EXPERIENCE REPORT
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Microbiological quality evaluation of dehydrated infant formulas after reconstitution and during storage in the lactary of a University Hospital

Avaliação da qualidade microbiológica de fórmulas infantis desidratadas após reconstituição e durante o armazenamento no lactário de um Hospital Universitário

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ABSTRACT

Introduction: Infant formulas (IF) fail to reproduce the immunological and digestibility properties of breast milk; however, they can meet the estimated nutritional needs when breastfeeding is prevented. Being rich in nutrients, artificial milk is an environment conducive to the growth of microorganisms. **Objective:** To perform microbiological analysis of IF intended for infants prepared in the lactary of a University Hospital of Rio de Janeiro, after reconstitution and storage. **Method:** 60 samples of IF reconstituted lactary were collected, immediately after preparation (time 0 h) and after 18 h storage under refrigeration (< 5°C), in two different shifts A and B. The Most Probable Number (MPN) for coliform growth at 35°C and 45°C and Plate Count of *Bacillus cereus* were calculated, and coagulase positive *Staphylococcus* (SCP) and qualitative analysis of the presence of *Salmonella* spp. were performed. **Results:** IF showed values of coliforms with growth at 35°C < 0.3 MPN /mL and *B. cereus* and SCP < 1 Colony Forming Unit (CFU)/mL, in addition to absence of *Salmonella* spp./25 mL at both times studied. **Conclusions:** There was no growth of the analyzed microorganisms in 100% of the samples, probably due to the adequate training of the food handlers of the studied hospital.

KEYWORDS: Infant Formulas; Lactary; Microbiological Analysis; Quality Control

RESUMO

Introdução: As fórmulas infantis (FI) não conseguem reproduzir as propriedades imunológicas e de digestibilidade do leite materno, entretanto conseguem atender às necessidades nutricionais estimadas quando há impedimento do aleitamento materno. Por ser rico em nutrientes, o leite artificial é um ambiente propício para o crescimento de microrganismos. **Objetivo:** Realizar análise microbiológica de FI destinadas a bebês preparadas no lactário de um Hospital Universitário do Rio de Janeiro, após reconstituição e armazenamento. **Método:** Foram coletadas 60 amostras de FI reconstituídas em lactário, logo após o preparo (tempo 0 h) e depois de 18 h de armazenamento sob refrigeração (< 5ºC), em dois plantões diferentes A e B. Foi realizada a técnica do Número mais Provável (NMP) para o crescimento de coliformes a 35ºC e a 45ºC, contagem em placa de *Bacillus cereus* e *Staphylococcus* coagulase positiva (SCP) e análise qualitativa de presença de *Salmonella* spp. **Resultados:** As FI apresentaram valores de coliformes com crescimento a 35ºC < 0,3 NMP/mL e de *B. cereus* e SCP < 1 Unidade Formadora de Colônia (UFC)/mL, além de ausência de *Salmonella* spp./25 mL, nos dois momentos estudados. **Conclusões:** Não houve crescimento dos microrganismos analisados em 100% das amostras, provavelmente devido ao treinamento adequado dos manipuladores de alimentos do lactário do hospital estudado.

PALAVRAS-CHAVE: Fórmulas Infantis; Lactário; Análise Microbiológica; Controle de Qualidade

INTRODUCTION

The World Health Organization (WHO) recommends exclusive breastfeeding during the first six months of life and, from that age onward, the gradual introduction of local and nutrient-rich food and the maintenance of breastfeeding up to two years of age or older[1]. Breastfeeding, however, can be prevented by temporary or permanent situations, such as in some infectious diseases (chickenpox, herpes with breast lesions, untreated tuberculosis, infection by the human immunodeficiency virus), as well as by the use of drugs that can be released by the breast alveolar epithelium and be harmful to the baby. Other factors are related to the occurrence of rare metabolic diseases in the baby, like phenylketonuria and galactosemia[2].

Infant formulas (IFs) are unable to reproduce the immunological and digestibility properties of breast milk, but they can meet estimated nutritional needs, according to the *Codex Alimentarius*[3]. Therefore, even if they do not replace the benefits of breastfeeding, IFs can be a good alternative when breastfeeding is not possible[4].

According to item I of article 6 of RDC n. 43, of September 1, 2011[5], IF for infants is the product, in liquid or powder form, used under prescription, specially manufactured to meet, alone, the nutritional needs of healthy infants during their first six months of life. In hospitals, these formulas are usually prepared in the breastfeeding unit, which is used to prepare, clean and distribute milk bottles and their substitutes for feeding newborns and pediatric patients[6].

Milk-based powdered IFs have also been linked to outbreaks of foodborne diseases (FBD), which have occurred mainly in hospitals, where this type of food is often administered to hospitalized children[7]. When considering the microbiological safety of IFs, it is important to note that cow milk, the basic component of most of them, has characteristics that make it an excellent culture medium for the multiplication of microorganisms, mainly due to the presence of emulsified fats, physiological concentrations of proteins, the content of salts and sugars, in addition to pH around 6.8[8].

