Investigation of Extended Spectrum B-Lactamases (ESBL)-Producing *Enterobacteriaceae* and *Cronobacter* Spp in Infant Formulas and Cereal-Based Foods for Children

Pınar CAVA GÜMÜŞ[*], İsmail Hakkı TEKİNER[**], Burcu ÇAKMAK[***], Zeynep TACER CABA[****], Haydar ÖZPINAR[*****]

Abstract

Aim: The extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* have attracted attention due to their ability of delaying or cancelling antimicrobial therapy. The foods for infants and children may occasionally contain pathogens such as ESBL-producing *Enterobacteriaceae*, including *Cronobacter* spp., because of inappropriate production, storage and handling conditions. This situation leads to a greatest risk of infection to infants and children through their consumption. The objective of this study was to investigate the occurrence of ESBL-producing *Enterobacteriaceae*, including *Cronobacter* spp., from a total of 115 samples of various foods from different brands for infants and children sold in Istanbul, Turkey (20 locally produced infant formula, 20 imported infant formula, 20 starch, 20 rice flour, 20 semolina, and 15 milk powder).

Methods: For isolation of *Enterobacteriaceae* ISO 21528-2:2004 was followed. The isolated colonies were identified by Mass Spectrometer. The identified colonies were then exposed to pre-enrichment at 35-37°C for 18-24 hours, the suspension was transferred to a Chromogenic ESBL selective media, and allowed for an incubation at 35-37°C for 18-24

Özgün Araştırma Makalesi (Original Research Article)
Geliş Tarihi: 11.01.2017 **Kabul Tarihi:** 06.03.2017
[*] Medical Specialist, Trainer, Solgar Vitamin & Herb, E-mail: pinar.cava@hotmail.com
[**] Assist. Prof. Dr., Istanbul Gelisim University, School of Applied Sciences, Department of Gastronomy, Istanbul, Turkey, E-mail: ihtekiner@gelisim.edu.tr
[***] Assist. Prof. Dr., Istanbul Esenyurt University, School of Health Sciences, Department of Nutrition and Dietetics, Istanbul, Turkey, E-mail: burcucakmak@esenyurt.edu.tr
[****] Assist. Prof. Dr., Istanbul Aydin University, Faculty of Engineering, Department of Food Engineering, Istanbul, Turkey, E-mail: zeynepcaba@aydin.edu.tr
[*****] Prof. Dr., Istanbul Aydin University, Faculty of Engineering, Department of Food Engineering, Istanbul, Turkey, E-mail: haydarozpinar@aydin.edu.tr

hours again. The presumptive ESBL producers were subjected to ESBL screening test using a combination of ceftazidime, cefotaxime, and cefpodoxime ± clavulanic acid. Finally, MIC values for ESBL confirmation were conducted by Merlin Micronaut-S beta-lactamase VII kit, the readings were automatically evaluated by Sifin Software integrated with a spectrometer. All procedures were performed according to the Guidelines of CLSI (2013).

Results: Of 18 ESBL suspected isolates (7 *P. agglomerans*, 1 *E. aerogenes*, 3 *E. cloacae/asburiae*, 2 *K. oxytoca*, 2 *L. adecarboxylata*, and 3 *C. sakazakii*), 5 isolates (*3 P. agglomerans*, 1 *E. cloacae/ asburiae*, and 1 *E. aerogenes*) were positive for ESBL-production, whereas none of *C. sakazakii* isolates was ESBL-producers.

Discussion and Conclusion: Some of the analysed infant formulas and cereal-based foods for children (locally produced infant formulas, rice flour, and starch) were determined to be posing a serious health risk for the infants and children due to contamination by ESBL-producing *Enterobacteriaceae*, except for *C. sakazakii* isolates.

Keywords: Cereal-based food, children, *cronobacter* spp., *enterobacteriaceae*, esbl, infant formula.

