J Food Sci Technol (March 2015) 52(3):1796–1801
DOI 10.1007/s13197-013-1162-0

ORIGINAL ARTICLE

Incidence and enterotoxigenic profile of *Bacillus cereus* in meat and meat products of Uttarakhand, India

Anita Tewari · S. P. Singh · Rashmi Singh

Revised: 20 August 2013 / Accepted: 25 August 2013 / Published online: 25 September 2013
© Association of Food Scientists & Technologists (India) 2013

Abstract The present investigation was undertaken to study the incidence and enterotoxigenicity of *Bacillus cereus* in raw meat and meat products. *B. cereus* was isolated from 29 (30.9 %) of the 94 samples analyzed. Recorded incidences of *B. cereus* from raw meat and meat products samples were 27.8 and 35 %, respectively. A high level of organism was found in cooked-meat (35 %) than raw meat samples (27.78 %) from 40 cooked-meat products and 54 raw meat samples analyzed. Screening of isolates by multiplex polymerase chain reaction revealed the overall distribution of various enterotoxin genes *hbl*DAC complex, *nhe*ABC complex, *cyt*K and *ent*FM as 55.2, 89.7, 41.4 and 93 %, respectively. The level of contamination with *B. cereus* was moderately higher in some samples but did not exceed the level which is sufficient to induce food poisoning. A relatively higher incidence of *B. cereus* in meat products, with the majority of isolates harboring all the enterotoxin genes can pose a potential public health threat.

Keywords *Bacillus cereus* · Meat · Meat products · Enumeration · Enterotoxin gene · PCR

A. Tewari (✉)
School of Public Health & Zoonoses, Guru Angad Dev Veterinary & Animal Sciences University, Ludhiana, Punjab 141004, India
e-mail: anita_vet@rediffmail.com

A. Tewari
e-mail: dranitatewari@gmail.com

S. P. Singh
Department of Veterinary Public Health & Epidemiology, Govind Ballabh Pant Agriculture and Technology, Pantnagar, Uttrakhand, India

R. Singh
Pt. Deen Dayal Upadhaya Pashu Chikitsa Vigyan Vishwa Vidhyalaya, Mathura, Uttar Pradesh, India

Introduction

B. cereus, a Gram-positive, rod shaped endospore-forming bacteria is an important cause of food-borne illness in humans and is frequently involved in food-borne outbreaks (Hall et al. 2001). It is a facultative anaerobic organism belonging to genus *Bacillus*.

B. cereus food poisoning occurs year-round and is without any particular geographic distribution. The genus *Bacillus* is ubiquitous in nature because it does not have complex nutrient requirements, it is frequently found in soils with low nutrients as well as on rice and straw (Kotiranta et al. 2000). Bacterial counts in excess of 10^5–10^6/g have been encountered in food suspected of causing illness (Goepfert et al. 1972). Food poisoning usually occur as a result of spore surviving cooking or pasteurization followed by germination and multiplication. The resistance of its spores to adverse environmental conditions has enabled it to get distributed widely in the environment (soil, dust and water). Because of its ubiquitous nature, it easily spreads to the foods of plant origin and through cross-contamination to other foods such as milk, meat and meat products (Granum 1994; Larsen and Jorgensen 1997). When food is not adequately refrigerated and in the absence of competitive flora, *B. cereus* grows well after cooking (Kramer and Gilbert 1989). Nowadays, animal origin foods like meat and meat products are the important part of the diet so the contamination and multiplication of *B. cereus* in raw meat and their products is of concern as public health hazard.

B. cereus is frequently associated with diarrheal and emetic types of food borne illness. Out of two, diarrheal type syndrome caused by enterotoxin (s), results in diarrhoea and the emetic type induces nausea and vomiting. Sometimes both types of symptoms are produced probably due to the synergistic effects of one or more enterotoxin(s) (Andersson et al. 1998). *B. cereus* produces emetic toxin (Agata et al. 1995) and four other enterotoxins: hemolysin BL or Hbl (Beecher and

Wong 1994), nonhemolytic enterotoxin or Nhe (Lindback et al. 2004), Cytotoxin K or *cyt*K (Lund et al. 2000) and enterotoxin FM or *ent*FM (Asano et al. 1997). The HBL, NHE complex and *cyt*K proteins are considered as the primary virulence factors in *B. cereus* diarrhea (Lund et al. 2000).

