours (p=0.425). The duration of diarrhoea was 3.12 days in *Saccharomyces boulardii* group whereas it was 3.58 days in the control group (p=0.080). The risk of diarrhoea lasting <7 days was more than 85% in two groups (p=0.347). No side effects were observed.

Ji *et al.* (2009) included in a randomised placebo-controlled trial 92 hospitalised Chinese children suffering from acute diarrhoea, aged from 2 months to 7 years. 46 children as control group received conventional treatment (ORS, oral montmorillonite, antibiotics if necessary), 46 children received *Saccharomyces boulardii* plus conventional therapy. The daily dose is not mentioned in the

abstract. The mean diarrhoea duration was 6.54±1.74 days in the control group and 5.720±1.67 days in the *Saccharomyces boulardii* group (t=2.30, p<0.05). On the 4th day, the patients in the *Saccharomyces boulardii* group passed 3.13±0.95 stools per day versus 3.74±0.91 stools per day in the control group (t=3.14, p<0.01). On the 7th day, the patients in the *Saccharomyces boulardii* group passed 1.74±0.93 stools per day versus 2.24±0.95 stools per day in control group (t=2.55, p<0.05). A statistically significant difference was observed in the number of stools on the 4th and 7th day favouring the subgroup that received early treatment (within the first 48 hours of the onset of diarrhoea) (t=3.90, 3.71, p<0.01).

Grandy *et al.* (2010) compared the effect of two probiotic products and placebo in addition to a rehydration therapy in the treatment of Bolivian children less than 2 years of age hospitalised for acute rotavirus diarrhoea in a randomised double-blind controlled clinical trial. Sample size was 20 per group and the outcomes were duration of diarrhoea, fever, vomiting and hospitalisation. 64 cases finished the protocol. 20 patients received placebo (GC group), 21 patients *Saccharomyces boulardii* (4×10^{10} lyophilised cells b.i.d.; GB group) and 23 patients a combination of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum* and *Saccharomyces boulardii* (1.375×10^7 lyophilised cells/dose), GARLB group. Median duration of diarrhoea in the GB group (58 hours) was shorter than in the GC group (84.5 hours, p=0.04), also the duration of fever (p=0.041).

In a randomised prospective open-label study, Eren *et al*. (2010) compared the clinical efficacy and cost/effectiveness of *Saccharomyces boulardii* with YF (yogurt fluid) in acute non-bloody diarrhoea in Turkish children. Group A (n=28) received 250 mg lyophilised *Saccharomyces boulardii* twice a day in children ≥2 years and 125 mg twice a day in children <2 years of age. Group B (n=27) received YF (a fluid extracted from yoghurt made by a ferment containing *Lactobacillus bulgaricus* and *S. thermophilus*. The statistical analysis showed no significant difference between group A and group B concerning duration of diarrhoea and duration of hospitalisation in both the PP ('per protocol') and ITT ('intention to treat') analyses.

Table 21: Results of Eren *et al.* (2010)

	Group A	**Group B**	**Significance**
Duration of diarrhoea in the ITT analysis (day)	4.45±2.46	5.38±3.14	p>0.05
Duration of diarrhoea in the PP analysis (day)	4.54±2.36	4.81±1.79	p>0.05

At day 3, the PP analysis showed a resolution of diarrhoea in 13 patients (46.4%) in group A and in 6 patients (22.2%) in group B (p=0.059). However, the ITT analysis showed a statistically significant result for group A compared with group B (p=0.033). At day 5, there was no significant difference in both analyses concerning resolution of diarrhoea.

Le Luyer *et al.* (2010) compared in a double-blind, randomised, controlled, multicentre study the efficacy of a specific adapted formula (lactose-free, high-mineral, low-osmolarity formula, containing rice and pectin, fortified with *Saccharomyces boulardii*) in the management of 70 infants (PP; 77 ITT) aged between 1 and 9 months suffering from acute diarrhoea with a standard formula used to feed healthy infants from birth. After a short phase of rehydration (oral or i.v.), 36 infants received the standard (control) formula, 34 infants received the modified formula. The duration of the diarrhoea was defined as the time needed until the occurrence of the first normal stool after the last liquid stool. The duration of diarrhoea from the time of inclusion was significantly reduced in the treated group (35.4±3.7 hours) versus the control group (67.1±5 hours); p<0.001). There were 15 infants with rotavirus in the treated group and 13 in the control group, however the

duration of diarrhoea did not depend on the presence or absence of rotavirus but only on the treatment. The average daily weight gain was significantly higher in the treated group compared with the control group (74.2±26.4 g versus 23.7±6.7 g; p<0.05). On average 156 mg per day *Saccharomyces boulardii* were consumed in the treatment group. Efficacy of *Saccharomyces boulardii* cannot be assessed objectively because it was administered in combination with a specific adapted formula. No adverse event was reported.

Corrêa *et al.* (2011) investigated in a randomised double-blind, placebo-controlled study the efficacy of *Saccharomyces boulardii* in 186 Brazilian children (6 to 48 months old) with acute diarrhoea. The children received twice per day 200 mg *Saccharomyces boulardii* or placebo for 5 days. Among the 176 children, who completed the trial, those treated with *Saccharomyces boulardii* (n=90) showed a reduction in diarrhoea duration (p <0.05) when compared with the placebo group (n=86).

Table 22: Results of Corrêa *et al.* (2011) I

		Patients with diarrhoea 3 days after beginning of intervention				
Analysis	Groups (nb patients)	Yes (%)	No (%)	*P*	RR	95% CI
Intention to treat	*Saccharomyces boulardii* (95)	29 (30.5)	66 (69.5)	0.001	0.54	0.38-0.66
	Placebo (91)	51 (56.0)	40 (44.0)			
Per protocol	*Saccharomyces boulardii* (90)	29 (32.3)	61 (67.8)	0.0006	0.54	0.38-0.77
	Placebo (86)	51 (59.2)	35 (40.8)			

The presence of rotavirus was detected in faecal samples from 162 patients. An exploratory analysis showed that the beneficial effect of *Saccharomyces boulardii* was observed essentially for patients presenting with rotaviral diarrhoea.

Table 23: Results of Corrêa *et al*. (2011) II

		Patients with diarrhoea 3 days after beginning of intervention					
Groups (nb patients)		Yes (%)	No (%)	*P*	RR	95% CI	*P*, ratio RR, 95% CI
Rotavirus positive (93)	*S. boulardii* (48)	14 (29.2)	34 (70.8)	0.0014	0.45	0.28-0.74	0.15
	Placebo (45)	29 (64.4)	16 (35.6)				0.60
Rotavirus negative (69)	*S. boulardii* (34)	14 (41.2)	20 (58.8)	0.395	0.76	0.46-1.26	0.30-1.20
	Placebo (35)	19 (54.3)	16 (45.7)				

Dalgic *et al*. (2011) evaluated the effectiveness of zinc, *Saccharomyces boulardii* (no further specification) and lactose-free formula, and their different combinations in the treatment of rotavirus diarrhoea in children from 1 to 28 months in Turkey. Time interval between onset of diarrhoea and hospitalisation had to be less than 96 hours. 480 children were enrolled in the prospective, single-blind and controlled trial and randomised to 8 groups, each with 60 patients. Group 1 received 250 mg *Saccharomyces boulardii* once daily, group 2 received zinc, group 3 received lactose-free formula, group 4 received *Saccharomyces boulardii* plus zinc, group 5 received *Saccharomyces boulardii* plus lactose-free formula, group 6 received zinc plus lactose-free formula, group 7 received *Saccharomyces boulardii* plus zinc plus lactose-free formula, control group 8 received only oral and/or parenteral rehydration solutions. No statistically significant differences were found in the time to resolution of fever after intervention between the treatment groups and the control group. The time to resolution of vomiting was significantly lower in group 4 compared with groups 1 and 5. The duration of diarrhoea was significantly reduced in groups 2 and 4 compared to control. A statistically significant difference in the duration of hospitalisation was observed for the groups 2 and 4 in comparison to the control group. No adverse effects were observed.

Riaz *et al*. (2012) analysed the efficacy and safety of *Saccharomyces boulardii* in acute childhood diarrhoea in a double-blind randomised controlled trial (RCT). 108 Indian children aged between 3 months and 5 years were included in the study, 54 in the *Saccharomyces boulardii* and 54 children in the placebo group. Six cases in the *Saccharomyces boulardii* group and 3 cases in the placebo group left the study. There were 5 and 4 treatment failures in the *Saccharomyces boulardii* and placebo group, respectively. The children received either 250 mg *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926) twice daily or placebo for 5 days or till the recovery whichever was earlier. Mean post intervention duration of diarrhoea was significantly (95% CI: -28.13 to -5.43) shorter in the *Saccharomyces boulardii* group (52.08±24.57 hours) as compared to placebo group (64.04±30.43 hours). The time of appearance of first semi formed stool in the *Saccharomyces boulardii* group (39.48±23.09 hours) was significantly (95% CI:-25.4 to -3.87) shorter than the placebo group (54.13±28.21 hours). Other parameters like total amount of oral rehydration solution, weight gain and number of stools do not differ significantly. No adverse effects were observed.

Erdoğan *et al.* 2012 compared the clinical effectiveness of *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926) and *Bifidobacterium lactis* in children aged 5 months-5 years who had been diagnosed with rotavirus gastroenteritis. The first group (25 children) received oral rehydration therapy and rapid refeeding with a normal diet with 282.5 mg per day *Saccharomyces boulardii*. The second group (25 children) received oral rehydration therapy and rapid refeeding with a normal diet with 30 mg per day *Bifidobacterium lactis*. The third group received only rehydration therapy and rapid refeeding with a normal diet. The mean duration time of diarrhoea was 6.6±1.7 days for the first group, 4.1±1.3 days for the second group, and 7.0 ± 1.6 days for the third group. The mean duration time for diarrhoea in the second group was significantly shorter than in the first and third groups ($p<0.001$). Vomiting rates had no significant difference in all groups.

Khan *et al.* 2012 performed a randomised controlled trial in 420 Pakistani children with acute watery diarrhoea, aged 2 months to 5 years. Group 1 (210 children) was given *Saccharomyces boulardii* (250 mg 'BD') and rehydration therapy, group 2 (210 children) rehydration therapy only for 5 days. Statistically significant differences in terms of stool consistency and frequency were noted in group 1 from 2nd day of treatment onwards. Group 1 also showed reduction in mean duration of diarrhoea by 1.1 days compared with group 2.

Burande 2013 performed a prospective, parallel, single-blind, randomised, controlled clinical trial in children with acute diarrhoea. 72 Indian children were randomised either to group I given 250 mg *Saccharomyces boulardii* twice a day, rehydration therapy and zinc or to group II given rehydration therapy and zinc only. One patient in each group were lost to follow up. Average time for recovery from loose motions was 3.4 days ± 1.4 days in group I, and 5.5 days ± 2.1 days in group II (statistical significance).

Shaikh *et al*. (2015) conducted a randomised comparative study in 100 patients with acute diarrhoea (age 3 months to 5 years) in Pakistan. Cases were given low osmolar ORS, zinc & *Saccharomyces boulardii* 250 mg twice daily for 3 days and controls were given low osmolar ORS and zinc. P-value ≤ 0.05 was considered as significant. The results showed that 51 patients were male and 49 patients were female. The overall mean age of study subjects was 26.73±12.65 months. 96 children were hospitalised. There were 50 children in each of the two groups. The results were evaluated according to 1st, 2nd and 3rd day post intervention. Among 50 patients of the case group, 18 patients were compliant. 49 patients had decrease in duration. The same results were observed in the decrease in frequency. Consistency was improved in 39 patients. The duration of hospitalisation was reduced in 36 patients. It was noted that improvement in the duration, frequency and consistency was mostly observed on 2nd day post intervention. There was no significant difference between the two groups regarding the mean number of stools after 24 hours of beginning of treatment. However, the results showed statistically significant gradual reduction in favour of probiotic group from 48 hours onwards. The mean number of stools remained comparable between probiotic group and control group on day 0 and day 1. However, in probiotic group, the mean number of stools was lower on day 2, day 3 and day 4 compared to control group. The information concerning study design and method is incomplete.

Das *et al*. (2016) studied the efficacy and safety of *Saccharomyces boulardii* (SB) in acute childhood rotavirus diarrhoea in India. In a double-blind, randomised controlled trial, 60 children (3 months to 5 years) with WHO-defined acute watery diarrhoea ($\geq$3 unformed or loose stools in the last 24 hours) and stool rotaviruspositive were randomised into intervention (n= 30) and control (n= 30) groups. The intervention group received SB in lyophilised powered form (2 times 250 mg per day) for 5 days. The children remained in hospital till improvement in their clinical condition, and, after discharge, were followed till 7 days. The primary endpoint "the median duration (hours)

of diarrhoea" (time (in hours) from the first to the last abnormal (loose or liquid) stools preceding normal stool return) was significantly shorter in the intervention group (60 vs. 89; 95% CI: -41.2 to -16.8). A significantly shorter duration of hospitalisation (74 vs. 91; 95% CI: -33.46 to- 0.54) was also seen in the intervention group, but no significant difference was seen for fever and vomiting. There was also no difference between the two groups in the proportion of children requiring parenteral rehydration and persistence of diarrhoea lasting beyond day 7. There was no report of any adverse event.

Table 24: Results of Das *et al.* (2016)

Outcome	***S. boulardii* group (n=30)**	**Placebo group n=28 (completed follow-up)**	**Difference (95% CI)**
Median (IQR) hours of diarrhoea[a]	60 (51-67)	89 (68-95)	-29 (-41.2 to -16.8)
Median (IQR) hours of hospitalization[a]	74 (64-90)	91 (76-105)	-17 (-33.46 to -0.54)
Median (IQR) duration of fever (hours)[a]	56 (48-67)	67 (55-81)	-11 (-23.04 to 1.04)
Median (IQR) duration of vomiting (hours)[a]	48 (39-56)	55 (43-61)	-7 (-16.41 to 2.41)
Proportion of children requiring parenteral rehydration[b]	2 (6.7)	5 (16.7)	0.36 (0.06 to 2.01)
Proportion of children having diarrhoea lasting beyond day 7[b]	1 (3.3)	4 (14.3)	0.21 (0.02 to 1.98)

a. The difference between the two medians and calculation of 95% CI (confidence interval) has been done by the method proposed by Bonett and Price.
b. Data expressed in odds ratio (OR) and 95% CI.

In a randomised controlled trial in Pakistan, Asmat *et al.* (2018) compared the efficacy of *Saccharomyces boulardii* and lactic acid producing probiotics in addition to usual treatment regimen to cure diarrhoea among children. Children at the age from 6 months to 5 years suffering from diarrhoea (<14 days) were randomly assigned to oral treatment with *Saccharomyces boulardii* or lactic acid producing bacterial probiotics for 5 days. The dose for probiotics was administered orally twice a day in 20 ml of water; dosage for less than one year of age was 150 mg and 250 mg for children older than two years, divided into two doses for each of *Saccharomyces boulardii* and lactic acid producing probiotics groups. Efficacy of both probiotics was assessed as per operational definition (frequency and consistency of stools per day). In addition to probiotics, all patients were treated with i.v. antibiotics (ceftriaxone) and oral rehydration therapy as per hospital protocol. Chi-square test was applied to compare the efficacy of both groups. Data were stratified for age, gender, gestational weight, duration of diarrhoea and socioeconomic status to deal with effect of modifiers. Post-stratification chi-square test was applied. P-value of <0.05 was considered as significant. 200 patients were randomly selected; 100 were treated with *Saccharomyces boulardii* while the other 100 were supplemented with lactic acid concomitantly along with conventional diarrhoea treatment. Results indicated that *Saccharomyces boulardii* treatment group has significantly higher efficacy rate (45%) compared to lactic acid producing probiotics (26%). According to the authors, this study showed that *Saccharomyces boulardii* has a better efficacy compared to lactic acid and may be adopted as a probiotic of choice.