Bebek Mamaları ve Tahıl Bazlı Çocuk Gıdalarında Genişlemiş Spektrumlu B-Laktamazlar (GSBL) Üreten *Enterobacteriaceae* ve *Cronobacter* Spp. Araştırılması

Öz

Amaç: Genişlemiş spektrumlu β-laktamaz (GSBL) üreten *Enterobacteriaceae* türleri antimikrobiyal tedavileri başarısız kıldıkları için artan şekilde ilgi görmektedirler. Yenidoğan ve çocuk gıdaları uygunsuz üretim, depolama ve hazırlama koşulları sebebiyle GSBL-üreten *Enterobacteriaceae* suşları, özellikle *Cronobacter* türleri dahil olmak üzere, patojen mikroorganizmalar içerebilmektedir. Bu tür gıdaların tüketilmesi yenidoğan ve çocuk sağlığı açısından son derece tehlikeli enfeksiyonlara yol açmaktadır. Bu çalışmada İstanbul ilinde satışa sunulan farklı markalara ait toplam 115 örnekte (20 yerli üretim bebek maması, 20 ithal bebek maması, 20 nişasta, 20 pirinç unu, 20 irmik ve 15 süt tozu) GSBL-üreten *Enterobacteriaceae* ve *Cronobacter* türlerinin varlıklarının araştırılması amaçlanmıştır.

Yöntem: Örneklerde *Enterobacteriaceae* türlerinin izolasyonu ISO 21528-2:2004 talimatları takip edilerek yapılmıştır. İzole edilen *Enterobacteriaceae* türleri Kütle Spektrometresi ile tiplendirilmiştir. Tiplendirmesi tamamlanmış izolatlar ön

zenginleştirme amaçlı 35-37°C/18-24 saat inkübe edildikten sonra, kromojenik GSBL seçici besiyerine transfer edilmiş ve tekrar 35-37°C/18-24 saat inkübasyona bırakılmıştır. İnkübasyon sonunda gelişen GSBL şüpheli koloniler seftazidim, sefotaksim ve sefpodoksim ± klavulanik asit içeren antibiyotik diskler kullanılarak incelenmiştir. İnceleme sonucu güçlü şüpheli GSBL pozitif olduğu belirlenen kolonilerin MİK değeri tespiti Merlin Micronaut-S beta-lactamase VII kiti kullanılarak yapılmış, MİK bulguları spektrometre tarafından ölçülmüş ve Sifin yazılımı ile otomatik olarak değerlendirilmiştir. GSBL tarama ve MİK tespiti CLSI (2013) talimatları takip edilerek yapılmıştır.

Bulgular: Mikrobiyolojik İnceleme sonucu elde edilen toplam 18 adet GSBL şüpheli izolat arasında (7 adet *P. agglomerans*, 1 adet *E. aerogenes*, 3 adet *E. cloacae/asburiae*, 2 adet *K. oxytoca*, 2 adet *L. adecarboxylata* ve 3 adet *C. sakazakii*), 5 adet izolatın (3 adet *P. agglomerans*, 1 adet *E. cloacae/ asburiae* ve 1 adet *E. aerogenes*) GSBL pozitif oldukları, diğer taraftan *C. sakazakii* izolatların GSBL üretmedikleri tespit edilmiştir.

Tartışma ve Sonuç: Yerli üretim bebek mamaları, pirinç unu ve nişasta örneklerinin, *C. sakazakii* izolatları hariç, GSBL-üreten *Enterobacteriaceae* türleri içerdikleri, bu sebeple bebek ve çocuk sağlığı açısından ciddi sağlık riski oluşturdukları görülmüştür.

Anahtar Sözcükler: Tahıl bazlı gıda, çocuk, *cronobacter* spp., *enterobacteriaceae*, gsbl, bebek maması.

Introduction

Incidence of increasing food-borne disease over recent decades seems be related mostly to the increase in diseases caused by microorganisms in food. Having a less well-developed immune system and lack of competing organisms in the gut flora, infants are more susceptible to infections by foodborne pathogens. The foods consumed by neonates and infants represent a rich source of nutrients from various origins. They may carry potential risk of being exposure to major food borne pathogens[1].