Cases of food poisoning in India have been reported by many workers. Hussain et al. (2007), reported an episode of gastrointestinal illness due to consumption of *Bacillus cereus* contaminated food in a fast food (Chola-puri) restaurant in India during 21 May 2006. In Kolkata (India), presence of this organism has been reported in 3.5 % cases of acute diarrhea over a 2-year period (between October 2006 and September 2008) (Banerjee et al. 2011). The incidence of *B. cereus* has been reported in various food products including meat and meat products by various researchers (Bachhil and Negi 1984; Bachhil and Jaiswal 1988; Willayat et al. 2007; Hafiz et al. 2012; Rao et al. 2012).

Still the accurate number of food poisonings caused by *B. cereus* in different countries is not known because it is not a reportable illness and is not always diagnosed (Kotiranta et al. 2000).

The aim of the present investigation was to study the incidence and level of contamination with *B. cereus* in meat and meat products. Besides, a multiplex polymerase chain reaction (PCR) was carried out to study the enterotoxin gene profile of isolates to determine their pathogenic nature.

Materials and methods

A total of 94 (n) samples of raw meat and meat products were collected from different part of northern India. A total of 54 raw meat [chicken (42), chevon (10), beef (2)] and 40 meat products were collected in sterilized containers from different meat shops and restaurants. Meat products were consisted of Butter chicken (5), Chicken curry (15), Chicken Kabab (6), Chicken sausage (2), Chevon momo (3), Chevon shami kabab (5), Chevon curry (2) and Fish curry (2). All the samples aseptically brought to the laboratory immediately after collection maintaining the proper cold chain for processing.

Isolation, identification and enumeration

All the samples were processed for isolation and enumeration of *B. cereus* as per the methodology of Rhodehamel and Harmon (1998) with suitable modifications. Raw meat and meat products were homogenized before being serially diluted. Ten grams of meat or meat product was taken in a flask containing 90 ml of peptone water (PW) to give a dilution of 1:10. Serial dilutions were prepared and 0.1 ml of each diluted sample was inoculated in agar plates by spreading evenly onto surface of each plate with sterile L-shaped spreader. Plates were incubated for 24 h at 30 °C. The typical eosin pink MYP colonies surrounded by precipitate zone indicating lecithinase production were presumptively identified to be *B. cereus* and enumerated with colony counter. Furthermore, a typical colony presumed to be *B. cereus* from each sample was transferred to nutrient agar slants and incubated for 24 h at 30 °C for further characterization. All the presumptive colonies of *B. cereus* so collected were subjected to morphological and biochemical tests for identification of species.

DNA template preparation

One typical colony from each of the isolates was picked up and inoculated in 5 ml of Brain Heart Infusion (BHI) broth and incubated overnight at 35 °C. The broth culture was subjected for DNA extraction using the Hi-Pura Bacterial and yeast genomic DNA purification kit (Hi-Media, Mumbai) as per the instructions of manufacturer. The DNA samples were diluted to a concentration of 50 ng/μl prior to its amplification.

Molecular characterization of *B. cereus* isolates

Primers and PCR assay for confirmation

The primers (Forward-BCJH- 5′TCATGAAGAGCC TGTGTACG3′; Reverse-BCJH- 5′CGACGTGTCAATTC ACGCGC3′) for detection of *gyr*B gene (encoding the subunit B protein of DNA gyrase) for differentiation and confirmation of *B. cereus* used in this study were got synthesized from M/S Aldrich, USA. PCR was performed for molecular characterization of *B. cereus* by using reaction condition as described by Park et al. (2007) with suitable modifications. A 25 μl PCR reaction mixture was made consisting of 0.5 μl DNA template (25 ng), 2.5 μl PCR buffer with MgCl2 (1×), 0.5 μl dNTPs (100 μM), 0.20 μl forword primer (460 pmol/μl), 0.40 μl reverse primer (230 pmol/μl) and 0.4 μl Taq DNA polymerase (1 U/μl).

The optimized PCR was setup in a 25 μl volume reaction mixture and the cycling conditions consisted of an initial denaturation at 94 °C for 5 min, 30 cycles of amplification with denaturation at 94 °C for 30 s, annealing at 63 °C for 30 s, an extension at 72 °C for 30 s, and final extension of the incompletely synthesized DNA at 72 °C for 5 min.

