

Original Research Article

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Prevalence and Characterization of *Bacillus cereus* in Raw Poultry Meat Sold at Retail Meat Outlets of Bikaner

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ABSTRACT

Keywords

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The present study was carried out to isolate *Bacillus cereus* from meat collected from Bikaner. In this study 50 raw poultry meat samples were collected from various retail meat outlets of Bikaner city. Out of 50 raw poultry meat samples, 14 (28%) samples were found positive for *Bacillus cereus* by culture and biochemical tests. The isolates showing positive reaction for the Catalase test, Voges Proskauer's test, Arginine test, Nitrate reduction test, Citrate utilization test and sugar tests like Glucose, Sucrose, Arabinose and Trehalose while negative with ONPG test, Mannitol and Malonate test were confirmed as *Bacillus cereus*. Phenotypically identified *B. cereus* isolates were further analysed by polymerase chain reaction technique. The 14 isolates were subjected to PCR. Out of 14, 12 (85.71%) isolates possessed *gyrB* gene and were confirmed as *B. cereus*. Hence it was concluded that the occurrence of *B. Cereus* in foods especially in raw meat as a meat contaminant and causes food poisoning which is a serious public health concern and need to be addressed.

Introduction

Food poisoning diseases are continuing problem of high magnitude in both, developed and developing countries. The pathogens transmitted through food contribute 30 percent to globally emerging infections (Carlin *et al.*, 2009). In all over the world, morbidity and mortality are attributed to gastroenteritis, due to food borne diseases which pose a serious threat to public health. Food borne pathogens are leading cause of illness and death in developing countries costing billions of dollars in medical care

(Fratamico, 2005) and it is well documented that contamination of food with pathogens is a major public health concern worldwide (Mead,1994). Microorganisms present in the meat may be harmful for human that cause spoilage and may be used as indicator organisms (Bhandre *et al.*, 2007).

Amongst the organisms responsible for causing foodborne diseases, *Bacillus cereus* has emerged as major foodborne pathogen during the last few decades and is often present in a variety of foods, such as starchy foods (rice), animal origin foods (meat, milk

and dairy products) and others like vegetables, spices, cake desserts etc. In India, occurrence of *B. cereus* has been reported from foods like milk (Garg *et al.*, 1977; Chopra *et al.*, 1980), meat (Bacchil and Jaiswal, 1988) and various other foods such as, fried rice, cereals and egg etc (Meena *et al.*, 2000).

Bacillus cereus is a spore-forming, aerobic to facultative anaerobic, Gram-positive, motile rod that can be isolated from a wide variety of different sites (Kotiranta *et al.*, 2000). It grows over a wide temperature range 10 to 48°C with an optimum range of 28 to 35°C. It also grows over a wide pH range from 4.9 - 9.3 and approximately 7.5% concentration of sodium chloride. Microscopically it may be seen in chains and macroscopically the colonies have a dull or frosted glass appearance on a nutrient agar plate (Lattuada & McClain, 1998).

Bacillus cereus is an ubiquitous organism encountered in many types of food. According to Food and Drug Administration of the United States of America, food poisonings caused by *Bacillus cereus* are presented in two different clinical syndromes i.e. diarrheal and emetic syndrome.

The primary diagnosis of *Bacillus cereus* food poisoning is done by isolation of the pathogen from specimens like faeces, vomitus and foods etc. Though Bacilli have been presumptively identified and characterized based on their morphology and biochemical profile yet use of these conventional as well as 16S DNA sequence analysis tests may be insufficient to differentiate *Bacillus cereus* from other members of *Bacillus cereus* group as these members share a quite significant degree of genetic and phenotypic similarities (Rhodehamel and Harmon, 2001). The organisms isolated from meat and meat products in different regions might have

enough diversity at genetic level and can be classified at genetic level by using molecular techniques like polymerase chain reaction. The species of the *Bacillus cereus* group have a high level of DNA homology (Seki *et al.*, 1978), but a specific sequence within *gyrB* gene can distinguish *Bacillus cereus* from the other members of the group (Yamada *et al.*, 1999). Therefore, the main objective of this study is to isolate *Bacillus cereus* by culture and biochemical tests and confirm by detection of specific gene *gyrB* from raw poultry meat samples.

Materials and Methods

The present study was conducted at the Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India

A total of 50 raw poultry meat samples were collected for the present study from retail meat outlets of Bikaner city. About 10-20 grams of raw meat samples were collected in sterilized test tubes and immediately brought to the laboratory under cold conditions. The samples were processed within 4-6 hours of collection. For isolation of this organisms all the samples were enriched in brain heart infusion and then processed for staining.

