Chapter 2

Bacillus cereus Isolates Obtained from Food Used in Infant and Child Nutrition[1]

Edip Urhan[2]

H. Yeşim Can[3]

Abstract

In the present study, a total of 80 sample materials were used (30 powdered baby formulas, 25 jarred baby foods, and 25 cereal-based products). The isolation of *Bacillus cereus* from the samples was performed with the classical culture technique. The isolates were confirmed as *B. cereus* with PCR targeting the hemolysin gene. Emetic (*ces*) and diarrheal toxin genes (*nhe, hbl, cytK*) were detected by multiplex PCR in confirmed isolates.

As a result of the study, 62.5% (50/80) of the samples were found to be contaminated with *B. cereus*. *B. cereus* could not be detected in jarred baby foods. *B. cereus* was detected at 83.3% (25/30) and 100% (25/25) in powdered baby foods and cereal-based products, respectively. Thirteen (26%) of 50 isolates confirmed by PCR carried at least one gene responsible for toxin synthesis. Only *nhe* gene was found in three (6%) isolates, and *nhe* and *cytK* genes were found together in 10 (20%) isolates. It was found that none of the isolates had *ces* and *hbl* genes and all isolates were able to form hemolysis on blood agar and two isolates were psychrotrophic. In conclusion, the presence of enterotoxigenic and psychrotrophic *B. cereus* strains in powdered baby formulas and cereal-based products may be risky for infants and young children whose immune systems and intestinal microbiota are not fully developed.

1 It was summarized from the master's thesis. This study was financially supported by Hatay Mustafa Kemal University with project number 21.YL.024.

2 Directorate of Mersin Veterinary Border Control Point, Republic of Türkiye Ministry of Agriculture and Forestry, edip.urhan@gmail.com, Orcid ID: 0000-0002-3765-8784

3 Assoc. Prof., Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, yesimcan@mku.edu.tr, Orcid ID: 0000-0002-5191-6268

https://doi.org/10.58830/ozgur.pub305.c1247

1. Introduction

Although babies need to receive breast milk for their healthy growth and development, ready-made formulas can also be used as an alternative source when breast milk is insufficient or is interrupted (WHO/FAO, 2004; Rahimi et al., 2013; WHO, 2021). Infant formulas are prepared in liquid or powder form to meet the nutritional needs of normal infants, can be used instead of breast milk when necessary, and with a composition close to breast milk (Seyrekbasan, 2000).

Although the composition of baby foods varies according to the age of use, it generally includes skimmed milk, skimmed milk powder, whey powder, lactose, vegetable oils, fish oil, starch, vitamins, minerals, emulsifiers, and stabilizers (Gökçay et al., 2012). Powdered infant formulas, dried dairy products, and other medicinal foods are produced by using similar techniques. In production, the first step is mixing the content, and after mixing the raw materials and additives, the mixture is pasteurized. After pasteurization, homogenization and standardization processes are performed in terms of oil, vitamin, and mineral content. Then, it is packaged with the appropriate packaging method and heat treatment (sterilization) is applied. The final stage is spray drying (Becker et al.,1994; WHO/FAO, 2004; Dalkılıç Kaya, 2011; Gökçay et al., 2012).

Previous studies show that the heat treatment applied during the production of baby foods and supplementary foods used in infant and child nutrition can destroy the vegetative forms of pathogenic bacteria that have the potential to be present in the raw material, but the spore forms are not inactivated and can maintain their viability. Milk and dried dairy products and starch contained in baby foods are suitable media for microorganisms (Shadlia-Matug et al., 2008; Bahçeci et al., 2018; Genç and Vural, 2021). Microorganisms likely to be found in foods used in infant and young children's nutrition were examined in three different categories by the World Health Organization and the Food and Agriculture Organization as *Cronobacter sakazakii*, and *Salmonella enterica*, which are known to cause infection in babies in category A, coliform group bacteria in category B, *B. cereus, Clostridium difficile, Clostridium perfringens, Clostridium botulinum, Staphylococcus aureus,* and *Listeria monocytogenes* are in C category (WHO/FAO, 2004). It was stated that *B. cereus* can be used as an indicator in determining the microbiological safety and contamination level of infant formulas (Pei et al., 2018).