Enterotoxin gene profile of isolates: multiplex PCR protocol

The primers used for the detection of enterotoxin genes (*hbl*ADC, *nhe*ABC, *cyt*K and *ent*FM) in the current study are described by Ngamwongsatit et al. 2008. Two sets of multiplex PCR were employed to amplify the genes under study. Briefly, in first reaction; *hbl*C, *hbl*D, *hbl*A and *cyt*K genes were amplified while in second reaction; *nhe*A, *nhe*B, *nhe*C and *ent*FM genes were targeted using specific primers.

In first set of reaction, 5 μl (50 ng/μl) aliquot of bacterial genomic DNA was combined with 3.0 μl of reaction buffer (2 μl of 1× PCR buffer, 1 μl of 1.5 mM MgCl2), 0.4 μl of deoxynucleoside triphosphate (dNTP) mixture (concentration of each dNTP, 200 μM), 6.0 μl of primers (0.4 μM for each of *hbl*C, *hbl*D, *hbl*A and 0.3 μM of *cyt*K), and 1 μl of Taq DNA polymerase (5 U/μl). Then after, the volume was made upto 20 μl by addition of triple glass distilled water in final mixture. The second set of reaction was similar to first set except having 3.2 μl of primers (0.2 μM for each of *nhe*A, *nhe*B, *nhe*C and *ent*FM). Both the sets of reactions were amplified under same cycling conditions. Amplification was performed in a thermocycler (GeneAmp PCR system 9700, Applied biosystems) involving an initial denaturation at 95 °C for 5 min., followed by 30 cycles of denaturation at 94 °C for 45 s, primer annealing at 54 °C for 1 min. and extension at 72 °C for 2 min. A final extension at 72 °C for 5 min. was also given. The PCR products were electrophoresed in 1.5 % agarose gel and analysed using Gel documentation system (Alpha Innotech).

Results

Among 94 samples, *B. cereus* was isolated from 29 (30.85 %) of the samples. The percent isolation recorded for raw meat and meat products samples was 27.8 and 35, respectively. All the isolates were motile and hemolytic on 5 % sheep blood agar. The counts of *B. cereus* ranged from 6.40×10^2 colony forming units/gram (cfu/g) (low level) to 3.04×10^4 cfu/g (medium level) in raw meat and in the meat products, the levels ranged from 8.40×10^2 cfu/g (low level) to 3.90×10^4 cfu/g (medium level). From cooked meat products, one each from butter chicken, chevon momo, chevon shami kabab and fish was found to reveal the presence of *B. cereus* and the number of bacteria was recorded to be 4.8×10^3, 1.52×10^3, 3.0×10^3 and 1.26×10^4 cfu/g, respectively. None of the sausage sample, however, was positive for detectable level of *B. cereus.* Six samples from chicken curry possessed variable level of *B. cereus* ranged from 8.40×10^2 to 3.90×10^4 cfu/g, while both samples of chevon curry possessed mean count of 9.19×103 cfu/g. The incidence and colony counts of *B. cereus* in different food samples are depicted in Table 1. All presumptive *B. cereus* isolates were subjected to PCR targeting *gyrB* gene by using species-specific primers. All isolates produced PCR product of 475 bp on agarose gel (Fig. 1), which was specific to *B. cereus*.

Enterotoxigenic profile

The isolates were screened by multiplex PCR for the presence of eight enterotoxin genes (*hbl*ADC complex, *nhe*ABC complex, *cyt*K and *ent*FM), having the predicted size of 1,018, 935, 884, 759, 695, 618, 565 and 486 for *hbl*D, *nhe*B, *hbl*A, *nhe*A, *hbl*C, *nhe*C, *cyt*K and *ent*FM, respectively (Fig. 2). On the basis of the presence or absence of enterotoxins, *B. cereus* isolates were divided into 6 groups (G1–G6). In Group-1(G1) there were 2 isolates having *cyt*K, *ent*FM, NHE and HBL complex but devoid of at least one gene of HBL complex. All isolates of Group-2 lacked *cyt*K gene. While 2 of G-2 isolates were devoid of at least one of the genes of NHE complex and remaining were lacking atleast one gene of HBL and NHE complex. Group 3 comprised of isolates having *cyt*K, *ent*FM and incomplete NHE complex. Members of the G4 were devoid of *cyt*K and HBL complex while isolates having cytK and entFM complex were placed in G5. Group 6 was occupied by isolates possessing only HBL complex. Of the total isolates, 2 (6.9 %), 12 (41.4 %), 9 (22.5 %), 3 (10.3 %), 1 (2.5 %) and 2 isolates (5 %) were categorized into G1, G2, G3, G4, G5 and G6, respectively (Table 2). The *ent*FM gene was the most common enterotoxin gene found in 27 isolates (93 %), followed by NHE complex

