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Original Research

Molecular Detection of Food Borne Pathogen *Bacillus cereus* in Ready to Eat Meat Products

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Abstract

Bacillus cereus is a causative agent for common foodborne outbreaks. In this study, we investigate the presence of food borne bacterial pathogen, Bacillus cereus in ready to eat meat products by cultural and molecular techniques and their antibiotic resistance profiles. Ready to eat meat products (n = 115) were procured from commercial outlets in and around Chennai. Enumeration and isolation of B. cereus was performed by conventional culture and molecular method targeting 185 bp of haemolysin (HL) gene. The isolates obtained were tested for antibiotic sensitivity against commonly used antibiotics. Out of 115 samples screened, 29 found to be positive with 25.2% incidence rate of Bacillus cereus in meat products. The obtained six isolates of B. cereus had 100% resistance against vancomycin and penicillin. The isolates were sensitive towards methicillin, streptomycin, tetracycline and chloramphenicol antibiotics. Hence, the occurrence of B. cereus in ready to eat meat products with the isolates being resistant to antibiotics may cause a serious public health concern and need to be addressed.

Keywords: *Bacillus cereus*, Food Pathogen, Molecular Detection, Meat Products

Introduction

Foodborne illness caused by bacterial pathogens is a common problem worldwide causing serious threat to public health. Both developing and developed countries suffer from increasing trend of food borne outbreaks. The pathogens transmitted through food contribute 30% to globally emerging food borne infections (Carlin *et al.*, 2009). Bacillus cereus is Gram positive, aerobic, motile, heat resistant pathogen and still grows after exhaustive cooking which causes spore germination (Granum and Lund 2006). Food poisoning from *B. cereus* is usually related to cooked rice, vegetables, meat products and milk products (Shiota *et al.*, 2010). Two different clinical symptoms of food poisoning caused by *B. cereus* are distinguished as emesis and diarrheic form (Kim *et al.*, 2010).The ubiquitous nature of B. cereus and the contamination of meat during meat production and processing make meat as an ideal medium for the growth of *B. cereus* and its toxin secretion. The psychotropic nature of certain *B. cereus* strains ensures the survival and multiplication of bacteria during meat processing. Thus, the meat industry often reports food poisoning outbreaks due to *B. cereus* (Granum et al., 1994)

Multiple drug resistant isolates of *B. cereus* due to production of beta lactamase cause a significant public health threat. Beta-lactamases, being one of the potential virulence factors make these strains resistant to penicillin, ampicillin and third generation cephalosporins (Cormian *et al.*, 1998). Indiscriminate use of antibiotics in food animals is the major cause in the development of antibiotic resistance. Hence, the need of the hour is to study the potential transmission of antibiotic resistant bacteria from food chains to humans (Faria – Reyes *et al.*, 2001). Under these circumstances, the present study was framed to investigate the incidence and molecular detection of enterotoxic strain and characterization of *B. cereus* in ready to eat meat products.

Materials and Methods

Samples Collection and Preparation

A total of 115 samples of meat, meat products were collected from different city markets from different regions of Chennai. All samples were analyzed for enumeration and isolation techniques of *Bacillus sps* and *Bacillus cereus* according to the method described in the Bacteriological analytical manual, AOAC International 1995.Mannitol egg yolk polymyxin media was as used as selective media for isolation of *B. cereus*. Presumptive colonies were confirmed as bacillus species by Gram staining, haemolysis on blood agar and biochemical characterization using Hi Bac KB 013, Himedia kit. Confirmation of *Bacillus cereus* isolates was done by polymerase chain reaction (PCR) targeting haemolysin (HBL) toxin gene. *Bacillus cereus* (ATCC 117783) was used as positive control in this study. Details of samples screened are given in Table 1.

