Original Research Article

Multidrug Resistant *Bacillus cereus* in Fresh Vegetables: A Serious Burden to Public Health

Bhavish Sood[1]*, Param Pal Sahota[1] and Mandeep Hunjan[2]

[1]Department of Microbiology, Punjab Agricultural University, Ludhiana-141004, India
[2]Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141004, India
*Corresponding author

A B S T R A C T

The frequent inhabitation of *B. cereus* in fresh vegetables can critically damage the public health and economics. Fresh vegetables from three sources; Village fields, Supermarkets and Street Vendors were examined for microbiological safety for raw consumption. Among the seven salad vegetables that are integral to daily diet intake were found with high Food MPN count of $>100g^{-1}$ (42.8-100%), total coliforms $>10ml^{-1}$ (75.6-98.5%), faecal coliforms $>10ml^{-1}$ (59.6-91.2%). The highest mean count of *B. cereus* (4.35 log cfu g^{-1}) was found in carrots sourced from Village fields). The ability of *Bacillus* isolates to lyse erythrocytes can directly be correlated with the expression of haemolytic enterotoxin. Antibiotic resistance of *Bacillus cereus* isolates to four or more antibiotics may bud a serious perturbation in treating associated illness. Effectiveness of aminoglycoside, fluoroquinone, tetracycline and glycopeptides during this study could be considered before empirical therapy against *B. cereus* infections. PCR analysis of nineteen *B. cereus* isolates showed the presence of haemolytic enterotoxin gene *hblA* (100%), *hblC* (73.6%) and *hblD* (89.4%) and that of non-haemolytic enterotoxin *nheA* (100%) and *nheC* (100%). All the isolates were devoid of *nheB* gene. Their toxigenicity can further increase the risk of illness due to raw consumption of vegetables for which important interventions should be implemented for decontamination.

Introduction

The consumption of fresh vegetables meets the demand of dietary nutrients and fiber. The growing trend of its raw consumption in the urban areas necessitates its concern in light of microbial safety. *Bacillus cereus*, a Gram-positive, aerobic-to-facultative, widely distributed environmentally and sporulating ability favours its prevalence in all food types. High incidence of *B. cereus* in raw milk, meat and vegetables in India were suspected in many outbreaks which go unrecognized (Valero *et al.,* 2002; Bedi *et al.,* 2005;

Willayat *et al.,* 2007; Altaf *et al.,* 2012). A wide variety of food poisoning is associated with emetic and diarrheal syndrome caused by *Bacillus cereus* (Toh *et al.,* 2004; Ceuppens *et al.,* 2011). Fresh water and decaying organic matter are one of the most frequent reservoirs of *Bacillus cereus* that cause the contamination of growing vegetables through soil leading to the transient colonization of the human intestine (Ghosh 1978; Jensen *et al.,* 2003).

Uniform resistance to certain antibiotics was observed in *Bacillus cereus* isolates (Bottone 2010) which can cause perturbation in the empirical therapy during outbreaks. Moreover, the niche colonization of bacteria make the effect more pronounced with horizontal transfer of such antibiotic resistance traits to other non-pathogenic bacteria (Fernández *et al.,* 2011).

The diarrheal type of food poisoning is most common type of food poisoning around the world. The two protein complexes from *B. cereus* responsible for virulence in diarrheal type are haemolysin BL (HBL) and non-haemolytic enterotoxin (NHE). The haemolysin BL consists of a binding component B and two lytic components L_1 and L_2 responsible for enterotoxigenicity of *B. cereus* (Beecher *et al.,* 1995). Three genes *hblA, hblD* and *hblC* are translated to B and L (L_1 and L_2) components respectively (Ryan *et al.,* 1997). Another non-haemolytic enterotoxin with three different proteins A, B and C are encoded by *nheA, nheB* and *nheC* genes, respectively (Granum *et al.,* 1999). This study scanned the frequency of genes coding for the two protein complexes (HBL and NHE) in indigenous isolates and antibiotic screening to reach the positive empirical outcome in treating *Bacillus cereus* infections.

Materials and Methods

Study area and sampling

The Buddha Nallah, a natural stream of Sutlej, traversing through Ludhiana city of Punjab state has high Total Dissolved solids >1000 mg/L, chlorides up to 400 mg/L, Chemical Oxygen Demand >400 mg/L, Biochemical Oxygen Demand 52-195 mg/L, Most Potable Number up to 2400+ per 100ml. Villages along the Buddha Nallah are known for vegetables growers. They use irrigation

pumps, river or streams for cultivation. Epidemiological surveillance study for the microbiological quality of fresh vegetables was routinely carried out during the vegetable of the season for the period of 2 and 1/2 years (July 2013-December 2015). Water samples from the irrigation pumps were collected according to the standard method of BIS (IS-10500:1991).

A total of 420 samples of salad vegetables such as carrot, radish, cucumber, tomato, cabbage, spinach and long melon were procured from village fields along Buddha Nallah. In comparison, 75 samples from supermarkets and 230 samples from street vendors were also collected.

Enumeration of *Bacillus cereus*

The fresh vegetable, 25grams of sample was taken and washed with autoclaved water so as to omit any environmental contamination. Vegetable sample was chopped with the help of sterile knife into 2-3cm pieces and transferred to the 225ml water blank. It was shaken vigorously for uniformity and serial dilutions of the suspension were spread onto *Bacillus cereus* Agar Base supplemented with Polymyxin B sulphate (50000Unit/L) and sterile Egg Yolk Emulsion (25ml/L) (HiMedia Laboratories Pvt. Ltd., Mumbai) [IS-5887(Part 6):1999] plates in triplicates for each dilution and incubated at 37°C for 24-48 hours. Following incubation, all colonies on dishes containing 30-300 colonies were counted per dilution and log cfu g^{-1} was calculated. The presumed colonies were verified by complete phenotypic characterisation.