Table 1 Incidence and counts of *Bacillus cereus* in meat samples

Source of sample (no. of sample)	No. of positive samples (%)	Counts (cfu/g)[1]
Raw meat (chicken, chevon, beef) (54)	15 (27.8)	6.40×10^2–3.04×10^4
Meat products (40)	14 (35.0)	8.40×10^2–3.90×10^4
Total (94)	29 (30.6)	–

[1] Colony forming unit/g

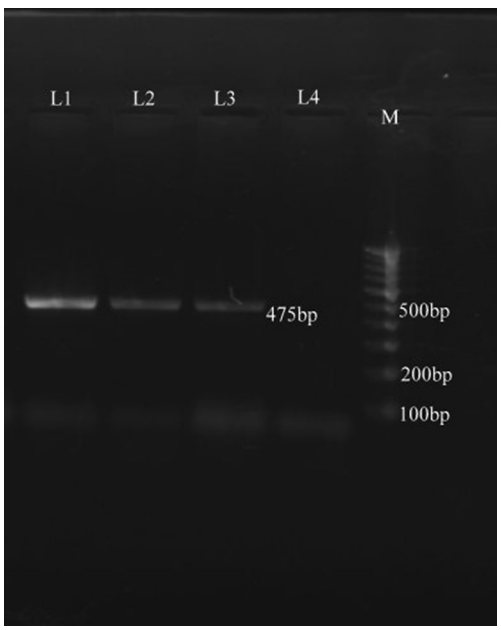

Fig. 1 Molecular characterization of *B. cereus* by using *gyr*B gene (475 bp). *L1*—positive control of *B. cereus*. *L2, L3*—*B. cereus* isolates of meat origin, *L4*—negative control, *M*—marker (100 bp DNA ladder)

