Assiut Vet. Med. J. Vol. 38 No. 75, October 1997

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Assiut Regional Laboratory,
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INCIDENCE OF BACILLUS CEREUS IN SOME FOOD STUFFS WITH THE SPECIAL REFERENCE TO ITS PRODUCTION OF THERMONUCLEASE ENZYME IN ASSIUT CITY

(With 3 Tables)

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(Received at 15/7/1997)

مدى تواجد ميكروب الباسيللس سيريس فى بعض أنواع الأغذية بمدينة أسيوط مع الإشارة إلى انتاجيته لأنزيم السيرمونيوكليز

نوال خليل

تضمن البحث فحص ٢٠٠ عينة من بعض أنواع الأغذية فى مدينة أسيوط بواقع ٤٠ عينة لكل من اللانشون والسجق والجبن القريش والايس كريم والزبادى وذلك لمعرفة مدى تواجد ميكروب الباسيلس سيريس بها باستخدام طريقة الفرد السطحى على مستنبت KG. وأثبتت النتائج عن تواجد الميكروب فى عينات اللانشون والسجق والجبن القريش والايس كريم بنسب ٥٠٪، ٥٧٪، ٣٧,٥٪، ٨٥٪، على التوالى وكان متوسط العدد الكلى للياسيليس سيريس ٣,٨ × ١٠^٥، ٥ × ١٠^٤، ١,٥٢ × ١٠^٣، ٤,٥ × ١٠^٤ على التوالى. لم نتمكن من عزل الميكروب من أى من عينات الزبادى. وقد شمل الجزء الثانى من البحث تعين أنزيم السيرمونيوكليز الثابت والمحطم بالحرارة. وشمل الجزء الثالث من البحث تأثير المضادات الحيوية على العترات المعزولة من الميكروب وأوضحت النتائج أن جميع العترات أظهرت حساسية بنسبة ١٠٠٪ لكل من باستراسين والكلور مفينكول والارثرومايسين والجنناميسين والكاناميسين ونيومايسين والاسترتبومايسين والنتراسيكلين. وعلى عكس ذلك جميع العترات غير حساسة للكولستين والبنسلين. هذا وقد نوقشت الأهمية الصحية للميكروب والشروط الواجب مراعاتها لانتاج أغذية سليمة.

SUMMARY

A total of 200 random samples of meat and milk products (40 samples of lunchoen, susage, kareish cheese, ice cream and yoghurt) were collected from Assiut city. The samples were examined

bacteriologically to enumerate and isolate *Bacillus cereus* using surface platirig technique on KG agar plates. *Bacillus cereus* was found in 20 (50%); 23(57%); 15(37.5%) and 34(85%) samples of lunchoen, susage, kareish cheese and ice cream with a mean values of 3.8×10^5, 5×10^4, 1.52×10^3 and 4.5×10^4 cfu/g or ml, respectively. *Bacillus cereus* could not be isolated from yoghurt. 55.43% of isolated *Bacillus cereus* produce thermolabile Deoxyribonuclease at PH (9) while 14.13% produce it at pH (6.7). On the other hand thermostable Deoxyribonuclease activated only at pH(9) with incidence of 3.26%. All *Bacillus cereus* strains were highly senstive to bacitracin, chlormephenicol, erythromycin, gentamycin, kanamycin, neomycin, streptomycin and tetracycline, whereas all isolates were resistant to colistin and pencillin. The puplic health importance and sanitary measures to minimize contamination of food stuffs with *Bacillus cereus* are discussed.

Key words: *Food stuffs - Bacillus cereus - Thermonuclease enzyme*

INTRODUCTION

Bacillus cereus is a large Gram positive bacillus resembling *Bacillus anthracis*, except that it is motile and lacks the glutamic acid capsule. Also, *B.cereus* is one of the most important heat resistance aerobic sporeforming bacteria, which belong to the genus Baccillus (Griffith, 1992).

B.cereus is widely distributed in the environment, the organism can be introduced into food from soil, air, water, equibment and workers.

The unique combination of both thermoduric and psychrotrophic properties of *B.cereus* represent recurrent problems of meat and milk industries. The organism associated with defects such as off-flavours, sweet curdling and spoilage of both meat and milk products due to proteniase, lipase and phospholipase produced by B.cereus and this led to economic losses (Giffel et al., 1995).