Each raw poultry sample was streaked on Nutrient agar, and plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hr at 37°C. After 24 hr incubation these isolated colonies were culture on Mannitol egg yolk Polymyxin (MYP) agar plates for isolation of *Bacillus cereus* and incubated for 24 hours at 32°C. The growth was examined for the colonial morphology and pigmentation and different types of colonies were sub-cultured on separate nutrient agar plates in order to

obtain a pure culture. *Bacillus cereus* isolates were gram positive, catalase positive, Mannitol negative and producing rough pinkish colonies with zone of precipitation around the colonies on Mannitol egg yolk Polymyxin agar

The confirmation of the *Bacillus cereus* isolates as were done by using Gram's staining, and a set of 12 biochemical tests provided in HiBacillus™ identification kits for *Bacillus cereus* were used.

The genotypic characterization was carried out by PCR which was used for detection of species specific *gyrB* gene by following methodologies described by Park *et al.*, (2007). The optimized PCR was carried out in a final reaction volume of 25µl and the cycling conditions consisted of an initial denaturation at 94°C for 5 minutes, 35 cycles of amplification with denaturation at 94°C for 60 seconds, annealing at 63°C for 30 seconds, an extension at 72°C for 45 seconds and final extension of the incompletely synthesized DNA at 72°C for 7 minutes. The primers used for detection of *gyrB* gene for characterization of *B. cereus* isolates were synthesized by Xcelris Lab Ltd. Ahmedabad, Gujrat, India. The confirmation and resolution of the PCR product was done by agarose gel electrophoresis using 1% agarose gel.

Results and Discussion

In the present study out of 50 raw poultry meat samples processed 14(28%) samples were found positive for *Bacillus cereus* (Figure-1). Scheimann (1978) reported that 16.96% meat samples to be positive for *Bacillus cereus* which is lower than present study. Sooltan (1987) observed 6.9% samples and Konuma (1988) found 16.3% samples to be positive for *Bacillus cereus* which is also lower than present investigation. Bedi *et al.*, (2004) isolated 56.3% *Bacillus cereus* from

the raw meat samples collected from the areas in and around Ludhiana, Punjab which is just double to our present study, while Smith *et al.*, (2004) reported 45% poultry samples to be positive for *Bacillus cereus* which is higher than present study. Tewari *et al.*, (2015) isolated 27.8% raw poultry samples positive in different parts of northern India which is almost similar to that of present investigation. Abd-El-Tawab *et al.*, (2015) reported 38.33% of *Bacillus cereus*, which is also higher than present study. Bashir *et al.*, (2017) recorded 28% *Bacillus cereus* in poultry meat samples collected from the cities of Jammu which is in complete agreement to our study. Acun *et al.*, (2018) isolated 12% *Bacillus cereus* which is lower whereas, Osman *et al.*, (2018) reported 50% *Bacillus cereus* in poultry meat samples which is higher than present investigation. Gdoura-Ben Amor *et al.*, (2018) observed lower prevalence of *Bacillus cereus* (9.4%) while Jawad *et al.*, (2018) reported much higher prevalence (50%) than present investigation. Solanki *et al.*, (2019) also observed 20% prevalence in the poultry samples collected from Gujarat; whereas, Zaki and Hadad (2019) isolated 8.6% *Bacillus cereus* which are lower than that reported from the present investigation.

In the present study all the isolates that were Gram's positive, catalase positive and pink colonies on Mannitol egg yolk polymyxin agar (MYPagar) were considered as *Bacillus cereus*. All *Bacillus cereus* strains gave opaque zone around the colony on MYP agar. For confirmation of *Bacillus cereus* HiBacillus™ identification kits were used. The isolates showing positive reaction for the Catalase test, Voges proskauer's test, Arginine test, Nitrate reduction test, Citrate utilization test and sugar tests like Glucose, Sucrose, Arabinose and Trehalose while negative with ONPG test, Mannitol and Malonate test were confirmed as *Bacillus cereus*. All the isolates confirmed as *Bacillus*

cereus showed 100% positive reaction with the Citrate utilization test, Nitrate reduction test, Arabinose and Trehalose sugar test. Arginine and Sucrose showed 92.8% positive reaction while Catalase and Glucose test showed 85.71% positive reaction. Voges Proskauer's test showed 78.57% positive reaction. Mannitol test showed 100% negative

reaction while ONPG and Malonate test showed 85.71% negative reaction (Figure1-3). Variability in the results might have occurred due to presence of different types of strains of *Bacillus cereus*. The detailed results of various biochemical tests performed for *Bacillus cereus* are shown in table 1.

