j.Egypt.net.med.Assoc 76, no 3. 365 – 373 (2016)

UPDATED PREVALENCE OF *BACILLUS CEREUS* ISOLATED FROM MEAT, MEAT PRODUCTS AND CHICKEN PRODUCTS IN EGYPT

By

Gamal, M.A.[1], Mohamed, H.M.H. [2], Elhariri, M. [3] and Mohamed, Kh.F.[4]

[1] Ph.D. Researcher in Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

[2]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

[3]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

[4]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

ABSTRACT

Randomly, 150 different meat &meat products and chicken products samples [15 rice kofta, 15 oriental sausage, 20 raw meat, 20 minced meat, 20 meat lunchon, 20 chicken panne, 20 chicken nuggets and 20 chicken burger] were collected from super markets and local markets in Cairo, Giza, and Alexandria governorates, EGYPT. Morphological and biochemical identification of bacterial isolates revealed *B.cereus* with total incidence of 18.66%. While the individual incidence was 15% in raw meat, 20% in minced meat, 20% in meat lunchon, 35% in chicken panne, 25% in chicken nuggets, 25% in chicken burger and 0% in both oriental sausage and rice kofta.

Key words:

(Meat- Meat products- Chicken products-*B.cereus*).

INTRODUCTION

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs **(Fratamico *et al.,* 2005).** Contaminated raw meat is one of the main sources of food-borne illness **(Podpecan *et al.,* 2007).**Meat products such as minced meat, beef burger, sausage and luncheon are highly demined due to their high biological value, reasonable price, and agreeable taste and easy during serving **(Soliman, 1999)**. Meat products are considered excellent media for the growth of many microorganisms including *B. cereus*. On the other hand, meat products constitute public health hazard where bacteria are responsible for unfavorable changes or pathogenic microorganisms can lead to infection and intoxication **(Kozareva *et al.,* 1982).**In processed

poultry products presence of *B. cereus* is due to surviving of spores from raw poultry, spores from added ingredients and contamination with either spores or cells during processing. The greater degree of contamination found on processed poultry compared to raw poultry meat, is a consequence of synergic action of multiple factors. Ingredients commonly added to meat products, such as spices, seasonings, and protein supplements, have been found to contain *B. cereus*. Also, the packing materials used in food industry prove to be a source of *B. cereus* **(Floristean *et al.,* 2007).** *B.cereus* food poisoning is a major concern worldwide. This bacterium is an aerobic spore-former commonly found in soil. It can be isolated from raw meat, processed foods and vegetables and entered into the food chain either through contaminated food or water. Food poisoning from the past outbreaks include boiled and fried rice, vegetables, cooked meats, soups, and raw vegetable sprouts **(Food and Drug Administration, 2012).** *Bacillus cereus* group consists of six closely related species: *B anthracis, B. cereus, B. mycoides, B. thuringiensis, B. pseudomycoides* and *B. weihenstephanensis*. They share common properties and strong genetic similarity, especially in the sequence of 16S rRNA, the number of rRNA operons, their organization, localization and ability to produce numerous toxins, responsible for pathogenicity and food poisonings **(Tourasse *et al.,* 2011)**.*B. cereus* is a Gram positive rod-shaped aerobic, endospore-forming bacterium. *B.cereus* causes two types of food-borne intoxications. One type is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6 hours. This is the "short-incubation" or emetic form of the disease. The second type is manifested primarily by abdominal cramps and diarrhea with an incubation period of 8 to 16 hours. This type is referred to as the "long-incubation" or diarrheal form of the disease **(Granum, 2009).**There is no enough data about the prevalence of *Bacillus cereus* in Egypt thus; the current study was designed to shed light on the following points: 1- Isolation and identification of *B. cereus* affecting meat, meat products and chicken products in EGYPT.2- Investigating the prevalence of *B. cereus* in meat, meat products and chicken products in EGYPT.

MATERIAL AND METHODS

Sampling:

A total number of 150 different meat, meat products and chicken products samples (15 rice kofta, 15 oriental sausage, 20 raw meat, 20 minced meat, 20 meat lunchon, 20 chicken panne, 20 chicken nuggets and 20 chicken burger) were collected from super markets

and local markets in Cairo, Giza, and Alexandria governorates, EGYPT, in 2015 and 2016 to be analyzed for *B. cereus* detection and isolation. The collected samples werestored in ice box to keep them in a fresh state, and then transferred to the Microbiology laboratory (Department of Microbiology), Faculty of Veterinary Medicine, Cairo University. The collected samples were either ready packed frozen products or public fresh products sold in local markets.