Phenotypic characterization

The isolates were verified as *Bacillus cereus* by their size, shape, Gram's staining, simple staining, motility and colony morphology,

biochemical tests, virulence based tests and further confirmed with molecular tools. Antibiotic susceptibility was also evaluated using the Kirby–Bauer disc diffusion method on Mueller Hinton agar with 25 antibiotics belonging to 15 different classes; β-Lactams, Fluoroquinolones, Aminoglycoside, 1st Generation Cephalosporins, 2nd Generation Cephalosporins, 3rd Generation Cephalosporins, Chloramphenicol, Tetracycline, Metronidazole, Macrolide, Sulphonamides, Lyncosamides, Glycopeptides, Nitrofurans and Carbapenems.

Bacterial genome extraction

Genomic DNA was extracted from colonies that were identified as *Bacillus cereus* with the help of Invitrogen Easy DNA® Isolation Kit (Invitrogen Inc.) as per the manufacturer's protocol from selected bacterial strains grown in nutrient broth for overnight at optimal temperature. DNA was eluted into 100μl 1X TE buffer (Invitrogen Inc.). Quantity and quality of DNA was checked on TECAN 2000 Nanoquant Plate. The DNA of all the samples was diluted to 25ng/μl by adding nuclease free water and stored at -20°C. Alternatively, DNA quality was checked on 0.8% agarose gel. A single sharp band of DNA signified high quality of DNA. DNA of each sample was diluted to 25ng per μl of nuclease free water (Promega Inc.) before the PCR assay.

PCR assay

Uniplex PCR was performed with reaction volume of 30 μl for each sample, that contained 1.5 mM of 25mM Mgcl$_2$ (Promega Inc.), 1X Go Taq TM buffer (Promega Inc.), 0.2 mM of dNTPs (Promega Inc.), 0.5μM of each primers (Table 1), 2U of GoTaqTM DNA polymerase (Promega Inc.) and 50 ng/μl of the DNA template. The final volume of the reaction was adjusted with Nuclease free Water (Promega Inc.). The PCR conditions consisted of a pre-incubation step for 5 min at 94°C, 35 cycles of denaturation at 94°C for 30sec, annealing step for 30sec (Table 1) with extension time of 40sec at 72°C and a termination step with a final extension for 7min at 72°C. After amplification, the product was loaded onto the 1.5% agarose gel, visualized under UV light and photographed using SYNGENE gel documentation system with Gene snap software programme.

Results and Discussion

MPN Index of vegetables and irrigation water

During the Food MPN analysis from the village field samples, cucumber samples showed high prevalence (91%) of MPN index >100/g followed by spinach (84%), long melon (83.8%), carrot (72.7%), cabbage (63.4%) and radish (61.1%) (Table 2). The MPN index for tomato was not determined due to the acidic properties of the vegetable which gives false positive result.

In samples collected from Supermarkets, high MPN index of >100/g was found in 83.3% of the radish samples followed by cucumber and long melon (75%), spinach (71.4%), cabbage (44%) and 42.8% in carrot (Table 2).

With respect to samples sourced from street vendors, all carrot samples were found to have >100/g of MPN index while spinach samples showed lowest prevalence of 70% samples with >100/g of MPN index.

73.33% salad, ready to eat samples and water contaminated with total and faecal coliform bacteria used in the kitchen of various restaurants from Chittagong city, Bangladesh were reported in 2011. The presence of faecal coliform in supply water indicates direct or indirect contamination from faecal origin.

Manure is commonly applied to fields in order to dispose off animal waste and to fertilize soils. Enteric pathogens can survive for prolonged periods of time in animal faeces (Hutchison *et al.,* 2004) and may serve as potential inoculum onto plants in the field. *Bacillus cereus*, a spore former can persist in soil for many years and can even withstand pasteurization at 70°C for hours in cattle manure (Marañón *et al.,* 2006). Therefore, microbial assessment of the manure should gain immense importance in stringent sanitation management strategies as these bacteria are opportunistic and become virulent during animal or human contact.

Similarly, water for irrigation has utilized faecal coliform as an indicator of acceptable quality. Total coliform and fecal coliform MPN count was determined to be >10/ml in most of the samples with highest positive percentage of 98.5 and 91.2 for cucumber and spinach respectively (Table 2). The Indian environment ministry recommended desirable limit of faecal coliform at 1,000 MPN/100ml and a maximum permissible limit at 10,000 MPN/100ml for discharge of treated sewage into a water body or reuse for agriculture.

Our study has shown the imminence of contaminated water used for irrigation. The total coliform as well as faecal coliform were found above the desirable limit. It was observed that all vegetable growing areas (village fields) around Buddha Nallah have contaminated ground water with high MPN index of >10/ml (1000/100ml) (Table 2) which is undesirable for irrigation purpose. Crop irrigation and application of pesticides with contaminated water also are considered as primary sources of inoculum in the field (Brandl, 2006). Water can predispose the coliform to the fields if the ground water is mixed with the sewage, runoff water in from immediate vicinity of livestock and use of untreated manure into the fields. Owing to dissemination of pathogens into the field,

greater risk may establish which aids reaching the pathogens to their threshold of virulence with increase in nutrient abundance.

