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RESEARCH ARTICLE

Presence of *Enterobacter sakazakii* in Milk Powder, Whey Powder and White Cheese Produced in Konya

Mukadderat GÖKMEN * Kemal Kaan TEKİNŞEN ** Ümit GÜRBÜZ **

* Province Control Laboratory, 42090 Konya - TURKEY
** Department of Food Hygiene and Technology, Faculty of Veterinary, University of Selcuk, 42075 Konya - TURKEY

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Summary

Enterobacter sakazakii has been caused foodborne illnesses through consumption of a variety of foods such as milk powder, cheese, sausage, particularly infant foods. In this study, the presence of *E. sakazakii* were investigated in 60 milk powder, 50 whey powder and 50 white cheese. ISO/TS 22964 (IDF/RM 210) method was used for detection *E. sakazakii*. *E. sakazakii* was identified from milk powder 5% (3/60), white cheese 4% (2/50). *E. sakazakii* was not detected in whey powder. According to Commission Regulation (EC) and Turkish Food Codex Microbiological Criteria Communique *E. sakazakii* must not present in infant formulae, formulae for special medical purposes and follow-on formulae. Also in many countries, *E. sakazakii* infections associated with most of the milk-based products that have been reported. In conclusion, the results indicate that milk powder and white cheese produced some dairy plants in Konya presents a risk in terms of human health and the necessary precaution will have to be taken to carry out effective sanitary practices in the plants.

Keywords: *E. sakazakii, Milk powder, Whey powder, White cheese*

Konya'da Üretilen Süt Tozu, Peynir Altı Suyu Tozu ve Beyaz Peynirde *Enterobacter sakazakii* Varlığının Araştırılması

Özet

Enterobacter sakazakii süt tozu, peynir, sucuk ve özellikle bebek mamaları gibi çeşitli gıdaların tüketilmesiyle gıda kaynaklı hastalıklara neden olmaktadır. Bu çalışmada 60 süt tozu, 50 peynir altı suyu tozu ve 50 beyaz peynir *E. sakazakii* varlığı yönünden araştırıldı. *E. sakazakii*'nin tespiti için ISO/TS 22964 (IDF/RM 210) metodu kullanıldı. Süt tozu örneklerinin %5 (3/60)'inde ve beyaz peynir örneklerinin %4 (2/50)'ünde *E. sakazakii* identifiye edildi. Peynir altı suyu tozu örneklerinde ise *E. sakazakii* tespit edilemedi. Commission Regulation (EC) ve Türk Gıda Kodeksi Mikrobiyolojik Kriterler Tebliği'ne göre bebek mamaları, özel tıbbi amaçlı formüller ve devam mamalarında *E. sakazakii* bulunmaması gerektiği belirtilmekte, pek çok ülkede *E. sakazakii* enfeksiyonlarının çoğunlukla süt bazlı ürünler ile ilişkili olduğu rapor edilmektedir. Sonuç olarak, bulgular Konya'da bazı işletmelerde üretilen süt tozu ve beyaz peynirin insan sağlığı açısından risk oluşturduğunu ve işletmelerde etkili sanitasyon uygulamalarının gerçekleştirilebilmesi için ilgili tedbirlerin alınması gerekliliğini ortaya koymaktadır.

Anahtar sözcükler: *E. sakazakii, Süt tozu, Peynir altı suyu tozu, Beyaz peynir*

INTRODUCTION

The ubiquitous microorganism *Enterobacter sakazakii,* a gram-negative, non-spore-forming, rod-shaped, oxidase, lactose and sorbitol negative, facultative anaerobe, motile by peritrichous flagella bacterium, is a member family [1-3]. This organism was known as 'yellow pigmented *Enterobacter cloacae*' until 1980, afterward it was designated as *E. sakazakii* due to differention of biochemical characteristic from *E. cloacae* [4]. *E. sakazakii* is an opportunistic pathogen in new-born. *E. sakazakii* infections are important cause of life-threatening cases of

İletişim (Correspondence)
☎ +90 332 2233568
✉ kktekinsen@selcuk.edu.tr

meningitis (58%), sepsis (17%), and necrotizing enterocolitis (29%) in premature and full-term infants. Mortality rates of 40-80% have been described and survivors often suffer from neurological sequelae [5,6].

Food and food ingredients may be contaminated with *E. sakazakii* under unsuitable conditions of hygiene and contaminated equipment [7]. Powdered milk formula is important source of *E. sakazakii* infections [2,8]. This bacterium is resistance to drying and acid pH, heat, biofilm formation and persistence on food preparation surfaces. Due to new-born infections *E. sakazakii* is noticed as associated with infant formula and milk powder [4,9]. Infant formula is produced from ingredients that may include milk, milk derivatives, soy protein isolates, carbohydrates, fats, minerals, vitamins and some food additives [10]. Formula preparation equipment contaminated by *E. sakazakii* has been demonstrated to have caused two outbreaks [11,12], but the original source of *E. sakazakii* was not determined in either case. Environmental swabbing of formula preparation areas in the course of outbreak investigations has not demonstrated *E. sakazakii* in the general environment. *E. sakazakii* has been identified in the environments of milk powder production facilities and other food production facilities, as well as in households [13]. Not all infants with *E. sakazakii* infection have been exposed to powdered infant formula, and *E. sakazakii* infections can also occur in adults [6]. Thus, although an environmental source of *E. sakazakii* infection other than infant formula has not been strictly identified, other sources undoubtedly exist [10].

