

**Infant feeding practices and probiotics effects on
nutritional recovery and morbidity of severely
malnourished infants in Democratic Republic of Congo**

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DEDICATION

To Almighty God who has done great wonders for me!

To my dear wife Aline and our children: Lionel, Laetitia, Anael and Anaïs;

To my parents Fabien Kambale and Mélanie Nziavake;

To my parents-in-law Alexandre Ntibonera and Adolphine Mutwedu;

To my brothers and sisters: Ildephonse, Alain, Colette, Adèle, Jean-Claude, Pascal,

Alex, Georges, Mamy and Toussaint;

To all my nieces and nephews;

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
AMR	Antimicrobial resistance
ATP	Adenosine triphosphate
BMIAZ	Body mass index-for-age z-score
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CFU	Colony-forming unit
ChroSAM	Chronic disease outcomes after severe acute malnutrition
CI	Confidence interval
CIES	Comité Institutionnel d'Éthique de la Santé
CMAM	Community-based Management of Acute Malnutrition
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
EBF	Early initiation of breastfeeding
EED	Environmental enteric dysfunction
ESBL-E	Extended spectrum beta lactamases producing Enterobacteriaceae
FANTA	Food And Nutrition Technical Assistance
FAO	Food and Agriculture Organization
FMT	Fecal microbiota transplantation
GAM	Global acute malnutrition
GI	Gastrointestinal
GM	Gut microbiota
HAZ	Height-for-age z-score
HCAZ	Head circumference-for-age z-score
HFIAS	Household Food Insecurity Access Scale
HIV	Human immunodeficiency virus
IPC	Infection Prevention and Control
IYCF	Infant and Young child feeding
K ⁺	Potassium
LAZ	Length for age z-score
LBW	Low birth weight
LMICs	Low-and middle-income countries
MAM	Moderate acute malnutrition
MAZ	Microbiota-for-age z-score
MDCF	Microbiota-directed complementary foods
MDR	Multidrug resistance
MGRS	Multicentre Growth Reference Study

MUAC	Mid-upper arm circumference
Na ⁺	Sodium
NCHS	National Center for Health Statistics
NTS	Non-typhoid Salmonella
OptiMA	Optimizing MAInutrition treatment
OTUS	Operational Taxonomic Units
PCR	Polymerase Chain Reaction
ProbiSAM	Probiotics for severe acute malnutrition
PRONUT	Pre and PRObiotics in the treatment of severe acute malNUTrition):
PruSAM	Probiotics for Uncomplicated Severe Acute Malnutrition
RNA	Ribonucleic acid
RR	Risk ratio
rRNA	Ribosomal ribonucleic acid
RUSF	Ready-to-use supplementary foods
RUTF	Ready-to-use therapeutic foods
SAM	Severe acute malnutrition
SCFA	Short chain fatty acids
SD	Standard deviation
SSA	Sub-Saharan Africa
TB	Tuberculosis
UCB	Université Catholique de Bukavu
UNICEF	United Nations of International Children's Emergency Fund
USA	United States of America
WAZ	Weight-for-age z-score
WHO	World Health Organization
WHZ	Weight-for-height z-score
WLZ	Weight-for-length z-score

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SUMMARY OF THE THESIS

Context

Malnutrition is a major public health concern, affecting around 45 million children worldwide. Malnutrition increases incidence, severity and case fatality of common infections, and underlying 35% of all child deaths globally. Recent evidence has shown that malnutrition is not due to food insecurity alone, but reflects a combination of factors, including an important role of the gut microbiota, which fails to develop properly during the first two years of life in malnourished children. Dietary interventions alone are insufficient to comprehensively reduce the burden of child malnutrition and fail to address the persistent infectious burden of the disease. With the advancement in knowledge of the key role of gut microbiota disruption in childhood malnutrition, several interventions targeting the gut microbiota have emerged as promising treatment modalities.

Objectives of the thesis

- To assess infants' feeding practices and factors associated with appropriate minimum acceptable diet in a general population sample from South Kivu;
- To identify the main infectious diseases experienced by children with severe acute malnutrition (SAM); and
- To assess the effect of probiotics on the main childhood killer infectious diseases (pneumonia and diarrhea), and on nutritional recovery in children with SAM

Methods

The thesis reports the results of four studies: two cross-sectional studies, one systematic review with meta-analysis, and one randomized controlled trial (RCT).

Except for the systematic review, all studies were conducted in South Kivu province, (Eastern DRC), in 3 Health Zones: Ibanda (urban area) and Kabare (rural area) Health Zones for the first cross-sectional study; and Kadutu Health Zone (urban area) for the second cross-sectional study and the RCT. Selection of these Health Zones was based on demographic, epidemiological, accessibility, and logistical criteria.

In the first cross-sectional study assessing infants' feeding practices in urban and rural areas of South Kivu, the sample size was 750 mother-infant's pairs (375 in each area). The second study assessed the main infectious diseases experienced by children with SAM. An exhaustive sampling was used. The systematic review included 15 RCTs. Finally, in the 4th study assessing the effect of probiotics on diarrhea, pneumonia and nutritional recovery, the sample was 400 infants (200 in probiotics group and 200 in placebo one).

Findings

Main findings are summarized as follows:

- Less than 30% of infants consumed flesh foods, dairy products, eggs, fruits and vegetables, predominantly in rural areas;
- The rate of minimum acceptable diet was 33%;
- Mothers living in urban area [Adjusted Odds Ratio (AOR) 2.39; 95% CI 1.43, 3.85], those who had postnatal care visits (AOR 1.68; 95% CI 1.12, 2.97), those with secondary or post-secondary education level (AOR 1.83; 95% CI 1.20, 2.77), and those with good household socioeconomic level (AOR 1.72; 95% CI 1.14, 2.59) were more likely to give recommended minimum acceptable diet compared to their counterparts;
- Pneumonia (16%), bacteremia (6%), gastroenteritis (5%) and malaria (5%) accounted for 32% of confirmed diagnosis in children suffering from SAM;
- For children suffering from diarrhea, the number of days of disease was lower in probiotics [4.11 (95%CI: 3.37, 4.51)] compared with placebo group [6.68 (95%CI: 6.26, 7.13)] ($p<0.001$);
- For children aged 16 months or older, the risk of diarrhea was lower in probiotics [75.6% (95%CI: 66.2, 82.9)] compared with placebo group [95.0% (95%CI: 88.2, 97.9)] ($p<0.001$), but no significant difference of the risk for the youngest;
- In probiotics group, nutritional recovery happened earlier: At the 6th week, 40.6% of the infants were waiting for nutritional recovery, contrasting with 68.7% of infants in placebo group; but the nutritional recovery rate at the 12th week was similar between groups;
- Probiotics had no effect on pneumonia incidence;

Conclusions

Our thesis research has shown that the minimum acceptable diet rate remained low among infants, especially among mothers with low school attendance and those non-attending prenatal care. As part of nutrition optimization among those at risk, the nonadherence to minimum acceptable diet should urgently be addressed through appropriate and widespread counseling.

This thesis further demonstrates an increased incidence of pneumonia, gastroenteritis, bacteremia and malaria among children with SAM. Since data are lacking on pathogens and their antimicrobial susceptibility in children with SAM in DRC, future research should fill this gap to distinguish children with SAM who require antimicrobial therapy and the type of antimicrobial that is appropriate.

Finally, this PhD research has shown that probiotics significantly reduced the number of days of diarrhea, the risk of diarrhea in older infants (>16 months), and the time to reach nutritional recovery. Future research is needed for economic analysis of scaling up probiotic's use nationally and internationally, and exploration of local microbiota-directed complementary foods.

Chapter I: INTRODUCTION

1.1 Definition of malnutrition

The World Health Organization (WHO) defines malnutrition as deficiency, excess or imbalance in a person's intake of energy and/or specific nutrients in relation to their requirements [1]. The term malnutrition covers two broad groups of conditions: undernutrition and overnutrition. In this thesis, we will use the term 'malnutrition' to mean undernutrition.

1.2 Assessment of nutritional status

Energy and/or specific nutrient intake, requirements and expenditure are very rarely directly assessed. Instead, practice is based on anthropometric measures. Anthropometry is an easy, quick, inexpensive, and non-invasive method that provides detailed information on different components of body structure, especially muscular and fat components [2]. Anthropometric measurements commonly used for children include weight, height, mid-upper arm circumference (MUAC), and head circumference. Bilateral pitting edema, a clinical indicator, is often assessed along with anthropometry. These measurements are used to calculate nutritional indices compared to international standard reference ranges.

Since the late 1970s, the National Center for Health Statistics/World Health Organization (NCHS/WHO) growth reference has been recommended for international use [3]. The meticulous evaluation of NCHS/WHO reference to the early 1990s by a group of experts reported several limitations: (i) The data used to construct this reference covering birth to three years of age came from a longitudinal study of children of European ancestry from a single community in the USA; (ii) These children were measured every three months, which is inadequate to describe the rapid and changing rate of growth in early infancy; (iii) the statistical methods available at the time the NCHS/WHO growth curves were constructed were too limited to correctly model the pattern and variability of growth [4-6]. As a result, the NCHS/WHO curves did not adequately represent early childhood growth.

To address these limitations, the WHO implemented, between 1997 and 2003, the WHO Multicentre Growth Reference Study (MGRS). The MGRS collected primary growth data and related information from 8,440 healthy breastfed infants and appropriately fed children from diverse ethnic backgrounds and cultural settings (Brazil, Ghana, India, Norway, Oman and USA), and raised in optimal conditions [7]. Following the MGRS, The WHO published in 2006 new Child Growth Standards for infants and children up to the age of 5 years [8].

The individual's nutritional indices relative to the population mean are expressed as z-scores, which describes how far and in what direction an individual's measurement is from the median of the WHO Child Growth Standards. A z-score is in the normal range when it is between -2 and +2. When it falls outside of the normal range, it indicates a nutritional issue. MUAC is compared to cutoffs that apply to all children in a specific age range. The main nutritional indices are weight-for-age z-score (WAZ), height-for-age z-score (HAZ), weight-for-height z-score (WHZ), body mass index-for-age z-score (BMIAZ), and head circumference-for-age z-score (HCAZ). Table 1 summarizes the major nutritional disorders [7]:

Table 1: Common nutrition conditions, anthropometric measurements, and cutoffs

Nutritional issue	Condition	Description	Measure or index	Global	Moderate	Severe
Acute malnutrition (wasting)	Occurs with rapid weight loss, inadequate weight gain, or bilateral pitting edema.	Low weight-for-height (WHZ)	WHZ or BMIAZ	< -2	-3 to -2	< -3
			MUAC (in mm)	< 125	≥ 115 to < 125	< 115
			Bilateral pitting edema	-	-	If present
Stunting	Reflects chronic undernutrition; increases risk of poor cognitive and motor development and death	Low height-for-age (HAZ)	HAZ	< -2	-3 to -2	< -3
Underweight	Can indicate stunting, acute malnutrition, or both	Low weight-for-age (WAZ)	WAZ	< -2	-3 to -2	< -3
Overweight/Obesity	Reflects high levels of body fat; increases risk of non-communicable diseases	High WHZ	WHZ or BMIAZ	-	+2 to +3 (overweight)	> +3 (Obese)
Microcephaly	May indicate abnormal brain development	Small head size-for-age	HCAZ	< -2	-3 to -2	< -3

In the past, there has been increased focus on the use of MUAC as a single screening tool for severe acute malnutrition (SAM), assuming that children with a low WHZ and normal MUAC have lower risks of morbidity and mortality. Previous reports suggested that MUAC was a better predictor of mortality, was easier to perform, and was not as affected by dehydration [9]. Recent evidence demonstrated that WHZ may detect another 40% of children with SAM who have normal MUAC values [10]. Moreover, WHZ scores are better correlated with several biochemical indices of malnutrition associated with clinical morbidity [10]. Thus, the combination of both MUAC and WHZ is optimal for sensitivity and identifying children at greatest risk [10, 11].

1.3 Classification of severe acute malnutrition (SAM)

SAM is classified by 2 distinct clinical entities [12]:

- Non-edematous SAM (marasmus), characterized by severe wasting: MUAC < 115 mm or a WHZ < -3 according to the 2006 WHO growth standards in children aged 0–59 months;
- Kwashiorkor, characterized by nutritional bilateral pitting edema;

The term “marasmic kwashiorkor”, has been used to describe children with both wasting and edema [19]. Figure 1 illustrates these 3 clinical forms of SAM.



Figure 1: Categories of severe acute malnutrition. From left to right: marasmus, kwashiorkor and marasmic kwashiorkor. © Richard Mbusa Kambale (Written parental permission was obtained for all photographs of children in this thesis)

Marasmus is the term derived from the Greek word *marasmos*, which means withering or wasting. Marasmus is characterized by wasting of body tissues, particularly muscles and subcutaneous fat, and is usually a result of severe restrictions in energy intake. Children younger than 5 years are the most involved because of their increased caloric requirements and increased susceptibility to infections [12].

The term kwashiorkor, first used by Williams in 1933, is taken from the Kwa language of Ghana and means "the sickness of the weaning" or "the deposed child" [13]. Prevalence of kwashiorkor is thought to be highest in central and southern Africa [14]. Edema is its distinguishing characteristic. It is assessed by pressing down firmly on the third to fourth tarsal bones on the dorsum of the foot for 3 to 5 seconds and then assessing for pitting edema for 2 to 3 seconds [15]. The signs distinguishing marasmus from kwashiorkor are summarized in Table 2.

Children with kwashiorkor were previously thought to have worse outcomes compared with those with marasmus, but more recent large studies demonstrate that children with kwashiorkor tend to have better outcomes, perhaps due to the additional burden of higher rates of HIV and diarrheal dehydration among those with marasmus [16-18].

Table 2: Distinguishing features of marasmus versus kwashiorkor

	Marasmus	Kwashiorkor
Weight and Height	Low WHZ (severe wasting) Low HAZ (stunting) if also chronically malnourished	WAZ is variable Low HAZ (stunting) if also chronically malnourished
Head	Appears large relative to body, with staring eyes	Moon face (rounded prominence of the cheeks), with or without facial edema. May appear plump
Extremities	Emaciated arms, thighs, and buttocks, with loose folds due to subcutaneous fat loss	Edema: • Mild (1+): involves only the feet • Moderate (2+): involves the feet and legs and/or the upper limbs • Severe (3+): generalized edema or moderate plus facial edema
Behavior	Typically irritable	Typically apathetic May be irritable when handled
Abdomen	-	Distended, dilated intestinal loops; Hepatomegaly
Skin	Thin; Dry skin	Thin; dry and peeling skin with areas of hyperkeratosis and hyperpigmentation (dermatosis of kwashiorkor).
Hair	Thin, sparse hair that is easily plucked	Dry, dull and hypopigmented hair that falls out or is easily plucked. Hypopigmentation may occur in stripes if malnutrition has been intermittent ("flag sign").

HAZ: Height-for-age z-score; WAZ: Weight-for-age z-score; WHZ: Weight-for-height z-score

SAM is further classified according to the presence or absence of medical complications [12]:

- Uncomplicated SAM: It refers to the child who has no clinically obvious acute infections or other medical complications, and has a good appetite, determined by an "appetite test" during the initial evaluation.
- Complicated SAM: It refers to children with clinical features of infection or metabolic and electrolyte disturbance, severe edema, or poor appetite.

1.4 SAM epidemiology

1.4.1 Status of acute malnutrition worldwide

Malnutrition is an important public health concern. Although trends in child malnutrition have declined substantially in the past 30 years, more than 45 million (6.7% of children under 5 worldwide) children are wasted. Among the 45 million wasted children, 14 million are severely wasted (Figure 2). SAM is disproportionately prevalent in low- and middle-income countries (LMIC), with Africa and Asia bearing 21% and 71% respectively of SAM such malnutrition in children under 5 as illustrated in Figure 2 and 3.

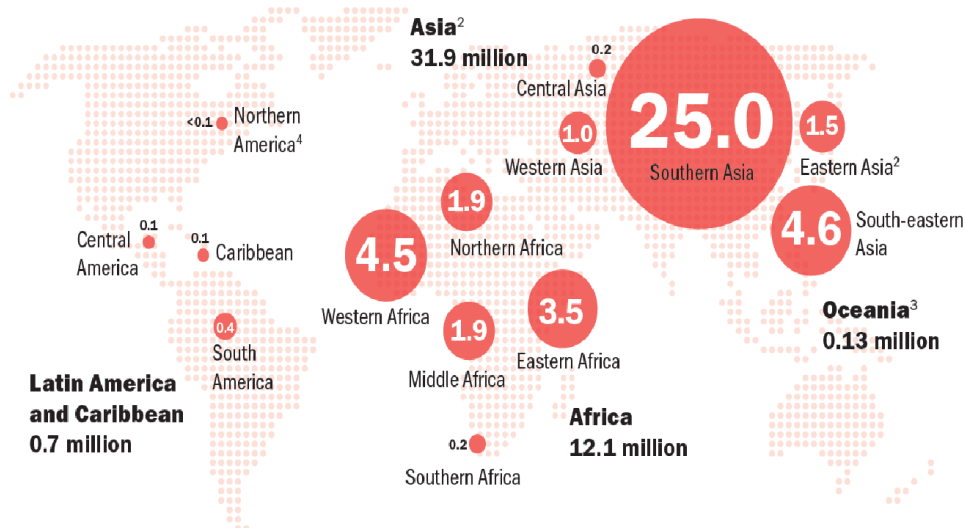


Figure 2: Number of children under-five affected by wasting. Source: UNICEF, WHO, World Bank Group Joint Malnutrition Estimates, 2021 edition.

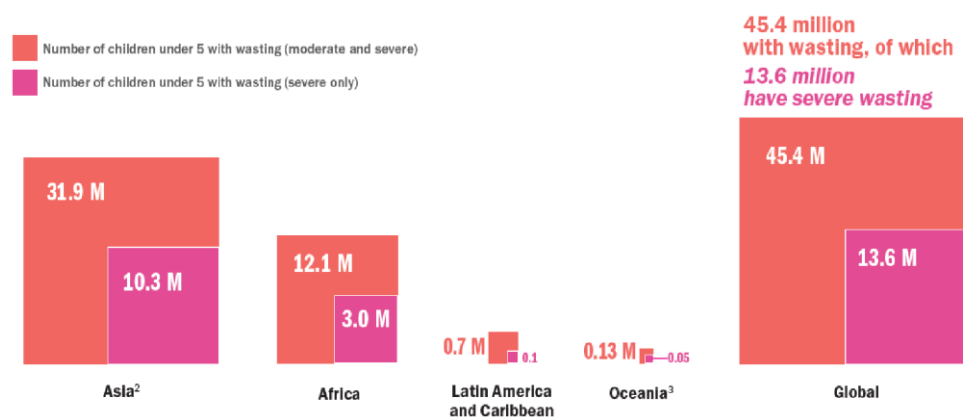


Figure 3: Number of children under-five affected by wasting and severe wasting. Source: UNICEF, WHO, World Bank Group Joint Malnutrition Estimates, 2021 edition.

The Integrated Food Security Phase Classification is a common global scale for classifying the severity and magnitude of food insecurity and malnutrition [20]. The recommended thresholds according to the Integrated Food Security Phase Classification of acute malnutrition are:

- Acceptable: Global Acute Malnutrition (GAM), ie proportion of children either with SAM or Moderate Acute Malnutrition (MAM), is < 5%
- Alert: GAM is 5-9.9%
- Serious: GAM is 10-14.9%
- Critical: GAM is 15-29.9%
- Extremely critical: GAM is ≥30%

The prevalence of acute malnutrition is geographically very unbalanced, South Asia having the highest prevalence of wasting of any sub-region worldwide. Five countries worldwide experience critical thresholds of acute malnutrition: Southern Sudan (22.7%), Djibouti (21.5%), India (17.3%), Yemen (16.4%) and Sudan (16.3%) (Figure 4).

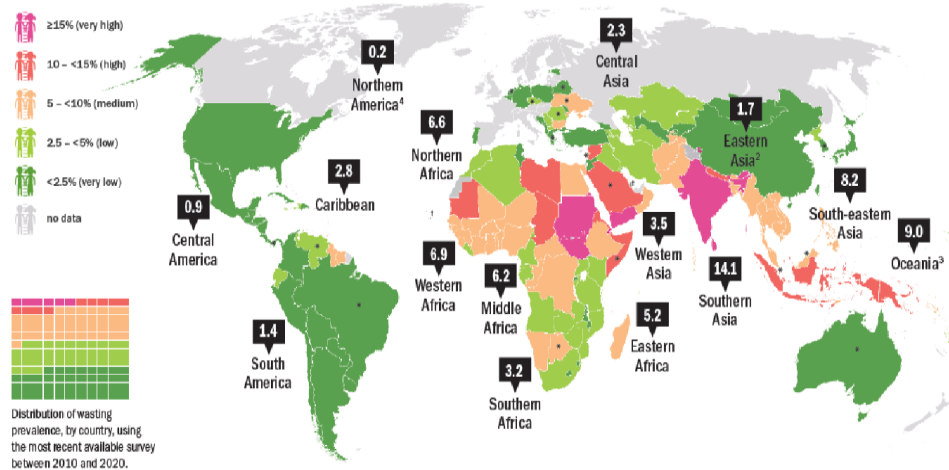


Figure 4: Prevalence of wasting. Source: UNICEF, WHO, World Bank Group

1.4.2 Malnutrition and infections

A classic monograph, published in 1968 by Scrimshaw in *“Interactions of Nutrition and Infection”*, suggests a vicious cycle between nutrition and infection: malnutrition resulted in infections; infections resulted in malnutrition by anorexia, malabsorption, and diversion, loss, and increased requirements of nutrients [21]. Currently, within this vicious infection-malnutrition cycle, a more nuanced understanding is emerging of the roles of the environment, burden of exposure to pathogens because of crowding or poor water and sanitation, gut microbiota, chronic intestinal inflammation, mucosal barrier loss and immune function (Figure 5) [22].

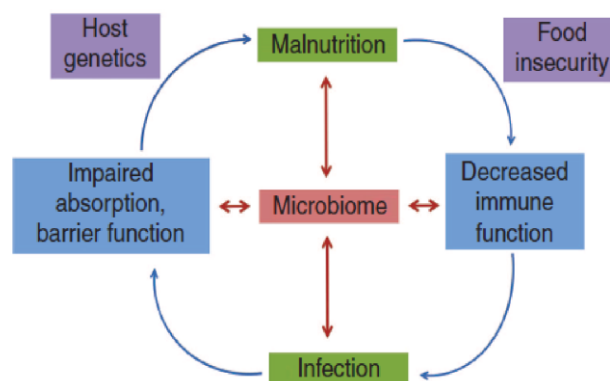


Figure 5. A growing understanding of a “vicious cycle”: interactions between malnutrition, infection, and intestinal dysfunction. Source: Walson JL, Berkley JA. The impact of malnutrition on childhood infections. Curr Opin Infect Dis. 2018;31(3):231-236.

Malnutrition results in increased incidence, severity and case fatality of common infections, risks continue beyond acute episodes resulting in significant post-discharge mortality [23-25]. Several epidemiological studies have documented a high prevalence of diarrhea, pneumonia, and systemic infections, including bacteremia with *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella* spp, *Salmonella* spp and other *Enterobacteriaceae* [26-31]. Golden reported that kwashiorkor is precipitated by infection, particularly diarrhea, measles, tuberculosis, and malaria [32].

1.4.3 Malnutrition and in-patient mortality

The immediate consequences of SAM are life threatening, as a child with SAM is approximately nine times more likely to die than a non-malnourished child [33]. SAM directly contributes to nearly one million children under 5 years of age deaths annually worldwide. It is also an important underlying contributor to many other child deaths, especially those due to diarrhea and pneumonia (Figure 6) [34]. These two are the leading complications of SAM associated with increased morbidity, longer hospitalization, and mortality [26-28]. Complicated SAM is typically associated with an inpatient mortality risk of 10-40% in health centers in sub-Saharan Africa (SSA) [26-31].

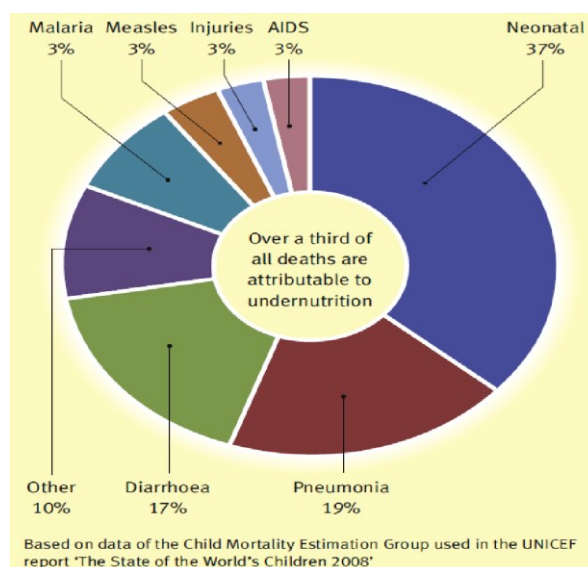


Figure 6: Specific causes of mortality in children under five years of age. Source: Walton E and Allen S. Malnutrition in developing countries. Pediatrics and Child Health, 2011; 21(9): 418-424.

1.4.4 Mortality following in-hospital management of SAM

Emerging data suggest that there is high ongoing mortality risk following hospital discharge, and discharge from outpatient treatment programs. Indeed, several recent studies have shown high post-discharge mortality following in-hospital management of diarrhea-SAM, HIV infection-SAM, and pneumonia-SAM comorbidities respectively [23-25]. Furthermore, a recent study reported mortality rates between 0.6% and 10.4%, 6 to 24 months after discharge from community-based therapeutic feeding centers following nutritional rehabilitation for uncomplicated SAM [35, 36].

1.4.5 Long-term effects of SAM

The effects of malnutrition are not just short term and confined to the affected individual; they may also have important long-term and intergenerational consequences (figure 7).

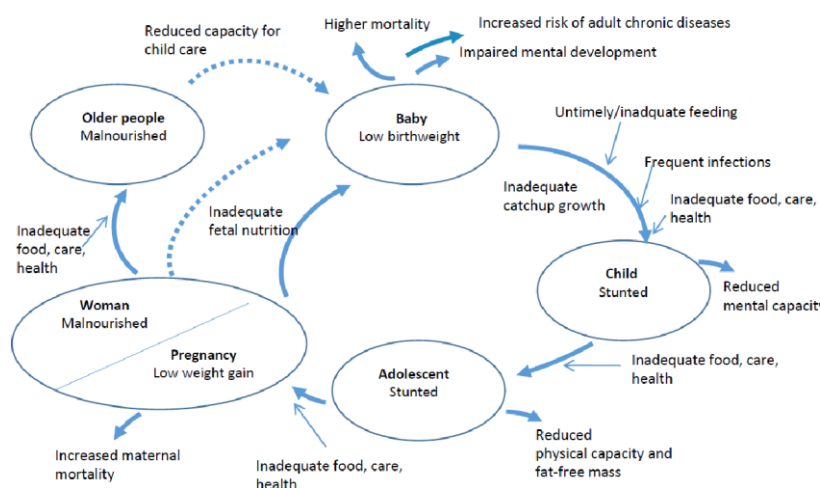


Figure 7: Life course and intergenerational effects of malnutrition. Source: World Nutrition Situation, 4th Report

Long-term effects of SAM have been investigated in several studies. In the ChroSAM (Chronic Disease Outcomes after Severe Acute Malnutrition) study, a cohort of Malawian SAM-survivor children were followed up seven years after inpatient SAM treatment and compared with sibling and community controls. SAM survivors had lower height-for-age compared with controls, although with evidence of catch-up growth. Associated functional impairments were found, such as poorer physical strength, poorer physical capacity, and lower school achievement, compared to controls [37].

Evidence suggests that malnutrition in early childhood may affect language, intelligence quotient, cognition, and social emotions, hamper later development, and have a negative impact on human capital and economic growth [38-40]. Other long-term effects of malnutrition, including shorter adult height linked to childhood stunting, higher propensity for stunted children to become overweight or obese adults, metabolic and cardiovascular diseases, have been described [33].

Intergenerational effects on linear growth have been also documented. Intergenerational influences are directly responsible for perpetuating the intergenerational cycle of growth failure. This involves young girls who grow poorly and become stunted women who are more likely to have small babies; these babies, if girls, will continue the cycle [41].

Thus, the burden of child malnutrition remains a significant threat to global public health in the short and long term.

1.5 SAM pathophysiology

In both marasmus and kwashiorkor, normal physiology is perturbed by a process called “reductive adaptation”. Reductive adaptation is the physiological response of the body to malnutrition i.e systems slow down and do less in SAM in order to allow survival on limited nutrient resources especially calories. This process affects every physiological function in the body, mobilizes energy and nutrient reserves, and decreases energy and nutrient demands [42]. It is initially beneficial and allows the organism to maintain homoeostasis. However, as the severity of nutritional insult increases, it limits the body’s ability to respond to stresses such as infection. Reductive adaptation’s process affects the cell, organ, and system (figure 8) [42].

- At the cell level: cellular Na⁺/K⁺-ATPase pumps that maintain fluid, electrolyte and substrate levels are impaired in children with SAM. There is an increased membrane permeability and reduced Na⁺/K⁺-ATPase activity, resulting in sodium leakage into cells and loss of potassium out of the cells and into the urine [43, 44].
- At the organs and system level:
 - Central nervous system: malnutrition leads to a reduction in the number of neurons, synapses, dendritic arborizations and myelination, resulting in a decrease in brain size. The cerebral cortex is thinned, brain growth is slowed, resulting in delayed global and motor functions [45]. The effects on the developing brain may be irreversible after the age of 4 years [45].

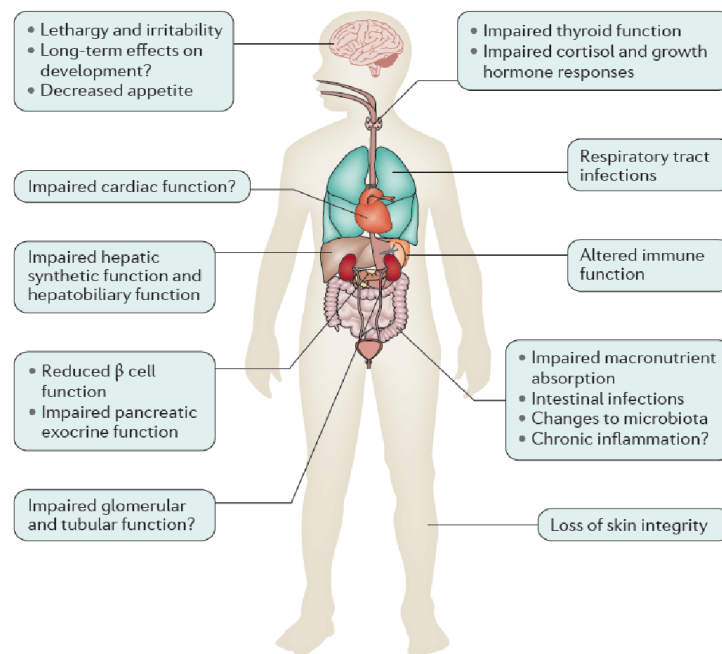


Figure 8. Organ system involvement in severe malnutrition. Source: Bhutta et al. Severe childhood malnutrition. Nat Rev Dis Primers 2017;3:17067.

- **Cardiovascular system:** Cardiac myofibrils are thinned with impaired contractility. Bradycardia and hypotension are also common in severe cases. The combination of bradycardia, impaired cardiac contractility, and electrolyte imbalances predisposes to arrhythmias [46, 47].
- **Lungs:** children treated for SAM do not experience negative effects regarding later lung function [48].
- **Liver:** A striking feature of kwashiorkor is the presence of hepatic steatosis (that is, fatty liver). In addition, an increase in fatty acid release from adipose tissue that could be taken up by the liver has been observed [49, 50]. There is severely reduced hepatic metabolism and excretion of toxins, as well as reduced hepatic synthesis of proteins including albumin and production of abnormal metabolites of amino acids [51].
- **Gastrointestinal (GI) tract:** malnutrition exhibits environmental enteric dysfunction (EED), a state of intestinal pathology characterized by blunted villi, inflammation, and reduced barrier

function. EED is associated with reduced nutrient absorption and hence faltering growth phenotypes. The direct cause of EED is unknown. It is hypothesized that infection and subclinical pathogen carriage contribute to EED and malnutrition. Bacterial overgrowth and pancreatic atrophy lead to fat malabsorption [52-54].

- Genitourinary system: Glomerular filtration rate, and the capacity of the kidney to excrete sodium, excess acid, or a water load are greatly reduced. Urinary tract infections are common [55].
- Endocrine system: SAM leads to reduced levels of triiodothyronine, insulin, insulin-like growth factor and increased levels of growth hormone and cortisol [56].
- Metabolic changes in SAM: Reduced secretion of insulin contributes to a catabolic state. In marasmus, the reduced secretion of insulin leads to a lipolytic and proteolytic response and the release of free fatty acids and amino acids into the bloodstream. Free fatty acids are taken up by muscle tissue for oxidation. Both free fatty acids and amino acids are also partially taken up by the liver and are used for ATP production and the synthesis of essential proteins and glucose. In kwashiorkor, this adaptive response is disturbed, which causes a reduced release of free fatty acids from adipose tissue and amino acids from muscle tissue [57, 58].
- Immune system: SAM leads to atrophy of the thymus, lymph nodes and tonsils. There are reduced CD4 with normal CD8-T lymphocytes, loss of delayed hypersensitivity, impaired phagocytosis, and reduced secretory immunoglobulin A [59]. Impaired immune and inflammatory systems lead to vulnerability to infections. Pneumonia, tuberculosis, enteric infections, and malaria are common complications in SAM patients [60, 61].
- Skin and glands: In marasmus, the skin and subcutaneous fat are atrophied, which causes loose skin folds. Many signs of dehydration are unreliable: the eyes may appear sunken because of loss of subcutaneous fat in the orbit; the child may have dryness of mouth and eyes as well as reduced sweat production because of skin atrophy [62].