Occurrence of *Bacillus cereus*

Out of 420 vegetable samples collected from village fields, 246 (58.6%) were positive for *Bacillus cereus* which was higher than its percent occurrence in supermarket samples (30/75, 40.7%). The percent contamination increased by 7.9 % in vendor samples.

Cucumber samples from village fields had shown highest percent contamination by *B. cereus* (75%) followed by 50% in case of radish and long melon from supermarkets and 77.77% in Long melon collected from street vendors (Fig. 1).

Up to 30% food samples were reported positive for *B. cereus* in processing plant (Kim *et al.,* 2016) (raw material, salads in washing steps, and final products) which were substantially less than the results found in this study. The high contamination of irrigation water, Bacillus spores in soil and use of untreated animal manure is the suggestive of this high prevalence of *Bacillus cereus* in vegetables.

The high percentage and high mean count of *Bacillus cereus* in samples collected from vendors can occur at any step during packaging, storage, transportation, poor handling and vending operations. Spores being hydrophobic in nature may stick to the vegetable surface (Anderson *et al.,* 1998) and germinate whenever the ideal conditions are set. Furthermore, the rise in population of bacteria may occur due to the spore germination into vegetative phase on the surface/internal part of the vegetable during temperature abuse, nutrient availability and sprinkling of water over the fresh produce to retain its freshness and consumer acceptability during vending operations.

Table.1 List of primers used for targeting species specific virulent genes of *Bacillus cereus*

Gene	Primer sequence (5'→3')	Product size (bp)	Annealing temp.	Reference
hblA	F-GTGCAGATGTTGATGCCGAT R-ATGCCACTGCGTGGACATAT	320	56°C	Banerjee *et al* (2011)
hblD	F- AATCAAGAGCTGTCACGAAT R-CACCAATTGACCATGCTAAT	430	52°C	-do-
hblC	F- AATGGTCATCGGAACTCTAT R-CTCGCTGTTCTGCTGTTAAT	750	52°C	-do-
nheA	F- TACGCTAAGGAGGGGCA R- GTTTTTATTGCTTCATCGGCT	500	52°C	-do-
nheB	F- CTATCAGCACTTATGGCAG R-ACTCCTAGCGGTGTTCC	770	52°C	-do-
nheC	F- CGGTAGTGATTTGCTGGG R-CAGCATTCGTACTTGCCAA	582	52°C	-do-

Table.2 Microbial analysis of different vegetables among three sources

Vegetables	Source	Water quality Index			
		Mean MPN index of food[b] >100/g	Total coliforms[c] >10/ml	Faecal coliforms[c] >10/ml	*Bacillus cereus* count[a] (log cfu g^{-1})
Carrot	Village field	72.7	90.1	68.1	4.35
	Supermarket	42.8	ND	ND	2.68
	Street vendors	100	ND	ND	2.75
Radish	Village field	61.1	86.5	70.1	3.06
	Supermarket	83.3	ND	ND	2.67
	Street vendors	94.1	ND	ND	2.91
Cucumber	Village field	91	98.5	74.6	2.69
	Supermarket	75	ND	ND	2.56
	Street vendors	82.3	ND	ND	3.01
Tomato	Village field	ND	75.6	64.9	3.09
	Supermarket	ND	ND	ND	2.58
	Street vendors	ND	ND	ND	2.79
Cabbage	Village field	63.4	86.5	59.6	2.37
	Supermarket	44.4	ND	ND	2.68
	Street vendors	86.3	ND	ND	2.88
Spinach	Village field	84	96	91.2	3.12
	Supermarket	71.4	ND	ND	2.55
	Street vendors	70	ND	ND	3.62
Long melon	Village field	83.8	98.3	79	2.73
	Supermarket	75	ND	ND	2.08
	Street vendors	80	ND	ND	3.11

a- level of significance at $p<0.05$
b- Desirable limit is 100/g
c- Desirable limit is 10/ml
ND= Not Determined (low pH of Tomato gives false positive results, true representative of water sample for vegetables washing could not be produced)

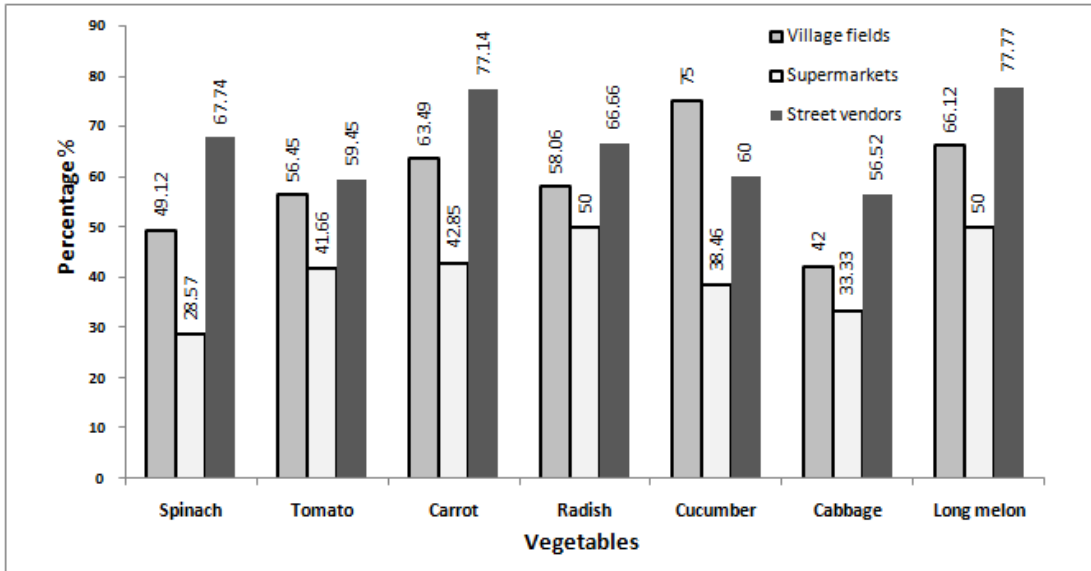**Fig.1** Percent incidence of *Bacillus cereus* among vegetables and sources