Isolation and identification of isolates:

Each sample was incubated for 24-48 hours at 30 ºC in nutrient broth, brain heart infusion broth and tryptic soy broth under complete aseptic conditions. A loopful from the inoculated broth was streaked on MYP agar and tryptic soy agar then the plates were incubated at 27ºC and 30 ºC for 24-48 hours **(Hwang and Park, 2015).** The suspected colonies were examined for their colonial morphology. Typical colonies were picked up from each plate and were subcultured again on MYP and tryptic soy agar. Then, they were incubated at 27 ºC and 30 ºC for 24-48 hours for purity and maintained for further investigations. The suspected colonies were examined for their colonial morphology, microscopical examination and biochemical reactions. Suspected colonies were plated on MYP agar and tryptic soy agar. Smears from the suspected colonies from pure culture were stained with Gram stain and examined microscopically. Suspected colony with a typical colonial appearance and morphological characteristics was picked up and kept into semi-solid agar medium for preservation of the isolates for further investigation. For motility, the isolates were stabbed in semisolid agar tubes (0.4%) and incubated at 30ºC aerobically for 24 hours. Diffuse zone of growth spreading from the line of inoculation is suspected for motility.

RESULTS

On MYP (mannitol egg-yolk polymyxin) agar:

B. cereus colonies after incubation of plates for 24-48 hr at 30 °C were characteristic pink colonies, non-fermenting mannitol and surrounded by the zone of white precipitate.

Microscopic examination:

Gram stained smears from suspected colonies showed Gram-positive, facultative anaerobic sporeformer. Endospores were ellipsoidal/cylindrical, central/terminal. Sporangia not swollen or, only very slightly swollen, by endospores as shown in Photo (1). Dimensions of vegetative cells were typically 1.0- 1.2 µm by 3.0-5.0 µm. The ellipsoidal spores were formed in a central or paracentral position without swelling the sporangium. Only 28 isolates were

confirmed by separate conventional biochemical tests. As shown in (Table1), all the 28 isolates were positive reactions for catalase test, Voges Proskauer, nitrate reduction, Lecithinase activity, glucose fermentation and surose fermentation. All isolates gave negative reactions with tests of Indole, Urea hydrolysis, and Mannitol fermentation.The results showed that 28 out of 150 samples were positive for *B.cereus* (with total prevalence of 18.66%). The individual prevalence among different meat ,meat products and chicken products was 15% in raw meat, 20% in minced meat, 20% in meat lunchon, 35% in chicken panne, 25% in chicken nuggets, 25% in chicken burger and 0% in both oriental sausage and rice kofta as shown in (Table 2) , Fig.(1).

DISCUSSION

Microbial infections are a leading cause of morbidity and mortality in humans worldwide. Every year, microbial infections cause more than 100 million illnesses worldwide **(Alwan, 2010)**. Microorganisms control in meat products is the major concern in the preparation of high quality foods **(Jo *et al.,* 2004)**. The hygienic state of animals prior, during and after slaughter can be critical to the finished product quality **(Satin, 2002)**. During slaughtering process the meat is exposed to many sources of *Bacillus cereus* contamination **(Lawrie, 1998)**. The incidence of *Bacillus cereus* is higher in cooked and processed (ground beef) meat than in raw meat samples **(Mosupye and Von Holg, 2000)**. *B. cereus* has been incriminated in several foodborne outbreaks involving cereals products (including rice), pasta, meat, vegetables and milk **(European Food Safety Authority, 2007)**. Some ingredients have also been identified as important sources of contamination with *B. cereus*, such as texturing agents, liquid egg, herbs and spices **(Agata *et al.,* 2002)**.This increase in the processed poultry products infection might be attributed to the presence of bacterium which is due to surviving of spores from raw poultry, spores from added ingredients and contamination with either spores or cells during processing. The greater degree of contamination found on processed poultry compared to raw poultry meat, is a consequence of synergic action of multiple factors. Ingredients commonly added to meat products, such as spices, seasonings, and protein supplements, have been found to contain *B. cereus* **(Te Giffel *et al.,* 1996)**. Also, the packing materials used in food industry prove to be a source of *B. cereus* **(Pirttijarvi *et al.,* 2000)**.Concerning the results of morphological and biochemical identification of retrieved isolates, it was revealed that *B.cereus* isolated from meat, meat products and chicken products