The *B. cereus* microorganism can be found in various environmental sources in the form of spores, it is resistant to adverse environmental

conditions and can maintain its vitality for a long time (Wong et al., 1988; Reyes et al., 2007; Stenfors Arnesen et al., 2008; Tallent et al., 2012; Gdoura-Ben Amor et al., 2019). By synthesizing two different toxins of diarrheal and emetic types, it causes two different foodborne infections (emetic syndrome and diarrheal syndrome) (Kotiranta et al., 2000; Stoeckel et al., 2013). Diarrheal enterotoxins are responsible for the diarrheal syndrome and are heat-sensitive and are produced by bacteria while growing in the intestinal tract. The diarrheal form is characterized by diarrhea and abdominal pain. The proteins responsible for the diarrheal syndrome are hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K (Park et al., 2009; Logan, 2012). The emetic syndrome is characterized by nausea, vomiting, and abdominal cramps, similar to food intoxication caused by *Staphylococcus aureus*, due to the incubation period and observed symptoms (Tewari and Abdullah, 2015; Abraha et al., 2017). Emetic toxin is heat-stable and synthesized in foods. The emetic toxin is designated as "cereulide" and is encoded by the *ces* gene (Sánchez-Chica et al., 2021).

B. cereus is a natural contaminant of raw milk and can often be found in raw milk in spore form, although at low levels (Wong et al., 1988). Dried dairy products may contain *B. cereus* spores (Becker et al., 1994; Pei et al., 2018) and it has been reported that *B. cereus* is the main contaminant in infant formulas (Rahimi et al., 2013).

The purpose of this study was as follows: i) to determine the presence of *B. cereus* in foods used in infant and child nutrition (powdered baby formulas, jarred baby foods, and cereal-based products), ii) to determine the hemolysis-forming ability and psychrotrophic characteristics of isolates, iii) to detect emetic and diarrheal toxin genes (*ces*, *nhe*, *hbl*, *cytK*) in isolates by multiplex PCR method.

2. Material and Method

2.1. Collection of Samples

In this study, 30 powdered baby formulas from different brands (20 imported, 10 domestic formulas), 25 jarred baby foods, and 25 cereal-based products (packaged or unpackaged) that consisted of flour, starch, rice flour, and semolina were investigated. A total of 80 samples were used as the study material. The samples were collected and analyzed under aseptic conditions from the provinces of Hatay and Mersin in March-April-May 2021.

2.2. Isolation and Identification of *B. cereus*

B. cereus isolation was performed in the samples with the classical culture technique (Tallent et al., 2012). Ten grams of each sample were weighed homogeneously into sterile polyethylene bags under aseptic conditions and 90 ml of Tryptic Soy Broth containing 10 mg/ml concentration of polymyxin B was added to the samples. Then, the pre-enrichment broths were incubated at 30°C for 24 hours under aerobic conditions. A loopful of samples was taken from the pre-enriched samples and inoculated on Brilliance *Bacillus cereus* agar. The plates were incubated at 30°C under aerobic conditions for 24-48 hours. Colonies that formed turquoise green pigmentation on the agar at the end of the incubation were considered suspicious for *B. cereus*. Suspicious colonies were confirmed by PCR by targeting the hemolysin gene for *B. cereus* (Wang et al., 1997).

2.3. DNA Extraction

DNA extraction was performed according to the protocol recommended by the commercial nucleic acid extraction kit (Vivantis, Malaysia). Extracted DNA samples were stored at -20°C until PCR.

2.4. Determination of Hemolysis Ability

The obtained isolates were activated in Brain Hearth Infusion Broth, inoculated on blood agar by the streak plating method, and incubated at 37°C under aerobic conditions for 24-48 hours. At the end of the incubation, the development of a greenish or clear zone of hemolysis around the colonies was considered positive.

