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INCIDENCE, BIOCHEMICAL CHARACTERISTICS AND ANTIBIOTIC RESISTANCE OF *Enterobacter sakazakii* ISOLATED FROM MILK AND DAIRY PRODUCTS

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ABSTRACT

During the course of this study, 235 samples of raw milk, dried milk and some local dairy products were collected from eight Egyptian governorates and examined for the presence of the emergent pathogen *Enterobacter sakazakii*. Out of 235 examined samples of milk and dairy products, 16 suspect isolates of *Ent. sakazakii* were recovered, of which only 6 isolates were confirmed as belonging to this pathogen. Confirmed *Ent. sakazakii* isolates were recovered from dried milk (2 isolates), stored Domiati cheese (2 isolates) and fresh Domiati cheese (2 isolates). Other examined samples including those of raw milk, Ras cheese, Kariesh cheese, and yoghurt were free of the pathogen. Biochemical characterization of *Ent. sakazakii* isolates using the Api 20E miniaturized identification system showed that these isolates had the typical biochemical traits previously reported for this species. These isolates expressed, however, variable reactions in the ornithine decarboxylase and voges-proskauer tests. The isolates were found to be susceptible to 11 antibiotics including ampicillin (10 μg), ciprofloxacin (5μg), chloramphenicol (30 μg), nalidixic acid (30 μg), tetracycline (30 μg), spiramycin (100 μg), carbenicillin (100 μg), penicillin (100 unit), colistin sulphate (10 μg), cephradine (30 μg) and gentamicin (10 μg). Overall, these results confirmed the association of *Ent. sakazakii* with dried milk and certain dairy products such as Domiati cheese, but at generally limited incidence rates. The current results also show that it is possible to eradicate *Ent. sakazakii* using currently available generations of antibiotics.

INTRODUCTION

Enterobacter sakazakii is an emergent pathogen that was named after the Japanese bacteriologist Riichi Sakazaki in recognition of his work on enteric bacteria (Lehner and Stephan 2004). It was referred to as yellow-pigmented *Ent. cloacae* until 1980, but has been re-named following the emergence of genetic and physiological studies suggesting its re-classification as a new species (Grimont and Grimont 1992). *Ent*. *sakazakii* has been implicated in several outbreaks and sporadic cases of meningitis, especially in neonates and infants, yet it has been also reported to be associated with adults and elderly people (Farber 2004; Mullane *et al*. 2007). Its infections are generally associated with high mortality rates of 20-50%.

Previous studies have reported that *Ent. sakazakii* was particularly associated with dried foods including dried milk and infant dried milk formulas (Nazarowec-White and Farber 1997; Van Acker *et al*. 2001). However, these studies were mostly carried out in Western countries involving the examination of food products produced and distributed there. Recently, El-

Sharoud *et al*. (2008) have detected *Ent. sakazakii* in dried milk and soft cheese samples collected from Mansoura city and villages in its vicinity. Nevertheless, this study did not include milk and dairy product samples produced and/or distributed in other Egyptian governorates. Furthermore, little is generally known about the biochemical characteristics and antibiotic resistance of *Ent. sakazakii* isolated from foodstuffs. The present study was thus designed to examine the presence of *Ent. sakazakii* in milk and dairy products produced and/or distributed in 8 Egyptian governorates as to allow more proper assessment of its association with milk and dairy products consumed in this country. The study also considered characterizing *Ent. sakazakii* isolates recovered from these products in terms of their biochemical activities and antibiotic resistance.

MATERIALS AND METHODS

Collection of milk and dairy product samples

A total of 235 samples of milk and milk products were collected from 8 Egyptian governorates namely El-Dakahlia, El-Gharbia, Alexandria, Cairo, El-Sharqia, El-Fayoum, Port Said and Domiatta. These samples included 45 raw milk samples, 66 samples of dried milk, 36 samples of fresh Domiati cheese, 38 samples of stored Domiati cheese, 15 samples of Ras cheese, 20 samples of Kariesh cheese and 15 samples of yoghurt. Samples were aseptically transferred to sterile plastic bags, placed in an ice box, and transported to the laboratory within 2 h of collection.