Table 25: clinical studies in children with acute unspecific diarrhoea

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Chapoy, 1985 Chapoy, 1986	Controlled	Chapoy 1985: *S. cerevisiae* CBS 5926 (no information on number of viable cells) Chapoy 1986: *S. cerevisiae* CBS 5926 (≥1.8 x 10^{10} viable cells/g) Dosage: 2 times 250 mg and rehydration treatment or only rehydration treatment Duration: 5 days	38 French children (2 weeks to 30 months) *S. boulardii*: n= 19 Control: n= 19	Gastroenteritis with acute diarrhoea	Results of Day 1 and Day 4 were compared and calculated as mean±SD Frequency (days), consistency (watery, soft, normal), weight (g), transit time (hours, assessed by the carmin red method) of stools Significant reduction in the number of stools ($p<0.01$), weight and consistency of stools ($p<0.05$), and transit time ($p<0.05$) could be confirmed by statistical evaluation of all 19 children in the *S. cerevisiae* group	Student's t-test, chi-square test	Small European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Cetina-Sauri and Sierra Basto, 1989 and 1991	Multicentre, randomised, placebo-controlled, double-blind	200 mg *S. cerevisiae* CBS 5926 (no information on strain and number of viable cells are given in the reference, according to Corrêa *et al*. (2011), who used the same product: 200 mg contain 4 x 10^9 viable cells) every 8 hours or placebo and rehydration therapy Duration: 4 days	130 Mexican children; (*S. boulardii* n=65; placebo n=65) Age: 3 months-3 years	Acute diarrhoea	Frequency (comparison between the two treatment groups=efficacy) and consistency (effective, ineffective) of stools after 4 days; clinical cure rate (efficacy was defined as return to 4 bowel movements or deterioration of symptoms) Frequency of stools decreased and the difference between the two groups became statistically significant at 48 and 96 hours ($p<0.05$) Clinical cure rate after 48 hours was higher in the *S. boulardii* group (48 hours: ≈37%;	Student's t-test, chi-square test	Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					96 hours: ≈87%) than in the placebo group (48 hours: ≈7%; 96 hours: ≈43%) Judgement of effectiveness showed significant results too ($p<0.01$)		
Treatment of acute unspecific diarrhoea Urganci *et al*., 2001	Placebo-controlled, double-blind	*S. boulardii* (*S. cerevisiae* CBS 5926; ≥1.8 x 10^{10} viable cells/g) 250 mg per day *S. boulardii* or placebo together with rehydration therapy	100 Turkish children *S. cerevisiae* n=50 (24 boys, 26 girls). Mean age= 11.5±7.1 months Placebo n=50 (22 boys, 28 girls). Mean age= 10.1±5.1 months	Acute, non-bacterial diarrhoea lasting more than 48 hours	Significant differences were found regarding numer of stools after 48 hours ($p<0.01$) and 96 hours ($p<0.05$) and percentage of cases cured after 48 hours ($p<0.01$) and 96 hours ($p<0.05$) in *S. cerevisiae* treated children	Student's t-test for two independent samples, chi-square test	Randomisation not mentioned Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
			Age: 2-29 months				
Treatment of acute unspecific diarrhoea Kurugöl and Koturoglu, 2005	Randomised placebo-controlled, double-blind	250 mg per day *S. boulardii* (*S. cerevisiae* CBS 5926, no information on number of viable cells) or placebo together with rehydration therapy Duration: 5 days	200 Turkish children out of 232 enrolled children Age: 3 months-7 years	Acute diarrhoea (liquid, mucous of bloody stools passed at least twice as frequently than usual for a minimum of 24 hours before admission but not for longer than 7 days	Time from start of treatment until appearance of the first normal stool: significant reduction in the *S. boulardii* group compared to the placebo group (4.7±2.5 vs 5.5±3.2, p=0.03). Medians of average stool frequency after the second day of treatment were significantly lower in the *S. boulardii* group than in the placebo group (p=0.003). Weighted mean difference = WMD (fixed) 95% CI: -1.00 day	Student's t-test, chi-square test	Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					(-1.35, -0.65), Vandenplas *et al*., 2008		
Treatment of acute unspecific diarrhoea Billoo *et al*., 2006	Randomised controlled	*S. cerevisiae* CBS 5926, about 2 x 10^{10} viable cells/g (250 mg b.i.d.) with rehydration therapy or rehydration therapy only Duration: active treatment 5 days, follow-up for 2 months	100 Pakistani children (2 months-12 years) (50/50)	Acute watery diarrhoea of mild to moderate severity	By day 3 frequency of stools reduced to 2.8 and 4.4 stools per day, respectively (p=0.01) and by day 6 it reduced to 1.6 (*S. boulardii* group) and 3.3 (control group), p=0.001 Duration of diarrhoea was 3.6 days in *S. boulardii* group whereas it was 4.8 days in control group (p=0.001) WMD (fixed) 95% CI: -1.26 day (-1.73, -0.79), Vandenplas *et al*., 2008	Student's t-test	Non-European study population, significant results
Treatment of acute	Prospective single-blind	*S. cerevisiae* CBS 5926: 5 x 10^{9} live micro-	571 Italian children aged between 3-36	Acute diarrhoea (3 or more outputs of loose or	*S. boulardii* had no effect on duration of diarrhoea, stool outputs and consistency compared to	Chi-square test, Mann-Whitney U test	Single-blind, European study population

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
unspecific diarrhoea Canani *et al*., 2007	randomised controlled	organisms/dose twice per day Group 1: rehydration solution alone Group 2: *Lactobacillus rhamnosus* strain GG Group 3: *S. boulardii* Group 4: Bacillus clausii Group 5: mix of *L. delbrueckii* var *bulgaricus*, *Streptococcus thermophiles*, L. *acidophilus* and *Bifidobacterium bifidum*	months randomised 558 children received study intervention: Group 1: n=91; drop-out 2 Group 2: n=98; drop-out 1 Group 3: n=87; drop-out 2 Group 4: n=100; drop-out 1 Group 5: n=94; drop-out 1 Group 6: n=88; drop-out 1	liquid stools a day)	rehydration therapy alone in contrast to *Lactobacillus rhamnosus* and the combination preparation None of the preparations had a significant effect on the secondary outcomes fever, vomiting and hospital admissions WMD (fixed) 95% CI: -0.11 day (-0.48, 0.26), Vandenplas *et al*., 2008		negative outcome for *S. boulardii*

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		group 6: *Enterococcus faecium* SF68 Duration: 5 days					
Treatment of acute unspecific diarrhoea Ozkan *et al*., 2007	Randomised double-blind, placebo-controlled	*S. cerevisiae* CBS 5926, no information on number of viable cells (250 mg b.i.d.) or placebo in addition to oral rehydration therapy Duration: 7 days	27 Turkish children (6 months - 10 years) Verum=16 (9 male, 7 female) Mean age=23.4±6.6 months Placebo=11 (6 male, 5 female) Mean age=17.6±4.6 months	Acute diarrhoea	Both groups experienced reduced daily stool frequency, the decrease being significantly greater in group 1 on days 3 and 4 compared with group 2 Group 1 demonstrated significant increases in serum immunoglobulin A and decreases in C-reactive protein levels on day 7 Percentage of CD8 lymphocytes on day 7 was significantly higher in group 1 than group 2	Chi-square test, Fisher's exact test, Mann Whitney U-test, Wilcoxon's signed rank test	Small non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Villarruel *et al*., 2007	Randomised double-blind, placebo-controlled	Children under 1 year: 1 times 250 mg *S. boulardii* (no further specification) Children over 1 year: 2 times 250 mg per day or placebo in addition to oral rehydration solution (ORS) Duration of therapy: 6 days 72 patients had a follow-up for one month (verum 35; placebo 37)	100 children Argentinian/ Belgian (?) (3-24 months) Verum=44 Placebo=44 12 drop-outs	Acute mild to moderate diarrhoea	Primary endpoints: number of stools on day 4 and 7 were significantly smaller in the *S. boulardii* group than in the placebo group Patients were also less likely to have diarrhoea on the 7th day or diarrhoea lasting more than 7 days in the *S. boulardii* group compared to the placebo group WMD (fixed) 95% CI: -1.46 day (-2.68, -0.25), Vandenplas *et al*., 2008	Student's t-test. ANOVA	European or non-European study population, significant results
Treatment of acute	Randomised, double-blind,	500 mg per day *S. boulardii* (no further	202 Indian or Indonesian children <3	Infectious gastroenteritis, diarrhoea,	Duration of diarrhoea was 66.57±52.52 hours in the control group	Not mentioned.	Only one page long publication available. Non-

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
unspecific diarrhoea Vandenplas *et al*., 2007	placebo-controlled	specification) or placebo in addition to ORS for 5 days	years old included. 188 children (93 verum, 95 placebo) (age range 3-33 months) completed	duration before inclusion 1-5 days	versus 53.65±38.74 hours (difference ~13 hours or 20% of duration) in the verum group (p=0.05) 86% in *S. boulardii* group versus 74% in placebo group were cured on day 3 (p=0.04) Number of cured patients on day 5 did not differ (97% vs 90%, p=0.133)		European study population, significant results
Treatment of acute unspecific diarrhoea Htwe *et al*., 2008	open, controlled	*S. boulardii* (250 mg b.i.d., no further specification) with rehydration therapy or rehydration therapy only. Duration: 5 days	100 children in Myanmar (3 months-10 years) Verum=50 Control=50	acute watery diarrhoea with a duration of less than 7 days Diarrhoea was defined as passing three or more loose stools per day	mean duration of diarrhoea was 3.08 days in the *S. boulardii* group and 4.68 days (p<0.05) in the control group Stools had a normal consistency on day 3 in 38 (76%) of 50 patients in the *S. boulardii* group compared with 12 (24%)	Chi-square test	Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
				(loose stool is a stool that takes the shape of the container)	of 50 in the control group (p=0.019) On day 2, 27 (54%) of 50 had less than three stools per day in the *S. boulardii* group compared with 15 (30%) of 50 in the control group (p=0.019) WMD (fixed) 95% CI: -1.60 day (-2.03, -1.17), Vandenplas *et al*., 2008		
Treatment of acute unspecific diarrhoea Shen *et al*., 2008	Multicentre, randomised, controlled	*S. boulardii* (n=75) or dioctahedral smectite as control (n=62)	137 Chinese children (1 month-8 years)	Acute diarrhoea	Improvement rate of diarrhoea after 48 hours: 84% in the *S. boulardii* group and 69.35% in control group (p=0.041) Improvement rate after 72 hours: 60% and 53.22%, respectively (p=0.425)	Not mentioned.	Abstract only. Non-European study population, active-controlled, dosage not given, superiority of *S. boulardii* in improvement of diarrhoea after 48 hours, otherwise

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					Duration of diarrhoea: 3.12 days in *S. boulardii* group and 3.58 days in the control group (p=0.080) risk of diarrhoea lasting <7 days was more than 85% in two groups (p=0.347)		comparable results
Treatment of acute unspecific diarrhoea Ji *et al*., 2009	Randomised controlled	Conventional treatment (ORS, oral montmorillonite, antibiotics if necessary), or *S. boulardii* (no further specification) plus conventional therapy duration: unspecified	92 Chinese children (2 months-7 years) Verum=46 Control=46	Acute diarrhoea	4th day: *S. boulardii* group 3.13±0.95 stools per day versus 3.74±0.91 stools per day in control group (t=3.14, p<0.01) 7th day: *S. boulardii* group 1.74±0.93 stools per day versus 2.24±0.95 stools per day in control group (t=2.55, p<0.05)	Not exactly mentioned: Student's t-test, chi-square test	Abstract only. Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Grandy *et al*., 2010	Randomised double-blind, placebo-controlled	In addition to a rehydration therapy: 21 patients (GB group): *S. boulardii* (4 x 10^{10} lyophilised cells b.i.d.) 23 patients (GARLB group): a combination of *L. acidophilus*, *L. rhamnosus, Bifidobacterium longum* and *S. boulardii* (1.375 x 10^{7} lyophilised cells/dose) b.i.d. 20 patients (GC group) placebo Duration: 5 days	76 Bolivian patients 12 patients were excluded from analysis (other etiologic agents, urinary infection, pneumonia, edema, kwashiorkor, severe vomiting) 64 analyzed patients (1-23 months) 36 male, 28 female	Acute rotavirus diarrhoea: presence of at least 3 bowel movements more than the normal number for the child and/or presence of watery stools per day, plus latex test positive for rotavirus	Median duration of diarrhoea in the GB group (58 hours; IRQ 41) was shorter than in the GC group (84.5 hours (IRQ 94), p=0.04), also the duration of fever (p=0.041) Median duration of diarrhoea in the GARLB group (60 hours, IRQ 40) was shorter than in the GC group (84.5 hours) without statistical significance (p=0.06) IRQ= interquartile range	Kruskall-Wallis testing, Mann-Whitney U Test, chi-square test	Non-European study population, small study groups, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Eren *et al*., 2010	Randomised controlled, open	*S. cerevisiae* CBS 5926 (no information on number of cells) Group A (n=28): 250 mg *S. boulardii* twice a day in children ≥2 years and 125 mg twice a day in children <2 years of age Group B (n=27) yogurt fluid (YF: a fluid extracted from yoghurt made by a ferment containing *Lactobacillus bulgaricus* and *S. thermophiles*) Duration: until diarrhoea resolved	67 Turkish children (5 months-16 years) 12 drop-outs (extraintestinal infections, violation of the fermented milk restriction, secondary hemophagocytic syndrome, celiac disease) 55 children (36 boys, 19 girls; mean age=21±28.2 months)	Acute diarrhoea (presence of 3 or more liquid or loose stools per day lasting for less than 14 days)	No significant difference between group A and B concerning duration of diarrhoea and duration of hospitalisation in the PP and ITT analyses At day 3: PP analysis showed a resolution of diarrhoea in 13 patients (46.4%) in group A and in 6 patients (22.2%) in group B (p=0.059) ITT analysis showed statistically significant result for group A compared with group B (p=0.033) At day 5: no significant difference in both analysis concerning resolution of diarrhoea	Independent t-test, chi-square test, Mann-Whitney U tests, and Wilcoxon sign test	Small non-European study population, open design, active-controlled, ITT analysis showed statistically significant result in resolution of diarrhoea for *S. boulardii*, otherwise comparable results

<table>
<tr><th>Type (aim) and objective(s) of Study
Reference</th><th>Study Design and Type of Control Study duration</th><th>Test Product(s): (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)</th><th>Number of Subjects (including age, sex, drop-out)</th><th>Healthy Subjects or Diagnosis of Patients (inclusion criteria)</th><th>Outcomes (primary and secondary endpoints)</th><th>Statistical analysis (e.g. ITT yes/no, CI 95%)
Quality score (e.g. Jadad score)</th><th>Comments on clinical relevance of results</th></tr>
<tr><td>Treatment of acute unspecific diarrhoea
Le Luyer et al., 2010</td><td>Randomised controlled, double-blind, multicentric</td><td>Lactose-free, high-mineral, low-osmolarity formula, containing rice and pectin fortified with S. boulardii versus standard formula after a short phase of rehydration therapy oral or i.v.
In average:
156 mg per day S. boulardii
Duration of treatment: 6 days</td><td>77 (ITT):
-38 treatment group
-39 control group
70 (PP):
-36 treatment group
-34 control group
Age:1-9 months</td><td>Acute diarrhoea (presence of 3 or more liquid or loose stools during the last 24 hours)</td><td>Duration of diarrhoea from time of inclusion was significantly reduced in the treated group (35.4±3.7 hours) versus the control group (67.1±5 hours)
Average daily weight gain was significantly higher in the treated group compared with the control group (74.2±26.4 g versus 23.7±6.7 g; p<0.05)
<table>
<tr><td>No of stools</td><td>treat-ment</td><td>control</td></tr>
<tr><td>Begin</td><td colspan="2">6.6±0.4</td></tr>
<tr><td>Day 4</td><td>0.9±0.4</td><td>2.8±0.7</td></tr>
<tr><td>Day 5</td><td>1.1±0.5</td><td>2.3±0.7</td></tr>
<tr><td>Day 6</td><td>0.2±0.2</td><td>1.3±0.7</td></tr>
</table>
p<0.005
no adverse event</td><td>Mean value with standard deviation, variance analysis, "somme des carrés", Kruskal-Wallis-test</td><td>Efficacy of S. boulardii cannot be assessed because it was administered in combination with a specific adapted formula. Good tolerability</td></tr>
</table>