Skin manifestations of kwashiorkor include dryness, atrophy, generalized hyperkeratosis, and hyperpigmentation. The fragile skin peels off in an irregular manner, revealing underlying hypopigmentation (“flaky paint” or “peeling paint” dermatosis),

particularly over the buttocks and limbs. The most common skin finding is the shiny, varnished-looking nature of hyperpigmentation. The hair is sparse, dry, and brittle, with a reddish yellow hue. There are alternate bands of normal and pale hair (flag sign), reflecting periods of good and poor nutrition. The nail plate exhibits thinning, fissuring, ridging, or prominent koilonychias [63, 64]. These cutaneous and hair manifestations are due to low methionine levels that impair sulphation of the keratin [65].

- Basal metabolism: The basal metabolic rate is slowed by approximately 30%. Both heat generation and heat loss are impaired, so that the child becomes hypothermic in a cold environment and hyperthermic in a hot environment. Physical activity and growth are all reduced [66, 67].

1.6 SAM pathogenesis

It is still unknown why some children develop edematous malnutrition. Multiple theories have been advanced to explain the edema in edematous malnutrition:

- It was previously thought that marasmus was the result of inadequate energy intake whereas kwashiorkor was the result of inadequate protein specifically [68]. However, this has since been disproved as evidence has shown that some patients have edematous malnutrition without hypoalbuminemia, others develop edematous malnutrition despite adequate proportion of protein in the diet (e.g., in exclusively breastfed infants), and others recover from edematous malnutrition with supportive care even without enhancing the protein content of the diet [69-72]. In animal models, hypoalbuminemia can be induced by a diet low in protein and high in monosaccharides and disaccharides, but edema is rarely observed [73, 74]. Moreover, the degree of hypoalbuminemia and recovery upon nutritional management in children with kwashiorkor correlates poorly with the degree of edema or the speed of its resolution [75].
- Oxidative stress: Children with SAM have reduced levels of antioxidants, including vitamin E and glutathione, compared with well-nourished, and this reduction is more pronounced in children with kwashiorkor [76, 77]. Thus, excessive oxidant stress has been postulated to have a role in the pathophysiology of kwashiorkor [32]. However, a large randomized trial of an antioxidant mixture (riboflavin, vitamin E, selenium and N-acetylcysteine) in Malawi failed to show decreased rates of kwashiorkor, leading to the conclusion that antioxidant depletion may be a consequence rather than a cause of kwashiorkor [78].

- Other theories, including reduced production of sulfated glycosaminoglycans [79], altered aflatoxin metabolism [80-82], and increased inflammatory reactivity [83, 84] have also been proposed as causes of edematous malnutrition, but they were not successful.
- Microbiome: Emerging evidence suggests the role of gut microbiome in metabolic disease states and in normal intestinal structure and function. The gut microbiome plays a critical role in the integrity of the intestinal barrier, nutrient absorption, metabolic homeostasis, and immune maturity, particularly in the early stages of life. Disruption of the intestinal microbial environment via antibiotics, suboptimal nutrition, or unhygienic environments has been causally related to child malnutrition. This concept will be developed further in section 1.8 "gut microbiota and malnutrition".

1.7 Causes of malnutrition

Understanding the causes of malnutrition may have relevance to the possible resultant long-term effects. There is no single cause of malnutrition. Causes of malnutrition may range from individual, families, district, country, regions, and global level. In 1990, the United Nations Children's Fund (UNICEF) introduced a conceptual framework for the causes of child malnutrition that identified three categories of determinants: the basic determinants at the societal level, the underlying determinants at the household and community levels, and the immediate causes at the individual level (figure 9) [85].

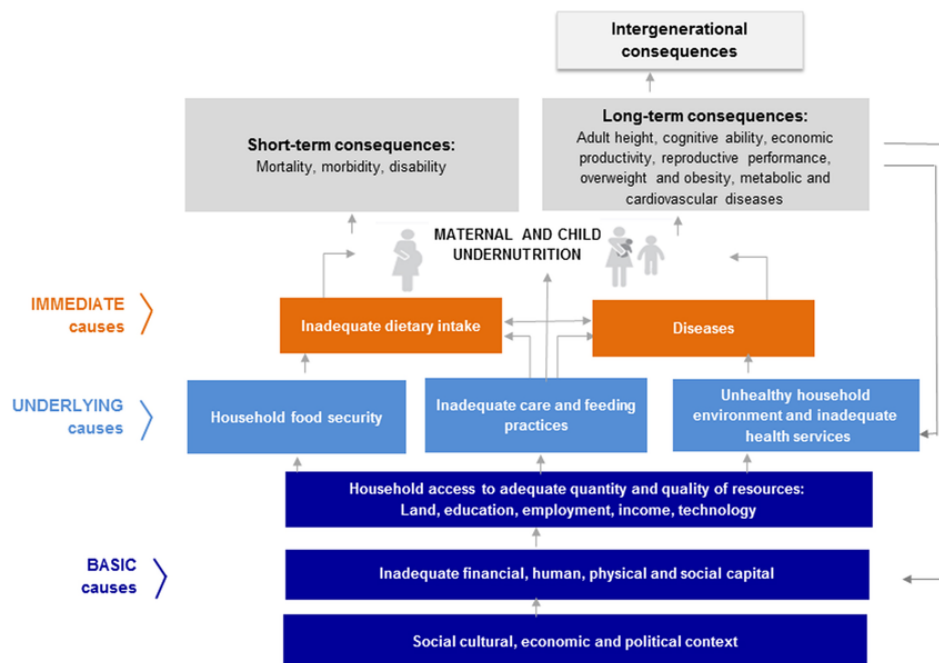


Figure 9: Conceptual framework for the causes of child malnutrition. Source: UNICEF. Improving Child Nutrition: The achievable imperative for global progress. United Nations Children's Fund; 2013. p. 4.

The immediate-level causes of malnutrition include inadequate dietary intake and diseases. Adequate food intake and freedom of disease are the immediate conditions for good nutrition. On an immediate level, malnutrition results from an imbalance between the required amount of nutrients by the body and the actual amount of nutrients introduced or absorbed by the body. In 2002, the World Health Organization (WHO) and the UNICEF adopted the global strategy for Infant and Young Child Feeding (IYCF), including: (i) early initiation of breastfeeding within 1 h of birth; (ii) exclusive breastfeeding for the first 6 months of life and (iii) introduction of nutritionally adequate and safe complementary (solid) foods at 6 months together with continued breastfeeding up to 2 years of age or beyond [86]. Inadequate dietary intake and diseases are caused by food insecurity, inadequate care for women and children, insufficient health services, and unsanitary environments [87]. Reduced dietary intake, reduced absorption of macro- and/ or micronutrients, increased losses or altered requirements, and increased energy expenditure, lead to immediate malnutrition [88].

Underlying causes encompassing household food insecurity, inadequate care and feeding practices, and unhealthy household environment and inadequate health services. These underlying issues are caused by conflict, inadequate education,

poverty, gender inequality, inadequate infrastructure, and other basic issues [87]. Household food insecurity is one of the main drivers of malnutrition and is a specific problem for displaced people [33].

Basic causes encompass three essential parts of resources: human resource (people, knowledge, skill, and time), economic resource (assets, lands, income, and others), and organizational resources (formal and informal institutions, extended families, and childcare organizations) [87]. Other aspects of these causes include poverty, lack of information, political and economic insecurity, war, lack of resources at all levels, unequal status of women, and/or natural disasters [87].

1.8 Gut microbiota and malnutrition

1.8.1 Overview of the human gut microbiota

The human gastrointestinal (GI) tract harbors an abundant and diverse microbial community, gathering more than 100 trillion microorganisms, a number 10 times greater than the total number of human cells in the body. The microbes that inhabit the GI tract are mainly divided into bacteria, viruses, fungi, and protozoa. This microbial community is referred to as the gut microbiota. Bacteria are most abundant. This group includes more than 1,000 different species of bacteria, with approximately 100 species comprising up to 99% of the total population [89]. The number of bacteria progressively increases along the small intestine, from approximately 10^4 colony-forming units (CFU) per gram of luminal content in the jejunum to 10^7 CFU at the ileal end, where gram-negative aerobes and some obligate anaerobes are predominant. In the colon, anaerobes are predominant and bacterial counts reach around 10^{12} CFU per gram of luminal content. Thus, the colon is the most densely populated microbial habitat known on earth [90].

Scientists have been interested in gut microbiota for years. However, one of the major challenges in the relevant research has been the ability to culture these microorganisms. In the early years of studying the gut microbiota, culture-based methods were used [91-93]. Due to the anaerobic nature of most bacteria in the human gut, culture-based techniques uncovered only 10-25% of the diversity in the gut microbiota [93, 94].

In recent years, new technologies have allowed researchers to phylogenetically identify and/or quantify components of the gut microbiota by analyzing nucleic acids (DNA and RNA) directly extracted from stool. Two techniques are widely used to characterize the gut microbiome: 16S rRNA amplicon sequencing and shotgun metagenomics [95]. Sequencing of 16S rRNA amplicons targets marker genes for taxonomic classification. 16S rRNA sequences can be clustered into Operational Taxonomic Units (OTUs) based on sequence similarities. OTUs are assigned to

taxonomic groups at different levels and can then be used to characterize the microbiota by estimating α - (within-sample) and β - (between-sample) diversity indices, composition, and richness [96]. Metagenomics (whole metagenome shotgun sequencing) allows a more specific taxonomic and functional identification through massive and parallel sequencing of whole "microbiota genomes" [97]. Superior to amplicon sequencing, whole metagenome shotgun sequencing allows direct functional profiling of microbial communities, as it is not limited by the amplification of a single marker gene [98].

Taxonomically, bacteria are classified according to phyla, classes, orders, families, genera, and species. Overall, the healthy gut microbiota is predominantly constituted by the phyla *Firmicutes* and *Bacteroidetes*, representing 90% of gut microbiota, followed by the phyla *Actinobacteria*, *Verrucomicrobia*, and *Proteobacteria* [99]. Examples of taxonomic gut microbiota composition are illustrated in Figure 10.



Figure 10 : Taxonomic gut microbiota composition. Source: Rinninella et al. What is the Healthy Gut Microbiota Composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019;7(1):14.

1.8.2 Development of the human gut microbiota

The development of the microbiota is generally believed to begin from birth. However, this concept is challenged by several studies in which microbes were detected in womb tissues, such as the placenta [100-102].

The mode of delivery affects the microbiota composition. The vaginally delivered infants' microbiota contain a high abundance of *lactobacilli* during the first few days, a reflection of the high load of *lactobacilli* in the vaginal flora [103, 104]. In contrast, in cesarean section (C-section), the newborn's gut microbiota is first colonized by

the mother's skin microbiota and the environment. This microbiota is depleted and delayed in the colonization of the *Bacteroides* genus, but colonized by facultative anaerobes such as *Clostridium* species [105, 106]. Whilst the fecal microbiota of 72% of vaginally delivered infants resembles that of their mothers' fecal microbiota, in babies delivered by C-section, this percentage is reduced to only 41% [107].

In early stages of development, the microbiota is generally low in diversity and is dominated by two main phyla, *Actinobacteria* and *Proteobacteria* [108]. During the first year of life, the microbial diversity increases, and the microbiota composition converges towards a distinct adult-like microbial profile with temporal patterns that are unique to each infant [109]. By around 2.5 years of age, the composition, diversity, and functional capabilities of the infant microbiota resemble those of adult microbiota [110]. This programmed maturation of the infant microbiome assembly, which is driven primarily by early life feeding practices, is strongly predictive of healthy child growth

1.8.3 Factors influencing gut microbiota

Several intrinsic and extrinsic factors can influence the composition of the gut bacteria at different stages: fetal, infancy, and childhood stage (Figure 11).

1.8.3.1 Phase 1: Fetal stage

- **Maternal diet during pregnancy:** Newborns' gut bacterial colonization begins in utero. The maternal gut microbiota is a major source of healthy microbiota for the infant, which persists through the first weeks of life. Gut microbial composition varies with the diet and health status of the host, and these factors during pregnancy can affect the maternal gut microbiota, which in turn can affect the infant in utero and even post birth [111].
- **Maternal health during pregnancy:** Mothers share their microbes and metabolites with the fetus in utero, during delivery and lactation. Thus, maternal health during pregnancy affects the development of the fetus. Studies have shown that offspring of mothers suffering from any type of diabetes or being overweight/obese are at risk of developing diabetes and obesity in later life [112, 113]. It is hypothesized that maternal diabetes and obesity influence the offspring's risk for developing chronic metabolic diseases, through changes in the maternal microbial composition during pregnancy and lactation [114].
- **Vaginal health:** The vaginal microbiome plays a role in the health of the mother-newborn pair due to its direct contact with the fetus, and its possible role in colonizing the placenta. A recent study reported that the vaginal

microbiome of women with healthy ongoing pregnancies has a relatively lower richness and diversity, along with a high abundance of *Lactobacillus* with a lower prevalence of *Mycoplasma* and *Ureaplasma*, which is otherwise related to preterm birth and low birthweight [115].

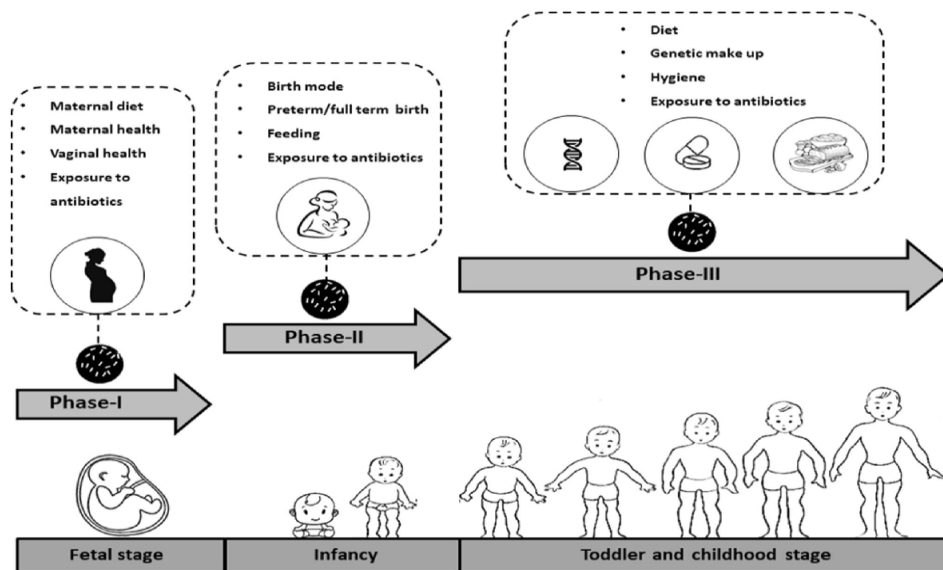


Figure 11. Factors influencing the gut microbiome at different stages (fetal, infancy, and childhood stage). Source: Kumbhare et al. Factors influencing the gut microbiome in children: from infancy to childhood. J Biosci. 2019;44(2):49.

- **Maternal exposure to antibiotics:** Exposure to antibiotic therapy and its modulating effects on the human microbiome may begin in utero and continue throughout critical growth and development stages. Gonzalez-Perez et al. showed that among mothers treated with antibiotics during pregnancy and lactation, there were profound alterations in the composition of the mothers' and infants' gut microbiota. *Streptococcus* spp. dominated the gut microbiota of treated mothers, while *Enterococcus faecalis* predominated in the infants' gut [116]. In another study, Miyoshi et al. found that the use of broad-spectrum antibiotics by pregnant women and infants at an early age can lead to changes in gut microbial composition and increase the risk of colitis in susceptible offspring by affecting a critical stage in their microbial and immune development [117].

1.8.3.2 Phase 2: Early infancy

- **Mode of delivery:** The mode of delivery has a crucial impact on the type of microbiota ingested by the infant during birth. A 16S RNA pyrosequencing study performed by Dominguez-Bello et al. showed that infants delivered vaginally had bacterial communities dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp. that resembled their own mother's vaginal microbiota. On the other hand, infants delivered by C-section had bacterial communities dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium*, which are more similar to the bacteria found on the skin surface [118].
- **Preterm and full-term birth:** The pattern of gut microbial colonization in premature infants in an intensive care setting varies when compared with that of healthy, term and breastfed infants. Studies comparing gut microbial colonization of preterm and full-term infants suggest that preterm infants show high levels of facultative anaerobes such as *Enterococcus*, *Enterobacter* and *Lactobacillus*, and lower levels of strict anaerobes like *Bifidobacterium*, *Bacteroides* and *Atopobium*, when compared with term infants [119-121]. *Proteobacteria* and *Firmicutes* are among the phyla that dominate in preterm infants when compared to full-term infants [122].
- **Feeding:** Studies have shown that formula-fed infants are more often colonized with *Escherichia coli*, *Bacteroides*, and *Clostridium difficile* compared with breastfed infants [123]. Regarding *Actinobacteria* abundance, *Bifidobacterium* spp. have been associated with breastfeeding and formula milk [124, 125]. However, breastfed infants generally harbor a more complex and diverse *Bifidobacterium* microbiota than formula-fed infants [124]. Breastfed infants are provided with microbiota having more than a two-fold increase in the number of *Bifidobacterium* cells compared to formula-fed infants [125]. Breastfed infants have more beneficial gut microbiota, with a higher richness and diversity of *Bifidobacterium* spp. and a lower number of *C. difficile* and *E. coli* than formula-fed infants [123].
- **Use of antibiotics in neonates:** Pérez-Cobas et al. explored the effect of antibiotics with different modes of action on the composition of the human gut microbiota and demonstrated that antibiotic treatments alter the composition of the gut microbiota with an abundance/appearance of some species, and a decrease/disappearance of other species [126]. Broad-spectrum antibiotics lead to an imbalance between *Firmicutes* and *Bacteroidetes*. Bacterial diversity decreases as well as the abundance of these bacteria during treatment. The alteration of microbiome composition depends on the antibiotic class, dose, period of exposure, pharmacological action, and target bacteria [127].

1.8.3.3 Phase 3: childhood

- **Diet:** The introduction of solid foods and the termination of milk-feeding/weaning coincide with major gut microbiota changes. The introduction of solid foods to breastfed infants results in an increase in *enterobacteria* and *enterococci*, as well as colonization by *Bacteroides* spp, *Clostridium* and *Streptococcus*. In contrast, formula-fed infants do not show such an abrupt transition in gastrointestinal flora upon introduction of outside solid foods. This is because, from birth, formula-fed infants are exposed to a diet that includes certain complex compounds, which establishes the microbial community necessary to support feeding habits [128].

The type of diet also affects the community structure of the gut microbiota. De Filippo et al. compared the fecal microbiota of European children and that of children from a rural African village of Burkina Faso, consuming a western diet and a diet rich in fiber, respectively. They observed that children with the high-fiber diet had significantly increased levels of *Bacteroidetes* and decreased levels of *Firmicutes*. These findings were accompanied by higher short-chain fatty acid (SCFA) levels in the rural diet of children than in the European children with an abundance of a distinctive set of bacteria from the genus *Prevotella* and *Xylanibacter*, known to be responsible for fermenting cellulose and xylan to liberate energy [129].

Since diet has an influence on gut microbiota's composition, nutritional disorders such as obesity, severe acute malnutrition and anorexia nervosa are linked to an alteration of the gut microbiota mirroring the physiopathology of the nutritional disorder [129].

- **Exposure to antibiotics during childhood:** Use of antibiotics has several short- and long-term impacts on the ecology of the normal gut microbiota. The use of broad-spectrum antibiotics allows growth of the pathogen *C. difficile*, which causes infections and severe diarrhea [130]. In a study to assess the effects of multiple antibiotic treatments in the first three years of life, Yassour et al. measured the diversity and richness of the microbiota at the species and strains level and reported that antibiotic-treated children had less diverse and more unstable gut microbial composition compared to untreated children [131]. In a cohort study of 142 Finnish children aged 2 to 7 years, Korpela et al. reported that macrolide use was associated with long-lasting changes in microbial composition and reduced diversity. The abundance of *Bifidobacterium* and *Bacteroides*, factors influencing the gut microbiome in children and antibiotic resistance, were restored within a year

after the antibiotic course. However, the abundance of certain other genera and diversity did not recover until two years after treatment. The penicillin use did not have a severe influence on the microbiota, suggesting the variable influence of different antibiotics [132].

- **Hygiene:** Several studies suggest that early-life exposure to germs through contact with pets or older siblings, as well as a larger family size, enhances the rate of maturation of the microbiome [133], and acts as a shield against the risk of developing allergic disease and asthma [134].
- **Genetic makeup:** Host genetic interactions act as a part of a complex network of factors affecting the microbial composition. The number of specific bacteria found in the gut microbiota is influenced in part by the genetic makeup of the host in ways that affect host metabolism and ultimately can affect health [135]. Family members have been found to have more similar microbiota communities than unrelated individuals, and the gut microbiota is more similar in monozygotic than in dizygotic twins [135].

1.8.4 Functions of gut microbiota

The gut microbiota performs essential functions in maintenance of health: metabolic, protective, and structural roles (figure 12).

1.8.4.1 Metabolic functions

The GI microbiota is crucial to the de novo synthesis of essential vitamins which the host is incapable of producing. Lactic acid bacteria are key organisms in the production of vitamin B12 [136]. *Bifidobacteria* are main producers of folate [137]. Further vitamins, which gut microbiota have been shown to synthesize in humans, include vitamin K, riboflavin, biotin, nicotinic acid, pantothenic acid, pyridoxine and thiamine [138].

The gut microbiota has a positive impact on lipid metabolism by suppressing the inhibition of lipoprotein lipase activity in adipocytes. In addition, *Bacteroides thetaiotaomicron* has been shown to increase the efficiency of lipid hydrolysis by regulating the expression of a colipase required by pancreatic lipase for lipid digestion [139].

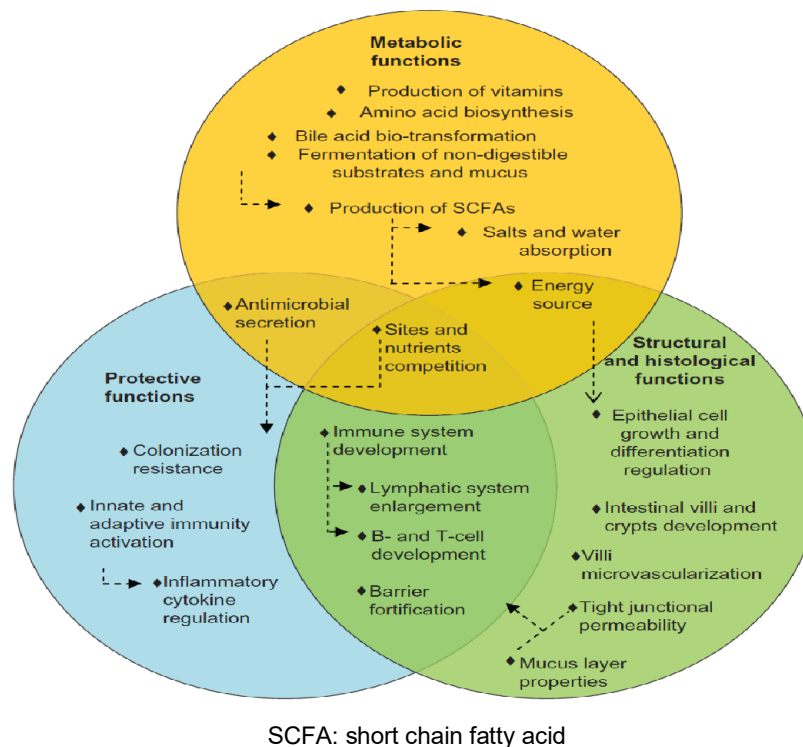


Figure 12. Main beneficial functions of the human gut microbiota. Source: Prakash S et al. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. *Biologics*. 2011;5:71-86.

The gut microbiota is also enriched with an efficient protein metabolizing machinery that functions through microbial proteinases and peptidases in tandem with human proteinases. Several amino acid transporters on the bacterial cell wall facilitate amino acid entry from the intestinal lumen into the bacteria, where several gene products convert amino acids into small signaling molecules and antimicrobial peptides [140].

Gut microbiota also plays an important role in the cometabolism of bile acids with the host. In humans, 95% of bile acids are reabsorbed at distal ileum. The 5% of unabsorbed primary bile acids are then bioconverted or deconjugated into secondary bile acids by bile salt hydrolases secreted by several colonic microbiota such as *Clostridium perfringens* and *Clostridium scindens*. They are then partially reabsorbed in the colon and transported to the liver for conjugation. Unabsorbed secondary bile acids are excreted by the host [141]. Secondary bile acids possess antimicrobial effects which alter microbial cell membrane integrity, causing spillage of intracellular contents, thus inhibiting growth of bile acid-intolerant microbes [142].

Gut microbiota is important in fermenting unabsorbed starch and soluble dietary fiber. The fermented end products exist in the form of SCFA. The major SCFA produced are acetate, propionate, and butyrate [143]. SCFAs, particularly butyrate, are a primary energy source for colonic epithelial cells. It is estimated that SCFAs provide 10% of total dietary energy intake in humans. Butyrate and propionate regulate intestinal physiology and immune function. Acetate acts as a substrate for lipogenesis and gluconeogenesis [144].

Recent studies suggest that the human gut microbiota also is involved in the degradation of various polyphenols consumed in the diet. Polyphenolic secondary metabolites are present in a variety of plants, fruits, and plant-derived products (tea, cocoa, wine). Polyphenols, which generally remain inactive in the diet, are biotransformed into active compounds after removal of the sugar moiety by the intestinal microbiota, among other factors. The final active products are absorbed through the portal vein and move to other tissues and organs, providing antimicrobial and other metabolic actions [145].

An increasing body of evidence has now provided sufficient insights on the role of the gut microbiota on xenobiotic and drug metabolism. Clayton et al. has shown that a gut microbial metabolite p-cresol can reduce the capacity of the liver to metabolize acetaminophen due to competitive inhibition of hepatic sulfotransferases [146]. The example of microbiome induced drug metabolism is the microbial β -glucuronidase induced deconjugation of the anticancer drug irinotecan that can contribute to its toxicities such as diarrhea, inflammation, and anorexia [147].

1.8.4.2 Protective functions

Another important role of human microbiota is colonization resistance where indigenous microbiota confers protection to host against colonization of pathogenic invaders and prevention against overgrowth of pathogenic microbiota members (pathobionts). Colonization resistance has been recognized since at least the 1950s [148]. Three main mechanisms of commensal bacteria-driven protection against intestinal pathogens were recently described:

1.8.4.2.1 Commensal bacteria outcompete pathogens for nutrients

In the "nutrient niche" hypothesis in 1983, Freter stated that "the populations of most indigenous intestinal bacteria are controlled by one or a few nutritional substrates that a given strain can use most efficiently" [149]. Subsequent experiments confirmed the hypothesis that, for some bacteria, substrate limitation was a key

determinant of their successful colonization of the gut [150, 151]. For example, in *E. coli*, the ability to utilize one sugar changed the competitive balance between otherwise identical strains in the gut [152]. The murine enteric pathogen *Citrobacter rodentium* colonizes the intestinal lumen of germ-free mice, but in the presence of non-pathogenic *E. coli*, *C. rodentium* is outcompeted for available carbohydrates and is eliminated from the intestinal lumen [153]. Perturbation of the microbiota disrupts the intestinal ecosystem and enables pathogens to access resources that would otherwise be consumed by commensal bacteria. Antibiotic-mediated disruption of the microbiota, for instance, results in elevated levels of free sialic acids, which two distinct intestinal pathogens, *Salmonella typhimurium* and *C. difficile*, catabolize and use as an energy source to expand [154].

1.8.4.2.2 Microbiota-derived metabolic by-products directly inhibit pathogens.

Commensal bacteria evolve defense mechanisms that directly inhibit the survival ability of pathogens. Most prominent among these mechanisms include a class of peptides, bacteriocins, which are antimicrobial factors produced by bacteria that target a narrow spectrum of competing bacteria [155].

Metabolic byproducts from commensal bacteria have the potential to inhibit pathogen growth. SCFAs produced by commensal bacterial fermentation of ingested complex carbohydrates can inhibit the growth of enteropathogenic bacteria [156].

Commensal bacteria harbor a diverse assortment of bacteriophages, viruses that infect bacteria, which, in part, constitute the virobiota of the gut. Bacteriophages can help shape the bacterial communities in the intestine and in turn the ability of pathogens to establish infection. The microvirome, the genes derived from the virobiota, encodes a broad range of functions, and can influence the relative representation of different bacterial species within the intestine. The mechanisms employed by bacteriophages to support host bacteria remain largely unknown [157].

Bacteria have also evolved defense mechanisms that involve direct attack against heterologous bacterial species. The bacterial type VI secretion system utilizes a multicomponent organelle to pierce the cell wall of neighboring cells and transport effector proteins into the targeted eukaryotic or prokaryotic cell [158].

1.8.4.2.3 Commensal bacteria stimulate host immune cells and drive basal expression of host defense factors

Commensal bacteria also contribute to pathogen clearance by activating host defense mechanisms that maintain physical separation between the microbial world of the gut lumen and the host. Thus, a complex network of immune and non-immune cells acts in concert following detection of microbial molecules by innate immune receptors to regulate host defenses that limit bacterial dissemination, repair the intestinal epithelial barrier, and maintain intestinal homeostasis [159]. Tonic stimulation of host defenses by commensal bacteria benefits the host and resident bacteria by inhibiting the expansion of invasive pathogens and preserving the environmental niche for non-invasive commensal species [160].

1.8.4.3 Structural and histological functions

Currently, there is a body of evidence supporting a role for the gut microbiota in maintaining the structure and function of the GI tract. *B. thetaiotaomicron* induces the expression of the small proline-rich protein 2A, which is required for the maintenance of desmosomes at the epithelial villi [161, 162]. In addition, the *Lactobacillus rhamnosus* GG strain produces two soluble proteins (p40 and p75) which can prevent cytokine-induced apoptosis of intestinal epithelial cells in a manner dependent on the epithelial growth factor receptor and the protein kinase C pathway [163].

The gut microbiota also contributes to the structural development of the gut mucosa by inducing the transcription factor angiogenin-3 involved in the development of the intestinal microvasculature [164]. This hypothesis is also supported by a significant reduction in the villi capillary network in germ-free mice, which may impair digestion and nutrient absorption. Further evidence for the role of the gut microbiota in maintaining structure and function is obtained in germ-free mice, which exhibit lower gut surface area [165], thin villi [166], increased cell cycle time [167] and impaired peristalsis [168]. The gut microbiota may also modulate mucosal glycosylation patterns that constitute microbial attachment sites both at the cell surface and subcellular level.

1.8.5 Gut microbiota alterations in childhood malnutrition

Until a few decades ago, inadequate dietary intake was the main recognized cause of malnutrition. Although malnutrition is often a consequence of inadequate food intake, the etiology of childhood malnutrition cannot be explained simply by limited access to macro- and micro-nutrients. Malnutrition appears to be the result of a

complex set of determinants. Recent metagenomics studies in malnourished children from Bangladesh, India and sub-Saharan Africa provided evidence that dysbiosis, an altered microbiota composition, was causally related to malnutrition [169-174].

The development and maturity of the gut microbiota is disrupted by childhood malnutrition. A metric of microbiome maturity, termed the microbiota-for-age Z score (MAZ), has been derived from a cohort of healthy growing infants in Bangladesh and validated in humans and experimental animals. In this cohort study, Subramanian et al. further showed that acutely malnourished children have lower relative microbiota maturity and diversity index (α -diversity) than healthy controls. In addition, several taxa positively related to relative microbiota maturity were found to be depleted in all phases of acute malnutrition [175].

The link between microbiota immaturity and malnutrition was also demonstrated by Blanton et al [176]. In a study to test the hypothesis that perturbations in the normal development of the gut microbiota were causally related to malnutrition, they showed a positive correlation between the MAZ and WHZ and WAZ. Furthermore, MAZ at 12 months was found to be positively related to WHZ and WAZ at 18 months, suggesting that MAZ may be a predictor of future growth.

Several studies in children from sub-Saharan Africa, India and Bangladesh have reported taxonomic and functional alterations of the gut microbiota of children suffering malnutrition. In a cross-sectional study of Bangladeshi children, Monira et al. [170] showed that malnutrition was associated with a less diverse microbiota, characterized by a higher number of *Proteobacteria* and a lower number of *Bacteroidetes* compared to healthy controls. In addition, a higher abundance of *Klebsiella* and *Escherichia* was found in the gut microbiota of malnourished children [170]. Consistent with these findings, *Proteobacteria* were also found to dominate the gut microbiota of malnourished children hospitalized in Uganda [171]. Furthermore, Tidjani et al. suggested that the kwashiorkor microbiota was associated with lower β -diversity and specifically lower anaerobic diversity; and enriched in potentially pathogenic *Proteobacteria*, *Fusobacteria* and *Streptococcus gallolyticus* [174]. In a cross-sectional study of Indian children with varying nutritional status, Ghosh et al. reported that the abundance of the genera *Escherichia*, *Streptococcus*, *Shigella*, *Enterobacter*, and *Veillonella* increased with deteriorated nutritional status [169]. Time series from Malawian twin pairs discordant for kwashiorkor evidenced a decrease in the relative abundance of *Actinobacteria* in children with kwashiorkor but not in their healthy cotwins after 2 weeks of RUTF treatment. Microbiota transfer from discordant twins to gnotobiotic mice exhibited 37 species that differed between the gnotobiotic mice with kwashiorkor and the healthy

microbiota [177]. In a meta-analysis of 5 studies including children from Niger, Senegal, Malawi, Bangladesh, and India, Million et al. evidenced a dramatic depletion of obligate anaerobes in malnutrition, regardless of age, sex, and recruitment center. Furthermore, malnutrition was associated with depletion of several species from *Bacteroidaceae*, *Eubacteriaceae*, *Lachnospiraceae* and *Ruminococcaceae* families, and enrichment of several aerotolerant species with potential pathogenic effects such as *E. coli*, *Enterococcus faecalis* and *Staphylococcus aureus* [178].

In summary (Table 3), the available evidence indicates that childhood malnutrition is associated with gut microbiota immaturity, with decreased bacterial diversity, and increases in *Proteobacteria*, *Bacteroidetes* and anaerobic *Firmicutes*, and as such, this is a functional target that should be explored in the fight against malnutrition.

