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Occurrence of *Enterobacter sakazakii* in some meat products sold in Assiut governorate

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SUMMARY

A total number of 75 samples including 25 samples each of luncheon, minced frozen beef and sausage were collected from Assiut city (Upper Egypt) & examined bacteriologically for the presence of *E. sakazakii* and other Enterobacteriaceae genera & species by using the FDA enrichment procedure. In addition, a polymerase chain reaction (PCR) method for the rapid detection of *E. sakazakii* was adapted by using a set of primers designed from a region of the 16S ribosomal RNA gene. *E. sakazakii* was detected in 7 (28%) of sausage, but none of the luncheon and minced frozen beef samples were positive for *E. sakazakii*. Moreover, one false positive isolate was observed with FDA method. In contrast, PCR was highly sensitive and specific to *E. sakazakii*. *E. cloacae* were isolated from all samples, while *E. agglomerans* was detected only in luncheon samples. Also, 43 isolates related to family Enterobacteriaceae could be isolated including *Citrobacter freundii, Escherichia coli, Hafnia alvei, Klebsiella pneumoniae, K.oxytoca, K.ozaenae, K. planticola and Serratia marcescens.* The public health importance of *E. sakazakii* and suggested control measures were discussed.

***Key words**: Enterobacter sakazakii, luncheon, minced beef, sausage, PCR & FDA.*

INTRODUCTION

Enterobacter sakazakii (*E. sakazakii*) comprises a group of Gram-negative, motile, rod-shaped bacteria belonging to the family Enterobacteriaceae, genus *Enterobacter*. In the past, this organism was referred to as a "yellow- pigmented *Enterobacter cloacae*". In 1980, *E. sakazakii* was designated as a unique species on the basis of differences from *E. cloacae* in pigment production, biochemical

reaction and DNA relatedness (Farmer et al., 1980).

Several species within the genus *Enterobacter* have been recognized as important causative agents of hospital-acquired infections. *E. sakazakii* has been implicated in some outbreaks causing several outbreaks or sporadic cases of either severe neonatal meningitis or necrotizing enterocolitis in premature babies (Simmons et al., 1989; Van Acker et al., 2001). Although most documented cases involved infants, reports describe infections in adults as well (Lai, 2001). Overall, case-fatality rates have varied considerably with rates as high as 80 % in some instants. A mortality rate of 40 to 80 % has been recorded and in many cases neonates die within days of birth. Moreover, in surviving patients with meningitis, severe neurological sequlae such as ventriculitis, brain abscess, hydrocephalus and retarded neural development can occur (Willis and Robinson, 1988).

Recent taxonomic analyses have determined that *E. sakazakii* comprises a number of genomospecies, and it has been proposed that *E. sakazakii* may be reclassified as a novel genus, "Cronobacter" (Beuchat et al., 2009). Cawthorn et al. (2008) recommended the use of accurate methods for rapid detection and identification of this group of micro-organisms, since even low cell numbers have been reported to cause disease. In a recent review described the ubiquitous nature of the organism in food other than infant formula, *E. sakazakii* could be isolated from plant food and food ingredients like cereal, fruit and vegetables, legume products, herbs and spices as well as from animal food sources like milk, meat, fish and products made from these foods. The spectrum of *E. sakazakii*-contaminated food covers both raw and processed food. The kind of processing of *E. sakazakii*-contaminated food was not restricted to dry products. Fresh, frozen, ready-to-eat, fermented and cooked food products as well as beverages and water suitable for the preparation of food, were found to be contaminated by *E. sakazakii* (Friedemann, 2007).

Few researchers reported the mechanisms of pathogenicity of potential virulence factors of *E. sakazakii*. As Gram-negative organisms, *Enterobacter* species possess endotoxin and therefore they are considered opportunistic pathogens. They have been implicated in a broad range of clinical syndromes as infections of skin, soft tissue, respiratory tract, urinary tract and gastrointestinal tract (Sanders and Sanders, 1997). *E. cloacae* and *E. aerogenes* are well-known causes of nosocomial infections (Farmer and Kelly, 1992). Several strains of *E. cloacae* genetically related to *E. sakazakii* can produce exotoxin, aerobactin and hemagglutination (Biering et al., 1989; Sanders and Sanders, 1997).