Table.1 Results of biochemical tests for *Bacillus cereus* using HiBacillus™ identification kits

S. No.	TEST	POSITIVE		NEGATIVE	
		Number	%	Number	%
1.	Malonate	02/14	14.29%	12/14	85.71%
2.	Voges proskauer's	11/14	78.57%	03/14	21.43%
3.	Citrate utilisation	14/14	100%	0/14	0%
4.	ONPG	02/14	14.29%	12/14	85.71%
5.	Nitrate reduction	14/14	100.00%	-	-
6.	Catalase	12/14	85.71%	02/14	14.29%
7.	Arginine	13/14	92.8%	01/14	7.2%
8.	Sucrose	13/14	92.8%	01/14	7.2%
9.	Mannitol	0/14	0%	14/14	100%
10.	Glucose	12/14	85.71%	02/14	14.29%
11.	Arabinose	14/14	100%	0/14	0%
12.	Trehalose	14/14	100%	0/14	0%

Fig.1 Isolation of *Bacillus cereus* on Mannitol egg yolk Polymyxin agar from Poultry meat Samples

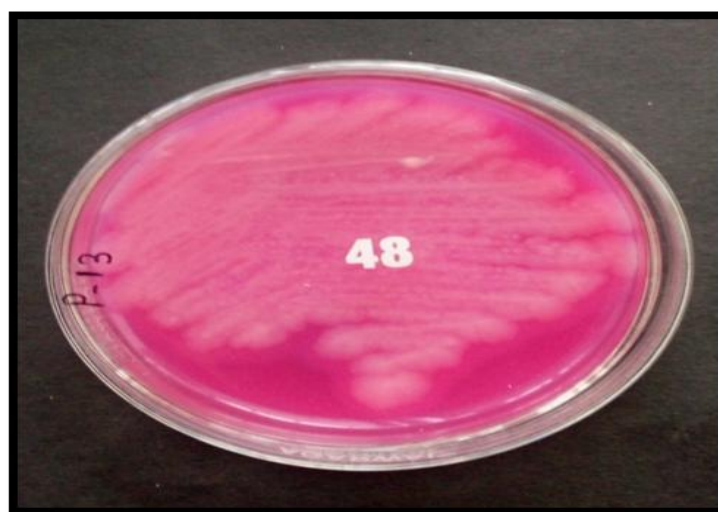
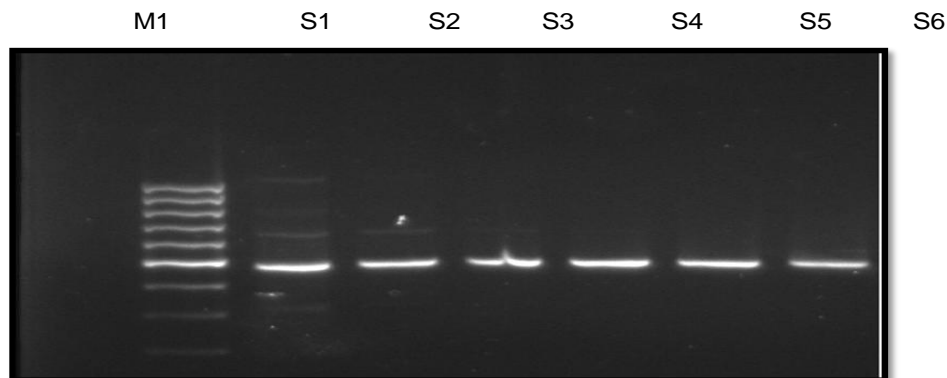


Fig.2 Results of biochemical tests for *Bacillus cereus* obtained from HiBacillus™ commercial kits



Fig.3 Polymerase Chain Reaction based on species specific primer



M1 -100bp DNA ladder (Marker)
S1, S2, S3, S4, S5, S6 – Samples

All the *Bacillus cereus* isolates were screened for the presence or absence of species specific *gyrB* gene using PCR technique. The PCR was standardized for the detection of gene, *gyrB*, following the methodology as described by Mudasir Bashir *et al.*, (2017) and Park *et al.*, (2007) with suitable modifications.

In the present study out of 50 raw poultry meat samples, 14 samples were found positive for *Bacillus cereus* (28%) by culture and HiBacillus™ identification kits. A total of 12 samples (85.71%), out of 14 were found

positive by Polymerase Chain Reaction through amplification of *Bacillus cereus* specific 475bp amplicon (sequence within *gyrB* gene). A specific sequence within *gyrB* gene can distinguish *Bacillus cereus* from the other members of the group (Yamada *et al.*, 1999). Almost similar results reported by La-Duc *et al.*, (2004) and Bashir *et al.*, (2017) that characterized *Bacillus cereus* group phylogenetically and found 84.6% isolates positive by PCR. Rather *et al.*, (2012) and Tewari *et al.*, (2015) confirmed 98.3% and 100% isolates targeting species specific *gyrB*

gene by using PCR which is higher than present investigation.

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