Table 3: Summary of main findings from studies of microbiota alterations in childhood malnutrition

Study, subject, country	Design	Method of analysis	Malnutrition criteria	Diet intervention	Findings
Chen 2021 [179]; 123 children with MAM and SAM between 12-18 months; Bangladesh	Randomized controlled trial	V4-16S rRNA gene sequencing	WHO criteria	Microbiota-directed complementary food-2 (MDCF-2) compared with RUSF	MDCF-2 produced better outcomes with regards to wasting and stunting than RUSF did. MDCF-2 was associated with <i>Faecalibacterium prausnitzii</i> , <i>Dorea formicigenerans</i> , <i>Ruminococcus gnavus</i> and a member of <i>Clostridiales</i>
Castro-Mejia 2020 [180]; SAM (n=400) and Healthy (n=22) children between 5-59 months; Uganda	Prospective study nested in a randomized, double-blind, placebo-controlled trial	16S rRNA amplicon sequencing (V3-V4 regions)	WHO criteria	RUTF with or without probiotics	The GM of children with SAM showed distinct composition over admission (e.g. <i>Klebsiella</i> spp., and <i>Enterobacteriaceae</i> spp.), discharge (e.g. <i>Clostridiaceae</i> spp., <i>Veillonella dispar</i>) and follow-up (e.g. <i>Lactobacillus ruminis</i> , <i>Blautia</i> spp., <i>Faecalibacterium prausnitzii</i>), reaching similar β - and α -diversity as healthy individuals. Children with diarrhea had reduced distribution of <i>Bacteroidaceae</i> , <i>Lachnospiraceae</i> , increased <i>Enterobacteriaceae</i> and <i>Moraxellaceae</i> , and lower α -diversity. Children suffering from edematous SAM had a diminished proportion of <i>Prevotellaceae</i> , <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> and a higher α -diversity when compared to non-edematous SAM.
Gehrig 2019 [181]; 343 children with SAM between 6-36 months; Bangladesh	Double-blind, randomized, four-group, parallel assignment interventional trial	V4-16S rRNA gene sequencing	WHO criteria	MDCFs compared with RUSF	<i>Enterococcus avium</i> , <i>Escherichia fergusonii</i> , <i>Bifidobacterium pseudocatenulatum</i> , <i>Streptococcus constellatus</i> and <i>Streptococcus pasteurianus</i> were characteristic of malnourished microbiota. MDCF-2 was shown to increase the relative abundance of <i>F. prausnitzii</i> , <i>Clostridiales</i> sp., and decrease the abundance of <i>B. longum</i> . MDCF-2 shifted the plasma proteome of MAM toward a healthy one and improved growth parameters in MAM
Raman 2019 [182]; 36 members of the Bangladesh birth cohort; Bangladesh	Sparse random forests-derived model	V4-16S rDNA amplicons	WHO criteria	No intervention	15 bacteria taxa were associated with different degrees of undernutrition (SAM and MAM). Termed "eco group", the 15 bacteria taxa identified were <i>S. thermophilus</i> , <i>F. prausnitzii</i> (514940 and 851865), <i>Bifidobacterium</i> , <i>E. coli</i> , <i>Dialister</i> , <i>B. longum</i> , <i>S. gallolyticus</i> , <i>Prevotella</i> , <i>P. copri</i> (840914 and 588929), <i>Clostridiales</i> , <i>E. rectale</i> , <i>L. ruminis</i> and <i>E. faecalis</i> .

Blanton 2016 [176]; Twin pairs concordant for healthy growth from Malawian cohort; Malawi	Longitudinal and GM transplantation from healthy or malnourished donors to GF mice	16S rRNA amplicon sequencing (V4 region)	WHO criteria	GF mice fed with representative Malawian diet with low caloric and nutrient density	Positive correlation between MAZ and WHZ and WAZ. 25 age discriminatory taxa were defined which are also growth discriminatory. Combination of malnourished microbiota transplantation and deficient Malawian diet transmitted impaired growth phenotype to mice. Cohousing of healthy and malnourished microbiota recipient mice resulted in invasion of taxa- from the healthy donor's microbiota into the malnourished microbiota recipient mice and prevented growth impairments. Improved growth by adding growth discriminatory taxa to the malnourished microbiota recipient mice.
Dihn 2016 [183]; LBW, persistently stunted (n=10) vs. Controls with normal birth weight (n=10); India	Longitudinal Birth Cohort	16S rRNA amplicon sequencing (V4 region)	LBW: <2500 g Persistent stunting: HAZ < 2 at least 6 of 8 three monthly periods	It mainly consists of rice, lentils and vegetables. No difference in EBF and weaning between groups.	No difference in α -diversity indices or in the rates of their increase with the age between cases and controls. Significant increase in β -diversity in cases compared to controls at the 12 months, but not at other time points. Taxa is enriched in the cases <i>Prevotella stercora</i> , <i>Prevotella copri</i> , <i>Desulfovibrio</i> and <i>Catenibacterium</i> and <i>Campylobacteriales</i> .
Kristensen 2016 [171]; 6-24 months old hospitalized children with edematous (n=54) vs. no edematous (n=33) SAM; Uganda	Cross-sectional	DGGE and 16S rRNA amplicon sequencing (V3-V4 regions)	WHO criteria	F75 and F100 and RUTF after stability	α -diversity significantly higher in edematous children compared to no edematous. β -diversity revealed a minor significant difference between edematous and no edematous children. No significant difference in the abundance of specific genera were observed between SAM types.
Tidjani Alou 2016 [174]; Children with kwashiorkor (n=10, mean age 13 months) vs. healthy controls (n=5, mean age 25 months); Senegal and Niger	Cross-sectional	Culturomics in 18 conditions: colonies identified using MALDI-TOF/MS and 16S rRNA amplicon sequencing (V3-V4 regions)	WHO criteria	No intervention	Decreased β -diversity and anaerobic diversity and increased Proteobacteria and Streptococcus gallolyticus in malnourished groups. 12 species with possible probiotic effects identified representing missing repertoire in Kwashiorkor patients: Firmicutes (<i>Anaerostipes caccae</i> , <i>Bacillus licheniformis</i> , <i>Bacillus subtilis</i> , <i>Intestinimonas butyriciproducens</i> , <i>Lactobacillus parabuchneri</i> , <i>Lactobacillus perolens</i> , <i>Lactobacillus vaccinosercus</i> , <i>Terrisporobacter glycolicus</i>), Bacteroidetes (<i>Alistipes indistinctus</i> , <i>Bacteroides salyersiae</i>) and Actinobacteria (<i>Bifidobacterium adolescentis</i>)

Million 2016 [178]; <60 months old children with a varying nutritional status from Niger (n=34) And Senegal (n=52). For the meta-analysis: SAM (n=107) vs controls (n=77) from 5 studies (Bangladesh, India, Malawi, Niger and Senegal)	Case control and meta-analysis	16S rRNA amplicon sequencing (V3-V4 regions)	WHO criteria	In Niger: children were recruited immediately on diagnosis, before any nutritional supplements were administered. In Senegal: an energy milk drink consisting of milk, oil, and sugar was given.	Total gut bacterial concentration and <i>Methanobrevibacter Smithii</i> were significantly depleted in SAM whereas gut redox potential was significantly higher. Relative depletion of obligate anaerobes and enrichment of aerotolerant organisms in SAM. SAM was associated with anaerobic depletion regardless of age, sex and recruitment center. The meta-analysis: Depletion of several obligate anaerobes, including several Firmicutes (<i>Eubacteriaceae</i> , <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> , <i>Erysipelotrichaceae</i>), Bacteroidetes (<i>Bacteroidaceae</i>) and Actinobacteria (<i>Eggerthella</i> , <i>Coriobacteriaceae</i>). Conversely, some aerotolerant bacteria were enriched in SAM, including <i>Enterococcus faecalis</i> , <i>E. coli</i> , and <i>S. aureus</i> , which all represent potential pathogens.
Gough 2015 [184]; Stunted children from Malawian twin pairs cohort (case n=10, control n=8) and Bangladesh cohort (case n=6, control n=5); Malawi and Bangladesh	Secondary case-control data analysis of longitudinal twin cohorts	Malawian Cohort: whole genome shotgun sequencing datasets Bangladesh Cohort: Relative OTUs abundance	WHO criteria	Only available for Bangladesh cohort: Peanut-based RUTF (n=32) vs locally produced rice-lentil based therapeutic foods (Khichuri-Halwa) (n=32) intervention	Less diverse gut microbiota with greater covariance network density is associated with stunting severity. Increase in the relative abundance of <i>Acidaminococcus</i> sp. is associated with lower future linear growth in both cohorts. In the Malawi cohort: <i>Prevotella</i> , <i>Bacteroides</i> , <i>Eubacterium</i> and <i>Blautia</i> showed the largest decrease in relative abundance in cases vs controls. In the Bangladesh cohort: <i>Lactobacillus</i> , <i>Olsenella</i> , <i>Dorea</i> , <i>Blautia</i> , and unclassified genera in the <i>Coriobacteriaceae</i> and <i>Enterococcaceae</i> showed the largest decrease in relative abundance in cases vs controls.
Subramanian 2016 [175]; 64 children with SAM between 6 and 20 months; Bangladesh	Randomized intervention study	16S rRNA amplicon sequencing (V4 region)	WHO criteria	Peanut-based RUTF (n=32) vs locally produced rice-lentil based therapeutic foods (Khichuri-Halwa) (n=32) intervention	WHZ scores significantly and inversely correlated with relative microbiota maturity and MAZ scores. Age discriminatory taxa depleted across all phases of SAM were <i>Faecalibacterium prausnitzii</i> , <i>Clostridium</i> sp., <i>Dorea formicigenerans</i> , <i>Ruminococcus</i> sp 5 1 39BFAA, <i>Ruminococcaceae</i> sp., <i>Catenibacterium mitsuokai</i> , <i>Haemophilus para influenzae</i> . Lower age adjusted Shannon Diversity Index at enrollment, during the treatment and follow up periods of children with SAM compared to healthy.

Ghosh 2014 [169]; 5-60 months old children with varying nutritional status (n=20); India	Cross-sectional	Whole genome sequencing	WHO criteria	No intervention	Taxa characterized by <i>Escherichia</i> , <i>Streptococcus</i> , <i>Shigella</i> , <i>Enterobacter</i> , <i>Veillonella</i> increased in their abundance with the decreasing nutritional index. <i>Roseburia</i> , <i>Faecalibacterium</i> , <i>Butyrivibrio</i> had positive correlations with nutritional index.
Smith 2013 [177]; Monozygotic and dizygotic twin pairs (n=317 and < 3 years old); Malawi,	Longitudinal (follow-up to 36 month) and GM transplantation from twin pairs discordant for kwashiorkor to GF mice	16S rRNA amplicon sequencing (V4 region) and shotgun pyrosequencing	WHO criteria	SAM treated with RUTF and MAM treated with RUSF. GM transplantation to GF mice: Malawian diet vs. RUTF intervention.	Children with kwashiorkor manifested a decrease in Actinobacteria with the introduction of RUTF, unlike their healthy cotwins. GM transplantation to GF mice: Combination of fecal transplantation of kwashiorkor co-twin's microbiota and Malawian diet resulted in greater weight loss in 3 weeks. 30 species-level taxa significantly changed in kwashiorkor microbiota transplant recipients. Species characteristic for a kwashiorkor related microbiota were <i>Bilophila wadsworthia</i> (Proteobacteria, Desulfovibrio) and <i>Clostridium innocuum</i>
Monira 2011 [170]; Healthy (n=7) and malnourished (n=7) children between 2 and 3 years; Bangladesh	Cross-sectional	16S rRNA amplicon sequencing (V5-V6 regions)	WHZ $\geq 100\%$ for healthy and <70% for malnourished children	Both groups received usual Bangladeshi foods (rice, meat, fish, milk, egg, fruits and vegetables).	Higher microbiota diversity in healthy children (higher number of OTUs). Bacterial population of the phyla Proteobacteria and Bacteroidetes accounted for 46% and 18% in malnourished children; 5% and 44% in healthy children respectively. <i>Klebsiella</i> and <i>Escherichia</i> were 174-fold and 9-fold higher in malnourished children.

EBF: Exclusive breastfeeding; GF: Germ-free; GM: Gut microbiota; HAZ: height-for-age z-score; LBW: Low birth weight; MAM: Moderate Acute Malnutrition; MAZ: microbiota-for-age Z-score; MDCFs: Microbiota-directed complementary food; OTUs: Operational Taxonomic Units; rDNA: Recombinant DNA; rRNA: Ribosomal ribonucleic acid; RUSF: Ready-to-use supplementary food; RUTF: Ready-to-use therapeutic food; SAM: Severe Acute Malnutrition; WAZ: weight-for-age z-score; WHO: World Health Organization;

1.9 Treatment programs for childhulienood SAM

Previously, all children with SAM were managed as inpatients, regardless of whether clinical features of infection (or other complications) were present [185]. This historical system of universal inpatient management was plagued by limited access, poor outcomes, and high costs. Over the last several years, a revolution in the case management of SAM has occurred with the introduction and widespread acceptance of RUTF, a mixture of peanuts, sugar, oil, and powdered milk, supplemented with a vitamin and mineral mixture [186]. These therapeutic foods have several novel features, including that they resist bacterial contamination because they contain very little water, do not require cooking, are very energy dense (5.5 kcal/g), and can be produced locally [187]. These characteristics make them ideally suited for use in places in which food insecurity is common and hygiene is poor.

In 2007, the WHO and the United Nations endorsed a community-based model for the management of SAM. In this model, children with uncomplicated SAM are treated at home with RUTF with periodic visits at a decentralized health center, ideally in proximity to where children live [188]. Community-based management of acute malnutrition (CMAM) is safe and effective for most of these children, increases access to care by avoiding travel and reducing costs, decreases the risk of nosocomial infection, and decreases the likelihood that the caretaker will take the child home (or even abandon the child) against medical advice before therapy is complete [12, 189].

1.9.1 Triage

For a child with SAM, triage to inpatient versus outpatient care settings depends on the child's age, appetite, presence or absence of medical complications, and access to follow-up. A child with SAM is eligible for CMAM if he (i) is aged six months or older; (ii) passes an "appetite test", which assesses whether the child is able to consume approximately 30 grams of RUTF in a supervised, calm setting under the direct observation of clinical staff; (iii) has no signs or symptoms suggesting acute complications; (iv) is already on effective HIV treatment if the child is HIV infected; (v) has a reliable caretaker who can provide frequent, closely supervised feedings at home; and (vi) is able to return to the feeding center for regular follow-up where progress can be monitored, medical complications addressed, counseling and major health messages reinforced, and additional RUTF provided. Otherwise, he should be hospitalized for inpatient care [12, 15, 186].

1.9.2 Community-based management of acute malnutrition (CMAM)

The cornerstone of CMAM is the use of RUTF, a soft, semi-solid paste that is easily consumed by children at least six months of age. Originally developed in the 1990s, RUTF has become the de facto standard of care, although no specific formulation is universally endorsed as long as all nutritional and safety standards are met [184]. The most widely available commercial source of RUTF is marketed as Plumpy'Nut, but it is also increasingly produced locally in more than a dozen countries. In children with uncomplicated SAM, the dosage is 175 kcal/kg/day. RUTF is usually supplied in foil sachets containing 500 kcal of RUTF [187].

Several other additional interventions, such as vitamin A and/or folic acid supplementation, antihelminthic medications such as albendazole or mebendazole, antimalarial medication, measles vaccination and routine antibiotics (amoxicillin 40 to 45 mg/kg twice daily, seven-day course), are variably included in several CMAM regimens [12, 15].

Children should return for follow-up every 1-2 weeks, or sooner, if complications arise or the child's condition is worsening. At each follow-up visit, weight, height, MUAC, edema, and medical complications are assessed. Treatment should continue until they have no more edema or until WHZ ≥ -2 or until MUAC ≥ 125 mm [12, 15].

Most children recover within 10-12 weeks, and about half recover within 6 weeks if there are no social or compliance concerns and no acute infections that complicate their care. Discharge criteria are WHZ ≥ -2 and no edema for at least one to two weeks, or MUAC ≥ 125 mm and no edema for at least one to two weeks. The mother or caregiver should be able to provide food and recognize when diarrhea, fever, respiratory distress, and other common symptoms warrant medical follow-up. Ideally, a health worker should be available in the community to provide follow-up of the child and support for the mother [12, 15].

Children who are losing weight (other than water weight from decreasing edema) or those who are not making steady progress over two to three weeks during follow-up, or those who do not achieve their anthropometric goals within 12 weeks should be referred for inpatient care [12, 15].

1.9.3 Management of complicated SAM

The protocol for management of complicated SAM consists of three major phases:

- Initial stabilization: It is a critical time that focuses on recognizing and treating life-threatening conditions, including hypoglycemia, hypothermia, dehydration, electrolyte imbalance, infection, and micronutrient deficiencies. The core of this phase is slow, cautious feeding with F-75 formula every few hours, progressing to F-100 formula as recovery proceeds and when they are able to tolerate this increased solute load.
- Rehabilitation phase: During this phase, the child's caregiver is trained to continue care at home and any social problems are addressed. Emotional and sensory stimulation of the child are continued throughout the initial and rehabilitation phases.
- Follow-up: In this phase, the physical, mental, and emotional development of the child are monitored after discharge.

These phases are coordinated in a 10-step protocol (figure 13).

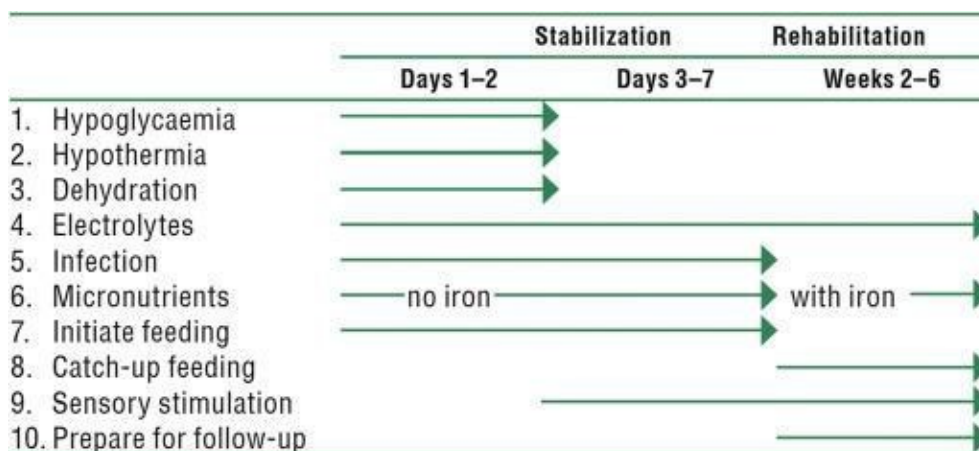


Figure 13. Ten-step inpatient management protocol for severe acute malnutrition. Source: Ashworth A et al. *Guidelines for the inpatient treatment of severely malnourished children*. Geneva: World Health Organization, 2003.

In the best circumstances, around 75% of children are expected to recover from SAM and <10% mortality should be achievable [190]. However, the success of current therapies for SAM underestimates overall mortality as those who recover are at continued risk for relapse (relapse rate after SAM treatment varying from 0% to 37%) [191] and death in the long term, especially those who are HIV-infected [23, 24].

1.9.4 Other components of the malnutrition management

The cornerstone of malnutrition management suggested that the disorder is solely treated and prevented by nutrition. However, recent evidence suggests that the 10 most effective nutritional interventions, if scaled up to 90% in high-risk countries, would reduce stunting by only 20% and wasting by 60% [192]. Therefore, childhood malnutrition is increasingly viewed as a complex disorder driven by several nutritional, infectious, physiological, and economic factors. Each of these factors needs to be addressed to effectively reduce the mortality and morbidity associated with this disease. With the advancement in knowledge of the key role of gut microbiota disruption in childhood malnutrition, several interventions targeting the gut microbiota have emerged as promising treatment modalities.

1.9.5 Microbiota-targeted interventions to address malnutrition

1.9.5.1 Microbiota-directed complementary foods (MDCF)

Legumes contain resistant starch that is readily fermented by the gut microbiome. The fermentation of dietary fiber produces SCFA and other metabolites, which play essential roles in maintaining the integrity of the intestinal barrier. Several studies have assessed locally sourced legumes as sustainable interventions to prevent child malnutrition. Cowpeas and beans have shown small but significant benefits in improving length-for-age Z-score and EED biomarkers, respectively, in RCT conducted in Malawian children, which may be partially mediated through the fermentation of resistant starch by the microbiome [193, 194].

Recent data has provided promising evidence for the potential of MDCF to improve child growth. Gehrig et al. [181] conducted a pilot RCT to compare the efficacy of MDCF with the traditional rice- and lentil-based ready-to-use supplementary food (RUSF) used in the treatment of Moderate Acute Malnutrition (MAM). The 1-month pilot trial tested 3 MDCF formulations with different proportions of 4 ingredients (peanut flour, chickpea flour, soy flour, and banana) among 12- to 18-month-old Bangladeshi children with MAM. Findings suggested that such microbiota-targeted foods enhanced biomarkers of growth, bone formation, neurodevelopment, and immune function in both experimental animals and undernourished children [181]. However, the study was not sufficiently large or long enough to confirm these positive effects.

Based on these observations, Chen et al conducted a large RCT in which 118 children ages 12 to 18 months with MAM were randomly assigned to receive the MDCF-2 (n=59) or a standard therapeutic food (n=59). The MDCF2 contained a mixture of chickpeas, soy, bananas and peanuts, ingredients that authors had discovered in earlier preclinical models to improve the gut microbiome. The standard therapeutic food was rice- and lentil-based and contained about 20% more calories per serving than the MDCF-2. The children received 25 grams of their assigned therapeutic foods twice daily for three months. The children's height, weight and MUAC were measured at regular intervals throughout the intervention period and for one month after cessation of treatment. Findings suggested that the rate of change in the children's weight and their MUAC were significantly greater in the group receiving the MDCF-2 compared with the standard therapeutic food; this growth superiority was sustained even a month after the children had stopped receiving the nutritional intervention. Furthermore, receipt of MDCF-2 was linked to the magnitude of change in levels of 70 plasma proteins and of 21 associated bacterial taxa that were positively correlated with the weight-for-length Z score [195].

Microbiota-targeted therapies hence pose potential as promising alternatives to standard therapeutic and complementary foods in settings with high prevalence of child malnutrition.

1.9.5.2 Antibiotics

The use of antibiotics in all cases of SAM was adopted into WHO guidelines in 1999 [12]. The rationale behind antibiotic treatment for all children with SAM lies in the observation that children with SAM have high rates of underlying bacterial infection, infection is an important contributor to mortality, and overt signs of infection are often lacking [12, 196]. Large intervention studies of antibiotic therapy following treatment for SAM in Malawi (2,767 children) [18], Kenya (1,778 children) [25], and Niger (2,412 children) [197], have provided conflicting evidence for their benefits in recovering growth. However, a large meta-analysis found that antibiotic use increased height by 0.04 cm/month and weight by 23.8 g/month in children in LMIC [198]. Furthermore, in a meta-analysis of both Malawi and Niger trials, the rate of nutritional recovery was higher in children treated with amoxicillin plus standard care, compared with placebo plus standard care [199].

The efficacy of empiric antibiotics is also supported by retrospective studies, which found that children who received amoxicillin as part of outpatient management of SAM were 1.5 to 2 times more likely to achieve nutritional recovery compared with those who did not receive antibiotics [16, 200]. Although empiric use of antibiotics may increase the prevalence of antibiotic resistance, the nutritional and mortality benefits generally still justify this practice [201].

1.9.5.3 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT), also called stool/fecal transplantation or fecal bacteriotherapy, is the administration of a solution of fecal matter from a donor into the intestinal tract of a recipient to directly change the recipient's microbial composition and confer a health benefit [202, 203]. The process usually involves first selecting a donor without a family history of autoimmune, metabolic, and malignant diseases and screening for any potential pathogens. The feces are then prepared by mixing with water or normal saline, followed by a filtration step to remove any particulate matter. The mixture can be administered through a nasogastric tube, nasojejunal tube, esophagogastroduodenoscopy, colonoscopy, or retention enema [203].

The first known description of the use of feces as therapy was described by Ge Hong in fourth-century China for the treatment of a variety of conditions [204]. At that time, he orally administered human fecal suspension to treat patients who had food poisoning or severe diarrhea [205]. In 1958, Eiseman et al. described the use of fecal enemas as a treatment for pseudomembranous colitis, marking the introduction of FMT into mainstream medicine [206].

FMT is yet untested in child malnutrition; however, an ongoing pilot study of FMT in children aged 12 to 60 months with malnutrition with failure to respond nutritionally will provide critical data on the feasibility and safety of this treatment for SAM (<https://clinicaltrials.gov/ct2/show/NCT03087097>).

1.9.5.4 Probiotics, prebiotics, and synbiotics

The joint Food and Agriculture Organization (FAO) and WHO define probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [206]. Prebiotics have been consensually defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit [207], whereas synbiotics are a mixture of probiotics and prebiotics [208]. The major mechanisms of action of probiotics include enhancement of the epithelial barrier, increased adhesion to intestinal mucosa, and concomitant inhibition of pathogen adhesion, competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances and modulation of the immune system [209].

Probiotics, prebiotics, and synbiotics have gained significant attention following discovery of their potential ability to modulate the gut microbiota of malnourished

children [210, 211]. A systematic review to assess the effects of probiotics on child growth suggests that probiotics might have more significant effects improving child nutritional status and growth among malnourished children and those living in LMICs [212]. In well-nourished children with acute diarrhea, recent meta-analyses evidence suggests that probiotics are effective in reducing the duration of hospitalization [213]. Investigations of strain-specific effects have led to recommendations of 3 main strains: *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii*, and *Limosilactobacillus reuteri* DSM 17938 [214]. In children with malnutrition, a recent meta-analysis has shown that probiotics are effective in reducing the hospital stay duration by approximately 3 days [215].

Probiotics' effects have also been investigated in two large RCTs among children with SAM. In the ProbiSAM study, a randomized, double-blind, placebo-controlled trial in Uganda to assess the effect of probiotics on diarrhea during in- and outpatient treatment of children with SAM, Grenov et al. [216] found no difference between groups in the duration of diarrhea during hospitalization (primary outcome), but the intervention group had fewer days with diarrhea during outpatient treatment. Mortality after hospital discharge was similar in the intervention (3/147; 2%) and control groups (4/145; 3%).

In an earlier double-blind efficacy RCT in Malawi (PRONUT study) to assess the clinical and nutritional efficacy of a probiotic during in- and outpatient treatment of children with SAM, Kerac et al. [217] reported no difference between groups in nutritional cure, but there was a trend toward reduced mortality in the intervention group following discharge from the index hospitalization (14% compared with 19%; $p=0.05$). In a meta-analysis of these two trials, there was evidence that probiotics reduced mortality (RR: 0.72; 95% CI: 0.51, 1.00; $p=0.049$) [218].

Implementing and scaling up the use of probiotics in LMIC to address childhood malnutrition requires several logistical considerations: The need for vigilant safety monitoring, due to the effects of malnutrition on the child's immune system; strengthening local manufacturing and storage capacities to ensure product viability despite infrastructure, financial, and climatic challenges; using existing distribution networks to ensure accessibility to those most in need of the treatment; and to gain acceptability by cultures that might not be inclined to add live microbes to dietary and medical practices [219].

Furthermore, the use of probiotics as therapies for child malnutrition must carefully consider probiotic strain-specificity and the ability of strains to colonize the dysfunctional intestine of these children [219]. Twelve species, including 9 *Firmicutes*, 2 *Bacteroidetes*, and 1 *Actinobacteria* have been proposed as potential probiotics due to their representativeness of a healthy flora: *Alistipes indistinctus*,

Anaerostipes caccae, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacteroides salyersiae*, *Bifidobacterium adolescentis*, *Intestinimonas butyriciproducens*, *Lactobacillus perolens*, *Lactobacillus parabuchneri*, *Lactobacillus vaccinoferus*, *Terrisporobacter glycolicus*, and *Weissella confus* [174].

Chapter 2: AIMS AND HYPOTHESIS

As mentioned in the introduction, malnutrition is associated with a web of risk factors, with inadequate dietary intake and infectious diseases being the main factor in LMIC. Approximately 100,000 deaths due to malnutrition in children under 5 could be prevented each year if infant and young child feeding (IYCF) practices were adequate. In this context, assessing IYCF practices and its modifiable risk factors should be prioritized. However, there is a lack of data regarding IYCF practices in the Eastern part of the DRC. The first aim of this thesis was to assess infants' feeding practices, and factors associated with appropriate complementary feeding.

Infection diseases and malnutrition have always been intricately linked. In addition to an increased incidence of infectious disease, children with SAM are at significantly higher risk of more severe disease and suffer significantly more acute and long-term morbidity and mortality when infected. The second objective of this research was to identify the main infectious diseases experienced by children with SAM.

During the last decade, probiotics and prebiotics have been investigated for their potential to improve digestive functions and to mitigate the effects of infectious and inflammatory conditions. Unfortunately, these studies have been mainly conducted in high-income countries and in well-nourished children, so that in LMIC, data regarding the effects of probiotics in malnourished children are scarce. Therefore, the third objective of the thesis was:

- To assess, through a systematic review, the effects of probiotics on the main childhood killer infectious diseases (pneumonia and diarrhea), and on the nutritional recovery of malnourished children;
- To assess, through a randomized controlled trial, the effect of probiotics on the main childhood killer infectious diseases (pneumonia and diarrhea), and on nutritional recovery in children treated for uncomplicated SAM in the community.

We therefore formulated the following hypotheses:

1. Infants' feeding practices are suboptimal in South Kivu;
2. In children with SAM, the same childhood killer infectious diseases (pneumonia, diarrhea, septicemia and malaria) are also involved;
3. Probiotics are effective in reducing diarrhea, pneumonia and improving nutritional recovery in children with SAM.

Chapter 3: GENERAL METHODS

3.1 Study design and setting

3.1.1 Study design

The first paper of this thesis gives the finding of a community-based cross-sectional study conducted in August 2019. The second paper was written from a retrospective hospital-based cross-sectional study, performed on medical records of children with MAS followed as inpatients at Saint Joseph Nutritional Center from July 2017 to December 2018. The third paper is a systematic review with a meta-analysis, including 15 RCTs searched from 1990 to May 2020, and assessing the effects of probiotics and synbiotics on diarrhea, pneumonia, and nutritional recovery in malnourished children. The last paper is a RCT conducted from August 2021 to May 2022.

3.1.2 Settings

Except for the third paper, all studies were conducted in South Kivu province, located in Eastern DRC. South Kivu province shares borders with Rwanda, Burundi and Tanzania. The landscape includes mountains (eastern north part), the Central Basin (western part) and a vast plain at the southern east part. The South Kivu province covers an area of 69,130 km² with an estimated population of 8,187,055 inhabitants in 2021, representing a density of 118 inhabitants/km². It encompasses 8 rural territories and 4 towns, among which Bukavu the capital (transformation of the word 'bu'nkafu' meaning cow farm in Shi language). The city of Bukavu has a population of 1,054,206 inhabitants for 60 km², representing a density of 17,571 inhabitants/km². The population of South Kivu province is distributed across 34 Health Zones: 3 urban Health Zones in Bukavu (Bagira, Kadutu and Ibanda), and 31 rural Health zones. (Figure 14 and 15). The Health Zone is a geographical area contained within the limits of an administrative territory, comprising a population of at least 100,000 inhabitants.

These studies were conducted in three Health Zones: Ibanda (urban) and Kabare (rural) Health Zones for the first study, and Kadutu Health Zone (urban) for the second and the fourth study. Selection of these health zones was based on demographic, epidemiological, accessibility, and logistical criteria. Furthermore, in these sites there is availability of a strong network of community health workers and the existence of an experienced team having participated in previous nutritional surveys.

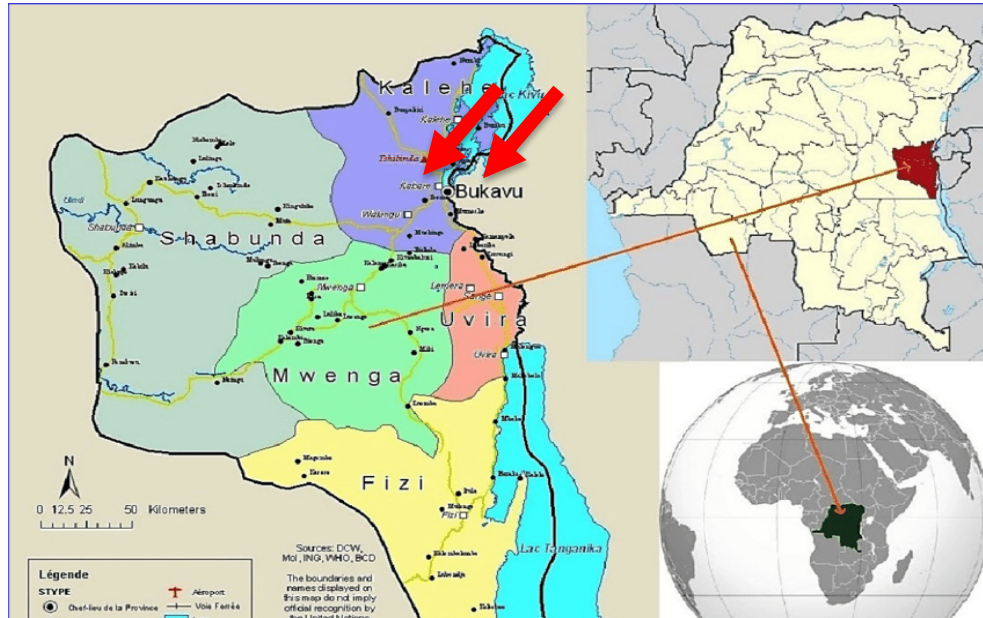


Figure 14: Administrative map of South Kivu (study sites are outlined by red arrows). Source: United Nations Office for the Coordination of Humanitarian Affairs, UNOCHA (ochadrc@un.org www.rdc-humanitaire.net)



Figure 15: Map of South Kivu with Ibanda and Kadutu (urban site), and Kabare (rural site), outlined by red arrows. Source: United Nations Office for the Coordination of Humanitarian Affairs, UNOCHA (ochadrc@un.org www.rdc-humanitaire.net)

The Ibanda Health Zone is located in the city of Bukavu, in the South Kivu province of DRC. Bounded on the north by Lake Kivu and on the east by Rwanda, Ibanda Health Zone is an area with mountainous terrain, clay soil, grassy vegetation and a tropical highland climate. At the last census in 2018, it had a recorded population of 452,608 with a population density of 32 per km². Infants < 24 months of age were estimated at 10,275. It is a cosmopolitan city, inhabited by various ethnic groups originating from both South Kivu and other DRC provinces.