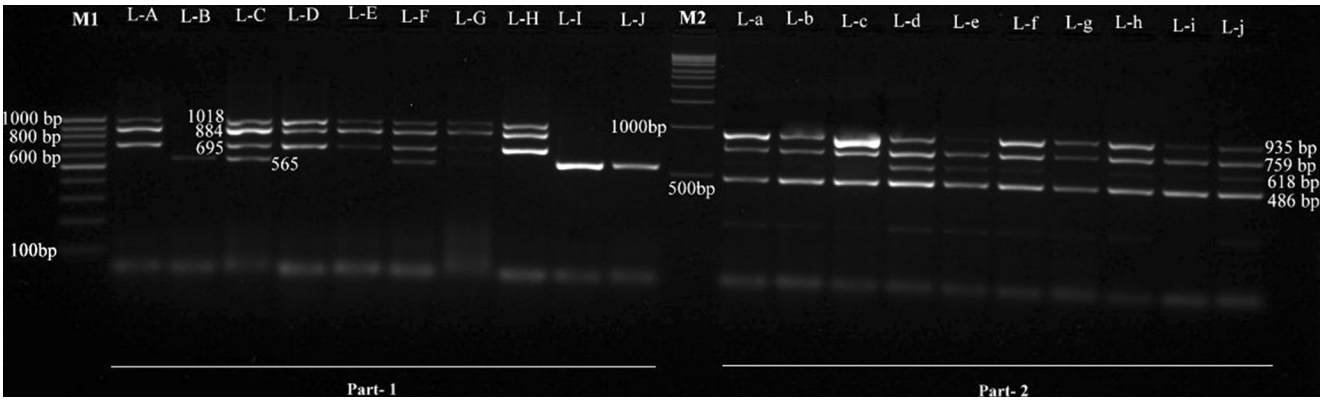

Fig. 2 Agarose gel electrophoresis of multiplex PCR products amplified from genomoic DNA of *Bacillus cereus* for grouping on the basis of enterotoxin gene; *Part 1*—primers used for *cyt*K,*hbl*C, *hbl*A and *hbl*D genes and *Part 2*—primers used for *ent*FM, *nhe*C, *nhe*A and *nhe*B genes. *M* presents 100 bp plus DNA ladder; *L1 to L7* present the isolates of *B. cereus* (Group 1 to Group 6). The polymerase chain reaction products in base pairs in figure indicate the enterotoxin gene i.e. 1,018, 935, 884, 759, 695, 618, 565, 486 indicates *hbl*D, *nhe*B, *hbl*A, *nhe*A, *hbl*C, *nhe*C, *cyt*K and *ent*FM, respectively

and HBL complex with 26 (89.7 %) and 16 isolates (55.2 %), respectively but both the NHE and HBL complex were lacking none, one or two genes of respective complex. The *cyt*K gene was detected in only 12 isolates (41.4 %) of *B. cereus* (Table 3).

Discussion

The isolates were confirmed as *B. cereus* based on various morphological and biochemical tests as described by Rhodehamel and Harmon (1998), and the results were in accordance with previous reports (Sharma et al. 2003; Chitov et al. 2008). *B. cereus* was isolated from 29 of the 94 food samples screened making an overall percent incidence of 30.6 %, almost similar incidence was reported by Schlegelova et al. (2003) who found 28 % of meat samples positive for *B. cereus*. Willayat et al. (2007) and Das et al. (2009) found 23.5 % and 36.7 % contamination level of *B. cereus*, respectively. While, Kamat et al. (1989), found 80 % of chicken and meat products contaminated with *B. cereus*. This variation might be because of better and advanced hygienic practices followed in meat shops and restaurants in recent times.

Analysis of raw and cooked mutton samples by the Willayat et al. (2007) revealed that the extent of contamination of *B. cereus* was reported 30 % and 15 % in raw and cooked mutton, respectively. However, in the current study, incidence of *B. cereus* in cooked meat (35 %) samples was higher than raw meat samples (27.78 %). This finding was is in accordance with the previous reports (Bachhil and Negi 1984; Konuma et al. 1988; Bachhil and Jaiswal 1988). The possible reason behind this could attributed to the ambient temperature food storage and/or improper cooking of food before consumption which might favour the germination of endospores leading to rapid increase in the total count (Gilbert et al. 1974; Byran et al. 1981). In agreement of this, Rao et al. (2012) conducted one study to identify microbiological hazards and assess their exposure associated with consumption of poultry based street food (chicken fried rice, chicken noodles, boiled noodles and boiled rice) served in different localities of Hyderabad, India. They observed that rice and noodles were kept at room temperature for about 5–6 h which was a critical control point for microbial contamination.

Overall counts of *B. cereus* in raw meat and meat products was from low to medium level i.e. it ranged from 6.40×10^2 cfu/g to 3.04×10^4 cfu/g in raw meats, while from 8.40×

Table 2 Distribution of enterotoxic isolates of *B. cereus* in differ*ent* groups

Group	Gene/genes present	Number of isolates	% isolates in each group
G1	HBL, *ent*FM, *cyt*K, NHE complex	2[a]	6.9
G2	HBL complex, *ent*FM, NHE complex	12 (2[b] + 10[c])	41.4
G3	*ent*FM, *cyt*K, NHE complex	9 (1+8[b])	22.5
G4	*ent*FM, NHE complex	3	10.3
G5	*cyt*K, *ent*FM	1	2.5
G6	HBL complex	2	5
		Total=29	

[a] Lacked atleast one gene of HBL complex

[b] Lacked atleast one gene of NHE complex

[c] Lacked atleast one gene of NHE & HBL complex

Table 3 Distribution of enterotoxin genes in total isolates

Gene	Isolate having the gene	Total number of isolate	%
NHE	26 (20 IC)	29	89.7
HBL	16 (12 IC)	29	55.2
*ent*F	27	29	93
*cyt*K	12	29	41.4

IC incomplete complex *i.e.* lacking two or three gene of respective complex

10^2 cfu/g to 3.90×10^4 cfu/g in meat products. In support of this, Rather et al. (2012) reported similar level of contamination in raw meat but found higher in meat products. Thus, if carcasses are not stored properly, the population of *B. cereus* may multiply to dangerous levels exceeding 1×10^6 cfu/g within the short span of time. The products prepared from such highly contaminated meats may contain a large number of *B. cereus* cells, which can be hazardous (Johnson 1984). Similarly, Rao et al. (2012) conducted one study to identify microbiological hazards in poultry based street food (chicken, chicken fried rice, chicken noodles, chicken manchuria and chilly chicken) served in different localities of Hyderabad, India. He reported that *B. cereus* and *S. aureus* were the most prevalent pathogenic bacteria isolated with the level of contamination from 2.4×10^3 to 2.6×10^3 cfu/g.