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
Treatment of acute unspecific diarrhoea Corrêa *et al.*, 2011	Randomised placebo-controlled, double-blind	*S. cerevisiae* CBS 5926; 1 capsule contains 200 mg lyophilised *S. boulardii* (4 x 10^9 viable cells) 200 mg *S. boulardii* twice a day or placebo duration: 5 days	186 Brazilian children (6-48 months): Verum=95 Placebo=91 10 drop-outs: Verum=3 and placebo=2 due to need for antibiotic treatment Verum=2 and placebo=3 because of withdrawal during the trial	Acute diarrhoea within 72 hours before hospitalisation. Diarrhoea was defined as a change in bowel habits with a diminution of stool consistency and 3 or more evacuation per day	Therapy with *S. boulardii* showed a reduction in diarrhoea duration ($p<0.05$) when compared with the placebo group Exploratory analysis showed that the beneficial effect of *S. boulardii* was observed essentially for patients presenting with rotaviral diarrhoea	Yates continuity-corrected chi-square test	Non-European study population, significant results
Treatment of acute unspecific diarrhoea Dalgic *et al.*,	Randomised single-blind, controlled	1) 250 mg *S. boulardii* once per day alone (no	480 Turkish children (1-28 months)	Episode of ≥3 watery or looser-than normal stools within 24 hours	Duration of diarrhoea (from start of treatment until the first normal stool) was significantly reduced in groups 2 and	Chi-square test, ANOVA	Non-European study population, single-blind, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
2011		further specification) 2) Zinc alone 3) Lactose-free formula alone 4) *S. boulardii*+zinc 5) *S. boulardii* +lactose-free formula 6) Zinc+lactose-free formula 7) *S. boulardii*+zinc+ lactose-free formula 8) Only oral and/or parenteral rehydration	60 patients in each group 288 boys, 192 girls Mean age: 13.71±6.21 months 470 partially breast-fed, 10 received cow's milk-based formula only	and/or forceful vomiting Time interval between onset of diarrhoea and hospitalisation <96 hours, stool positive for rotavirus antigen Mild-moderate dehydration	4 compared to control ($p<0.05$) Time to resolution of vomiting was significantly lower in group 4 compared with groups 1 and 5 Statistically significant difference in the duration of hospitalisation was observed for the groups 2 and 4 in comparison to the control group No statistically significant differences were found in the time to resolution of fever after intervention between the treatment groups and the control group No adverse event		for *S. boulardii*+zinc

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		Duration: minimum 5 days.					
Treatment of acute unspecific diarrhoea Riaz *et al*., 2012	Randomised placebo-controlled, double-blind	*S. cerevisiae* CBS 5926 (no information on number of cells): 250 mg twice per day or placebo Duration: 5 days or till recovery whichever was earlier	108 Indian children (3 months-5 years), 54 in each group 6 children in the *S. boulardii* group and 3 in the placebo group left the study Treatment failures: 5 in the *S. boulardii* group, 4 in placebo group	acute onset diarrhoea (less than 48 hours)	duration of post intervention diarrhoea (defined time from enrolment to recovery, Recovery and discharge criterion was defined as passage of 3 consecutive semi formed stools or no stools for 12 hours) significantly (95% CI=-28.13 to -5.43) shorter in the *S. boulardii* group (52.08±24.57 hours) as compared to placebo group (64.04±30.43 hours)	Student's t test. Mann-Whitney U test, chi-square test, Fisher's exact test	Non-European study population, significant results
Treatment of acute	Prospective randomised, controlled	Group I (25): 282.5 mg per day *S. cerevisiae* CBS 5926 (no	75 Turkish children	rotavirus gastroenteritis with 3 or more times of	Mean duration time of diarrhoea. Group I: 6.6±1.7 days	SPSS 19 programme for windows, chi-square test,	Non-European study population, small study groups, treatment

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
unspecific diarrhoea Erdoğan *et al.*, 2012		information on numer of cells) + rehydration therapy Group II (25): 30 mg *Bifidobacterium lactis* + rehydration therapy Group III (25): rehydration therapy only	between (5 months-5 years) Group I: 21.6±11.5 months, 11 boys, 14 girls Group II: 22.1±14 months, 12 boys, 13 girls Group III: 19.1±13.3 months, 14 boys, 11 girls	watery diarrhoea per Day in the last 48 hours	Group II: 4.1±1.3 days Group III: 7.0±1.6 days Significance in group II compared with group I and group III Vomiting rates had no significant difference in all groups	repeated ANOVA test	with *S. boulardii* shortened the duration of diarrhoea, significance questionable
Treatment of acute unspecific diarrhoea Khan *et al.*, 2012	Randomised controlled	Group I (210): 250 mg BD *S. boulardii*+rehydration therapy Group II (210): rehydration therapy alone	420 Pakistani children (2 months-5 years) Group I: 107 children 1-12 monts; 85 children 1-3	Acute watery diarrhoea	Stool consistency and frequency: the improvement was significantly rapid in group I compared with group II	Student's t-test, chi-square test	Non-European study population, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		duration of treatmen: 5 days	years, 18 children 3-5 years; 107 boys, 103 girls Group II: 107 children 1-12 months; 79 children 1-3 years; 24 children 3-5 years; 109 boys, 101 girls		Mean duration of diarrhoea in group I was 3.43 days, compared with 4.5 days in group II ($p<0.05$)		
Treatment of acute unspecific diarrhoea Burande, 2013	Prospective, parallel, single-blind, randomised, controlled	Group I: 250 mg *S. boulardii* twice per day (lyophilised powder in a sachet weighing 282 mg equivalent to 250 mg of yeast)+rehydration therapy	100 Indian patients screened; 72 patients randomised Group I=36; Group II=36, 1 drop-out per group (lost to follow up)	Acute diarrhoea (≥ unformed stool in last 24 hours with duration of <48 hours)	Average time for recovery from loose motions: Group I: 3.4±1.4 days. Group II: 5.5±2.1 days (Z value = 4.9) Statistical significance	Not exactly mentioned, Student's t-test	Non-European study population, single-blind, significant results

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		Group II: rehydration therapy only In addition, zinc 10 mg per day for children<6 months, and 20 mg per day for children >6 months for 14 days Duration of treatment with *S. boulardii* 5 days	Group I: Mean age: 11.46±8.64 months, 17 boys, 18 girls Group II: Mean age 13.55±12.84 months, 14 boys, 21 girls		Vomiting (11 patients in group I, and 8 patients in group II) Average time of recovery in group I 2.5±1.2 days, and in group II 3.3±1.2 days (p<0.01, two-tailed unpaired Student t-test)		
Treatment of acute unspecific diarrhoea Shaikh *et al.*, 2015	Randomised controlled study, no further information is given	Cases: low osmolar ORS, Zinc & *S. boulardii* 250 mg twice per day for three days Controls: low osmolar ORS and Zinc	100 children in Pakistan (3 months-5 years, mean age 26.73±12.65 months) 51 male and 49 female patients	acute diarrhoea	Primary outcome variables: duration, frequency, consistency of stools and duration of hospitalisation according to 1st, 2nd, and 3rd day post intervention: case group: 49 patients had decrease in duration and frequency.	SPSS-version 17 (statistical package for social sciences). Chi-square test P-value ≤0.05 was considered as significant	Non-European study population The information concerning study design and method is incomplete Statistical significance in

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
		Treatment duration: 3 days	96 children were hospitalised 50 children in each of the two groups		Consistency was improved in 39 patients. The duration of hospitalisation was reduced in 36 patients. Improvement in the duration, frequency, and consistency was mostly observed on 2nd day post intervention No significant difference between the two groups for the mean number of stools after 24 hours of beginning of treatment. Statistically significant gradual reduction in favour of probiotic group from 48 hours onwards The mean number of stools remained comparable between probiotic group and		favour of probiotic group from 48 hours onwards

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					control group on day 0 and day 1. In probiotic group the mean number of stools was lower on day 2, day 3 and day 4 compared to control group 2		
Treatment of acute unspecific diarrhoea Das *et al.*, 2016	Double-blind, randomised controlled. One centre in India. Duration of follow-up: 7 days	SB group: 250 mg *S. boulardii* in lyophilised powdered form b.i.d. for 5 days Control group: placebo Treatment duration: 5 days	60 Indian children (3 months to 5 years) enrolled, each group 30	Acute rotavirus diarrhoea of <48 hours duration, (diarrhoea: ≥3 unformed or loose stools in the last 24 hours)	Primary endpoint: Duration (in hours) of acute diarrhoea: median duration (hours) of diarrhoea was significantly shorter in the SB group (60 vs. 89; 95% CI: -41.2 to -16.8) Secondary endpoints: Significantly shorter duration of hospitalisation, no significant difference in fever duration, vomiting duration, parenteral	SPSS software (version 20.0 Chicago, IL, USA) Chi-square test, Mann-Whitney U test, Bonett and Price Intention to treat analysis was used for the primary outcome. P-value <0.05	Non-European study population, significant results partially hospitalised children, small groups

Type (aim) and objective(s) of Study **Reference**	**Study Design and Type of Control Study duration**	**Test Product(s):** (herbal preparation, pharmac. form; Dosage Regimen; Route of Admin.; Treatment duration)	**Number of Subjects** (including age, sex, drop-out)	**Healthy Subjects or Diagnosis of Patients** (inclusion criteria)	**Outcomes** (primary and secondary endpoints)	**Statistical analysis** (e.g. ITT yes/no, CI 95%) **Quality score** (e.g. Jadad score)	**Comments on clinical relevance of results**
					rehydration requirement and persistence of diarrhoea lasting beyond day 7	was taken as significant	
Treatment of acute unspecific diarrhoea Asmat *et al*., 2018	Randomised controlled	A) *S. boulardii* <1 year: 150 mg >2 years: 250 mg Each divided in 2 daily doses B) Lactic acid producing bacterial probiotics: same dosage as A) All patients: i.v. antibiotics (ceftriaxone)+oral rehydration Treatment duration: 5 days	200 children in Pakistan *S. boulardii*: n= 100 6 months-3 years: 61% 4-5 years: 39% Male: 48% Female: 52% Lactic acid: n= 100 6 months-3 years: 62% 4-5 years: 39% Male: 57% Female: 43%	Children of both genders from 6 months to 5 years of age, suffering from diarrhoea (less than 14 days) (diarrhoea: ≥3 unformed or loose stools in the last 24 hours)	Efficacy rate of both probiotics was assessed as per operational definition (frequency and consistency of stools per day) *S. boulardii*: 45% Lactic acid producing bacterial probiotics: 26% p= 0.004	Comparison of efficacy: chi-square Post-stratification chi-square (Age, gender, gestational weight, duration of diarhoea, socioeconomic status)	Non-European study population. Unblinded. Incomplete methodology Dosage inconsistent, concomitant administration of antibiotics in both groups. *S. boulardii* not further specified. Significant results

Meta-analyses

Szajewska *et al.* (2007) performed a meta-analysis in order to assess the effectiveness of *Saccharomyces boulardii* in treating acute infectious diarrhoea in children. Five randomised-controlled trials (310 participants in the verum group and 309 in the control group) met the inclusion criteria: Billoo *et al.* (2006), Cetina-Sauri and Sierra Basto (1994), Hafeez *et al.* (2002), Kurugöl and Koturoglu (2005) and Villarruel *et al.* (2007; not published up to the time of meta-analysis). Combined data from four randomised-controlled trials showed that *Saccharomyces boulardii* significantly reduced the duration of diarrhoea compared with control: weighted mean difference (WMD) -1.1 day, 95% CI: -1.3 to -0.83). Cetina-Sauri and Sierra Basto (1994) did not report the duration of intervention. Four studies (not Kurugöl and Koturoglu (2005)) provided information of stool frequency at various time intervals. The meta-analysis showed a reduction in the frequency of diarrhoea for those treated with *Saccharomyces boulardii* compared with the control at all-time intervals studied, except on day 1. The authors concluded that there exists a moderate clinical benefit of *Saccharomyces boulardii* therapy in otherwise healthy infants and children with acute gastroenteritis, mainly a shorter duration of diarrhoea. However, it should be noted that studies with methodological limitations are included in this meta-analysis and that the included studies were carried out mainly in non-European countries.

Vandenplas *et al.* (2008) updated the above-mentioned meta-analysis including Canani *et al.* (2007) and Htwe *et al.* (2008). Based on the pooled results of six RCTs involving 756 children (treatment group: n=377; control group: n=379), *Saccharomyces boulardii*, as compared to placebo or no intervention, reduced the duration of diarrhoea by 0.92 day (22 hours; WMD -0.92, CI: -1.11 to -0.74).

Szajewska and Skórka (2009) updated their meta-analysis 2007 including Canani *et al.* (2007), Htwe *et al.* (2008) and Vandenplas *et al.* (2007). The meta-analysis of seven RCTs (treatment group: n=470; control group: n=474) showed a reduction in the duration of the diarrhoea (WMD -1.08 day, 95% CI: -1.64 to -0.53, random effects model) in those treated with *Saccharomyces boulardii* as compared with placebo.

Pan *et al.* (2012) made a systemic review about clinical RCTs focused on *Saccharomyces boulardii* in treating acute childhood diarrhoea. The following selection criteria were used to identify published studies for inclusion in the meta-analysis: study design-RCTs; population - children with acute diarrhoea; intervention - *Saccharomyces boulardii* versus placebo or no additional intervention; outcome variable – duration of diarrhoea, stool frequency and adverse effects. Eight articles (Billoo *et al.* (2006), Canani *et al.* (2007), Hafeez *et al.* (2002), Htwe *et al.* (2008), Ji *et al.* (2009), Kurugöl and Koturoglu (2005), Shen *et al.* (2008), Villarruel *et al.* (2007)) were included: 978 children with acute diarrhoea (*Saccharomyces boulardii* group: 487; control group: 491). According to the Jadad score, Billoo *et al*. (2006), Canani et al. (2007), Hafeez *et al.* (2002), Htwe *et al.* (2008), Kurugöl and Koturoglu (2005) and Villarruel *et al.* (2007) were regarded as high quality literature (Jadad score 4), and Ji *et al.* (2009) and Shen *et al.* (2008) as low quality literature (Jadad score 2). A meta-analysis of the eight studies showed a reduction in the duration of diarrhoea (MD: -0.92 day, 95% CI: -1.32 to -0.52) for those treated with *Saccharomyces boulardii* as compared with placebo. The included studies were heterogeneous. Five studies (Billoo *et al.* (2006), Hafeez *et al.* (2002), Ji *et al.* (2009), Shen *et al.* (2008), Villarruel *et al.* (2007)) provided information of stool frequency at various time intervals. The analyses showed a reduction in stool frequency of stools for those treated with *S. boulardi* as compared with the control on day 3, day 4 and day 7. Adverse effects associated with *Saccharomyces boulardii* were not reported. The authors concluded that the therapeutic effects of *Saccharomyces boulardii* was demonstrated in children with acute diarrhoea, but the clinical trials included were of small samples with methodological limitations.