The main microbiological changes related to powdered milk and its byproducts, like powdered IFs, occur due to accidental contamination during or after the reconstitution of the product[9]. One of the factors generally associated with contamination is the handling process, that is, dilution of these formulas in inappropriate environments as well as non-validated nor controlled processes[7].

FBDs affect the well-being and health of many individuals, with greater severity in impaired people, including hospitalized children[10]. One of the most frequent exogenous sources of epidemic outbreaks in neonatal Intensive Care Units (ICUs) includes milk-based IFs[11]. The inadequate preparation and handling of reconstituted IFs provides ideal conditions for the multiplication of pathogenic microorganisms, which substantially increases the risk of FBDs. However, the risk of disease can be reduced if good practices are adopted in the preparation and handling of IFs[12].

Considering the several factors that can change the microbiological patterns of IFs reconstituted in a breastfeeding unit, such as hygiene-health conditions and storage period, in addition to the relevance of feeding in the lives of hospitalized babies, investigating the microbiological quality of this food after the handling and storage process is necessary to minimize these risks and guarantee the quality of the product.

In view of the above, the objective of the present study was to perform a microbiological analysis of infant formulas for babies, prepared at the breastfeeding unit of a University Hospital in the city of Rio de Janeiro, Brazil, after reconstitution and storage.

METHOD

During 15 weeks (from April to August 2018), we collected 60 samples of IF intended for babies, reconstituted in the breastfeeding unit of a University Hospital in the city of Rio de Janeiro.

Collection and analysis were performed four times a week – in different shifts, A and B –, after the IF preparation processes (time 0 h) and after storage for 18 h in a refrigerator in the ward (time 18 h). The samples were transported under refrigeration (< 5ºC) and immediately processed at the Clinical Bacteriology Laboratory (LABAC).

Detection of *Salmonella* spp.

The search for *Salmonella* spp. was performed by the classical culture method of presence/absence. This qualitative method consisted of three stages, pre-enrichment in 1% peptone water (Bacteriological Peptone – OXOID, LTD., Basingstoke, Hampshire, England), selective enrichment in Rappaport Vassiliadis broth (OXOID) and selective differential plating on XLD agar (OXOID) for detection of typical colonies. The *S.* Typhimurium ATCC 14028 strain and the *Escherichia coli* ATCC 25922 strain were used as positive and negative controls of the test, respectively.

Pre-enrichment of 25 mL of the sample was made in 225 mL of 1% buffered peptide water (OXOID) at 35 ± 2ºC / 24 h. Afterward, aliquots of the pre-enriched culture were inoculated in Rappaport Vassiliadis broth (OXOID) followed by incubation at 42ºC – 43ºC/18 to 24 h. Some of this broth was streaked on XLD Agar (OXOID) and incubated at 35 ± 2ºC/18 to 24 h.

Determination of coliforms at 35ºC

The determination of coliforms at 35ºC was performed using the Most Probable Number technique (MPN). The test was conducted in three series of three tubes each (3 x 3) containing Lauryl Sulfate Broth (OXOID) with inverted Durham tubes. 25 ml of analytical unit of each sample were measured and homogenized in

225 ml of 0.1% peptone water (OXOID). The dilutions used were 10^0, 10^{-1} and 10^{-2}. The positive tube was the one that presented turbidity and gas production. The *E. coli* ATCC 25922 strain and the *Bacillus cereus* ATCC 14579 strain were used as positive and negative controls of the test, respectively.

Determination of *B. cereus*

The determination of *B. cereus* was performed by the method of Plate Count by Surface Plating. 100 µL (0.1 mL) of the 10^0 dilution were transferred to the MYP agar surface (OXOID). The plates were incubated at 35ºC for 24 h. The *B. cereus* ATCC 14579 strain and the *E. coli* ATCC 25922 strain were used as positive and negative controls of the test, respectively.

Determination of *Staphylococcus* coagulase-positive (SCP)

The determination of SCP was performed by the method of Plate Count by Surface Plating. 100 µL (0.1 mL) of the 10^0 dilution were transferred to a Vogel Johnson agar surface (OXOID). The plates were incubated at 35ºC for 24 h. Presumptive colonies were subjected to the coagulase test. With the aid of a sterilized bacteriological loop, a fresh culture aliquot was inoculated into 0.5 mL of rabbit plasma and incubated in a water bath at 35 ± 2ºC. The reading was made after 4 h of incubation. Negative cases were reassessed after 24 h of incubation. The *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 strains were used as positive and negative controls, respectively.

Quantitative and qualitative analyses were performed following the protocol of the American Public Health Association[13]. The results were described in percentages and compared with the standards established by current legislation[14].

RESULTS AND DISCUSSION

The results of the microbiological analysis have shown that the microorganisms studied were not detected in 100% of the samples, immediately after reconstitution (time 0 h) and after 18 h of storage. We also emphasize that there was no growth of characteristic colonies in the *B. cereus* determination test and no growth in the Lauryl Sulfate Broth in the coliform determination test at 35ºC.