2.5. Determination of Psychrotrophic Characteristic

The confirmed isolates were activated in Brain Hearth Infusion Broth and then plated onto Tryptone Soy Agar by the streak plating method and incubated under aerobic conditions at 7°C in a refrigerated incubator. The development of growth in the plates was checked at 24, 48, and 72 hours and finally on the 7th day.

2.6. Investigation of Toxin Genes with Multiplex PCR Method

The presence of diarrheal toxin genes (*nhe, hbl, cytK*) and emetic toxin gene (*ces)* in isolates confirmed as *B. cereus* were found with multiplex PCR method. For this purpose, the amplification conditions and primer pairs recommended by Ehling-Schulz et al. (2005, 2006) were used (Table 1).

Table 1. Primer sequences and amplification conditions used in the study.

Genes	**Primer sequences (5'- 3')**	**Product (bp)**	**Reference**
Hemolysin gene	F; CTGTAGCGAATCGTACGTATC R; TACTGCTCCAGCCACATTAC	185	Wang et al. (1997)
Hbl	F; GTA AAT TAI GAT GAI CAA TTTC R; AGA ATA GGC ATT CAT AGA TT	1091	Ehling-Schulz et al. (2006)
Nhe	F; AAG CIG CTC TTC GIA TTC R; ITI GTT GAA ATA AGC TGT GG	766	
cytK	F; ACA GAT ATC GGI CAA AAT GC R; CAA GTI ACT TGA CCI GTT GC	421	
Ces	F; GGTGACACATTATCATATAAGGTG R; GTAAGCGAACCTGTCTGTAACAACA	1271	Ehling-Schulz et al. (2005)

bp: base pair, F: forward, R: reverse

3. Results

As a result of the study, 62.5% (50/80) of the samples were found to be contaminated with *B. cereus*. *B. cereus* was detected at levels of 83.3% (25/30) and 100% (25/25) in powdered baby formulas and cereal-based products, respectively. *B. cereus* was found as 80% (16/20) in imported powdered baby formulas, and 90% in domestic baby powdered formulas (9/10). However, *B. cereus* could not be detected in jarred baby foods.

A total of 50 isolates were confirmed as *B. cereus* with PCR by targeting hemolysin gene (Figure 1). It was observed that all isolates were able to produce hemolysis on blood agar. Also, two isolates (4%, 2/50) were found to be psychrotrophic. In 13 isolates (26%), at least one gene responsible for enterotoxin synthesis was detected and the isolates were found to have the highest number of *nhe* gene. It was also found that three isolates carried only the *nhe* gene (6%, 3/50), and 10 isolates carried the *nhe and cytK* genes (20%, 10/50) together (Figure 2). It was found that the isolates did not carry the *ces* and *hbl* genes.

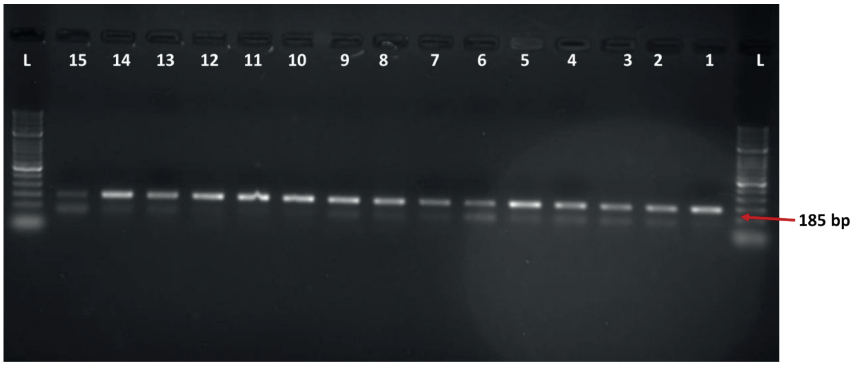

Figure 1. Agarose gel electrophoresis image of the obtained *B. cereus* strains [L; DNA ladder (100 bp), 1: Positive control, 2-15: *B. cereus* positive isolates (185 bp)].