Fig.2 Percent frequency of antibiotic resistance exhibited by *Bacillus cereus* isolates
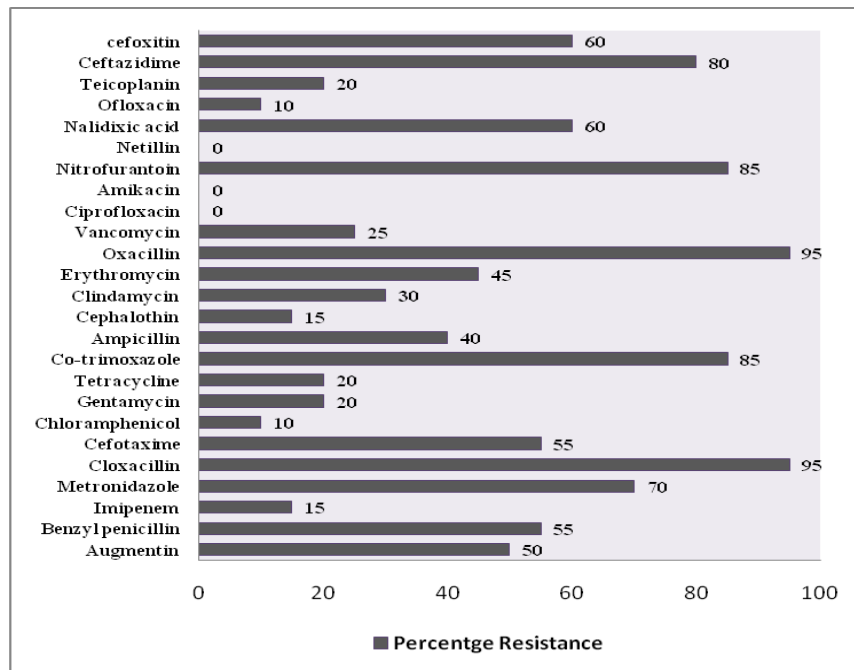
Fig.3 Electrophoretic gel imaging of *hblA* gene (320bp fragment) showing M lane= 1kbp ladder (Invitrogen); Lanes 2-5=*Bacillus cereus* isolates showing the *hblA* gene and Lane 1= Positive control of *Bacillus cereus* MTCC 430
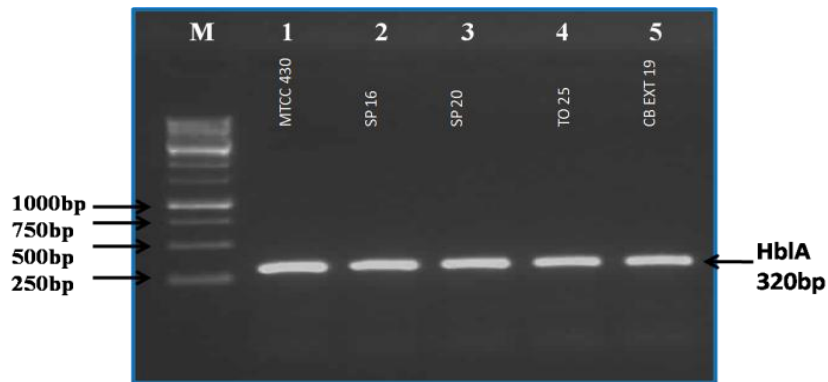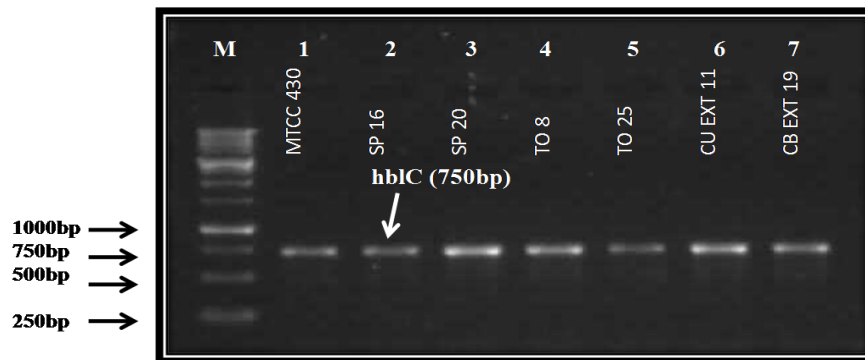

Fig.4 Electrophoretic gel imaging of *hblC* gene (750bp fragment) showing M lane= 1kbp ladder (Invitrogen); Lanes 2-7=*Bacillus cereus* isolates showing the *hblC* gene and Lane 1= Positive control of *Bacillus cereus* MTCC 430

