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Randomised clinical trial: a Lactobacillus GG and micronutrient-containing mixture is effective in reducing nosocomial infections in children, vs. placebo

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SUMMARY

Background
Nosocomial infections are a major public health issue and preventative strategies using probiotics and micronutrients are being evaluated.

Aim
To investigate the efficacy of a mixture of Lactobacillus GG and micronutrients in preventing nosocomial infections in children.

Methods
A randomised, double-blind, placebo-controlled trial was conducted in hospitalised children. Children (6 months to 5 years of age) received Lactobacillus GG (6×10^9 CFU/day) together with vitamins B and C and zinc or placebo, for 15 days, starting on the first day of hospitalisation. The incidence of gastrointestinal and respiratory nosocomial infections after discharge was determined by follow-up telephone call at 7 days. After 3 months, another telephone call estimated the incidence of further infections during follow-up.

Results
Ninety children completed the follow-up. Of 19/90 children with a nosocomial infection (20%), 4/45 children (9%) were in the treatment group and 15/45 (33%) in the placebo group ($P = 0.016$). Specifically, 2/45 (4%) children in the treatment group vs. 11/45 (24%) children in the placebo group ($P = 0.007$) presented with diarrhoea. The duration of hospitalisation was significantly shorter in the treatment group (3.9 days $\pm$ 1.7 vs. 4.9 $\pm$ 1.2; $P = 0.003$). At the follow-up, a total of 11/45 (24.4%) children in the treatment group had at least one episode of infection compared to 22/45 (48.9%) in the placebo group ($P = 0.016$).

Conclusion
A mixture containing Lactobacillus GG and micronutrients may reduce the incidence of nosocomial infections, supporting the hypothesis that this may represent a valid strategy to prevent nosocomial infections.

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INTRODUCTION

Nosocomial infection is an infection acquired by a patient in the course of a hospital admission. The definition of nosocomial infections includes infections occurring >48 h after admission and <48 h after discharge.[1] Despite recent scientific and technological progress, nosocomial infections remain a major problem in public health. Nosocomial infections complicate treatment, prolong hospital stay and increase hospitalisation costs. Infection control has an important role in paediatric hospitals as the incidence of nosocomial infections in children in developed countries ranges from 5% to 44%, with gastrointestinal infections and respiratory infections being predominant.[2–4] Children are susceptible to infections that are preventable in older patients through vaccination or previous exposure. Specific factors, such as the lack of immune proteins, the spread of secretions and the exchange of contaminated toys while in hospital, are associated with an increased risk of nosocomial infection.[5]

Current measures for the prevention of nosocomial infections in the paediatric setting are often ineffective. Probiotics may prevent common infections in high-risk environments, such as day care units. Studies have shown that probiotics are effective in a range of clinical conditions, particularly in the treatment and prevention of acute diarrhoeal disorders such as acute gastroenteritis and antibiotic associated diarrhoea.[6–12] However, these conclusions are controversial and the statistical evidence is weak.[13–16] A randomised clinical trial, conducted by Hojsak *et al.*, demonstrated a significant reduction in the risk of gastrointestinal infections (RR = 0.40), the number of episodes of diarrhoea and vomiting, and the duration of symptoms, in hospitalised children treated with Lactobacillus GG, compared with patients receiving placebo.[17] Moreover, there was a significant reduction in the risk of respiratory tract infections (RR = 0.38) and their duration (RR = 0.40). However, another trial reported that hospitalised children receiving *Bifidobacterium lactis* and *Lactobacillus reuteri* have a similar incidence of nosocomial infections compared to the control group.[18, 19]

Randomised controlled trials have shown that zinc supplementation during acute diarrhoea reduces the duration, severity and incidence of further diarrhoeal episodes.[20–24] Malik *et al.*, found that a 2-week course of prophylactic zinc supplementation may reduce diarrhoea induced morbidity in infants aged 6–11 months, during a 5-month follow-up period.[25]

We performed a randomised, double-blind, placebo-controlled trial with the aim of evaluating the role of a mixture containing Lactobacillus GG, vitamins B and C and zinc in the prevention of nosocomial gastrointestinal and respiratory tract infections within a paediatric hospital setting.