Cronobacter spp. and other *Enterobacteriaceae* can cause foodborne illnesses from a variety of foods, including infant foods[2-4]. *Cronobacter* spp. causes severe illness in susceptible neonates and infants. Even though infections associated with *Cronobacter* spp. have low frequencies, the infections, such as necrotizing

enterocolitis (NEC), septicemia, and meningitis can be fatal[5]. *Cronobacter spp*. has a more than 40% mortality rate in infected infants. Survivors suffer from severe neurological complications[6]. In Europe and the United States of America (USA), outbreaks of *Cronobacter* spp. have been reported associated with contaminated powdered infant formula[6-8]. Specific international attention by leading organizations of the United Nations such as Food and Agriculture Organization (FAO) and World Health Organization (WHO) has been given to the safety of food for infants and young children. These organizations have completed two international risk assessments on the specific topic of *Cronobacter* spp. prevalence to provide risk management guidance at the international level[9,10]. Widespread using of antibiotics causes emergence of antibiotic resistant bacteria. Antimicrobial resistance to gram-negative bacteria is increasing. The common resistance mechanism in Gram-negative bacteria is the β-lactamase production.[11] Extended-spectrum β-lactamases (ESBLs) are bacterial enzymes that might have resistance to most β-lactam antibiotics such as penicillin and cephalosporins. Moreover, because of their plasmid-associated genes, ESBLs might confer co-resistance to other antibiotics[12]. ESBL-producing *Enterobacteriaceae* are a threat to health care and increasing worldwide especially over the past decade. ESBL-producing *Enterobacteriaceae* prevalence has increased[13,14].

ESBL-producing *Enterobacteriaceae* have been isolated from different food products and animals. This is evidence that microorganisms can be transferred from animal to human[13]. When compared with infection due to susceptible strains, infections due to ESBL-producing *Enterobacteriaceae* are associated with a delay in initiation of appropriate antibiotic therapy that consequently increases patients' mortality, morbidity and treatment costs[14,15].

Antibiotic resistant *Cronobacter* spp. have been reported in some isolates from food samples, although most isolates are susceptible to commonly used antimicrobial agents[16-18]. With their less well-developed immune system and lack of competing organisms in the gut flora, infants are more susceptible to infections by foodborne pathogens. Neonatal deaths from ESBL producing *Cronobacter* spp. have been reported in the previous studies[7].

Infant formulas might be a source of contamination for clinical outbreaks of neonatal infections. A variety of powdered infant formula environmental samples from milk powder factories presented a possible risk. Moreover, the blenders and cleaning brushes that are used to prepare infant food in hospital kitchens have been reported as places where *Cronobacter* spp. has previously been isolated. Different possible sources of *Cronobacter* spp., need to be well understood[19].

Cereals are the common ingredients in infant and follow-up formulas. Prevalence of ESBL-producing *Enterobacteriaceae Cronobacter* spp. in some cereal commodities such as oat, barley and others was reported previously [4,5,20].

In this study, we aimed to investigate the occurrence of ESBL-producing *Enterobacteriaceae* and *Cronobacter* spp., in 115 samples of various foods from different brands for infants and children sold in Istanbul, Turkey.

Material and Methods

Material

Reference cultures

ESBL-negative strain *E. coli* ATCC®25922 and ESBL-positive strain *K. pneumoniae* ATCC®700603 were used for testing controls.

Sampling

A total number of 115 samples from different brands of infant formulas and cereal consumed by neonates and children were collected from different markets and public bazaars in İstanbul, Turkey. 34.8% of the samples were infant formulas (20 locally produced and 20 imported from abroad) and 65.2% were cereal - based infant formulas (20 starch, 20 rice flour and 20 semolina) and 15 milk

powders. These cereal-based formulas are used for preparation of domestic infant formulas.