The importance of *B.cereus* which may be present in meat and milk products (especially lunchoen, susage, kareish cheese, ice cream and yoghurt) is its harmful effect on human when consumed, therefore *B.cereus* is recognized as one of the potential organisms for human food poisioning [christiansson, (1992), Baker and Griffths (1993) and Granum et al. (1993)].

Two different forms of food poisioning have been attributed to consumption of contaminated food with *B.cereus*, a rapid onest emetic form

which is characterized by acute attack of nausea, vomiting and abdominal cramps, which occurs within 30 mins. to 5 hours, heat-stable enterotoxin is responsible for emetic form while the second diarrheal form occurs within 8 - 16 hours after ingestion of contaminated food, this is due to heat-labile entero toxin produced during multiplication of *B.cereus* in situ. This form is characterized by diarrhea, nousea and abdominal cramps (Patrick et al., 1994). The number of such organisms required to produce disease in man is generally of order 10^6 - 10^8, however in compromised consumers a much smallar dose (1.2×10^3) may cause illness (Gianella and Brasile, 1979).

The purpose of this investigation is to study the incidence and enumaration of *B.cereus* in lunchoen, susage, kareish cheese, ice- cream and yoghurt sold in Assiut city.

Testing the isolated strains of *B.cereus* for production of thermolabile and thermostable Deoxyribo nucleas. Study the antibiotic sensitivity of isolated *B.cereus* strains. Discuss the public health importance of *B.cereus*.

MATERIAL and METHODS

Two hundred randum samples including Lunchoen, susage, kareish cheese, ice-cream and yoghurt (each 40 samples) were collected from super markets, markets, dairy shops and groceries in Assiut city. The samples were transferred in sample cases and delivered promptly to the laboratory.

Preperation of samples:

In a sterile morter, to 10 grams of thoroughly mashed sample (lunchoen, susage and kareish cheese) 90 ml of peptone water 1% were added and thourougly mixed to make a dilution 1:10, on the other hand ice-cream samples were melted in a thermostatically controlled water bath (40C°±1 C°) for 15 minutes and well mixed. For yoghurt samples each was thoroughly mixed and 10ml or g were added to 90ml peptone water 1% to make a dilution 1 :10, from the supernatant fluid 1ml was taken by using sterile pipette to make 10-fold serial dilutions technique.

From the prepared samples 10-fold serial dilutions up to 10^{-4} were made, using sterile peptone water.

Enumeration and isolation of *B.cereus*:

One tenth ml amount of each of the prepared dilutions was carefully transferred and evenly spread over a dry surface of KG agar plates as recommended by Kim and Goepfert, 1971. Following

incubation at 37 C° for 24h, KG plates were examined for typical colonies, which were dry flat and surrounded by wide cloudy zones.

Colonies presumed to be *B.cereus* were transferred to nutrient agar slants. Gram's stain and motility test were performed on each isolate. Confirmatory tests were based on carbohydrate utilization, nitrate reduction, and production of acetymethyl- carbinyl according to Cowan and Steel (1974). Numbers of confirmed *B.cereus* / g or ml were counted and recorded.

Surveying the isolated strains of B.cereus for production of thermolabile and thermostable Deoxyribonuclease at pH (6.7) and pH (9):

Toluidine blue -O- DNA agar (TDA).was prepared and 15ml. quantities were pipetted into *plates*. After solidification, wells of 2mm. diamter were cut in the agar, and agar cuts were removed with a metal canula. Over night brain-heart infusion broth cultures (before and after heating, in a boiling waterbath, for 15 minutes) were added to the wells using micropipette and incubated at 37C°. positive results were indicated by development of bright pink zones arround wells after 3 and 24 h. of incubation (Lachia, *et al* 1971).

Antibiotic sensitivity of isolated *B.cereus* strains:

The diffusion method described by Cruickshank *et al* (1975) was performed using different types of sensitivity discs obtained from Bio Merieux France.

RESULTS

The results are presented in Tables (1-3).

DISCUSSION

The results given in table (1) showed that out of 40 samples of Lunchoen, *B.cereus* could be isolated from 20 (50%) with a maximum count of 3×10^6 /g, while the minimum count was 2×10^3 /g with a mean value of 3.8×10^5.Similar observation were reported by Hefnawy et al. (1984) and Lotfi et al. (1988), on the other hand Ashmowee (1994) could detecte the organism in Lunchoen samples with high incidence 92%. The mean count /g was nearly similar count.

