

Detection of Food Poisoning (*Bacillus cereus*) Pathogen in Cooked and Refrigerated Rice Samples

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Abstract. Rice is a staple food of Pakistan. It is being contaminated with several food poisoning causing bacterial and mold contaminants. In this study 100 different rice samples were collected from local market of Karachi city. The presence of *Bacillus cereus* vegetative cell and survival of their spores were quantitatively analyzed after cooking and refrigeration. From the study it was observed that out of 100 rice samples, 25% cooked/refrigerated samples were positive for the presence of *B. cereus* spores, even there were few samples which showed increase of count due to improper (cooking and refrigeration) which causes the germination and proliferation of spores into vegetative cells under favorable conditions. The detection of increased count of *B. cereus* even after cooking and refrigeration treatments is very alarming since cooking is supposed to be best treatment given to the raw food. Different rice dishes are being frequently consumed by the general population and are also available on different shops (as biryanis or fried rice), hence, detection of *B. cereus* in cooked rice samples will be useful to control any outbreak of food poisoning cases especially in summer seasons.

Keywords: *Bacillus cereus*, food poisoning, cereulide toxin, rice sample

Introduction

Microbiological analysis of cereal grains which includes total count of molds, yeast, *Escherichia coli*, *Bacillus cereus* and *Salmonella* sp. is usually carried out to determine the hygienic conditions/quality of rice, which gets contaminated during growth, harvesting and processing (Haque and Russell 2005; Anderson, 1995). Usually endospore forming bacteria, yeast and mold are considered as normal flora of rice besides *Aspergillus* sp., which has also become frequent contaminant of rice due to heavy rain in eastern, south and western regions of Pakistan.

According to Rajkovic *et al.* (2006) rice is frequently associated with *B. cereus* related food poisonings. *B. cereus* is an endospore-forming, gram positive, facultative anaerobe which is commonly isolated from food, which produces various virulent factors or enterotoxins. *B. cereus* is known to cause two types of foodborne diseases namely diarrhoea and emesis. Diarrhoeal disease is associated with edible items and has an incubation period of 8-16 h. Symptoms associated with diarrhoea include abdominal pain and severe watery diarrhoea (Kramer and Gilbert, 1989). The diarrhoeal enterotoxins are produced at an optimum temperature

of 32 °C to 37 °C, it adapts well in a wide range of pH from 4.9-9.3 and salt concentration up to 7.5% (Batt and Tortorello, 2014). It resembles the foodborne illness caused by *Clostridium perfringens* (Ankolekar *et al.*, 2009).

While the emetic type is parallel to *Staphylococcus aureus* food poisoning and is linked to farinaceous foods especially cooked rice. Nausea, vomiting and malaise are the symptoms of the emetic disease which occurs rapidly (0.5-5 h) after consumption of contaminated food. *B. cereus* can grow quickly and produces emetic toxins in very short period of time in cooked and other ready to eat foods at room temperature (Agata *et al.*, 2002). The emetic type illness is characterized by nausea and vomiting starting after ≤ 1 to 5 due to consumption of rice which is rich in starch content. Variety of foods including cooked meat, poultry and dishes which are rich in protein contents and involved in diarrhoeal type of illness (Clavel, 2004; Lund, 1996).

According to Koohy-Kamaly-Dehkordy *et al.* (2013) the spices could also contaminate the food. Naeumayr *et al.* (1983) has found that *B. cereus* spores make up 50% of mesophilic aerobic plate counts in spices. This is because spores could survive the drying process and long storage conditions (ICMSF, 2005). Many foods such as gravies, sauces, or stews can be contaminated

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due to the added spices which are store houses for spore forming bacteria, and under favorable conditions, they germinate, proliferate and sometimes also produce enterotoxins (Banerjee and Sarkar, 2003). *B. cereus* in its spore and vegetative forms, is widely found in soil. Spices or rice contaminated with spores of *B. cereus* had also been a cause of several outbreaks of the diarrhoeal syndrome in Hungary during 1960's. The spores in spices and other food items could survive cooking temperatures which germinates when cooled improperly, and the nutrients in dish support the growth of the resulting vegetative cells (Kramer and Gilbert, 1989).

Rice is the most consumed cereal in the world and has been reported as an important factor in food poisoning caused by *B. cereus* (Granum, 2000). The preparation of many rice dishes requires the addition of spices during cooking and right after cooking, which makes it logical to test the growth of *B. cereus* (spores) in rice before and after cooking.

It is important for the restaurant and food market to provide safe foods to consumers. The aim of this study is to evaluate the incidence of *B. cereus* in various rice samples collected from the local markets of metropolitan city. The survival of *B. cereus* spores after heating or refrigerating treatments of the cooked rice samples was further investigated.

Materials and Methods

Procurement of sample. 100 different rice samples were collected from the local markets of Karachi which includes branded and local samples.