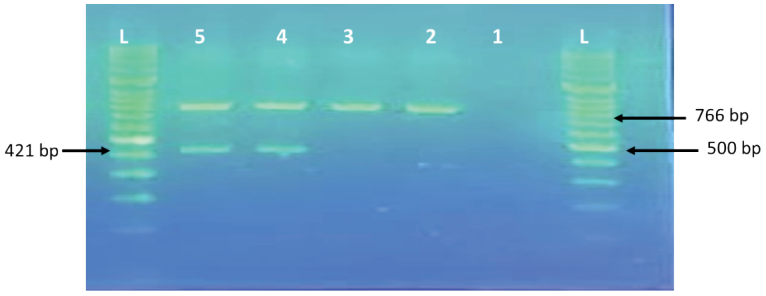

Figure 2. Presence of toxin genes in *B. cereus* positive isolates [L; DNA ladder (100 bp), 1: Negative control, 2-3: Positive for only *nhe* gene (766 bp), 4-5: Positive for both *cytK* and *nhe* genes (421 bp, 766 bp)].

4. Discussion

When the studies performed in our country and abroad were reviewed, Özdemir (2003) reported that *B. cereus* was present in 46.6% of the pasteurized milk offered for sale in Ankara. Can et al. (2022) reported that they detected this bacterium in 12 (34.2%) of 35 raw milk samples. Yıbar et al. (2017) found *B. cereus* at the level of 3.8% (2/53) and 26% (13/26) in raw milk and pasteurized milk samples, respectively.

In the study conducted by Te Giffel et al. (1997) in the Netherlands, *B. cereus* was isolated from 133 (40%) of 334 pasteurized milk samples. In the study conducted by Ahmed et al. (1983) in the United States, 400 milk and its products collected from different retail outlets for 5 months were analyzed for the presence of *B. cereus*. Although *B. cereus* was detected in a total of 103 samples (25.75%), it was found that raw milk and pasteurized milk were contaminated with *B. cereus* at 9% and 35% levels, respectively.

It was reported that the higher presence of *B. cereus* in pasteurized milk compared to raw milk may be due to the germination of spores due to the heat treatment applied in pasteurization. These studies show that pasteurized milk can often be contaminated with *B. cereus*. Competitive microflora is suppressed by the heat treatment applied in pasteurized milk production and due to the absence of competitive microflora in the product, *B. cereus* spores can easily develop as germs (Ahmed et al., 1983; Wong et al., 1988).

In a total of 30 powdered baby formulas analyzed, *B. cereus* was detected at a rate of 83.3%. In this context, when the level of *B. cereus* was examined in baby foods, contrary to the present study findings and at lower levels, Seyrekbasan (2000) stated that *B. cereus* was detected in 1 (2.5%) of a total of 40 imported powdered infant formulas belonging to various companies, Ergün et al. (2002) in 2 (4%) of 50 powdered baby foods, Bahçeci et al. (2018) reported that *B. cereus* was detected in 2 (10%) of a total of 20 imported or local baby foods. Unlike our study findings, Vural and Genç (2022) reported that *B. cereus* could not be detected in a total of 30 samples (15 of infant formula, 15 of follow-on formula) collected from the markets in Diyarbakır.

B. cereus could not be detected in a total of 25 jarred baby foods in our study. Similar to our study findings, Ergün et al. (2002) reported that *B. cereus* could not be detected in a total of 10 jarred baby foods. Unlike our study findings, Vural and Genç (2022) stated that *B. cereus* was was detected in 13.3% (2/15) of fruit-based jarred baby foods.