Out of 40 samples of susage *B.cereus* could be isolated from 23 (57%) with a count of 5×10^5 /g as a maximum, 7×10^2 /g as a minimum

and a mean value of 5×10^4 Table (1). These results were agreed with that reported by El-said (1992) and Lotfi et al. (1988). High incidence (92%) was reported by Ashmawee (1994) while low one (28%) was reported by Hefnawy et al. (1984).

From this study the incidence of *B.cereus* in meat products pointed that the meat products contained high *B.cereus* count and this may be attributed to contamination of flesh used for manufacture of such products. Mincing machines, grinders, equipments and knives considered a main source of contamination of meat during processing. Moreover addition of spices, which often contain a large number of spore formers, to meat led to marked increase in bacterial population. Unsatisfactory hygienic measures during preparation, handling and distribution, may play a role in the contamination (Hefnawy *et al* (1984). Lotfi et al. (1988); El-said (1992) and Ashmawee (1994).

In case of kareish cheese *B.cereus* was isolated from 15(37.5%) of examined samples, with a maximum count of 3×10^4/g and a minimum count of 2×10^2/g. The mean value was 1.52×10^3/g. (Table 1). This is somewhat agree with the incidence of (45%) reported by Ell-Boudy (1985), while low incidence (18% and 9.09%) were reported by Saad (1985) and Aman and Ahmed (1997) respectively.

Regarding results of ice-cream samples *B.cereus* could be isolated from 34(85%) of examined samples, in counts ranging from 10 to 9×10^4/ml with a mean value of 4.5×10^4/ml. The results obtained are nearly similar to that reported by Abdel Haleem (1995), while incidence of 38% and 40% were reported by Al-ashmawy et al. (1996) and Rangasamy et al. (1993) respectively. Low results (11.36% and 16%) were reported by Ahmed (1980) and El-Bagoury (1992).

B.cereus couls not be isolated from examined samples of yoghurt. These results are similar to those obtained by Ahmed *et al* (1983) and Saad (1985). The failure of detecting these organisms in yoghurt may be due heating of milk during manufacturing and, or from the inhibitory effect of lactic acid bacteria on *B.cereus* (Saad, 1985).

Generally, *B.cereus* can gain access to dairy products from different sources during its manufacturing, handling and directly from contaminated ingredients of animal (milk). *B.cererus* gain entery into milk by variety of routes. Contamination via the udder can occur either by infection with *B.cereus* causing acute mastitis (Jones and Turnbull 1981). or by sticking direct from soil, bedding, dung, fodder, pasture and water (Becker and Terplan 1989). Other possible sources may be milk handling equipment,

transport vessels and processing equipment (Heddehem and Vlaemynck 1992).

The incidence of deoxyribonuclease (DNase) producing *B.cereus* recovered from 92 samples of meat and milk products was shown in (Table 2). The incidence of thermolabile DNase postive *B.cereus* at pH (9.0) was greatest in lunchoen 13(65%), followed by ice-cream 20(58.8%), susage 12(52.2%) and kareish cheese 6(40%) while thermolabile DNase positive *B.cereus* at pH(6.7) was 20%, 14.71%, 13.04% and 10% in case of kareish cheese, ice-cream, susage and lunchoen, respectively.

Goepfert et al. (1972) mentioned that the pathogenic character of *B.cereus* is due to a toxic protein like component, secreted by the organism during growth, this exotoxin was described to be thermolabile. Moreover thermostable DNase was recovered only on 2(8.7%) susage and 1(6.7%) kariesh cheese at pH (9).

The production of thermolabile and thermostable DNase by *B.cereus* from milk and meat products were reported by Saad (1985) and Lotfi et al. (1988).

Results in this study indicate that some strains of *B.cereus* are capable to produce thermostable DNase. Moreover, *B.cereus* thermostable DNase production was activated at pH 9 than pH 6.7 and this was similar to that of staphylococcal thermostable DNase. So the production of the enzyme by *B.cereus* should be viewed with concern.