Kabare Health Zone is in the Kabare administrative zone of South Kivu province in the DRC. It is situated 17 km from the city of Bukavu. It is an area with high plains and hills at elevations between 900 and 1900 m, with a tropical highland climate. At the last census in 2018, it had a recorded population of 213,882 with a very high population density of 856 per km². Infants under 24 months of age were estimated at 12,800.

Kadutu Health Zone is in the city of Bukavu. It has similar geographical, climatic, and demographic features to those of Ibanda Health Zone. It has an area of 15km² and a density of 30,000 inhabitants per km² for a total of 450,000 inhabitants. Children under-five are estimated at 85,000.

3.2 Study population and sampling procedure

In the 1st and 4th studies, the study population was all infants 6 to 24 months of age, whereas in the 2nd and 3rd studies, it was children 6 to 59 months of age.

For the first study assessing infants' feeding practices in urban and rural areas of South Kivu, the sample size was calculated using the 52% prevalence of minimum acceptable diet, estimated based on a study conducted in another setting [220]. Aiming at an absolute precision of 5% at the 95% confidence level, a design effect of 1.5, and allowing for 20% refusals and incomplete questionnaires, the required sample size was 750. Thus, we projected a sample of 375 in each area.

For the second study assessing the main infectious diseases experienced by children with SAM, all children admitted to the Nutritional Center during the study period were included.

For the 4th study assessing the effect of probiotics on diarrhea, a sample of 178 children in probiotics and placebo groups respectively was calculated to provide the study with 80% power at 5% significance level to detect a 1-day reduction in the number of days with diarrhea. Allowing for a 10% of attrition rate, 200 infants were recruited per arm. Assuming that the standard deviation of days with diarrhea in Outpatient Therapeutic Center was 3 days, it would be possible to detect a 1-day reduction in days with diarrhea.

3.3 Data sources and collection

A structured and pre-tested paper-based questionnaire was used at enrolment of participants. Research assistants, nurses practitioners, and nutritional nurses, assisted by community health workers, collected data during house-to-house visits (first study) or in Outpatient Therapeutic Centers (fourth study). For data quality control, the questionnaire was first developed in French and translated to local language (Swahili or Shi language) and then back-translated to French by an independent translator for consistency. Training was given to this research staff for 2 days.

According to the study, the following data were collected for each child:

- Sociodemographic: age, gender, sibling size, household size, marital status, mother's education level, household's socioeconomic level, number of living parents/cares, birth order, polygamy) (all studies);
- Past medical history: pregnancy and neonatal history (pregnancy term, route of delivery, birthweight, birth asphyxia), significant illnesses, contact with TB/measles, known/suspected HIV or TB, immunization (all studies);
- Dietary History: IYCF practices (duration of exclusive/supplementary breastfeeding, use of infant formula/non-human milk, age, and diet at weaning), current diet with number and types of meals) (first study);
- History of presenting complaint: appetite, recent weight change/swelling of feet, diarrhea (frequency, consistency, presence of blood), fever, cough (second and fourth study);
- Anthropometric data: weight, length/height, head circumference, MUAC, head circumference, nutritional indexes (WAZ, HAZ, WLZ), presence or absence of edema (second and fourth study)
- Biological data: blood count, blood glucose, hemoglobin (second and fourth study)
- Infectious disease data: malaria, HIV, septicemia, pneumonia, infectious diarrhea, urinary tract infection (second and fourth study)
- Study product compliance and adverse events (fourth study)

Household Food Insecurity Access Scale (HFIAS) was constructed according to the Food And Nutrition Technical Assistance (FANTA) indicator guide [221]. Education level was defined as unschooled, primary, secondary, and post-secondary (university). This variable was categorized into two classes: low (for mother's unschooled or primary school), good (for those having secondary school or university).

To assess infants' feeding practices, we used 4 indicators:

- Introduction of solid, semi-solid or soft foods: Children 6–7 months who received solid, semi-solid or soft foods at least once on the day preceding the survey date [222].
- Minimum meal frequency: Breastfed children 6–23 months who were fed a minimum recommended number of times in the previous day of the survey. Minimum is defined as 2 times for breastfed infants 6–8 months, 3 times for breastfed children 9–23 months, 4 times for non-breastfed children 6–23 months [222].
- Minimum dietary diversity: Children 6–23 months who received in the previous day of the survey four or more food groups out of seven food groups: (i) grains, roots, and tubers; (ii) legumes and nuts; (iii) dairy products (milk, yogurt, cheese); (iv) eggs; (v) flesh foods (meat, fish, poultry and liver/organ meats); (vi) vitamin-A-rich fruits and vegetables; and (vii) other fruits and vegetables [222].

WHO considers the cut-off of at least 4 of the above 7 food groups above because the consumption of foods from at least 4 food groups would mean that in most populations the child had a high likelihood of consuming at least one animal source food and at least one fruit or vegetable that day, in addition to a staple food (grain, root or tuber) [222].

- Minimum acceptable diet: Children 6–23 months old who met age-specific minimum recommended diet diversity and minimum recommended meal frequency and consumed a source of dairy (or were breastfed) on the previous day of the survey [222].

The minimum acceptable diet indicator was expressed as a dichotomous variable categorized as “adequate minimum acceptable diet” and “inadequate minimum acceptable diet”. A child who met both the minimum dietary diversity and the minimum meal frequency was categorized as “adequate minimum acceptable diet”, and a child who did not meet either or both the minimum dietary diversity and the minimum meal frequency as “inadequate minimum acceptable diet”.

Regarding anthropometry, the child's age was determined based on the date of birth (obtained either from birth certificate, child health record booklet or baptismal card) and the date of the survey. Weight was measured using an electronic baby scale

(SECA® type 435) with a precision of 10g. It was recorded twice and the mean value was used in the analyses. If the difference between the two measures exceeded 0.2 kg, the child was weighed again. The scale was checked for accuracy with standard weights after about every 200 measures. Recumbent length was measured to the nearest 0.1 cm with subjects in a lying position. The MUAC (assessed in children 6-59 months of age) was measured in centimeters, to the nearest 0.1cm, using standard MUAC measuring tape for children. The head circumference was measured as the maximum occipito-frontal circumference using a non-stretchable tape measure. The presence of edema was assessed by pressing firmly with the thumb for at least 2 seconds on each extremity (dorsum of the foot, behind the medial malleolus, lower calf above the medial malleolus). Three grades of edema severity were distinguished: edema + (mild edema), located to both feet/ankles; edema ++ (moderate edema), situated on feet and lower legs, hands, or lower arms; and edema +++ (severe edema), generalized to feet, legs, hands, arms, and face. All these parameters were measured according to the methods recommended by WHO 1995 [3].

The age, gender, presence or not of bilateral pitting edema, and infants' anthropometric parameters were imported into WHO Anthro plus software, which then calculated WAZ, LAZ and WLZ and identified outliers: < - 6 and > 5 for WAZ, < - 6 and > 6 for LAZ and < - 5 and > 5 for WLZ. These values were excluded and the infant measured again.

3.4 Brief overview of the statistical analysis

Analyses were performed using SPSS 25.0 (SPSS, Chicago, Illinois). WHO Anthro plus 1.0.4 (WHO, Geneva, Switzerland) was used to calculate anthropometric Z scores by applying 2007 WHO References. Normally distributed continuous variables were presented as means $\pm$ standard deviation, whereas non-normally distributed variables were presented as medians (interquartile range), qualitative variables as crude counts and percentages.

Globally, all comparisons between groups were performed using non-parametric tests: Chi-square test (with Cook's correction) for dichotomous variables, Mann-Whitney rank test for continuous variables, and Kolmogorov-Smirnov test for continuous and ordinal variables.

Other statistical methods have been used to meet specific objectives of this dissertation. They are detailed in the respective papers. A p value of < 0.05 was considered statistically significant.

3.5 Ethical considerations

The study was performed in accordance with the principles in the Declaration of Helsinki of 1975 as revised in 1983. Ethical approval was granted by the Université catholique de Bukavu Ethics Committee, n° UCB/CIES/NC/08/2018, for the first and the second study. For the fourth study, ethical approval was granted by the Université catholique de Bukavu Ethics Committee n° UCB/CIES/01/05/2020) and the Comité d’Ethique Hospitalo-Universitaire of the Université catholique de Louvain and Cliniques Universitaires Saint-Luc (Brussels/Belgium, 04-01-2021). Children were enrolled after verbal and written consent had been provided by their caregivers. Caregivers were made aware of their right to withdraw from the study at any time. Moreover, in the fourth study, an independent Data Safety Monitoring Board, including one statistician and one pediatrician, regularly monitored serious adverse events during the study.

Chapter 4: RESULTS

4.1 Minimum acceptable diet among children aged 6–23 months in South Kivu, Democratic Republic of Congo: a community-based cross-sectional study.

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RESEARCH ARTICLE

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Minimum acceptable diet among children aged 6–23 months in South Kivu, Democratic Republic of Congo: a community-based cross-sectional study



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Abstract

Background: Suboptimal child nutrition remains the main factor underlying child undernutrition in Democratic Republic of Congo (DRC). This study aimed to assess the prevalence of minimum acceptable diet and associated factors among children aged 6–23 months old.

Methods: Community-based cross-sectional study including 742 mothers with children aged 6–23 months old was conducted in 2 Health Zones of South Kivu, Eastern DRC. WHO indicators of Infant and Young Child Feeding (IYCF) regarding complementary feeding practices were used. Logistic regression analysis was used to quantify the association between sociodemographic indicators and adequate minimum acceptable diet for both univariate and multivariate analysis.

Results: Overall, 33% of infants had minimum acceptable diet. After controlling for a wide range of covariates, residence urban area (AOR 2.39; 95% CI 1.43, 3.85), attendance postnatal care (AOR 1.68; 95% CI 1.12, 2.97), education status of mother (AOR 1.83; 95% CI 1.20, 2.77) and household socioeconomic status (AOR 1.72; 95% CI 1.14, 2.59) were factors positively associated with minimum acceptable diet.

Conclusion: Actions targeting these factors are expected to improve infant feeding practices in South Kivu.

Keywords: Complementary feeding, Infants, Minimum acceptable diet, Nutrition, South Kivu

Introduction

Sustainable Development Goals (SDG) 2 and 3 call for an ending all forms of undernutrition, and all preventable deaths under 5 years of age by 2030 [1]. Worldwide, 5.4 million children under-five still die each year, 80% of them in sub-Saharan Africa and South Asia. Almost half of these deaths occur among undernourished children [2, 3].

Although these deaths are the result of a complex set of determinants, poor breastfeeding practices and inadequate complementary feeding play a major role [4]. Therefore, optimal infant and young child feeding (IYCF) practices rank among the most effective interventions to improve child health.

In 2002, the World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF) adopted the global strategy for IYCF, including: (i) early initiation of breastfeeding within 1 h of birth; (ii) exclusive breastfeeding for the first 6 months of life and (iii) introduction of

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nutritionally-adequate and safe complementary (solid) foods at 6 months together with continued breastfeeding up to 2 years of age or beyond [5].

The Democratic Republic of Congo (DRC) is still recovering from years of war and political upheaval and continues to face significant humanitarian challenges. Since 2016, the long-running crisis in Eastern regions has forced some 4.8 million internally displaced persons to flee from their villages and lose their agricultural livelihoods and jobs. The number of food-insecure people almost doubled from 7.7 million in 2017 to 13.1 million in 2018, making access to food a daily struggle for a significant part of the Congolese population [6]. The health and nutritional status of the children under 5 years of age have been impacted by these years of armed conflict and instability: approximately 42% are stunted, 6% are wasted, 23% are underweight, and 47% are anemic (< 11.0 g/dl); Among children 6–23 months, only 18 and 37% receive the WHO recommended minimum dietary diversity and meal frequency each day, respectively; Furthermore, the under-5 mortality rate is 70 per 1000 live births [7]. Thus, undernutrition and suboptimal IYCF practices remain serious public health concerns in DRC.

To address inadequate IYCF practices, the Ministry of Public Health has developed a program named “Infant and Young Child Feeding in Emergency”. This program aimed to provide concise, practical guidance on how to ensure appropriate IYCF in emergencies [8].

To date, there has been no published report on infant complementary feeding practices in South Kivu, a city that has the highest stunting rate among children under 5 years of age in DRC (48%) [7]. Yet growing evidence suggests that suboptimal IYCF practices may be a major contributor to the high prevalence of stunting in Eastern DRC [9–11]. Therefore, context-specific information on infant complementary feeding practices is needed as eating habits differ according to the tribes and customs in DRC. This study sought to assess the prevalence of minimum acceptable diet, as well as identify associated factors of adequate minimum acceptable diet, among children under 2 years of age in two health zones in South Kivu, Eastern DRC. Apart from a better understanding of the realities of infant complementary feeding practices, this study could generate evidence of a better practice of IYCF in an economic, political, social, and health context of precariousness, particularly to the context of the DRC.

Methods

Study design and setting

This community-based cross-sectional study was conducted in August 2019 in 2 Health Zones of South Kivu (Eastern DRC): Ibanda Health Zone, an urban area, and Kabare Health Zone, a rural one. The Health Zone is a

geographical area contained within the limits of an administrative territory, comprising a population of at least 100,000 inhabitants. A Health Area is a geographical area consisting of a group of villages (in rural areas) or streets (in urban areas) with a population size of 10,000 inhabitants [12]. Ibanda Health Zone was selected by simple random sampling from the 3 Health Zones of Bukavu urban city (Kadutu, Bagira, Ibanda); Kabare Health Zone was selected also by simple random sampling from 5 surrounding rural areas of Bukavu (Nyantende, Walungu, Kabare, Katana, Miti-Murhesa). Each of these 2 selected Health Zones encompasses 16 Health Areas.

The Ibanda Health Zone is located in the municipality of Ibanda, city of Bukavu, in the South Kivu province of DRC. Bounded on the north by Lake Kivu and on the east by Rwanda, Ibanda Health Zone is an area with mountainous terrain, clay soil, grassy vegetation and a tropical highland climate. At the last census in 2018, it had a recorded population of 452,608 with a population density of 32 per km². Infants < 24 months of age were estimated at 10,275. The main activities are small-scale trading and administration. Secondary activities are subsistence agriculture, small livestock farming and artisanal fishing. The staple food is cassava, cereals (maize, rice and sorghum), other tubers (taro, sweet and white potatoes) and bananas. These foods are generally served with vegetables, fish, beans and meat, which helps to balance the family dish. On average, two to three meals are consumed per day [13].

Kabare Health Zone is located in the Kabare administrative zone of South Kivu province in the DRC. It is situated 17 km from the city of Bukavu. It is an area with high plains and hills at elevations between 900 and 1900 m, with a tropical highland climate. At the last census in 2018, it had a recorded population of 213,882 with a very high population density of 856 per km². Infants under 24 months of age were estimated at 12,800. The population of this area is devoted to agriculture, livestock and small-scale trade. Bananas, cassava, taro, sweet and white potatoes, corn and sugar cane are the main crops grown there. However, the soil is not very fertile, and most of the population of the area leaves the fields to sell labor in the city of Bukavu [14].

Eligibility criteria

The study included mothers with children aged 6–23 months old with the following characteristics: (i) Infants aged between 6 and 23 months; (ii) residence in the study area; (iii) infants without chronic debilitating illnesses such as cerebral palsy, congenital heart disease, Down syndrome, cleft lip/palate; and (iv) parental consent.

Sample size

The sample size was calculated using Emergency Nutrition Assessment 2011 software, considering an absolute precision of 5% at the 95% confidence level, a design effect of 1.5 and a 52% prevalence of minimum acceptable diet, estimated based on a study conducted in Western Indonesia [15]. Allowing for 20% refusals and incomplete questionnaires, the required sample size was 750. Thus, we projected a sample of 375 in each Health Zone.

Sampling procedure of the study

In each Health Zone, a complete list of households with an infant between 6 and 24 months of age was obtained through a prior survey. This list served to identify and create sampling frame and apply the systematic random sampling to select the study samples, in order to assure the selection of representative sample from each health area.

Ten health extension workers and one public health professional were recruited as data collector and supervisor respectively. Data were collected in a separate room, in the absence of other relatives. We used face-to-face interview during house-to-house visit from mothers who had an infant between 6 and 24 months of age using structured questionnaire. The questionnaire contained the information on sociodemographic characteristics of participants and infant feeding practices as proposed by WHO. To determine household socioeconomic status, a household wealth score was computed as proposed by Bangirana et al. [16] based on the material possession of several items (electricity, radio, bike, motorbike, car) and on several characteristics of the house such as material used for the walls and for the roof. The wealth score of a household was obtained by summing points attributed to the above items and ranged from 1 to 11. We determined the child's age based on the date of birth (obtained either from birth certificate, child health record booklet or baptismal card) and the date of the survey. The mother's school level was subdivided into two classes: low (for mothers unschooled or primary school), and good (for those having secondary school or university). The household socioeconomic status was categorized into two classes: good (for households with medium or high socioeconomic level), and low.

For data quality control, the questionnaire was first developed in French and translated to local language (Swahili and Mashi language) and then back-translated to French by an independent translator for consistency. French version of the survey is provided in the [Supplementary Appendix](#). Training was given to health extension workers and supervisor for 2 days. The questionnaire was pre-tested in

19 (5%) mothers in Ibanda Health Zone and 19 (5%) mothers in Kabare Health Zone, which were not included in actual study, to assess the content and approach of the questionnaire. To assure the quality of the data, the supervisor and investigator closely reviewed the data collection technique on daily basis, reviewed the filled questionnaire for completeness and returned any incomplete questionnaire to the data collectors for correction. There was also debriefing every day.

Operational definitions

Introduction of solid, semi-solid or soft foods

Children 6–7 months who received solid, semi-solid or soft foods at least once on the day preceding the survey date [17].

Minimum meal frequency

Breastfed children 6–23 months who were fed a minimum recommended number of times in the previous day of the survey. Minimum is defined as 2 times for breastfed infants 6–8 months, 3 times for breastfed children 9–23 months, 4 times for non-breastfed children 6–23 months [17].

Minimum dietary diversity

Children 6–23 months who received in the previous day of the survey four or more food groups out of seven food groups: (i) grains, roots and tubers; (ii) legumes and nuts; (iii) dairy products (milk, yogurt, cheese); (iv) eggs; (v) flesh foods (meat, fish, poultry and liver/organ meats); (vi) vitamin-A-rich fruits and vegetables; and (vii) other fruits and vegetables [17]. WHO considers the cut-off of at least 4 of the above 7 food groups above because the consumption of foods from at least 4 food groups would mean that in most populations the child had a high likelihood of consuming at least one animal-source food and at least one fruit or vegetable that day, in addition to a staple food (grain, root or tuber) [17].

Minimum acceptable diet

Children 6–23 months old who met age-specific minimum recommended diet diversity and minimum recommended meal frequency and consumed a source of dairy (or were breastfed) in the previous day of the survey [17]. The minimum acceptable diet indicator was expressed as dichotomous variable categorized as “adequate minimum acceptable diet” and “inadequate minimum acceptable diet”. A child who met both the minimum dietary diversity and the minimum meal frequency was categorized as “adequate minimum acceptable diet”, and a child who did not meet either or both the minimum dietary diversity and the minimum meal frequency as “inadequate minimum acceptable diet”.

Statistical data analyses

Data were entered and analyzed using Statistical Package for the Social Sciences for Windows version 25 (SPSS Inc. Version 25.0, Chicago, Illinois). Characteristics of mothers and infants were summarized as mean and standard deviation (SD) for continuous variables with a normal distribution, or as median and range for continuous variables with a non-normal distribution, and as number or percentages for categorical variables. Normality of continuous variables was explored visually (Q-Q plots and histogram) and numerically (Shapiro-Wilk and Kolmogorov-Smirnov tests). Proportions were compared by using either the χ^2 or the Fisher exact test. The socio-demographic variables were selected on the basis of previous literature. They were imported into the multiple regression model on the basis of a value $p \leq 0.25$ and/or on the basis of biological plausibility. The unadjusted and adjusted odds ratios with their 95% confidence intervals were used to measure the association between the variables and minimum acceptable diet. The significance level was fixed at 0.05.

Ethical considerations

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Ethical Committee of the Université Catholique de Bukavu (UCB/CIES/NC/08/2018). Informed consent was obtained from all study participants as well as from infants' parents.

Results

The study included 742 mothers with children aged 6–23 months old (371 in Kabare Health Zone and 371 in Ibanda Health Zone), yielding a 98.9% response rate. Their sociodemographic characteristics are summarized in Table 1.

Eighty percent of households had a low socioeconomic level, and 55.8% of mothers had a low educational level. Low socio-economic and maternal educational levels were more prevalent in rural areas than in urban ones ($p < 0.05$). The postnatal care follow-up rate was 85.0%, higher in rural areas than in urban ones ($p < 0.05$).

Complementary feeding practices are summarized in Fig. 1 and Table 2. The majority, 727 (98%) and 631 (85%) infants received cereals-roots-tubers and legume-based foods respectively. The consumption of animal foods was low as only 31% consumed flesh foods and only 12% consumed eggs. Consumption of dairy products, eggs, fruits and vegetables was significantly lower for rural infants compared to urban ones ($p < 0.05$). About 64% of infants had minimum meal frequency, 34% had minimum dietary diversity, and 33% had

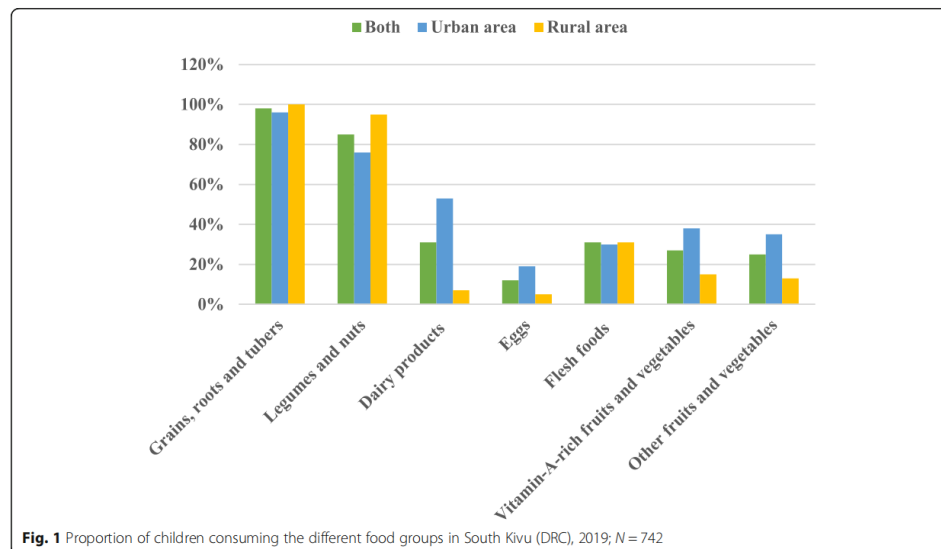
Table 1 Sociodemographic characteristics of the study participants

Characteristics	Total (N = 742)
Age (in months), mean (Standard Deviation)	16 (7.8)
6–12 months, n (%)	252 (34.0)
13–23 months, n (%)	490 (66.0)
Sex of the child	
Male, n (%)	381 (51.3)
Female, n (%)	361 (48.7)
The child lives with	
Both biological parents, n (%)	691 (93.1)
The mother only, n (%)	51 (6.9%)
Age of mothers age (in months), median (range)	26 (17–41)
Interval between last and before last births (months), mean (SD)	29 (17)
Place of birth	
Hospital, n (%)	224 (30.2)
Health Center, n (%)	467 (62.9)
Home delivery, n (%)	51 (6.9)
Attendance at postnatal visits	
Yes, n (%)	631 (85.0)
No, n (%)	111 (15.0)
Household socioeconomic status	
High, n (%)	15 (2.0)
Medium, n (%)	133 (18.0)
Low, n (%)	594 (80.0)
Mother educational status	
Maternal education more than secondary, n (%)	450 (60.6)
Maternal education less than secondary, n (%)	292 (39.4)
Polygamy	
No, n (%)	466 (62.8)
Yes, n (%)	276 (37.2)
Number of household members, median (range)	6 (4–14)
Sibling size, mean (Standard Deviation)	5 (3)

minimum acceptable diet, with rates higher in urban area than in rural one for all these indicators ($p < 0.05$).

The multivariate analysis showed that residence area, attendance at postnatal care, household socioeconomic status, and maternal education status were statistically associated with minimum acceptable diet.

Mothers living in urban area were 2.36 times [Adjusted Odds Ratio (AOR) 2.39; 95% CI 1.43, 3.85] more likely to provide minimum acceptable diet to their child when compared to their counterparts. Similarly, higher odds of receiving minimum acceptable diet was observed among mothers who had postnatal care visits (AOR 1.68; 95% CI 1.12, 2.97). Likewise, mothers with



secondary and post-secondary education were 1.83 times (AOR 1.83; 95% CI 1.20, 2.77) more likely to give recommended minimum acceptable diet compared to their counterparts. Finally, higher odds of receiving minimum acceptable diet was observed among mothers with good household socioeconomic status (AOR 1.72; 95% CI 1.14, 2.59) (Table 3).

Discussion

This study sought to assess the prevalence and associated factors of adequate minimum acceptable diet among children 6–23 months. Findings revealed that the prevalence of minimum acceptable diet was 33%. Residence in urban area, good maternal education status, good household socioeconomic status, and attendance to postnatal consultations were factors that may increase the minimum acceptable diet practices.

The prevalence of minimum acceptable diet found in this study is close to that reported in Nepal (29%) [18]

and China (27%) [19]. Minimum acceptable diet prevalence ranging from 8 to 18% have been observed in India, Bhutan, Ethiopia and Malawi [20–23], while in Tanzania [24], Bangladesh [25], and Indonesia [15] they were 38, 42, and 48% respectively. The differences in health services, including health education and advice on breastfeeding and complementary feeding during pre-natal and postnatal care, and region-dependent feeding cultural practices could be the factors explaining these differences.

The acute nature of emergency situations such as those observed in Eastern DRC challenges optimal feeding practices. They may increase the likelihood of not breastfeeding, as well as the risks of artificial feeding and inappropriate complementary feeding, with potentially devastating short- and long-term implications on malnutrition, illness and mortality [26].

In our report, urban area appeared better in several aspects of complementary feeding practices than rural

Table 2 Proportions of children meeting the WHO complementary feeding indicators in South Kivu (DRC), 2019, N = 742

Variable	Total n (%)	Urban n (%)	Rural n (%)
Introduced solid, semi-solid or soft foods (children 6–7 months)	76/100 (76.0)	41/53 (77.3)	35/47 (74.5)
Took minimum meal frequency (children 6–23 months) **	474/742 (63.9)	294/397 (74.0)	180/345 (52.2)
Took minimum dietary diversity (children 6–23 months) **	250/742 (33.7)	179/397 (45.0)	71/345 (20.6)
Took minimum acceptable diet (children 6–23 months) **	245/742 (33.0)	155/397 (39.0)	90/345 (26.0)
Consumed iron-rich foods (children 6–23 months) **	372/742 (50.1)	227/397 (57.2)	145/345 (42.0)

*: $p < 0.05$; **: $p < 0.01$

Table 3 Factors associated with no minimum acceptable diet in South Kivu (DRC), 2019, *N* = 742

Variables and categories	Minimum acceptable diet		COR (95%CI)	AOR (95%CI)
	Inadequate, n (%)	Adequate, n (%)		
Residence Health Zone				
Rural	289 (83.5)	56 (16.5)	1	1
Urban	186 (53.5)	185 (46.5)	5.13 (3.18–6.29) *	2.39 (1.43–3.85) *
Place of delivery				
Home	29 (58.8)	21 (41.2)	1	1
Health facility	472 (62.2)	220 (31.8)	0.64 (0.21–1.34)	
Attended PNC				
No	92 (82.9)	19 (17.1)	1	1
Yes	409 (64.9)	222 (35.1)	2.63 (1.25–4.89) *	1.68 (1.12–2.97) *
Socioeconomic status				
Low	434 (72.9)	160 (27.1)	1	1
Good	64 (44.8)	79 (55.2)	3.35 (2.28–4.88) *	1.83 (1.20–2.77) *
Maternal education				
Less than secondary	242 (82.9)	50 (17.1)	1	1
Secondary and above	258 (57.5)	192 (42.5)	3.60 (2.50–5.49) *	1.72 (1.14–2.59) *
Polygamous family				
Yes	226 (81.9)	50 (18.1)	1	1
No	275 (59.0)	191 (41.0)	3.14 (2.24–4.67) *	
Parents live with children				
No	33 (67.3)	16 (32.7)	1	1
Yes	468 (67.6)	225 (32.4)	0.99 (0.81–1.27)	
Household size				
> 10	77 (72.0)	30 (28.0)	1	1
5–10	238 (66.2)	122 (33.8)	1.24 (0.76–2.03)	
< 5	186 (67.4)	89 (32.6)	0.96 (0.69–1.34)	

AOR Adjusted Odds Ratio, CI Confidence interval, COR Crude Odds Ratio, PNC Postnatal care

**p* < 0.05

area. This might be the result of cumulative effect of a series of more favorable conditions, including better socioeconomic and educational conditions, in turn leading to better caring practices for children and their mothers. A study analyzing Demographic and Health Surveys (DHS) from 36 developing countries revealed that for the same reasons, the prevalence of malnutrition was higher in rural than in urban areas [27].

The association between attendance at postnatal care and adequate minimum acceptable diet is consistent with study findings in East African region and India [28–32]. Mothers' education on complementary feeding practices during these visits is the factor underlying this association. In DRC's health system, children's growth monitoring is conducted in every health facility according to the national nutrition program. In the outreach clinics, health workers monitor the weight of children using growth charts, and provide nutrition education to

mothers or caregivers of children. These activities also provide an opportunity to discourage harmful traditional beliefs that might inhibit child feeding practices, and to early recognition of signs of undernutrition, any illness and manage them accordingly [33].

Our findings show that more than 85% of the mothers attended at postnatal consultations. Surprisingly, the prevalence of minimum acceptable diet remained low. The low household socioeconomic status could be the main explanatory factor. Indeed, our study showed that about 80% of households had a low socio-economic status. This finding emphasizes the role of socio-economic status on feeding practices. Households with high socioeconomic status are more likely to be food secure, thus they can afford to provide the minimum acceptable diet to their children [34]. Several studies have also shown that improved household wealth has a significant effect on adequate complementary feeding practices [31, 35–38].

Consistent with studies conducted in China [19] and Ethiopia [39], the current study also found that mothers with secondary and post-secondary education had higher odds of adequate minimum acceptable diet. The possible reason might be that education is directly linked to women's autonomy, changes in traditional beliefs and women's control over household resources [40]. Hence educated mothers are more likely to understand information provided through health and nutritional programmes regarding complementary feeding practices [41].

Three systematic reviews [42–44] examined the effectiveness of infant's complementary feeding interventions. The socioeconomic status of caregivers, maternal education level, and postnatal care have been identified as main factors that may influence infant's complementary feeding practices. Nevertheless, the results of the 3 systematic reviews demonstrated that interventions that provide education to mothers will significantly improve complementary feeding knowledge and practices. When population is food secure, educational strategies alone can improve feeding practices. The case is different in the context of food insecurity or political trouble.

Study limitations

Our study has several limitations. Firstly, the recall bias may be possible and affect the validity of the results. However, this bias was minimized since most data related to infant's complementary feeding practices were based on a 24-h recall method. Another limitation is the social desirability bias. Finally, the cross-sectional design does not allow for causal inference.

Conclusion

In the context of South Kivu (DRC), our findings reveal a low prevalence of adequate minimum acceptable diet among infants between 6 and 23 months of age. Overall, the residence area, mother's educational status, household wealth, and postnatal care attendance were factors positively associated with adequate minimum acceptable diet. These findings suggest policy-makers to focus on improving and making effective behavior change, strengthening education of mothers, as well as improving complementary feeding practices for children through food security, health care utilization, and improved household socioeconomic status.

Abbreviations

AOR: Adjusted odds ratio; CI: Confidence interval; DRC: Democratic Republic of Congo; IYCF: Infant and young child feeding; MICS: Multiple indicator cluster survey; OR: Odds ratio; PNC: Postnatal care; SPSS: Statistical package for the social sciences; WHO: World Health Organization; UNICEF: United Nations Children's Fund

Supplementary Information

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Additional file 1.

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Authors' contributions

RMK and DVDL designed the study and reviewed the manuscript for important intellectual content. SN, BRC, AB and KCM were responsible for collecting the data. GAN supervised the team of surveyors. GAN and JBK analyzed the data. RMK wrote the manuscript. RMK and DVDL critically revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by the Ethical Committee of the Université Catholique de Bukavu (UCB/CIES/NC/08/2018). Informed consent was obtained from all study participants as well as from infants' parents.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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4.2 Severe acute malnutrition in children admitted in an Intensive Therapeutic and Feeding Centre of South Kivu, Eastern Democratic Republic of Congo: Why do our patients die?

PLOS ONE

RESEARCH ARTICLE

Severe acute malnutrition in children admitted in an Intensive Therapeutic and Feeding Centre of South Kivu, Eastern Democratic Republic of Congo: Why do our patients die?

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Abstract

Background

Severe acute malnutrition (SAM) remains a serious public health concern in low- and middle-income countries. Little is known about treatment outcomes of child inpatients in Intensive Therapeutic and Feeding Units. This study aimed to assess treatment outcomes of SAM and identify factors associated with mortality among children treated at Saint Joseph Nutritional Center, South Kivu, Eastern Democratic Republic of Congo.

