



Microbiological Evaluation of Foods for Special Medical Purposes of Children

Pınar Mursaloğlu Kaynar^{1,a}, Elçin Günaydın^{2,b,*}

¹Ankara Medipol University, Vocational School of Health Services, Ankara, Türkiye

²Kastamonu University, Veterinary Faculty, Department of Microbiology, Kastamonu, Türkiye

*Corresponding author

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ABSTRACT

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Foods for special medical purposes are specially produced or formulated with the intention of regulating children's diets for specific nutrition applications, and used under medical surveillance. These food should not offer a microbiological risk to human health as well as their compositions. The purpose of this study was the microbiological evaluation of foods for special medical purposes used in children's diet. For this evaluation, eleven imported foods for special medical purposes that were sold in Ankara-Turkey were analyzed in terms of *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *Escherichia coli*, *Escherichia coli* O157, *Enterobacteriaceae*, *Listeria monocytogenes*, and *Bacillus cereus*. The bacterial examinations were done according to each related EN ISO standart methods. In result of the examination, the microbial growth was not observed on the food samples. In conclusion, the samples investigated have no microbiological risk. Also, consumers should examine their expiration dates during purchase because the expiration dates declared on the foods have showed their microbiological stability.

^a pınarmkaynar@yahoo.com ^{id} <https://orcid.org/0000-0002-8451-1777>

^b gelcin75@gmail.com ^{id} <https://orcid.org/0000-0002-5247-7578>



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Introduction

Foods for particular medical purposes are used to regulate the diet of children with specific diseases, disorders, or medical conditions who are under the care of a doctor. These foods contain essential requirements on their composition such as fat, carbohydrate, sugar, vitamins and minerals (Jampilek et al., 2019). Foods for special medical purposes are used for nutritional problems in all world countries. In Turkey, these foods are defined as specially produced or formulated and used under medical supervision to regulate the diets of patients for special nutritional practices. (Turkish Food Codex Regulation on Microbiological Criteria, 2011). These foods are prepared for patients who have usual foodstuffs/certain nutrition value of the foodstuffs/their metabolites intake, digest, absorb, metabolize, limited of body intake, weakened, or impaired and, for people whose dietary management cannot be only provided with the normal diet modification/the other food/together useage of both. Production, preparation, processing, preservation, storage and transportation of such foods in accordance with the technique and hygienically are required (Turkish Food Codex Regulation on Microbiological Criteria, 2011; Munblit et al., 2020). Medical nutrition products, which are included in dietary foods for special medical purposes are suitable for use as alone a source of nutrition for children

one year of age and three above years of age, provide full and balanced nutrition. As well as importance of the energy and nutrient values on the labels, these foods should be microbiologically safe. At the present time; food poisoning, intoxication or infection can be caused by eating foods that are not suitable microbiologically (Al-Mamun et al., 2018). Therefore, microbiological evaluation of various foods has been performed (Berthold-Pluta et al., 2019; Cai et al., 2019). Especially, infant formula which are included in dietary foods for special medical purposes have been evaluated microbiologically (Jones et al., 2019; Zhuang et al., 2019). The purpose of this study was to determine the microbiological examination of foods for special medical purposes used in children's nutrition, and to measure their compatibility with the relevant legislations.

Material and methods

Material

Eleven samples of 200 mL liquid imported foods (coded P1 to P11) for special medical purposes used in the children's diet were taken from pharmacies in Ankara-Turkey (Table 1).

Tabel 1. Physical and organoleptic examinations of food for special medical purposes samples

Code	Taste	Colour	Flavour
P1	Vanilla	Cream	Specific
P2	Strawberry	Cream	Specific
P3	Chocolate	Brown	Specific
P4	Vanilla	Cream	Specific
P5	Strawberry	Cream	Specific
P6	Chocolate	Brown	Specific
P7*	Strawberry-Raspberry	Pink	Specific
P8*	Apricot	Cream	Specific
P9*	Banana	Cream	Specific
P10*	Vanilla	Cream	Specific
P11*	Chocolate	Brown	Specific

*for children over the age of three

Methods

These foods were analyzed for the presence of *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *E. coli*, *E. coli* O157, *Enterobacteriaceae*, *L. monocytogenes*, and *B. cereus*. The bacterial examinations were done according to each related EN ISO standart methods. The methods were explained in detail below.