were identified as *B.cereus*. These results agree with those reported by **(Stenfors Arnesen *et al.,* 2008)**.In respect to the results of total and differential prevalence among *B. cereus* isolates in meat, meat products and chicken products, the present study revealed that prevalence of *B. cereus* isolates was 18.66% among the examined meat and meat products and chicken products, While the individual prevalence among meat &meat products was 12% (11/90) and 28% (17/60) in chicken products. Such results were nearly agreed with those reported by **(Floristean *et al.,* 2007)** who reported prevalence percentage of 22.5%. The differential prevalence were less than that reported by **(Floristean *et al.,* 2007)** who reported that the processed poultry products infection was 40% (12/30). In this study, *Bacillus cereus* isolated from chicken panne, chicken nuggets and chicken burger at an incidence of 35 %, 25% and 25 % respectively .These results were higher than that observed by **(Samaha *et al.,*2012)** who isolated *B. cereus* from chicken panne and chicken nuggets at an incidence of 0 and 8 %, respectively. These results in chicken nuggets and chicken panne may be attributed to the use of contaminated additives, seasonings and spices with *B. cereus* spores. With regard to meat products, **(Konuma *et al.,* 1988)** founded similar incidence levels to ours of *B. cereus* in meat products (18.3%) and raw meat (6.6%) from a total of 1963 samples collected from four slaughterhouses, four meat processing factories and 12 retail meat outlets in six prefectures of Japan. The results given in (Table 2) reflected the presence of *B.cereus* in 3 samples from 20 raw meat samples with an incidence of 15%. Concerning the minced meat samples, it was found that out of 20 examined samples; *B.cereus* was isolated from 4 samples with an incidence of 20%. The results showed that from 20 of oriental sausage samples, 0 samples were positive with an incidence of 0%. The given results reflected the presence of *B.cereus* in 4 samples out of 20 meat luncheon samples with an incidence of 20%. The obtained findings proved to be less than those reported by **(Eid *et al.,* 2008)** who found that, the incidence of *B.cereus* in sausage was 30%, respectively. The obtained results were less than that recorded by **(El- Ghamry, 2004)** who found that, the incidence of *B.cereus* in minced meat was 55%, respectively. The obtained results were lower than the results reported by **(Khalil, 1997)**; who found that, the incidence of *B.cereus* in meat luncheon was 50%. The obtained results revealed that, the meat products contained high percentages of *B.cereus* and this may be attributed to contamination of flesh used for manufacture, mincing machine,

grinders, equipments and knives also considered as source of contamination of meat during processing **(El-Mossalami, 1994)**.

CONCLUSION

Total prevalence of *B.cereus* infection was relatively higher in poultry products (35% in chicken panne, 25% in chicken nuggets and 25% in chicken burger) than in meat and meat products in Cairo, Giza, and Alexandria Governorates, EGYPT.

REFERENCES

Agata, N.; Ohta, M. and Yokoyama, K. (2002): Production of Bacillus cereus emetic toxin (cereulide) in various foods. International Journal of Food Microbiology, 73: 23-27.

Alwan, A. (2010): Global Status Report on Non-Communicable Diseases; World Health Organization: Geneva, Switzerland, 2011.

Eid-Amal, M.; Eleiwa-Nesreen, Z.H. and Zaky-Eman, M.S. (2008): Prevalence of Bacillus cereus in some ready-to-eat meat products. 9th vet Med. Zag. Conference. 20-22 August Port-Said.

EL-Ghamry-Sanya, R. (2004): Incidence and public health importance of Bacillus cereus in meat and some meat products. MVSc. Thesis Meat Hygiene, Fac. Vet. Med., Zagazig University.

EL-Mossalami, M.K. (1994): Occurrence, species deferring and enterotoxin buildings assets of aerobic spore formers in various meat products. Ph.D. Thesis, the Veterinamedizin at Freie Universitat, Berlin. Feiner, G. 2006. Meat products handbook: Practical science and technology. CRC Press, Cambridge, England. pp. 73-74, 112-113.