Sample preparation, isolation and identification of *Enterobacteriaceae*

For isolation of *Enterobacteriaceae* ISO 21528-2:2004 was followed.[21]10 grams of each sample were added to 90 ml Maximum Recovery Diluent (MRD) (LAB103,UK). For isolation 1 ml sample was inoculated to violet red bile glucose agar (VRBGA)(LAB88, UK) and incubated at 37°C for 24 hours. Oxidase testing was done for VRBGA-positive isolates by Merck Bactident®Oxidase Kit. Oxidase negative isolates plated on Bromcresol Purple (BCP) glucose agar for confirmation of *Enterobacteriaceae* and, then streaked onto Nutrient Agar plates for identification by Vitek® MS (bioMérieux, France). After incubation at 37 °C for 24 hours, changes in color from purple to yellow of the BCP glucose agar (Conda, Catno 1320, Spain) were accepted as gram negative enteric bacteria. Oxidase negative/glucose positive colonies were accepted as *Enterobacteriaceae*.

Screening and Confirmation of ESBLs and Minimal Inhibitory Concentration (MIC)

Gram negative enteric bacteria isolates inoculated to ESBL selective - agar (Liofilchem 610629, Italy) were identified for investigation of ESBL producing isolates. After incubation at 37°C for 24 hours, positive strains were adjusted to McFarland 0.5 standard and used to inoculate Mueller Hinton Agar (LAB39, England) surface with a cotton swab for antibiotic susceptibility test. The antibiotic susceptibility test was performed using an agar disc diffusion test based on ceftazidime (CAZ), cefotaxime (CTX), cefpodoxime (CPD) with/without clavulanic acid (CV) according to CLSI 2013 criteria[22].

Micronaut-S beta-lactamase VII Plate (MerlinDiagnostika, Germany) was used for the confirmation of ESBL-production. A 50 µL aliquot of 0.5 McFarland-standardized microbial suspension of the isolate was initially vortexed in 10mL of

Mueller Hinton Broth (Merck, Germany). Subsequently, 100 µL of this suspension was pipetted in to each well of the plate. Then, the plate was incubated overnight at 37^0C. The readings were obtained by using a Thermofisher Multiskan FC Spectrometer, and the MIC analysis was automatically done by MCN6 Software (Sifin, Germany).

Results and Discussion

Bacterial growth was observed on VRBGA from 18 (16%) of the 115 infant formulas and cereal product (starch, rice flour, semolina) samples tested. According to results, ready to use import infant formulas and milk powders were free of any bacteria but domestic infant formulas and cereal-based infant formulas were contaminated with *Enterobacteriaceae*. The isolates were identified by Vitek® MS.

P. agglomerans (n=3) was isolated from domestic infant formulas and *P. agglomerans* (n=4), *E. aerogenes* (n=1), *E. cloacae/asburiae* (n=3), *K. oxytoca* (n=2), *L. adecarboxylata* (n=2) were isolated from cereal-based infant formulas. *C. sakazakii* were found as 5% (n=1) in starch, 5% (n=1) in rice flour, and 5% (n=1) in semolina.Details of identified *Enterobacteriaceae*were presented in Table 1.

Table 1. Types of *Enterobacteriaceae* isolated from the samples

Sample	**No. of isolates on VRBGA (n)**	**Type**
Domestic Infant Formulas	3	*P. agglomerans*
Starch	1	*C. sakazakii/maloniticus*
Rice Flour	2	*P. agglomerans*
	1	*C. sakazakii*
	1	*P. agglomerans*
	1	*E. aerogenes*
	3	*E. cloacae/asburiae*
Semolina	1	*C. sakazakii*
	2	*K. oxytoca*
	2	*L. adecarboxylata*
	1	*P. agglomerans*

After the isolates were identified by Vitek® MS, antibiotic susceptibility was tested using an agar disc diffusion test based on CAZ, CTX, and CPD with/without CV according to the CLSI (2013) criteria22. *P. agglomerans* (n=2) isolated from domestic infant formulas, *E. cloacae/ asburiae* (n=1), *E. aerogenes* (n=1) isolated from rice flour and *P. agglomerans* (n=1) isolated from starch were positive for ESBL. But, no *C. sakazakii* contained ESBL. The ESBL positive isolates and zone diameters were given in Table 2.