Fig.5 Electrophoretic gel imaging of *hblD* gene (430bp fragment) showing M lane= 1kbp ladder (Invitrogen); Lanes 1-18=*Bacillus cereus* isolates showing the *hblD* gene and Lane 19= Positive control of *Bacillus cereus* MTCC 430
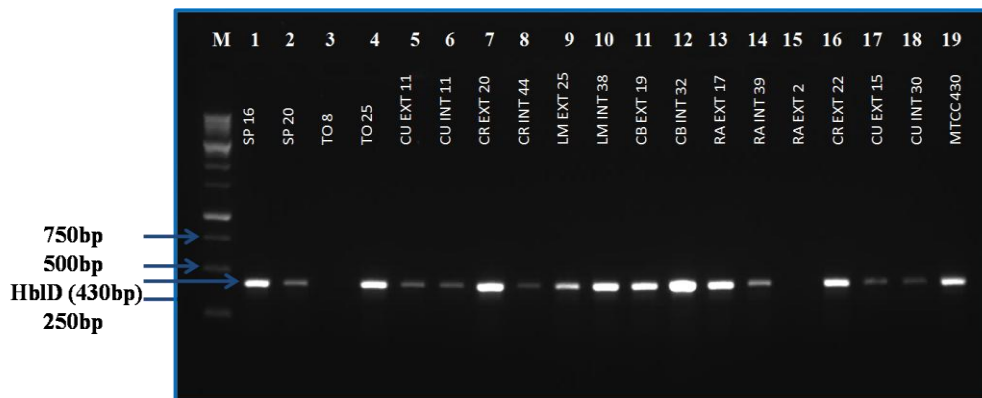
Fig.6 Electrophoretic gel imaging of *nheA* gene (500bp fragment) showing M lane= 1kbp ladder (Invitrogen); Lanes 2-18=*Bacillus cereus* isolates showing the *nheA* gene and Lane 1= Positive control of *Bacillus cereus* MTCC 430
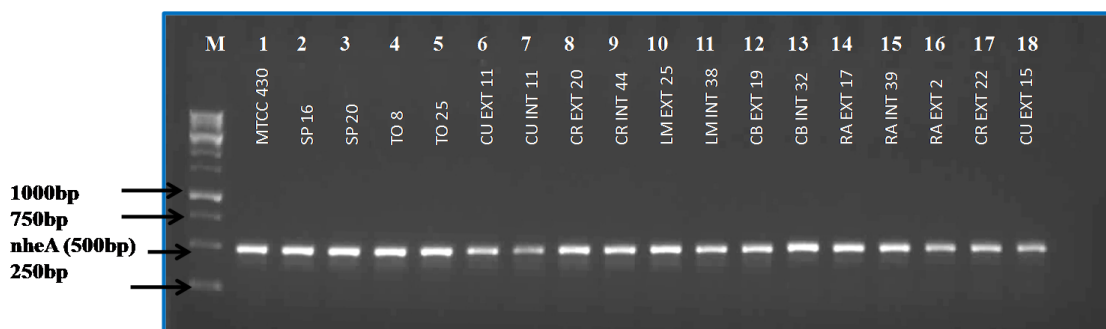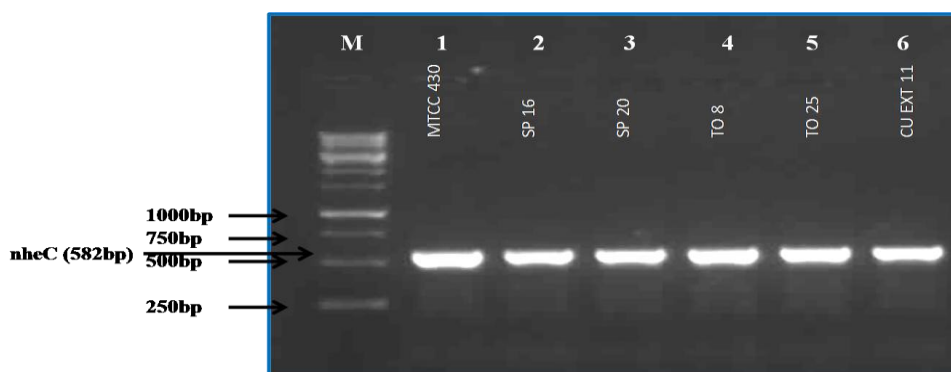

Fig.7 Electrophoretic gel imaging of *nheC* gene (582bp fragment) showing M lane= 1kbp ladder (Invitrogen); Lanes 2-6=*Bacillus cereus* isolates showing the *nheC* gene and Lane 1= Positive control of *Bacillus cereus* MTCC 430

Enumeration of *Bacillus cereus*

Bacillus cereus was found in low mean count (2.37 log cfu g^{-1}) only in cabbage samples from village fields, rest of the vegetables supported better growth of *B. cereus* (2.69-4.35 log cfu g^{-1}) (Table 2).

In case of supermarket samples, the mean count was found highest in radish and cabbage (2.68 log cfu g^{-1}) and lowest in long melon (2.08 log cfu g^{-1}). Spinach samples from street vendor on the other hand, were found most contaminated (3.62 log cfu g^{-1}) by *B. cereus* than other vegetables tested. *B. cereus* didn't proliferate much in carrot samples (2.75 log cfu g^{-1}) (Table 2).

Vegetables irrigated with river water had shown 32.7% contamination with *Bacillus* species (Weldezgina and Muleta, 2016). *B. cereus* was reported to be present in 27.3–30.8 % of food samples in processing plant (raw material, salads in washing steps, and final products) (Kim *et al.,* 2016).

