Sudan Journal of Science and Technology (2014) 15(2): 34-39

Sudan Journal of Science and Technology
Journal homepage: http://jst.sustech.edu/

Enterotoxigenicity of *Bacillus cereus* isolated from minced meat

Awadelkarim, A. B. [1*] and Suleiman, K. M. [2]

1. Department of Para-clinical studies. Faculty of Veterinary Science. University of West Kordofan, P. O. Box 20, Sudan
2. Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, Post code: 13314, Sudan

*Corresponding author: Amr Babiker Awadelkarim, E mail: amru_nimr@yahoo.com

ARTICLE INFO

Article history
Received:2 July 2014
Accepted: 25 July 2014
Available online: 1st March 2015

Keywords:
Bacillus cereus,
minced meat,
Enterotoxigenicity.

ABSTRACT

The aim of the study was to investigate the enterotoxigenicity of *Bacillus cereus* isolated from raw minced beef samples, fifty beef samples were collected from butcher shops in Omdurman city, Sudan. Ten Bacillus *cereus* were isolated and identified by standard bacteriological methods. Culture filtrates of the isolates were used to test for enterotoxigicity using the rabbit ileal loop assay. Eight of the *Bacillus cereus* isolates were found to be enterotoxigenic and hence raw beef meat could be a source of food poisoning to humans consuming raw or ill-cooked meat.

INTRODUCTION

Bacillus cereus is known to cause two different types of food poisoning which are characterized by either emesis or diarrhea (Granum, 2001; Ryaa *et al.*, 1997). Emetic syndrome is manifested by rapid onset (1-5 hrs) of nausea, vomiting and malaise. The toxin is produced at the late exponential or stationary growth phase of cells at the optimum temperature of 25 to 30°C (Adams and Moss, 1995). The emetic toxin is probably a peptide, termed "cereulide" (Agata, et al., 1994). The molecular mass of this toxin is less than 10 KD. The cereulide is stable in the digestive tract and induces emesis (Shinagawa and Suzuki, 1994; Mikami *et al.*, 1995). The toxin is highly heat-stable at 126°C for 90 minutes. The toxin also can withstand extremes of pH from 2 to 11 and the proteolytic enzymes trypsin and pepsin (Hughes *et al.*, 1988; Tumbull *et al.*, 1990). This form is diagnosed by isolation of *Bacilly Cereus* from incriminated food.

The onset of illness caused by diarrhoeal toxin is about 8 to 16 hours after consumption of the food contaminated with *Bacillus cereus*, and lasts for between 12 to 24 hours. Symptoms include predominantly diarrhoea and abdominal pain and, occasionally, vomiting. The symptoms

generally resemble those of *Clostridium perfringens* (Notermans and Batt, 1998).

The diarrhoeal enterotoxin can be destroyed by normal cooking temperatures at 56°C for 5 minutes or be inactivated at pH value lower than 4 and higher than 11. The toxin can also be degraded by pepsin, trypsin and hymotrypsin; this diarrheal form is diagnosed by isolation of the organism from stool and food (Kramer and Gilbert, 1989).

Beecher and Macmillan (1990) described a tripartite hemolysin, Hemolysin BL (HBL) produced by *Bacillus cereus*; a three-component enterotoxin consists of a binding component B and two lytic components L1 and L2, and is considered as a primary virulence factor in diarrheal type, it causes vascular Permeability and necrosis in rabbit skin. The presence of all three components is necessary for the toxin activity (Lindback and Granum, 2006).

Bacillus cereus food poisoning can be caused by either ingesting large numbers of bacterial cells and/or spores in contaminated food (diarrhoeal type) or by ingesting food contaminated with pre-formed toxin (emetic type). Transmission of this disease results from consumption of contaminated foods, improper food handling/storage and improper cooling of cooked foodstuffs (Schneider *et al.*, 2004).

Infection of *Bacillus cereus* is not commonly reported because of its usually mild symptoms, but previous study demonstrates a fatal case due to liver failure after the consumption of pasta salad is described and demonstrates the possible severity of the emetic syndrome (Katelijne *et al.*, 2005)

The most widely used method to evaluate the diarrheal response of bacterial toxins is the rabbit ileal loop assay (Notermans and Batt, 1998).

The purpose of the present study was to investigate the enterotoxigenicity of *Bacillus cereus* isolated from raw minced meat using the rabbit ileal loop assay.