Dinleyici *et al*. (2012) performed a meta-analysis which studied 11 RCTs with a total of 1,306 children (651 in the *Saccharomyces boulardii* group and 655 as controls). According to the authors, all of these studies have been performed with the same *Saccharomyces boulardii* strain by the same company (*Saccharomyces cerevisiae* CBS 5926). The included studies are Billoo *et al.* (2006), Canani *et al.* (2007), Dalgic *et al.* (2011), Eren *et al.* (2010), Grandy *et al.* (2010), Hafeez *et al.* (2002), Htwe *et al.* (2008), Kurugöl and Koturoglu (2005), Riaz *et al.* (2012), Vandenplas *et al.* (2007) and Villaruel *et al.* (2007). *Saccharomyces boulardii* significantly reduced the duration of acute infectious diarrhoea as compared with controls. The pooled WMD was -0.99 days (approximately 24 hours, 95% CI: -1.40 to -0.58). Based on the results of nine RCTs involving 1,128 children (Cetina-Sauri and Sierra Basto (1994), Corrêa *et al.* (2011), Eren *et al.* (2010), Hafeez *et al.* (2002), Htwe *et al.* (2008), Kurugöl and Koturoglu (2005), Riaz *et al.* (2012), Vandenplas *et al.* (2007), Villaruel *et al.* (2007)), *Saccharomyces boulardii* could significantly reduce the risk of diarrhoea on the third day of illness (RR: 0.52, 95% CI: 0.42-0.65).The authors stated that all included trials had a number of methodological limitations e.g. small sample size, different definition of diarrhoea. However, more than 80% of these studies have a follow-up and intention-to treat analysis. They concluded, nevertheless, that this analysis gives a strong evidence that *Saccharomyces boulardii* has a clinically significant benefit in the treatment of acute infections diarrhoea in infants and children. This benefit has been replicated worldwide and shown in developed and developing countries. Furthermore, treatment with *Saccharomyces boulardii* is safe in children with acute diarrhoea.

This statement is further supported by another systematic review and network meta-analysis by Florez *et al.* (2018). The aim was to determine the comparative effectiveness and safety of pharmacological and nutritional interventions for reducing the duration of acute diarrhoea and gastroenteritis in children. For this purpose, a total of 174 studies (32,430 children) proved eligible. Studies were conducted in 42 countries of which most were low- and middle-income countries (LMIC). Most interventions analysed (except vitamin A, MN (micronutrients), prebiotics and kaolin-pectin) showed evidence of superiority to placebo in reducing diarrhoea. With moderate to high quality of evidence, *Saccharomyces boulardii*+zinc and smectite+zinc demonstrated the best combination of evidence quality and magnitude of effect, while symbiotics, loperamide and zinc proved being the best single interventions, and loperamide was the most unsafe. According to the authors, the effect of zinc, *Saccharomyces boulardii*+zinc and smectite+zinc might only be applied to children in LMIC. Results suggest no further role for studies comparing interventions against no treatment or placebo, or studies testing loperamide, MN, kaolin-pectin, vitamin A, prebiotics and diluted milk.

Padayachee *et al.* (2019) published a systematic review to assess the efficacy and safety of *Saccharomyces boulardii* in the treatment of AGE (acute gastroenteritis) in the paediatric population. Ten of 190 articles were selected for final inclusion: Billoo *et al.* 2006, Burande 2013, Corrêa *et al.* 2011, Dalgic *et al.* 2011, Erdoğan *et al.* 2012, Eren *et al.* 2010, Htwe *et al.* 2008, Kurugöl and Koturoglu 2005, Ozkan *et al.* 2007, Riaz *et al.* 2012 (see Table 25). Overall, the results indicate that *Saccharomyces boulardii* shortened the duration of AGE caused by rotavirus (in days), when compared with the control/placebo group, with the included studies displaying little/no heterogeneity. In addition, no adverse effects were associated with the use of this yeast probiotic in treating AGE in otherwise healthy children. Therefore, the results of this systematic review indicate that there is a potential benefit associated with the use of *Saccharomyces boulardii* to treat AGE in the paediatric patient. However, owing to factors such as small sample sizes, unclear and inconsistent quality of methodology, and reporting bias, owing to source of funding and support, a definitive conclusion and recommendation for the use of a specific probiotic like *Saccharomyces boulardii* to be used as treatment or treatment adjunct for AGE in the paediatric hospitalised patient cannot yet be made. In order to offer specific treatment guidelines, future research initiatives investigating the subject of the benefits/harm associated with the use of *Saccharomyces boulardii* must therefore endeavour to consist

of larger RCTs that minimise heterogeneity associated with study participants enrolled, clearly predefine aetiologies e.g. GE or AGE, minimise methodological variability (e.g. blinding), standardise the presentation in which the intervention is offered, and investigate single-strain probiotic. In addition, secondary outcomes like length of hospital stay and cost-effectiveness can also be investigated.

Uncontrolled studies

Amorissani Folquet *et al.* (2011) conducted an open, observational study in the 3 West African countries Togo, Benin and Côte d'Ivoire. 331 children aged between 1 month and 15 years old (mean age 25.6 months), presenting with acute diarrhoea (at least 3 loose or liquid stools in a 24-hour period, which started less than 4 days before enrolment) were included. In addition to a rehydration therapy, *Saccharomyces boulardii* was administered for 5 days at a dose of 250 mg (no further specification given) in the morning and evening. The patients were predominantly male. 292 patients could be analysed. The mean duration of the diarrhoea after enrolment was 2.64 days (SD: 1.09). In all but 5 patients, duration of diarrhoea was less than 5 days. The mean daily number of stools on day 2 was 2.43. *Saccharomyces boulardii* was well tolerated (excellent or good) in 93% cases. One child developed mild transient constipation, which did not require discontinuation of the treatment.

Prevention of AAD in children

The efficacy of *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea (AAD) has also been investigated in children. Turck *et al*. (2003) investigated the incidence and risk factors of oral AAD in an outpatient paediatric population. 650 children with an age range from 1 month to 15.4 years were included and in 11% of them AAD occurred. An even higher rate was observed in children <2 years (18%) and the administration of amoxicillin/clavulanate had a higher risk of AAD (23%) as compared to other antibiotics.

Benhamou *et al*. (1999) investigated the efficacy of *Saccharomyces boulardii* in the prevention of antibiotic-induced diarrhoea in children (age 1 to 5 years) (n=327) receiving antibiotic treatment during 8 days because of infections of the respiratory tract. The study was controlled and double-blind. As comparative agent, diosmectite (n=289) was chosen.

Erdeve *et al.* (2004) investigated the efficacy of *Saccharomyces boulardii* in 653 children (age: 1-15 years) in a randomised, controlled clinical study. Patients were treated with the SAM (antibiotics sulbactam-ampicillin) or AZT (azithromycin) as monotherapy or in combination with *Saccharomyces boulardii* (250 mg per day). 466 patients in total completed the study and were included into the statistical evaluation: SAM monotherapy (n=117), SAM/*Saccharomyces boulardii* (n=117), AZT monotherapy (n=105), AZT/*Saccharomyces boulardii* (n=127). AAD occurred in 18.9% of the patients receiving antibiotic monotherapy and in 5.7% of the patients with the combination of antibiotic and probiotic. ($p<0.05$; chi-square test). In patients receiving AZT, the use of *Saccharomyces boulardii* showed no statistically significant effect on the development of diarrhoea, whereas in the SAM group a statistically significant effect was observed. While 25.6% of the children on antibiotic SAM-monotherapy developed diarrhoea, only 5.7% of the children receiving combined treatment experienced diarrhoea ($p<0.05$; chi-square test). The rate of SAM-associated diarrhoea was highest in the age group 1-5 years.

In 2005, Kotowska *et al*. performed a double-blind, randomised placebo-controlled clinical trial in order to investigate if *Saccharomyces boulardii* prevents AAD in children. A total of 269 children (age 6 months to 14 years) with otitis media and/or respiratory tract infections were included in the trial and received standard antibiotic treatment, plus 250 mg of *Saccharomyces boulardii* (n=132) or a placebo (n=137) orally twice daily for the duration of antibiotic treatment. Analyses were based on allocated treatment and included data from 246 children. Patients receiving *Saccharomyces boulardii* had a lower

prevalence of diarrhoea (definition: ≥3 loose or watery stools per day for ≥48 hours occurring during or up to 2 weeks after the antibiotic therapy) than those receiving placebo (8% vs. 23%, RR: 0.3, 95% CI: 0.2–0.7). *Saccharomyces boulardii* also reduced the risk of AAD (definition: diarrhoea caused by *Clostridium difficile* or otherwise unexplained diarrhoea) compared with placebo [3.4% vs. 17.3%, RR: 0.2, 95% CI: 0.07–0.5]. No adverse events were observed. As AAD may occur up to 2 months after the end of antibiotic treatment, the follow-up interval in this study was short and some cases of AAD may have been missed.

Shan *et al*. (2013) investigated the efficacy of *Saccharomyces boulardii* in the treatment and prevention of AAD. A total of 333 hospitalised children with acute lower respiratory tract infection (age 6 months to 14 years) were enrolled in a 2nd phase open randomised controlled trial. During the 1st phase, all children received i.v. antibiotics. They were randomly allocated to antibiotic treatment alone (B: n=166) or combination treatment with antibiotic and *Saccharomyces boulardii (*500 mg per day, A: n=167) and followed for 2 weeks. Diarrhoea was defined as ≥3 loose/watery stools per day during at least 2 days, occurring during treatment and/or up to 2 weeks after antibiotic therapy had stopped. AAD was considered when diarrhoea was caused by *Clostridium difficile* or when stool cultures remained negative. In the 2nd phase of the study, patients from group B who were treated with the antibiotic alone and developed diarrhoea were randomly allocated to two sub-groups: group B1 (*Saccharomyces boulardii* + ORS) and group B2 (ORS alone). Data from 283 patients were available for analysis. Diarrhoea prevalence was lower in group A than in group B (7.9% vs. 29.2%; RR: 0.27, 95% CI: 0.1-0.5). *Saccharomyces boulardii* reduced the risk of AAD (4.3% vs. 19.4%; RR: 0.22, 95% CI: 0.1-0.5). When group B patients developed diarrhoea (n=42), *Saccharomyces boulardii* treatment during 5 days (group B1) resulted in lower stool frequency ($p<0.05$) and higher recovery rate (91.3% in group B1 vs. 21.1% in B2; $p<0.001$). The mean duration of diarrhoea in group B1 was shorter (2.31±0.95 vs. 8.97±1.07 days; $p<0.001$). No adverse effects related to *Saccharomyces boulardii* were observed.

Casem (2013) performed a randomised clinical trial between June and October 2012 in the Philippines. 140 patients aged 6 months to 18 years with paediatric community acquired pneumonia (PCAP) and on i.v. or oral antibiotics within 24 hours of enrolment received either the standard antibiotic treatment alone (control n=71, aged 3.54±3.42 years) or antibiotic treatment plus 250 mg *Saccharomyces boulardii* (treatment group n=69, aged 4.19±3.09) twice a day for the entire duration of treatment. Analyses were based on treatment. 16 patients from the control group and 11 patients from the treatment group presented diarrhoea without reaching statistical relevance ($p=0.391$). The treatment group had a shorter duration of diarrhoea than the control group ($p=0.032$). *Saccharomyces boulardii* was generally well tolerated and there was no documented or reported adverse event.

A randomised, open, parallel study was conducted in an Indian tertiary care hospital by Jindal *et al*. (2017). 600 children, in the age group of 6 months-12 years, receiving beta-lactam antibiotics for various ailments like otitis media, tonsillitis, urinary tract infections etc. were included in the study. All these children did not have diarrhoea. 300 out of 600 children were also given *Saccharomyces boulardii* sachets b.i.d. (4-6 billion CFUs daily) for 7 days along with beta-lactam antibiotic. 72 [24%] out of 300 patients in the control group developed diarrhoea whereas only 16 [5.3%] out of 300 in the treatment group developed diarrhoea. The results were stastistically significant [$p <0.001$] as calculated by chi-square test.

In a meta-analysis, Johnston *et al*. (2007) investigated the efficacy and safety of probiotics for the prevention of AAD. Clinical studies included in their meta-analysis were those performed by Benhamou *et al.* (1999), Erdeve *et al*. (2004) and Kotowska *et al*. (2005) (see table 26). They concluded that the current data are promising but inconclusive, and that there is insufficient evidence to recommend the use of *Saccharomyces boulardii* for co-administration at this time. According to the meta-analysis of

Szajewska *et al*. (2006) on the use of probiotics in the prevention of AAD in children only the clinical study conducted by Kotowska *et al.* (2005) was included. According to the authors probiotics reduce the risk of AAD in children. For every 7 patients that would develop diarrhoea during antibiotic treatment one fewer will develop AAD if also receiving probiotics.

Johnston *et al.* (2011) again reviewed the data concerning probiotics for the prevention of paediatric antibiotic-associated diarrhoea. The same clinical studies with regard to *Saccharomyces boulardii* were included in this review (Benhamou *et al*. (1999), Erdeve *et al*. (2004) and Kotowska *et al*. (2005) - see table 26). The authors drew the following conclusion: *"Despite heterogeneity in probiotic strain, dose, and duration, as well as in study quality, the overall evidence suggests a protective effect of probiotics in preventing AAD. Using 11 criteria to evaluate the credibility of the subgroup analysis on probiotic dose, the results indicate that the subgroup effect based on dose (≥5 billion CFU per day) was credible. Based on high-dose probiotics, the number needed to treat (NNT) to prevent one case of diarrhoea is seven (NNT 7, 95% CI: 6 to 10). However, a GRADE analysis indicated that the overall quality of the evidence for the primary endpoint (incidence of diarrhoea) was low due to issues with risk of bias (due to high loss to follow-up) and imprecision (sparse data, 225 events). The benefit for high dose probiotics (Lactobacillus rhamnosus or Saccharomyces boulardii) needs to be confirmed by a large well-designed randomized trial. More refined trials are also needed that test strain specific probiotics and evaluate the efficacy (e.g. incidence and duration of diarrhoea) and safety of probiotics with limited losses to follow up. It is premature to draw conclusions about the efficacy and safety of other probiotic agents for pediatric AAD. Future trials would benefit from a standard and valid outcome to measure AAD."*

Szajewska and Kolodziej (2015b) published an update of their 2005 meta-analysis. Concerning children, they evaluated Kotowska *et al*. (2005) and, in addition, Shan *et al*. (2013), Erdeve *et al*. (2004), Casem (2013), Bin *et al*. (2015) and Zhao *et al*. (2014). In children compared with placebo or no treatment, *Saccharomyces boulardii* reduced the risk of diarrhoea from 20.9% to 8.8% (RR: 0.43, 95% CI: 0.30-0.60; NNT: 9, 95% CI: 7-12). Without Bin *et al*. (2015) and Zhao *et al*. (2014), who studied antibiotics as part of eradication therapy (see below), RR was 0.36 (95% CI: 0.21-0.61). Subgroup analysis, based on age, showed that the administration of *Saccharomyces boulardii* reduced the risk of *C. difficile*-associated diarrhoea in children (2 RCTs, n=579, RR: 0.25, 95% CI: 0.08-0.73). However, the wide confidence interval calls for caution. One major limitation is that the methodological quality of included trials varied. Only Kotowska *et al*. (2005) was at low risk of bias. Definition of AAD and/or diarrhoea differed. The optimal dose of probiotics, including *Saccharomyces boulardii*, and the duration of treatment have not been established. One important question remains according to the authors: whether the use of *Saccharomyces boulardii* shall be considered in all subjects receiving antibiotics or only in select populations. This will require clinical judgement.

A current Cochrane review (Guo *et al.* 2019) concluded that overall evidence suggests a moderate protective effect of probiotics for preventing AAD (NNTB: 9, 95% CI: 7 to 13). Subgroup analysis indicated that high dose (≥5 billion CFUs per day) is more effective than low probiotic dose (<5 billion CFUs per day) with moderate certainty evidence). Besides Benhamou *et al.* (1999), Erdeve *et al*. (2004) and Kotowska *et al. (*2005), the following studies with *Saccharomyces boulardii* were included: Shan *et al*. 2013, and Jindal *et al*. (2017); Zhao *et al.* 2014 and Bin *et al.* 2015 (both *Helicobacter pylorii* infection).

However, the authors concluded the benefit of high dose probiotics (e.g. *Lactobacillus rhamnosus* or *Saccharomyces boulardii*) needs to be confirmed by a large well-designed multicentre randomised trial. Only the study of Kotowska *et al.* (2015) was categorised as 'low risk of bias', the other studies were categorised as 'high risk of bias'. Adverse event rates were low, and no serious adverse event were attributed to probiotics.