METHODS

Population

Ninety previously healthy children between 6 months and 5 years of age admitted to an Italian paediatric ward with any cause, between January 2014 and January 2015, were eligible for this study. We excluded children with a history of preterm birth, children with immunodeficiency diseases, chronic severe illnesses, and genetic or metabolic disorders and children who had been administered probiotic and/or prebiotic products before enrolment (within 7 days of hospitalisation). A 7-day period of washout of probiotic and/or prebiotic was chosen because the study was performed during the peak season of rotavirus, and other viral acute gastroenteritis. A longer length of no pre/probiotic consumption period might reduce the sample size. However, the form for the collection of data also included a question on any assumption of pre/probiotics within 1 month before enrolment.

Specifically, during the enrolment period of 1 year, 1172 children were admitted to the paediatric hospitals involved in the study; 493 were excluded for prematurity, severe chronic illness, immunodeficiency, genetic or metabolic disease; 549 were excluded for age <6 months or >5 years, or use of probiotics and/or prebiotics, and 40 children refused enrolment.

Study design

The study design was a prospective, randomised, double-blind, placebo-controlled trial. The tested product contained Lactobacillus GG (3×10^9 CFU/dose), vitamin B (B1 1.10 mg, B2 and B6 1.40 mg, B12 1.25 μg), vitamin C (40 mg) and zinc (5 mg). The placebo was a mixture of the same colour, weight, smell and taste as the intervention product, however, it did not contain Lactobacillus GG, vitamins B or C or zinc. The Lactobacillus GG product and the placebo were packaged in identical bottles. The product and the placebo were kindly supplied by the 'Dicofarm'. Research staff and patients were unaware of the nature of the product administered to participants. Paediatric residents were also involved in the study during their clinical rotation.

Intervention

A total of 90 children (mean age 25 months, 50 male) were enrolled and randomly assigned to two groups (intervention group = 45; placebo group = 45). All enrolled children completed the follow-up. Patients were randomly assigned to the treatment or control group using a 1:1 allocation ratio. Randomisation was based on computer-assisted permuted-block scheme, with fixed blocks size of six by a statistician.

They received either the product or the placebo twice daily for 15 days starting from the day of hospital admission. Patients were not allowed to receive any other product containing probiotics or prebiotics for the duration of the study. For each enrolled child, the onset of symptoms suggestive of respiratory or gastrointestinal nosocomial infection was recorded during the hospital stay. In addition, two follow-up calls were performed 7 days and 3 months after discharge. By means of the phone call at the 7th day after discharge, we evaluated the incidence of nosocomial infections considered as the episodes of diarrhoea, vomiting and respiratory symptoms initiated within 2 days starting from the hospital discharge. The phone call at the 3rd month was used to assess the incidence of gastrointestinal and respiratory infections during the follow-up period that included all the episodes initiated after the first 2 days post discharge.

Primary and secondary outcomes

The primary outcomes were the incidence of gastrointestinal and respiratory nosocomial infection, and the incidence of gastrointestinal and respiratory tract infections occurring during the 3-month follow-up period. Patients with at least one episode of infection were considered as incident cases. The secondary endpoints were the duration of hospitalisation for nosocomial infections and the duration of symptoms for each episode of infection both nosocomial and during the follow-up period. A nosocomial infection was defined as the appearance of symptoms of gastrointestinal or respiratory infection in the period of time between 48 h after hospital admission and 48 h after hospital discharge.[1] The symptoms to define a gastrointestinal nosocomial infections were diarrhoea and/or vomiting associated or not to fever and abdominal pain. The symptoms to define a respiratory infection were: cough, runny nose, increased breathing rate etc. associated or not to fever.

All enrolled children with respiratory infection were routinely investigated for respiratory syncytial virus using a commercially available *in vitro* immunochromatographic assays for the qualitative detection of respiratory syncytial virus fusion protein antigen on nasal swab. Faecal samples of all children with gastrointestinal symptoms were routinely investigated for Rotavirus by searching the facal antigen using an enzyme-linked immunosorbent assay-based test. Influenza virus (detected by a commercially available immunochromatographic assays for influenza A and B virus nucleoproteins), Adenovirus (searched by an enzyme-linked immunosorbent assay-based test) and Salmonella (searched by standard faecal culture) were tested in selected patients basing on clinical and epidemiological criteria.

Parents of participants were specifically instructed during the informed consent process to record any symptom of respiratory or gastrointestinal infection occurring after discharge from hospital.

Paediatric residents entered the data regarding product consumption, infections and adverse effects in the patient's study diary.