Methods

A retrospective hospital-based cross-sectional study was conducted on medical records of 633 severely malnourished children followed as inpatients at Saint Joseph Nutritional Center from July 2017 to December 2018. Data were entered, thoroughly cleaned and analyzed in SPSS version 25. Univariable and multivariable logistic regression model were fitted to identify factors associated with mortality.

Results

Among 633 patients admitted with SAM, 13.1% were lost to follow-up and 9.2% died while in hospital. Children with late referral to the health facility (> 14 days) after the onset of main external malnutrition signs had 2.03 times higher odds of death than those referred less

than 14 days [AOR = 2.03 at 95%CI (1.12, 3.68)]. The odds of death was 1.91 times higher for children with MUAC < 115 mm than for those with MUAC $\geq$ 115 mm [AOR = 1.91 at 95% CI (1.05, 3.50)]. Children infected with HIV were 3.90 times more likely to die compared to their counterparts [AOR = 3.90 at 95% CI (2.80, 9.41)].

Conclusion

Particular emphasis should be placed on partnering with communities to improve information on malnutrition signs and on critical importance of early referral to the health system. While HIV incidence in DRC is still low (0.21%), its impact on mortality among severely malnourished children is increased due to the limited access to HIV testing and antiretroviral therapy.

Introduction

Tremendous progress has been made worldwide in child survival in recent decades. Among children under-5, nine percent of children died in 1990 compared to four percent in 2017. The under-five mortality rate fell from 93 deaths per 1,000 live births in 1990 to 39 in 2017 [1].

Despite all this progress, 5.4 million children under-5 still die each year, 80% of them in sub-Saharan Africa and South Asia. Almost half of these deaths occur among malnourished children [2–4]. Malnutrition is a serious public health concern in low- and middle-income countries, and is the focus of the first World Health Organization (WHO) Millennium Development Goal. In its acute form, it affects about 50 million children under-5 worldwide, including 48 million in Africa and Asia [4].

Acute malnutrition is subdivided into 2 categories: (i) Moderate Acute Malnutrition (MAM), defined as mid-upper arm circumference (MUAC) between 115 and 125 mm in children aged 6–59 months; or a weight-for-height/length z-score between -2 and -3 according to the 2006 WHO growth standards; with absence of edema; (ii) Severe Acute Malnutrition (SAM), defined by two distinct clinical entities: (a) non-edematous malnutrition (marasmus), defined as MUAC < 115 mm in children aged 6–59 months; or a weight-for-height/length z-score < -3 according to the 2006 WHO growth standards; (b) edematous malnutrition (kwashiorkor), defined by bilateral pitting edema [5].

SAM is further classified according to the presence or absence of medical complications. Uncomplicated SAM is defined as SAM with no apparent signs of infection nor other signs requiring hospitalization, and with adequate appetite. Complicated SAM is defined as SAM with clinical signs of infection, metabolic disorders, severe edema, hypothermia, vomiting, severe dehydration, profound anemia or lack of appetite [5].

SAM impairs life, cognitive and neurodevelopmental prognosis of children, especially in low- and middle-income countries. The shorter-term prognosis of SAM depends on the type of malnutrition. In uncomplicated SAM, the mortality rate is < 5% while it varies from 10 to 40% in complicated SAM [6]. Further, previous reports have shown high post-discharge mortality following in-hospital management of diarrhea-SAM, HIV infection-SAM and pneumonia-SAM comorbidities respectively [7–9]. The FuSAM (Follow Up of Severely Acute Malnourished Children) study reported a mortality rate of 25% over a year after the SAM treatment among children followed in inpatient-based SAM treatment centre in Blantyre (Malawi). The risk of mortality after hospital discharge was fourfold higher for HIV-SAM

compared with HIV-negative children with SAM [10]. In addition, ChroSAM (Chronic disease Outcomes after Severe Acute Malnutrition) study, which followed Malawian children with SAM, seven years post-discharge in the same centre, reported that children had poorer growth, body composition and physical function compared with siblings and community controls. These parameters are all risk factors of future cardiovascular and metabolic disease [11].

According to World Food Program (WFP), more than 6 million people are in a situation of acute food insecurity and livelihood crisis in the Democratic Republic of Congo (DRC) [12]. The combination of persistent violent armed conflicts, massive populations displacements, poor or inexistent infrastructures and widespread deterioration of productive assets have significantly affected food security in the DRC, and particularly in Eastern provinces, over the past two decades [12]. The impact of all these on children under-5 in the South Kivu province are alarming: the stunted rate of 48%, the wasting rate of 3% and the under-5 mortality rate of 4% [13].

Since 2000, joint efforts of the WHO and the United Nations Children's Fund (UNICEF) allowed the standardization of the management of acute malnutrition which improved outcomes and prevented complications [14, 15]. Following these recommendations, the Ministry of Public Health of DRC has implemented the Integrated Management of Acute Malnutrition programme (Prise en Charge Intégrée de la Malnutrition Aigüe, PCIMA) [16]. PCIMA approach encompasses three components: (i) community outreach and mobilization; (ii) outpatient management of SAM without medical complications; and (iii) inpatient management of SAM with medical complications. Children with complicated SAM are managed in Intensive Therapeutic and Feeding Unit, which is one of the units of the pediatric department.

This study aimed to describe the treatment outcomes and to identify risk factors associated with mortality for a cohort of children admitted with SAM in a Nutritional Center located in the South Kivu province of DRC.

Methods

Study setting

This study was performed at Saint Joseph Nutritional Centre (SJNC), which is an Intensive Therapeutic and Feeding Unit included in the department of Pediatrics, of the provincial reference hospital (Hôpital Provincial Général de Référence de Bukavu, HPGRB). HPGRB hospital is the main healthcare facility in Bukavu, a city of more than 700,000 inhabitants and is the teaching hospital of the Université Catholique de Bukavu (U.C.B.).

The SJNC is specialized in the management of children severely acutely malnourished referred mainly from rural and urban settings surrounding Bukavu. It includes an intensive care unit (5 beds), a transition unit (15 beds), a nutritional rehabilitation unit (35 beds) and an isolation unit (5 beds). Children are admitted to the intensive care unit or transition unit according to the severity of medical complications, or to the nutritional rehabilitation unit for cases with mostly nutritional problems. Children with contagious diseases are admitted to the isolation unit. The staff is constituted by 3 medical doctors, including a pediatrician, 7 nurses and 1 nutritionist. The Centre admits an average of 50 to 60 cases of malnutrition monthly. The average length of stay is approximately 3 weeks.

The SJNC is normally intended to manage complicated SAM. Sometimes, MAM and uncomplicated SAM cases are referred to the SJNC for nutritional management because the health centers intended to manage those cases are confronted with stockouts in therapeutic supplies. In addition, in SJNC, children admitted for complicated SAM are often accompanied by other siblings, themselves with MAM or uncomplicated SAM. Thus, in the SJNC, the majority of cases are complicated SAM and some cases of uncomplicated SAM and MAM.

Within SJNC, therapeutic consumables are provided by the National Nutrition Programme, supported by UNICEF. However, the implementation of WHO recommendations is hampered by many frequent stockouts due to the weakness of the national distribution system. Locally, International Committee of the Red Cross and WFP provide therapeutic milk formula (F-75 milk and F-100 milk) and Ready-to-Use Therapeutic Foods (RUTF), but these interventions remain insufficient to cover the nutritional consumables needs. During all the study period, SJNC was without the standard supplies. During these long periods of shortage, health caregivers use whole milk / maize-sorghum-soya (MASOSO)-vegetal oil-sugar porridge preparations in SAM management. This preparation is constituted of whole milk (in initial stabilization phase), and MASOSO (60g), vegetable oil which is derived from palm oil (10g) and sugar (10g) (in nutritional rehabilitation phase). Eighty grams of MASOSO vegetable oil-sugar flour mixture provides 357.3 Kcal. Vitamin and mineral supplements are also given when whole milk is used during this stockouts period.

Study design, period and population

We conducted a retrospective hospital-based cross-sectional study on the medical records of malnourished children admitted to SJNC from July 2017 to December 2018. During this period, 834 children from 1 month to 18 years of age were admitted to the Centre. Among these, 201 (24%) were excluded from this analysis because they presented a moderate form of malnutrition (MAM). In total, we included 633 severely malnourished children, among which 612 were classified as complicated and 21 uncomplicated.

Inclusion and exclusion criteria and clinical management

The study included medical records of SAM children between 1 month and 18 years of age. SAM in infants under 6 months of age was defined as weight-for-length z-score < -3 , or the presence of bilateral pitting edema. In children between 6–59 months of age, it was defined as weight-for-height/length z-score < -3 , or MUAC < 115 mm, or presence of bilateral edema. In children between 5–18 years of age, it was defined as weight-for-height $< 70\%$ of the median (National Center for Health Statistics reference) or presence of bilateral edema. Children under one month of age and those with other causes of edema, such as renal or cardiac conditions were excluded.

At SJNC, children are treated according to WHO therapeutic guidelines for complicated SAM [15] adapted by PCIMA 2016 [16]. Standard inpatient management of SAM involves two phases: (i) initial stabilization when life-threatening complications are treated; (ii) nutritional rehabilitation when catch-up growth occurs. Dietary treatment includes 8 daily meals of F-75 milk for stabilization in intensive care or transition unit, followed by F-100 milk or RUTF (plumpy nut) for nutritional rehabilitation in cases exiting intensive care prior to discharge. These formulations are specifically conceived for the dietary treatment of severe malnourished patients and contain 75 Kcal/100 ml, 100 Kcal/100 ml and 500 Kcal/92g, respectively for F-75 milk, F-100 milk and plumpy nut sachet. Amoxicillin, 50–100mg/kg in two doses per day per os is given systematically, or parenteral ceftriaxone 100mg/kg once daily in cases of suspected severe or complicated infectious syndrome. Treatment is modified based on indications such as non-improvement of clinical condition and/or results of bacterial culture and antibiotic sensitivity testing.

Children are discharged from the Intensive Therapeutic and Feeding Unit if they fulfilled the following criteria: adequate appetite and consumption of RUTF; rising weight curve; no edema; no signs of infection; not under active treatment with injectable antibiotics; mother able to attend regular weekly outpatient sessions at Outpatient Feeding and Therapeutic Unit.

Data collection and variables definition

The data were collected using a survey form. The dependent variable was the treatment outcome, encompassing the recovery rate, loss to follow-up rate, average daily weight gain, median length of stay and mortality rate. These were compared to Sphere Standards: mortality rate > 75%, default rate < 15%, average daily weight gain > 10%, length of stay between 14–42 days, and lost to follow-up rate < 10%. The independent variables were socio-demographic and variables related with clinical condition at admission and follow-up.

Fever, hypothermia, tachypnea, bradypnea, tachycardia, bradycardia, hyperleukocytosis, severe anemia and hypoglycemia were defined in reference to pediatric standards [17, 18]. Diarrhea was defined as at least three watery stools in less than 24 hours, with or without blood [19]. Severe dehydration was considered as the presence of 2 or more of the following: lethargy, sunken eyes, drinks poorly or not able to drink and skin pinch goes back very slowly [20]. Malaria was defined as a thick blood film positive for malaria parasites, any density. Bacteremia was defined as a laboratory-confirmed bloodstream infection with a pathogen cultured from one or more blood cultures [21]. Culturing more than 10^5 colonies/mL of a single organism in the urine culture (with clean catch midstream urine sample) was considered as Urinary Tract Infection (UTI) [22]. Infectious diarrhea was diarrhea due to an infectious etiology. Due to the unavailability of early virologic diagnostic tests, HIV infection was defined either on the basis of positive HIV serology after 18 months of age or on the basis of clinical signs suggestive of HIV infection before 18 months, according to the WHO classification in children born to HIV-positive mothers [23]. Blood count, thick blood film for malaria, urinalysis and/or urine culture, ova and parasite examination and/or stool culture were often performed at admission.

Three grades of edema severity were distinguished: edema + (mild edema), located to both feet/ankles; edema ++ (moderate edema), situated on feet and lower legs, hands or lower arms; and edema +++ (severe edema), generalized to feet, legs, hands, arms and face [15].

Nutritional recovery was considered when severely acute malnourished children achieved the target weight $\geq 85\%$ of weight-for-height/length z-score at discharge. Additionally, edema for consecutive 10 days should not be observed.

The average daily weight gain and the median length of stay were calculated only for cured discharged children. The average daily weight gain (in g/kg/day) was calculated differently according to whether it was marasmus or kwashiorkor. In children with marasmus, it was calculated using the formula:

$$\frac{\text{Weight (in grams) at discharge} - \text{Weight (in grams) at the beginning of the feeding process}}{\text{Weight (in kg) at the beginning of feeding process} \times \text{length of stay (in days)}}$$

[24–26]

In children with kwashiorkor, it was calculated by the formula:

$$\frac{\text{Weight (in grams) at discharge} - \text{Weight (in grams) at the edema resolution}}{\text{Weight (in kg) at the edema resolution} \times \text{length of stay (in days)}}$$

[24–26]

The threshold used was the one proposed by WHO: poor (average daily weight gain < 5g/kg/d), moderate (average daily weight gain between 5–10g/kg/d) and good (average daily weight gain > 10g/kg/d) [24–26].

The threshold used to assess the mortality rate was the one proposed by WHO: unacceptable (mortality rate > 20%), poor (mortality rate between 11–20%), moderate (mortality rate between 5–10%) and acceptable (mortality rate < 5%) [24–26].

Loss to follow-up was defined by child absence for more than 3 days while in treatment [24–26].

Statistical analysis

The data were checked for completeness and inconsistencies, entered, coded, cleaned and analyzed using SPSS for Windows Version 25 (SPSS Inc. Version 25.0, Chicago, Illinois). Severely malnourished descriptive characteristics were summarized as mean and standard deviation for normal continuous variables, or as median and range for non-normal continuous variables, and as number or percentages for categorical variables. For categorical variables, we compared proportions using the chi-square or Fischer exact test; for continuous variables, medians were compared using the Wilcoxon rank-sum test.

To study the factors associated with mortality, we conducted univariable and multiple logistic regression analyses. The variables were imported into the multiple regression model on the basis of a value $p \leq 0.25$ and/or on the basis of biological plausibility. The unadjusted and adjusted odds ratios with their 95% confidence intervals were used to measure the association between the variables and mortality.

Ethical considerations

Ethical approval was granted by the Ethics Committee of the Université Catholique de Bukavu, DRC. The Ethical Committee waived the requirement for informed consent. All data were fully anonymized before we accessed them.

Results

We analyzed medical records of 633 severely malnourished children admitted to SJNC during study period. Their characteristics are summarized in Table 1. SAM was more prevalent among children aged 6 to 59 months (512/625; 81.9%) compared to those combined under 6 months and over 59 months ($p < 0.05$). Three hundred and thirty-two (51.2%) children were male and the sex-ratio (M/F) was 1.1. More than half of the severely malnourished children (358/618; 58.0%) came from urban areas. Overall, edematous malnutrition was the most prevalent form of SAM, found in 408 (65.3%) children.

Four hundred (63.2%) children were brought to health facility for consultation more than 2 weeks after the onset of main external malnutrition signs (thinning, edema or yellow-coloured hair). The median time between the onset of main external malnutrition signs and the referral to health facilities was 36 days (range: 7–85). It was more important for children coming from rural areas (42 days) than those coming from urban ones (22 days) ($p < 0.05$).

The anthropometric parameters of children late referred to the health facility (more than one month) were severely deteriorated (MUAC < 110 mm for non-edematous malnutrition and edema +++ for edematous malnutrition), compared to those of children early referred to the health facility ($p < 0.05$).

Four hundred and twenty (74.0%) children had received antimicrobials before admission to SJNC, including 175 (30.9%) at home. As shown in Fig 1, ampicillin and gentamycin were the most commonly administered antimicrobials at health centre (26%), while amoxicillin was the most commonly administered antimicrobial at home (14%). No child with uncomplicated SAM received antimicrobials before admission to SJNC.

Fig 2 shows the distribution of main infectious diagnoses of severely malnourished children at SJNC. Overall, respiratory tract infections (16%), bacteremia (6%), infectious diarrhea (5%) and malaria (5%) accounted for 32% of confirmed diagnosis.

Table 1. Baseline characteristics of the study population.

Characteristics	n (%)	
Age (months), median (range) (n = 625)		23 (1–192)
< 6	28 (4.5)	
6–24	335 (53.6)	
25–59	177 (28.3)	
> 59	85 (13.6)	
Gender (n = 627)		
Male	332 (52.9)	
Female	295 (47.1)	
Health area of provenance (n = 618)		
Rural	260 (42.0)	
Urban	358 (58.0)	
History of malnutrition (n = 604)		
No	403 (66.7)	
Yes	201 (33.3)	
Time between the onset of main external malnutrition signs and first visit to health facilities (days), median (range) (n = 594)		36 (7–85)
Child's immunization status (n = 550)		
Child fully immunized	355 (64.5)	
Child not fully immunized	195 (35.5)	
Type of SAM (n = 625)		
Non-edematous malnutrition	217 (34.7)	
Edematous malnutrition	408 (65.3)	
Edema +++	73 (11.7)	
Edema ++	255 (40.8)	
Edema +	80 (47.5)	
Antimicrobial before admission to SJNC/HPGRB (n = 568)		
None	148 (26.0)	
At home	175 (30.9)	
At the health centre	245 (43.1)	
Clinical and biological signs at admission (n = 544)		
Fever	64 (11.7)	
Hypothermia	18 (3.3)	
Tachypnea	222 (40.8)	
Bradypnea	8 (1.5)	

(Continued)

Table 1. (Continued)

Characteristics	n (%)
Tachycardia	203 (37.3)
Bradycardia	31 (5.7)
Hyperleukocytosis/neutrophilia	41 (7.5)
Severe anemia	43 (7.9)
Hypoglycemia	72 (13.2)

HPGRB: Hôpital Provincial Général de Référence de Bukavu; MUAC: mid-upper arm circumference measure; SAM: severe acute malnutrition; SD: standard deviation; SJNC: Saint Joseph Nutritional Center.

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The average daily weight gain was 9.2 g/kg/day, higher in children with marasmus (10.3g/kg/day) than in children with kwashiorkor (8.2g/kg/day) ($p < 0.05$). The median length of stay was 17 days (range: 8–28), longer in children with kwashiorkor (20 days) than in those with marasmus (11 days) ($p < 0.05$). Fifty-eight (9.2%) children died. As shown in Table 2, SJNC outcomes variables were comparable with that of international sphere standard ($p > 0.05$).

Variables associated with mortality at multivariable logistic regression included time between the onset of main external malnutrition signs and first consultation, MUAC and HIV infection. Severely malnourished children with delayed presentation to the health facilities (> 14 days) after the onset of main external malnutrition signs were 2.03 times higher odds of

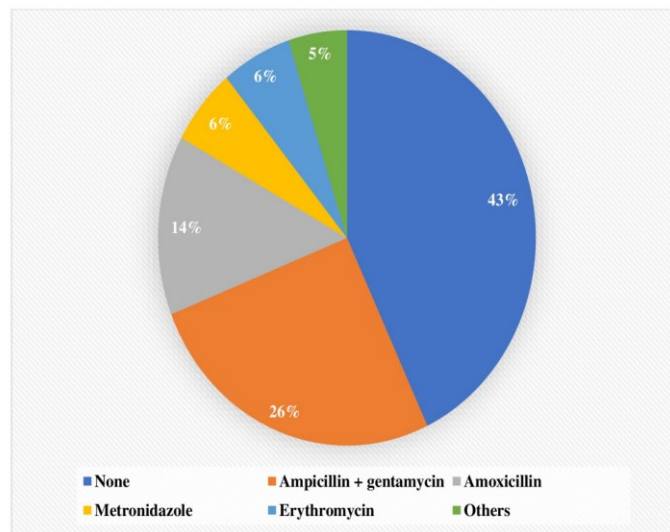


Fig 1. Antimicrobials used in severely malnourished children before their admission to the SJNC/HPGRB hospital (n = 568).

<https://doi.org/10.1371/journal.pone.0236022.g001>

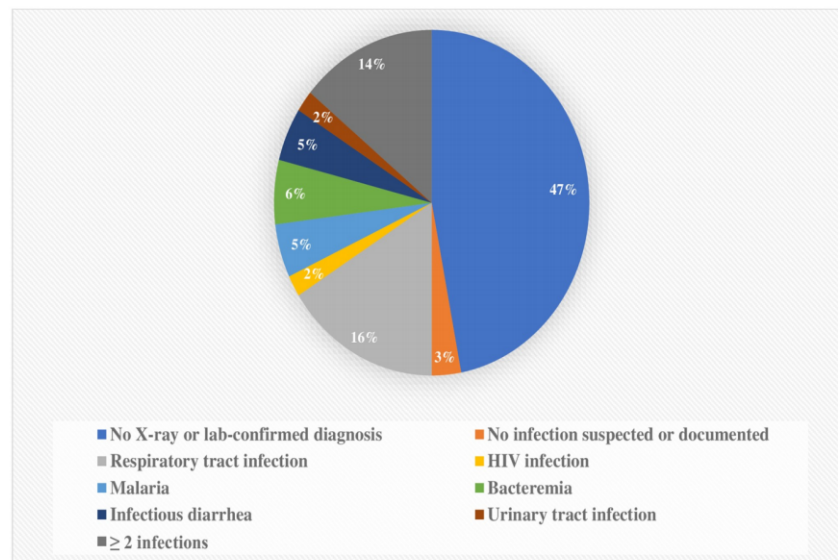


Fig 2. Main infectious diagnoses of severely malnourished children at SJNC/HPGRB hospital, June 2017–December 2018 (N = 633).

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Table 2. Performance indicators of SJNC/HPGRB and sphere standard references.

Performance indicator	Indicator of SJNC/HPGRB hospital	International sphere standards reference	
		Acceptable	Alarming
Recovery rate, n (%)	492 (77.7) *	> 75%	< 50%
Mortality rate, n (%)	58 (9.2)	< 10%	> 20%
Default rate, n (%)	83 (13.1) *	< 15%	> 25%
Average daily weight gain (SD) (g/kg/d)	9.2 (2.1) *	> 10%	< 5%
Length of stay (days), median (range)	17 (8–28) *	14–42	> 42
Lost to follow-up rate, n (%)	58 (9.2) *	< 10%	> 20%

*p > 0.05;

HPGRB: Hôpital Provincial Général de Référence de Bukavu (The Provincial Reference Hospital); SD: Standard Deviation; SJNC: Saint Joseph Nutritional Centre.

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death than those referred less than 14 days [AOR = 2.03 at 95%CI (1.12, 3.68)]. The odds of death was 1.91 times higher for children with MUAC < 115 mm than for those with MUAC ≥ 115 mm [AOR = 1.91 at 95% CI (1.05, 3.50)]. Severely malnourished children infected with HIV were 3.90 times more likely to die compared to their counterparts [AOR = 3.90 at 95% CI (2.80, 9.41)] (Table 3).

Discussion

The findings of this study provide preliminary data on factors associated with the mortality of severely malnourished children admitted as inpatients in the context of a food security crisis.

Table 3. Bivariate and multivariable analysis of factors associated with mortality of severely malnourished children at SINC/HPGRB hospital, July 2017–December 2018.

Variables and categories	Treatment outcome		OR (95%CI)	AOR (%CI)
	Dead n (%)	Cured n (%)		
Health area of provenance				
Rural	19 (11.2)	150 (88.8)	1.19 (0.66–1.88)	
Urban	38 (10.1)	340 (89.9)	1	
Time between the onset of main external malnutrition signs and first visit to health facility (days)				
< 7	7 (7.9)	82 (92.1)	0.36 (0.14, 0.93) **	
7–14	8 (21.6)	29 (78.4)	1	
> 14	25 (6.3)	375 (93.7)	3.46 (1.68, 7.12) ***	2.03 (1.12, 3.68) *
Temperature				
Hypothermia	6 (30.0)	14 (70.0)	4.5 (2.19, 9.56) ***	
Fever	4 (6.5)	58 (93.5)	0.97 (0.35, 2.65)	
Normal	30 (6.7)	420 (93.3)	1	
Breathing rate				
Bradypnea	4 (50.0)	4 (50.0)	6.5 (2.93, 14.4) ***	
Tachypnea	13 (6.1)	201 (93.9)	0.79 (0.41, 1.52)	
Normal	23 (7.7)	276 (92.3)	1	
Type of SAM				
Edematous malnutrition	7 (2.2)	308 (97.8)	0.14 (0.06, 0.31) ***	
Non-edematous malnutrition	34 (15.7)	183 (84.3)	1	
MUAC				
< 115 mm	27 (9.7)	250 (90.3)	2.21 (1.09, 4.47) **	1.91 (1.05, 3.50) *
≥ 115 mm	10 (4.4)	217 (95.6)	1	1
Severe dehydration				
Yes	14 (10.9)	115 (89.1)	1.58 (0.85–2.92) *	
No	27 (6.9)	367 (93.1)	1	
Hyperleukocytosis/Neutrophilia				
Yes	6 (14.6)	35 (85.4)	4.76 (1.03, 22.45) **	
No	2 (3.1)	63 (96.9)	1	
HIV infection				
Yes	5 (35.7)	9 (64.3)	5.28 (2.44, 11.46) **	3.9 (2.80, 9.41) *
No	34 (6.8)	464 (93.2)	1	1
Bacteremia				
Yes	5 (13.2)	33 (86.8)	1.81 (0.75–4.34) *	
No	36 (7.3)	459 (92.7)	1	
Respiratory tract infections				
Yes	8 (7.8)	94 (92.2)	1.02 (0.49–2.15)	
No	33 (7.7)	398 (92.3)	1	
Diarrhea with dehydration				
Yes	23 (9.9)	209 (90.1)	1.63 (0.89–2.94) *	
No	18 (6.1)	227 (93.9)	1	
Malaria				
Yes	30 (90.9)	3 (9.1)	0.98 (0.88–1.09)	

(Continued)

Table 3. (Continued)

Variables and categories	Treatment outcome		OR (95%CI)	AOR (%CI)
	Dead n (%)	Cured n (%)		
No	447 (92.5)	36 (7.5)	1	

*p < 0.25;

**p < 0.05;

***p < 0.01;

AOR: adjusted odd-ratio; HIV: human immunodeficiency virus; MUAC: middle upper arm circumference; OR: odd-ratio; SAM: Severe acute malnutrition; SJNC: Saint Joseph Nutritional Centre

<https://doi.org/10.1371/journal.pone.0236022.t003>

Despite the unavailability of therapeutic supplies (RUTF, F-75 and F-100) at SJNC and their substitution by whole milk / MASOSO-vegetal oil-sugar preparations, the average daily weight gain of our study population was comparable with that of international sphere standard.

Several recent studies showed the non-inferiority of cereal-based formulas (maize, sorghum, soya) mixed with vegetable oil and sugar, in comparison to WHO conventional preparation, in the diet of severely malnourished children. A study conducted in North Kivu (Eastern DRC) comparing the efficacy and safety of two recipes (cow milk with porridge made of maize, soybean, vegetal oil and sugar versus WHO conventional preparation) among two groups of severely malnourished children, reported no significant difference between the two groups in terms of weight changes, edema resolution, gastrointestinal tolerability and clinical outcome over 21 days ($p > 0.05$) [27].

A non-inferiority, randomized controlled clinical trial was conducted in a rural area of South Kivu (Eastern DRC) to compare the efficacy of soya-maize-sorghum RUTF with that of standard peanut paste-based RUTF for treating SAM. Intention-to-treat and per-protocol analyses showed non-inferiority of soya-maize-sorghum RUTF compared with peanut paste-based RUTF for the recovery rate, weight gain and length of stay in children < 24 months of age [28].

Another non-inferiority individually randomized controlled efficacy clinical trial was conducted in Malawi to compare the efficacy of milk-free soya, maize, and sorghum enriched with crystalline amino acids to the standard peanut and milk RUTF. Intention-to-treat and per-protocol analyses showed non-inferiority for recovery rates and length of stay in children aged 24–59 months and 6–23 months between these two RUTFs [29].

The nutrient density of peanut and milk RUTF equivalent to WHO F-100 milk [14] ensures high rates of recovery and weight gain and low fatality rates [30, 31]. Nevertheless, peanut and milk RUTF high milk content (25%) makes it very expensive for sustainable use in low-and middle-income countries. About 50% of peanut and milk RUTF is still produced in high-income countries and imported into low-and middle-income countries where it is needed. The high cost of current standard RUTF (47\$ USD for each child treated) remains, therefore, the main reason it is unavailable in resource-poor settings [32, 33]. The recipes based on soya, maize, and sorghum, vegetable oil and sugar has shown great promise and has the potential to provide a substantially lower-cost alternative, with adequate nutritional recovery.

The death rate found in our study was close to what is observed in similar settings. It remains, however, high compared to the PCIMA standards. Indeed, in non-emergency situations, PCIMA defines a mortality rate < 5% as an acceptable threshold [16]. This high death rate could be due to several barriers, often described in rural areas of low-and middle-income countries, including delayed referral to health facilities, mothers' lack of awareness of the signs of malnutrition, self-medication, or difficulties in accessing the health centre due to lack of

transport. WHO often attributes infant or maternal mortality in low-and middle-income countries to the phenomenon known as "three delays": (i) delay in deciding to seek care, (ii) delay in identifying and reaching health facility, and (iii) delay in receiving adequate and appropriate treatment [34].

As in this study, MUAC as predictor of mortality in children with SAM has been described elsewhere [35–39]. Several studies [40–44] showed that MUAC may be used in preference over weight-for-height z-score for predicting the risk of death in under-5 children. In addition, these studies documented that use of MUAC < 115 mm alone may be better than weight-for-height z-score < -3 in identifying children at high risk of death. Recent evidence based on meta-analysis highlights that children with a weight-for-height z-score < -3 have about the same or higher risk of death as children with a MUAC < 115 mm. They both are at substantial risk of death, and neither should be omitted from protocols aimed at diagnosis and treatment of all SAM cases [45].

Our data showed a negative influence of HIV-malnutrition comorbidity on mortality. Delayed diagnosis and antiretroviral treatment (ART) initiation are factors that worsen these children's life-threatening prognosis. Indeed, for most mothers and children admitted to SJNC, HIV status is often unknown before admission. Secondly, almost all health facilities in Eastern DRC do not yet have early virologic diagnostic tests for HIV infection. HIV infection diagnosis is established either on the basis of positive HIV serology after 18 months of age or on the basis of clinical signs suggesting HIV infection before 18 months of age, according to WHO classification for children born to HIV-positive mothers. Finally, when HIV infection is diagnosed, ART is started only after these children have been stabilized nutritionally, according to the guidelines of the national AIDS care programme in DRC.

Recent WHO guidelines recommend early ART for all children, regardless of their clinical and immune status [46]. The rationale behind early ART for all children is that (i) early ART decreases significantly the mortality rate and improves better growth response than deferred ART (waiting for the CD4 count falls below 15%) [47]; (ii) early ART mitigates the negative effects of HIV infection on growth and pubertal and nervous system development [48–54] and (iii) it promotes immune recovery [55].

About 50% of admission diagnoses were not confirmed by radiography or laboratory tests. This is a major handicap in the framework of epidemiological surveillance. Where available, laboratory confirmation of clinical diagnoses is important to improve the accuracy of surveillance systems. In addition, although tuberculosis (TB) is common in immunodeficiency situations, including malnutrition, no children had a confirmed diagnosis of TB. In SJNC, treatment for TB is guided by a presumptive diagnosis based on the Keith-Edward score [56]. The factors contributing to uncertainties concerning incidence of tuberculosis in severely malnourished young children is related to the challenge in confirming a diagnosis: (i) difficulties in obtaining high quality specimens; (ii) children commonly have a poor bacillary count and many are negative on culture; (iii) the lack of mycobacterial culture facilities in settings where tuberculosis and malnutrition are endemic [57]. The Xpert MTB/RIF assay, a recent, rapid diagnostic test for the detection of *M. tuberculosis* and rifampicin resistance, has demonstrated encouraging results in the diagnosis of pulmonary tuberculosis in children [58]. Unfortunately, it is the appanage of only a few countries in low-income countries.

Study limitations

The main limitation of study is its retrospective nature. Some medical records had incomplete data, increasing potential for selection bias.

Conclusion

Our study reflects the current nutritional situation of the South-Kivu province in DRC, with a high burden of children affected by malnutrition. Particular emphasis should be placed on partnering with communities to improve recognition of the signs of malnutrition and on the critical importance of early entry into the health system. While HIV incidence in DRC is still low (0.21%) [59], its impact on mortality among severely malnourished children is increased due to the limited access to HIV testing and antiretroviral therapy.

Supporting information

S1 Data.

(XLSX)

S2 Data.

(DOCX)

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4.3 Effects of probiotics and synbiotics on diarrhea in undernourished children: Systematic review with meta-analysis

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Meta-analyses

Effects of probiotics and synbiotics on diarrhea in undernourished children: Systematic review with meta-analysis



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SUMMARY

Background: Undernutrition predisposes children to a greater incidence and duration of diarrhea. No review and meta-analysis have yet been conducted to assess effectiveness of probiotics and synbiotics in undernourished children.

Aims: To assess the effectiveness of probiotics and synbiotics on diarrhea in undernourished children.

Methods: Randomized, double-blind, placebo-controlled trials evaluating the effects of probiotics and synbiotics on diarrhea in undernourished children were searched from 1990 to May 2020. Recommendations of the Cochrane Handbook and the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement were followed.

Results: The systematic review identified 15 trials with 6986 patients. The meta-analysis revealed that treatment with probiotic or synbiotic reduced significantly both the duration of diarrhea [Weighted mean difference (WMD) = −1.05 day, 95% CI (−1.98, −0.11)] and the hospital stay duration [Standard mean difference (SMD) = −2.87 days, 95% CI (−5.33, −0.42)], especially in specific patient subsets. In both groups, similar rates of vomiting and nutritional recovery were observed. No probiotics or synbiotics-related adverse effects were reported. Subgroup analyses showed that probiotic and synbiotic treatment were more effective in reducing risk of diarrhea in outpatients [Risk ratio (RR) = 0.86, 95%CI (0.75–0.98)].

Conclusion: This meta-analysis supports the potential beneficial roles of probiotics and synbiotics on diarrhea in undernourished children.