Table 26: Clinical studies on the prevention of acute antibiotic-associated diarrhoea (AAD) with *Saccharomyces boulardii* in children

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Prevention of AAD Benhamou *et al*., 1999	Randomised, controlled, double-blind	*S. boulardii* 226 mg per day 4.5 billion CFU per day vs. Diosmectite 6 g per day (1-2 years), 9 g per day (>2 years)	Included: n=779 Completed: *Saccharomyces boulardii* n= 327 Diosmectite n=289	Children (1-5 years) with respiratory tract infection receiving antibiotics during 8 days	Occurrence of diarrhoea (>3 liquid stools per day): *S. boulardii*: 7.6% Diosmectite: 5.5%		Only abstract in English Length of observation period not reported Probably too short in order to allow a reliable statement with regard to a prevention of AAD High rate of withdrawals
Prevention of AAD Erdeve *et al*., 2004, 2005	Randomised. No details given	1. Sulbactam-ampicillin+*S. boulardii* (n=117) 2. Sulbactam-ampicillin (n=117) 3. Azithromycin + *S. boulardii* (n=127)	n=653 patients Age: 1-15 years 466 completed	Children with antibiotic treatment (ampicillin-sublactam or azithromycin)	Incidence of diarrhoea: >3 watery stools per day Sublactam/ampicillin :5.7% 25.6% p<0.05	Chi-square test Significant difference only for the sulbactam/ampicillin group	High rate of discontinuations: 28.7% Duration of follow-up unknown

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
		4. Azithromycin (n=105) Dosage *S. boulardii*: 250 mg per day No details given			azithromycin: 5.5% 11.4% p>0.05		
Prevention of AAD Kotowska *et al*., 2005	Double-blind, randomised, placebo-controlled	Antibiotic therapy with 250 mg *S. boulardii* b.i.d. vs. antibiotic therapy with placebo	*S. boulardii* group n=132 Mean age: 58.8 months Placebo: n=137 mean age: 55.8 months	Children (6 months-14 years) with acute otitis media and short-term antibiotic treatment within 24 hours of enrolment, out- and in-patients	Frequency of diarrhoea (≥3 watery/loose stools per day for ≥48 hours occurring during or 2 weeks after antibiotic therapy) and AAD (*C. difficile*, unexplained) *S. boulardii*: 7.5%(diarrhoea) 3.4% (AAD) Placebo: 23% (diarrhoea) 17.3% (AAD)	Chi-square test, Fisher's exact test Significant reduction of diarrhoea caused by amoxicillin/ clavunate and cefuroxime i.v.	Only 2 weeks of follow-up discontinuations: *Saccharomyces boulardii* n=13 Placebo n=10

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
Prevention of AAD Shan *et al*., 2013	Open, randomised, controlled Follow-up: 2 weeks after the end of antibiotic therapy	Group A: 2 times 250 mg *S. boulardii* (*S. cerevisiae* CBS 5926) during antibiotic therapy Group B: Antibiotic therapy alone	Group A: n=167 Mean age: 49.8 months Group B: n=166 Mean age: 48.7 months	Hospitalised children (6 months-14 years) with acute infections of lower respiratory tract requiring i.v. antibiotic therapy	Incidence of diarrhoea (≥3 watery/loose stools per day for ≥48 hours occurring during or 2 weeks after antibiotic therapy) and AAD (*C. difficile*, unexplained) Group A: 7.9% (diarrhoea) 4.3% (AAD) Group B: 29.2% (diarrhoea) 19.4% (AAD)	Chi-square test. Significant reduction of diarrhoea caused by amoxicillin/ clavunate and i.v. cefuroxime	Open design follow-up of 2 weeks only Wide-age range Mainly younger children affected by diarrhoea
Prevention of AAD Casem, 2013	Randomised, controlled	Treatment group: 250 mg *S. boulardii* b.i.d. in addition to antibiotics	Treatment group: n=69 Drop-out: 3	Patients aged 6 months to 18 years with paediatric community acquired pneumonia	Primary outcome: 16 patients from the control group and 11 patients from the treatment group presented diarrhoea	Chi-square test or Fisher's exact test was used to compare differences between groups	Open, not placebo-controlled design Follow–up of 2 weeks only Wide-age range

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
		Control group: antibiotics only	Control group n=71 Drop-out: 3	(PCAP) and on IV or oral antibiotics within 24 hours of enrolement	(3 or more loose or watery stools (MBSFS 4 or 5) per day, which lasts for a minimum of 48 hours) without reaching statistical relevance (p=0.391) Secondary outcome: The treatment group had a shorter duration of diarrhoea than the control group (p=0.032) MBSFS=modified Bristol stool form scale	All statistical tests were two-tailed and were performed at the 5% level of significance A p value of <0.05 % was considered significant	Performed in the Philippines
Prevention of AAD Jindal *et al*., 2017	Randomised, open, parallel study in Indian out-patients	Treatment group: 250 mg *S. boulardii* b.i.d. (4-6 billion CFUs daily) for 7 days and antibiotics	Treatment group: n=300	Children aged 6 months to 12 years with urinary tract infection,	Treatment group: 16 [5.3%] diarrhoea. Subgroups (each 60 patients): Amoxyclav= 2 Cefpodoxime= 4	SPPS software version 17.01 using chi-square test	Open, not placebo-controlled design Performed in India No definition of diarrhoea is given

Study, Reference	**Study design, controls, duration**	**Test Products** (preparation, pharm. form, dosage, route of admin.)	**Number of Subjects**	**Type of Subjects**	**Outcomes** (primary, secondary endpoints)	**Statistical analysis**	**Clinical relevance**
		(Co-amoxyclav, Cefpodoxime, Cefdinir, Cefixime and Cephalaxin) Control group: antibiotics only	Control group: n=300	otitis media, tonsillitis	Cephalaxin= 2 Cefixime= 8 Cefdinir= 0 Control group: 72 [24%] diarrhoea Subgroups (each 60 patients): Amoxyclav= 26 Cefpodoxime= 12 Cephalaxin= 14 Cefixime= 16 Cefdinir= 4 p<0.001		Follow-up is not defined Beta-lactam antibiotics

Prevention of acute antibiotic-associated diarrhoea (AAD) caused by triple therapy of *Helicobacter pylori* infection in children

Clinical studies have been performed in children with *Saccharomyces boulardii* in addition to standard triple therapy for eradication of *H. pylori*.

The randomised, open clinical study by Hurduc *et al*. (2009) investigated the efficacy of *Saccharomyces boulardii* on the eradication rate of *H. pylori*. *H. pylori* infection was identified in 90 of 145 children (62%) and it correlated positively with age ($p < 0.002$) and inversely with socioeconomic status ($p < 0.005$). These 90 children (range 3-18 years) received standard triple treatment (omeprazole/esomeprazole, amoxicillin, clarithromycin) for 7-10 days. The intervention group (48 patients) received additional therapy with *Saccharomyces boulardii* 250 mg b.i.d. Apart from efficacy, the rate of side-effects was investigated and, in the *Saccharomyces boulardii* group, a significant reduction of the incidence of adverse reactions was observed (30.9% vs. 8.3%). The incidence of AAD and diarrhoea is not stated.

In an open randomised clinical study, Bin *et al.* (2015) randomised 194 *H. pylori* positive children (age: 22 months to 16 years) to triple therapy (omeprazole, clarithromycin, amoxicillin or, in case of penicillin allergy, omeprazole, clarithromycin, metronidazole) alone (n=92) or triple therapy plus *Saccharomyces boulardii* (n=102). *Saccharomyces boulardii* was administered at a dosage of 2 times 250 mg per day (*Saccharomyces cerevisiae* CBS 5926). The incidence of diarrhoea was the primary outcome of the study. Diarrhoea was defined as an increase in the frequency of bowel movements (>3 per day) or decrease in stool consistency (Bristol stool scale 5 or 6). In case of diarrhoea, montmorillonite powder, a natural clay (3 g three times daily), was administered orally to patients in either group without interrupting treatment with *Saccharomyces boulardii*. In the group receiving concomitant treatment with *Saccharomyces boulardii* 12 children (11.76%) experienced diarrhoea, in the control group with triple therapy alone 28.26% of the children (n=26). This difference was statistically significant ($p<0.05$).

In the meta-analyses on *Saccharomyces boulardii* as concomitant therapy during eradication therapy for *H. pylori* by Szajewska *et al.* (2010 and 2015a), the number of children included in clinical studies were assessed as not sufficient. Since then, only one clinical study (Bin *et al.* 2015) has been published. As this study, however, has been conducted in China, it remains open if the data presented also apply for European children.

Feng *et al.* (2017) aimed to identify the best probiotic supplementation in triple therapy for pediatric population with *Helicobacter pylori* infection in a systematic review and network meta-analysis. Children without symptoms and with gastrointestinal symptoms have been reported to have a seroprevalence rate of 15.7 and 40%, respectively. 29 trials (3,122 participants) involving 17 probiotics regimens were identified; among them, the five trials with *Saccharomyces boulardii* are: Zhang 2013 (publication not available), Zhang 2012 (publication not available), Zhao *et al*. (2014) [in Chinese, only abstract in English], Zhang 2015 (seems to be Bin *et al.,* 2015), Hurduc *et al.* (2015). Compared with placebo, probiotic-supplemented triple therapy significantly increased *H. pylori* eradication rates and reduced the incidence of total side effects. However, there were several limitations to the meta-analysis, e.g. diversity of antibiotics in triple therapy, confirmation of *H. pylori* eradication, different administration time of probiotics and different design of trials.

***Clostridium difficile*-associated disease (CDD)**

Over a period of 10 consecutive months, Buts *et al.* (1993) studied 19 eligible children (7 boys, 12 girls; median age 8 months; 2 months to 11 years) who presented with enteral symptoms lasting for >15 days and who had solely *C. difficile* in stools with positive cytotoxin B assay. *Saccharomyces boulardii* (ATCC 74012, *Saccharomyces cerevisiae* CBS 5926) was given orally in a lyophilised form

over 15 days (250 mg 2 times per day for infants <1year, 3 times per day for children 1-4 years of age, and 4 times per day for those >4 years of age). Within 1 week of treatment, enteral symptoms and physical findings resolved in 18 patients (95%) with marked decreases (p <0.001) in the number of stools, frequency of colic episodes and total duration of colics per day. Clearing of toxin B was observed within 15 days of therapy in 16 cases (85%), whereas eradication of *C. difficile* from stools was complete after 1 month in 14 (73%). A clinical and bacteriological relapse occurred in two patients (11%), which resolved rapidly with a second 15-day course of *Saccharomyces boulardii*.

Other kinds of diarrhoea in children

Gaon *et al.* (2003) evaluated the effect of *Lactobacillus* and *Saccharomyces* on persistent diarrhoea (more than 3 stools per day for the last consecutive 14 days or more) in Argentinian children in a randomised, double blind, placebo-controlled study. 89 children, aged 6-24 months, were randomly distributed to receive pasteurised cow milk containing *Lactobacillus* (10^{10}-10^{12} CFU/g, n=30), or lyophilised *Saccharomyces boulardii* (reconstituted in sterile distilled water at a concentration of 0.1 g/ml (1 g powder per ml contained 10^{10} CFU of *Saccharomyces boulardii*), n=30) or pasteurised cow milk as placebo (n=29); on each diet 175 g was given twice a day for a 5-day period. Patients with mild or moderate dehydration were rehydrated *ad libitum* orally or by gastric tube for 4 to 6 hours before starting the study. Number of stools, duration of illness and frequency of vomiting were considered. Enteric pathogens were isolated from stools in 40% of the patients, 27% had rotavirus. *Lactobacillus* and *Saccharomyces* significantly reduced the number of stools on day 5 (p<0.001) and diarrhoeal duration (p<0.005). Similarly, both significantly (p<0.002) reduced vomiting as compared with placebo. There was no difference between treatments depending on rotavirus status. In conclusion, *Lactobacillus* and *Saccharomyces* were both effective in the management of persistent diarrhoea in children.

Savaş-Erdeve *et al.* (2009) assessed the efficacy and safety of adding *Saccharomyces boulardii* to antibiotic treatment for amebiasis-associated acute diarrhoea in Turkish children aged from 1 to 15 years. 45 children in group I received metronidazole orally for 10 days, while 45 children in group II additionally received 250 mg lyophilised *Saccharomyces boulardii* (5 x 10^6 living microorganisms, *Saccharomyces cerevisiae* CBS 5926). The median duration of acute diarrhoea was 5 (1-10) days in group I and 4.5 (1-10) in group II (p=0.965). The median number on stools on follow-up and duration of bloody diarrhoea, fever, abdominal pain and vomiting were similar in the two groups. *Saccharomyces boulardii* was well tolerated without any side effects. Addition of *Saccharomyces boulardii* did not seem to be more effective than metronidazole alone.

Dinleyici *et al.* (2009) evaluated the clinical efficacy of *Saccharomyces boulardii* in addition to metronidazole as compared to metronidazole alone in 50 Turkish children with acute bloody diarrhoea caused by amebiasis in a prospective, randomised, open label study. Group A and B (each n=25) were treated with metronidazole (30 mg/kg twice daily), but *Saccharomyces boulardii* (250 mg twice daily, *Saccharomyces cerevisiae* CBS 5926) during the 7 days was added to group B patients. Group A was composed of 25 children (12 girls, 13 boys, mean age 11.7±2.1 years), group B of 25 children, too (14 girls, 11 boys, mean age 10.9±2.2 years). Duration of bloody diarrhoea was significantly longer in group A (72.0±28.5 vs. 42.2±17.4 hours, p<0.001). On day 5, amebic cysts had disappeared in all children in group B, whereas in group A, amebic cysts were still present in 6 children (p<0.05). On day 10, all children were cured, and cysts had disappeared in all. The addition of *Saccharomyces boulardii* to metronidazole in amebiasis significantly decreased duration of (bloody) diarrhoea and enhanced clearance of cysts.

Dinleyici *et al.* (2011) compared the natural evolution of diarrhoea (no treatment-group C) to the efficacy of *Saccharomyces boulardii* (250 mg b.i.d., *Saccharomyces cerevisiae* CBS 5926-group B) or metronidazole (group A) (30 mg/kg b.i.d.) in Turkish children with gastrointestinal symptoms

(abdominal pain, diarrhoea, nausea-vomiting, flatulence) for more than two weeks and positive stool examination for *Blastocystis hominis* in a randomised single-blinded clinical trial. Group A was composed of 15 children (7 girls, 8 boys, mean age 94.6±37.4 months), group B of 18 children (7 girls, 11 boys, mean age 99.1±43.8 months) and group C of 15 children (8 girls, 7 boys, mean age 90.2±46.7 months). The primary end points were clinical evaluation and result of microscopic stool examination at day 15. Clinical cure was observed in 77.7% in group B, in 66.6% in group A and in 40% in control group C (p<0.031, between *Saccharomyces boulardii* and control). Disappearance of the cysts from the stools on day 15 was 80% in group A, 72.2% in group B and 26.6% in group C (p=0.011 between group A and C; p=0.013 between group B and C). At the end of the first month after inclusion, clinical cure rate was 94.4% in group B and 73.3% in group A (p=0.11): parasitological cure rate for *B. hominis* was very comparable between both groups (94.4% vs. 93.3%, p=0.43).