The antibiotic susceptibility results revealed that 10 % of domestic infant formulas (n=2), 10% of rice flour (n=2), and 5% of semolina (n=1) were positive for ESBL-producing bacteria. It showed that microbiologic quality criteria of domestic infant formula samples were lower than the samples of imported infant formula[23].

Similarly, our findings also revealed that the local infant formulas were contaminated by ESBL-producing *Enterobacteriaceae* in consistent with previous literature findings[23].

Cronobacter spp. have been isolated from various food product such as infant formulas, cereal-based foods, vegetables. *Cronobacter* spp. have been isolated from infant formula and semolina samples[4]. In another study conducted in Ankara, Turkey, *Cronobacter* spp. from 4 (rye flour, rice flour, fennel and oat flour) of 12 food ingredients were tested[24].

Table 2. Screening and confirmation results of ESBL suspected isolates

Samples	Antibiotic susceptibility for ESBL	Confirmatory Test Zone Diameter (mm)						Type of Enterobacteriaceae
		CPD	CPD CV	CAZ	CAZ CV	CTX	CTX CV	
Domestic Infant Formula-1	positive	30	30	23	32	35	35	*P. agglomerans*
Domestic Infant formula-2	positive	22	28	27	32	22	27	*P. agglomerans*
Rice Flour-1	positive	13	19	26	32	30	36	*E. cloacae/ asburiae*
Rice Flour-2	positive	21	26	24	30	30	36	*E. aerogenes*
Starch-1	positive	22	23	30	-	28	33	*P. agglomerans*

Another study investigated the microbiological safety of infant formulas and other foods for children. *Cronobacter* spp. from samples of infant formula and cereal based follow-up formula was detected[1]. Another recent study has found *Cronobacter* spp. was available in cereal-based products and this had the highest prevalence in other samples[6].

In our study, no *Cronobacter* contamination was detected in the infant formulas. But, it detected in starch, rice flour and semolina samples.

Pantoea spp. are opportunistic pathogens and cause urinary infections, blood sepsis, fatal infections and are associated with outbreaks in neonatal units[25,26]. Mardaneh and Dallal (2013) investigated 125 infant formulas for *P. agglomerans* and they isolated these bacteria from 8 of these samples. In this study *P. agglomerans* were found in 3 of domestic infant formula samples. Its inherent capability to remain viable and grow well at room temperature was previously stated as a possible cause of its prevalence[23]. They also assessed this microorganism for antibiotic resistance according to the CLSI criteria. Their results showed that 50% of isolates were resistant to antibiotics. In this present

study, ESBL-producing *P. agglomerans* was also isolated from infant formula and semolina samples, while *E. cloacae/asburiae* and *E. aerogenes* were isolated from rice flour. Our findings showed that ESBL-producing *Enterobacteriaceae* can be isolated from infant formula and cereal-based infant formula samples and this might be a potential risk to infant health and the success of antimicrobial therapy.

Extended spectrum beta-lactamases (ESBL) are a growing health concern worldwide. The local, regional, national, and international epidemiological studies for ESBL-producing *Enterobacteriaceae* and their encoding genes in the foods and in various geographical locations, including Turkey remain incompleted[3]. The Turkish authorities undertake that the use of antibiotics in the food animals to make them grow faster and/or to prevent disease can not be controlled effectively because the economic benefits of food-animals sector ignore the human and animal health[27]. On the other hand, some studies have been performed in this field in Turkey. Ondes and Ozpinar (2016) determined the occurrence of ESBL-producing *Enterobacteriaceae* in cubed beef samples, and Tekiner and Ozpinar (2016) detected ESBL-producing *Enterobacteriaceae* and the characteristics of their encoding genes from raw chicken meat, raw cow milk, and raw cow milk cheese sold in Turkey.