The contamination levels of *B. cereus* in cucumbers, carrots and peppers ranged from 2-3.89 log cfu g^{-1} which are in agreement to this study. These levels were sufficient to initiate growth of *B. cereus* strains and spoilage of the vegetables.

The study depicts that cropping vegetables in area around Buddha Nallah are highly

contaminated with *B. cereus* and is a serious health burden.

Phenotypic characterization

All Bacillus strains were haemolytic in nature and could hydrolyse Esculin, reduce nitrate, ferment malonate and glucose.

Bacillus cereus isolates from fresh vegetable samples were found resistant to Cloxacillin (5mcg) (95%), Oxacillin (1 mcg) (95%), Co-Trimoxazole (25 mcg) (85%), Nitrofurantoin (300 mcg) (80%), Ceftazidime (30 mcg) (80%), Metronidazole (5 mcg) (70%), Nalidixic Acid (30 mcg) (60%), Cefoxitin (30 mcg) (60%), Benzyl penicillin (2 mcg) (55%) and Cefotaxime (30 mcg) (55%). The other antibiotics showed low resistance (<45%) to the isolates tested but no resistance to Netillin (30 mcg), Amikacin (30 mcg) and Ciprofloxacin (5 mcg) (Fig. 2).

The antimicrobial resistance pattern in 35 *Bacillus cereus* strains and found high resistance to β-lactam antibiotics, including ampicillin, cefepime, oxacillin, and penicillin while completely susceptible to gentamycin, clindamycin, vancomycin, ciprofloxacin, imipenem (94%), chloramphenicol (89%), tetracycline (54%) and erythromycin (97%) (Chon *et al.,* 2012). Results showed that three antibiotics named netillin, amikacin (aminoglycoside) and ciprofloxacin were most efficacious with no resistance pattern found for the tested *B. cereus* isolates. Furthermore, only 20-25% resistance was observed when subjected to glycopeptides (Teicoplanin and Vancomycin) and tetracycline which corroborates its application in any future complications in bacterial infection.

Results in this study are in conformity with the finding of many researchers (Wong *et al.,* 1988; Weber *et al.,* 1988; Bottone, 2010;

Ikeda *et al.,* 2015; Tatsinkou-Fossi *et al.,* 2016) except for nalidixic acid in which no resistance was reported in previous studies. Most *B. cereus* isolates are resistant to penicillins and cephalosporins as a consequence of β-lactamase production (Bottone 2010). The study, however, contradict the findings in which more than half of the isolates were resistant to clindamycin and all to ampicilin (Ikeda *et al.,* 2015). High resistance to the multiple antibiotics could represent high mortality and can pave an opportunity for epidemic outbreaks which are tough to circumvent. During Bacillus infections such as Blood stream infection (BSI) (Ikeda *et al.*2015) and gastrointestinal-tract infections[11], susceptibility pattern of aminoglycosides, fluoroquinones, tetracycline and glycopeptides could be considered along with the empirical therapy.

The multiple drug resistance was observed with MAR index for all the *Bacillus cereus* isolates in the range of 0.16-0.68 with an average of 0.41. The only *Bacillus cereus* strain with <0.2 MAR index was isolated from Spinach sample while others were having MAR of >2.0. All the *Bacillus cereus* isolates were found resistance to not less than 4 antibiotics and the resistance count increased up to 17 antibiotics.

The percentage occurrence of multiple antibiotic resistances was highest (25%) with 0.32 index followed by 20% isolates with MAR 0.68 (not shown). None of the isolate was found complete resistant to all the antibiotics tested. High MAR index clearly reflects the indiscriminate use of antibiotics in non-strategic healthcare services. More elaborately, multiple resistances could be due to the expression of resistance genes present in the bacteria or resistance genes channelled from other bacterial species through horizontal gene transfer. Conveyance of these

genes by plasmid was elucidated for tetracycline resistance in *Bacillus cereus* (Rather *et al.,* 2012) which means that these genes significantly affect the resistant phenotype. The source of bacteria is also the concerning factor for example; clinical samples of *S. aureus* (Rajaduraipandi *et al.,* 2006) and *E. coli* (Hora and Ali, 2014) were reported to be multiple drug resistant.

Molecular detection of *Bacillus cereus* and its virulence gene distribution

Nineteen isolates were identified for virulence genes present in *B. cereus* with positive control of *B. cereus* MTCC430. The haemolysin gene *hblA* (320bp) (Fig. 3)*, hblC* (750bp) (Fig. 4) and *hblD* (430bp) (Fig. 5) were found in 100% (19), 73.6% (14), 89.4% (17) isolates respectively. Two of the non-haemolytic enterotoxin genes *nheA* (500bp) (Fig. 6) and *nheC* (582bp) (Fig. 7) were present in all the isolates tested while no *nheB* (770bp) gene was detected at all.

Similar haemolytic enterotoxin gene profiling was reported from RTE vegetables (Chon *et al.,* 2015; Banerjee *et al.,* 2011) and that of non-haemolytic enterotoxin gene of *B. cereus* isolated from milk (Banerjee *et al.,* 2011; Abbas *et al.,* 2014). These enterotoxins are related to outbreaks of food borne disease; enterotoxin-T has been classified as enterotoxin on the basis of genetic and structural relationship with bacterial enterotoxins (Agata *et al.,* 1995). Production of both Hbl and Nhe is believed to be restricted to members of the *B. cereus* group (From *et al.,* 2005).