European Food Safety Authority. (2007): The community summary report on trends and sources of Zoonoses, Zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. The EFSA Journal, 130**:** 3-352.

Floristean, V.; Carmen Cretu and Carp-Carare, M. (2007): Bacteriological characteristics of Bacillus cereus isolates from poultry. Bulletin USAMV-CN, 64/2007 (1-2).

Food andDrugAdministration(FDA) (2012): Bacillus cereus. Downloaded from http://www.fda.gov/ Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm 070875.

Fratamico, P.M.; Bhunia, A.K. and Smith, J.L. (2005): Foodborne pathogens in Microbiology and Molecular Biology, Caister Academic press, Wymondham Norfolk, UK. Pp. 270-275.

Granum, P.E. (2009): Bacillus cereus. In Food Microbiology Fundamentals and applications 2nd edition. Edited by: Doyle MP, Beuchat LR, Montville TJ. Washington D C, ASM Press, 373-381.

Granum, P.E. and Lund, T. (1997): Bacillus cereus and its food poisoning toxins. FEMS Microbiol. Lett, 157: 223-228.

Hwang, J.Y. and Park, J.H. (2015): Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of Bacillus cereus isolated from infant formulas and ready-to-eat foods. Department of Food Science and Biotechnology, Gachon University, Sungnam 461-701, republic of Korea. J. Dairy Sci., 98:1-9.

Jo, C.; Lee, N.Y.; Kang, H.J.; Shin, D.H. and Byun, M.W. (2004): Inactivation of food-borne pathogens in marinated beef rib by ionizing radiation. Food Microbiol, 21: 543-8.

Khalil, B.G. (1997): Incidence of Bacillus cereus in some food stuffs with special references to its production of thermonouclease enzyme in Assuit City. Assuit Vet. Med. J. 75: 55-63.

Konuma, H.; Shinagawa, K.; Tokumaru, M.; Onoue, Y.; Konno, S.; Fujino, N.; Shigehisa, T.; Kurata, H.; Kuwabara, Y. and Lopes, C.A.M. (1988): Occurrence of Bacillus cereus in meat products, raw meat and meat product additives. Journal of Food Protection, 51 (4): 324-326.

Kozareva, M.; Enikova, R.; Smova, B. and Lordanova, M. (1982): Proteolytic activity of conditionally pathogenic microorganisms causing food poisoning. Vopr- Pitan., 1:53.

Lawrie, R.A. (1998): Lawrie's Meat Science (6th ed.). Wood-head Publishing Limited, Cambridge.

Mosupye, F.H. and Von Holg, A. (2000): Microbiological hazard identification and exposure assessment of street food vending in Johannesburg, South Africa. Int. J. Food Microbiol, 61: 137- 45.

Pirttijarvi, T.S.; Andersson, M.A. and Salkinoja Salonen, M.S. (2000): Properties of Bacillus cereus and other bacilli contaminating biomaterial-based industrial processes. International Journal of Food Microbiology, 60 (2-3): 231-239.

Podpecan, B.; Pengov, A. and Vadnjal S (2007): The source of contamination of ground meat for production of meat products with bacteria Staphylococcus aureus. Slov. Vet. Res., 44: 24-30.

Samaha, I.A.; Ibrahim, H.A.A. and Hamada, M.O. (2012): Isolation of Some Enteropathogens from Retailed Poultry Meat in Alexandria Province.J. Vet. Science, VOL. 37 NO.1: (17-22).

Satin, M. (2002): Use of irradiation for microbial decontamination of meat: situation and perspectives. Meat Sci., 62: 277- 83.

Soliman-Salwa, R. (1999): Risk of street food vending on public health. M.V.Sc. Thesis, Fac. Vet., Cairo University.

Stenfors Arnesen, L.P.; Fagerlund, A. and Granum, P.E. (2008): From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol. Rev., 32: 579-606.

Te Giffel, M.C.; Beumer, R.R.;Leijendekkers,S.and Rombouts, F.M.(1996): Incidence of Bacillus cereus and Bacillus subtilis in foods in the Netherlands. Food Microbiology, 13 (1):53-58.