ESBL-producing *Enterobacteriaceae* causes infections and these infections are associated with delaying the initiation of antimicrobial therapy, increasing morbidity and mortality of the patients when compared with susceptible bacterial infections[14,28]. Prevalence of ESBL producing bacteria is becoming more common, and creating a risk for the success of antimicrobial therapy.

Conclusion

The present study focused on ESBL-producing *Enterobacteriaceae* and *Cronobacter* spp. in both samples of infant formula and cereal-based infant formula in Turkey and reported the situation of ESBL producing Gram negative enteric bacteria, in particular*, C. sakazakii* from domestically produced infant

formulas as well as cereal-based infant formulas in Turkey. In this study, it was observed that the ESBL-producing *Enterobacteriaceae* strains are resistant to antibiotics. However, widespread usage of antibiotics might result in the emergence of antibiotic resistant bacteria. Babies' insensitivity to antibiotic treatment and infant formula are among the most common sources of antibiotic resistant bacteria for babies. In conclusion, some of the analysed infant formulas and cereal-based foods for children (locally produced infant formulas, rice flour, and starch) were determined to be posing a serious health risk for the infants and children due to contamination by ESBL-producing *Enterobacteriaceae*, except for *C. sakazakii* isolates.

REFERENCES

1. Kim SA, Oh SW, Lee YM, Imm JY, Hwang IG, Kang DH, Rhee MS. Microbial contaminationof food products consumed by infants and babies in Korea. *Lett ApplMicrobiol*. 2011;53: 532–538.
2. Ondes N, Ozpinar H. Occurrence of ESBL-producing Enterobacteriaceae in cubed beef samples. *KafkasUniv Vet Fak Derg*. 2016; 22(1): 79-83.
3. Tekiner IH, Ozpinar H. Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. *Braz J Microbiol*. 2016; 47(2): 444-51.
4. Shaker R, Osaili T, Al-Omary W, Jaradat Z, Al-Zuby M.Isolation of *Enterobacter sakazakii* and other *Enterobacter* spp. from food and food production environments. *Food Control*. 2007;18: 1241-1245.
5. Holy O, Forstythe S. Cronobacter spp. as emerging causes of healthcare-associated infection. *J Hosp Infect*. 2014;86: 169-177.
6. Li Y, Chen Q, Zhao J, Jiang H, Lu F, Bie X, Lu Z. Isolation, identification and antimicrobial resistance of *Cronobacter* spp. isolated from various foods in China. *Food Control*. 2014; 37:109-114.
7. Caubillia-Barron J, Hurrel E, Townsend S, Cheetham P, Loc-Carrillo C, Fayet O. Genotypic and phenotypic analysis of *Enterobacter sakazakii*

strains from an outbreak resulting fatalities in a neonatal intensive care unit in France. *J ClinMicrobiol*.2007;45(12):3979-3985.