Overall Review/Meta-analysis

Feizizadeh *et al*. (2014) showed in their review and meta-analysis that *Saccharomyces boulardii* is safe and has clear beneficial effects in children with acute diarrhoea when administered in addition to rehydration therapy. However, the authors stated that additional studies using head-to-head comparisons are needed to define the best dosage of *Saccharomyces boulardii* for diarrhoea with different causes. The authors identified 22 articles, which met the inclusion criteria, although these studies varied in the definition and cause of diarrhoea, the termination of diarrhoea, inclusion and exclusion criteria, their methodological quality and the reported outcomes. 2,440 patients between 1 month to 15 years of age were included (1,225 interventions, 1,215 controls). For most of the studies, the daily dosage of *Saccharomyces boulardii* was 250-750 mg (10^9 to 10^{10} CFU). Duration of intervention was 5 to 10 days. The pooled data of 17 studies, which reported duration of diarrhoea (Cetina-Sauri and Sierra Basto, 1994; Urganci *et al.*, 2001; Hafeez *et al.*, 2002; Kurugöl and Koturoglu, 2005; Billoo *et al.*, 2006; Canani *et al.*, 2007; Vandenplas *et al.*, 2007; Villarruel *et al.*, 2007; Htwe *et al.*, 2008; Savaş-Erdeve *et al.*, 2009; Dinleycici *et al.*, 2009; Grandy *et al.*, 2010; Dalgic *et al.*, 2011; Erdoğan *et al.*, 2012; Khan *et al.*, 2012; Riaz *et al.*, 2012; Burande, 2013), showed that *Saccharomyces boulardii* significantly reduced the duration of diarrhoea (mean difference [MD] -19.7 hours, 95% CI: -26.05 to -13.34). Subgroup analysis according to cause of diarrhoea showed the duration of diarrhoea was reduced in all 3 subgroups, including rotavirus, *Entamoeba histolytica* and nonspecific cause. Subgroup analysis based on hospitalisation indicated that using *Saccharomyces boulardii* reduced duration of mild diarrhoea more than severe diarrhoea.

The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Infectious Diseases updated in 2014 the evidence-based guidelines for the management of acute gastroenteritis (AGE) in children in Europe (Guarino *et al*., 2014). Concerning treatment, oral rehydration therapy should be used as first-line therapy for the management of children with AGE (strong recommendation, moderate quality evidence). With regard to probiotics, these guidelines endorse the document developed by the ESPGHAN Working Group on Probiotics and Prebiotics (Szajewska *et al*., 2014), which provided recommendations for the use of probiotics for the treatment of AGE in infants and children. Probiotics reduced the duration of approximately 1 day. However, probiotic effects are strain-specific, so the safety and clinical effects of one probiotic microorganism should not be extrapolated to other probiotic microorganisms. The use of the following probiotics may be considered in the management of children with AGE in addition to rehydration therapy: *L. rhamnosus* GG (low quality evidence, strong recommendation), *Saccharomyces boulardii* (low quality evidence, strong recommendation), based on a consistent amount of evidence in various settings.

Irritable bowel syndrome (IBS) in children

Chouraqui *et al*. (1995, abstract) investigated the effectiveness of *Saccharomyces boulardii* at a dosage of 25 mg/kg b.w./day (*Saccharomyces cerevisiae* CBS 5926) versus placebo treatment in infants with irritable bowel syndrome who did not improve after one month of dietary modifications. Disease severity was rated by means of a clinical score including gastrointestinal symptoms and signs. During the first month of treatment, the infants were randomly assigned to *Saccharomyces boulardii* or placebo. For the following 2 months, all patients received *Saccharomyces boulardii*. 41 children (mean age: 22.7 months) were eligible, of whom 20 responded to dietary modifications. 18 of the remaining 21 patients were included in the study (*Saccharomyces boulardii*: n=8; placebo: n=10). After the first month of treatment, the clinical score improved by 59% with *Saccharomyces boulardii* and 24% with placebo ($p<0.01$). Two months later, when both groups received *Saccharomyces boulardii*, no difference was observed. According to the authors, this trial demonstrates the effectiveness of oral *Saccharomyces boulardii* in the treatment of infants with IBS who do not respond to dietary modifications alone. *Saccharomyces boulardii* seems to be effective after one month of administration. Due to the low number of patients included and missing details of the study (e.g. diagnostic criteria, statistical evaluation, clinical score), it cannot be accepted as evidence that *Saccharomyces boulardii* is effective in the treatment of IBS in children.

4.4. Overall conclusions on clinical pharmacology and efficacy

Pharmacology

Based on bibliographic data, a protective mechanism of *Saccharomyces cerevisiae* CBS 5926 against pathogens causing diarrhoea is supposed. Experimental data suggest different mechanisms (luminal and trophic actions as well as mucosal anti-inflammatory signaling effects). It is assumed that the effects are tied to the viability of the yeast cells.

In a review, Terciolo *et al*. (2019) summarised the possible impact of *Saccharomyces boulardii* on various gastrointestinal and systemic diseases associated with intestinal epithelial barrier defects. Through anti-inflammatory, anti-secretion, pro-migratory and adhesive effects, *Saccharomyces boulardii* preserves and restores intestinal barrier functions. Possibly, a yeast-induced general metabolic activation may enhance the barrier function by the acceleration of the enterocyte turnover.

Human pharmacokinetic studies show that following oral administration of *Saccharomyces boulardii* steady-state levels are reached within 3 days. With a half-life of 6 hours in healthy volunteers, *Saccharomyces boulardii* is eliminated from the gastrointestinal tract within 7 days after cessation of administration. There was no evidence that cells of *Saccharomyces cerevisiae* CBS 5926 cross the gastrointestinal wall in healthy humans when administered orally.

Acute unspecific diarrhoea

The use of *Saccharomyces boulardii* has to be regarded as well-established according to the Directive 2001/83/EC. In addition, the period of 30 years required for the establishment of an EU herbal monograph/entry to the EU List of herbal substances, preparations and combinations thereof for use in traditional herbal medicinal products (HMPC guideline on the assessment of clinical safety and efficacy in the preparation of EU herbal monographs for well-established and traditional herbal medicinal products, EMA/HMPC/104613/2005 Rev.1) has elapsed for the preparation containing $>1.8 \times 10^{10}$ viable cells/g lyophilisate.

One randomised double-blind, placebo-controlled, multicentre clinical trial was conducted in adults with a positive statistically significant result for the treatment group (Höchter *et al.*, 1990). The daily dose was 3 times 200 mg for the first 2 days and then 3 times 100 mg *Saccharomyces cerevisiae* CBS 5926

(≥1.8 x 10^{10} viable cells/g). A second randomised controlled study in adults compared two varying *Saccharomyces* preparations (*Saccharomyces cerevisiae* CBS 5926 (at least 10^{10} live micro-organisms/g according to the reference)), however, without a placebo control.

Numerous randomised studies were conducted in children all over the world. Only in the study of Canani *et al.* (2007) *Saccharomyces boulardii* had no effect on duration of diarrhoea, stool outputs and consistency as compared to rehydration therapy alone. In the study of Dalgic *et al.* (2011) *Saccharomyces boulardii* had only an effect on diarrhoea duration when given together with zinc.

Table 27: Summary of clinical studies in children with acute unspecific diarrhoea

Study	**Country**	**Age**	**Patient number under *Saccharomyces***	**Patient number under control**	**Daily dose of *Saccharomyces***
Chapoy, 1985/1986	France	2 weeks-30 months	19	19 only rehydration	2 times 250 mg (*S. cerevisiae* CBS 5926>1.8 x 10^{10} viable cells/g lyophilisate)
Cetina-Sauri and Sierra Basto, 1989/1991	Mexico	3 months-3 years	65	65 placebo	3 times 200 mg (*S. cerevisiae* CBS 5926; 2 x 10^{10} cells/g)
Urganci *et al*., 2001	Turkey	2-29 months Mean age 10.8±0.9 months	50	50 placebo	250 mg *S. cerevisiae* CBS 5926 (≥1.8 x 10^{10} viable cells/g)
Biloo *et al*., 2006	Pakistan	2 months-12 years	50 Mean age 18.3±20.33 months	50 only rehydration Mean age 26.01±23.37 months	2 times 250 mg (*S. cerevisiae* CBS 5926, about 2 x 10^{10} alive cells/g)
Corrêa *et al.*, 2011	Brasilia	6-48 months	95	91 placebo	2 times 200 mg (*S. cerevisiae* CBS 5926; 2 x 10^{10} cells/g according to reference)
Kurugöl and Koturoglu, 2005	Turkey	3 months-7 years	100	100 placebo	250 mg (*S. cerevisiae* CBS 5926, number of cells unknown)
Ozkan *et al*., 2007	Turkey	6 months-10 years	16 Mean age 23.4±6.6 months	11 placebo Mean age 17.6±4.6 months	2 times 250 mg (*S. cerevisiae* CBS 5926, number of cells unknown)

Study	**Country**	**Age**	**Patient number under *Saccharomyces***	**Patient number under control**	**Daily dose of *Saccharomyces***
Villarruel *et al*., 2007	Argentina/ Belgium (?)	3-24 months	44	44 placebo	<1 year: 1 time 250 mg >1 year: 2 times 250 mg
Vandenplas *et al*., 2007	India/ Indonesia	<3 years Age range 3-33 months	93	95	500 mg per day
Htwe *et al*., 2008	Myanmar	3 months-10 years	50	50 only rehydration	2 times 250 mg
Shen, 2008	China	1 month-8 years	75	62 dioctahedral smectite	(?)
Ji *et al*., 2009	China	2 months-7 years	46	46 only conventional therapy	(?)
Grandy *et al*., 2010	Bolivia	<2 years	21	20 placebo 23 combination of probiotics	2 times 4 x 10^{10} lyophilised cells/dose
Eren *et al*., 2010	Turkey	5 months-16 years (mean age 21.2±28.2 months)	28	27 yogurt fluid	<2 years: 2 times 125 mg >2 years: 2 times 250 mg *S. cerevisiae* CBS 5926, number of cells unknown
Le Luyer *et al*., 2010	France (?)/ Lebanon	1 month-9 months	38	39 standard formula	156 mg
Riaz *et al*., 2012	India	3 months-5 years	54	54 placebo	2 times 250 mg (*S. cerevisiae* CBS 5926, number of cells unknown)
Erdoğan *et al*., 2012	Turkey	5 months-5 years	25	25 Bifidobacterium lactis 25 rehydration therapy only	282.5 mg per day (*S. cerevisae* CBS 5926, number of cells unknown)

Study	**Country**	**Age**	**Patient number under *Saccharomyces***	**Patient number under control**	**Daily dose of *Saccharomyces***
Khan *et al*., 2012	Pakistan	2 months -5 years	210	210 rehydration therapy only	250 mg BD
Burande, 2013	India	<6 months-(?)	35 Mean age 11.46±8.64 months	35 rehydration therapy and zinc only Mean age 13.55±12.84 months	2 times 250 mg
Shaikh *et al*., 2015	Pakistan	3 months-5 years	50+low osmolar ORS, zinc	50 low osmolar ORS and zinc	2 times 250 mg
Das *et al*., 2016	India	3 months-5 years	30	30 placebo	2 times 250 mg *S. boulardii* in lyophilised powdered form
Asmat *et al*., 2018	Pakistan	6 months-5 years	100 (61: 6 months-3 years; 39: 4-5 years) In addition to i.v. antibiotics (ceftriaxone)+ oral rehydration	100 lactic acid producing probiotics in addition to i.v. antibiotics (ceftriaxone)+oral rehydration	150-250 mg *S. boulardii*
Canani *et al*., 2007	Italy	3-36 months	87	91 only rehydration 98 *Lactobacillus rhamnosus* GG 100 *Bacillus clausii* 94 mix	2 times 250 mg (*S. cerevisiae* CBS 5926; >2 x 10^{10} cells/g lyophilisate)
Dalgic *et al*., 2011	Turkey	1-28 months	240 60 *S. boulardii* alone 60 *S. boulardii* + zinc 60 *S. boulardii* + lactose-free formula	240 60 zinc alone 60 lactose-free formula alone 60 zinc + lactose-free formula 60 only oral and/or	1 time 250 mg

Study	Country	Age	Patient number under *Saccharomyces*	Patient number under control	Daily dose of *Saccharomyces*
			60 *S. boulardii* + zinc + lactose-free formula	parenteral rehydration	

Neglecting Canani *et al.*, 2007 and Dalgic *et al.*, 2011, nine placebo controlled, nine controlled (rehydration or standard therapy) and three active controlled studies were conducted. All together 1,293 children between 1 months and 16 years of age received *Saccharomyces boulardii* (485 children between 1 month and 3 years of age; 554 children between 2 months and 7 years; 191 children between 2 months and 12 years; 28 children between 5 months and 16 years, Burande 2013 35 children with unspecified age). The administered daily dose was 150 mg to 500 mg *Saccharomyces.* Predominantly 2 times 250 mg was given.

The meta-analysis (Szajewska and Skórka, 2009) of seven RCTs (treatment group: n=470; control group: n=474) showed a reduction in the duration of the diarrhoea (WMD -1.08 day, 95% CI: -1.64 to -0.53, random effects model) in those treated with *Saccharomyces boulardii* compared with placebo.

A meta-analysis (Pan *et al.*, 2012) of eight studies showed a reduction in the duration of diarrhoea (MD: -0.92 day, 95% CI: -1.32 to -0.52) for those treated with *Saccharomyces boulardii* compared with placebo.

Feizizadeh *et al*., 2014 showed in their review and meta-analysis that *Saccharomyces boulardii* is safe and has clear beneficial effects in children, who have acute diarrhoea, when administered in addition to rehydration therapy.

In their review, Guarino *et al*. (2015) drew the conclusion that acute gastroenteritis (diarrhoea) is the original and probably the best established indication for probiotics and that they have obtained "conclusive" evidence of efficacy of selected strains including *Saccharomyces boulardii*. The effect is reduction of gastroenteritis by approximately 24 hours. The authors believe therefore that there are now no reasons to omit this active treatment in children with acute gastroenteritis in addition to rehydration. This is supported by solid compelling and authoritative indications by many agencies and institutions.

Recently, Chen *et al*. (2018) published guidelines on evidence-based indications for the management of children with acute infectious diarrhoea in Chinese paediatric population. The guideline was developed by an expert working group composed of paediatric gastroenterology, paediatric infectious disease and epidemiology experts, under the organisation of the Academic Group of Paediatric gastroenterology of Chinese Pediatrics Association. Recommendations were based on a comprehensive thorough literature review in relevant databases including PubMed, Cochrane, EMBASE, China Biomedical Database (CBM) and Chinese Journal Full-text Database, up to June 2013. According to this guideline, several probiotics have curative effects on the treatment of acute infectious diarrhoea in children, especially for watery diarrhoea caused by viral infection (evidence level A). *Saccharomyces boulardii* can shorten the duration of acute infectious diarrhoea in children and reduce the duration of hospital stay (evidence level A).

It can be concluded that *Saccharomyces cerevisiae* therapy in addition to rehydration therapy in otherwise healthy infants and children with acute unspecific diarrhoea has a moderate clinical benefit (mainly a shorter duration of diarrhoea), even if some studies have methodological limitations and the studies were carried out mainly in non-European countries. This conclusion is supported by recently

published reviews/meta-analyses and guidelines based on a consistent amount of evidence in various settings.

Infants from 6 months to 2 years should be treated under the care of a doctor, only. Elder children with unspecific acute diarrhoea can be treated for two days. If the symptoms do not improve or worsen, a doctor has to be consulted.

The use in infants younger than 6 months is not recommended, because an exact analysis of data from existing literature concerning this special age group is not possible. In the first months of life, the intestinal flora changes, especially when nutrition of the infant's evolves from breast feeding to different nutrition (Lentze, 2013). Therefore, more precise study results concerning efficacy and safety are necessary to recommend a treatment in this age group.

Based on the clinical data available (for adults: mainly Höchter *et al*., 1990; for children: mainly Chapoy 1985/1986, Villarruel *et al*., 2007; supported by studies with Non-European patient groups) and supported by the marketing overviews (for adults and children e.g. No. 2, 4, 7, 11, 14, 22 in Table 2), the following posology is recommended:

Children (6 months to 11 years): Single dose: 250 mg dried yeast, frequency of administration: 1-2 times
Adolescents, adults and elderly: Single dose: 250 mg dried yeast, frequency of administration: 2 times. The medicinal product should contain at least 1.8×10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g dried yeast, rounded up to 2×10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g dried yeast (see information given in section 1.1).

Prevention of antibiotic-associated diarrhoea (AAD)

A variety of clinical studies and meta-analyses have been published with regard to the effects of *Saccharomyces boulardii* in the prevention of AAD in both children and adults. As the selection of clinical studies for the meta-analyses had been based on different criteria (e.g. age group, study design, indication), different assessments on the efficacy of *Saccharomyces boulardii* in this indication were given by the authors.