The study protocol was approved by the Ethics Committee 'Carlo Romano' of the University of Naples Federico II. Written informed consent was obtained from the parent of each child included in the study. This trial was formally registered as a clinical trial at Clinical Trial.gov (NCT02558192).

Statistical analysis

Statistical analysis was performed using R statistical computing software (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics, frequency and percentages or mean ± s.d., were used to describe the baseline characteristics of patients in the two groups.

The association between the treatment group and the primary outcomes was assessed using Chi-square test and was further quantified by estimating the odds ratio (OR) with the corresponding 95% confidence intervals (95% CI). To evaluate whether breastfeeding and antibiotics usage modulated the relative efficacy of probiotics on the primary end points, the Breslow–Day chi-square statistics was used as recommended.[26] A *t*-test was used to evaluate the differences between the treatment and placebo group in case of numerical variables. Kaplan–Meier (KM) curves were used to estimate the probability of remaining free of infection and the univariate hazard ratio (HR) was computed using the Cox Proportional Hazard model. Statistical analysis was planned on the intention-to-treat basis. All tests were two-sided, and a $P < 0.05$ was deemed significant.

The sample size of the study was calculated on the assumption of a 35% incidence of nosocomial infection and by considering as clinically relevant an absolute reduction in the risk of infection equal to 25%. A sample size of 43 subjects in each group was deemed sufficient to detect such a reduction, if truly present, with a power of 80% and a two-sided significance level of 5%. Allowing for a dropout rate of 5%, 45 patients were enrolled in each group.

RESULTS

Characteristics of population

There was no clinically significant difference between the groups, in terms of age, gender, weight, height, body mass index (BMI) and the educational level of parents (Table 1). The most common reason for hospitalisation in all patients was an acute infection. By a check on the RCT form, none of the enrolled children assumed probiotics or prebiotics within 1 month before the enrolment.

Table 1 | Baseline characteristics of enrolled patients

Variable	Intervention* (n = 45)	Placebo (n = 45)
Age, months, mean ± s.d.	33.8 ± 17.4	34.7 ± 17.2
Female gender, n (%)	21 (47)	19 (42)
Exclusive brestfeeding, n (%)	18 (40.9)	15 (35.7)
Duration of brestfeeding in months, mean ± s.d.	10.1 ± 6.1	11.1 ± 5
Weight in kg, mean ± s.d.	12.2 ± 3.8	12.4 ± 4.2
Height in cm, mean ± s.d.	86.4 ± 20.1	86.8 ± 16.9
BMI as kg/m^2, mean ± s.d.	18.09 ± 5.43	16.42 ± 1.79
Reason for hospitalisation, n (%)		
Acute gastroenteritis	9 (20)	8 (17.8)
Respiratory tract infection	17 (37.8)	12 (26.7)
Fever	12 (26.7)	16 (35.6)
Other	7 (15.5)	9 (20)

s.d., standard deviation; BMI, body mass index.

* LGG+micronutrients

Incidence of nosocomial infection

Ninety-two per cent of enrolled patients were allocated to hospital wards with an average of four patients per room (±1 s.d.). Each room contained a sink and diaper disposal units.

A total of 19/90 children presented with an episode of nosocomial infection (20%). The number of children with nosocomial infection was significantly lower in the intervention group compared to the placebo group (4/45 vs. 15/45, OR = 0.2; 95% CI: 0.06–0.65; P = 0.008; Table 2).

The relationship between treatment and incidence of nosocomial infection was homogenous by breastfeeding (Breslow–Day chi square = 1.26, P = 0.262) and antibiotic usage (Breslow–Day chi square = 3.05, P = 0.081).

This was particularly evident for gastrointestinal infections. Only 2/45 (4.4%) children in the treatment group vs. 11/45 (24.4%) children in the placebo group developed a gastrointestinal nosocomial infection (OR = 0.14; 95% CI: 0.03–0.69; P = 0.016). No difference in the incidence of respiratory tract nosocomial infections was observed between the two groups (2/45 vs. 4/45; P = 0.407; Table 2).

There were significant differences between the two groups in the secondary endpoints related to nosocomial infections. The mean duration of hospitalisation was significantly lower in patients in the intervention group compared with the placebo group (3.9 days ± 1.6 vs. 4.9 ± 1.2 days, respectively; P = 0.001; Table 3). The duration of hospitalisation was significantly shorter for gastrointestinal nosocomial infections in the treatment group compared to the placebo group (1.5 days vs. 4.4 days; P = 0.0017); however, no difference was observed for respiratory infections.