Weighing of the samples and preparation of dilutions

First of all, initial suspensions of the samples were prepared according to EN ISO 6887-1 (2017). For this purpose, each sterile stomacher bags containing 90 mL of sterile peptone saline (8.5 g NaCl (Merck), and 1.0 g peptone (Merck), 1000 mL distilled water) were used and 10g of each sample was placed into each sterile stomacher bags and totally homogenized.

Detection and enumeration of *Enterobacteriaceae* in the samples

The detection and enumeration of *Enterobacteriaceae* in the samples was performed according to EN ISO 21528-2 (2017). One mL of the initially prepared dilutions was parallelly transferred petri dishes, added into each Petri dish approximately 15 ml of the Violet Red Bile Glucose (VRBG) Agar (Merck). Inoculated agar plates were incubated at 37±1 °C, for 24±2 h. After incubation, biochemical analyses such as oxidase reaction and glucose fermentation were performed for suspicious colonies which were pink to red or purple, with or without precipitation haloes

Detection and enumeration of *B. cereus* in the samples

The EN ISO 7932 (2004) method was used for the detection and enumeration of *B. cereus* in the food samples. One mL of the initial suspension was dispersed on three dishes with MYP Agar medium (Oxoid), duplicate preparations were made using six plates, and then incubation was carried out at 30±1 °C for 21±3 h. Pink colonies were included in the conventional technique as suspicious colonies after incubation if they were surrounded by an opaque zone because of high lecithinase production in the medium or if they were not surrounded by an opaque zone because of low lecithinase production. For the confirmation of the suspicious colonies, hemolysis test was performed on Sheep Blood Agar (Merck), and incubation was performed at 30±2 °C for 24±2 h. A hemolysis zone on the Sheep Blood Agar (Merck) medium was interpreted as a positive reaction after incubation.

Determination of coagulase-positive staphylococci in the samples

EN ISO 6888-1 (1999) and EN ISO 6888-1:1999/A1 (2003) were used. First, 1 mL of the dilutions prepared was cultured into Baird-Parker Agar medium (Merck) and allowed to incubate at 35±1 °C for 48 ± 2 h. First, Baird-Parker Agar medium (Merck) was cultured with 1 mL of the produced dilutions and incubated at 35±1 °C for 48 ± 2 h.. Following incubation, coagulase was used to confirm the presence of typical colonies, which were black or grey, shiny, convex, and bordered by a clear zone.

Determination of the presence of staphylococcal enterotoxin in samples

With the purpose of determining the presence of staphylococcal enterotoxins, 10 g of each sample were homogenized in 10 mL of 0.85% sterile peptone salt solution (Merck) before being centrifuged at 4 °C for 30 min. After centrifugation, a sterile filter with a 0.20 m diameter (Sartorius) was used to filter the supernatant. Following filtering, the manufacturer-recommended staphylococcal enterotoxin kit procedure (SET-RPLA, Oxoid) was carried out.

Determination of *E. coli* in the samples

The ISO 16649-2 method (2001) was used to determine *E. coli* in the samples. Duplicate plates were inoculated with 1 mL of the initial suspension, and TBX Agar medium (Himedia) was poured into each duplicate plates. The plates were then incubated at 44±1 °C for 18–24 hours. Typical blue colony β- glucuronidase-positive *E. coli* colonies on the medium were counted after incubation.