Many of the clinical studies, which have been published so far, indicate that the incidence of AAD tends to be lower when *Saccharomyces boulardii* is administered concomitantly. Nevertheless, it has to be emphasised that the study designs employed show a great variety with regard to e.g. dosage of *Saccharomyces boulardii*, study duration and follow-up, selection of patients (age, inpatient or outpatient), choice of antibiotic, or definition of diarrhoea. Therefore, the data available are very inhomogeneous and do not represent strong and sufficient evidence to support a general efficacy of *Saccharomyces boulardii* in the prevention of AAD. The effective dosage and the duration of treatment as well as the patient groups at a particular risk are not sufficiently investigated.

A reliable assessment of efficacy is only possible if risk factors for patients for the development of AAD have been identified. Although the risk for AAD seems to be increased in hospitalised patients (26-60% according to McFarland, 2006), the clinical study by Pozzoni *et al*. (2012) reported a diarrhoea rate of 13.3% only in elderly, hospitalised patients receiving placebo during treatment with various antibiotics. Thus, it does not seem justified to recommend *Saccharomyces boulardii* for general administration in the prevention of AAD, since in about 90% of the patients, diarrhoea does not occur while on antibiotic therapy.

This assessment is in agreement with Suardi *et al*. (2013). In their review on probiotics in the prevention of AAD in adults, they summarise as follows: "*In spite of some evidence consensus statements favoring the use of probiotics in the prevention of AAD is lacking, due to the difficulties in analyzing the available studies. The differences of sample sizes with heterogeneous populations, the*

differences in the given dose of probiotics agents, the differences in the amounts of viable organisms administered represent the main factors impacting the correct interpretation of the promising results found in these studies. Moreover, study designs are not homogeneous as well as endpoints and objectives that do not include assessments of possible adverse events. In conclusion, albeit some promising results on the efficacy of probiotics in the prevention of AAD and C. difficile associated diarrhoea, there are no evidence-based guidelines regarding probiotics for this use. Adequately powered, double blind, randomized controlled trials are needed to assess the efficacy of specific probiotic strains."

Guarino *et al.* (2015) also state that AAD, in contrast to acute gastroenteritis (diarrhoea), is a less clear indication for probiotics, and even if efficacy is supported by several randomised clinical trials and meta-analyses, there is no official recommendation. The question if probiotics should be prescribed to children under antibiotics to prevent an episode of diarrhoea has no answer yet. Maybe a selective use of active intervention based on the antibiotic involved and especially the child's age and underlying condition may be considered as the best advisable approach.

The latest Cochrane review (Guo *et al.,* 2019) concerning children also concludes that the benefit of high dose probiotics (e.g. *Lactobacilus rhamnosus* or *Saccharomyces boulardii*) needs to be confirmed by a large well-designed multicentre randomised trial. Most of the included studies were categorised as 'high risk of bias'.

Prevention of antibiotic-associated diarrhoea caused by triple therapy in *Helicobacter pylori* infection

The use of the strain *Saccharomyces cerevisiae* CBS 5926 in prevention of diarrhoea caused by triple therapy in *H. pylori* infection cannot be regarded as well-established according to the Directive 2001/83/EC and the HMPC guideline on the assessment of clinical safety and efficacy in the preparation of EU herbal monographs for well-established and traditional herbal medicinal products' (EMA/HMPC/104613/2005 Rev.1).

So far, the administration of *Saccharomyces cerevisiae* for the prevention of diarrhoea during triple therapy for infection with *H. pylori* has not been established in Europe. Medicinal products for prevention of diarrhoea due to such triple therapy are not available in EU Member States.

Thus, although a great number of clinical studies have included patients undergoing triple therapy and have indicated a tendency of a reduction of diarrhoea, the requirements for a well-established use are not met (at least one decade of well-established medicinal use).

In addition, the studies included in this assessment report have several methodological shortcomings. Only two studies (Cremonini *et al.,* 2002a, Cindoruk *et al.,* 2007) have a double-blind study design; however, the number of patients included was low (43 and 124 patients, respectively). Differences were also observed with regard to the duration of triple therapy (Cremonini *et. al.*, 2002a: one week; Cindoruk *et al.,* 2007: 2 weeks) and the dosage of administered *Saccharomyces boulardii* ranged from 500 mg to 1,000 mg *Saccharomyces cerevisiae* daily (2 times 500 mg, 3 times 250 mg, 2 times 250 mg) in the studies assessed. Besides, most of the studies did not investigate diarrhoea as primary parameter and thus a definition of diarrhoea was not given. Accordingly, the rates of diarrhoea incidence under triple therapy are very diverse ranging from 6% (Song *et al*., 2010) to 43.1% (Lee *et al*., 2011). Thus, there is a great uncertainty about the real incidence. Definite factors have not been described for the group of patients, who may benefit from concomitant treatment with *Saccharomyces cerevisiae* during *H. pylori* eradication therapy. In summary, a general recommendation cannot be given in this indication. Another important aspect is that that the efficacy of standard triple treatment for *H. pylori* eradication has decreased and many new treatments have been introduced to improve eradication rates (Li *et al*., 2015). Geographical differences have to be taken into account.

Paediatric population

Up to now, the data available for the use in children and adolescents are insufficient, too.

Some open randomised clinical studies in children were performed.

Hurduc *et al*. (2009) only treated 48 children (range from 3-18 years) with *Saccharomyces cerevisiae* in addition to triple therapy. The incidences of AAD and diarrhoea are not stated; only adverse reactions in general are reported.

Bin *et al.* (2015) conducted their study in China and it remains open if the data presented also apply for European children.

The publications of further studies conducted in China mentioned in Feng *et al.* (2017) are not available.

In conclusion, a well-established use for *Saccharomyces cerevisiae* CBS 5926 in the prevention of diarrhoea due to triple therapy in *H. pylori* infections appears justified neither in adults nor in children.

***Clostridium difficile*-associated disease (CDD)**

The data available in literature at that time do not support a well-established use of *Saccharomyces boulardii* as an adjunct to standard antibiotic treatment with vancomycin or metronidazole for the secondary prevention of CDD. Statistically significant results, which demonstrate a reduction of CDD recurrences in patients with active CDD, have only been observed in a low number of highly selected patients and thus cannot be used as a basis for a general recommendation. Consequently, clinical studies with a sufficiently high number of patients receiving different categories of antibiotic drug regimen and with an adequate duration of follow-up are needed.

Prevention of diarrhoea associated with tube-feeding

So far, clinical experience with the use of *Saccharomyces boulardii* in prevention of nutrition-related diarrhoea in tube-fed patients is very limited. Only a low number of patients has been included in clinical studies. The dosages applied ranged from 1 g per day (Bleichner *et al*., 1997) to 2 g per day (Tempé *et al*., 1983, Schlotterer *et al*., 1987). Thus, a well-established use of *Saccharomyces boulardii* in tube-fed patients cannot be supported, especially since up to now the recommended daily dosage is 750 mg/1.5 l nutrient solution per day. This posology, which is recommended in current SPCs, is not supported by adequate data.

Furthermore, it has to be taken into account that a risk for immunocompromised patient cannot be excluded when administered *Saccharomyces cerevisiae* (see below). Tube-fed patients are often immunocompromised patients. Therefore, a well-performed benefit-risk evaluation is needed, which is only possible when well-designed studies are available, that also investigate dose-response relationships.

Treatment of irritable bowel syndrome (IBS)

A well-established use of *S. boulardii* in the treatment of IBS is not recommended. The clinical data available do not comply with the recommendations of the CHMP guideline on the evaluation of medicinal products for the treatment of IBS (CHMP, 2014).

There is only one older double-blind randomised clinical study from 1983 (Maupas *et al*.) which showed a superiority of treatment with *Saccharomyces boulardii* regarding the following items: opinion of physician/patient, number and consistency of stools. Other clinically relevant signs of IBS such as abdominal pain, distension, dyspepsia, however, were not improved by *Saccharomyces boulardii* as compared to placebo.

Another study (Choi *et al*., 2011) showed that *Saccharomyces boulardii* was superior to placebo only with regard to quality of life. According to the guideline of the CHMP (2014), the primary outcome in clinical studies for the evaluation of medical products for the treatment of IBS consists of abdominal pain and consistency/frequency of stool. Such evidence of efficacy for the IBS indication is yet to be provided.

There is no medicinal product available on the European market with a tradition of 30 years in this indication, making a 'traditional use' acceptance unlikely. Furthermore, IBS cannot be diagnosed by patients themselves, as diagnosis is mainly based on the exclusion of underlying organic causes that can be done only by a medical practitioner.

Prevention of traveller's diarrhoea

Two randomised double-blind clinical trials have been performed, which assessed the efficacy of *Saccharomyces boulardii* for the prevention of traveller's diarrhoea. There seems to be a dose-dependent preventive effect and the administration of high dose *Saccharomyces boulardii* (1,000 mg per day) showed the best anti-diarrhoea results. Nevertheless, the prophylactic efficacy of *Saccharomyces boulardii* was influenced by the patient's strict adherence to the dosage instructions. Furthermore, regional differences were observed. Concluding from this, further investigations are needed to find out which individuals would obtain the highest benefit from the preventive administration of *Saccharomyces boulardii*. A general recommendation cannot be given for this indication, as the preventive efficacy is also affected by geographical factors, which are not completely understood at present. Another remaining question relates to optimal dosing. Different dosages have been administered and varying effects have been observed. With regard to the clinical study by Kollaritsch *et al*. (1993), another point of criticism is the high rate of drop-outs. From 3,000 subjects included into the study, only 1,016 questionnaires could be used in the analysis of efficacy; so there are doubts if the results reported are representative.

In conclusion, more information is needed for recommending a WEU for the prevention of traveller's diarrhoea. Based on the data and medicinal products available, the period requested for the traditional use has elapsed for the medicinal use of preparations containing $>1.8 \times 10^{10}$ viable cells/g lyophilisate in adolescents and adults. The following posology is recommended:

Adolescents and adults: single dose: 250 mg dried yeast; daily dose: 1-2 times daily equivalent to 250-500 mg. The treatment should start 5 days before departure. The treatment should be consequently maintained during the travel.

The posology of 3 times 100-150 mg does not have a tradition of 30 years, because the medicinal product is only available since 1995.

Acne

None of the clinical studies performed confirms an efficacy of *Saccharomyces cerevisiae* in the treatment of acne. Controlled clinical studies demonstrating efficacy of *Saccharomyces cerevisiae* as compared to other therapies using objective parameters for assessment are not available. Thus, a well-established use would not be accepted here.

Based on the data available, however, the period requested for the traditional use of *Saccharomyces cerevisiae* for the adjuvant treatment of chronic acne has elapsed for the preparation containing $>1.8 \times 10^{10}$ viable cells/g lyophilisate. The indication should be restricted to uncomplicated mild acne. If acne is accompanied by a high rate of inflammatory lesions and scarring occurs, a doctor should be consulted.

Supported by the marketing overviews and the available clinical experience, the following posology is recommended:

Adolescents and adults: 250 mg 3 times daily. The use is recommended for a period of 3 months at least, considering the precautions and warnings mentioned above.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

Saccharomyces cerevisiae has been used as a probiotic for a long period of time. Numerous clinical trials have shown that the yeast has expressed a positive safety profile in adults and children.

5.2. Patient exposure

In her review on *Saccharomyces boulardii* in adults, McFarland (2010) summarises that 2,963 patients were included in clinical trials with different gastrointestinal indications and thus provide data on safety. According to her, the following adverse events were associated with *Saccharomyces boulardii*: thirst (n=5 patients), constipation (n=8 patients). These adverse events were reported in a clinical study with CDD patients (McFarland *et al*., 1994). In their review on the efficacy and safety of *Saccharomyces boulardii* for the prevention and treatment of gastrointestinal disorders, Kelesidis and Pothoulakis (2012) mentioned that adverse effects have not been observed in any of the clinical trials performed.

On the basis of the long-standing use in many Member States, a significant exposure can be assumed.

According to information received following the call for market overview at the beginning of the assessment, preparations containing *Saccharomyces boulardii* have been on the European market at least since 1968.

5.3. Adverse events, serious adverse events and deaths

In the above-mentioned clinical investigations, no signs of acute toxicity and serious adverse events have been observed.

Hypersensitivity reactions (pruritus, urticaria, localised or generalised exanthema, angioneurotic oedema, dyspnoea, anaphylactic shock) have been reported in the German pharmacovigilance database. The frequency is not known.

Furthermore, in some cases gastrointestinal complaints like flatulence, nausea and abdominal pain were reported. As these symptoms could also be signs of the underlying disease diarrhoea, it is difficult to assess if these symptoms are adverse events.
During the single PSUR assessment (PSUSA/00009284/201702), the lead assessor listed constipation and flatulence as frequently reported adverse drug reactions. The MAH reported that, in the assessed studies, constipation occurred significantly more frequently with *Saccharomyces boulardii* in comparison to placebo. The actual frequency cannot be objectively estimated, due to fact that the majority of cases were reported in the self-medication context and given the very low reporting of such effect in the clinical studies.

Based on this, flatulence and constipation are considered relevant undesirable effects with unknown frequency.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

According to literature, there is a risk of fungemia, which is of clinical relevance especially in patients with severe general or intestinal disease having an indwelling catheter. According to the review of Enache-Angoulvant and Hennequin (2005), 92 cases of invasive infections with *Saccharomyces* have been identified in literature, 40% of them were caused by *Saccharomyces boulardii* (n=37). They also found out that patients infected with *Saccharomyces boulardii* were more likely than patients infected with *Saccharomyces cerevisiae* to have digestive tract disease (58% vs. 6%; $p<0.01$), to have i.v. catheters (83% vs. 29%; $p<0.0001$), and to be hospitalised in an intensive care unit (32% vs. 0.05%, $p<0.01$). As from the 37 patients affected by *Saccharomyces boulardii* infection, 5 did not take a probiotic containing *Saccharomyces boulardii* at the time of diagnosis, the authors concluded that nosocomial acquisition is probable with catheters being a likely portal of entry due to possible contamination through hand transmission. Simulation tests performed by Hennequin *et al*. (2000) showed that opening a packet of *Saccharomyces boulardii* lead to massive air contamination and consecutively contaminated surrounding inert surfaces and the skin of a simulated patient. Moreover, the hands of the person, who opened the packet, were highly and persistently contaminated despite vigorous hand washing. According to the authors, *Saccharomyces boulardii* fungemia is an underestimated nosocomial and iatrogenic infection and the potential benefit has to be evaluated for each patient.

Venugopalan *et al*. (2010) mention the following factors, which constitute excessive and undue risk for development of *Saccharomyces* fungemia during probiotic administration: the patient's immunocompromised state during critical illness, the potential for live yeast spore contamination of healthcare workers' hands during preparation of the probiotic capsule for administration, and introduction of live yeast from contaminated hands to catheter sites.

Accordingly, in the Clinical Practice Guidelines for *Clostridium difficile* infection in adults (Cohen *et al*., 2010), it is stated: *"The administration of Saccharomyces boulardii has, however, been associated with fungemia in immunocompromised patients and in patients with central venous lines, and it should be avoided in critically ill patients."*

The review by Didari *et al*. (2014) revealed that, although probiotics are helpful, they do not always seem to be safe. Through interference with commensal microflora, they can result in opportunistic performances in the host due to bacteraemia and fungemia. The experts concluded that the main observed adverse effects of probiotics were sepsis, fungemia and GI ischemia. Generally, critically ill patients in intensive care units, critically sick infants, postoperative and hospitalised patients and patients with immune-compromised complexity were the most at-risk populations. While the overwhelming existing evidence suggests that probiotics are safe, complete consideration of risk-benefit ratio before prescribing is recommended. With regard to *Saccharomyces boulardii* the authors mentioned 30 case reports of fungemia in preterm infants and adults with underlying disease. One of the reported fungemia occurred in an infant, who had not received *Saccharomyces boulardii* but was in the cot adjacent to an infant with fungemia after probiotics digestion. In four controlled clinical trials (Costalos *et al.,* 2003, Bleichner *et al.*, 1997, Schneider *et al.*, 2005, McFarland *et al.*, 1994) no significant adverse effects such as fungemia or sepsis were reported. *Saccharomyces boulardii* was well tolerated. Didari *et al.* recommended to avoid the administration of *Saccharomyces boulardii* in patients with central venous catheter and patients with synthetic cardiac valve replacement.