At a higher level than our study findings, Pei et al. (2018) found that 92.4% of 6656 powdered infant milk and follow-on formula samples collected from markets in China were contaminated with *B. cereus*. At lower levels than our study, in Colombia, Sánchez-Chica et al. (2021) detected *B. cereus* in infant milk by 11% (8/75), Becker et al. (1994) detected *B. cereus* to be 54% of 194 infant milk and follow-up formulas, Rahimi et al. (2013) isolated *B. cereus* from 84 (42%) of 200 infant formulas sold in Iran, Reyes et al. (2007) reported that *B. cereus* was isolated in 175 (45.9%) of a total of 381 dried milk products (milk-rice, milk-semolina, milk-rice-cereal samples, milk powder, milk pudding).

As a result, in studies that were conducted in our country and abroad, the presence of *B. cereus* in baby foods was determined at lower (Becker et al., 1994; Seyrekbasan, 2000; Ergün et al., 2002; Rahimi et al., 2013; Bahçeci et al., 2018; Sánchez-Chica et al., 2021; Vural and Genç, 2022) or higher levels (Pei et al., 2018) than in our study. This may be because of the number of samples was different, the characteristics of the samples (composition,

production technique, packaging condition) collection of samples from different geographical places, the different methods used in the isolation and identification of the bacterium.

In the present study, all cereal-based products were found to be contaminated with *B. cereus* (100%, 25/25). Unlike our study, Vural and Genç (2022) could not detect *B. cereus* in a total of 15 cereal-based supplementary foods. Bahçeci et al. (2018) determined it in 1 (5%) of a total of 20 grain-based foods, and the sample found positive was unpackaged semolina. *B. cereus* was found at the level of 11% in 155 grain-based samples (wheat flour, corn starch) analyzed in Colombia (Sánchez-Chica et al., 2021), and in 25% of the total 293 grain samples (rice, barley) analyzed in Korea (Park et al., 2009), these results are much lower than our study findings.

In this study, it was determined that 4% of 50 isolates identified as *B. cereus* were psychrotrophic. Higher than our study findings, Te Giffel et al. (1997) reported that 53% of 106 isolates were obtained from pasteurized milk samples, and Can et al. (2022) reported that 66.6% of 12 *B. cereus* isolates isolated from raw milk were psychrotrophic. However, contrary to our study findings, Reyes et al. (2007) stated that none of the 28 enterotoxigenic *B. cereus* strains isolated from dried dairy products were psychrotrophic.

It was found in our study that 13 (26%) of 50 isolates confirmed as *B. cereus* carried at least one gene responsible for toxin synthesis. Although it was determined that three isolates carried only *nhe* gene (6%), and 10 isolates carried the *nhe* and *cytK* genes (20%), the *ces* and *hbl* genes were not detected in any isolates. Similar to our study findings, Reyes et al. (2007) reported that 28 (29.8%) of 94 isolates obtained from dried dairy products were enterotoxigenic.

Gdoura-Ben Amor et al. (2019), Park et al. (2009), and Sánchez-Chica et al. (2021) identified enterotoxin genes (*nhe*, *hbl*, *cytK*) at higher levels than our study. Sánchez-Chica et al. (2021) reported that 100%, 63.4%, and 62% of 52 isolates confirmed as *B. cereus* isolated from various food samples in powder form (baby milk, milk powder, wheat flour, corn starch) had *nhe, hbl,* and *cytK* genes, respectively. Gdoura-Ben Amor et al. (2019) reported the highest number of *nhe* (100%), *bceT* (60.9%), *hbl* (52.2%), and *cytK* (39.1%) genes in 23 isolates in cereal products, respectively. Park et al. (2009) reported that 73 *B. cereus* isolates obtained from various cereal-based samples had the most *nhe* (99%), *hbl* (84%), and *cytK* (55%) genes, respectively.

Contrary to our study findings and at a higher level, Te Giffel et al. (1997) found that 76% of 37 isolates isolated from pasteurized milk samples carried the *hbl* gene. Unlike our study, Rahimi et al. (2013) found the most *entFM* gene (61.9%) in 84 isolates obtained from infant formula.