Table 1: Enumeration and isolation of *Bacillus sps* and *Bacillus cereus*

S. No	Sample details	No. of samples screened	Mean CFU/g	No. of positive samples for *Bacillus sps.*(%)	No. of positive samples for *Bacillus cereus* (%)
1	Chicken products (cutlet, nuggets, sausage, pakoda, pattices, khabab, kheema, meat balls and raw chicken)	43	3.5×10^5	48.80 (21/43)	20.0 (9/43)
2	Mutton products (Mutton balls and raw mutton)	24	4.6×10^5	45.8(11/24)	20.8 (8/24)
3	Pork products (Sausage, Ham andbacon)	21	2.2×10^4	66.7(14/21)	33.3 (7/21)
4	Beef products (sausage, cubes, meat balls and kheema)	27	5.9×10^5	59.2(16/27)	18.5 (5/27)

DNA Extraction

DNA was extracted as per Kwasaki *et al*. (2005) with slight modification. Overnight culture (1ml) was centrifuged at 10000 rpm for 3 minutes, the pellet was suspended in 1ml of tris – EDTA buffer (TE buffer pH-8), centrifuged at 10000 rpm for 3 minutes. The final pellet was suspended in 20µl of Nucleuses free water (NFW) and boiled for

10 minutes, centrifuged at 10000 rpm for 3 minutes and supernatant was used as DNA template for PCR.

Polymerase Chain Reaction and Sequencing

The presumptive colonies confirmed by culture methods were subjected to PCR targeting haemolysin gene. To amplify the 185 bp portion of HBL toxin gene the primers ith the following sequence Forward Primer 5'CTGTAGCGAATCGTACGTATC 3' and Reverse Primer 5'ACTGCTCCAGCCACATTAC 3' (Wang *et al.*, 1997) were used. The amplified products were checked in agarose gel electrophoresis (Fig 1). The PCR purified samples (n =5) (Qiagen gel extraction kit, Germany) were subjected to sequencing (Eurofins Genomics India Pvt. Ltd., Bengaluru, India) and analysed using DNA Star Laser gene V. 7.0 software. The phylogenetic tree was constructed with 1000 bootstrap value using a Maximum Likelihood method with MEGA software version 6.0.

Antibiotic Susceptibility Test

All the isolates of *Bacillus cereus* were tested for their antibiotic susceptibility pattern using disc diffusion method against a panel of ten antibiotics. Isolates were inoculated in Muller – Hinton broth and incubated at 37°C for 16 – 24 hrs. Turbidity of the overnight culture was adjusted to 0.5 McFerland standard and spread evenly over the Muller Hinton Agar (MHA) plates and the disc containing the specific concentrations of 10 antibiotics (HiMedia laboratories Pvt. Ltd., Mumbai) were placed on the surfaces of the agar plates and incubated at 37°C for overnight. The diameter of the inhibition zones were measured as per the manufacturer's instructions.

Results and Discussion

In the present study, a total of 115 samples were subjected to standard microbiological procedure for detection and biochemical characterization of *B. cereus*. About 62 samples showed characteristic colonies in selective medium (Plate 1) and pure colonies obtained were subjected to various biochemical tests. Only 29 samples showed characteristic biochemical feature for B. cereus. All the 29 presumptive isolates were positive for catalase, nitrate reduction and Voges Proskauer tests but negative for oxidase test. These results were in accordance to the reports of Priest et al., 1988.

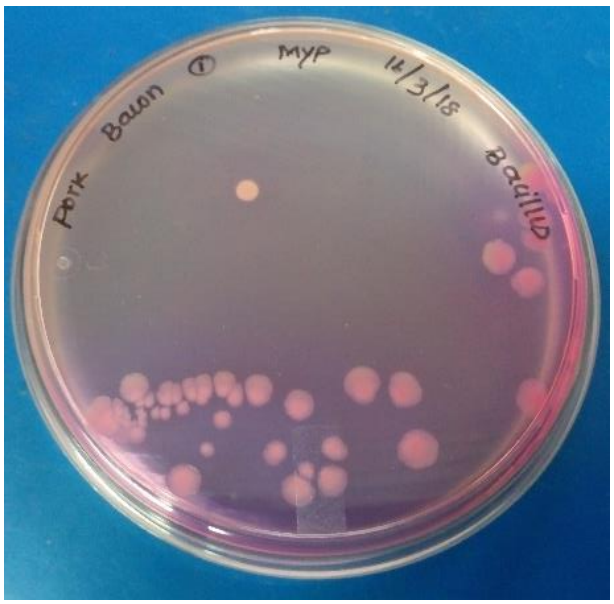

Plate 1: Colony morphology of *Bacillus spp* from pork product in Mannitol Egg yolk Polymixin Agar

