Studies on the Incidence of *Bacillus cereus* in Milk, Milk Products and Farm Environment

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**ABSTRACT**

The present study was carried out to screen the milk, milk products and environmental samples for the incidence and count of *Bacillus cereus*. Seventy five samples each of raw milk, farm water, feed and soil were collected from various sources viz., college dairy farm, private organized dairy farms and small milk producers. Fifty samples of each pasteurized, UHT and flavoured milks, whole milk powder and burfi samples were also collected in ice packs from branded and unbranded manufacturers. Out of 25 samples each of raw milk, water, feed and soil samples collected from college dairy farm, private organized dairy farm and small producers from villages, the incidence of *B. cereus* ranged from 40% - 80% and counts of *B. cereus* ranged from $3.6 \times 10^3$ to $6.20 \times 10^5$ cfu/ml. Out of 25 samples each of pasteurized, ultra high temperature (UHT) and flavoured milks, milk powder and burfi collected from branded and unbranded sources the incidence of *B. cereus* ranged from 16% to 88%. *B. cereus* counts were from $2 \times 10^2$ to $5.25 \times 10^5$ cfu/ml, respectively. The higher incidence of *Bacillus cereus* is an indicative of unhygienic conditions and environmental pollution in and around dairy farms.

**Keywords:** *Bacillus cereus*, incidence and count, milk, milk products.

**Introduction**

*Bacillus cereus* is an important foodborne pathogen of worldwide economic significance and widely distributed in air, soil and water (Claus and Berkeley, 1986). *B. cereus* has been recognized as an agent of food poisoning since long (Hauge, 1995). Concerns over *B. cereus* contamination have increased over the past few years, because of rapidly expanding market of chilled foods that may be pasteurized, but still contain viable spores (Guinebretiere *et al.*, 2002). Due to heat and acid resistance of its spores, *B. cereus* is not eliminated by pasteurization or normal sanitation procedure and is therefore a major problem in mass catering. It also causes spoilage defects like sweet curdling and bitty cream in milk due to proteinases, lipases and phospholipases enzymes (Meer *et al.*, 1991). The incidence of *B. cereus* foodborne illness, accounted for 2% of total foodborne outbreaks reported by CDC during 1973-1987 (Bean and Griffiths, 1990). But raised nearly to 4% according to data of Food and Consumer Safety Authority (Duynhoven *et al.*, 2005).

In India, *B. cereus* has been isolated from a variety of foods including milk, milk products, vegetable soups and sauces, rice, noodles, pasta, pastry, fish, meat, chicken, environmental water samples and feed samples (Sharma *et al.*, 2003). All age groups are believed to be susceptible to *B. cereus* food poisoning, however young children are more severely affected (ICMSF, 1996). The concentrations of viable *B. cereus* in foods ranging from $10^3$ to $10^{10}$ cfu/ml have been implicated in foodborne disease outbreaks (Anderson *et al.*, 1995).
Sufficient data is not available on the incidence of *B. cereus* in dairy products and environmental samples especially urban dairies present in and around Hyderabad. The present study was undertaken to know the incidence and total viable count of *B. cereus* in milk, milk products and environmental samples from urban dairies by screening through cultural methods.

**Materials and Methods**

**Sample collection**

Twenty five samples each of raw milk, farm water, feed and soil samples were collected from college dairy farm, private organized dairy farms and from small milk producers from villages in and around Hyderabad city. Twenty five samples of each pasteurized, UHT and flavoured milks, whole milk powder and burfi were also collected in ice packs from branded and unbranded manufacturers. The soil samples of 50 g were collected aseptically with a sterilized spoon down to a depth of 10 cm from the surface in a UV sterilized polythene bags. The samples were stored at 4°C, whereas the samples like dehydrated milk products, feed and soil samples were stored at room temperature and processed within 3-4 h after their collection.

**Processing of samples**

The samples of raw milk were subjected to heating at 80°C for 10 min in order to destroy non-sporing organisms. Whereas, pasteurized and flavoured milks were directly taken for dilution. One mliter of milk samples was transferred to 9 ml of the Butterfield’s phosphate-buffered dilution water (pH 7.2) to make 1 in 10 dilutions. In case of UHT milk, 25 ml was added to 225 ml Butterfield’s phosphate-buffered dilution water (pH 7.2) and blended for 2 min at 18000-20000 rpm.

Twenty-five grams of burfi was homogenized in 225 ml of buffered peptone water. The milk powders were rehydrated by soaking 10 g in 90 ml of sterile distilled water and shaking at 160 rpm for 30 min at room temperature (Wong et al., 1988).

The procedure described by Christiansson et al. (1999) was followed in case of soil and feed samples. Twenty five grams of sample was added to 225 ml peptone water into glass flask and shaken at 300 rpm for 15 min. Then, 100 ml of liquid was transferred to sterile 100 ml cylinder and allowed for 2 min for sedimentation. Later, 20 ml of liquid was taken into a 100 ml flask and heat treated for 72 °C for 5 min. The water samples were heat treated at 72 °C for 5 min before analysis.