Effects of the treatment in the follow-up period

A total of 33/90 (37%) children had at least one episode of infection during the 3-month follow-up period; of these, 11/45 (24.4%) children were in the intervention group and 22/45 (48.9%) children were in the placebo group (OR: 0.34; 95% CI: 0.14–0.84; P = 0.016). Neither breastfeeding

Table 2 | Effect of treatment on the occurrence of nosocomial infections

Outcome	Intervention* (n = 45)	Placebo (n = 45)	OR (95% CI)	P
Overall nosocomial infections, n (%)†	4 (8.8)	15 (33.3)	0.2 (0.06–0.65)	0.008
Nosocomial gastrointestinal infections, n (%)†	2 (4.4)	11 (24.4)	0.14 (0.03–0.69)	0.016
Nosocomial upper respiratory tract infections, n (%)†	2 (4.4)	4 (8.8)	0.48 (0.08–2.75)	0.407

* LGG+micronutrients.

† Number and percentages of children with at least one episode of infection.

Table 3 | Effect of treatment on the occurrence of vomiting/diarrhoea episodes and on the duration of hospital stay

Outcome	Intervention* (n = 45)	Placebo (n = 45)	OR (95% CI)	P
Nosocomial vomiting episodes, n (%)†	10 (22.2)	9 (20)	1.14 (0.42–3.15)	0.796
Nosocomial diarrhoeal episodes, n (%)†	11 (24.4)	12 (26.7)	0.89 (0.35–2.3)	0.809
Duration of hospital stay, mean ± s.d.	3.9 ± 1.6	4.9 ± 1.2	–	0.003

s.d., standard deviation.

* LGG+micronutrients

† Number and percentages of children with at least one episode of vomiting/diarrhoea.

(Breslow–Day chi square = 0.03, P = 0.852) nor antibiotic usage (Breslow–Day chi square = 0.67, P = 0.413) acted as effect modifier of this association.

The main effect was observed for gastrointestinal infections with 5/45 children in the intervention group showing at least one episode of gastrointestinal infection compared to 14/45 children in the placebo group (OR: 0.29; 95% CI: 0.08–0.84; P = 0.020). No difference in the incidence of respiratory tract infections was observed (6/45 children in the intervention group vs. 8/45 children in the placebo group; P = 0.56).

A total of 40 infectious episodes were recorded during the 3-month follow-up period: 13/40 (32%) infectious episodes occurred in the intervention group and 27/40 (60%) episodes in the placebo group. The mean incidence of infections during the follow-up period was 0.28 ± 0.08 episodes/child/3 months in the treatment group compared to 0.6 ± 0.1 episodes/child/3 months in the placebo group (P = 0.02). Again, the effect was significant for gastrointestinal infections in the intervention group compared with placebo (0.11 ± 0.04 vs. 0.33 ± 0.07, respectively; P = 0.016), whereas no difference was found in the incidence of respiratory tract infections between the two groups (0.17 ± 0.06 vs. 0.28 ± 0.09; P = 0.43; Figure 1).

Furthermore, the mean duration of symptoms over the 3-month follow-up period was significantly reduced in the intervention group compared to the placebo group (3.9 ± 1.6 vs. 4.9 ± 1.2 days respectively, P = 0.03; Figure 2).

Cox regression analysis showed a 56% reduction in the risk of infections in patients in the intervention group compared to the placebo group (HR = 0.44; 95% CI: 0.22–0.89; P = 0.023). The corresponding KM curves representing the probability of remaining infection-free are shown in Figure 3.

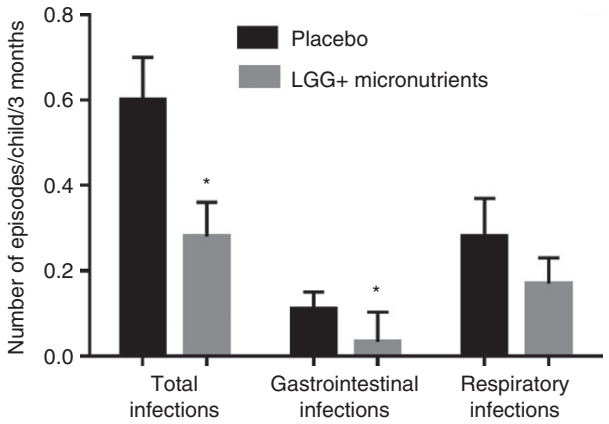

Figure 1 | During a 3-month follow-up period the incidence of infections in children supplemented with LGG and micronutrients was significantly reduced compared to placebo group (P = 0.02). The main effect was observed in the incidence of gastrointestinal infections (P = 0.016) whereas no difference was observed in the incidence of respiratory infections. * P < 0.05.