Determination of *E. coli* O157 in the samples

ISO 16654 (2001) and EN ISO 16654:2001/A1 (2017) were carried out. Each sample, weighing 25 grams, was homogenized with 225 ml of modified Trytone Soya Broth (mTSB+N, Merck), novobiocin, and incubated at 41.5 °C for 6 to 18 hours to enrich for *E. coli* O157. After enrichment, immune-magnetic separation (IMS, LabM) was applied. The samples were then placed onto MacConkey (CT-SMAC) (Oxoid) agar medium and allowed to incubate for 18–24 hours at 37 °C. The suspicious, translucent, and light-yellowish brown colonies that formed after incubation were verified using indole and serological confirmation assays.

Determination of *Salmonella* spp. in the samples

The EN ISO 6579-1 method (2017) was used to identify *Salmonella* spp. in the samples. Pre-enrichment was performed by placing 25g of each sample into sterile stomacher bags, homogenizing the samples with 225 mL of sterile buffered peptone water (Merck), and incubating the samples at 34–38 °C for 18–2 hours. Following pre-enrichment, 0.1 ml and 1 ml of each pre-enrichment culture were inoculated into each Rappaport Vassiliadis Broth (Biokar) and Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn broth) (Biokar), respectively. Inoculated Rappaport Vassiliadis Broth (Biokar) and Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn broth) were incubated at 41.5±1 °C for 24±3 h and at 37±1 °C for 24±3 h, respectively. Following enrichment, 10 µl inocula from each enrichment culture were streaked onto selective agar, Xylose Lysine Deoxycholate (XLD) Agar (Merck) and incubated at 37±1 °C for 24±3 h. Black-centered and colorless suspicious colonies growing in the medium were examined for usage of glucose, non-usage of lactose, non-usage of sucrose, gas formation from glucose, formation of H₂S, urea hydrolysis, L-lysine decarboxylase activity, and the indole test in order to confirm the colonies isolated after incubation. For serological confirmation analysis, the agglutination control, O antigens, Vi antigens, and H antigens were used.

Determination of *L. monocytogenes* in the samples

The EN ISO 11290-1 method (2017) was used for this purpose. In the pre-enrichment step, 225 mL of Half Fraser Broth (LabM) were used to homogenize 25g of each sample placed into sterile stomacher bags, and incubated for 24-26 h at a temperature of 30±1 °C. After pre-enrichment, the sample was put into Fraser Broth (Biokar) and incubation was performed at 37±1 °C for 24±2 h. Following enrichment step, the samples were inoculated on Agar *Listeria* according to Ottaviani and Agosti (Merck) medium and Oxford *Listeria* Selective Agar medium (Merck), and incubated at 37±1 °C for 48±2 h. Blue-green colonies surrounded by an opaque halo on Agar *Listeria* according to Ottaviani and Agosti medium and grayish green colonies with collapsed centers, surrounded by a black halo were accepted as presumptive *L. monocytogenes* colonies. For the purpose of confirming presumed *L. monocytogenes* colonies, beta-hemolysis, L-rhamnose, D-xylose, the catalase test, Gram staining, and the Microbact *L. monocytogenes* identification kit (Oxoid) were utilized.

Eleven foods used in the diet of children for medical purposes were examined physically and organoleptically. In resulting of the physical and organoleptic examination of the samples; 7 of 11 samples were cream, 3 of 11 samples were brown and one of 11 sample was pink. Also, the food samples examined were liquid and had their specific flavours. Results of the physical and organoleptic examinations of the samples were given Table 1. In this study, expiration dates and nutrition facts labeled on food samples were also examined.

Food for special medical purposes samples were evaluated in terms of microbiology as *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *E. coli*, *E. coli* O157, *Enterobacteriaceae*, *L. monocytogenes*, and *B. cereus*. It was not found, in results of tested *Enterobacteriaceae*, coagulase positive

staphylococci, and *L. monocytogenes* for these food samples, atypical and typical colonies grown on their used media. It was determined that one yellow colony grown on MYP agar medium for 2 of 11 samples (P6 and P11 samples) while atypical/typical colonies of *B. cereus* not grown on this medium for the other samples. For P11 sample, seven white colony was grown up on XLD Agar medium. No colonies have observed in this medium for the other samples. In the result of confirmation analyses, it was determined that *Salmonella* spp. and *B. cereus* were not found in these samples. No staphylococcal enterotoxin was also detected. Furthermore, neither *E. coli*, nor *E. coli* O157 colonies were detected in any of the samples.