RDC n. 54, of November 12, 2012[15], based on the recommendations of the WHO guide[12], establishes that, when preparation in advance is necessary, the reconstituted IF must be cooled to a temperature lower than 5ºC, for a maximum of 24 h. In the breastfeeding unit of the present study, IFs were stored for 18 h at most. For this reason, they were analyzed at time 0 and time 18 h, in order to evaluate the growth of microorganisms during this period. The refrigerator used for storage was kept at temperatures below 5ºC and monitored twice a day. The staff in charge of handling the IFs received regular training on dressing and cleaning their hands and the benchtops where the reconstitution occurred, which contributed to the adequacy of the IFs. In addition, the formulas were reconstituted manually with the aid of easy-to-clean utensils.

Horita et al.[16], in a study done in breastfeeding units of six hospitals, also obtained 100% of their samples, with regard to coliforms at 35ºC, in line with RDC n. 12, of January 2, 2001[14]. However, this result is in disagreement with the findings by Rossi et al.[17], who analyzed 30 samples of reconstituted IFs stored under refrigeration for up to 24 h in a public hospital breastfeeding unit and found a high estimate of coliforms at 35ºC. This can be explained by the fact that, in the study of this group, the IFs were processed in a blender, which is an appliance that may have a high microbial load.

With regard to contamination during the use of equipment, Miyahira et al.[18], in a study conducted at a university hospital in Rio de Janeiro, found that the blender and mixer used in food preparation were vehicles of contamination by *Staphylococcus* spp. This result reinforces the importance of properly cleaning utensils and equipment before production processes.

In the breastfeeding unit of the Sul de Minas Gerais University Hospital, Momesso et al.[19] analyzed 16 samples of IF and the tools that had been used in their reconstitution. Although the blender and the spoon had a high microbial load, all formulas were suitable for consumption. Maurício et al.[20] used 21 IF samples to research microbiological quality. They found that 19% of them were unfit for human consumption, due to the growth of coliforms at 35ºC above the maximum allowed by the current legislation (RDC n. 12/2001[13]). The presence of a large number of total coliforms is an indication that the conditions of preparation, storage and facilities are in inadequate health conditions[21].

In a study published in 2014, Reginato et al.[22], when investigating the growth of aerobic mesophilic microorganisms, coliforms at 35ºC, coliforms at 45ºC and SCP, observed that 53.8% (n = 26) of the IFs analyzed were unfit for consumption. When conducting research on hospital breastfeeding, Guerra et al.[23] found no contamination by *Salmonella* spp. in the analyzed samples (n = 8). However, these results do not mean the IFs are suitable for consumption, since other potentially pathogenic bacteria, like SCP and *B. cereus*, were found in 25% of the samples.

When analyzing 90 samples of IF reconstituted at the Pedro Ernesto University Hospital, in the city of Rio de Janeiro, in two shifts of employees (A and B), Carneiro et al.[24] noticed a difference in contamination between the two shifts. Coliform count at 35ºC > 10 MPN/mL was observed in 64.4% (n = 29) of the IFs reconstituted by shift A, whereas on shift B growth was in compliance with the legislation.

Salles and Goulart[25], in a study carried out in two hospital breastfeeding units (A and B), evaluated the microbiological conditions of reconstituted IFs and of the hands of the staff. They found that 58.3% and 50.0% of the preparations from breastfeeding units A and B, respectively, were unfit for consumption.

The literature describes the direct relationship between food contamination and staff training[26]. Research done in Canada has shown that the knowledge of the food-handling staff about the concept of safe food was greater when they had received specific training[27].

We emphasize that the frequent supervision and constant training on Good Practices of the staff participating in our study may have influenced the microbiological quality of the IFs. Additionally, the ward in question had low staff turnover, which favors the formation of a specialized team. Finally, the water used for the reconstitution of the IFs was filtered and boiled. The hospital's quality control department periodically performed microbiological water analysis.

Because of their composition, IFs are a good culture medium for the growth of microorganisms. The review of existing microbiological standards in the current legislation, provided for by the National Health Surveillance Agency, through Public Inquiry n. 542 of 2018[28], is essential to ensure the microbiological quality of IFs. Because of the immunological conditions of hospitalized neonates, opportunistic infections become frequent. Therefore, hygiene-health control must be actively enforced in breastfeeding units in order to avoid these consequences.

CONCLUSIONS

The results we obtained have shown that there was no growth of the microorganisms surveyed in 100% of the analyzed samples, both at the time of preparation, and after 18 h of storage in a refrigerator, giving the babies one less microbiological risk factor.

The training and supervision of breastfeeding units' staff are extremely important for the hygiene-health control of the production process, which contributes to the microbiological quality of the final product.

Furthermore, the scientific literature demonstrates that cleaning the equipment used for reconstitution of infant formulas is directly related to a decrease in food contamination.

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Authors' Contributions

All authors participated in the conception, planning (study design), acquisition, analysis, interpretation of data and writing of the paper. All authors approved the final draft of the paper.

Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.