Isolation of *Ent. sakazakii* from milk and dairy products

The method used for isolating *Ent. sakazakii* from the collected milk and dairy product samples was based on the detection method of the Food and Drug Administration (FDA) described by Nazarowec-White and Farber (1997). The isolation of *Ent. sakazakii* involved five consecutive steps: pre-enrichment, enrichment, plating, preliminary confirmation and identification. All samples except raw milk and dried milk samples were pre-enriched in buffered peptone water (BPW) medium by mixing 25 g sample with 225ml BPW, followed by incubation at 36^0C for 24 h. Dried milk samples were pre-enriched by suspending 10 g powder sample in 100 ml sterile distilled water. Raw milk samples were directly enriched by inoculating 10g milk into 90 ml Enterobacteriaceae Enrichment (EE) broth. Ten milliliters of the pre-enrichment mixture of each sample was transferred into 90 ml of EE-broth, followed by incubation at 36°C for 24h. Loopfuls from each enrichment broth were used to streak the surface of Druggan-Forsythe-Iversen (DFI) agar plates that were then incubated at 36˚C for 24 h. Three to five suspect blue-green colonies from each plate were picked up and streaked on the surface of DFI for purification. Purified isolates were then tested for the production of yellow pigment on tryptone soy Agar (TSA) incubated at 36^oC for 24 h. Isolates producing yellow or orange growth on TSA were considered as potential *Ent. sakazkaii.* All media used in the isolation and confirmation of the *Ent. sakazakii* isolates were obtained from Oxoid, Basignstoke, UK.

Identification and biochemical characterization of *Ent. sakazakii* isolates using the Api-20E System

Potential *Ent. sakazakii* isolates were further subjected to identification using the Api-20E miniaturized system (bioMerieux, Marcy l'Etoile, France). This system includes strips of 20 microtubes per each one. These microtubes contain small amounts of dehydrated media for performing the following 20 testes: orthonitrophenyl galactosidase (ONPG), arginine dehydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), citrate utilization (CIT), hydrogen sulfide production (H_2S), urease (URE), tryptophane deaminase (TDA), indole production (IND), Voges-Proskauer (VP), gelatin liquefaction (GEL), fermentation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY) and arabinose (ARA). The system was used for the identification and biochemical characterization of the *Ent. sakazakii* isolates according to the manufacturer's instructions and Benson (1990).

Examining the antibiotic susceptibility of *Enterobacter sakazakii*

The antibiotic susceptibility of *Ent. sakazakii* isolates recovered from dairy products in this study was assessed using the Kirby-Bauer disc-diffusion method (Fuchs 1987) as follows. An overnight culture of each isolate was used to inoculate 5 ml sterile 0.85% NaCl (saline) solution. The turbidity of the inoculated saline was adjusted as to approximate that of 0.5 MacFarland standard. An inoculum of the resultant bacterial suspension was taken and adequately spread over the surface of Muller-Hinton agar (MHA) plates using a sterilized cotton-tipped applicator. Eleven antibiotic-impregnated discs were then placed onto the inoculated MHA plates with an antibiotic dispenser. The antibiotic test panel consisted of the following agents: ampicillin (10 μg), ciprofloxacin (5μg), chloramphenicol (30 μg), nalidixic acid (30 μg), tetracycline (30 μg), spiramycin (100 μg), carbenicillin (100 μg), penicillin (100 unit), colistin sulphate (10 μg), cephradine (30 μg) and gentamicin (10 μg). MHA plates were then incubated for 24 h at 36^0 C and the diameters of the inhibition zones around antibiotic discs were measured with calipers. Isolates were scored as susceptible (S), intermediate (I), or resistant (R) to examined antibiotics according to the standards of the British Society for Antimicrobial Chemotherapy (2008). Both the MHA medium and antibiotic-impregnated discs were obtained from Oxoid, Basingstoke, UK.

RESULTS AND DISCUSSION

Incidence of *Ent. sakazakii* in milk and dairy products

Two hundreds and thirty five (235) samples of milk and milk products were collected from 8 Egyptian governorates and examined for the presence of *Ent. sakazakii.* Table 1 shows the number of collected samples and the locations, from which they were gathered. The method used for isolating *Ent. sakazakii* from the collected milk and dairy products samples was based on the FDA detection method with some modifications as shown in the Materials and Methods section. This method was applied to ensure the

recovery of "injured cells" of *Ent. sakazakii* from the examined milk and dairy product samples (Gurtler and Beuchat, 2005). Injured cells are live but weakened cells generated by the exposure of microorganisms to sub-lethal food preservation treatments (Wu, 2008). These cells are sensitive to selective agents used in the differential, selective media employed for the detection of the organism in question. Therefore, the detection of injured cells requires an initial pre-enrichment stage in a nutritious, nonselective medium such as BPW. This pre-enrichment stage allows injured cells to resuscitate and proliferate as healthy intact cells. Since the reconstitution of dried milk in water provides a nutritious, nonselective medium of liquid milk, dried milk samples were pre-enriched by dissolving samples in distilled water. Raw milk samples were not pre-enriched since they there were not exposed to heat or other preservation treatments that could result in the generation of injured cells. Following pre-enrichment, samples were subjected to selective enrichment in the EE broth to inhibit competing bacteria and allow *Ent. sakazakii*, if present, to grow to levels that could be detected thereafter on the DFI agar.