MATERIALS AND METHODS

Study area: The study was conducted in Omdurman city, Khartoum State, Sudan.

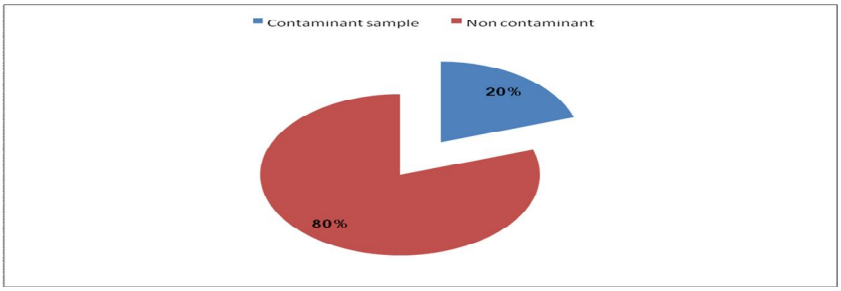
Sampling and cultivation: Fifty minced meat samples were collected in sterile screw-capped bottles from 10 different butcher shops and were transported to the laboratory on ice in a thermos-flask. Two grams of each sample was suspended in 5 ml sterile normal saline which was used to streak blood agar plates that were incubated at 37º C for 24 hours. Smears from suspected colonies were stained by Gram's Method and examined microscopically. Isolates that were haemolytic, Gram positive, spore forming and catalase positive were further identified by conventional biochemical tests.

Enterotoxigenicity of *Bacillus cereus* Isolates: Two rabbits (1 Kg each) were used in the test and it was performed as described by (Beecher *et al.,* 1995). Each rabbit was sedated with ketamin (30 mg/kg) and Xylazine (5 mg/kg) intramuscularly. Animals were then secured in the dorsal recumbence and a mid line incision was made in the abdominal wall and the small intestine was exposed and flushed with sterile saline before ligation. The ileum was segmented and tied off to form 6 loops each 5 cm long, separated from one another by 2 cm blank loops. Culture filtrates of the *Bacillus cereus* isolates were prepared in brain heart infusion broth over night at 37º C aerobically and filtration through millipore filter (0.22 µm) (Beecher and Wong., 1994). Five ileal loops in each rabbit were injected intraluminally with 1 ml of sterile culture filtrate and the sixth loop was injected with 1 ml sterile brain heart infusion broth as a negative control. The ileum was then returned into the abdominal cavity and the incision was sutured. After a holding period of 7 hours rabbits were sedated as above and

the abdominal cavities were opened for examination. The gross appearance of the loops was noted, and fluid inside the loops was measured, RIL activity was reported as the ratio of fluid volume (in milliliters) to loop length (in centimeters) (V/L). Responses were considered positive when V/L was ≤ 0.5. Results were considered applicable when negative control was negative, results didn't give these criteria were eliminated from the study.

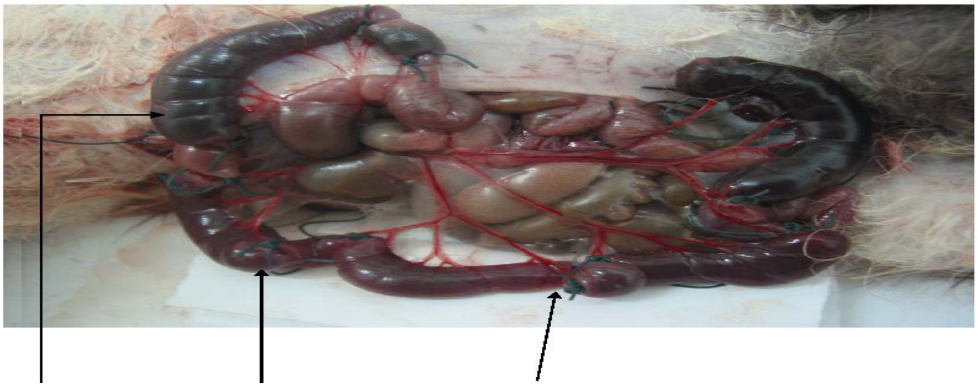
RESULTS

Ten strains of *Bacillus cereus* were isolated from the fifty minced meat samples and were identified by colonial and biochemical features (Figure 1). These were grown under aerobic conditions on 5% sheep blood agar at 37°C, *B. cereus* colonies were dull gray and opaque with a rough matted surface and zones of beta-hemolysis.