It is evident from (Table 3) that all isolates of *B.cereus* were highly sensitive to bacitracin, chlormephenical, erythromycine, gentamycin, kanamycin, neomycin, streptomycin and tetracycline. On the other hand, all isolates were resistant to colistin and pencillin. These results were in agree with that reported by Al-Ashmay et al. (1996) and Chopra et al. (1980).

It is apparent from the results obtained in this study that meat and milk products sometimes could contain *B.cereus* strains with various levels of contamination. Furthermore if the conditions are suitable, multiply rapidly and produce sufficient toxin to induce symptoms of food poisoning. Therefore, the effective measures should be applied to reduce the number of *B.cereus* in the ingredient through; proper heating (temperature/time) during preparation and filling of the product, adequate refrigeration temperature of the product after the preparation, during distribution and in the house hold and finally prevention of the contamination of raw product by the equipment in relation to scale of production.

REFERENCES

Abdel-Haleem, A.A (1995): Microbiological evalution and sanitary improvement of ice-cream. Ph.D. (Milk Hygiene) Fac. Vet. Med. Assiut-univ.

Ahmed, S.H (1980): Studies on acute gastrointestinal infections in Assiut Governorate. Ph.D. Thesis, Fac. of Med. Assuit univ.

Ahmed, A.A.H, Moustafa, M.K. and Marth, E.H. (1983): Incidence of *Bacillus cereus* in milk and some milk products. J. Food Protection 46(2) 126-128.

AL-Ashmawy, A.M.;Ell-Boudy, A.A; El-Gamal, A.M and Youssef SH.M. (1996): Occurrance and enumeration of *Bacillus cereus* in Egyptian dairy desserts. Assiut Vet. Med.J. vol 36 No 71; 117-124.

Aman, I.M. and Ahmed, H.F. (1997): Incidence and survival of some foodborne pathogens in milk and cheese. J.Egypt Vet. Med. Ass. 57, No 1: 151-167.

Ashmawee, M.M. (1994): Predominance of some Food poisoning microorganisms in meat products. Ph.D. Thesis (Meat Hygiene). Fac. Vet. Med. Assiut univ.

Baker, M.J and Griffiths, W.M (1993): Prodictive modling of psychrotrophic *Bacillus cereus*. J. Food Prot. 56,8, 684-688.

Becker, H. and Terplan (1989): Bacillus cereus occurrence and significance in dried milk products. Molkereitechnik 82-83 pp 77-78.

Chopra, P.; Singh, A and Kalar, M.S. (1980): Occurrence of *B.cereus* in milk and milk products. Indian J. Dairy Sci. 33: 248-252.

Christiansson, A. (1992): The toxicology of *Bacillus cereus* in milk and milk products. Bulletin IDF 275, 30-35.

Cowan, S.T. and Steel, K.J. (1974): Mannual for identification of medical bacteria 2nd Ed. Cambridge. Cambridge University press.

Cruickshank, R.; Duguid, J.P.; Marmion, B.R and Swain R. H.A (1975): Medical Microbiology 12th Ed. Vol, 11, Churchill Living stone, Edinburg, London and NewYork.

El-Bagoury. A.R.M. (1992): Incidence and public health importance of food poisoning causative organisms in milk and some dairy products in Kaliobia Governorate. Ph. D. Thesis Fac. of Vet. Med. Zagazig Univ. "Benha Branch".

Ell-Boudy, A.A (1985): studies on sporeforming food poisoning organisms in milk and some milk products. M.V. sc Thesis (Milk hygiene)Fac. Vet. Med. Alex. Univ.

El-Said, S.A. (1992): Studies on susage marketed in sharkia. Ph D. Thesis (Meat Hygiene). Fac. Vet. Med. Zagazig Univ.

Gianella, R.A. and Brasile, L. A (1979): A hosptial Food borne outbreak of diarrhoea *caused* by *Bacillus cereus.* J. Infect. Diseases 139, 366.

Giffel, M.C,; Beumer, R.R.; Slaghuis, B.A. and Rombouts, F.M. (1995): Occurrance and characterization of (psychrotrophic) *Bacillus cereus* on farms in the Netherlands. Nether. Milk and Dairy J. 49, 125-138.

Goepfert; J.M.; Spira, W.M. and kim, H.U. (1972): Bacillus cereus food poisoning organism. A review. J. Milk food Technol. 35: 213-227.