Results presented in table (2) show that a total of 11 *Ent. sakazakii* suspect isolates were recovered from the examined 235 milk and dairy products. These isolates produced blue-green colonies on the DFI agar and appeared as Gram-negative short rods when examined by microscopy. These isolates were further tested for their ability to produce yellow pigment on tryptone soy agar (TSA). All of them were positive, producing yellow growth on TSA, which is one of the main identification traits of *Ent. sakazakii* (Brenner and Farmer 2005; Hoffmann *et al*. 2005). Therefore, all of the 11 isolates were considered as potential *Ent. sakazakii* isolates and were further subjected to biochemical identification using the Api 20E miniaturized system. This identification system confirmed that 6 out of 11 isolates were *Ent. sakazakii* with the rest of 5 isolates being identified as *Escherichia vulneris* and *Pseudomonas oryzihabitans.* These results suggest that the development of blue-green colonies on DFI that can also produce yellow growth on TSA is to be considered as a preliminary indicator of the presence of *Ent. sakazakii* in examined samples, and further biochemical testing must be performed in order to confirm the identity of the isolated organisms.

Confirmed *Ent. sakazakii* isolates were found to be associated with dried milk (2 isolates), fresh Domiati cheese (2 isolates) and stored Domiati cheese (2 isolates) (Table 2). Other examined samples including those of raw milk, Ras cheese, Kariesh cheese and yoghurt were free of the pathogen (Table 2). The incidence rate of *Ent. sakazakii* in the examined samples were generally limited (2.6%), which indicated that the pathogen was not widely present in milk and dairy products distributed and/or produced in Egypt. The presence of *Ent. sakazakii* in contaminated samples of dried milk, fresh Domiati cheese and stored Domiati cheese was also limited being 3.3%, 5.6% and 5.3%, respectively (Table 2). However, given the wide use of dried milk in the preparation of dairy products and wide consumption of Domiati cheese in Egypt, beside severe consequences of infections caused by *Ent.*

sakazakii, these limited incidence rates should be considered of concern to the safety of these products.

Table 1: Locations and numbers of examined milk and dairy product samples.

Samples	Governorate								Total
	El-Dakahlia	El-Gharbia	Alexandria	Cairo	El-Sharqia	El-Fayoum	Port Said	Domiatta	
Raw milk	12	13	2	4	5	1	4	4	45
Dried milk	24	11	6	12	5	2	2	4	66
Fresh Domiati cheese	11	6	3	4	3	2	2	5	36
Stored Domiati cheese	9	8	2	2	6	2	5	4	38
Ras cheese	5	2	1	2	1	1	1	2	15
Kariesh Cheese	4	3	2	2	3	1	4	1	20
Yoghurt	4	1	2	1	3	1	1	2	15
Total									235

Table 2: Numbers of suspect and confirmed isolates of *Enterobacter sakazakii* recovered from milk and dairy products.

Samples	Number of samples	Number of suspect isolates*	Number of isolates producing Yellow-pigment on TSA	Number of confirmed isolates using the Api 20E system	Number of positive dairy products samples (%)
Raw milk	45	0	0	0	0 (0%)
Dried milk	66	6	6	2	2 (3.3%)
Fresh Domiati cheese	36	3	3	2	2 (5.6%)
Stored Domiati cheese	38	2	2	2	2 (5.3%)
Ras cheese	15	0	0	0	0 (0%)
Kariesh Cheese	20	0	0	0	0 (0%)
Yoghurt	15	0	0	0	0 (0%)
Total	235	11	11	6	6 (2.5%)

* **Suspect isolates are those that produced blue- green colonies on the DFI agar and were found to be G-negative short rods.**