E. sakazakii has been isolated from a range of foods including cheese, fermented bread, tofu, sour tea, cured meats, minced beef and sausage meat [4]. According to Commission Regulation (EC) [14] and Turkish Food Codex [15], Microbiological Criteria Communique *E. sakazakii* must not present (0/10 g and 0/25 g, respectively) in infant formulae, formulae for special medical purposes and follow-on formulae. The objective of this study was to investigate the presence of *E. sakazakii* in milk powder, whey powder and white cheese produced in Konya.

MATERIAL and METHODS

Samples

In this study, total of 160 samples from three different milk products (60 milk powder, 50 whey powder and 50 white cheese) were used. The samples were obtained different retail market and dairy products factory in Konya and transported to the laboratory in insulated cooler boxes immediately. Samples were stored at 4°C until analyses. The presence of *E. sakazakii* were examined in milk powder, whey powder and white cheese.

Detection of E. sakazakii

ISO/TS 22964 (IDF/RM 210) [16] method was used for detection *E. sakazakii*. To prepare the pre-enrichment dilution, 25 g sample was added to 225 ml Buffered Peptone Water (BPW). Pre-enrichment dilution was incubated at 37±1°C for 18±2 h. After incubation of the inoculated pre-enrichment medium, 0.1 ml of the obtained culture was transferred into 10 ml Modified Lauryl Sulphate Broth (mLST) (Oxoid CM 1133)/ Vancomycin (Oxoid DR 595) medium. This medium was incubated at 44±0.5°C for 24±2 h. After incubation of the inoculated mLST-Vancomycin medium, a loopful (ca. 10 µ) was streaked onto the surface of the ChromoCult® *Enterobacter sakazakii* Agar (Merck 1.0087). The plate was incubated 44±1°C for 24±2 h. Five typical turquoise colonies was streaked on Tryptone Soya Agar (TSA) (Oxoid CM 131) and TSA plates was incubated at 25±1°C for 48±4 h [16]. After incubation, one yellow colony which selected from each TSA plate was confirmed with API 20 E kit [17] and VITEC 2 compact GN card according to the manufacturer's instructions [18] *(Fig. 1)*.

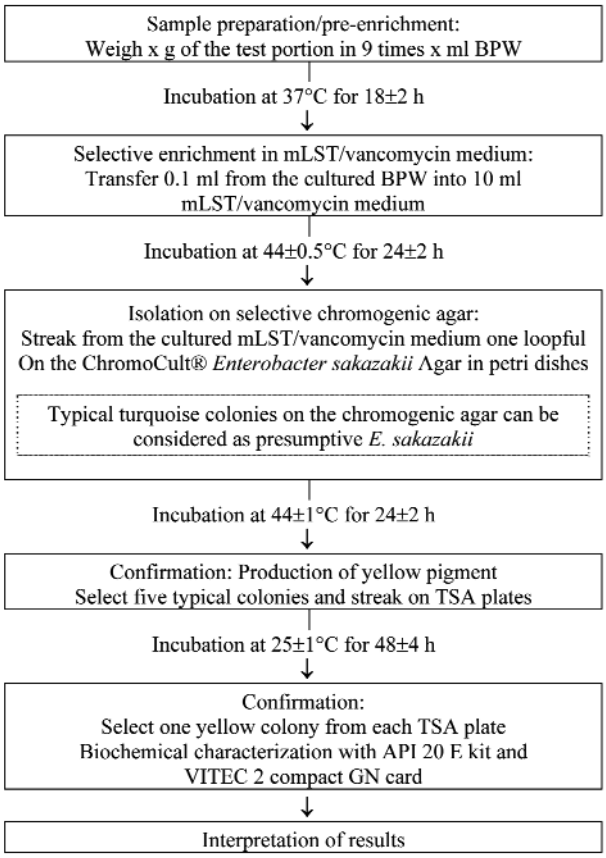

Fig 1. Identification method flow scheme of *E. sakazakii*
Şekil 1. *E. sakazakii*'nin identifikasyon metodu akış şeması

RESULTS

In this study, three different milk products total 160 samples (60 milk powder, 50 whey powder and 50 white cheese) were tested. *E. sakazakii* was isolated from 3/60 (5%) milk powder, 2/50 (4%) white cheese. But none of the samples contained *E. sakazakii* in whey powder. The summarized results of presence of *E. sakazakii* in milk powder, whey powder and white cheese are shown in *Table 1*.