Meat and meat products may serve as a potential medium for many bacterial pathogens including *B. cereus*. In comparison with other microorganism, *B. cereus* has diversified characteristics like aerobic and anaerobic growth, psychrotropic (4°C) and thermophilic nature (50°C). Hence, the chances of its presence would be high in meat and meat products if temperature were abused. In our study, the overall prevalence rate of *Bacillus cereus* in various

meat products is 25.2%. The chicken, mutton, pork and beef products showed 20%, 20.8%, 33.3% and 18.5% prevalence of *B.cereus* respectively. Bachhil and Jaiswal (1988) reported the incidence of *B. cereus* in 35 % of fresh buffalo meat, 100 % of kababs and 30 % of curry sample, with a mean counts of 9.65×10^4, 1.07×10^3 and 6.4×10^2 *B. cereus*/g, respectively. Contamination of *B. cereus* is also reported by Yadava (2004) from variety of foods from fish (40 %) and chicken and meat products (80 %) by Kamat *et al.* (1989). Analysis of 200 raw and cooked mutton samples by Willayat *et al.* (2007) revealed the presence of *B. cereus* in 47 samples and the prevalence of 30 % and 15 % respectively. In another study, Tewari *et al.* (2013) reported 30.9 % overall incidence of *B. cereus* in various meat and meat products, while the recorded incidences of *B. cereus* from raw meat and meat products samples were 27.8 and 35 %, respectively. The reason behind the variation in the prevalence level may be due to improved hygienic practices in respective meat production and packaging procedures.

Further, these 29 isolates were confirmed by PCR targeting HL toxin gene that produced 185 bp amplified products (Fig 1) indicating that the ready to eat meat products can be a significant source of B. *cereus* contamination with toxin production.

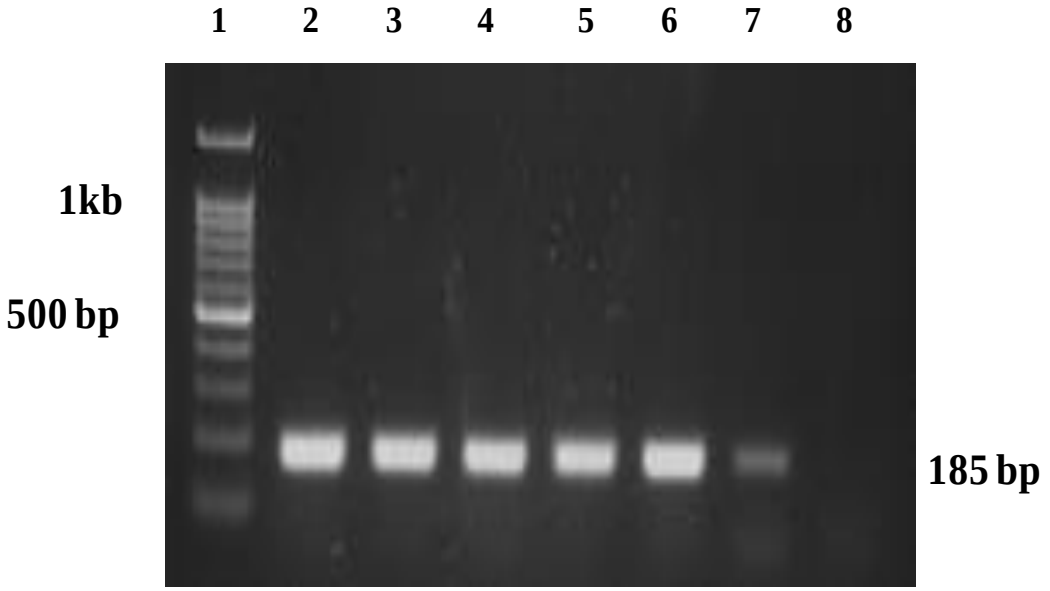

Figure 1: Agarose gel (2%) picture showing 185 bp PCR amplicon of HL gene.
Lane 1: 100 bp ladder, Lane 2: *Bacillus cereus* (ATCC 117783),
Lane 3-7: *Bacillus cereus* isolates and Lane8:Non template control NTC)

It is essential to devise a rapid technique to detect and differentiate the emerging pathogens from their closely related species including *B. thuringenesis* and *B. mycoides.* Here, we used HL toxin gene specific primers for detection and sequencing analysis for specific identification of *B. cereus.* Sequences of five isolates of *Bacillus cereus* obtained from meat products were submitted to Genbank and accession number obtained as MH 547207 (chicken nuggets), MH 547206 (beef cubes), MH 547205 (pork), MH 547204 (meat balls) and MH 547203 (chicken kheema).The overall sequence identity between the present study isolates and the other isolates reported in the NCBI database CP009968 was about 96-99% indicating the conserved nature of the *Bacillus cereus* sequences.