The most frequently mentioned method of entry of *Enterobacter* in patients with bacteremia was ingestion (Weischer and Kolmos, 1992). There has been no epidemiological evidence for a minimal infectious dose. The recent (FAO \ WHO, 2004) draft risk assessment for *E. sakazakii* provides a fail-safe estimate of the infectivity per organism, assuming that all infected servings contain only one organism.

The International Commission for Microbiological Specifications for Foods (ICMSF, 2002) has ranked *E. sakazakii* as severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration. Due to the raised awareness of the organism and its implied role as an emerging food- borne pathogen are of concern to clinicians, the food industry and consumers. The high mortality rate, coupled with the lack of ecological information about this organism made the need for this study.

Polymerase chain reaction (PCR) detection methods are now widely used in microorganism identification. PCR provides a powerful tool for the rapid, specific and sensitive detection of foodborne pathogens and is considered a reliable alternative to traditional bacteriological methods (Malorny and Wagner 2005). In this respect, Lehner et al. (2004) designed a set of primers based on the 16S rRNA phylogenetic analysis sequences, which allows the identification of *E. sakazakii* strains by PCR.

As there is no researches about the occurrence of *E. sakazakii* in meat and meat products in Egypt and there is an increase interest of this pathogen as public health significance, therefore, the objectives of the current study were to determine the incidence of *E. sakazakii* and other Enterobacteriaceae species in commercial meat products including luncheon, minced frozen beef and sausage and to apply biochemical and genetic methods to characterize *E. sakazakii* isolates.

MATERIAL AND METHODS

Collection of samples:

A total number of 75 random samples of different commercial samples of meat products represented by 25 each of luncheon, minced frozen beef and sausage were collected from retail supermarkets and groceries in Assiut city. The collected samples were transferred directly to the laboratory in an ice box for bacteriological examination.

Preparation of samples:

At the laboratory, frozen samples were thawed in refrigeration for overnight. Each sample was aseptically and carefully freed from its casings and mixed thoroughly in sterile mortar.

Isolation and identification of *E. sakazakii* and other Enterobacteriaceae species:

The procedure of FDA (2002) for detection, isolation and identification of *E.*

sakazakii and other Enterobacteriaceae sp. in food samples was adapted as follows:

Pre-enrichment procedure:

Ten grams of each sample was weighed and homogenized in 90 ml of sterile buffered peptone water by Colworth stomacher (Lab. Blender, 400) and incubated overnight at 36°C.

Enrichment procedure:

Ten milliliters of the incubated sample was added to bottles contained 90 ml of Enterobacteriaceae enrichment broth (Oxoid CM317). The bottles were then incubated at 36°C for 14 – 16 hrs.

Selective plating:

A loopful of the enriched culture was streaked onto four quadrants of duplicate violet red bile glucose agar (Oxoid CM485) plates. After incubation at 36°C for 18 – 24hrs, typical colonies appear purple and surrounded by a purple halo of precipitated bile acids. Five presumptive colonies were streaked onto trypticase soy agar (Oxoid CM131) and incubated at 25°C for 48 – 72hrs. The yellow pigmentation on TSA is a characteristic feature of *E. sakazakii*.

Identification of isolates:

The revealed isolates were identified biochemically using conventional tests according to Nazarowec-White and Farber (1997) including catalase test, carbohydrates fermentation tests, citrate utilization, indol production, methyl red, oxidase test, Triple Sugar Iron, , and then Voges-Proskauer test.