DISCUSSION

Nosocomial infections are the most frequent and severe complication of hospitalisation. Furthermore, they have significant repercussions on healthcare costs and are indicators of the quality of service provided to in-patients.

Despite the significant impact of nosocomial infections, surveillance systems and strategies to minimise their consequences often remain ineffective. Studies have shown that prevention of infections results in lower healthcare costs and improved services and that further preventive measures are necessary to minimise the risk of hospital-acquired infections. One of the potential strategies for the prevention of nosocomial infections is the use of probiotics. While there is strong evidence of their efficacy in different clinical conditions, such as acute gastroenteritis and antibiotic associated diarrhoea,[10, 11] the role of probiotics in the prevention of nosocomial infections is controversial and the results are not uniform.

Our aim was to evaluate the effects of a mixture containing Lactobacillus GG, zinc and vitamins in the prevention of nosocomial infection in children. The

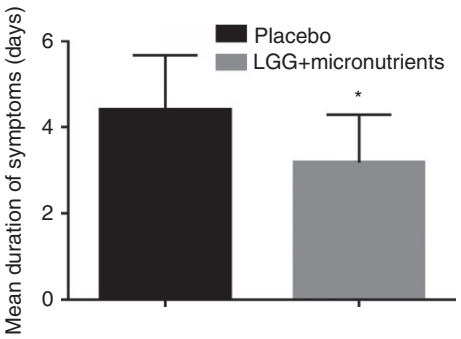

Figure 2 | The mean duration of symptoms of infection during the follow-up period was significantly shorter in children who received LGG and micronutrients compared to children received placebo ($P = 0.03$). * $P < 0.05$.

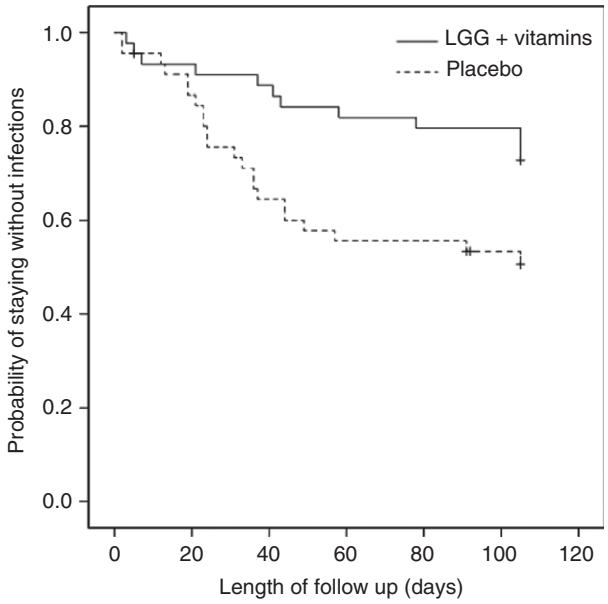

Figure 3 | Cox regression analysis showed a 56% reduction in the risk of infections in patients in the treatment group compared to the placebo group (HR = 0.44; 95% CI: 0.22–0.89; $P = 0.023$).

relatively high incidence of nosocomial infection observed is explained by the target population, and timing of the study which was conducted during the peak season of rotavirus, influenza and respiratory syncytial virus infections, the most frequent nosocomial infections in infants and young children. Our study shows that treatment with probiotics and micronutrients, started within 24 h of hospital admission and continued for 2 weeks, reduces the incidence of nosocomial infections. This finding was statistically significant only in the context of gastrointestinal infections. The lack of a statistically significant difference in the incidence of respiratory tract infections may be explained by the small number of enrolled children. We calculate the sample size basing on the primary outcome that was the reduction in nosocomial infections regardless the site of the infection. To establish the effect on respiratory infection the number needed to enrol should be higher and further larger scale studies are needed to further explore this option.