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1. Introduction

Globally, an estimated 200 million children younger than 5 years of age suffer from undernutrition, contributing to 45% of all child deaths every year, and 11% of the total global disability-adjusted-life-years worldwide [1,2]. Undernutrition leads to long term consequences in impairing the cognitive and physical development of children, and the economic productivity of individuals and societies [3].

Diarrhea continues to be a leading cause of death in children younger than 5 years of age in low- and middle-income countries, and a leading cause of morbidity worldwide. It leads to a high rate of hospitalization, and is associated with an economic burden inflicted by parents' absence from work [4]. Furthermore, diarrhea has long been linked to a reciprocal cycle of malnutrition and infection among vulnerable children in low- and middle-income countries [5]. This highlights the importance of developing safe, efficient and multifactorial intervention strategies to address malnutrition and enteric infections as major public health challenges [6].

The relationship between gut microbiota, diarrhea and undernutrition has attracted attention in recent years. Undernutrition predisposes children to a greater incidence and duration of diarrhea, and it can be triggered or worsened by significant

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diarrhea [7]. Diarrhea leads to reduced absorption of carbohydrates, protein, potassium, zinc and other nutrients, further contributing to undernutrition [8]. Breastfeeding, food and water, sanitation and hygiene are major protective factors against undernutrition and diarrhea [9]. Early depletion in gut *Bifidobacterium longum*, and later, the absence of the healthy mature anaerobic gut microbiota leads to deficient energy harvest, vitamin biosynthesis and immune protection, and is associated with diarrhea, malabsorption and systemic invasion by microbial pathogens. These two events represent the main steps in gut microbiota alteration, and are associated with undernutrition [9].

Germ-free animal studies have provided evidence of the mechanisms by which gut immaturity contributes to undernutrition through interaction with the diet. In a study involving 377 Malawian twin pairs in the first three years of life, Smith et al. [10] characterized the gut microbiome of twin pairs discordant for kwashiorkor during and after treatment with ready-to-use therapeutic food. A model of gnotobiotic mice was used to test the effectiveness of the same nutritional intervention, following transplantation of frozen fecal communities from three twin pairs, with one twin from each pair suffering from kwashiorkor, into germ-free mice. The animals were then fed the low-caloric density and nutrient-deficient diet similar to that of Malawian children. After 3 weeks, their diet was switched to the high energy, micronutrient rich ready-to-use therapeutic food for 2 weeks and then switched back to the initial Malawian diet. A significant difference in weight change between mice transplanted with healthy and kwashiorkor fecal communities was observed. Furthermore, a more dramatic change in body weight when switching between diets in kwashiorkor mice than in the healthy control mice was reported. This study suggested clinically important differences in the gut microbiota of children who develop kwashiorkor versus their healthy twin, which may determine the response to dietary change. Additionally, the study indicated that gnotobiotic mice, transplanted with the fecal communities of different groups, might be a valuable model in devising individualized dietary interventions.

Recently, Gehrig et al. [11] have integrated preclinical gnotobiotic animal models with human studies to understand the contributions of perturbed gut microbiota development to childhood undernutrition and to identify new microbiota-directed therapeutic approaches. They identified a set of proteins that distinguish the plasma proteomes of healthy children from those with severe acute malnutrition. This finding allowed them to develop microbiota-directed complementary foods that shifted the plasma proteome of children with moderate acute malnutrition toward that of healthy individuals, including proteins involved in linear growth, bone development, neurodevelopment, and immune function.

The past decade has seen a new era in medical science with increased use of probiotics and synbiotics. Probiotics are “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” [12]. Prebiotics have been consensually defined as a substrate that is selectively utilized by host microorganisms conferring a health benefit [13], whereas synbiotics are a mixture of probiotics and prebiotics [14]. Probiotic microorganisms act via a variety of means, including modulation of immune function, production of organic acids and antimicrobial compounds, interaction with resident microbiota, interfacing with the host, improving gut barrier integrity and enzyme formation. Prebiotics effects include defense against pathogens, immune modulation, mineral absorption, bowel function, metabolic effects and satiety [15].

In well-nourished children, meta-analyses have shown that probiotics and synbiotics may reduce the risk of diarrhea, as well as the duration of diarrhea and hospitalization [16–18]. Furthermore, other studies have reported that probiotics and synbiotics may also

enhance child growth by preventing infections and micronutrient deficiencies, as they have been shown to improve the absorption of certain nutrients (calcium, zinc and vitamin B12) [19,20] and reduce the risk of anemia [21].

Although the last few years have seen data emerge from many probiotics and synbiotics human studies, a search of the Cochrane Library, PubMed, and Scopus did not reveal any review and meta-analysis in undernourished population. This review aims to assess the effectiveness of probiotics and synbiotics (any specified strain or dose), compared with placebo, in the management of diarrhea in undernourished children.

2. Materials and methods

This systematic review was conducted according to the Cochrane Handbook for systematic reviews of intervention guidelines, the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement, and the Cochrane statistical methods guidelines [22,23]. It did not involve any animal, cell or human experimental research.

2.1. Search strategy and selection criteria

The Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library), MEDLINE, and EMBASE databases were searched, with no language restriction nor geographical limits. The search strategy was conducted with Medical Subject Headings (MeSH) and search text word terms outlined in Table 1. Furthermore, reference lists of all included studies and review articles identified by the search were also checked to identify other relevant studies.

2.2. Eligibility and exclusion criteria

Only randomized controlled trials (RCTs) were eligible for inclusion. Participants were undernourished children. Childhood undernutrition encompassed various clinical forms, including stunting (low height-for-age < −2 Standard deviation (SD)), wasting (low weight-for-age < −2 SD) and underweight (low weight-for-height < −2 SD). Patients in the experimental groups received probiotics or synbiotics at any type, dose and duration. Subjects in the control group received placebo or no additional intervention. The primary outcome measures were the incidence of diarrhea; the duration of diarrhea, the duration of hospitalization; and the number of episodes of diarrhea. The secondary outcome measures were the duration of vomiting; the adverse effects of probiotics/synbiotics; and the nutritional recovery.

Exclusion criteria were: (i) studies in well-nourished children; (ii) studies no related to study outcomes; (iii) studies related to neonates or adult persons; (v) studies in animals; and (vi) no RCTs.

Table 1
Search terms used in search strategy.

- 1) Malnutrition OR malnourished OR undernutrition OR underweight
- 2) Severe acute malnutrition OR kwashiorkor OR marasmus OR stunting OR wasting
- 3) Protein-energy malnutrition OR child nutrition disorders OR infant nutrition disorders
- 4) OR 1-3
- 5) Randomized controlled trial OR randomized OR randomly OR trial
- 6) Controlled clinical trial OR clinical trial as topic OR placebo
- 7) OR 5-6
- 8) Probiotics OR probiotics and prebiotics OR Synbiotics OR fermented milk products
- 9) 4 AND 7 AND 8
- 10) Limit 9 to human

2.3. Selection of studies and data extraction

Two review authors (RMK and FIN) independently screened all studies by title and abstract. Studies fulfilling the inclusion criteria have been selected. Disagreements were resolved by discussion between the 2 authors. Two investigators (GAN and JBK) independently extracted the data from each included study. The following data were extracted: author, year of publication, country, age group, patient source, type of undernutrition, sample size, experimental and control treatments, dose, and duration of intervention. Data regarding main outcome indicators included the following: incidence of diarrhea, duration of diarrhea, duration of hospitalization, and number of episodes of diarrhea per child year. Secondary outcome data included duration of vomiting, adverse effects of probiotics and synbiotics, and nutritional recovery. If the study data were unclear, we contacted the trial authors to obtain clarification.

2.4. Risk of bias assessment

Risk of bias was assessed independently by 2 authors (GAN and JBK), and disagreements were resolved by discussion between the 2 authors, and, when necessary, by consulting a third review author (RMK). The Revised Cochrane risk-of-bias tool for randomized trials (RoB2) was used. The risk of bias parameters included the type of randomization method (selection bias), allocation concealment (selection bias), blinding of participants and personnel

(performance bias), blinding of outcome assessment (detection bias) and incomplete outcome data (attrition bias). Each trial was graded as 'yes', 'probably yes', 'no', 'probably no', or 'no information' with respect to the abovementioned aspects, representing a high risk of bias, a low risk of bias, or some concerns bias, respectively [24].

2.5. Rating the quality of evidence

The GRADE (Grading of Recommendations, Assessment Development and Evaluations) approach was used to assess the quality of the supporting evidence for main outcomes. GRADE pro software (<https://gdt.grade.pro.org>) [25] was used to import the data from Review Manager 5.3 (RevMan 5.3) to create "summary of findings" tables. These tables provided outcome-specific information concerning the overall quality of evidence derived from the included studies, the magnitude of the effects of the interventions examined and the sum of the available data on the outcomes that were considered. The quality of the evidence was categorized as high, moderate, low or very low based on consideration of the risk of bias, the directness of evidence, consistency and precision of the estimates.

2.6. Statistical analysis

Statistical analyses were performed using RevMan 5.3 software. Regardless of heterogeneity between the pooled studies, we

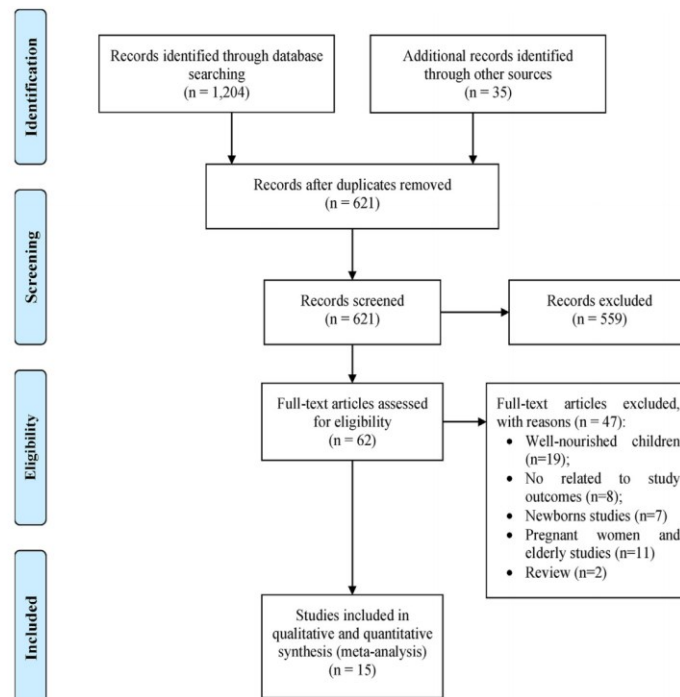


Fig. 1. PRISMA flow diagram for selection of studies.

Table 2
Characteristics of the included studies

Study	Country	Age range (month)	Patient source	Type of undernutrition	n (exp/control)	Intervention	Control	Dosage (CFU/day)	Duration
Aflatoonian et al., 2020 [36]	Iran	24–59	Outpatients	Underweight	69 (37/32)	Symbiotic Probiotics: <i>Lactobacillus acidophilus</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium infantis</i> , <i>Bifidobacterium breve</i> , and <i>Streptococcus thermophilus</i> Prebiotic: Fructo-oligosaccharide <i>Lactobacillus rhamnosus</i> GG	Placebo sachet daily which similar taste, smell, and appearance with symbiotic sachet	1.0×10^9	30 days
Kara et al., 2019 [30]	Turkey	6–59	Outpatients	Underweight, Stunting and wasting	100 (50/50)	<i>Bifidobacterium animalis</i> subsp <i>lactis</i> and <i>Lactobacillus rhamnosus</i> <i>Bifidobacterium animalis</i> subsp <i>lactis</i> and <i>Lactobacillus rhamnosus</i> <i>Lactobacillus casei</i>	No treatment	1.0×10^9	3 months
Grenov et al., 2017a [29]	Uganda	6–59	Inpatients	Wasting	400 (200/200)	<i>Bifidobacterium animalis</i> subsp <i>lactis</i> and <i>Lactobacillus rhamnosus</i>	One sachet daily containing 1g of maltodextrin without probiotic	1.0×10^{10}	8–12 weeks
Grenov et al., 2017b [29]	Uganda	6–59	Outpatients	Wasting	327 (164/163)	<i>Bifidobacterium animalis</i> subsp <i>lactis</i> and <i>Lactobacillus rhamnosus</i>	One sachet daily containing 1g of maltodextrin without probiotic	1.0×10^{10}	8–12 weeks
Sur et al., 2011 [31]	India	12–59	Outpatients	Underweight	3585 (1802/1783)	<i>Lactobacillus casei</i>	The same nutrient drink daily without probiotic	6.5×10^9	12 weeks
Sawazal et al., 2010 [37]	India	12–36	Outpatients	Wasting ad stunting	422 (205/217)	Symbiotic Probiotic: <i>Bifidobacterium lactis</i> HN019 Prebiotic: Oligosaccharide <i>Lactobacillus rhamnosus</i> GG	The same milk without Symbiotic	1.9×10^7	1 year
Basu et al., 2009a [28]	India	Unclear	Inpatients	Underweight	371 (186/185)	<i>Lactobacillus rhamnosus</i> GG	ORS without probiotics	10^{12} CFU	Minimum period of 7 days
Basu et al., 2009b [28]	India	Unclear	Inpatients	Underweight	373 (188/185)	<i>Lactobacillus rhamnosus</i> GG	ORS without probiotics	10^{10} CFU	Minimum period of 7 days
Kerac et al., 2009 [38]	Malawi	5–168	Inpatients and outpatients	Wasting	795 (399/396)	Symbiotic Probiotics: <i>Pediococcus pentosaceus</i> , <i>Leuconostoc mesenteroides</i> , <i>Lactobacillus paracasei</i> ssp <i>paracasei</i> and <i>Lactobacillus plantarum</i> Prebiotic: oat bran, inulin, pectin, and resistant starch <i>Lactobacillus rhamnosus</i> GG	RUTF without Symbiotic	1×10^{11}	10 weeks
Basu et al., 2007 [32]	India	>24	Inpatients	Underweight	235 (117/118)	<i>Lactobacillus rhamnosus</i> GG	Readymade disposable poly packs of ORS without <i>Lactobacillus rhamnosus</i> GG	6×10^7	Minimum 7 days or till diarrhea has stopped
Agarwal 2002a [27]	India	6–59	Inpatients and outpatients	Underweight	102 (52/50)	<i>Lactococcus lactis</i> , <i>Lactococcus lactis cremoris</i> , and <i>Leuconostoc mesenteroides cremoris</i>	Ultra-heat-treated yoghurt preparation	1×10^8	3 months
Agarwal 2002b [27]	India	6–59	Inpatients and outpatients	Underweight	98 (48/50)	<i>Lactobacillus casei</i> , <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>	Ultra-heat-treated yoghurt preparation	1×10^8	3 months
Saran 2002 [33]	India	24–59	Outpatients	Stunting	100 (50/50)	<i>Lactobacillus acidophilus</i>	an isocaloric supplement (two biscuits each)	1×10^8	6 months
Oberhelman et al., 1999 [34]	Peru	6–24	Outpatients	Underweight	204 (99/105)	<i>Lactobacillus rhamnosus</i> GG	Identical-appearing placebo capsule administered in identical fashion.	3.7×10^{10}	15 months
Raza et al., 1995 [35]	Pakistan	1–24	Inpatients	Underweight	40 (21/19)	<i>Lactobacillus rhamnosus</i> GG	Microcrystalline cellulose with identical appearance mixed in 10 ml of ORS twice a day	1×10^{10}	2 days

CFU: Colony-forming units; exp: exposed; ORS: Oral Rehydration Salts; RUTF: ready-to-use therapeutic food.

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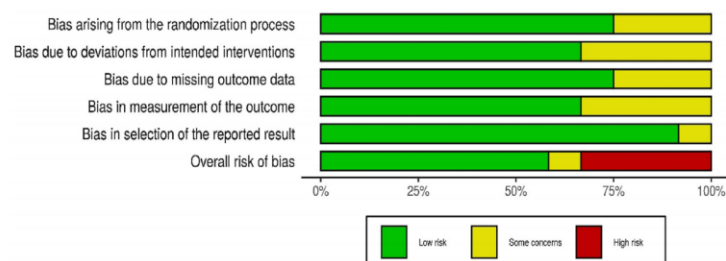


Fig. 2. Bias risk assessment of the RCTs on the effects of probiotics and synbiotics in undernourished children with diarrhea.

used a random-effects model to synthesize all data. We sought missing data from the trial authors. Outcome measures were analyzed on an Intention-to-treat population. Continuous outcome variables were assessed with Mean Differences (MDs) and 95% confidence intervals (CIs), and dichotomous outcomes were evaluated with aggregated risk ratios (RRs) and 95% CIs. We calculated the rate ratio of episode rates (episodes per child year) of diarrhea between two groups and the standard error of rate ratio according to the Cochrane Handbook for Systematic Reviews of Interventions [23]. We used the generic inverse variance data to pool these studies.

Statistical heterogeneity was evaluated with the χ^2 test with significance being set at P value < 0.1 . The degree of heterogeneity among the studies was measured by the I^2 statistic. An I^2 statistic $< 40\%$ was considered to be a low level of heterogeneity, 41% – 60% a moderate level, 61% – 90% a substantial heterogeneity, and $> 90\%$ a considerable heterogeneity [26]. We did not assess the presence of publication bias in this review, due that funnel plots are difficult to detect with small numbers of studies (i.e. less than 10) in systematic reviews.

Subgroup analyses were conducted to evaluate the influence of patient source, type of undernutrition, combination of probiotics, dose, and duration of intervention on the main overall outcome indicators. We did not perform a sensitivity analysis since only a few studies were included in each subgroup.

3. Results

3.1. Study selection

A total of 1239 studies were included after an initial search, of which 1181 studies did not fulfil the inclusion criteria as determined by screening the titles and abstracts. Sixty-two potential trials were selected for full-text assessment, and 47 studies were excluded. A total of 15 RCTs were included in the meta-analysis. The detailed selection process is presented in Fig. 1.

3.2. Characteristics of the included studies

A total of 6986 participants (3618 in the experimental group and 3368 in the control group) in 15 RCTs were identified in the literature. Two experimental arms in the study of Agarwal et al. [27] were listed separately to exhibit different strains of probiotics, which were marked as Agarwal 2002a and Agarwal 2002b. Similarly, two experimental arms in the study of Basu et al. [28] were listed separately to exhibit different doses of probiotics, which were marked as Basu 2009a and Basu 2009b. In the study of Grenov et al.

[29], two experimental arms were also listed separately to exhibit different management and follow-up, which were marked as Grenov 2017a and Grenov 2017b. Eleven trials were conducted in Asia, three in Africa and one in South America. Patients were followed up as outpatients (7 RCTs), inpatients (5 RCTs) and both (3 RCTs). Twelve studies [27–35] presented the results of probiotic interventions, whereas synbiotic interventions were reported in 3 trials [36–38]. Six trials used a mixture of probiotics including 2 to 7 strains [27,29,36,38], while 8 studies used a single probiotic strain [28,30–35,37]. *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus*, *Pediococcus* and *Leuconostoc* were the 6 genera of probiotics used in the trials. *Lactobacillus* was the most commonly genus of probiotics used (13 RCTs), including the *Lactobacillus rhamnosus* GG strain in 9 RCTs. The daily dose of probiotics in these trials ranged from 1.9×10^7 to 1×10^{12} colony-forming units (CFU). Other detailed characteristics of the included RCTs are presented in Table 2.

3.3. Risk of bias assessment

Most trials were at risk of bias for at least one of the domains (see Fig. 2). Only 72% of trials adequately generated their randomization sequence; Fifty-six percent adequately concealed allocation, and 61% blinded participants, study personnel, and outcome assessors. There were incomplete outcome data in almost 28% of trials. Overall, only five studies were considered to be at low risk of bias with regard to adequate randomization, allocation concealment, blinding, and follow-up.

3.4. Main outcome indicators

3.4.1. Incidence of diarrhea

A total of 6 studies [29–31,33,36] with 1932 individuals reported the incidence of diarrhea. A pooled analysis of data from these studies revealed that, compared with placebo, probiotics and synbiotics did not reduce significantly the risk of diarrhea [RR 0.87, 95% CI (0.74, 1.02)]. There was a substantial heterogeneity among studies ($P < 0.1$, $I^2 = 78\%$) (Fig. 3A).

3.4.2. Duration of diarrhea

Nine trials [27–29,32–34] were included in the pooled analysis of the effects of probiotics and synbiotics on the duration of diarrhea, including 1094 undernourished children allocated to treatment groups, and 900 undernourished children allocated to control groups. The pooled results suggest that probiotic and synbiotic supplementation can reduce the duration of diarrhea in undernourished children with diarrhea [WMD -1.05 day, 95% CI

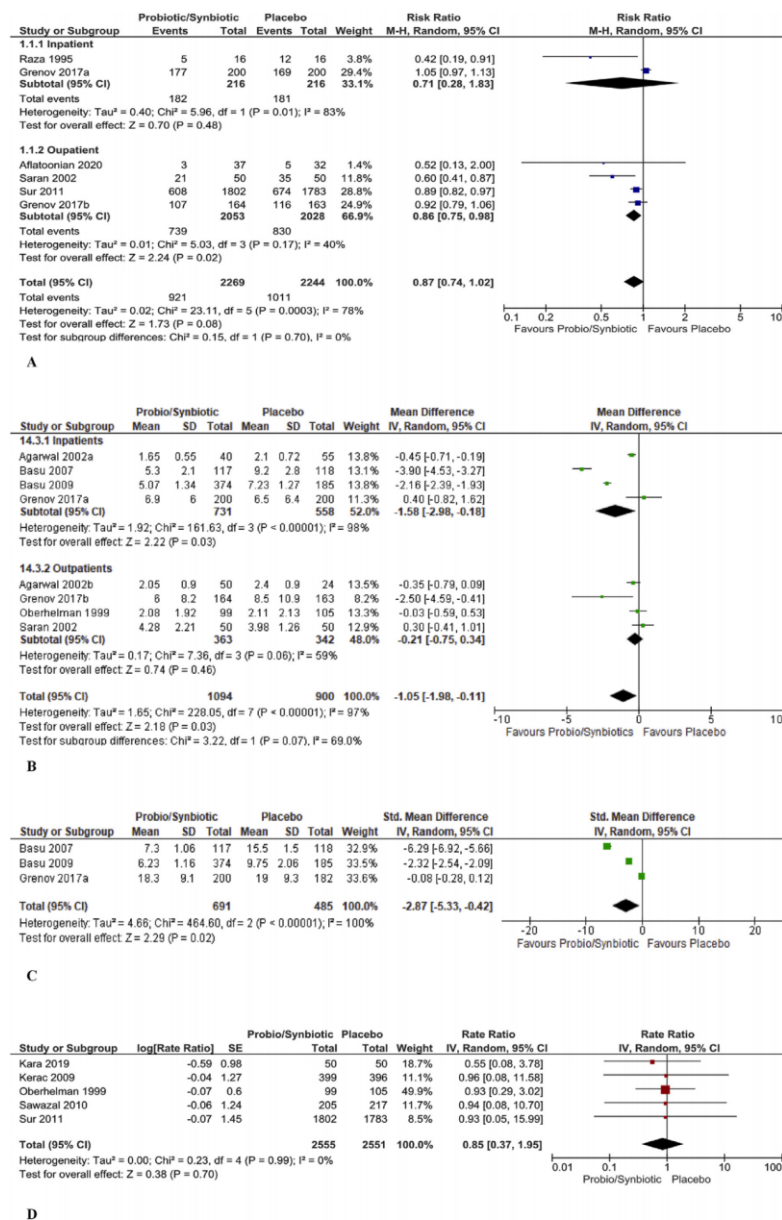


Fig. 3. A: Meta-analysis results for probiotics and synbiotics on incidence of diarrhea in undernourished children. B: Meta-analysis results for probiotics and synbiotics on duration of diarrhea in undernourished children. C: Meta-analysis results for probiotics and synbiotics on duration of hospitalization in undernourished children. D: Meta-analysis results for probiotics and synbiotics on number of episodes of diarrhea in undernourished children.

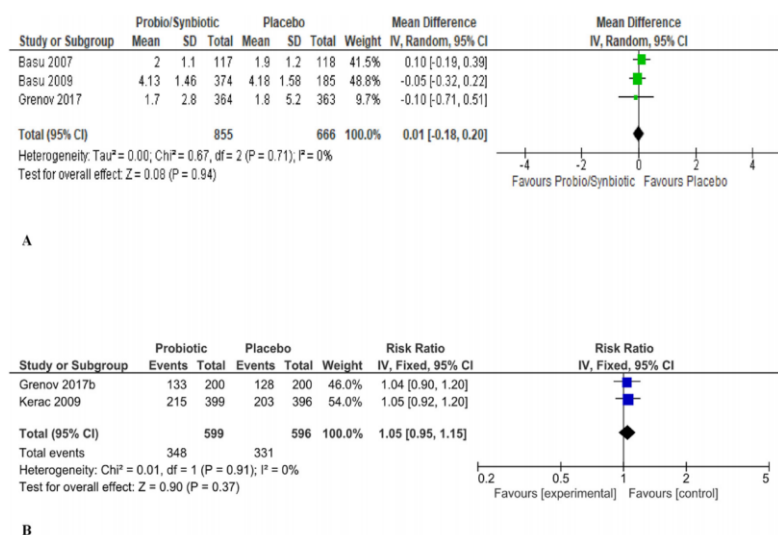


Fig. 4. A: Meta-analysis results for probiotics and synbiotics on duration of vomiting in undernourished children. B: Meta-analysis results for probiotics and synbiotics on nutritional recovery in undernourished children.

($-1.98, -0.11$)). There was a considerable heterogeneity among trials ($I^2 = 96\%$, $P < 0.1$) (Fig. 3B).

3.4.3. Duration of hospitalization

Four studies [28,30,32] were included in the meta-analysis of the effects of probiotics and synbiotics on the duration of hospitalization, including 691 undernourished children assigned to treatment groups and 485 undernourished children assigned to control groups. The aggregated results suggest that probiotic and synbiotic supplementation can considerably reduce the duration of hospitalization in undernourished children with diarrhea [Standard Mean Difference (SMD) = -2.87 days, 95% CI ($-5.33, -0.42$)], and the heterogeneity among trials was considerable ($I^2 = 98\%$, $P < 0.1$) (Fig. 3C).

3.4.4. Number of episodes of diarrhea

Five trials [29,31,34,37,38] reported the total number of episodes of diarrhea, including 2555 undernourished children in the probiotic and synbiotic group, and 2551 undernourished children in the placebo group. Pooled analyses showed that across these studies, the episode rates of diarrhea were not statistically significant between groups [rate ratio 0.85, 95%CI (0.37, 1.95)]. There was no statistically significant heterogeneity between studies ($P = 0.99$; $I^2 = 0\%$) (Fig. 3D).

3.5. Secondary outcome indicators

3.5.1. Duration of vomiting

A total of 5 studies reported the duration of vomiting, including 855 individuals in the probiotic and synbiotic group, and 666 individuals in the placebo group. Compared with the placebo group, no statistically significant reduction in the duration of vomiting was

noted with the probiotic and synbiotic group [WMD -0.01 day, 95% CI ($-0.18, 0.15$)]. There was no statistically significant heterogeneity between studies ($P = 0.49$, $I^2 = 0\%$) (Fig. 4A).

3.5.2. Adverse effects

Data regarding probiotics and synbiotics-related adverse events were collected in twelve trials [27–32,35,37,38]. In eleven of them, adverse events were not observed. In one trial [35], authors reported one case of myoclonic jerks of the limbs among two children, one receiving *Lactobacillus casei* and one receiving placebo.

3.5.3. Nutritional recovery

Two trials [29,38] reported the nutritional recovery, including 599 undernourished children in the probiotic and synbiotic group and 596 undernourished children in the placebo group. Pooled analyses showed that there was no statistically significant difference between groups [RR 1.05, 95% CI (0.95, 1.95)]. There was no statistically significant heterogeneity between studies ($P = 0.91$, $I^2 = 0\%$) (Fig. 4B).

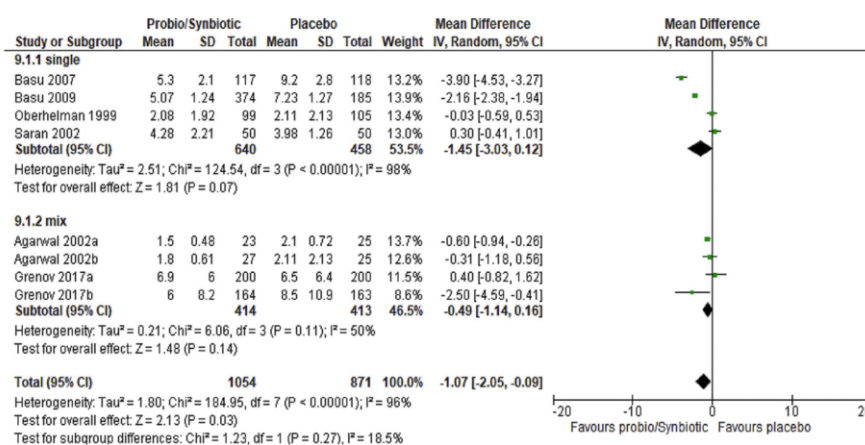
3.6. Subgroup analyses

Subgroup analyses were conducted based on sociodemographic and intervention features such as patient source, type of under-nutrition, combination of probiotics, dose, and duration of intervention. Patient source explained the statistically heterogeneity concerning incidence of diarrhea. Indeed, the test of subgroup differences (outpatients versus inpatients) showed that there was no statically significant heterogeneity ($P = 0.70$, $I^2 = 0\%$). Compared with placebo, probiotics and synbiotics reduced significantly the risk of diarrhea in outpatients [RR 0.86, 95%CI (0.75–0.98)] (Fig. 3A).

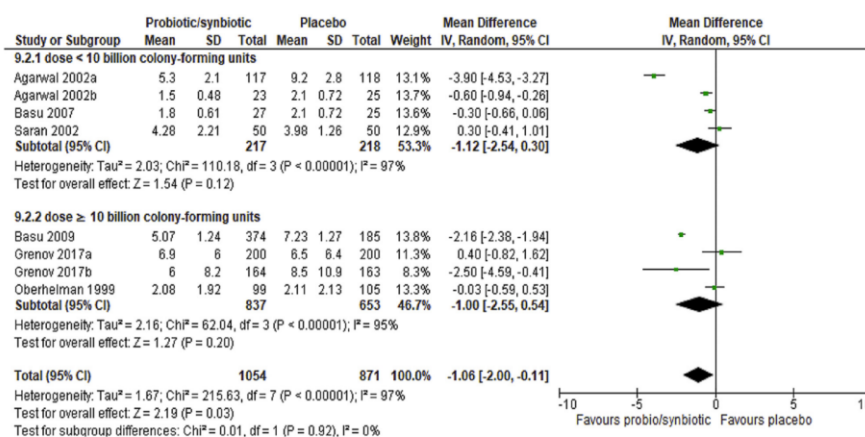
Regression analysis between the duration of diarrhea and probiotic type and dose revealed that different types and doses of probiotic/synbiotic contributed to the heterogeneity. The test of subgroup differences (single versus combination of probiotics) showed that there was no statically significant heterogeneity ($P = 0.27$, $I^2 = 18.5\%$) (Fig. 5A). Similarly, the test of subgroup differences (<10 billion CFU versus ≥ 10 billion CFU) showed that there was no statically significant heterogeneity ($P = 0.86$, $I^2 = 0\%$) (Fig. 5B).

4. Discussion

This systematic review identified 15 trials that randomized 6986 undernourished children with diarrhea in a control group with an experimental group treated with probiotic or synbiotic. The analysis revealed that treatment with probiotic or synbiotic reduced significantly both the duration of diarrhea and the hospital stay duration. In both groups, similar rates of vomiting and nutritional recovery were observed. No probiotics or synbiotics-related



A



B

Fig. 5. A: Subgroup analysis regarding duration of diarrhea: single versus combination of probiotics. B: Subgroup analysis regarding duration of diarrhea: dose of probiotics 10^{10} C: Subgroup analysis regarding duration of diarrhea: duration of intervention. D: Subgroup analysis regarding duration of diarrhea: types of undernutrition.

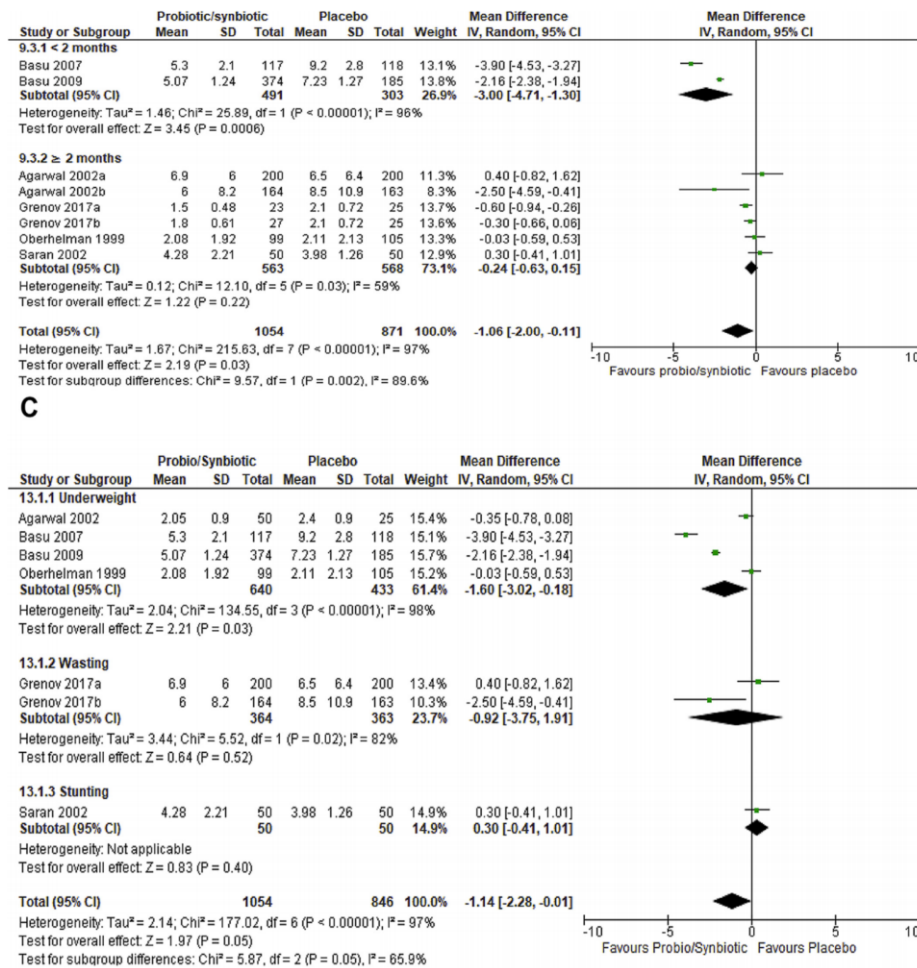


Fig. 5. (continued).

adverse effects were reported. Subgroup analyses showed that probiotic or synbiotic treatment was more effective in reducing risk of diarrhea in outpatients.