In this study *B. cereus* strains (94.7%) showed continuous haemolytic patterns. The presence of *hblA* gene in all strains can be correlated with this pattern. *B. cereus* causes self-limiting (24–48 h) food-poisoning syndromes (a diarrheal type and an emetic type) (Tewari and Abdullah, 2015).

The diarrheic syndromes in patients are suspected to occure due to three toxins: hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe) and cytotoxin K (CytK) that cause intestinal fluid secretion, probably by several mechanisms, including pore formation and activation of adenylatecyclase enzymes (Guinebretière and Broussolle, 2002; Jalalpour 2012).

B. cereus contaminated food; emetic type of food poisoning has been largely associated with the consumption of rice and pasta, while the diarrheal type is transmitted mostly by milk products, vegetables and meat (Murray *et al.,* 2006; Logan and Rodrigez-Diaz, 2006).

Bacillus cereus was found in high count in vegetables and showed their virulence in terms of lysis of erythrocytes and presence of gene encoding for haemolytic and non-haemolytic enterotoxin. Both the patterns along with the resistance in antibiotic response can further complicate the availability of possible interventions against illness by these bacteria. The presence of enterotoxin of haemolytic as well as non-haemolytic type may contribute to the Bacillus infections which should be assessed by in-vitro toxicity assay so that empirical intervention could be adopted at the earliest.

The present study surveillance of raw vegetables from village fields around Buddha Nullah has revealed the high health concerns due to contamination with *B. cereus.* The recovered isolates were found haemolytic which can be established by presence of haemolytic genes in their genome. Along with, genes encoding for non-haemolytic enterotoxin were also detected. The expression of such genes in the mammalian cells and resistance to many antibiotics could potentially cause illness which will be difficult to circumvent. The data in study provides useful information for public health

management policies. The proper sanitation strategies should be established so as to prevent the outbreaks related to consumption of fresh vegetables.

References

Abbas, B.A., Khudor, M.H. and Saeed, B.M. (2014). Detection of *hbl, nhe* and *bceT* Toxin Genes in *Bacillus cereus* Isolates by Multiplex PCR. *International Journal of Current Microbiology and Applied Sciences* 3(11):1009-1016.

Agata, N., Ohta, M., Arakawa, Y. and Mori, M. (1995). The *bceT* gene of *Bacillus cereus* encodes an enterotoxic protein. *Microbiology* 141(4):983-988.

Altaf, M.S., Iqbal, A., Ahmad, M., Hussain, S.A., Ahmad, R. and Willayat, M.M. (2012). Study of enterotoxigenicity of *B.cereus* emetic strain by skin vasopermeability reaction in rabbits and poultry. *International Journal of Pharma and Bio Sciences* 3(2):166-172.

Anderson, A., Granum, P.E. and Ronner, U. (1998). The adhesion of *Bacillus cereus* spores to epithelial cells might be an additional virulence mechanism. *International Journal of Food Microbiology* 39:93-99.

Banerjee, M., Nair, G.B. and Ramamurthy, T. (2011). Phenotypic and genetic characterization of *Bacillus cereus* isolated from the acute diarrhoeal patients. *Indian Journal of Medical Reserch* 133(1):88-95.

Bedi, S.K., Sharma, C.S., Gill, J.P.S., Aulakh, R.S. and Sharma, J.K. (2005). Incidence of enterotoxigenic *B. cereus* in milk and milk products. *Journal of Food Science and Technology* 42(3):272-275.

Beecher, D.J., Schoeni, J.L. and Wong, A.C. (1995). Enterotoxic activity of hemolysin BL from *Bacillus cereus*. *Infection and Immunology* 63:4423–4428.

Bottone, E.J. (2010). *Bacillus cereus*, a Volatile Human Pathogen. *Clinical Microbiology Reviews* 23(2):382-398.

Brandl, M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety 1. *Annual Review of Phytopathology* 44:367-392.

Ceuppens, S., Rajkovic, A., Heyndrickx, M., Tsilia, V., Van de Wiele, T., Boon, N. and Uyttendaele, M. (2011). Regulation of toxin production by *Bacillus cereus* and its food safety implications. *Critical Reviews in Microbiology* 37:188-213.

Chon, J.W., Kim, J.H., Lee, S.J., Hyeon, J.Y. and Seo, K.H.(2012). Toxin profile, antibiotic resistance, and phenotypic and molecular characterization of *Bacillus cereus* in Sunsik. *Food Microbiology* 32:217-222.

Chon, J.W., Yim, J.H., Kim, H.S., Kim, D.H., Kim, H., Oh, D.H., Kim, S.K. and Seo, K.H. (2015). Quantitative Prevalence and Toxin Gene Profile of *Bacillus cereus* from Ready-to-Eat Vegetables in South Korea. *Foodborne pathogens and disease* 12(9):795-799.

Fernández, L., Breidenstein, E.B. and Hancock, R.E. (2011). Creeping baselines and adaptive resistance to antibiotics. *Drug Resistance Updates* 14(1):1-21.

From, C., Pukall, R., Schumann, P., Hormazabal, V. and Granum, P.E. (2005). Toxin-producing ability among *Bacillus* spp. outside the *B. cereus* group. *Applied and Environmental Microbiology* 71:1178-1183.

Ghosh, A.C. (1978). Prevalence of *Bacillus cereus* in the faeces of healthy adults.

Journal of Hygiene (Lond.) 80:233-236.