Similar to our study findings, the *ces* gene responsible for emetic syndrome could not be detected in *B. cereus* isolates in the studies of Gdoura-Ben Amor et al. (2019) and Sánchez-Chica et al. (2021). Also, consistent with our study findings, the detection of the highest number of *nhe* genes in different studies (Park et al., 2009; Gdoura-Ben Amor et al., 2019; Sánchez-Chica et al., 2021) supports that nhe is the enterotoxin most responsible for diarrheal syndrome (Stenfors Arnesen et al., 2008).

5. Conclusion

As a result, it can be speculated that the presence of enterotoxigenic and psychrotrophic *B. cereus* in the powdered baby formulas and cereal-based products examined may be dangerous in the nutrition of infants and young children whose immune systems and intestinal microflora are not fully developed. Maximum attention must be paid to the implementation of national and international food quality safety systems at all stages from production to consumption of the foods used in the nutrition of this age group.

References

Abraha A, Bikila T, Alemu S, Muktar Y. *Bacillus cereus* isolation and load from raw cow milk sold in markets of Haramaya District, eastern Ethiopia. Food Contam 2017; 4: 15.

Ahmed AA, Moustafa MK, Marth EH. Incidence of *Bacillus cereus* in milk and some milk products. J Food Protect 1983; 46 (2): 126-128.

Bahçeci T, Çakmak Sancar B, Özpınar H. Bebek beslenmesinde kullanılan gıdaların mikrobiyolojik kalitelerinin araştırılması. Aydın Gastronomy 2018; 2(1):15-20.

Becker H, Schaller G, von Wiese W, Terplan G. *Bacillus cereus* in infant foods and dried milk products. Int J Food Microbiol 1994; 23(1): 1-15.

Can HY, Elmalı M, Karagöz A, Dişli HB. Psychrotrophic properties, toxigenic characteristics, and PFGE profiles of *Bacillus cereus* isolated from different foods and spices. Cienc Rural 2022; 52(4): 1-11.

Dalkılıç Kaya G. Süt ürünleri ve bebek mamalarında *Enterobacter sakazakii (Cronobacter* spp.) varlığının araştırılması ve gelişmesine sıcaklık ve şeker çeşitlerinin etkisi. Doktora Tezi. İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü, İstanbul, 2011.

Ehling-Schulz M, Svensson B, Guinebretiere MH, Lindbäck T, Andersson M, Schulz A, Fricker M, Christiansson A, Granum PE, Märtlbauer E, Nguyen-The C, Salkinoja-Salonen M, Scherer S. Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology (Reading). 2005; 151(Pt 1): 183-197.

Ehling-Schulz M, Guinebretiere MH, Monthan A, Berge O, Fricker M, Svensson B. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. FEMS Microbiol Lett 2006; 260(2): 232-240.

Ergün Ö, Aksu H, Arun ÖÖ, Çolak H. Ülkemizde satılan bebek ve çocuk mamalarında gıda zehirlenmesine neden olan önemli bazı mikroorganizmaların varlığı üzerine araştırmalar. Gıda 2002; 27(4): 253-257.

Gdoura-Ben Amor M, Jan S, Baron F, Grosset N, Culot A, Gdoura R, Gautier M, Techer C. Toxigenic potential and antimicrobial susceptibility of *Bacillus cereus* group bacteria isolated from Tunisian foodstuffs. BMC Microbiol 2019; 19(1): 196.

Genç E, Vural A. Bebek ve küçük çocuk gıdalarında bakteriyel sağlık riskleri. Turk Mikrobiyol Cem Derg 2021; 51(1): 1-10.

Gökçay G, Eren T, Devecioğlu E. Bebek mamalarındaki katkı maddeleri. Çocuk Derg 2012; 12(2): 60-65.

Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes Infect 2000; 2(2): 189-198.