The above results are consistent with those reported by El-Sharoud *et al*. (2008), who could detect *Ent. sakazakii* at generally limited incidence rates in dried milk and soft cheese samples collected from Mansoura City, Egypt. Iversen and Forsythe (2004) also detected the pathogen in 3 out of 72 samples of dried milk and 2 out of 62 samples of cheese samples collected from the UK, other European countries, Asia, South Africa and USA. The contamination of dried milk powder with *Ent. sakazakii* had been early reported by Postupa and Aldova (1984), Muytjens *et al*. (1988) and Heuvelink *et al*. (2002). Interestingly, the early paper of Farmer *et al*. (1980) defining *Ent. sakazakii* included the NCTC 8155 that was originally cultured from dried milk. Several outbreaks of disease have been also attributed to the contamination of infant dried milk formulas with *Ent. sakazakii* (Iversen and Forsythe 2004). Together, this confirms the fact that while the manufacture of dried milk products involves the use of high temperatures, these products are not sterile and may involve contaminating microorganisms. However, there is still no

clear explanation of the association of *Ent. sakazakii* with dried milks (Breeuwer *et al*. 2003). Nazarowec-White and Farber (1997) examined the heat resistance of *Ent. sakazakii* in reconstituted infant milk formulas, and reported that this pathogen was a highly thermotolerant organism among the Enterobacteriaceae. However, the pathogen was found not to be able to even survive pasteurization. A recent study by Arku *et al*. (2008) showed that 4 strains of *Ent. sakazakii* inoculated into 35% reconstituted skim milk were able to survive spray-drying in a min-spray drier. All strains were detected in the resultant milk powders for at least 12 weeks. One concern over these results is the reconstitution of such an high level of skim milk powder (35%) that provided an elevated level of milk total solids that could protect the organism against spray drying.

The presence of *Ent. sakazakii* in dried milks suggests, however, that this pathogen has the ability to withstand dry environment and/or high osmotic stress. This may also explain its presence in Domiati cheese, whose manufacture involves the addition of relatively high salt levels. The use of dried milk for elevating total solid content of milk used for the making of Domiati cheese may also aid the transmission of *Ent. sakazakii* to this cheese variety. This could be aided by the application of substandard heat treatments during the preparation of cheese as in small production facilities.

The present results show that *Ent. sakazakii* was absent in raw milk, which was consistent with Muytjens and Kollee (1990) and El-Sharoud *et al*. (2008), who could not isolate the pathogen from raw milk samples collected from certain areas in Netherlands and Egypt, respectively. Neither was the pathogen detected in samples of fermented dairy products including Ras cheese, Kariesh cheese and yoghurt collected within the current study. This may indicate the sensitivity of *Ent. sakazakii* to acidic conditions. However, it is also possible that *Ent. sakazakii* was not originally present in the collected samples of these products.

Biochemical characteristics of *Ent. sakazakii* isolates recovered from milk and dairy products

The biochemical characteristics of *Ent. sakazakii* isolates recovered from dairy products in this study were examined using the Api 20E miniaturized system. As seen in table 3, all isolates were able to produce the □-galactosidase enzyme as indicated by their positive reaction in the ONPG test. They were also able to produce arginine dehydrolase (ADH), but could not express lysine decarboxylase (LDC) or ornithine decarboxylase (ODC). All isolates were able to use citrate as a sole carbon source (CIT), but could not produce H_2S, urease (URE), tryptophane deaminase (TDA) or indole (IND). Variable reactions were reported for the Voges-Proskauer (VP) test since 4 isolates (No 1, 2, 3 & 6) gave positive results and two isolates (No 4 & 5) were negative in this test. All isolates were negative in the GEL test and could ferment glucose (GLU), mannitol (MAN), inositol (INO), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY) and arabinose (ARA), but could not ferment sorbitol (SOR). All isolates were negative in the oxidase test.

Table 3: characteristics of confirmed *Enterobacter sakazakii* isolates.

Isolate No*	Test																			
	Orthonitrophenyl galactosidase (ONPG)	Arginine dehydrolase (ADH)	Lysine decarboxylase (LDC)	Ornithine decarboxylase (ODC)	Citrate utilization (CIT)	Hydrogen sulfide production (H2S)	Urease (URE)	Tryptophane deaminase (TDA)	Indole production (IND)	Voges-Proskauer (VP)	Gelatin liquefaction (GEL)	Fermentation of Glucose (GLU)	Fermentation of Mannitol (MAN)	Fermentation of Inositol (INO)	Fermentation of Sorbitol (SOR)	Fermentation of Rhamnose (RHA)	Fermentation of Sucrose (SAC)	Fermentation of Melibiose (MEL)	Fermentation of Amygdalin (AMY)	Fermentation of Arabinose (ARA)	Oxidase
1	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	-
2	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	-
3	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	-
4	+	+	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-
5	+	+	-	-	+	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	-
6	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+	-

*** Isolates 1 & 2 were recovered from dried milk.**
Isolates 3 & 4 were recovered from fresh Domiati cheese.
Isolates 5 & 6 were recovered from stored Domiati cheese.