Griffiths, M.W. (1992): Bacillus cereus in liquid milk and other milk products. Bulletin IDF 276, 36-39.

Granum, P.E.; Brynestad, S.; Sulivan, K.O. and Nissen, H. (1993): Enterotoxin from *Bacillus cereus* production and characterization. Nether. Milk and Dairy J. 47: 63-70.

Heddeghem. M and Vlaemynck, G (1992): Sources of contamination of milk with *Bacillus cereus* on the farm and in the factory. Bulletin IDF, 275: 19-22.

Hefnawy, Y,; Youssef, H.; Abdel-Rahman and Lotfi, A(1984): Occurrence of *Bacillus cereus* in selected meat products. Fleischwirtsch 64 (11), 1371-1372.

Jones, T.O. and Turnbull, P.C.B. (1981): Bovine mastitis caused by *Bacillus cereus* Vet. Rec., 108; 271-278.

Kim, H.U. and Goepfert, J.M. (1971): Occurrence of *Bacillus cereus* in selected dry food products. J. Milk food, Technol. 34: 12-15.

Lachia, R.V. F.; Genigeogis, C. and Hoeprich, P.D. (1971): Metachromatic agar-diffusion methods for detection of staphylococcal nuclease activity. Appl. Microbiol. 21: 585-587.

Lotfi, A.; Youssef, H.; El-Khateib, T; seddik, I; EL- Timawy, A. and Ali, G. (1988): Incidence of *Bacillus cereus* in meat products. Assiut Vet. Med. J. Vol. 20, No 39: 11-115.

Patrick, R.M; George S.K; Michael, A.P. and Ken S.R. (1994): Medical Microbiology. 2ed Ed. International student published ISBNO.

Rangasamy, P.N.; Lyer, M. and Roginski, H. (1993): Isolation and characterisation of *Bacillus cereus* in milk and dairy products manufactured in Victoria. The Australian J. of Dairy Technology 48: 93-95.

Saad, N.M. (1985): Occurrence of *Bacillus cereus* in milk and milk products in Assiut City. Ph D. Thesis Fac. of Vet. Med. Assiut Univ.

Table (1) Incidence and count of *Bacillus cereus* in examined samples of meat and milk products.

Products	No. of examined samples	Postive samples		Count / g or ml		
		No.	%	Minimum	maximum	Mean
Lunchoen	40	20	50	2×10^3	3×10^6	3.8×10^5
Susage	40	23	57	7×10^2	5×10^5	5×10^4
kareish cheese	40	15	37.5	2×10^2	3×10^4	1.52×10^3
Ice-cream	40	34	85	10	9×10^4	4.5×10^4
Yoghurt	40	0	0	0	0	0

Table (2) The incidence of isolated strains of *Bacillus cereus* producing deoxyribonuclease from meat and milk products.

Products	No. of strains tested	Deoxyribonuclease producing *Bacillus cereus*					
		Thermolabile				Thermostable	
		pH(9.0)		pH(6.7)		pH(9.0)	
		No.	%	No	%	No	%
Lunchoen	20	13	65	2	10	0	0
Susage	23	12	52.2	3	13.04	2	8.7
Kareish cheese	15	6	40	3	20	1	6.7
Ice-cream	34	20	58.8	5	14.71	0	0
Total	92	51	55.43	13	14.13	3	3.26

Table (3): Antibiotic susceptibility of 92 strains of *Bacillus cereus* isolated from meat and milk products.

Antimicrobial agent	Disk Content	Resistant		Intermediate		Senstive	
		No	%	No	%	No	%
Ampicillin	10 mcg	15	16.30	2	2.17	75	81.52
Bacitracin	10 iu	0	0	0	0	92	100
Chlormephenicol	30 mcg	0	0	0	0	92	100
Colistin sulphate	10 mcg	92	100	0	0	0	0
Erythromycin	15 mcg	0	0	0	0	92	100
Gentamycin	10 mcg	0	0	0	0	92	100
Kanamycin	30 mcg	0	0	0	0	92	100
Naladixic acid	30 mcg	14	15.55	2	2.17	76	82.6
Neomycin	30 mcg	0	0	0	0	92	100
Pencillin	10 iu	92	100	0	0	0	0
Streptomycin	10 mcg	0	0	0	0	92	100
Tetracycline	30 mcg	0	0	5	5.43	87	94.56