Fiqure 1: Frequency of B. cereus in raw minced meat

The isolates were tested for enterotoxigenicity by the ligated ileal loop assay in rabbits (Figure 2). Eight out of ten (80%) strains exhibited positive response and gave V/L ratios greater than 0.5.



Positive response negative response negative control

Figure 2: Enterotoxigenicity of B. cereus by (RIL) test

The greatest response seen in this study was 0.8. The two negative controls gave V/L ratios less than 0.5 (Table 1). The fluid in positive loops ranged from straw colored to red as a result of variable degrees of hemorrhages.

Table 1: Rabbit ileal loop assay (RILA) activity of various *Bacillus cereus* strains

No of *Bacillus cereus* strains	Fluid volume to length ratio
1	0.8
2	0.75
3	0.65
4	0.65
5	0.6
6	0.6
7	0.55
8	0.55
9	0.3
10	0.25
Negative control (1)	0.2
Negative control (2)	0.2

DISCUSSION

Justification of food poisoning is considered one of the main problems in Khartoum state, Sudan. Since there are 34 cases reported in 2010 from hospital admissions (Health-Sudan FMO, 2010). The World Health Organization reported it as one of the main cause for morbidity and mortality in developing countries (Olea *et al.,* 2012).

Result of the present study showed that the frequency of isolation of *Bacillus cereus* from raw beef samples was 20%. Fluid accumulation in rabbit ileal loops was detected in eight (80%) out of ten of the isolates. This high rate of enterotoxic *Bacillus cereus* in the isolates is in agreement with previous studies (Spira and Goepefert, 1972; Josef *et al.,* 2009). On the contrary, Turnbull, (1976) reported that only 2 of 11 isolates of *Bacillus cereus* from food poisoning investigations exhibited positive response in RIL assay.

The presence of some isolates of *Bacillus cereus* which didn't exhibit positive response in RIL tests might explain that not all B. cereus strains cause enterotoxaemia. Psychrotrophic strains grow very poorly at 37º C suggesting that they would be unable to grow in the ileum and probably not causing diarrhea, these observations need, however, to be confirmed. Psychrotrophic *Bacillus cereus* can grow well in foods kept at or below 7°C and may produce emesis even if the food is treated with heat since the emetic toxin is stable to heat while the diarrheal toxin can be destroyed upon heat treatment (Kevin *et al.,* 1998). Understanding the ability of *B. cereus* to grow at low temperatures will help to control multiplication in refrigerated food and prevent outbreaks of food-borne poisoning.

In conclusion, the present study illustrates that, most of the *Bacillus cereus* isolates from raw minced beef meat were found to be enterotoxigenic, which makes the raw beef meat is one of the most important sources of food poisoning to humans.

ACKNOWLEDGEMENTS

We acknowledge the staff of the Department of Microbiology, Faculty of Veterinary Medicine, and University of Khartoum for cooperation and assistance. My gratitude's are also extended to Dr. Nuha, Department of Surgery.

REFERENCES

Adams, M. R and Moss, M. O. (1995). *Bacillus cereus* and other

Bacillus species. *Food Microbiology, pp. 160- 164. The Royal Society of Chemistry*.

Agata, N., Mori, Ohta. M., Swam, S., Ohtani, I., and Isobe, M. (1994). A novel dodecadedepsptide, cereulide, isolated from *Bacillus cereus* cause vacuole formation in Hep –2 cells. *FEMS Microbiological Letter*, **121**: 31 – 34.

Beecher, Douglas. J., Schoeri, Jeanl and lee Wong Amyc. (1995). Enterotoxic Activity of hemolysin BL from *Bacillus cereus*. *Infection and Immunity,* **63**: 4423-8.

Beecher, D. J., and A. C. L. Wong. (1994). Identification of hemolysin BL producing *Bacillus cereus* isolates by a discontinuous hemolytic pattern in blood agar. *Appl. Environ. Microbiol*. **60**:1646–1651.

Health-Sudan FMo: Sudan Household Health Survey. Khartoum; 2010.

Beecher, D. J., and J. D. Macmillan. (1990). A novel bicomponent hemolysin from *Bacillus cereus*. *Infection and Immunity*, **58**:2220-2227.