Sample preparation. 50 gram of each sample aseptically weighed and mixed in 450 mL of phosphate buffer and blended for 2 min at 18000 rpm. This gives 1:10 dilution, and then further serial dilutions were made.

Enumeration of *B. cereus*. Spread plate method was used for the enumeration of *B. cereus*. For this purpose 0.1 mL of each dilution was evenly spread by using sterile glass rod spreader over the surface of pre-poured MYP agar plates in duplicate. Plates were incubated at 30 °C for 24-48 h.

Positive and negative control of MYP agar positive control. *B. cereus* (ATCC 11778) was streaked on MYP agar plate. Plates were incubated at 30 °C (pink coloured colonies with egg yolk precipitation) (Fig. 1).

Negative control. *E. coli* (ATCC 25922) was streaked on MYP agar plate. Plates were incubated at 30 °C for 24 h (no growth was observed).

Identification of *B. cereus* on MYP agar. *B. cereus* appeared as pink coloured colonies surrounded by zone of precipitation on MYP agar plates after 24 h. Plates with no growth were re-incubated for additional 24 h.

Confirmation of *B. cereus*. In order to confirm the presence of *B. cereus* the suspected colonies were picked from MYP agar plates and subjected to different confirmatory tests including microscopy, catalase test, anaerobic utilization of glucose, nitrate reduction, VP reaction and Mannitol fermentation (Table 1).

Cooked sample testing. After analyzing the samples for the presence of vegetative forms, the samples were cooked for twenty minutes and cooled at room temperature and then the samples were analyzed for the presence of spore by the Schaeffer-Fulton staining method and agar plating on MYP medium.

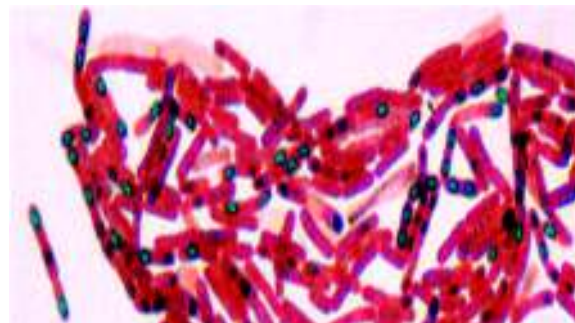


Fig. 1. Picture of vegetative cells with spores of *B. cereus*.

Table 1. Identification test results of suspected *Bacillus cereus* strains

Test performed	Result obtained
Gram reaction	Gram positive rods with spores
Glucose fermentation	Ferments glucose and produces acid anaerobically
Nitrate reduction	Reduces nitrate to nitrite
VP test	Positive
Mannitol fermentation test	Negative

Refrigerated sample testing. The cooked samples of rice were left at room temperature for 4-6 h and then the samples were refrigerated for 24h. Next day the samples were again tested for the surviving *B.cereus* spores by the Schaeffer-Fulton staining method and agar plating on MYP medium.

Results and Discussion

100 different rice samples including local and branded, were qualitatively and quantitatively analyzed after passing through different traditional treatments which includes cooking and refrigeration, respectively. It can be observed from the data shown in Table 2 that even after cooking 25% of the samples were detected with the presence of *B.cereus* (spores) and were not fit for human consumption. There are few samples (sample # 8,12,19,21 and 25) which gave increased count even after heating and refrigeration as compared to raw samples. It was due to the regrowth of the survived spores which in the presence of favorable growth conditions such as temperature (35°C) germinate and propagate very quickly and gave high count in the following samples.

Among 25% of cooked and refrigerated rice samples, 96% cooked samples showed the count of *B. cereus* below 10⁵cfu/g, while among refrigerated samples 92% of the sample gave positive *B. cereus* growth below 10⁵ cfu/g. While 4% of cooked and 8% of refrigerated rice samples showed more than 10⁵ cfu/g count. The detection of such large count of *B.cereus* spores in cooked and refrigerated rice samples, which has already been given conventional treatments, is very alarming because, rice dishes are very popular in Pakistan and other countries. The same survival and regeneration of *B.cereus* spores after giving ultrapasteurization (138 °C for 2 s), “superpasteurization” (96 °C for 13 s) was

Table 2. Count of *B. cereus* strains (with spores) in raw, cooked and refrigerated samples.

Rice Sample	≤10 ⁵ cfu/g out of 25 detected samples		≥10 ⁵ cfu/g out of 25 detected samples		Not detected
Raw	17	68%	8	32%	75%
Cooked	24	96%	1	4%	75%
Refrigerated	23	92%	2	8%	75%

also reported by Georgiana *et al.* (2009) in a study conducted on milk samples. It was reported by the author that the pasteurization temperature only inhibits the regrowth of *B.cereus* spores for 2 weeks at refrigerated temperature in highly sterilized conditions and under re contamination conditions the chances of regrowth of spores is very high along with toxin production.