These isolates *of B. cereus* from our study exhibited 100% resistance against vancomycin and penicillin (Plate 2).

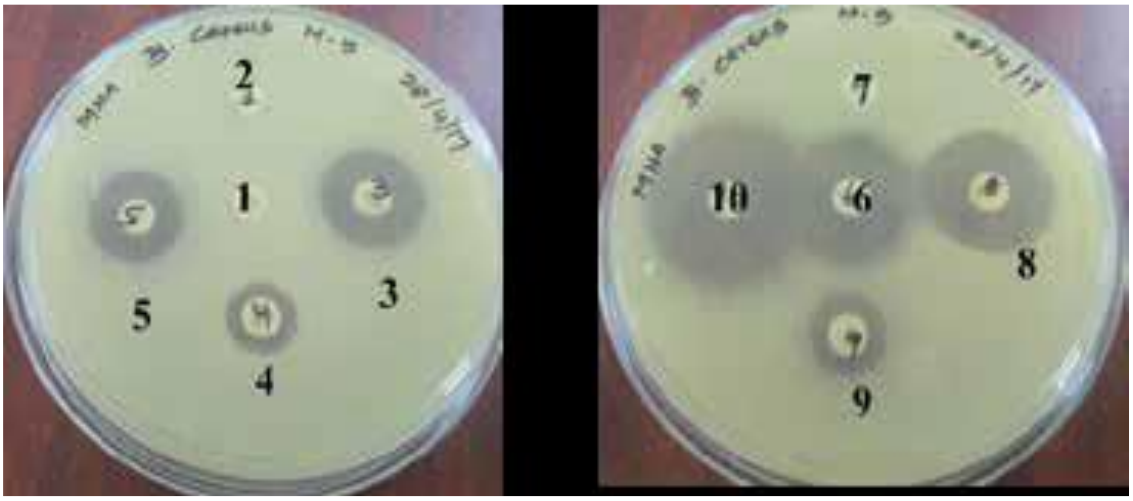

Plate 2: Antibiotic susceptibility of *Bacillus cereus.* 1. Vancomycin, 2. Penicillin, 3. Gentamicin, 4.Ampicillin, 5. Streptomycin, 6. Chloramphenicol, 7. Methicillin, 8. Tetracycline, 9.Erythromycin and 10. Ciprofloxacin

The isolates were sensitive towards methicillin, streptomycin, tetracycline and chloramphenicol antibiotics which are similar to the results of Luna *et al.,* 2007 who reported that *B.cereus* isolated were sensitive to erythromycin, cloramphenicol and tetracycline. From a study by author Reyad and Reda, 2017,who reported that isolates obtained from minced meat, burger, sausage, kofta, and luncheon showed resistance to penicillin G and sensitivity to oxacillin, clindamycin, vancomycin, erythromycin, gentamicin, ciprofloxacin, and ceftriaxone.

In this study, less than 50% resistance was observed towards ampicillin (28.5%) and ciprofolxin (19%). Similarly, Ali *et al*., 2017 from his study reported that, *B. cereus* isolates from ready to eat products showed high resistance to ceftriaxone (100%), vancomycin (87.5%), clindamycin (91.6%) and nalidixic acid (100%) and were sensitive towards ciprofloxacin (100%), streptomycin (91.6%) and chloramphenicol (83.4%).). Variation in the antimicrobial resistant pattern of the isolates may be due to variation in concentration of the antibiotics used, differences in the source of the isolates, drug resistance transfer and overall use of antibiotics. The extensive use of antibiotics in medical and veterinary practice may lead to antibiotic resistance microbial strains. Hence, it may be safe to use probiotics in animal feed and in foodstuffs intended for human use.

Conclusion

The present study provides cautious information that the ready to eat meat products can act as a potential source of food borne illnesses. The occurrence of B.cereus in ready to eat meat products with the isolates being resistant to antibiotics may cause a serious public health concern. Hence, routine and rapid screening of ready to eat food products at all levels of production and distribution in order to provide safe food to the consumers.

Conflict of Interests

The author expresses no conflict of interest with any other individual or organisation regarding the information discussed in the manuscript

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