The beneficial effects of probiotics or synbiotics in reducing the duration of diarrhea and the hospital stay duration highlighted in this meta-analysis is consistent with studies' findings conducted in well-nourished children with acute diarrhea [16–18,39]. Under-nutrition and diarrhea are major causes of morbidity and mortality among children worldwide. When they coincident, they place the child at increased risk of death and disability [40,41]. The situation is of particular concern in low-income countries where children

under three years old experience on average three episodes of diarrhea every year. Each episode deprives the child of the nutrition necessary for growth [42]. In resource-poor settings, the high diarrheal disease burden is related to increased transmission of disease pathogens through ingestion of fecally contaminated water or food [40]. Children living in deprived areas with poor sanitation, inadequate hygiene, and unsafe drinking water have greater exposure to enteric pathogens and an increased risk of morbidity and severity of diarrheal illnesses [43–45].

Diarrhea is a common complication in children hospitalized with undernutrition. Mortality for children who had diarrhea at any

Table 3
Rating the quality of evidence.

Certainty assessment		N° of patients					Effect		Certainty	Importance
N° of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Probiotics or synbiotics	Placebo	Relative (95% CI)	Absolute (95% CI)
Incidence of diarrhea										
6	randomized trials	serious ^a	serious ^b	not serious	not serious	none	921/2269 (40.6%)	1011/2244 (45.1%)	RR 0.87 (0.74–1.02)	59 fewer per 1 000 (from 117 fewer to 9 more)
Duration of diarrhea										
9	randomized trials	serious ^c	serious ^d	not serious	not serious	none	1104	1106	–	MD 1.03 lower (1.74 lower to 0.32 lower)
Duration hospitalization										
4	randomized trials	serious ^e	serious ^f	not serious	not serious	none	691	688	–	MD 4.05 lower (7.47 lower to 0.62 lower)
Episodes of diarrhea										
5	randomized trials	serious ^g	not serious	not serious	not serious	none	2555	2551	Rate ratio 0.85 (0.37–1.95)	per 1000 patient(s) per years
										⊕⊕⊕⊕ LOW
										⊕⊕⊕⊕ LOW
										⊕⊕⊕⊕ LOW
										⊕⊕⊕⊕ MODERATE

CI: Confidence interval; RR: Risk ratio; MD: Mean difference; a, c, e, and g: Downgraded one level for limitation in study design due to a high risk of attrition bias; b, d, and f: Downgraded one level for considerable heterogeneity.

CI: Confidence interval; RR: Risk ratio; MD: Mean difference; a, c, e, and g: Downgraded one level for limitation in study design due to a high risk of attrition bias; b, d and f: Downgraded one level for considerable heterogeneity.

time during their admission is significantly higher than the mortality without diarrhea as reported in studies' findings conducted in Kenya [46]. Severely undernourished children often experience long hospital stay duration ranged 2–4 weeks [47]. Both community- and nosocomially-acquired diarrhea are major challenges to the successful management of children hospitalized with severe undernutrition, as reported elsewhere [48,49].

Management of diarrhea and dehydration continue to be controversial issues in the treatment of malnutrition. No specific WHO guidelines exist to provide guidance on the treatment of diarrhea in children with undernutrition. The implementation of a program including both Water, Sanitation and Hygiene (WASH) improvement and probiotic supplementation as a public health strategy in low and middle-income countries could be an attractive approach. It would prevent the diarrhea occurrence, and reduce the diarrhea duration and the hospital stay duration [50]. WASH programs (including hand washing with soap, improved sanitation, and water supply) could interrupt specific pathways for fecal-oral transmission while probiotics could contribute to support a healthy microbiota, and theoretically potentiate a reduction in the risk of diarrhea in children. Hence, the combination of probiotics with WASH could be potentially beneficial in disrupting the vicious diarrhea-malnutrition circle and its consequences, particularly in high disease endemic areas.

In the subgroup analyses, no significant differences between combination or single-strain probiotics were observed in terms of incidence of diarrhea, duration of diarrhea and hospitalization. Chapman et al. showed the beneficial effects of mixtures of probiotics on diarrhea, respiratory tract infections and gut microbiota modulation [51]. However, Grandy et al. revealed no differences in the duration of diarrhea when they compared the use of a single probiotic (*Saccharomyces boulardii*) against a group receiving a combination of them [52]. Similarly, Grenov et al. reported no effect of mixtures of probiotics (*Bifidobacterium animalis subsp. lactis* and *L. rhamnosus* GG) on diarrhea in children with severe acute malnutrition [29], while Dubey et al. observed a reduction in the duration of diarrhea, stool frequency, and overall better recovery rates by incorporation of eight different strains [53]. The effect of combination versus single strain probiotics remain inconclusive. Negative effects related to competition among different probiotic strains may occur, and more trials are needed to evaluate how to combine probiotics so that they can act synergistically.

In poor settings, the cost of probiotics/synbiotics may limit their accessibility. In Uganda, dairy farmers and small-scale entrepreneurs were taught to convert their milk into a probiotic yoghurt using an innovative bacterial starter culture and basic equipment [54]. This self-support provides better cost-effectiveness. The implementation of such projects in poor settings could be one of the keys in the control of malnutrition and diarrhea.

The study presents several strengths. To our knowledge, this meta-analysis is the first to investigate probiotics or synbiotics effects for the treatment of diarrhea in undernourished children. We included 15 RCTs involving 6986 undernourished children and we performed a detailed subgroup analysis to explore the sources of heterogeneity between trials. Finally, we used the GRADE approach to assess the quality of evidence (Table 3).

There are several limitations of our study. First, the findings were limited by the available studies. The overall quality of studies was low, largely due to missing information regarding randomization procedures and missing outcome data, all of which affect internal validity. Second, the categories of undernourished individuals were not differentiated in trials. Indeed, the effectiveness of probiotics or synbiotics can be dramatically altered by the cause of undernutrition (lack of intake or lack of absorbance). Third, heterogeneity between individual trials was observed, related to

the different phenotypes of undernutrition, dose, duration and choice of strain of probiotics, and severe/moderate undernutrition phenotype.

5. Conclusion

Current evidence supports the potential role of probiotics and synbiotics in the management of diarrhea in undernourished children. A probiotic/synbiotic intervention capable of reducing durations of diarrhea and hospitalization in undernourished children, even with modest efficacy, would have an enormous impact in resource-poor settings. Its broad implementation should be envisaged in order to improve the prognosis of undernourished children.

Author contributions

RMK and DVDL conceptualized the study. RMK and FIN selected and screened studies. GAN and JBK extracted data from included trials. RMK was responsible of data analysis. RMK wrote the manuscript. DVDL and LBB critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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4.4 Probiotics for children with uncomplicated severe acute malnutrition (PruSAM study): a randomized controlled trial in the Democratic Republic of Congo

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Original Research Article

Probiotics for children with uncomplicated severe acute malnutrition (PruSAM study): A randomized controlled trial in the Democratic Republic of Congo

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ABSTRACT

Background: Severe acute malnutrition (SAM) contributes to nearly 1 million deaths annually worldwide, with diarrhea and pneumonia being the common morbidity associated with mortality.

Objectives: To assess the effect of probiotics on diarrhea, pneumonia, and nutritional recovery in children with uncomplicated SAM.

Methods: A randomized, double-blind, placebo-controlled study was conducted involving 400 children with uncomplicated SAM randomly assigned to ready-to-use therapeutic food (RUTF) either with ($n = 200$) or without ($n = 200$) probiotics. Patients received 1 mL daily dose of a blend of *Lacti-caseibacillus rhamnosus* GG and *Limosilactobacillus reuteri* DSM 17938 (dosage, 2 billion colony-forming units; 50:50) or placebo during 1 mo. They were simultaneously fed with the RUTF for 6 to 12 wk, depending on patients' recovery rates. The primary outcome was the duration of diarrhea. Secondary outcomes included diarrheal and pneumonic incidence, nutritional recovery, and transfer to inpatient care rate.

Results: For children with diarrhea, the number of days of disease was lower in the probiotic group (4.11; 95% CI: 3.37, 4.51) than that in the placebo group (6.68; 95% CI: 6.26, 7.13; $P < 0.001$). For children aged 16 mo or older, the risk of diarrhea was lower in the probiotic group (75.6%; 95% CI: 66.2, 82.9) than that in the placebo group (95.0%; 95% CI: 88.2, 97.9; $P < 0.001$), but no significant difference of the risk for the youngest. In the probiotic group, nutritional recovery happened earlier: at the 6th wk, 40.6% of the infants were waiting for nutritional recovery, contrasting with 68.7% of infants in the placebo group; but the nutritional recovery rate at the 12th wk was similar between the groups. Probiotics had no effect on pneumonic incidence and transfer to inpatient care.

Conclusions: This trial supports using probiotics for the treatment of children with uncomplicated SAM. Its effect on diarrhea could positively affect nutritional programs in resource-limited settings.

This trial was registered <https://pactr.samrc.ac.za> as PACTR202108842939734.

Keywords: diarrhea, probiotics, low-income country, uncomplicated severe acute malnutrition, infants

Abbreviations used: CMAM, Community-based Management of Acute Malnutrition; DRC, Democratic Republic of Congo; ProbiSAM, Probiotics for Severe Acute Malnutrition; PRONUT, Probiotics and prebiotics for severe acute malnutrition; PruSAM, Probiotics for uncomplicated severe acute malnutrition; RUTF, ready-to-use therapeutic food; SAM, severe acute malnutrition.

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Introduction

Severe acute malnutrition (SAM) affects 14 million children aged younger than 5 y worldwide and directly contributes to nearly 1 million deaths annually [1,2]. It is also an important underlying contributor to many other child deaths, especially those due to diarrhea and pneumonia [2]. These 2 are the leading complications of SAM associated with increased morbidity, longer hospitalization, and mortality [3–5]. Long-term effects of SAM include high postdischarge mortality after inpatient management; and poorer growth, body composition, and physical and cognitive function; and low probability of attaining a high level of education [6–10].

SAM in children is classified as uncomplicated or complicated. Uncomplicated SAM refers to the child who has no clinically obvious acute infections or other medical complications and has a good appetite. Complicated SAM refers to child who has clinical features of infection or metabolic and electrolyte disturbance, severe edema, or poor appetite [11].

Malnutrition is due to a complex set of determinants, such as inadequate food intake and infections [12]. Recently, a number of metagenomic studies in undernourished children from Bangladesh, India, and sub-Saharan Africa provided evidence that microbiota immaturity was involved in development or maintenance of acute malnutrition [13–18]. The growing evidence for a causal role of microbial dysbiosis in child malnutrition warrants intervention studies using microbiota-targeted therapies to prevent or treat undernutrition. Probiotics, that is, live microorganisms that confer a health benefit to the host when administered in adequate amounts [19], provide a potential opportunity to promote a healthy gut microbiota. In well-nourished children with acute diarrhea, recent meta-analyses evidence suggests that probiotics are effective in reducing the duration of hospitalization [20]. Investigations of strain-specific effects have led to recommendation of 3 main strains: *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii*, and *Limosilactobacillus reuteri* DSM 17938 [21]. In children with undernutrition, our recent meta-analysis has shown that probiotics could reduce significantly the hospital stay duration by approximately 3 d [22].

Probiotic effects have also been investigated in 2 large trials among children with SAM in Malawi [23] and Uganda [24], and no benefits of probiotic treatment on clinical outcomes or nutritional recovery were reported. Nevertheless, these studies demonstrated that probiotics were safe to use in children with immunodeficient SAM. Indeed, no cases of probiotic-related bacteremia were identified. Moreover, they showed trends toward reduced outpatient mortality and outpatient days with diarrhea in the probiotic group, suggesting possible positive role of probiotics in community-based management of children with SAM. Several bacterial species, such as *Lactobacillus* strains, have been previously proposed as probiotic candidates as an alternative to fecal transplantation to address children with SAM [25].

In this study, we aimed to assess the effect of a combination of probiotics, *Lactobacillus rhamnosus* GG and *Limosilactobacillus reuteri* DSM 17938, on diarrhea, pneumonia, and nutritional recovery in children treated for uncomplicated SAM in the community.

Methods

Study design and participants

The Probiotics for Uncomplicated Severe Acute Malnutrition (PrusAM) trial was a double-blind, individually randomized placebo-controlled trial. It was conducted in the Kadutu Health Zone, in the

South Kivu Province, eastern Democratic Republic of Congo (DRC). Kadutu Health Zone has 450,000 people, including 85,000 children aged younger than 5 y, with 24 health centers and 5 referral hospitals. Furthermore, it encompasses 5 outpatient therapeutic centers where children with uncomplicated SAM receive routine medical care and a take-home ration of RUTF once a fortnight, and 1 intensive therapeutic and feeding unit where all children with complicated SAM are managed. Both centers receive children of the same age category. The prevalence of acute malnutrition in this region was estimated to be 3% in 2018. The PrusAM trial was conducted in 2 of the 5 outpatient therapeutic centers. Selection of these 2 trial centers was based on demographic, epidemiologic, accessibility, and logistical criteria.

Children were eligible for inclusion if they were aged between 6 and 24 mo; had a diagnosis of SAM [mid-upper arm circumference of <115 mm, weight-for-length z-score (WAZ) ≤ -3 , and/or grade 1 or 2 nutritional edema]; passed an appetite test performed as per the national protocol [26]; and had no medical complications and their parent or guardian provided written informed consent. Exclusion criteria were a known allergy to RUTF, congenital or acquired disorders affecting growth, and grade 3 nutritional edema. Children were enrolled after their parent or guardian provided written informed consent. Although these ineligible children were excluded from the study, they were compassionately enrolled in the standard RUTF therapeutic feeding program, unless there is another contraindication such as peanut allergy. Those with complicated SAM were referred for inpatient care. After stabilization, they were returned to standard RUTF therapeutic feeding program.

Randomization, allocation concealment, and blinding

Children were randomly assigned in a 1:1 ratio, and in computer-generated blocks of 6, to receive probiotics or placebo. The randomization codes were created with a computerized random-number generator according to site; they were kept inside opaque, sealed, consecutively numbered envelopes and opened by a study team physician in a numerical order. The codes based on the block randomization table were provided to the study team by a designated person through telephone. All participants, caregivers, investigators, and staff involved in the study were blinded until the database was locked.

Intervention, monitoring, and follow-up

Treatment followed DRC national guidelines [26], which are based on standard international WHO [27] and Community-based Management of Acute Malnutrition (CMAM) guidelines [28]. Patients received 1 mL of study product daily in single dose, in addition to the standard treatment of uncomplicated SAM. This 1 mL was picked directly from the vial using a 1-mL graduated pipette provided in the box and administered before RUTF. Each milliliter of study product contained coconut oil with or without a combination of the 2 probiotic strains, at a dosage of 1 billion colony-forming units of each bacterium. *Lactobacillus* strains were chosen because they are one of the most widely used probiotic strains, which have been studied extensively in clinical trials and human intervention studies. Study product was administered for 1 mo, and the first dose was given at inclusion, at the centers. Probiotic products were manufactured by Ineldea, whereas the placebo products containing coconut oil were bottled locally in vials similar to those of probiotics. The appearance, taste, and smell of the products were indistinguishable, so patients were blind to their group allocation. Four very small letters printed on the right corner of the label was the only way to identify the correct group. According to the manufacturer's instructions, the product should be stored at ambient

temperature not exceeding 30 °C. In South Kivu, the mean temperature is between 20–25 °C. Probiotics were transported to the DRC in a single batch at an ambient temperature and were stored at an ambient temperature in South Kivu (Bukavu). Study products were received in Bukavu in August, 2021. The test to see how many colony-forming units were still viable on arrival was not performed. Regular quality control checks performed in December, 2021, and March, 2022, showed that we prescribed more than 10⁶/mL of live probiotic bacteria at the time of consumption according to recommendations [29].

Clinical history and examination, anthropometry, blood glucose and hemoglobin levels, rapid malaria tests, and HIV testing using 2 rapid antibody tests (Determine and Unigold) were systematically undertaken at admission. If malarial test showed positive results, an artemisinin-based combination therapy was prescribed. HIV-positive patients received further care according to national protocols, which included referral for antiretroviral medication. Children with suspected tuberculosis were referred to the hospital for diagnosis and management.

After admission into the study, all children received a 5-d course of amoxicillin, a single dose of vitamin A, an anthelmintic, a 2-wk ration of RUTF, and health and nutrition advice. The RUTF ration was calculated to provide 200 kcal/kg of bodyweight/d. Children with SAM normally need 15 kg of RUTF (1 carton) given over a period of 6 to 8 wk for recovery from malnutrition [30]. Considering the length of stay in the CMAM program in DRC (12 wk maximum) and the risk of RUTF sharing within and outside the household, 2 cartons of RUTF were planned per child. All these medications were directly administered to the child by the caregivers at admission, supervised by the nurse. Follow-ups were planned biweekly throughout a period of 12 wk, depending on the nutritional recovery of each child. Breastfeeding was encouraged throughout the study.

Routine procedures in each follow-up appointment included taking anthropometric measurements and vital signs and assessing and managing of signs of pneumonia, diarrhea, dehydration, presence of appetite, other signs of illness, and adverse events. Caregivers were given a stool diary in which they ticked every time their child passed stool. Stool frequency and consistency were checked at follow-up visits. Based on a photograph scale, stool was categorized as watery, abnormally loose, loose, or normal. A repeat 2-wk rations of RUTF was provided at the same dosage rate. RUTF were administered for 8–12 wk depending on each child's nutritional recovery.

Caregivers were contacted by telephone once a week to ask about the health progress of their infants and to remind them about visit dates. In case of missed follow-up, a home visit was performed to inquire about reasons for failure to return for a follow-up visit. Study product compliance was estimated using the following criteria: 1) verbal report from the caregiver; 2) examination of the amount of intervention remaining in the medication bottle; 3) the returned empty or unused RUTF sachets; and 4) differences between visit dates and number of sachets supplied. Infants were considered compliant to the study products if they fulfilled the abovementioned 4 criteria during the whole study period.

Infants identified at any point in the process who did not demonstrate an appropriate appetite, or had medical complications, or remained severely malnourished after 4 follow-up visits were referred for inpatient care. These infants were followed up until 12 wk to correctly allocate severity of hospital course or death to each study group.

Outcomes

The primary outcome was the number of days with diarrhea. Based on stool diary data, diarrhea was defined as the passage of 3 or more

loose or liquid stools per 24 h [31]. A diarrhea episode started when the diarrhea definition was fulfilled, and ceased when the child passed <3 loose or watery stools per day. If diarrhea re-appeared after <48 h, it was considered part of the same diarrhea episode, but only days with ≥3 loose or watery stools were counted as diarrhea days.

Secondary outcomes were, first, diarrheal incidence, defined as the proportion of infants with minimum 1 d of diarrhea, and second, pneumonic incidence, defined as the proportion of infants with pneumonia. Pneumonia was defined as the combination of signs, such as fever, tachypnea, focal crackles, or decreased breath sounds together, with characteristic radiologic abnormalities [31]. Third outcome was nutritional recovery. Infants were considered to have recovered when they were without edema, had a WAZ of −2 or higher, and mid-upper arm circumference of ≥125 mm at 2 consecutive follow-up visits with a maximum of 15 d between the visits. Finally, other outcomes such as weight gain, time to recovery, transfer to inpatient care, death rate, and RUTF and probiotic safety were assessed. Transfer to inpatient care included infants referred to inpatient care owing to medical complications.

Statistical analysis

Sample size

We calculated that a sample of 178 children in each group would provide the study with an 80% power at a 5% significance level to detect a 1-d reduction in number of days with diarrhea. Allowing for a 10% rate of loss to follow-up, 200 infants were recruited per arm. Assuming that the standard deviation of days with diarrhea in outpatient therapeutic center was 3 d, it would be possible to detect a 1-d reduction in days with diarrhea, which is similar to what is found in meta-analyses [22].

Data management and statistical analysis

Data were double-entered in EpiData v.3.1 (EpiData) and analyzed using SPSS 25.0 (SPSS). WHO Anthro plus 1.0.4 (WHO) was used to calculate anthropometric z-scores by applying 2007 WHO References. In each study trial, the age, sex, presence or absence of bilateral pitting edema, and anthropometric measurements of infants were imported into WHO Anthro plus software, which then calculated WAZ, length-for-age z-score, and weight-for-length z-score and identified outliers: <−6 and >5 for WAZ, <−6 and >6 for length-for-age z-score, and <−5 and >5 for weight-for-length z-score. These values were excluded and the infants subjected to measurements again. Household Food Insecurity Access Scale was constructed according to Food and Nutrition Technical Assistance indicator guide [32]. Except for the end points, all comparisons between the probiotic and placebo groups were performed using nonparametric tests: χ^2 test (with Cook correction) for dichotomous variables, Mann–Whitney rank test for continuous variables, and Kolmogorov–Smirnov test for continuous and ordinal variables. The number of days with diarrhea was modeled by a zero-inflated γ -Poisson regression. Presence or absence of diarrheal predisposition and number of days of diarrhea, if any, were simultaneously analyzed by the maximum likelihood method. The weight gain achieved at nutritional recovery was modeled using a γ distribution regression for the weight gain achieved, if any. Data regarding the time to reach nutritional recovery were truncated by the limitation of the time of observation and were thus analyzed by a survival statistical calculation method. A Rényi test was performed to assess the gap between the time curves waiting for nutritional recovery of the probiotic and placebo groups. To assess the influence of continuous variables on the time to reach nutritional recovery, the Kvaloy rank test

was used. CIs were measured at 95%. The regression results were transformations of logarithmic or logistic values, giving asymmetric CI values. This trial was registered at a Pan African Clinical Trials Registry as PACTR202108842939734.

Ethics

The study was performed in accordance with the principles in the Declaration of Helsinki of 1975 as revised in 1983. An independent Data Safety Monitoring Board, including 1 statistician and 1 pediatrician, regularly monitored serious adverse events during the study. Children were enrolled after verbal and written consent had been provided by their caregivers. Caregivers were made aware of their right to withdraw from the study at any time. Ethical approval was granted by the Université catholique de Bukavu Ethics Committee (Bukavu/DRC, UCB-CIES 01-05/2020) and the Comité d’Ethique Hospitalo-Universitaire of the Université catholique de Louvain and Cliniques Universitaires Saint-Luc (Brussels/Belgium, 04-01-2021).

Results

Between August 2021 and May 2022, 694 children with SAM were assessed for eligibility in the 2 trial centers. After the exclusion of

ineligible children, 400 children were randomly assigned to a study group. The total loss to follow-up was 7 in the probiotic group and 9 in the placebo group. Finally, 384 children (193 in the probiotic group and 191 in the placebo group) were included in the final analysis (Figure 1).

Baseline sociodemographic, anthropometric, and clinical characteristics were similar in both groups (Table 1). The median age was 16 mo (IQR: 11–20 mo), 212 (53%) infants were boys, 288 (72%) were currently breastfed, and 236 (59%) had edematous malnutrition (kwashiorkor). Forty-six (11.5%) infants showed positivity for malaria, and 7 (1.8%) were HIV seropositive. Moreover, 327 (81.8%) caregivers were from households with a low socioeconomic level, 231 (57.8%) had a low education level, 163 (40.8%) were currently pregnant, and 52 (13%) were undernourished.

Compliance to study product consumption was 98% and 96% for probiotics and placebo, respectively. Study products and RUTF were tolerated equally well in both groups. No cases of allergy or anaphylaxis were identified. During follow-up, 3 infants died: 1 infant in the probiotic group and 1 in the placebo group died at home due to unknown cause; and 1 infant in the placebo group died in hospital of septic shock. All were HIV-negative children.

Table 2 summarizes main study outcomes. Compared with that of the placebo group, the number of days with diarrhea was multiplied by

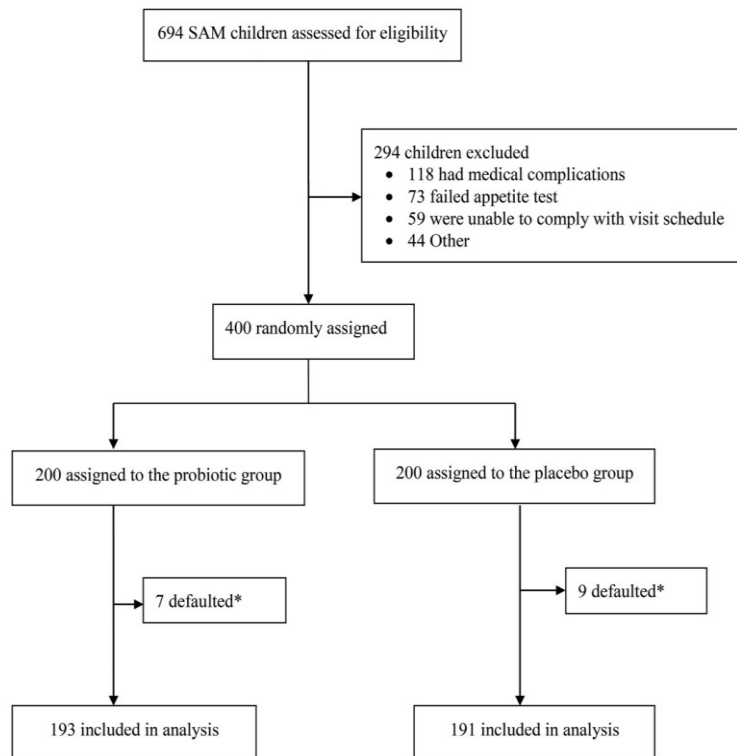


FIGURE 1. Trial profile. SAM, severe acute malnutrition. *Default was defined as 3 or more consecutive missed weekly visits. Children who defaulted did not recover and withdrew from trial follow-up before 12 wk.

TABLE 1
Baseline characteristics

	Probiotics (n = 200)	Placebo (n = 200)
Sociodemographic characteristics		
Age (y)	16 (12–20)	16 (11–20)
Male	107 (53.5)	105 (52.5)
Vaginal birth	182 (91.0)	185 (92.5)
Birth weight (g)	3035 (2900–3600)	3200 (2940–3700)
Born at full-term	194 (97.0)	195 (97.5)
Currently breastfed	142 (71.0)	146 (73.0)
Age of introduction of CFs (months)	5 (3–6)	5 (4–6)
No. siblings	3 (2–5)	3 (3–6)
Child lives with both parents	151 (75.5)	148 (74.0)
Mother education primary school or lower	116 (58.0)	115 (57.5)
Mother with undernutrition	25 (12.5)	27 (13.5)
Mother currently pregnant	83 (41.5)	80 (40.0)
Household size	7 (5–9)	7 (5–9)
Household with low socioeconomic level	162 (81.0)	165 (82.5)
Anthropometric characteristics		
Weight (kg)	6.7 (6.0–7.4)	6.6 (5.7–7.2)
Length (cm)	70 (67–73)	69.5 (65–73.1)
MUAC (mm)	118 (110–125)	117 (110–123)
Head circumference (cm)	45 (43–46)	45 (43–46)
Weight-for-age z-score	−3.6 (−4.2 to −3.1)	−3.6 (−4.4 to −3.1)
Length-for-age z-score	−3.3 (−4.2 to −2.5)	−3.6 (−4.3 to −2.6)
Weight-for-length z-score	−2.6 (−3.3 to −1.9)	−2.5 (−3.3 to −1.7)
Medical characteristics at admission		
Fever	12 (6.0)	11 (5.5)
Cough	138 (68.0)	133 (66.5)
Diarrhea	88 (44.0)	91 (45.5)
Vomiting	45 (22.5)	49 (24.5)
Had a malaria	22 (11.0)	24 (12.0)
Hemoglobin (g/dL)	9.1 (8.4–10.2)	9.0 (8.0–10.1)
HIV-positive children (serology)	4 (2.0)	3 (1.5)
Kwashiorkor (edematous malnutrition)	116 (58.0)	120 (60.0)

Values are given as n (%) or median (IQR).

CF, complementary food; MUAC mid-upper arm circumference.

0.615 (95% CI: 0.550, 0.689) in the probiotic groups, corresponding to a reduction of 38.5% (95% CI: 31.1, 45.0), regardless of age ($P < 0.001$). There was a trend toward a decreased risk of diarrhea in the probiotic group, but the bivariate analysis, including age and probiotics usage, showed a significant interaction, so that the probiotics had more influence on the risk for the oldest infants ($P < 0.001$). This observation required separated regressions by the age level. For children aged 16 mo (the median) or older, probiotics decreased the risk of diarrhea by 19.3% ($P = 0.006$) and, in case of diarrhea, decreased the duration by 34.3% ($P < 0.001$). For the youngest, the risk was not modified by the probiotics, but in case of diarrhea, the duration was reduced by 47.0% ($P < 0.001$). The magnitude of the effect on the duration of diarrhea was not significantly different in these 2 groups ($P = 0.099$), in contrast with the difference between the 2 groups regarding the risk ($P = 0.003$). With equal treatment, age increased the risk of diarrhea ($P < 0.001$) with only a slight increase in duration.

Overall, 292 (76.0%) infants enrolled in the trial recovered from SAM. There was no significant difference between groups in the frequency of nutritional recovery at 12 wk ($P = 0.77$), but the curves of

TABLE 2

The outcomes of infants with SAM randomly assigned to probiotics or placebo

	Probiotics (n = 193)	Placebo (n = 191)	P
Average duration of diarrhea, if any¹ (d)			
Whole population ²	4.11 (3.37, 4.51)	6.68 (6.26, 7.13)	<0.001
Half youngest infants ³	3.44 (2.88, 4.09)	6.48 (5.76, 7.29)	<0.001
Half oldest infants ⁴	4.45 (4.00, 4.96)	6.77 (6.26, 7.33)	<0.001
Risk of diarrhea (%)			
Whole population	61.6 (54.4, 68.2)	70.8 (63.9, 76.6)	0.063
Half youngest infants	45.9 (35.6, 56.3)	45.8 (36.0, 55.6)	0.99
Half oldest infants	75.6 (66.2, 82.9)	95.0 (88.2, 97.9)	<0.001
Still waiting for nutritional recovery at week 12 ⁵ (%)	17.8 (12.6, 23.7)	19.6 (11.1, 25.7)	0.58
Still waiting for nutritional recovery at year 6 (%)			
Whole population	40.6 (33.4, 47.6)	68.7 (61.4, 75.0)	<0.001
Infants with diarrhea	33.7 (24.1, 43.5)	70.0 (59.4, 78.3)	<0.001
Infants without diarrhea	34.2 (23.7, 45.1)	46.3 (32.7, 58.8)	0.22
Weight gain, if any (whole population) ^{6,7} (g/kg/d)	4.34 (4.09, 4.56)	4.01 (3.37, 4.51)	0.069
Frequency of pneumonia (%)	7.8 (4.4, 12.5)	9.4 (5.7, 14.5)	0.57
Transfer to inpatient care (%)	6.2 (3.3, 10.6)	5.2 (2.5, 9.4)	0.69

Value given in parentheses are 95% CII.

¹ The number of days with diarrhea was modeled by a zero-inflated γ -Poisson regression. Presence or absence of diarrhea predisposition and number of days of diarrhea were simultaneously analyzed by the maximum likelihood method.

² With probiotics, the mean of placebo is multiplied by 0.615 $\times$ 1.059 (95% CI: 0.550, 0.689).

³ With probiotics, the mean of placebo is multiplied by 0.530 $\times$ 1.114 (95% CI: 0.430, 0.655). Half youngest infants were defined as younger than the median (16 mo); $n = 184$.

⁴ With probiotics, the mean of placebo is multiplied by 0.657 $\times$ 1.071 (95% CI: 0.575, 0.752). Half oldest infants were defined as equal or above the median; $n = 200$.

⁵ Data regarding the time to reach nutritional recovery were truncated by the limitation of the time of observation and were thus analyzed by a survival statistical calculation method. A Rényi test was performed to assess the gap between the time curves waiting for nutritional recovery of the probiotics and placebo groups.

⁶ The weight gain achieved at nutritional recovery was modeled using a γ distribution regression for the weight gain achieved, if any.

⁷ With probiotics, the mean of placebo is multiplied by 1.083 $\times$ 1.043 (95% CI: 0.996, 1.117).

time hindsight while waiting for nutritional recovery were different ($P < 0.001$, Rényi test). In the probiotic group, nutritional recovery happened earlier for a large proportion of infants (Figure 2): At the 6th wk, 40.2% of infants were waiting for nutritional recovery, contrasting with 68.7% of infants in the placebo group. Logistic regressions showed a significant interaction between treatment allocation and presence of diarrhea on the percentage reaching nutritional recovery at 6 and 8 wk. The effect of probiotics was restricted to infants with diarrhea. In these children, probiotic use was associated with an earlier nutritional recovery ($P = 0.010$, Rényi test), whereas for infants without diarrhea, this effect was not demonstrated ($P = 0.43$, Rényi test). The age was not associated with weight gain ($P = 0.26$) nor with earlier nutritional recovery ($P = 0.35$, Kvaloy test). The weight gain,

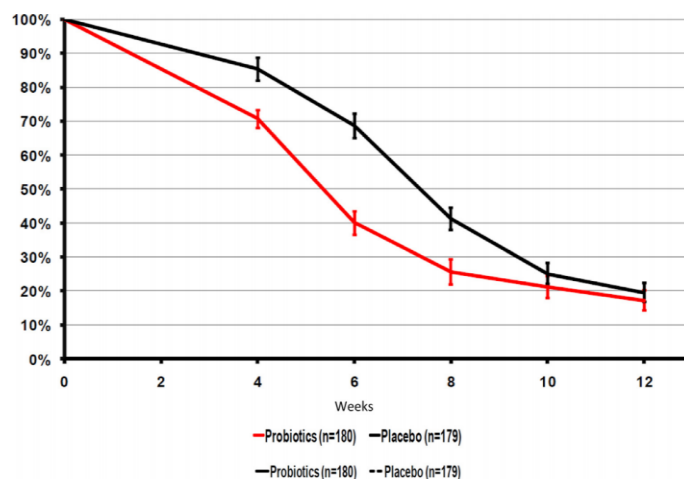


FIGURE 2. Time before nutritional recovery.

the risk of transfer to inpatient care, and the risk of death were similar between the placebo and probiotic groups.