Our study supports the efficacy of Lactobacillus GG, as demonstrated in previous studies.[17] Hojsak *et al.*, in a randomised, controlled, double-blind clinical trial, demonstrated that the administration of Lactobacillus GG results in a significant reduction in the risk of gastrointestinal infections as well as episodes of gastroenteritis lasting more than 2 days.[18] Similar results were observed following the administration of Lactobacillus GG to children admitted to a day care centre, another setting associated with an increased risk of common respiratory and intestinal infections.[27]

In a meta-analysis, Szajeska *et al.*, showed that the administration of Lactobacillus GG significantly reduced the incidence of nosocomial diarrhoea (2 RCTs, $n = 823$, RR = 0.37, 95% CI: 0.23–0.59) and of symptomatic rotavirus gastroenteritis (3 RCTs, $n = 1043$, RR = 0.49, 95% CI: 0.28–0.86) compared to placebo.[13] It is not clear whether the efficacy of the product in our study was entirely due to the probiotic or to one or more of the added micronutrients. There is evidence that Lactobacillus GG prevents nosocomial infections.[17] There is also evidence supporting the use of zinc in children with impaired nutritional status.[28] However, a recent research showed that daily zinc but not multivitamin supplementation reduces the burden of diarrhoea and respiratory tract infections in Tanzanian infants.[29] A recent study showed that the administration of a mixture of four probiotic strains together with vitamin C was able to reduce the burden of upper respiratory infection in children attending pre-school.[30]

Therefore, our findings support the strategy of prescribing probiotic and micronutrients in conditions of increased exposure to common childhood infections, such as hospital admission, however, the optimal choice and dose of bacteria and micronutrients, as well as the optimal duration of the intervention, remain unclear.

Finally, an interesting aspect of our study is that we enrolled children admitted to hospital, regardless of their specific illness, so our trial was conducted in a real setting of paediatric general hospital and the obtained results may be considered representative of a field trial.

Our data show that the duration of hospitalisation is reduced by 1 day in the treatment group compared to the placebo group. This result is important because it has not been shown in previous studies, although it has been reported that the administration of Lactobacillus GG in children hospitalised for diarrhoea results in earlier discharge from hospital.

However, unlike previous studies, we did not find any statistically significant difference in the incidence of respiratory tract infections. This could be explained by the fact that our study was powered to detect a reduction in the overall infection rate, and not specifically of gastrointestinal or respiratory tract infections.

Interestingly, we found that children in the treatment group had a lower incidence of gastrointestinal infections compared to children in the placebo group over the 3-month follow-up period. As shown in Figure 3 children treated with the mixture of Lactobacillus GG and micronutrients showed an increased probability of staying without infection after about 21 days. These data are relative to the infectious episodes during the 3 months of follow-up and not to the nosocomial infections.

Furthermore, symptoms duration and severity were reduced. This evidence of a prolonged effect in the prevention of infection, beyond the treatment period has been previously reported in children with cystic fibrosis[31] and is likely to be related to a long-term effect of Lactobacillus GG on immunity.

Limitations of the study

We are aware of a number of limitations in our study. Difficulties with patient enrolment have limited the sample size. We observed a reluctance to participate in the study, which is likely to be associated with a number of variables, such as a low educational level of the legal guardians and misinterpretation, including several warnings, in the informed consent form. The population enrolled largely comprised low school and junior high school graduates.

Another limitation is the lack of established protocols on dose and duration of probiotic treatment for our specific clinical context. We therefore adopted the probiotic dose recommended by the European Guidelines[32] for the treatment of acute gastroenteritis and set our own parameters for the timeframe of probiotic treatment.

CONCLUSIONS

The use of a mixture containing Lactobacillus GG and micronutrients is effective in reducing the incidence of nosocomial infections, specifically gastrointestinal infections. Treatment reduced the length of hospitalisation in a population of children in whom the main cause of admission was an infection itself. Furthermore, the effect on prevention of infectious diseases was observed beyond the duration of hospitalisation.

These results support the hypothesis that the administration of Lactobacillus GG and micronutrients provide a valid strategy in the prevention of hospital-acquired infections.

AUTHORSHIP

Guarantor of the article: Alfredo Guarino is the person who takes responsibility for the integrity of the work as a whole, from inception to published article.

Author contributions: Afredo Guarino and Eugenia Bruzzese designed the research study, and wrote the paper. Fedele Maria Cristina and Sara Viscovo performed the research and collected data. Antonietta Giannattasio, Claudia Mandato and Paolo Siani contributed to the design of the study and performed the research. Dario Bruzzese analysed the data. All authors approved the final version of the manuscript.

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