All of the *B. cereus* isolates carried at least one of the enterotoxin genes out of 4 encoding virulence factors investigated in this study, similar to findings of Yang et al. (2005). At least one gene of NHE complex was present in 26 isolates (89.7 %) of *B. cereus*, whereas, it was found in almost all tested isolates in previous study of Stenfors Arnesen et al. (2008). He also explained that because of the variability in the NHE operons, these unexpected results were found. The enterotoxigenic profile of the isolates revealed the presence of at least on gene of HBL complex (*hbl*DAC) in 16 isolates (55.2 %). Guinebretiere et al. (2002) reported 73 % level of HBL complex in samples of food poisoning. Das et al. (2009) reported that 71.4 % *B. cereus* (30 out of 42) isolates of fish origin were enterotoxigenic and all were positive to hbla gene specific PCR. Whereas Thaenthanee et al. (2005) found HBL complex in 65.5 % isolates of *B. cereus*. In the present study, the genes of HBL complex were found in lesser frequency than NHE complex, this finding was in accordance with Wong et al. (1988); Moravek et al. (2006) and Al-khatib et al. (2007).

According to the reports of Ngamwongsatit et al. (2008) and Vyletelova and Banyko (2008), the three genes of HBL and NHE complexes occur together in operon. But the present study indicates that the genes in an operon can occur independently of each other, as 12 of the isolates showed the absence of one or two genes in HBL complex, and similarly 20 of the isolates showed the absence of one or two genes in NHE complex (Table 3). Thus, the genotype and incidence of enterotoxin genes may vary in different geographical locations and source of origin as is reported by various authors above.

All isolates produced beta haemolysis on sheep blood agar. It was in general agreement with result of those of multiplex as 16 isolates out of 29 harbored at least one gene of HBL complex which indicated gene function. On contrary, 13 isolates lacked all genes of HBL complex, but interestingly, they produced positive haemolysis reaction on blood agar. There is a possiblity that such haemolytic effect might be produced by other toxins such as haemolysin I (Cereolysin O) (Coolbaugh and Williams 1978), haemolysin II (Miles et al. 2002), Haemolysin III (Baida and Kuzmin 1996) or *cyt*K (Lund et al. 2000) of *B. cereus*.

The *ent*FM gene was the common enterotoxin gene found in 27 (93 %) *B. cereus* isolates in this study, Ngamwongsatit et al. (2008) also found this gene in all tested isolates of *B. cereus*. The gene for *cyt*K was found in 12 (41.4 %) of the isolates whereas Wijnands et al. (2006), Ngamwongsatit et al. (2008), Chitov et al. (2008) and Rather et al. (2012) detected it in 50, 88, 70.40 and 65.98 % of their isolates, respectively.

The results of biochemical characterization and enterotoxin gene profile of isolates revealed that there is high probability of the potential transmission of enterotoxigenic bacteria to humans from the food chain, more particularly through contamination of raw meat and meat products.

Conclusion

An overall incidence of 30.9 % and a relatively higher incidence in meat products (35 %) pose a potential public health threat. The four enterotoxin genes of hemolysin BL, nonhemolytic enterotoxin, cytotoxin K and enterotoxin FM occur independently of each other, and the genes in operons (*hbl*CDA and *nhe*ABC), though closely associated, can also occur independently. Each isolates regardless of their origin harbored at least one of the enterotoxin genes indicating their pathogenic nature, which must be considered as serious health hazard.