Tourasse, N.J.; Helgason, E.; Klevan, A.; Sylvestre, P.; Moya, M.; Haustant, M.; Økstad, O.A.; Fouet, A.; Mock, M. and Kolstø, A.B. (2011): Extended and global phylogenetic view of the Bacillus cereus group population by combination of MLST, AFLP, and MLEE genotyping data. Food Microbiol, 28: 236 -244.

Photo (1): Microscopical appearance of *Bacillus cereus* with gram stain showing gram-positive spore forming cells.

Table (1): Characteristics of *B. cereus*.

Characteristics	Results
Motility	**(+ve) Motile**
Hemolysis	**(+ve) β hemolysis**
Catalase	**(+ve) gas bubble**
Indole	**(-ve)**
Voges Proskauer	**(+ve)**
Citrate utilization	**(+ve) color change**
Nitrate reduction	**(+ve)**
Urea hydrolysis	**(-ve) no color change**
Lecithinase activity	**(+ve)**
Glucose fermentation	**(+ve)**
Mannitol fermentation	**(-ve)**
Sucrose fermentation	**(+ve)**

N.B: +ve (Positive), -ve (Negative).

Table (2): The total prevalence of *B. cereus* isolates among 150 different examined meat and meat products and chicken products samples.

Source of samples	Number of examined samples	Positive *B. cereus* isolates	
		Number	%
Raw meat	20	3	15
Minced meat	20	4	20
Meat lunchon	20	4	20
Chicken panne	20	7	35
Chicken nuggets	20	5	25
Chicken burger	20	5	25
Oriental sausage	15	0	0
Rice kofta	15	0	0
Total	150	28	18.66

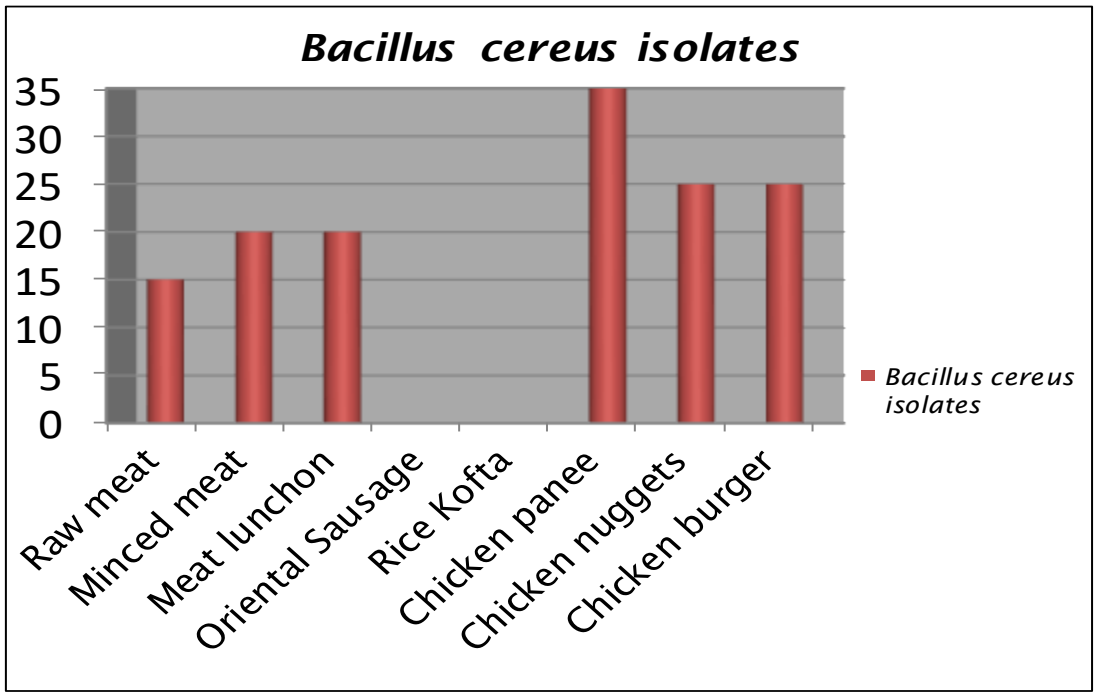

Fig. (1): The percentage of individual prevalence of *B. cereus* isolates among different examined meat and meat products and chicken products samples.