DNA Extraction: (Gutiérrez-Rojo and Torres-Chavolla, 2007)

For each assay, 1ml of culture was centrifuged at 15700xg at 4°C for 10 min. Genomic DNA was extracted using the rapid lysis method described by Laird et al. (1991). The pellet was suspended in 500 µl of lysis buffer containing 100-mM Tris-Hcl (PH 8.5), 5-mM ethylenediamine tetraacetic acid, 0.2 % sodium dodecyl sulfate (Sigma Chemical Co., St. Louis, MO), 200-mM Nacl and 200 mg/ml proteinase K (Fermentas). Samples were incubated at 55°Cfor 75 min., cooled on ice, and the total DNA was precipitated by the addition of an equal volume of ice-cold isopropanol (Lab-Scan). The samples were stored at -80°C for 30 min. The DNA pellet was washed twice in 70% ethanol (Lab-Scan), air-dried and suspended in 100 µl of DNAse-, RNAse-free distilled water (Roval Lab, Germany).

Oligonucleotides:

Previously described oligonucleotide primers that allow the amplification of a 929-bp DNA fragment from a region of the 16S ribosomal RNA gene in *E. sakazakii* species were used in this work (Lehner et al., 2004). Primer sequences used in the PCR are listed in Table 1.

Table 1: Species-specific PCR primers evaluated for the detection of *Enterobacter sakazakii*

Primer name	Primer sequence (5' to 3')	Target	Amplicon size (bp)	Reference
Esakf	GCT YTG CTG ACG AGT GGC GG	16S rRNA gene	929 bp	Lehner et al., 2004
Esakr	ATC TCT GCA GGA TTC TCT GG			

PCR reaction conditions: (Lehner et al., 2004)

For amplification, reaction mixtures (total volume 50 µl) containing primer at a concentration of 10 pM /each, using 10 x Taq reaction mixture, 2 U Taq polymerase (Fermentas) and 200 µM of dexoynucleotides (dNTPs) each were prepared. PCR was carried out in a thermal cycler (Techne Progene, Cambridge, UK) using the following conditions: initial DNA denaturation at 94°C for 2 min., followed by 29 cycles where each cycle consisted of a denaturation at 94°C for 30 sec, an annealing at 62 °C for 1 min. and an extension at 72°C for 90 sec. Cycling was completed by a final extension step at 72°C for 5 min. The reaction products were resolved on a 1.5 % agarose gel followed by ethidium bromide (0.5µg/ml) staining and examination under UV light. The 100 bp plus DNA ladder (Jena Bioscience, Germany) was used as molecular size marker.

RESULTS

The results of this study were illustrated in Tables (2, 3 & 4) and Fig. (1).

Table 2: Incidence of *E. sakazakii* in the examined meat products samples using FDA method.

Types of samples	No. of examined samples	No. of positive samples	% of *E. sakazakii*
Luncheon	25	0	0
Minced frozen meats	25	0	0
Sausage	25	7	28
Total	75	7	9.33

Table 3: Relationship among the conventional biochemical and molecular method for identification of *E. sakazakii* isolates