Table 1. *The results of presence of E. sakazakii in milk powder, whey powder and white cheese*

Tablo 1. *Süt tozu, peynir altı suyu tozu ve beyaz peynirde E. sakazakii analiz bulguları*

Sample	No. of Sample	No. of Positive Sample
Milk Powder	60	3 (5%)
Whey Powder	50	ND
White Cheese	50	2 (4%)

Figures in parentheses are the percent of samples
ND: Not detected

Table 2. *Presence of E. sakazakii in some food products and plants*

Tablo 2. *Bazı gıda ürünlerinde ve işletmelerinde E. sakazakii varlığı*

Sample	No. of Sample	No. of Positive Sample	Country	References
Infant formula powder	40	1 (3)	Netherland	Heuvelink et al.[19]
Infant milk formula	82	2 (2)	United Kingdom	Iversen and Forsythe [20]
Infant milk formula	8	2 (25)	Jordan	Shaker et al.[3]
Infant milk formula	50	7 (14)	South Africa	Cawthorn et al.[21]
Infant milk formula	35	2 (6)	Egypt	El-Sharoud et al.[22]
Dried infant foods	49	5 (10)	United Kingdom	Iversen and Forsythe [20]
Infant food formula	15	2 (13)	Jordan	Shaker et al.[3]
Milk powder	170	7 (4)	Netherland	Heuvelink et al.[19]
Milk powder	72	3 (4)	United Kingdom	Iversen and Forsythe [20]
Full-fat milk powder	10	-	Jordan	Shaker et al.[3]
Full-fat milk powder	15	-	Egypt	El-Sharoud et al.[22]
Skimmed milk powder	37	5 (14)	Egypt	El-Sharoud et al.[22]
Stored Domiatti cheese	10	-	Egypt	El-Sharoud et al.[22]
Fresh Domiatti cheese	10	4 (40)	Egypt	El-Sharoud et al.[22]
Ras cheese	10	-	Egypt	El-Sharoud et al.[22]
Kariesh cheese	10	-	Egypt	El-Sharoud et al.[22]
Cheese products	62	2 (3)	United Kingdom	Iversen and Forsythe [20]
Milk powder factory	23	2 (9)	Switzerland	Kandhai et al.[13]*
Milk powder factory	26	9 (35)	Switzerland	Kandhai et al.[13]*
Milk powder factory	11	1 (9)	Switzerland	Kandhai et al.[13]*
Milk powder factory	8	2 (25)	Switzerland	Kandhai et al.[13]*
Environmental, Milk factory	14	6 (43)	South Africa	Cawthorn et al.[21]
Environmental, Milk factory	1	1 (100)	Egypt	El-Sharoud et al.[22]

Figures in parentheses are the percent of samples
** From different factory*

DISCUSSION

E. sakazakii has been isolated from a wide range of environmental sources and from several foods of animal and plant origin. Infant formula and milk powder have been the most common vehicles implicated in neonatal *E. sakazakii* infections and also in many countries, *E. sakazakii* infections associated with most of the milk-based products that have been reported [10]. Presence of *E. sakazakii* in some food products and plants are shown *Table 2*.

At the present study, presence of *E. sakazakii* was determined in milk powder, whey powder and white cheese which are produced in Konya city of Turkey. As depicted in *Table 1*. *E. sakazakii* was detected in 5% of the milk powder samples and in 4% of the white cheese samples, respectively. But *E. sakazakii* was not detected in whey powder samples.

These results are in parallel with the findings of some previous reports [19,20,22] which pointed out the presence of *E. sakazakii* in 4% of the milk powder, 3% cheese products and 2-6% milk-based products (infant formula powder, infant milk formula) samples *(Table 2)*. The present findings suggest that the milk which is processed into dairy products may be contaminated with *E. sakazakii*. The presence of *E. sakazakii* in these products is unlikely since the liquid milk is normally pasteurized. It has been reported that pasteurization treatment is effective in the elimination of this pathogen [3]. Nazarowec-White and Farber [1] found that *E. sakazakii* was more heat-sensitive than other pathogenic organisms like *Listeria monocytogenes*. The results confirm the findings

of some researchers [13,21,22] who reported a high occurrence of *E. sakazakii* in enviroment of milk factory and milk powder factory. Thus, as seen on *Table 2* a number of researchers [3,20-22] reported a higher occurrence in some milk-based products (infant milk formula, dried infant foods, infant food formula), skimmed milk powder and fresh Domiatti cheese samples. On the other hand Shaker et al.[3] and El-Sharoud et al.[22] could not be detected *E. sakazakii* in full-fat milk powder and some cheese kinds (stored Domiatti, Ras and Kariesh cheeses). The variations on the findings may be attributable to the fact that milk to be processed may contain different levels of *E. sakazakii* according to different processing techniques and analysis methods. Moreover, differences in the hygiene and storage conditions at the dairies and retail points are other key factors on the variations of the results [3,22,23].

E. sakazakii was not isolated from any samples of whey powder *(Table 1)*. Although it is not detected in whey powder samples, each sample must be evaluated on itself. Cross contamination must be recognized; either directly or via surface and equipment from raw milk [3].

In conclusion, this study showed that *E. sakazakii* was found in milk powder and white cheese samples produced some dairy plants in Konya. The results indicate that the milk powder and white cheese represents a potential hazard for consumer. Thus the necessary precaution will have to be taken to carry out effective cleaning of tools and equipment and personal hygiene practices in the plants.

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