Granum, P.E., O'Sullivan, K. and Lund, T. (1999). The sequence of the non-hemolytic enterotoxin operon from *Bacillus cereus*. *FEMS Microbiology Letters* 177:225-229

Guinebretière, M.H. and Broussolle, V. (2002). Enterotoxigenic profiles of food-poisoning and food-borne *Bacillus cereus* strains. *Journal of Clinical Microbiology* 40(8):3053-3056.

Hora, S. and Ali, Z. (2015). A study of relationship between antibiotic resistance and molecular characteristics of *Escherichia coli* isolates obtained from different human clinical specimens against multiple antibiotic resistance (MAR) index in Bareilly (India) region. *International Journal of Pharmaceutical Sciences and Research* 3(9):3331-3336.

Hutchison, M.L., Walters, L.D., Moore, A., Crookes, K.M. and Avery, S.M. (2004). Effect of length of time before incorporation on survival of pathogenic bacteria present in livestock wastes applied to agricultural soil. *Applied and Environmental Microbiology* 70:5111-5118.

Ikeda, M., Yagihara, Y., Tatsuno, K., Okazaki, M., Okugawa, S., Moriya, K. (2015). Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections. *Annals of Clinical Microbioliology and Antimicrobials* 14(1):1.

Jalalpour, S. (2012). Food borne diseases bacteria; frequency antibiotic resistance bacteria in Iranian foods. *African Journal of Microbiology Research* 6(4):719-723

Jensen, G.B., Hansen, B.M., Ellenberg, J. and Mahillon, J. (2003). The hidden lifestyles of *Bacillus cereus* and relatives. *Environmental Microbiology* 5:631-640.

Kim, H.J., Koo, M., Hwang, D., Choi, J.H., Kim, S.M. and Oh, S.W. (2016). Contamination patterns and molecular typing of *Bacillus cereus* in fresh-cut vegetable salad processing. *Applied Biological Chemistry* 59(4):573–577.

Logan, N.A and Rodrigez-Diaz, M. (2006). *Bacillus* spp. and related genera. In: Gillespie, S.H. and Hawkey, P.M. (eds) Principles and practice of clinical bacteriology, 2nd edn. John Wiley and Sons Ltd, West Sussex, pp 139-158.

Maranon, E., Castrillon, L., Fernández, J. J., Fernández, Y., Peláez, A. I. and Sanchez, J. (2006). Anaerobic mesophilic treatment of cattle manure in an upflow anaerobic sludge blanket reactor with prior pasteurization. *Journal of Air and Waste Management Association* 56(2):137-143.

Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M.A. (2007). Manual of clinical microbiology, 9th edn. American Society of Microbiology Press, Washington DC.

Rajaduraipandi, K., Mani, K.R., Panneerselvam, K., Mani, M., Bhaskar, M. and Manikandan, P. (2006). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian Journal of Medical Microbiology* 24: 38-38.

Rather, M.A., Aulakh, R. S., Gill, J.P.S., Mir, A.Q. and Hassan, M.N. (2012). Detection and sequencing of plasmid encoded tetracycline resistance determinants (*tetA* and *tetB*) from food–borne *Bacillus cereus* isolates. *Asian Pacific Journal of Tropical Disease* 5(9):709-712.

Ryan, P.A., Macmillan, J.D. and Zilinskas, B.A. (1997). Molecular cloning and characterization of the genes encoding the L 1 and L 2 components of hemolysin BL from *Bacillus cereus*. *Journal of Bacteriology* 179:2551-2556.

Tatsinkou Fossi, B., Tatah Kihla Akoachere, J.F., Nchanji, G.T. and Wanji, S. (2016). Occurrence, heat and antibiotic resistance profile of *Bacillus cereus* isolated from raw cow and processed milk in Mezam Division, Cameroon. *International Journal of Dairy Technology* 68:1-9.

Tewari, A. and Abdullah, S. (2015). *Bacillus cereus* food poisoning: international and Indian perspective. *Journal of food Science and Technology* 52(5):2500-2511.

Toh, M., Moffitt, M., Henrichsen, L., Raftery, M., Barrow, K., Cox, J., Marquis, C and Neilan, B. (2004). Cereulide, the emetic toxin of *Bacillus cereus*, is putatively a product of nonribosomal peptide synthesis. *Journal of Applied Microbiology* 97:992-1000.

Valero, M., Hernández-Herrero, L.A., Fernández, P.S. and Salmerón, M.C. (2002). Characterization of *Bacillus cereus* isolates from fresh vegetables and refrigerated minimally processed foods by biochemical and physiological tests. *Food Microbiology* 19(5):491-499.

Weber, D.J., Saviteer, S.M., Rutala, W.A. and Tomann, C.A. (1988). In vitro susceptibility of *Bacillus* spp. to selected antimicrobial agents. *Antimicrobial Agents and Chemotherapy* 32:642-645.

Weldezgina, D. and Muleta, D. (2016). Bacteriological Contaminants of Some Fresh Vegetables Irrigated with Awetu River in Jimma Town, South Western Ethiopia.

Willayat, M.M., Sheikh, G.N. and Misgar, G.R. (2007). Prevalence of *B cereus* biotypes in raw and cooked mutton. *Journal of Veterinary Public Health* 5(2):123-125.

Wong, H.C., Chang, M.H. and Fan, J.Y. (1988). Incidence and Characterization of *Bacillus cereus* isolates contaminating dairy products. *Applied and Environmental Microbiology* 54(3):699-702.