The results of these reactions are generally consistent with previous reports on the biochemical characteristics of *Ent. sakazakii* (Brenner and Farmer 2005; Hoffmann *et al*. 2005). However, while it was reported that more than 80% of *Ent. sakazakii* strains gave positive reactions in the ODC and VP tests, all the 6 isolates examined in this study were ODC-negative and two of them were VP-negative. This suggests that an extent of diversity in biochemical characteristics could exist among *Ent. sakazakii* strains. Such diversity also occurs within the strains examined in this study given that 4 isolates (No 1, 2, 3 & 6) gave positive results in the VP test, while two of them (No 4 & 5) were negative in this test.

Antibiotic resistance of *Ent. sakazakii* isolates recovered from milk and dairy products

Studying the antibiotic resistance of foodborne *Ent. sakazakii* isolates is of interest since it shows which effective antibiotics could be used to treat patients admitted to hospitals with infections caused by this pathogen. Given that *Ent. sakazakii* causes meningitis, which is a rapidly fatal neural disease in neonates and that the host defenses in the cerebral fluid of neonates are limited, antibiotics are particularly required to eradicate infection with this pathogen (Willis *et al.* 1988). Therefore, the antibiotic resistance of the 6 *Ent. sakazakii* isolates recovered from dairy products in this study were examined against 11 antibiotics. This was carried out using the Kirby-Bauer disc-diffusion method (Fuchs 1987). Briefly, a standardized inoculum of each isolate was adequately spread over the surface of Muller-Hinton agar (MHA) plates, to which 11 antibiotic-impregnated discs were placed. MHA plates were then incubated at 36^0C for 24 h and the diameters of the inhibition zones around antibiotic-impregnated discs were measured. Table 4 shows the diameters of the inhibition zones and the interpretation of these results according to the British Society for Antimicrobial Chemotherapy (2008).

As seen in table 4, all isolates were susceptible to the 11 examined antibiotics including ampicillin (10 μg), ciprofloxacin (5μg), chloramphenicol (30 μg), nalidixic acid (30 μg), tetracycline (30 μg), spiramycin (100 μg), carbenicillin (100 μg), penicillin (100 unit), colistin sulphate (10 μg), cephradine (30 μg) and gentamicin (10 μg). These results are consistent with Farmer *et al.* (1980), who tested more than 100 isolates of *Ent. sakazakii* and found that all isolates were susceptible to gentamicin, chloramphenicol and ampicillin, 87% of these isolates were also susceptible to nalidixic acid, tetracycline and carbenicillin and 67% of the isolates were susceptible to colistin. However, they reported that all strains were resistant to penicillin, to which the 6 isolates examined in the present study were susceptible. Erickson and Kornacki showed that the reports of the antibiotic susceptibility of *Ent. sakazakii* during 1960-1999 indicated that the organism was typically susceptible to ampicillin, tetracycline, chloramphenicol, gentamicin, and the third-generation cephalosporins. However, a study of *Ent. sakazakii* isolates from patients at the University of Massachusetts Medical School in 1995–1996 revealed that these isolates were uniformly resistant to ampicillin, cefazolin, and extended-spectrum penicillins, and were not uniformly susceptible to the third-generation cephalosporins or the quinolones. Willis *et al.* (1988) suggested that in the majority of reported cases, a combination of ampicillin and gentamycin has been used in the treatment of *Ent. sakazakii* meningitis.

Table 4: Antibiotic resistance of *Enterobacter sakazakii* isolated from milk and dairy products.