Logan NA. *Bacillus* and relatives in foodborne illness. J Appl Microbiol 2012;112(3): 417-429.

Özdemir H. Pastörize sütlerde *Bacillus cereus*'un varlığı. Gıda 2003; 28(6): 611-614.

Park YB, Kim JB, Shin SW, Kim JC, Cho SH, Lee BK, Ahn J, Kim JM, Oh DH. Prevalence, genetic diversity, and antibiotic susceptibility of *Bacillus cereus* strains isolated from rice and cereals collected in Korea. J Food Prot 2009; 72(3): 612-617.

Pei X, Yang S, Zhan L, Zhu J, Song X, Hu X, Liu G, Ma G, Li N, Yang D. Prevalence of *Bacillus cereus* in powdered infant and powdered follow-up formula in China. Food Control 2018; 93: 101-105.

Rahimi E, Abdos F, Momtaz H, Baghbadorani ZT, Jalali M. *Bacillus cereus* in infant foods: Prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. Sci World J 2013; 292571.

Reyes JE, Bastías JM, Gutiérrez MR, Rodríguez Mde L. Prevelance of *Bacillus cereus* in dried milk products used by Chilean School Feeding Program. Food Microbiol 2007; 24(1): 1-6.

Sánchez-Chica J, Correa MM, Aceves-Diez AE, Castañeda-Sandoval LM. Genetic and toxigenic diversity of *Bacillus cereus* group isolated from powdered foods. J Food Sci Technol 2021; 58(5): 1892-1899.

Seyrekbasan BK. Türkiye'de kullanılan ithal bebek mamalarının mikrobiyolojik kalite kontrolleri üzerinde araştırmalar. Yüksek Lisans Tezi. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara, 2000.

Shadlia-Matug M, Aidoo KE, Candlish AAG, Elgerbi AM. Evaluation of some antibiotics against pathogenic bacteria isolated from infant foods in North Africa. *TOFSJ* 2008; 2: 95-101.

Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett 2008; 32(4): 579-606.

Stoeckel M, Westermann A, Atamer Z, Hinrichs J. Thermal inactivation of *Bacillus cereus* spores in infant formula under shear conditions. Dairy Sci Technol 2013; 93(2): 163-175.

Tallent SM, Kotewicz KM, Strain EA, Bennett RW. Efficient isolation and identification of *Bacillus cereus* group. J AOAC Int. 2012; 95(2): 446-451.

Te Giffel MCT, Beumer RR, Granum PE, Rombouts FM. Isolation and characterisation of *Bacillus cereus* from pasteurised milk in household refrigerators in the Netherlands. Int J Food Microbiol 1997; 34(3): 307-318.

Tewari A, Abdullah S. *Bacillus cereus* food poisoning: international and Indian perspective. J Food Sci Technol 2015; 52(5): 2500-2511.

Vural A, Genç E. Hygienic quality features in baby formulas, follow-on formulas, and some supplementary foods. Acta Vet Eurasia 2022; 48(2): 109-116.

Wang RF, Cao WW, Cerniglia CE. A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. J Appl Microbiol 1997; 83 (6): 727-736.

WHO. Fact sheets. Infant and young children feeding. 2021. Erişim: https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. Erişim tarihi: 17.02.2022.

WHO/FAO (World Health Organization/Food and Agriculture Organization of the United Nations). *Enterobacter sakazakii* and other microorganisms in powdered infant formula: Meeting report. Microbiological Risk Assessment Series 6, 2004. Erişim: https://www.who.int/publications/i/item/9789241562775. Erişim tarihi: 15.11.2021.

Wong HC, Chang MH, Fan JY. Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products. Appl Environ Microbiol 1988; 54 (3): 699-702.

Yıbar A, Çetinkaya F, Soyutemiz E, Yaman G. Prevalence, enterotoxin production and antibiotic resistance of *Bacillus cereus* isolated from milk and cheese. Kafkas Univ Vet Fak Derg 2017; 23(4): 635-642.