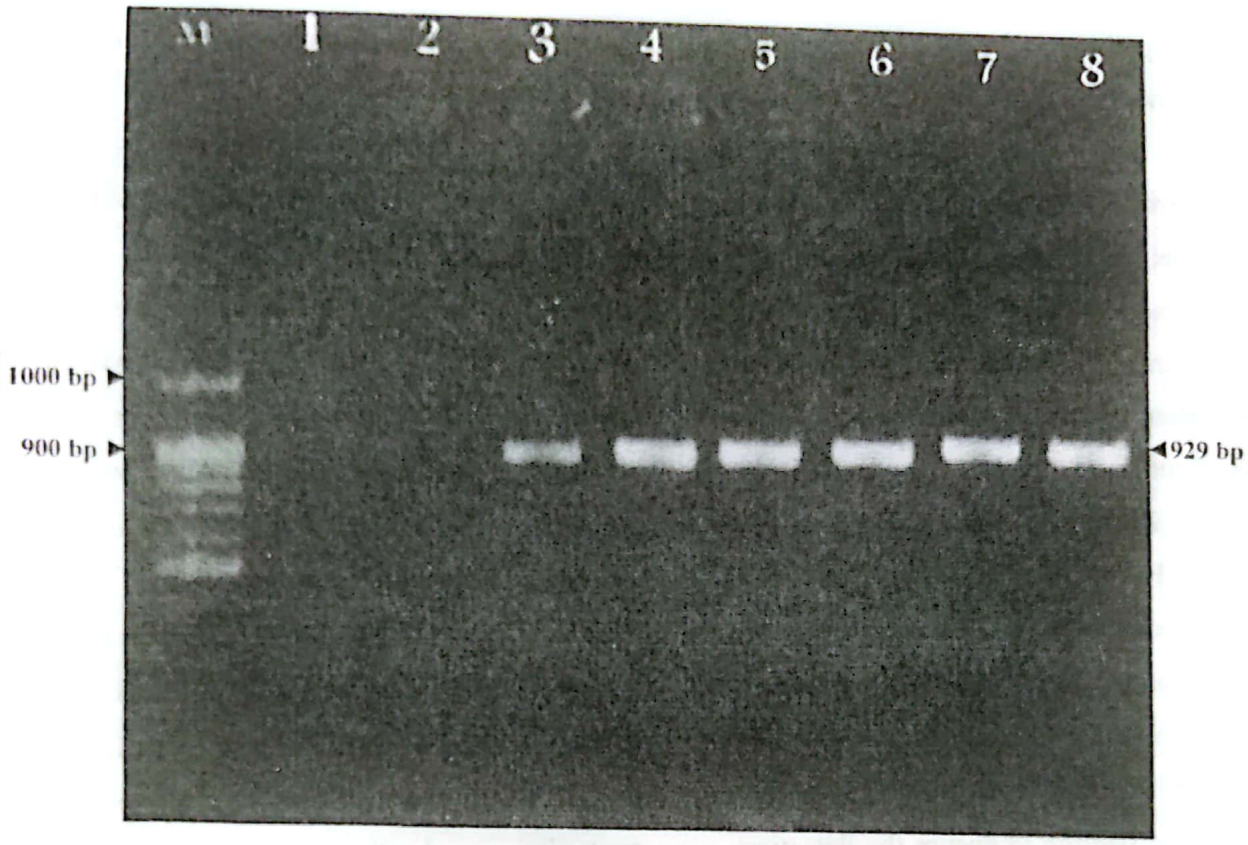
Types of samples	No. of examined samples	Conventional method		Molecular method	
		No.	%	No.	%
Luncheon	25	0	0	0	0
Minced frozen meats	25	0	0	0	0
Sausage	25	7	28	6	24

Table 4: Frequency distribution of Enterobacteriaceae species isolated from meat products.

Enterobacteriaceae species	Luncheon		Minced frozen meats		Sausage	
	No.	%	No.	%	No.	%
Citrobacter freundii	–	–	1	4	1	4
Enterobacter agglomerans	1	4	–	–	–	–
Enterobacter claocae	7	28	4	16	2	8
Escherichia coli	3	12	10	40	5	20
Hafnia alvei	–	–	–	–	1	4
Klebsiella pneumoniae	1	4	5	20	6	24
Klebsiella oxytoca	1	4	3	12	2	8
Klebsiella ozaenae	1	4	–	–	1	4
Klebsiella planticola	–	–	1	4	–	–
Serratia marcescens	–	–	1	4	–	–

No. = 25 for each product.

Fig (1): Agarose gel electrophoresis of amplification products obtained from genomic DNA of *E. sakazakii* stained with ethidium bromide.



Lane M: Molecular weight ladder (100 bp)
Lane 1: Negative control
Lane 2: Negative sample
Lane 3-8: *E. sakazakii* positive samples

DISCUSSION

E. sakazakii that designated as a unique species in 1980 (Farmer et al., 1980), has implicated as the causal organism in a rare but severe form of neonatal meningitis (Gallagher and Ball, 1991). A mortality rate of 50 to 75 % has been reported by Willis and Robinson (1988). Muytjens and Kollee (1990) investigated the occurrence of *E. sakazakii* more widely, but they could not isolate the organism from any environment they examined, which included surface water, soil, mud, rotting wood, grain, bird dung, rodents, domestic environments, cattle and untreated cow's milk.