References

Agata N, Ohta M, Arakawa Y, Mori M (1995) The bceT gene of *Bacillus cereus* encodes an enterotoxic protein. Microbiology 141:983–988

Al-Khatib M, Khyami H, Badran E, Shehabi A (2007) Incidence and characterization of diarrheal enterotoxins of fecal *Bacillus cereus* isolates associated with diarrhea. Diagn Microbiol Infect Dis 59: 383–387

Andersson MA, Mikkola R, Helin J, Eressson MC, Salkinoja SM (1998) A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin related depsipeptide ionophores. Appl Environ Microbiol 64: 1338–1343

Asano SI, Nukumizu Y, Bo H, Iizuka T, Yamamoto T (1997) Cloning of novel enterotoxin genes from *Bacillus cereu Bacillus thuringiensis*. Appl Environ Microbiol 63:1054–1057

Bachhil VN, Jaiswal TN (1988) *Bacillus cereus* in meats: incidence, prevalence enterotoxigenicity. J Food Sci Technol 25(6):371–372

Bachhil VN, Negi SK (1984) *Bacillus cereus* in meat and meat products: public health implications control. Indian J Public Health 28(2):68–69

Baida GE, Kuzmin NP (1996) Mechanism of action of hemolysin III from *Bacillus cereus*. Biochim Biophys Acta Biomembr 1284(2): 122–124

Banerjee M, Nair GB, Ramamurthy T (2011) Phenotypic & genetic characterization of *Bacillus cereus* isolated from the acute diarrhoel patients. Indian J Med Res 133:88–95

Beecher DJ, Wong ACL (1994) Improved purification and characterization of hemolysin BL, a haemolytic dermonecrotic vascular permeability factor from *Bacillus cereus*. Infect Immun 62:980–986

Bryan FL, Bartleson CA, Christopherson N (1981) Hazard analyses, in reference to *Bacillus cereus*, of boiled and fried rice in Cantonese-style restaurants. J Food Prot 44:500–512

Chitov T, Dispan R, Kasinrerk W (2008) Incidence and diarrheagenic pot*ent*ial of *Bacillus cereus* in pasteurized milk and cereal products in Thailand. J Food Saf 28:467–481

Coolbaugh JC, Williams RP (1978) Produnction and characterization of two haemolysis of *Bacillus cereus*. Can J Microbiol 3:255–273

Das S, Surendran PK, Thampuran N (2009) PCR-based detection of enterotoxigenic isolates of *Bacillus cereus* from tropical seafood. Indian J Med Res 129:316–320

Gilbert J, Stringerm F, Peacet C (1974) The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. J Hyg Cambr 73:433

Goepfert JM, Spira WM, Kim HU (1972) *Bacillus cereus*: food poisoning organism: a review. J Milk Food Technol 35:213–227

Granum PE (1994) *Bacillus cereus* and its toxins. J Appl Bacteriol 76: 61S–66S

Guinebretiere MH, Broussolle V, Nguyenthe C (2002) Enterotoxigenic profiles of food poisoning and food-borne *Bacillus cereus* strains. J Clin Microbiol 40:3053–3056

Hafiz Y, Iqbal A, Ahmad M, Wani N, Willayat MM (2012) Prevalence of *Bacillus cereus* in mutton tikka and chutney samples in different seasons in Kashmir Valley. J Pure Appl Microbiol 6(2):975–979

Hall JA, Goulding JS, Bean NH, Tauxe RV, Hedberg CW (2001) Epidemiologic profiling: evaluating foodborne outbreaks for which no pathogen was isolated by routine laboratory testing: United States, 1982–89. Epidemiol Infect 127:381–387

Hussain SA, Munshi ZH, Hussain I (2007) Food poisoning due to *Bacillus cereus*: a case report. J Vet Public Health 5(1):57–59

Johnson KM (1984) *Bacillus cereus* in food-borne illness—an update. J Food Prot 47:145–153

Kamat AS, Nerkar DP, Nair PM (1989) *Bacillus cereus* in some Indian foods, incidence and antibiotic, heat and radiation resistance. J Food Saf 10(1):31–41

Konuma H, Shinagawa K, Tokumaru M, Onoue Y, Konno S, Fujino N, Shigehisa T, Kurata H, Kuwabara Y, Lopes CAM (1988) Occurrence of *Bacillus cereus* in meat products, raw meat and meat product additives. J Food Prot 51(4):324–326

Kotiranta A, Lounatmaa K, Haapasalo M (2000) Epidemiology and pathogenesis of *Bacillus cereus* infections. Microbes Infect 2(2): 189–198