The emetic type of illness is caused by consumption of rice contaminated with *B.cereus* toxin which is known as cereulide. It is heat stable even at 121 °C for 90 min. pH stable range from 2-11, resistant to proteolysis and not antigenic (Ankolekar *et al.*, 2009) while according to Clavel *et al.* (2004) the lower count of *B. cereus* spores are required to cause diarrhoeal disease as compared to vegetative cells because the spores can survive in the gastric acid. Furthermore, according to Gilbert (1986) 10³cfu/g is the minimum range of *B.cereus* cells which produces sufficient emetic toxins to cause disease. Due to this reason the presence of *B.cereus* in the cooked and refrigerated foods are more important to be detected than in the raw food since the survived toxin present in the food could cause the illness.

Many outbreaks are reported in the past several years due to food poisoning caused by *B. cereus* spp. It is reported to be the third main reason of food poisoning cases in the world. For example according to Granum (1994) the lowest count 2×10² cfu/g of *B.cereus* spores was found in the food which caused outbreak of food poisoning in Norway, 117 outbreaks were reported in Hungary between 1960-1968, 50 outbreaks in Finland, 11 outbreaks in Netherland and 09 were recorded in Canada (Shinagawa1990; Gilbert 1979); USA (Bean and Griffin, 1990); UK, Scandinavia, Japan (Johnson *et al.*, 1984) and Norway (Kotiranta *et al.*, 2000). were also among the list of such food born outbreaks. Among normal population, elderly people or people suffering from severe diseases are more at risk to get infected with these food borne pathogens. According to Jaaskelainen *et al.* (2003) <8 µg/kg of body weight of cereulide toxin is required to cause serious outbreaks of emetic disease. While in European Union 14% of foods born out breaks were related to *Bacillus* species which also includes non-*cereus*, *Bacillus* species (EFSA, 2006). In 1992-2006 around 45 gastroenteritis cases were reported to health protection agencies caused by

Bacillus cereus species. Mehler *et al.* (1997) and Dierick *et al.* (2005) has reported the death of 17 year old Swiss boy and 07 years old Belgian girl due to liver failure caused by emetic food poisoning. According to Rajkovic *et al.* (2008), Cereulide the emetic toxin of *Bacillus cereus* is very heat stable even at 121 and 150 °C and under high alkaline conditions the inactive toxin can revert back to cause toxic effect while its inactivation time depends upon the concentration of the toxin present in the food. The more the concentration of toxin present, the more heating time is required to make toxin inactive. It is also reported to cause cellular damage and known to inhibit human natural killer cells of immune system (Paananen *et al.*, 2002; Shinagawa *et al.*, 1996). Usually diarrhoeal syndrome is related to meat, sauce and dairy products which are caused by three complex heat labile protein toxins. Usually *B.cereus* food poisoning is not easily distinguished from those caused by *S. aureus* intoxication. According to Stenforms *et al.* (2007) *B. cereus* diarrhoeal disease shows the same symptoms as *C. perfringens* type A food poisoning.

In a study conducted by Tahir (2012) it was reported that the rice samples collected from local markets of Lahore city were having heavy bacterial and mold contaminations which include *Aspegillus* sp due to the presence of high moisture content. This finding drew attention of agriculture authorities towards any untoward catastrophe related to this import quality agriculture commodity of Pakistan. Daily Times has reported that more than 250 flood victims in relief camp of Pakistan got severe food poisoning caused by *B.cereus* immediately after eating cooked rice provided to them in September (2010). Mohammad Saeed *et al.* (2007) has also reported severe outbreak of food poisoning caused by *B.cereus* to which out of 110 persons, 57 were severely ill during lunch in Pakistan Army Training Center within 3-5 h of incubation period.

Recently Maria *et al.* (2011) has reported sudden death of a 20 year old man after consuming meal of leftover of spaghetti with tomato sauce. From the clinical findings of food samples, significant count of 9.5×10^7 cfu/mL of *B. cereus* was found, the illness was of emetic type as cereulid toxin was present in significant amount in the food.

Conclusion

From the qualitative and quantitative analysis of raw, cooked and refrigerated rice samples for the presence of *B. cereus*, it can be concluded that the presence of *B. cereus* (spores/vegetative cells) especially in the cooked and refrigerated rice samples is very alarming. As the hygiene level of our society is already very low, there are more chances of any outbreak of food poisoning among general population even causing deaths of several people.

Proper measures must be taken by government food departments to keep track of *B.cereus* (vegetative/spores) especially in ready to eat food items such as biryanis, and other foods being sold in the markets so that any sudden outbreaks could be avoided. Now-a-days there is an increased trend of using ready to eat foods from different restaurants or food outlets which could be a possible source of any outbreak in the metropolitan city. Beside this, it is also important to mention that food should also be cooked properly and should not be left at room temperature as this may cause re-growth of survived spores of bacterial strains including *B.cereus*, resulting in production of toxins which may cause mild nausea to severe diarrhoea leading to sudden death.

Conflict of Interest. The authors declare no conflict of interest.

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