The present study gives an idea about the prevalence of *E. sakazakii* in luncheon, minced frozen meats and sausage. As outlined in the presented Table 2, only 7 out of the 25 sausage samples representing 28 % were contaminated by *E. sakazakii*. The presence of the pathogen in sausage is not surprise as Friedemann (2007) reviewed a

number of other reports outlining the presence of *E. sakazakii* on a range of meats including pork, camel, poultry and sausages. Such higher incidence in sausage samples reflects the contribution of other environmental cross contaminating sources during their processing. Also, the ability of *E. sakazakii* to form biofilms in manufacturing environments is likely to aid its persistence in the food factories providing a risk of contamination (Kandhai et al., 2004; Kim et al., 2006).

The FDA method utilizes yellow pigment production on TSA as criteria for identification of presumptive *E. sakazakii* isolates. However, it has been documented that these methods are not sufficiently sensitive and specific to ensure the detection and correct identification of *E. sakazakii* in food and environmental samples (Iversen et al., 2004). Therefore, in this study the suitability of yellow pigment production for *E. sakazakii* identification was evaluated on the isolates and which were identified by 16S rDNA amplification.

It is obvious from the achieved results in Table 3 that only six of the 7 yellow-pigmented isolates were confirmed to be *E. sakazakii* by PCR for a fragment of 929 bp of 16S rDNA a result that reflect the yellow pigment production is not restricted on *E. sakazakii* but to other members, this opinion came in close to that reported by Cawthorn et al., (2008) who described that the yellow pigment producing non-*E. sakazakii* isolates included members of the genera Acinetobacter and Pantoea, as well as other species of Enterobacter.

E. sakazakii was not the only species isolated from the examined samples, but also, other species of the same genus were detected in some of the samples, namely, *E. cloacae* and *E. agglomerans*. It is evident from Table (4) that *E. agglomerans* was only detected in luncheon samples. Additionally, it was also noticed that, *E. cloacae* constituted the highest prevalence (13 isolates) among all Enterobacter species even *E. sakazakii* (6 isolates). It was found that several strains of *E. cloacae* are genetically related to *E. sakazakii* and can produce exotoxins, aerobactin and haemagglutinin (Sanders and Sanders, 1997). Moreover, Enterobacter species can create community infections that are responsible for approximately half of all nosocomially acquired infections and have been recognized as important agents of other hospital-acquired infections (Hervas et al., 2001).

Regarding the results in Table (4), it is obvious that different species of Enterobacteriaceae were isolated in low incidence (4 %). While, the most prevalent isolates of Enterobacteriaceae were *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter cloacae*. Furthermore, it follows from the foregoing that contamination of the examined food samples

in this study with Enterobacteriaceae could be taken as an index of fecal pollution and could be attributed to the unsanitary practices and poor hygienic quality of ingredients used. It has been hypothesized that the reservoir for *E. sakazakii*, in addition to other coliforms as *Klebsiella oxytoca*, *K. pneumoniae*, *E. cloacae* and Citrobacter species, may be primarily environmental and from plant materials (Mossel and Struijk, 1995).

Conclusion

The sensitivity, specificity and accuracy of the FDA method were demonstrated to be lowered by the use of yellow pigment production as an identification criterion for *E. sakazakii* in different meat products. The current FDA method thus needs to be revised and other identification methods need to be incorporated that allow more rapid and specific detection of *E. sakazakii* (Iversen et al., 2008).

Good manufacturing practices and the implementation of Hazard Analysis Critical Control Point (HACCP) program in food manufacturing and food preparation should be done to improve quality and control pathogenic microorganisms contaminating foods. In addition, public health authorities and researchers are exploring ways to gain a better understanding of the ecology, taxonomy, virulence and other characteristics of *E. sakazakii* and on ways to eliminate and control its growth in foods and processing environments (Gurtler et al., 2005).

REFERENCES

Beuchat, L. R.; Kim, H.; Gurtler, J. B.; Lin, L. C.; Ryu, J. H. and Richards, G. M. (2009): *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. Int. J. Food Microbiol., 136 : 204-213.

Biering, G.; Karlsson, S.; Clark, N. C.; Jonsdottir, K. E.; Ludvigsson, P. and Steingrimsson (1989): Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. J. Clin. Microbiol., 27: 2054-2056.