Kramer JM, Gilbert RJ (1989) *Bacillus cereus* and other *Bacillus* species. In: Doyle MP (ed) Food borne bacterial pathogens. Marcel Dekker, New York, pp 21–77

Larsen HD, Jorgensen K (1997) The occurrence of *Bacillus cereus* in Danish pasteurized milk. Int J Food Microbiol 34:179–186

Lindback T, Fagerlund A, Roland MS, Granum PE (2004) Characterization of *Bacillus cereus* non-hemolytic enterotoxin (NHE). Microbiology 150:3959–3967

Lund T, Debuyser ML, Granum PE (2000) A new *cyt*otoxin from *Bacillus cereus* that may cause necrotic enteritis. Mol Microbiol 38:254–261

Miles G, Bayley H, Cheley S (2002) Properties of *Bacillus cereus* haemolysin II: a heptameric transmembrane pore. Protein Sci 11: 1813–1824

Moravek M, Dietrich R, Burk C, Broussolle V, Guinebretiere MH, Granum PE, Nguyenthe C, Martlbauer E (2006) Determination of the toxic potential of *Bacillus cereus* isolates by quantitative enterotoxin analyses. FEMS Microbiol Lett 257:293–298

Ngamwongsatit P, Buasri W, Pianariyanon P, Pulsrikarn C, Ohba M, Assavanig A, Panbangred W (2008) Broad distribution of enterotoxins genes (*hbl*CDA, *nhe*ABC, *cyt*K and *ent*Fm) among *Bacillus thuringiensis* and *Bacillus cereus* as shown by novel primers. Int J Food Microbiol 121:352–356

Park S, Kim H, Kim J, Kim T, Kim H (2007) Simultaneous detection and identification of *Bacillus cereus* group bacteria using multiplex PCR. J Microbiol Biotechnol 17(7):1177–1182

Rao VS, Kumar RN, Kashinath L, Bhaskar V, Polasa K (2012) Microbiological hazard identification and exposure assessment of poultry products sold in various localities of Hyderabad, India. Sci World J 2012:7. doi:10.1100/2012/736040

Rather MA, Aulakh RS, Gill JPS, Ghatak S (2012) Enterotoxin gene profile and antibiogram of *Bacillus cereus* strains isolated from raw meats and meat products. J Food Saf 32:22–28

Rhodehamel EJ, Harmon SM (1998) Bacteriological analytical manual online 8th edn. Revision. Chapter 14. U.S. Food and Drug Administration; 2001. Available from http://www.cfsan.fda.gov/~ebam/bam-14.html#authors

Schlegelova J, Brychta J, Klimova E, Napravnikova E, Babak V (2003) The prevalence of and resistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs. Vet Med 48(11):331–338

Sharma CS, Sharma DK, Gill JPS, Aulakh RS, Sharma J (2003) *Bacillus cereus* from foods of animal origin in India and its public health significance. Acta Vet Scand 44118

Stenfors Arnesen LP, Fagerlund A, Granum PE (2008) From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol Rev 32:579–606

Thaenthanee S, Wong ACL, Panbangred W (2005) Phenotypic and genotypic comparisons reveal a broad distribution and heterogeneity of hemolysin BL genes among *Bacillus cereus* isolates. Int J Food Microbiol 105:203–212

Vyletelova V, Banyko J (2008) Detection of HBL and NHE enterotoxin genes in *Bacillus cereus* using multiplex PCR. Paper presented at 12th International Symposium onMicrobial Ecology (ISME12). Cairns, Australia

Wijnands LM, Dufrenne JB, Rombouts FM, Veld PH, Leusden FM (2006) Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. J Food Prot 69(11): 2587–2594

Willayat MM, Sheikh GN, Misgar GR (2007) Prevalence of *Bacillus cereus* biotypes in raw and cooked mutton. J Vet Public Health 5(2): 123–125

Wong HC, Chang MH, Fan JY (1988) Incidence and characterization of *Bacillus cereus* isolates contaminating dairy products. Appl Environ Microbiol 54:699–702

Yang I-C, Yang-Chih D, Huang T-P, Huang Y-P, Wang J-Y, Pan T-M (2005) Establishment of a novel multiplex PCR assay and detection of toxigenic strains of the species in the *Bacillus cereus* group. J Food Prot 68(10):2123–2130