Discussion

This trial investigated the efficacy of probiotics on diarrhea, pneumonia, and nutritional recovery in infants with uncomplicated SAM in the CMAM. Probiotics reduced the number of days corresponding to 39% of the mean number of days with diarrhea.

In the Probiotics for Severe Acute Malnutrition (ProbiSAM) study [24], a randomized, double-blind, placebo-controlled trial in Uganda to assess the effect of probiotics on diarrhea during inpatient and outpatient treatment of children with SAM, the probiotics had no effect on diarrhea during inpatient treatment but a significant 26% reduction in days with diarrhea during the outpatient treatment. An earlier double-blind efficacy randomized controlled trial in Malawi [Probiotics and prebiotics for severe acute malnutrition (PRONUT) study] [23] to assess the clinical and nutritional efficacy of a probiotics during inpatient and outpatient treatment of children with SAM, reported trends toward less severe diarrhea in outpatient treatment in the probiotic group.

The discrepancy between our findings with ProbiSAM and PRONUT trials may be due to several factors. The first one is the patient population. Compared with the ProbiSAM and PRONUT trials in which children aged 6 to 59 mo and 5 to 168 mo were included, respectively, we included children aged 6 to 24 mo. Indeed, the life cycle contains windows of opportunity in which microbiota-targeted therapies may be more amenable to intervention. The 1000-d period represents a period of microbial assembly in which the microbiome could be targeted to optimize healthy composition [33].

The second is the type of children with SAM: ProbiSAM and PRONUT trials enrolled children with complicated SAM with multiple life-threatening conditions. Furthermore, the PRONUT study included a large proportion (45%) of HIV-infected children. More severe illness and a compromised gut barrier in children during hospitalization may have affected their ability to respond to probiotic treatment. In a follow-

up of the ProbiSAM trial, Castro-Mejia et al. [34] investigated gut microbiome evolution during rehabilitation from SAM and the effect of probiotics supplementation on the gut microbiome during inpatient and outpatient treatment of SAM. In addition, they assessed whether gut microbiome composition and development were linked to the observed reduction in days with diarrhea when administered probiotics during the randomized controlled ProbiSAM trial. The findings suggested that gut microbiome diversity and composition change over the course of rehabilitation from SAM and approach the gut microbiome of apparently healthy subjects as treatment progresses. Furthermore, probiotic treatment reduced the cumulative incidence of diarrhea during the outpatient phase, with the strongest effect in children where the administered probiotics could be detected in the gut microbiome [34].

Third one is the trial design. We performed a community-based trial to reduce the risk of possible contamination between the study groups. Indeed, in PRONUT study, freeze-dried symbiotic powder was factory-mixed into RUTF. The possible contamination between the groups by sharing RUTF while patients were living closely together during inpatient care could not be formally excluded.

Finally, a fourth factor worth considering are the antimicrobials used. Indeed, in PRONUT trial, all inpatients received co-trimoxazole, and 50% also had parenteral antibiotics. Children who were HIV seropositive continued on long-term co-trimoxazole prophylaxis after discharge, during and after outpatient treatment. However, in vitro testing showed sensitivity to co-trimoxazole in 2 of the 4 probiotic organisms used in this trial. Antibiotics might have decreased the viability of the probiotics and, hence, their efficacy.

This study found that in older infants, probiotic treatment reduced the risk and duration of diarrhea, and in infants with diarrhea, it resulted in a greater percentage of early nutritional recovery and probability of weight gain. The shorter time to reach the nutritional recovery, favorable to probiotics, is an interesting finding for low-income and middle-income countries. Indeed, these countries experience high prevalence of SAM among children aged younger than 5 y (13 million) contrasting with low treatment program coverage, with at least 75% of children with SAM aged 6–59 mo not accessing care [35,36]. The main reason

for low access to SAM treatment is related to CMAM programs considered to be expensive, and therefore largely underfunded, owing to the cost of RUTF [37]. The high cost and large quantity of RUTF administered during nutritional treatment in outpatient therapeutic centers [38] have prompted attempts to optimize product formulation and use. In a noninferiority, randomized controlled trial performed in DRC, Cazes et al. [39] assessed whether a single protocol for moderate and SAM management, using only RUTF and reducing the dose as the child improves [the optimising malnutrition treatment (OptiMA) strategy], could achieve similar or higher individual efficacy, increase coverage, and minimize costs compared with the current programs (standard strategy). The findings suggested that the OptiMA strategy was superior to the standard one regarding favorable outcomes (being alive, not acutely malnourished, and with no further episodes of acute malnutrition throughout the 6-mo observation period), time to nutritional improvement, and treatment cost.

In this study, as in most CMAM programs in DRC, 2 cartons of RUTF (\$92) were planned per infant for 12 wk of nutritional treatment. In addition, the cost of probiotics for 1 infant was \$36. Our study showed that a larger percentage of infants (>60%) in the probiotic group received only 1 carton of RUTF (\$46) because of the short time to reach nutritional recovery in this group (6–8 wk). Finally, the combined cost of RUTF and probiotics in the probiotic group was lower than the cost of RUTF in the CMAM program in DRC (\$82 and \$92, respectively). The overall cost of RUTF is a major cost driver in malnutrition treatment programs, and the global community is already struggling to pay for all RUTF that is needed. In addition to the individual benefits for children regarding the reduction of the number of days of diarrhea, the probiotic intervention seems to hold major advantages from a programming perspective because it allows more children to access treatment at a lower cost. Thus, the probiotic intervention and the OptiMA strategy seem to be promising strategies to facilitate increased coverage of SAM treatment in a resource-constrained environment.

The WHO [27] and a recent meta-analysis [40] support the use of oral amoxicillin in children with uncomplicated SAM because of the high risk of mortality and the significant reduction of mortality rate among children with uncomplicated SAM treated with antibiotics. Antibiotic-associated diarrhea is a potential consequence of prescribing broad-spectrum antibiotics for the outpatient treatment of children with SAM. Regarding the ambivalence of antibiotics in children with uncomplicated SAM, probiotics seem to be useful in this population because of its protective effect for preventing antibiotic-associated diarrhea, as suggested in a recent meta-analysis [41].

Implementing and scaling up the use of probiotics in low-income and middle-income countries to address childhood malnutrition requires several logistical considerations: The need for vigilant safety monitoring, owing to the effects of malnutrition on the child's immune system; strengthening local manufacturing and storage capacities to ensure product viability despite infrastructure, financial, and climatic challenges; using existing distribution networks to ensure accessibility to those most in need of the treatment; and gaining acceptability by cultures that might not be inclined to add live microbes to dietary and medical practices [42]. Many of these concerns might be circumvented by using locally sourced dietary approaches to target the gut microbiota and associated pathologies. Recent data have provided promising evidence for the potential of microbiota-directed foods to improve child growth. Gehrig et al. [43] conducted a pilot randomized controlled trial to compare the efficacy of microbiota-directed complementary food

with the traditional rice-based and lentil-based ready-to-use supplementary food used in the treatment of moderate acute malnutrition. Findings suggested that such microbiota-targeted foods enhanced biomarkers of growth, bone formation, neurodevelopment, and immune function in both experimental animals and undernourished children. Hence, microbiota-targeted therapies pose potential as promising alternatives to standard therapeutic and complementary foods in settings with a high prevalence of child undernutrition.

Our study has several strengths. First, the study was a randomized, double-blind controlled design, with low default rate after admission. Second, the follow-up and the compliance to study products and associated measures were excellent despite the challenging postconflict context. The study was implemented by well-trained and experienced research staff, and home visits were performed by well-involved and committed community health workers. Follow-ups were close and meticulous.

The study has some limitations. First, data on diarrheal etiology are lacking. Because diarrhea in children with SAM is triggered by infectious (bacterial, viral, fungi, or parasitic) or noninfectious (malabsorption or enteropathy) agents, probiotics are likely to have different effects based on these agents. Second is the lack of metagenomic and culturomic data. This approach could enable the analysis of the gut microbiota evolution during rehabilitation from SAM and the effect of probiotics supplementation on the gut microbiota. Third is the selection bias. Only 384 of the 694 subjects assessed for eligibility were analyzed, and they may have different characteristics from those of the unanalyzed or those lost to follow-up. Nevertheless, we believe that this would not have significantly altered our main conclusions because the characteristics at admission did not differ between the excluded/lost to follow-up and analyzed subjects. Fourth, the multiple secondary outcomes tests might lead to increase in type I error and should therefore be interpreted with caution. Finally, the study interventions were performed by well-trained and supervised medical staff. These features may not be generally representative of standard care provided in many outpatient therapeutic centers. Our findings should be confirmed in studies designed to reflect real-life conditions of nutritional programs.

In conclusion, the current findings support using probiotics for the treatment of children with uncomplicated SAM. Its effect in reducing the number of days of diarrhea and the time to reach nutritional recovery has potential for impact in resource-poor settings. Future research is needed for an economic analysis of scaling up probiotic use nationally and internationally, for exploration of local products (e.g., yogurts and fermented foods), which might have similar benefits, and to support breastfeeding, a free, widely available but much undersupported source of probiotic immunologically active compounds.

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The authors' responsibilities were as follows—RMK, LBB, DVdL: designed the research; DVdL: provided essential materials; RMK, JNN, JBK, ABZ, INF, BNM: conducted the research; EB, GAN, FZ: performed the statistical analysis; RMK: wrote the paper; DVdL, EB, FZ, LBB: critically revised the manuscript; RMK, EB, FZ, LBB, and DVdL: had primary responsibility for the final content; and all authors: read and approved the final version of the manuscript.

Conflict of interest

The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available on request pending application and approval.

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Chapter 5: GENERAL DISCUSSION

This thesis set out to explore infants feeding practices, the main infectious diseases experienced by children with SAM, and the effect of probiotics on diarrhea, pneumonia, and nutritional recovery in children with SAM in South Kivu, eastern DRC. The main findings of the thesis are summarized and discussed in the following sections.

5.1 Infants feeding practices in South Kivu

The DRC is still recovering from years of war and political upheaval, and continues to face significant humanitarian challenges: poverty is extraordinarily high (72% of the population is living in poverty); ongoing violence and insecurity, particularly in the east of the country, are contributing to persistent instability and high levels of displacement and undermining livelihoods and food security. About 26 million people are projected to become acutely food-insecure in 2023 [223]. It was in this context of post-conflict situations and food insecurity that this study was conducted.

Overall, infants' feeding practices in South Kivu remain suboptimal. Data suggest that only 42% of infants are exclusively breastfed up to 6 months of age [224], and only 32% of them have a minimum acceptable diet. The low minimum acceptable diet rate reported in our study is consistent with recent nutritional survey findings in Bukavu [225]. Additionally, consumption of flesh foods, dairy products, eggs, fruits, and vegetables remains significantly low.

Madzorera et al [226] examined the association between dietary diversity and treatment outcomes among children treated for uncomplicated SAM in Niger. Overall, diets in the study population showed limited diversity and were based on consumption of staples and plant-based foods, with lower consumption of dairy and eggs. There was no evidence that dietary diversity increased nutritional recovery at discharge. However, the consumption of eggs was protective against death [226].

The residence in rural areas, the nonattendance at postnatal care, the low household socioeconomic and the low maternal education were factors associated with inadequate minimum acceptable diet. Urban areas appeared better in several aspects of complementary feeding practices compared to rural ones. This might be the result of the cumulative effect of a series of more favorable conditions, including better socioeconomic and educational conditions, in turn leading to better caring practices for infants and their mothers. A study analyzing Demographic and Health

Surveys from 36 LMIC revealed that for the same reasons, the prevalence of malnutrition was higher in rural than in urban areas [227].

The association between nonattendance at postnatal care and inadequate minimum acceptable diet is consistent with study findings in the East African region and India [228–232]. Mothers' education on complementary feeding practices during these visits is the factor underlying this association. In DRC's health system, children's growth monitoring is conducted in every health facility according to the national nutrition program. In the outreach clinics, healthcare workers monitor the weight of children using growth charts and provide nutrition education to mothers or caregivers of children. These activities also provide an opportunity to discourage harmful traditional beliefs that might inhibit child feeding practices, and to early recognition of signs of malnutrition, any illness and manage them accordingly [233].

Our findings show that more than 85% of the mothers attended at postnatal care. Surprisingly, the minimum acceptable diet rate remained low. The low household socioeconomic status could be the main explanatory factor. Indeed, our study showed that about 80% of households had a low socioeconomic level. This finding emphasizes the role of socioeconomic status on feeding practices. Households with high socioeconomic level are more likely to be food secure, thus they can afford to provide the minimum acceptable diet to their children [234]. Several studies have also shown that improved household wealth has a significant effect on adequate complementary feeding practices [235–238].

Consistent with studies conducted in China [239] and Ethiopia [240], the current study also found that mothers with secondary and post-secondary education had higher odds of an adequate minimum acceptable diet. The possible reason might be that education is directly linked to women's autonomy, changes in traditional beliefs and women's control over household resources [241]. Hence educated mothers are more likely to understand information provided through health and nutritional programs regarding complementary feeding practices [242].

Three systematic reviews [243–245] examined the effectiveness of infant's complementary feeding interventions. The socioeconomic status of caregivers, maternal education level, and postnatal care have been identified as main factors that may influence infant's complementary feeding practices. Nevertheless, the results of the 3 systematic reviews demonstrated that interventions that provide education to mothers will significantly improve complementary feeding knowledge and practices. When the population is food secure, educational strategies alone can improve feeding practices. The case is different in the context of food insecurity or political trouble [246].

5.2 Infectious diseases experienced by children with SAM

Malnutrition and infectious diseases are tightly linked in a self-reinforcing cycle. In SAM, host defense mechanisms, both acquired (lymphocyte functions) and innate (macrophages and granulocytes) are affected. Diminished immune functions render SAM children more susceptible to infections [247]. In SAM, varying degrees of mucous membrane disruptions, abnormalities in protective secretions and nutrient deficiencies are observed. SAM compromises mucosal epithelial barriers in the gastrointestinal, respiratory and urogenital tracts. Thus, SAM predisposes to gastrointestinal, respiratory and urogenital infections [248, 249].

Infectious diseases also contribute to malnutrition through several mechanisms: prolonged diarrhea due to intestinal infection, leading to loss of nutrients; HIV/AIDS, TB and other chronic infections cause cachexia and anemia potentiating malnutrition; intestinal parasites cause anemia and nutrient deprivation [247]. Furthermore, immune response stimulation by infection increases the metabolic demands for energy and associated substrates, leading over time to adverse nutritional status and increased susceptibility to further infection [249]. In the other hand, infections alter the nutritional status, mediated by changes in dietary intake, absorption, nutrient requirements, and loss of endogenous nutrients. A metabolic consequence of infection is a decrease in serum iron, zinc, and vitamin A [247]. GI and respiratory tract infections have also been shown to cause malnutrition by causing a deficit in crucial body supplies of protein, energy, minerals, and vitamins [248].

Thus, for all these reasons, SAM children will experience an increased incidence, severity and case fatality of common infections compared to well-nourished children. Furthermore, children with complicated SAM will have a high ongoing risk of mortality after discharge from hospital [22].

In our cohort, bacteremia, pneumonia, gastroenteritis, and malaria were the most common infectious diseases among SAM children. This observation is consistent with other findings showing the high incidence of these infectious diseases in SAM children [26-31].

Because SAM leads to a high risk of infections, many of which are occult [194]; due to the high mortality rate reported among them (10-40%) [26-31], and the significant reduction of mortality rate and hospitalization episodes when they are treated with antibiotics [18, 196]; the WHO guidelines support the use of antibiotic treatment for

children with SAM: oral amoxicillin for children with uncomplicated SAM as outpatients; parenteral therapy beyond 2 days for inpatients if this is indicated by the clinical condition e.g. severe pneumonia or sepsis [12, 198, 201].

Bacteremia is a significant contributor to mortality. To estimate the prevalence and antimicrobial resistance (AMR) profile of community- and hospital- acquired bacteremia among hospitalized children with SAM, a recent longitudinal study was conducted by Andersen et al [250] in an intensive nutritional rehabilitation center in Madarounfa (Niger). Children aged 6 to 59 months admitted for inpatient treatment of complicated SAM (n=2187) had blood specimens drawn at admission to assess prevalence of community-acquired bacteremia. The prevalence of community-acquired bacteremia at admission was 9.1%, while the cumulative incidence of hospital-acquired bacteremia was 1.2%. Non-typhoid *Salmonella* (NTS) was the most common organism identified in over half (58%) of cases in community-acquired bacteremia, while *K. pneumoniae* (19%), *Acinetobacter baumannii* (16%), *E. faecalis* (13%), and *E. coli* (13%) were found in hospital-acquired bacteremia. In community-acquired bacteremia, 58% cases were resistant to amoxicillin-clavulanate, and 84% were resistant to ampicillin; 100% of hospital-acquired bacteremia cases were resistant to amoxicillin and amoxicillin-clavulanate. The risk of death was greatly elevated among children with hospital-acquired bacteremia (Risk Ratio, RR = 9.32) and among children with community-acquired bacteremia (RR = 2.67) [250].

An earlier ancillary study nested within a RCT in Niger was conducted by Grais et al [251] to test the role of routine 7-day amoxicillin therapy in nutritional recovery of children 6 to 59 months of age with uncomplicated SAM. Four hundred and seventy-two children were screened for rectal carriage of extended spectrum beta lactamases producing Enterobacteriaceae (ESBL-E) as well as their household siblings under 5 years old, at baseline, at first and fourth week after start of therapy. Among children exposed to amoxicillin, ESBL-E colonization was more frequent (53.7% versus 32.2%, adjusted risk ratio = 2.29, p= 0.001), and the risk of transmission to siblings was higher [251].

These data highlight the AMR to commonly used antibiotics for sepsis and the appearance of multidrug resistant (MDR) bacteria such as ESBLs and carbapenemases for treatment of both inpatients and outpatients.

Inappropriate use and overconsumption of antibiotics have led to an increase in AMR. This represents a threat to global health systems. In 2019, an estimated 5 million deaths were associated with bacterial AMR, including 1.3 million deaths attributable to bacterial AMR. At the regional level, the all-age death rate attributable to resistance was highest in western sub-Saharan Africa and lowest in Australasia.

The six leading pathogens for deaths associated with resistance (*E. coli*, *S. aureus*, *K. pneumoniae*, *S. pneumoniae*, *A. baumannii*, and *P. aeruginosa*) were responsible for one million deaths attributable to AMR and 4 million deaths associated with AMR [252].

In DRC, the management of malnutrition-infection comorbidity is particularly complex for several reasons: (i) most children are admitted to Nutritional Centers after having been exposed to amoxicillin at different levels: at home, at the pharmacy (without a prescription) and at the different healthcare centers; (ii) Amoxicillin is used systematically as prophylaxis in children with SAM according to WHO-guidelines; (iii) data on AMR are scarce and are exclusively available for well-nourished individuals [253-255]. This situation may contribute greatly to the appearance of MDR bacterial strains.

Microbiological surveillance should be implemented to tailor pediatric treatment guidelines for severely malnourished children to local pathogens and antimicrobial resistance patterns to increase the effectiveness of care and limit the emergence of new resistance. Therefore, interventions such as scaling up infection prevention and control (IPC), expanding mini-laboratories in nutrition centers, and multiplex Polymerase Chain Reaction (PCR)-based diagnostics for infectious diseases could improve microbiological diagnosis and targeted treatment. IPC is a practical, evidence-based approach preventing patients and health workers from being harmed by avoidable infections. It affects all aspects of health care, including hand hygiene, vaccination, surgical site infections, injection safety, antimicrobial resistance and how hospitals operate during and outside of emergencies [256]. The Mini-Lab is a transportable, self-contained, quality assured, stand-alone clinical bacteriology laboratory that can be operated by inexperienced technicians and used in low-resource settings [257]. The multiplex PCR is the simultaneous amplification of more than one target sequence in a single reaction tube using more than one primer pair. It allows the detection of viral, bacterial and/or other infectious agents in a single reaction tube. Thus, it reduces the cost and time required for the simultaneous detection of two, three or more pathogens in a sample [258].

Furthermore, in order to reduce inappropriate antibiotic use, WHO has developed practical guidance for the empirical use of antibiotics through the book "AWaRe" (Access, Watch, Reserve). AWaRe provides concise, evidence-based guidance on antibiotic selection, dose, route of administration, and duration of treatment for more than 30 of the most common clinical infections in children and adults in both primary care and hospital settings [259].

Pneumonia and malnutrition are two of the biggest killers in childhood. Chisti et al [26] conducted a systematic review to quantify the degree by which malnutrition increases the mortality risk in pneumonia, to identify potential differences in the etiology of pneumonia between children with and without SAM. They also assessed the validity of WHO-recommended clinical signs for the diagnosis of pneumonia in children with SAM. Findings suggest that children with pneumonia and SAM are at higher risk of death (reported relative risks ranged from 2.9 to 121.2). Commonly isolated bacterial pathogens were *K. pneumoniae*, *S. aureus*, *S. pneumoniae*, *E. coli*, and *H. influenzae*. The spectrum and frequency of organisms differed from those reported in children without SAM. There were very few data on the role of respiratory viruses and TB. WHO-recommended clinical signs (age-specific fast breathing and chest wall indrawing) were less sensitive as predictors of radiographic pneumonia in children with SAM [26].

Diarrhea remains the second leading cause of childhood deaths. When SAM and diarrhea coincide, the child is at increased risk of death and disability [260]. Malnutrition predisposes children to a higher incidence and duration of diarrhea, and severe diarrhea can trigger or worsen malnutrition. Diarrhea leads to reduced absorption of carbohydrates, protein, potassium, zinc, and other nutrients, which contributes to malnutrition. Significant water losses from diarrhea leads to dehydration, electrolyte imbalance (hypokalemia and hyponatremia), shock, decreased mental status, and ultimately death [261]. Enterotoxigenic *E. coli*, enteroaggregative *E. coli*, *Shigella* spp, *Salmonella* spp, *Campylobacter* spp, *E. histolytica*, and *Cryptosporidium* spp have been shown to be more common among children with SAM presenting with diarrhea, compared with well-nourished children [262].

Diarrhea caused by viruses, mainly rotavirus, accounts for most diarrhea episodes in resource-limited countries [263, 264]. The challenge in distinguishing viral from bacterial diarrhea and the unavailability of rotavirus vaccination leads to overtreatment of diarrhea with antimicrobials [264], resulting in the increase of AMR and gut microbiota imbalance.

Malaria and malnutrition in children are reported independently to be major causes of morbidity and mortality in LMIC [265]. Bacteremia co-infection with malaria among children with SAM was described by Andersen et al [250]. They reported the risk of community-acquired bacteremia significantly higher in children with malaria (RR = 1.47; 95% CI: 1.13, 1.93). Additionally, a co-occurring malaria infection was significantly more common among cases of NTS bacteremia (63.7%) than among cases of non-NTS bacteremia (41.7%, $p = 0.001$) [250]. Furthermore, in a systematic

review to assess evidence of interactions between malaria and malnutrition, Das et al [265] found that stunting was consistent with malaria severity, such as high-density parasitemia and anemia [266].

5.3 Effects of probiotics on diarrhea, pneumonia, and nutritional recovery in children with SAM

Macronutrient and micronutrient deficiencies certainly contribute to poor growth in infants. However, dietary interventions alone are insufficient to reduce the global burden of childhood malnutrition and to address the persistent infectious burden of the disease. Emerging evidence suggests that gut microbiota is involved in development or maintenance malnutrition. In addition, the gut microbiota accounts for the effect of diet on nutritional status. Therefore, these microbiome-nutrient interactions may help in the development of “microbiota-directed foods” to specifically target the impaired microbiome in child undernutrition.

Probiotics have been investigated for many years for their potential to improve digestive functions and to mitigate the effects of infectious and inflammatory conditions. In well-nourished children, meta-analyses evidence suggests that probiotics are effective in reducing the duration of hospitalization among children with acute diarrhea [213]; the risk of upper and/or low respiratory infections among children with acute respiratory infections [267, 268]; and in improving the weight gain among children [212].

Few studies have investigated the effects of probiotics in children with SAM. Two large RCTs, ProbiSAM trial in Uganda [216], and PRONUT in Malawi [217] reported no benefits of probiotics treatment on clinical outcomes (diarrhea, pneumonia, death) and nutritional recovery. A trend towards reduced outpatient mortality and outpatient days with diarrhea was observed in the probiotics group. The meta-analysis of these two RCTs showed that probiotics were effective in reducing mortality [218].

Our trial (PruSAM study) investigated the efficacy of probiotics on diarrhea, pneumonia, and nutritional recovery in infants with uncomplicated SAM in the community. Probiotics significantly reduced the number of days of diarrhea, and the risk of diarrhea in older infants (> 16 months). The time to reach nutritional recovery was earlier in the probiotic group, although at 12 weeks, the rate of nutritional recovery was similar. Probiotics had no effect on pneumonia incidence.

5.3.1 Implications for practice

5.3.1.1 Access to SAM treatment

Our findings showed that in older infants, probiotics treatment reduced the risk and duration of diarrhea, and in infants with diarrhea, it resulted in a greater percentage of early nutritional recovery and probability of weight gain. The shorter time to reach nutritional recovery, favorable to probiotics, is an interesting finding for LMIC. These countries experience high prevalence of SAM among under 5 years old children (13 million), contrasting with low treatment program coverage, with at least 75% of children with SAM 6–59 months old not accessing care [269, 270]. The main reason for low access to SAM treatment is related to CMAM programs considered to be expensive, and therefore largely underfunded, due to the cost of RUTF [271]. The high cost and large quantity of RUTF administered during nutritional treatment in Outpatient Therapeutic Centers [272] have prompted attempts to optimize product formulation and use.

In a non-inferiority RCT performed in DRC, Cazes et al [273] assessed whether a single protocol for moderate and SAM management, using only RUTF and reducing the dose as the child improves (The Optimising MAInutrition treatment, OptiMA strategy), could achieve similar or higher individual efficacy, increase coverage, and minimize costs compared with the current programs (Standard strategy). The findings suggested that the OptiMA strategy was superior to the standard one regarding favorable outcomes (being alive, not acutely malnourished, and with no further episodes of acute malnutrition throughout the 6-month observation period), time to nutritional improvement, and treatment cost [273].

5.3.1.2 SAM treatment cost

In this study, as in most CMAM programs in DRC, 2 cartons of RUTF (\$92) were planned per infant for 12 weeks of nutritional treatment. Additionally, the course of probiotics for one infant was \$36. Our study showed that a larger percentage of infants (>60%) in the probiotics group received only one carton of RUTF (\$46) because of the short time to reach nutritional recovery in this group (6-8 weeks). Finally, the combined cost of RUTF and probiotics in the probiotics group appear lower than the cost of RUTF in the CMAM program in DRC (\$82 vs \$92). The overall cost of RUTF is a major cost driver in malnutrition treatment programs and the global community is already struggling to pay for all RUTF that is needed. In addition to the individual benefits for children regarding the reduction of the number of days of diarrhea, the probiotics intervention appears to hold major advantages from a programming perspective as it allows more children to access treatment at a lower

cost. Thus, the probiotics intervention and the OptiMA strategy appear to be promising strategies to facilitate increased coverage of SAM treatment in a resource-constrained environment.

5.3.1.3 Antibiotic-associated diarrhea

Another potential consequence of the routine use of antibiotics for the treatment of children with SAM is antibiotic-associated diarrhea. Regarding the ambivalence of antibiotics in children with SAM, probiotics appear to be useful in this population because of its protective effect for preventing antibiotic-associated diarrhea, as suggested in a recent meta-analysis [274].

5.3.1.4 Implementing and scaling the use of probiotics

Implementing and scaling up the use of probiotics in LMIC to address childhood malnutrition requires several logistical considerations: The need for vigilant safety monitoring, the strengthening local manufacturing and storage capacities to ensure product viability despite infrastructure, financial, and climatic challenges; the use of existing distribution networks to ensure accessibility to those most in need of the treatment; and to gain acceptability by cultures that might not be inclined to add live microbes to dietary and medical practices [219].

Currently, probiotics are increasingly incorporated into foods. Indeed, the prevalence of non-dairy foods containing probiotics has steadily been on the rise, especially as consumers begin to experience “pill fatigue”. Thus, consuming probiotics in a food is perceived as a more natural way of receiving a daily dose [275].

Several studies were designed to supplement the diet of malnourished children with a formulation that contains locally available, culturally acceptable foods selected based on the ability of the ingredients to boost key growth-promoting gut microbes. The Argentine experience has documented that the consumption of yogurt containing the probiotic strain *Lactobacillus rhamnosus* CRL1505 improved natural defenses and prevented respiratory and intestinal infections, the infectious events of greatest relevance in childhood. These findings led to the implementation of a program called “Scholar Yogurito, the social probiotic” [276].

Recently, Chen et al conducted a trial including 118 Bangladeshi children with MAM to compare the effects of MDCF containing a mixture of chickpeas, soy, bananas and peanuts versus ready to use supplementary food (RUSF) containing rice- and lentil-based. The rate of change in the children's weight and their mid-upper arm circumferences were significantly greater in the group receiving the MDCF compared with the standard therapeutic food; this growth superiority was sustained even a month after the children had stopped receiving the nutritional intervention [196].

In sub-Saharan Africa, the "Fermented Food for Life" project to improve food and nutrition security by increasing local production, distribution and consumption of health-promoting probiotic fermented yogurt has been implemented in Kenya, Tanzania and Uganda. Malnourished children received 250 ml of special probiotic yogurt thrice a week. Furthermore, parents and caregivers were trained in making this probiotic yogurt so that they made it as a business to increase household income as well as improve family and community health [277]. By reaching vulnerable children, the project is impacting malnutrition, stunting and diarrhea that are common in these regions of Africa [277].

Further studies are needed to investigate the impact of these MDCF in reducing malnutrition-related morbidity and mortality. In addition, MDCF introduced after the six-month breastfeeding period recommended by the WHO has the potential for significant impact, but studies are needed. Finally, the benefits of MDCF during pregnancy to determine whether they can improve the gut microbiomes of the malnourished mothers and foster the transmission of healthy gut microbial communities to their infants and thus help break the devastating intergenerational cycle of malnutrition, need to be elucidated. Indeed, women are key actors in most LMIC food production systems, but they and their children bear the burdens of malnutrition, especially when exposed to food insecurity [278].

Chapter 6: CONCLUSIONS AND PERSPECTIVES

Reducing the occurrence of malnutrition, and when it is present, reducing its morbidity, remain the major challenges to be met in LMIC. Reducing the occurrence of malnutrition requires good infants feeding practices and control of infectious diseases. Our thesis research provided useful information concerning infants feeding practices assessed in the general population and stratified by urban and rural settings in South-Kivu. Overall, the minimum acceptable diet rate remains unacceptably low among infants, especially among mothers with low school attendance and those non-attending prenatal care. As part of optimizing nutrition in at-risk individuals, noncompliance with the minimum acceptable diet should be urgently addressed by appropriate and widespread counseling, targeting the microenvironment to identify households with high risk of developing malnutrition. In addition to the distribution of RUTF, cash transfer programs could also increase recovery from SAM and decrease default, non-response, and relapse rates during and following treatment. Financial support for household development is essential in food insecure areas to maximize the efficiency of SAM treatment programs.

Our research findings suggest an increased incidence of pneumonia, gastroenteritis, bacteremia, and malaria among children with SAM. In DRC, data are lacking regarding the pathogens and their susceptibility to antimicrobials in children with SAM. Future research should address this gap to distinguish children with SAM who need antimicrobial therapy from those who do not, and for those who do, the type of antimicrobial that fits the pathogen.

Finally, this PhD research has shown that probiotics significantly reduced the number of days of diarrhea, the risk of diarrhea in older infants (> 16 months), and the time to reach nutritional recovery. Future research is needed for economic analysis of scaling up probiotic's use nationally and internationally, exploration of local MDCF which might have similar benefits, and to support breastfeeding, a free, widely available, but much under-supported source of probiotics immunologically active compounds.

This PhD research has many prospects for future research:

First, real-life conditions studies. Our study interventions were carried out by well-trained and supervised medical staff. These features may not be generally representative of standard care provided in many Outpatients Therapeutic Centers. Our findings should be confirmed in operational studies, including studies in other settings with high prevalence of malnutrition with families at high risk of malnutrition.

Second, studies with diarrhea etiology. Diarrhea in children with SAM is triggered by infectious (bacterial, viral, fungi or parasitic) or noninfectious (malabsorption or enteropathy) agents.

Third, studies with metagenomic and culturomics data. This approach will enable the analysis of the gut microbiota evolution during rehabilitation from SAM and the impact of probiotics supplementation on the gut microbiota.

Fourth, microbiota-directed complementary food's studies (e.g. probiotics incorporated in yogurt) to assess: (i) their impact in reducing malnutrition-related morbidity and mortality; (ii) their effects in reducing SAM incidence if introduced after the six-month breastfeeding period recommended by the WHO; (iii) their benefits during pregnancy to determine whether they can foster the transmission of healthy gut microbiota to their infants and thus help to reduce the incidence of malnutrition.

Finally, the effects of probiotics according to several secondary outcomes: (i) The severity of acute gastroenteritis, assessed by the Vesikari score. Probiotics may have different effects depending on whether the acute gastroenteritis is severe or moderate; (ii) The exposure or not to antimicrobials before admission to the Nutritional Center; (iii) the effects of probiotics depending on whether the malnutrition is moderate or severe.

These findings have the potential to significantly improve the survival and well-being of malnourished children worldwide.

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