8. Himelright I, Harris E, Lorch V, Anderson M, Jomes T, Craig A. Enterobacter sakazakii infections associated with the use of powdered infant formula-Tennessee, 2001. *J Am Med Assoc*. 2002;287(17): 2204-2205.
9. FAO/WHO (2004) Enterobacter sakazakii and other microorganisms in powdered infant formula: Meeting report. Microbiological Risk Assessment Series No. 6, Rome, Italy.
10. FAO/WHO (2006) *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: Meeting report. Microbiological Risk Assessment Series No. 10, Rome, Italy
11. Oliphant CM, Eroschenko K. Antibiotic Resistance, Part 2: Gram-negative Pathogens. *J Nurse Practitioners*. 2015;11: 79-86.
12. Shakil S, Akram M, Ali SM, Khan AU. Acquisition of extended-spectrum b-lactamase producing *Escherichia coli* strains in male and female infants admitted to a neonatal intensive care unit: molecular epidemiology and analysis of risk factors. *J Med Microbiol*. 2010; 59: 948–954.
13. Tham J, Mats W, Eva M, Inga O. Prevalence of extended-spectrum beta-lactamase-producing bacteria in food. *Infect Drug Resist*. 2012; 5: 143-147.
14. Stewardson AJ, Renzi G, Maury N, Vaudaux C, Brossier C, Fritsch E, Pitet D, Heck M, Zwaluw KV, Reuland A, Laar T, Snelders E, Vandenbroucke-Grauls C, Kluytmans J, Edder P, Schrenzel J, Harbarth S. Extended-Spectrum β-Lactamase–producing Enterobacteriaceae in hospital food: A Risk Assessment. *Infect ContHosp Ep*. 2014;35(4): 375-383.
15. Wiklund S, Hallberg U, Kahlmeter G, Tammelin A. Living with extended-spectrum beta-lactamase: a qualitative study of patient experiences. *Am J Infec Control*. 2013;41: 723-727.
16. Chon JW, Song KY, Kim SY, Hyeon JY, Seo KH. Isolation and characterization of *Cronobacter* from desiccated foods in Korea. *J Food Sci*. 2012;77(7): 354-358.

17. Lee YD, Park JH, Chang H. Detection, antibiotic susceptibility and biofilm formation of *Cronobacter* spp. from various food from Korea. *Food Control*. 2012;24(1-2): 225-230.
18. Molly C, Cagney C, O'Brien S, Iversen C, Fanning S, Duffy G. Surveillance and characterisation by pulsed-field gel electrophoresis of *Cronobacter* spp. in farming and domestic environments, food production animals and retails foods. *Int J Food Microbiol*. 2009;136 (2): 198-203.
19. Kandhai MC, Heuvelink AE, Reij MW, Beumer RR, Dijk R, Van Tilburg JJHC, Van Schothorst M, Gorris LGM. A study into the occurrence of Cronobacter spp. in the Netherlands between 2001 and 2005. *Food Control*. 2010;21: 1127–1136.
20. Ben Said L, Jouini A, Klibi N, Dziri R, Alonso CA, Boudabous A, Ben Slama K, Torres C. Detection of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. *Int J Food Microbiol*. 2015; 203: 86–92.
21. Anon. (2004). International Standart ISO 21528-2:2004 (E) Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae*.
22. Anon. (2013). Clinical and laboratory standards institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. M100-S23.
23. Ergun F, Ergun O. Ülkemizde tüketime sunulan yerli ve ithal bebek mamalarının genel Mikrobiyolojik kriterleri ve bazı patojenlerin varlığı yönünden incelenmesi. *İstanbul Üniv. Vet. Fak. Derg*. 1999;25: 57-64.
24. Cetinkaya E, Josept S, Ayhan K, Forsythe SJ. Comparison methods for the microbiological identification and profiling of *Cronobacter* species from ingredients used in preparation of infant formula. *Mol Cell Probe*. 2012;27(1):60-64.
25. Kropp KA, Lucid A, Carroll J, Belgrudov V, Kelly WB, Templeton K, Smith C, Dickinson P, O'Driscoll A, Ghazal P, Sleator RD. Draft Genome Sequence of a *Pantoea*sp. Isolated from a preterm neonatal blood sepsis patient. *Genome Announ*. 2014;2(5): e00904-14.

26. Mardaneh J, Dallal MMS. Isolation, identification and antimicrobial susceptibility of *Pantoea (Enterobacter) agglomerans* isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. *Iran J Microb*. 2013;5(3): 263-267.
27. Yıbar A, Soyutemiz E. Antibiotics Use in Food-Producing Animals and Possible Residual Risk. *Atatürk Üniversitesi Vet Bil Derg*. 2013; 8(1): 97-104.
28. Meropol SB, Stange KC, Jacobs MR, Weiss JK, Bajaksouzian S, Bonomo RA. Bacterial colonization and antibiotic resistance in a prospective cohort of newborn infants during the first year of life. *Open Forum Infect Dis*. 2016;3(4): ofw221.4.