Nutrition is important for healthy growth and developments of children. For a good nutrition, it is significant to take the necessary energy and nutrients from various foods in the required amounts. Foods are expected to be important in terms of energy and nutrients as well as microbiological safety. Today, child deaths occur due to foodborne infections every year (Faour-Klingbeil and Todd, 2020; Lai et al., 2020). When disaster situations such as earthquakes and floods are encountered in our world, it is seen that it is important to use these foods in order to provide adequate and balanced nutrition for children. In this study, eleven sample of 200 mL liquid imported foods for special medical purposes used in the children's nutrition were examined for microbiological properties as *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *E. coli*, *E. coli* O157, *Enterobacteriaceae*, *L. monocytogenes*, and *B. cereus*. These foods which were purchased from pharmacies were coded, physical and organoleptic examination were applied (Table 1). Expiration dates and nutrition facts labeled on the food samples were also checked. In result of the checking, it was observed that expiration dates were effective. In this study, no *Salmonella* spp., coagulase positive staphylococci, staphylococcal enterotoxin, *E. coli*, *E. coli* O157, *Enterobacteriaceae*, *L. monocytogenes*, and *B. cereus* were also detected. Confirmation tests were applied for suspicious colonies of *Salmonella* spp. (P11) and *B. cereus* (P6 and P11). In resulting of the confirmation tests, these bacteria were not determined. It has been suggested that chocolate aromas of these samples may have caused the suspicious colonies which was observed. Deaths from food poisonings in the countries where foods cannot be properly produced and stored are higher in children. Various studies have been conducted to determine enteric infection risks of children through water and food (Aijuka et al., 2018; Knee et al., 2018). Therefore, microbiological examination of food for special medical purposes samples consumed by children is important. In this study, it was observed that results of microbiological examination of food for special medical purposes were suitable for use as alone a source of nutrition for children one year of age and three above years of age.

Additionally, these samples were evaluated microbiologically based on Turkish Food Codex Regulation on Microbiological Criteria (2011). *Salmonella* spp. (not detected/25g-mL), *Enterobacteriaceae* (<10-1 cfu/g-mL), and *L. monocytogenes* (not detected/25g-mL) in and *B. cereus* (<102 cfu/g-mL) in foods for special medical purposes are specified according to Regulation (Turkish Food Codex Regulation on Microbiological

Criteria, 2011). In this study, it was found that these foods complied with the Turkish Food Codex Regulation on Microbiological Criteria (2011). In 2011, an evaluation study was conducted on lack of cross contamination by species out of label and the amount of viable bacteria in the declared label on dietary supplements for medical use available on the Italian and European market (Toscano et al., 2013). Microbiological evaluation of various foods that children consumed fondly have been investigated (Bentancor et al., 2012). Besides, epidemiological studies on foodborne infections and intoxications have been conducted in different countries (Polański and Sadkowska, 2018; Bruyand et al., 2019; Marus et al., 2019). In a study conducted on microbiological safety of dietary supplements containing plant-derived ingredients, *Enterobacteriaceae* was found to be at a level exceeding of 10^3 cfu/g (Długaszewska et al., 2019). Ratajczak et al. (2015) emphasised that diet supplements, which were provided the missing element in a concentrated form, and also consumed by children, had to comply with the microbiological purity requirements.

Conclusion

Microbiological qualities of foods for special medical purposes as secure foods for children's consumption is very important. In conclusion of this study, investigated food samples for children met the general hygiene criteria, and ensured the food security and food safety. It was also revealed that these foods did not contain agents of gastrointestinal disease and/or food-based intoxication.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Pınar Mursaloğlu Kaynar Collecting samples, performing most of the analysis, writing some sections. Elçin Günaydın writing, performing *Salmonella* and *Listeria* analysis, editing.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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