Cawthorn, Donna-Mareé; Botha, Sharon and Corli Witthuhn, R. (2008): Evaluation of different methods for the detection and identification of *Enterobacter sakazakii* isolated from South African infant formula milks and the processing environment. Int. J. Food Microbiol., 127: 129-138.

Farmer, J. J. and Kelly, M. T. (1992): Enterobacteriaceae. In Barlows (ed) Manual of Clinical Microbiology. ASM, Washington, D. C.: 360-383.

Farmer, J. J.; Asbury, M. A.; Hickman, F. W.; Brenner, D. J. and the Enterobacteriaceae study Group (1980): *Enterobacter sakazakii*, new species of Enterobacteriaceae isolated from clinical specimens. Int. J. Syst. Bacteriol., 30: 569-584.

FDA (Food and Drug Administration) (2002): Isolation and enumeration of *Enterobacter sakazakii* from dehydrated powdered infant formula. Center for Food Safety and Applied Nutrition.

FAO / WHO (Food and Agriculture Organization / World Health Organization) (2004): *Enterobacter sakazakii* and other

microorganisms in powdered infant formula: Meeting Report, MRA series 6. FAO / WHO, United Nations.

Friedemann, Miriam (2007): *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). "A review" Int. J. Food Microbiol., 116: 1-10.

Gallagher, P. G. and Ball, W. S. (1991): Cerebral infractions due to CNS infection with *Enterobacter sakazakii*. Pediatr. Radiol., 21: 135-136.

Gurtler, J.B.; Kornacki, J.L. and Beuchat, L.R. (2005): *Enterobacter sakazakii*: A coliform of increased concern to infant health: A review. Int. J. Food Microbiol., 104:1-34.

Gutiérrez-Rojo, R. and Torres-Chavolla, E. (2007): A rapid polymerase chain reaction assay for *Enterobacter sakazakii* detection in infant milk formulas. Journal of Rapid Methods & Automation in Microbiology, 15 (4): 345-358.

Hervas, J. A.; Ballesteros, F.; Alomar, A.; Gil, J.; Benedi, V. J. and Alberti, S. (2001): Increase of Enterobacter in neonates sepsis: a twenty-two-year study. Pediatr. Infect. Dis. J., 20: 134-138.

International Commission on Microbiological Specification for Foods (ICMSF) (2002): Microbiological Testing in Food Safety Management (Vol.7) New York: Academic / Plenum Publisher.

Iversen, C.; Druggan, P. and Forsythe, S. (2004): A selective differential medium for *Enterobacter sakazakii*, a preliminary study. J. Food Microbiol., 96: 133-139.

Iversen, C.; Druggan, P.; Schumacher, S.; Lehner, A.; Feer, C.; Joosten, H. and Stephan, R. (2008): Development of a novel screening method for the isolation of Cronobacter spp. (*Enterobacter sakazakii*). Appl. Environ. Microbiol., 74: 2550-2553.

Kandhai, M. C.; Reij, M. W.; Gorris, L. G.; Guillaume-Gentil, O. and van Schothorst, M. (2004): Occurrence of *Enterobacter sakazakii* in food production environments and households. Lancet, 363: 39-40.

Kim, H.; Ryu, J. H. and Beuchat, L. R. (2006): Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. Appl. Env. Microbiol., 72: 5846-5856.

Lai, K. K. (2001): *Enterobacter sakazakii* infections among neonates infants, children and adults. Case reports and a review of the literature. Medicine, 80: 113-122.

Laird, P.W.; Zijderveld, A.; Linders, K.; Rudnicki, M. A.; Jaenischi, R.; and Berns, A. (1991): Simplified mammalian ADN isolation procedure. Nucleic Acids Res., 19:4293-4295.

Lehner, A.; Tasara, T. and Stephan, R. (2004):16S rRNA gene based analysis of *Enterobacter sakazakii* strains from different sources and development of a PCR assay for identification. BMC Microbiol., 4(43): 1-7.