Roy *et al.* (2017) described seven cases of *Saccharomyces* fungemia in two hospitals in India between July 2014 and September 2015. Two patients were premature neonates and five were adults. They were admitted in intensive care unit and were on probiotics containing *Saccharomyces boulardii* (except one adult patient). The probiotic intake for the two neonates was part of routine protocol for premature babies. In adult patients, probiotics were prescribed to either treat or prevent diarrhoea. The adult patient, who had no clear history of probiotics intake, might have acquired the agent by cross-contamination. Several hypotheses have been postulated on the acquisition of fungaemia from probiotics: translocation across the intestinal barrier or contamination of central or peripheral vascular lines from the hands of the healthcare workers when the sachets of probiotics are opened. The authors recommend avoiding this probiotic in critically ill or vulnerable patients, especially those with central venous catheter.

During the single PSUR assessment (PSUSA/00009284/201702), the CMDh reached the position that the marketing authorization(s) of products in the scope of this PSUSA should be varied (CMDh scientific conclusions and grounds for variation, amendments to the product information and timetable for implementation-PSUSA/00009284/201701, last updated: 01 December 2017).
This scientific conclusion was based on the data presented within the PSUR (periodic safety update report) under review, on the data in EudraVigilance database and available literature. The benefit-risk balance for use of *Saccharomyces boulardii* containing products in critically ill or immunocompromised patients was considered changed.

"*There were 19 cases reported with PT (preferred term) fungaemia during the interval period and 61 cases cumulatively. The search in EudraVigilance database overall revealed 10 fatal cases of fungaemia/fungal infection and sepsis associated with administration of Saccharomyces boulardii containing medicinal products where the causal association could not be ruled out. Moreover, there was also 1 fatal case of fungal infection and sepsis reported in a 48-year old patient, however no case narrative was provided, therefore the causality could not be established properly. Approximately half of the fatal fungaemia cases were reported in patients with CVC (central venous catheter) which has been already contraindicated. However, in rest of the fatal cases no CVC insertion was reported. In 1 fatal case of fungaemia insertion of CVC was explicitly ruled out by the reporter. Considering the known potential risk of fungaemia in critically ill patients and reported fatal cases in patients with no CVC insertion, the use of Saccharomyces boulardii in critically ill or immunocompromised patients should be contraindicated and relevant sections of the SmPC (sections 4.2, 4.3, 4.4 and 4.8) and PIL (patient information leaflet) should be updated accordingly*."

5.5.1. Use in children and adolescents

According to the data available, treatment of acute diarrhoea with *Saccharomyces cerevisiae* is adequate and well tolerated in infants >6 months, children and adolescents. However, the use in infants from 6 months to 2 years of age requires medical advice due to the disease itself. Severity of the diarrhoea with possible loss of water and electrolytes has to be supervised by a doctor.

If the symptoms persist longer than 2 days during the use of the medicinal product, a doctor or a pharmacist should be consulted for children above 2 years of age.

The use in infants below 6 months of age is not recommended.

5.5.2. Contraindications

Hypersensitivity to yeast, especially *Saccharomyces cerevisiae*.

Critically ill patients or immunocompromised patients (e.g. HIV-infection, organ transplantation, leukaemia, malignant tumours, radiotherapy, chemotherapy, long-term high dosage glucocorticoids treatment), and patients with central venous catheter because *Saccharomyces cerevisiae* CBS 5926 is a living microorganism, which can cause systemic fungemia under adverse circumstances.

5.5.3. Special Warnings and precautions for use

In case of diarrhoea, the most important therapeutic measure is rehydration therapy particularly in infants, children and elderly.

There have been very rare cases of fungemia (and blood cultures positive for *Saccharomyces* strains) reported mostly in patients with central venous catheter, critically ill or immunocompromised patients, most often resulting in pyrexia. In most cases, the outcome has been satisfactory after cessation of treatment with *Saccharomyces boulardii,* administration of antifungal treatment and removal of the catheter when necessary. However, the outcome was fatal in some critically ill patients (see sections 4.3 and 4.8).

As with all medicines made from living micro-organisms, special attention must be paid to the handling of the product in the presence of patients mainly with central venous catheter but also with peripheral catheter, even if not treated with *Saccharomyces boulardii*, in order to avoid any contamination by hands and/or the spread of microorganisms by air (see section 4.2).

As *Saccharomyces boulardii* is frequently used in self-medication, respective information has to be included in the informative texts (section 4.2): The risk of airborne contamination should also be considered in the presence of other critically ill or immunocompromised patients.

If during or shortly after the treatment with *Saccharomyces cerevisiae* CBS 5926 a microbiological examination of faeces is made, the results might be false positive. Therefore, the research laboratory should be informed adequately.

In case of traditional use for acne, a doctor has to be consulted, if acne is accompanied by a high rate of inflammatory lesions and scarring occurs.

5.5.4. Drug interactions and other forms of interaction

Interaction with concomitant administration of monoamine oxidase inhibitors (MAOI)

Several SmPCs indicate that concomitant use of MAOI with *Saccharomyces cerevisiae* can cause an increase of blood pressure. Probably this is based on the old preclinical data mentioned above and on theoretical considerations concerning the effects of these inhibitors.

MAOIs increase the central concentration of serotonin, norepinephrine and dopamine, but due to the unselective mechanism these inhibitors also have peripheral effects. When ingested orally, MAOIs inhibit the catabolism of dietary amines. When food containing tyramine is consumed concomitantly, the individual may suffer from increased blood pressure/hypertensive crisis.

Laux and Ulrich (2006) describe that 1,000 to 1,600 mg tyramine caused a 30 mm Hg increase of blood pressure in eight probands. These amounts are not reached with normal diet. But after administration of 20 mg tranylcypromine (MAOI) for 2 weeks, already 20-50 mg tyramine causes the same increase. These amounts can be reached with normal diet, exceptionally. The authors considered an amount of tyramine of 6 mg per meal as safe for most of the patients even if they are treated with tranylcypromine.

Microbial fermentation during manufacturing of foodstuff can lead to augmentation of biogenic amines. Caution is recommended with this kind of food, e.g. Marmite® yeast extract, in patients taking monoamine oxidase inhibitors (Blackwell and Marley, 1966).

Up to now there is no evidence that the specific strain of *Saccharomyces cerevisiae* CBS 5926 covered by this assessment report causes such interactions.

Interaction with concomitant administration of antimycotics

Elmer *et al*. (1995) studied the concomitant use of *S. boulardii* in 8 healthy volunteers receiving antifungals. *Saccharomyces boulardii* (*Saccharomyces cerevisiae* CBS 5926) was administered at 2 daily doses of 500 mg for 2 weeks. On days 7-14 an additional dose of either 50 mg per day or 100 mg per day fluconazole or 1,500,000 units per day nystatin was given. The authors showed that fluconazole with a high bioavailability did not affect steady-state levels of *Saccharomyces boulardii* when the ingestion of the two drugs was separated by 3 hours. In contrast, the concomitant use of nystatin, which is not absorbed, resulted in *Saccharomyces boulardii* levels below the limits of detection.

Therefore, a possible interaction with antimycotics when administered orally or systemically is to be considered.

5.5.5. Fertility, pregnancy and lactation

There are no data from use during pregnancy and lactation. Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

No fertility data available.

5.5.6. Overdose

No case of overdose has been reported.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

Not relevant.

5.5.8. Safety in other special situations

The use in critically ill, immunocompromised patients (e.g. HIV-infection, organ transplantation, leukaemia, malignant tumours, radiotherapy, chemotherapy, long-term high dosage glucocorticoids treatment), and in patients with central venous catheter is contraindicated. These contraindications are based on case reports of fungemia in such patients, often caused by nosocomial and iatrogenic infections. It is not evident whether cells of *Saccharomyces cerevisiae* CBS 5926 can cross the gastrointestinal wall in this special patient population, when administered orally.

5.6. Overall conclusions on clinical safety

In summary, the use of *Saccharomyces cerevisiae* could be evaluated as safe and well tolerated treatment under the conditions mentioned in this assessment report. Hypersensitivity reactions (pruritus, urticaria, localised or generalised exanthema, angioneurotic oedema, dyspnoea, anaphylactic shock) and gastrointestinal disorders (flatulence, constipation) have been reported. Use is

contraindicated in patients with hypersensitivity to the active substance. Treatment with *Saccharomyces cerevisiae* is contraindicated in immunocomprised patients and patients with central venous catheters.

6. Overall conclusions (benefit-risk assessment)

Acute unspecific diarrhoea

The use of the strain *Saccharomyces cerevisiae* CBS 5926 in acute unspecific diarrhoea could be regarded as well-established according to the Directive 2001/83/EC.

One randomised double-blind, placebo-controlled, multicentre clinical trial was conducted in adults with a positive statistically significant result for the treatment group (Höchter *et al.*, 1990). The daily dose was 3 times 200 mg for the first 2 days and then 3 times 100 mg *Saccharomyces boulardii*. A second randomised controlled study in adults compared two varying *Saccharomyces* preparations, however without a placebo control.

The recommended posology is as follows: single dose: 250 mg dried yeast containing at least 2 x 10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g; daily dose: 2 times daily equivalent to 500 mg.

Numerous randomised studies were conducted in children all over the world.

The recommended posology is as follows: single dose: 250 mg dried yeast containing at least 2 x 10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g; daily dose: 1-2 times daily equivalent to 250-500 mg.

In general, the use of *Saccharomyces cerevisiae* CBS 5926 is safe and well tolerated under the conditions described above. The use in critically ill or immunocompromised patients (e.g. HIV-infection, organ transplantation, leukaemia, malignant tumours, radiotherapy, chemotherapy, long-term high dosage glucocorticoids treatment) and patients with central venous catheter is contraindicated.

The use is not recommended in infants below 6 months of age as well as in pregnant and breast-feeding women due to insufficient data on safety and efficacy.

In summary, the benefit-risk assessment has to be regarded as positive.

Prevention of traveller's diarrhoea

No sufficient clinical evidence is available for the well-established use of the strain *Saccharomyces cerevisiae* CBS 5926 in prevention of traveller's diarrhoea. Two randomised double-blind clinical trials have been performed, in order to assess the efficacy of *Saccharomyces boulardii* for the prevention of traveller's diarrhoea. The results do not justify a general recommendation for this indication.

Based on the data available, the period requested by the Directive 2001/83/EC for the traditional use of *Saccharomyces cerevisiae* CBS 5926 in the prevention of traveller's diarrhoea has elapsed for the preparation containing >1.8 x 10^{10} viable cells/g lyophilisate.

Corresponding medicinal products have been in medicinal use throughout a period of at least 30 years, in EU Member States and *Saccharomyces cerevisiae* CBS 5926 can be used in this indication without the supervision of a medical practitioner for diagnostic purposes or for prescription or monitoring of treatment. The pharmacological effects or efficacy are plausible on the basis of long-standing use and experience.

The recommended posology is as follows: single dose: 250 mg dried yeast containing at least 2 x 10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g; daily dose: 1-2 times daily equivalent to 250-

500 mg. The treatment should start 5 days before departure. The treatment should be consequently maintained during the travel.

In general, the use of *Saccharomyces cerevisiae* CBS 5926 is safe and well tolerated under the conditions described above. The use in immunocompromised patients (e.g. HIV-infection, organ transplantation, leukaemia, malignant tumours, radiotherapy, chemotherapy, long-term high dosage glucocorticoids treatment) and patients with central venous catheter is contraindicated.

The use is not recommended in children under 12 years of age as well as in pregnant and breast-feeding women due to insufficient data.

Uncomplicated mild acne

No sufficient clinical evidence is available for the well-established use of the strain *Saccharomyces cerevisiae* CBS 5926 in the treatment of acne. Thus, a well-established use according to the Directive 2001/83/EC could not be accepted here.

Based on the data available, the period requested by the Directive 2001/83/EC for the traditional use of *Saccharomyces cerevisiae* CBS 5926 as an adjuvant in the treatment of uncomplicated mild acne in adolescents and younger adults has elapsed for the preparation containing $>1.8 \times 10^{10}$ viable cells/g rounded up to 2×10^{10}. If acne is accompanied by a high rate of inflammatory lesions and scarring occurs, a doctor has to be consulted. For such a traditional use, the requirements of the Directive 2001/83/EC are fulfilled.

The recommended posology is as follows: 250 mg dried yeast containing at least 2×10^{10} viable cells *Saccharomyces cerevisiae* CBS 5926/g, daily dose: 3 times daily equivalent to 750 mg. The use is recommended for a period of 3 months at least considering the precautions and warnings mentioned.

In general, the use of *Saccharomyces cerevisiae* CBS 5926 is safe and well tolerated under the conditions described above. The use in immunocompromised patients (e.g. HIV-infection, organ transplantation, leukaemia, malignant tumours, radiotherapy, chemotherapy, long-term high dosage glucocorticoids treatment) and patients with central venous catheter is contraindicated.

Due to indication, the use in children under 12 years and elderly is not relevant.

The use is not recommended in pregnant and breast-feeding women due to insufficient data on safety.

Discussions on the classification of *Saccharomyces cerevisiae*

Intensive discussions took place in the HMPC, especially on the nature and classification of a product containing *Saccharomyces cerevisiae* CBS 5926 as an herbal or a biological product and consequerntly its eligibility to the traditional use registration procedure.

The HMPC sent a request for legal interpretation about the application of Directive 2004/24/EC to living yeast cells (fungi) such as *Saccharomyces cerevisiae* (strain CBS 5926) to the European Commission, explaining the divergent views in the Committee.

Some HMPC members considered *Saccharomyces cerevisiae* CBS 5926 as a "herbal substance" based on the definition from Directive 2001/83/EC which mentions fungi as herbal substances.

Other members considered *Saccharomyces cerevisiae* CBS 5926 as a "biological active substance" since:

- the current herbal guidelines on quality are not fully suitable and applicable, moreover GACP or GMP annex 7, and Ph. Eur. herbal monographs do not cover unicellular living yeast or freeze-dried living fungal cells;

- such medicines are assessed in many Regulatory Agencies in accordance with guidelines for biologicals when used as medicinal products and the Live Biotherapeutic Products general monograph published by EDQM in the European Pharmacopoeia (monograph 3053);

- their specific manufacturing process is evaluated as per the quality requirements for biological medicinal products and inspected according to the GMP annex 2 for biological medicinal products.

The European Commission clarified that the classification of medicinal products falls under the responsibility of Member States based on all the characteristics of a particular product. Based on data provided by applicants, Member States may authorise a particular medicine under a traditional herbal medicine registration, 'well-established use' application or full marketing authorisation application and the product may be authorised differently in different Member States.

Differences in medical approach, regulatory classifications, healthcare systems and patient self-management in the EU were noted with regard to the use of *Saccharomyces cerevisiae.* In the absence of absolute majority in favour of adopting a monograph, the HMPC issued a public statement.

HMPC overall conclusion taking into account the classification, definition and nature of the active substance

Following intensive discussions in the Committee, taking into account that 'fungi' in general are included in the 'herbal substance' definition of the Directive 2001/83/EC and on the other hand living yeast are covered by the Live Biotherapeutic Products general monograph of the European Pharmacopoeia, no absolute majority required for adoption of the monograph was achieved.

Therefore, having regard of its Rules of Procedure, the HMPC concluded that the following requirement for the establishment of an EU herbal monograph on traditional and well-established herbal medicinal products containing Saccharomyces cerevisiae CBS 5926 *is not fulfilled:*

- the requirement laid down in Article 1 of Directive 2001/83/EC on the definition of 'herbal substance' (despite the existence of data on the safety, efficacy and historical data on the medicinal uses within the EU of products containing Saccharomyces cerevisiae CBS 5926.)

Annex

List of references