Isolates No.**	Antibiotics*										
	AMP10	CIP5	TET30	SP100	NA30	C30	CAR100	P100	CT10	CE30	CN10
1	20*** (s)****	34 (s)	22 (s)	14 (s)	26 (s)	30 (s)	34 (s)	23 (s)	15 (s)	20 (s)	23 (s)
2	24 (s)	36 (s)	20 (s)	15 (s)	25 (s)	30 (s)	33 (s)	20 (s)	14 (s)	19 (s)	24 (s)
3	24 (s)	34 (s)	22 (s)	16 (s)	24 (s)	32 (s)	34 (s)	20 (s)	16 (s)	18 (s)	24 (s)
4	26 (s)	34 (s)	22 (s)	13 (s)	25 (s)	26 (s)	34 (s)	21 (s)	13 (s)	18 (s)	22 (s)
5	27 (s)	34 (s)	21 (s)	13 (s)	25 (s)	26 (s)	34 (s)	25 (s)	14 (s)	21 (s)	24 (s)
6	24 (s)	33 (s)	21 (s)	14 (s)	25 (s)	32 (s)	30 (s)	18 (s)	14 (s)	18 (s)	20 (s)

*** AMP10: Ampicillin (10 μg), CIP5: Ciprofloxacin (5 μg), TET30: Tetracycline (30 μg), SP100: Spiramyacin (100 μg), NA30: Nalidixic acid (30 μg), C30: Chloramphenicol (30 μg), CAR100: Carbenicillin (100 μg), P100: Penicillin (100 unit), CT10: Colistin Sulphate (10μg), CE30: Cephradine (30 μg), CN10: Gentamicin (10μg).**

**** Isolates 1 & 2 were recovered from dried milk.**
Isolates 3 & 4 were recovered from fresh Domiati cheese.
Isolates 5 & 6 were recovered from stored Domiati cheese.

***** Diameters of inhibition zones around antibiotic discs in mm. **** S: sensitive.**

Taken the present results together with the previous studies on the antibiotic resistance of *Ent. sakazakii*, it could be concluded that it is possible to eradicate this pathogen using currently available generations of antibiotics. However, it will still be important to develop measures to avoid the transmission of this pathogen via foods rather than relaying on antibiotics to treat its infections.

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التواجد والصفات الكيموحيوية والمقاومة للمضادات الحيوية لميكروب الانتيروبكتر ساكازاكي المعزول من اللبن ومنتجاته

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تم في هذه الدراسة تجميع ٢٣٥ عينة من اللبن الخام والمجفف وبعض المنتجات اللبنية المحلية وذلك من ثمانية محافظات مصرية، وتم تحليل هذه العينات من حيث وجود الميكروب الممرض المتعرف عليه حديثاً انتيروبكتر ساكازاكي، ومن مجموع ٢٣٥ عينة تم عزل ١٦ عزلة مشكوك فيها من الميكروب، وتم تأكيد ٦ عزلات فقط منها على أنها انتيروبكتر ساكازاكي، ولقد تم الحصول على هذه العزلات من اللبن المجفف (٢ عزلة) والجبن الدمياطي الخزين (٢ عزلة) والجبن الدمياطي الطازج (٢ عزلة)، بينما خلت باقي العينات الخاصة باللبن الخام والجبن الرأس والجبن القريش واليوجورت من الميكروب، ولقد تم دراسة الصفات الكيموحيوية لعزلات انتيروبكتر ساكازاكي باستخدام نظام Api 20E ووجد أن لها نفس الصفات النموذجية والسابق التعرف عليها في دراسات أخري حول هذا الميكروب، إلا أن عزلات انتيروبكتر ساكازاكي المتحصل عليها في الدراسة الحالية أعطت نتائج متباينة في اختبار نزع مجموعة الكربوكسيل من الحمض الأميني أورنثين وكذلك في اختبار الفوجس بروسكور، ولقد أظهرت هذه العزلات حساسية لعدد ١١ مضاد حيوي وهي الامبسلين (١٠ ميكروجرام)، السبروفلوكسين (٥ ميكروجرام)، الكلورأمفنيكول (٣٠ ميكروجرام)، حمض الناليدكسك (٣٠ ميكروجرام)، التتراسيكلين (٣٠ ميكروجرام)، السبراميسين (١٠٠ ميكروجرام)، الكاربنسلين (١٠٠ ميكروجرام)، البنسلين (١٠٠ وحدة)، سلفات الكولستن (١٠ ميكروجرام)، السيفارايدين (٣٠ ميكروجرام) والجنتاميسين (١٠ ميكروجرام)، وبصفة إجمالية فإن نتائج هذه الدراسة تؤكد ارتباط ميكروب انتيروبكتر ساكازاكي باللبن المجفف ومنتجات الألبان مثل الجبن الدمياطي وإن كان هذا الميكروب قد وجد بنسبة محدودة في العينات المختبرة، كذلك فإن هذه الدراسة توضح أنه من الممكن التغلب على هذا الميكروب باستخدام الأجيال الحالية المتاحة من المضادات الحيوية.