Malorny, B. and Wagner, M. (2005): Detection of *Enterobacter sakazakii* strains by real-time PCR. J. Food Prot. 68 (8):1623–1627.

Mossel, D.A.A. and Struijk, C.B. (1995): *Escherichia coli*, otras Enterobacteriaceae e indicadores adicionales como marcadores de la calidad microbiologica de los alimentos. Microbiologia SEM, 11: 75-90.

Muytjens, H. L. and Kollee, L. A. (1990): *Enterobacter sakazakii* meningitis in neonates: a causative role of formula. Pediatr. Infect. Dis. J., 9: 372-373.

Nazarowec-White, M. and Farber, J. M. (1997): Incidence, survival and growth of *Enterobacter sakazakii* in infant formula. J. Food Prot., 60(3): 226-230.

Sanders, W. E. and Sanders, C. C. (1997): Enterobacter spp: Pathogens poised to flourish at the turn of the century. Clin. Microbiol. Rev., 10: 220-241.

Simmons, B. P.; Gelfand, M. S.; Haas, M.; Metts, L. and Feruson, J. (1989): *Enterobacter sakazakii* infection in neonates associated with intrinsic contamination of powdered infant formula. Infect. Control Hosp. Epidemiol., 10: 398-401.

Van Acker, J.; De Smet, F.; Muyldermans, G.; Bougatef, A.; Naessens, A. and Lauwers, S. (2001): Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J. Clin. Microbiol., 39: 293-297.

Weischer, M. and Kolmos, H. J. (1992): Reprospective 6-year study of Enterobacter bacteraemia in a Danish university hospital. J. Hosp. Infect., 20: 15-24.

Willis, J. and Robinson, J. E. (1988): *Enterobacter sakazakii* meningitis in neonates. Pediatr. Infect. Dis., 7: 196-199.

حدوث ميكروب الإنتيروباكتر ساكازاكى فى بعض منتجات اللحوم المباعة فى محافظة أسيوط

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نظرا لخطورة ميكروب الإنتيروباكتر ساكازاكى على الصحة العامة ، لذلك تضمنت هذه الدراسة فحص عدد ٧٥ عينة (٢٥ عينة لكل من اللانشون واللحوم المستوردة المفرومة والسجق) تم جمعها من المحلات والسوبرماركت المختلفة بمحافظة أسيوط لمعرفة مدى تلوثها بهذا الميكروب بإستخدام الطريقة التقليدية لعزله.

كما تضمنت الدراسة الكشف عن ميكروب الإنتيروباكتر ساكازاكى بإستخدام أحدث الطرق التشخيصية و ذلك بإجراء إختبار البلمرة المتسلسل (PCR)، بإستخدام مجموعة من البادئات الخاصة (specific primers) المحضرة صناعيا و معروف نظام تسلسلها النووى لتكبير جزء متخصص من منطقة (16S ribosomal RNA gene) الخاصة بهذا الميكروب

. وقد أسفرت النتائج عن تواجد الإنتيروباكتر ساكازاكى بنسبة ٢٨ % فى عينات السجق بينما لم يتم عزل الميكروب من عينات اللانشون واللحوم المستوردة المفرومة. وباستخدام تقنية تفاعل انزيم البلمرة المتسلسل (PCR) تم تأكيد وجود الميكروب فى ستة عينات من السجق بنسبة ٢٤ %. وبجانب الإنتيروباكتر ساكازاكى أمكن كذلك عزل ميكروبات الإنتيروباكتر كلواكا (E.cloacae) والأجلوميرانز (E. agglomerans) من العينات السابقة بنسب مختلفة. ذلك بالاضافة الى عدد ٤٣ عترة أخرى من الميكروبات المعوية تم عزلها بنسب مختلفة وهم :

Citrobacter freundii, Escherichia coli, Hafnia alvei, Klebsiella pneumoniae, K. oxytoca, K. ozaenae, K. planticola and Serratia macescens.

وقد تم مناقشة مدى خطورة ميكروب الإنتيروباكتر ساكازاكى على صحة وسلامة الإنسان والطرق الواجب اتباعها للحد من تلوث منتجات اللحوم بهذا الميكروب.