Dierick, K., Van, Coillie. E., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M and Mahillon, J. (2005). Fatal family outbreak of *Bacillus cereus* associated food poisoning. *Journal of Clinical Microbiology*. **43**(8):4277–4279.

Granum, P. E. (2001). *Bacillus cereus.* In M. P. Doyle (ed.), *Food Microbiology: Fundamentals and Frontiers*, 2nd ed. American Society for Microbiology, Washington, D.C. p. 373-381

Hughes, S., Bartholomew, B., Hardly, J. C and Karmer, J. M. (1988). Potential Application of Hep– 2 cell assay in the investigation of *Bacillus cereus* emetic syndrome food poisoning. *FEMS Microblogical letters* .**25**: 7 – 12.

Josef, B., Jiri, S., Petr, P., Jaroslav, On. Vladimír, B., Alois, C and Tomas, B. (2009). The Occurrence of Enterotoxigenic Isolates of *Bacillus cereus* in Food stuffs. *Czech Journal of Food Science,* **27**(4): 284–292.

Kramer, J. M and Gilbert, R.J. (1989). *Bacillus cereus* and other Bacillus species. In: M. P. Doyles (ed.), Food borne Bacterial Pathogens, Marcel Dekker, New York. pp. 21-70.

Katelijne, D., Els, Van. C., Izabela, S.,Geert, M., Hugo, D., Agnes M., Guy, H., Ludo, F., Marc, H., and Jacques, M. (2005). Fatal Family Outbreak of *Bacillus cereus*-Associated Food Poisoning. *Journal of Clinical Microbiology*. **43**(8): 4277-4279

Kevin, P. F., Ralf , M., Felix von, S., Gordon, S. A. B. Stewart., and Siegfried, S. (1998). Discrimination of Psychrotrophic and Mesophilic Strains of the *Bacillus cereus* Group by PCR Targeting of Major Cold Shock Protein Genes. *Appllied Environmental Microbiology,* **64**(9): 3525-3529.

Lindback, T and Granum, P. (2006). Detection and Purification of *Bacillus cereus* Enterotoxins. In: Adley, C.C. *Food-Borne Pathogens: Methods and Protocols*. Totawa, Humana Press. p. 15-24.

Mikami, T., Horikawa, T., Murakami, T., Sato, N., Ono, Y., Matsumoto, T., Yarnakawa, A., Murayama, S., Katagiri, S and Suzuki, M. (1995). Examination of toxin production from environmentai *Bacillus cereus* and Bacillus thuringiesis. *J. Phannaceutical Society of Japan.* **115**(9):742-748.

Notermans, S and Batt, C. A. (1998). Risk assessment approach for food –

borne *Bacillus cereus* and its toxin. *J App. Microbial*. **84**:51-61.

Olea, A., Díaz, J., Fuentes, R., Vaquero, A and García, M. (2012). Foodborne disease outbreaks surveillance in Chile. Unbound Medline.

Ryan, P. A., Macmillan, J. D., Zilinskas, B. A., (1997). Molecular cloning and characterization of the genes encoding the L1 and L2 components of hemolysin BL from *Bacillus cereus*. J. Bacteriol. **179**:2551-6

Shinagawa, K and Suzuki, M. (1994). an improved method for detecting cytostatic toxin (emetic toxin) of *Bucillus cereus* and its application to food samples. *EMS Microbiol. Lett.* **11**:953-58.

Spira, W. M and Goepfert J. M. (1972). Bacillus cereus induced fluid accumulation in rabbit ileal loops. *Appllied Microbial*. **24**:341-84.

Schneider, K. R., Parish, M. E., Goodrich, R. M and Cookingham, T. (2004). Preventing Foodborne Illness: *Bacillus cereus* and Bacillus anthracis. Series of the Food Science and Human Nutrition Department, No: FSHN04-05. Institute of Food and Agricultural Sciences, University of Florida.

Tumbull, P. C. B., Krarner, J and Melling, J. (1990). Bacillus. In: M. T. Parker and B. 1. Duerden (eds.), Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8th. ed., 2: 188-210.

Turnbull, P. C. B. (1976). Studies on the production of enterotoxins by *Bacillus cereus*. *J. Clin. Path*. **29**:941-8