**B. cereus count**

To determine the counts of *B. cereus* serial 10-fold dilutions were prepared using buffered phosphate diluent or peptone water up to a dilution of 10⁵. From the homogenate of each dilution, 0.1 ml was surface-spread on *B. cereus* selective agar (BCSA) with added polymyxin B and egg yolk (Holbrook and Anderson, 1980). The plates were incubated at 37°C for 24-48 h to facilitate the development of turquoise to peacock blue colonies indicative of *B. cereus*. The colonies were counted using digital colony counter.

All presumptive colonies of *B. cereus* were collected and subjected to biochemical tests for differentiation and confirmation. The motility, rhizoid growth, hemolysis and crystal toxin production tests were conducted to differentiate *B. cereus* group of organisms. The tests and identification of species were carried out as described by FDA (1998).

**Results and Discussion**

The incidence and mean counts of *B. cereus* in different samples collected from various sources are presented in Table 1 and 2.

Out of 25 raw milk samples each collected from college dairy farm, private organized dairy farms and small producers from villages, *B. cereus* was found to be present in 15 (60%), 17 (68%) and 19 (76%) samples, respectively. The mean counts of *B. cereus* in raw milk of these sources were 4 x 10⁴ (range: 3.25 x 10³ - 5.60 x 10⁵), 4.6 x 10⁵ (3.2 x 10⁴ - 5.4 x 10⁶) and 5.25 x 10⁶ (4 x 10⁵- 6.2 x 10⁷) cfu/ml, respectively.

Almost similar incidences (66.6%) and viable counts (10⁴ cfu/ml) of *B. cereus* in raw milk collected from organized milk shops were reported by Bedi et
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Table 1: Incidence and count of Bacillus cereus in raw milk, water, feed and soil collected from college farm, private organized dairy farm and small producers from villages

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>College farm</th>
<th>Private organized dairy farm</th>
<th>Small producers from villages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number positive</td>
<td>Mean count (cfu/ml)</td>
<td>Number positive</td>
</tr>
<tr>
<td>Raw milk (25)</td>
<td>15 60</td>
<td>4 \times 10^4</td>
<td>17 68</td>
</tr>
<tr>
<td>Water (25)</td>
<td>10 40</td>
<td>2.8 \times 10^3</td>
<td>13 52</td>
</tr>
<tr>
<td>Feed (25)</td>
<td>16 64</td>
<td>3.6 \times 10^3</td>
<td>18 72</td>
</tr>
<tr>
<td>Soil (25)</td>
<td>17 68</td>
<td>4.2 \times 10^4</td>
<td>18 72</td>
</tr>
</tbody>
</table>

Table 2: Incidence and count of Bacillus cereus in pasteurized milk, UHT milk, flavoured milk, milk powder and burfi collected from branded and unbranded sources.

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>BRANDED</th>
<th>UNBRANDED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurized milk (25)</td>
<td>18 72</td>
<td>22 88</td>
</tr>
<tr>
<td>UHT milk (25)</td>
<td>6 24</td>
<td>8 32</td>
</tr>
<tr>
<td>Flavoured milk (25)</td>
<td>4 16</td>
<td>7 28</td>
</tr>
<tr>
<td>Milk powder (25)</td>
<td>10 40</td>
<td>12 48</td>
</tr>
<tr>
<td>Burfi (25)</td>
<td>12 48</td>
<td>14 56</td>
</tr>
</tbody>
</table>

al. (2005). The higher incidence and counts in raw milk collected from rural producers is probably due to poor hygiene and sanitary conditions (Bedi et al., 2005), while improved hygienic and sanitary conditions in the college dairy and organized private dairy farms might be the reason for decreased incidence and counts of B. cereus (AIFST, 2003).

The incidence with mean counts of B. cereus in pasteurized milk samples from branded and unbranded sources was 72% (1.7 \times 10^3 cfu/ml) and 88% (3 \times 10^4 cfu/ml), respectively. The higher incidence in unbranded sources was due to improper handling and unhygienic conditions during storage and distribution. Higher incidence (100%) and counts (10^3 cfu/ml) in unbranded pasteurized milk were reported by Chitov et al. (2008).

About 24% and 37% UHT milk samples collected from branded and unbranded sources were positive for B. cereus. Almost similar incidence rates i.e. 25% and 34.17% reported by Martin et al. (1997) and De Rezende (1998), respectively. High incidence viz., 50% and 48% of B. cereus in UHT milk was reported by Kramer and Gilbert (1989) and ICMSF (1996), respectively. The B. cereus counts in UHT milk in the present study were 2 \times 10^2 and 2.6 \times 10^3 cfu/ml in branded and unbranded sources, respectively. Higher total viable counts (6.57 \times 10^5 cfu/ml) in UHT milk was reported by Martin et al. (1997), whereas very low counts (10 cfu/ml) by Vyletelova et al. (2002). The higher incidence in the local unbranded flavour milk powder may be due to its production in small rooms' maintained unhygienically without taking proper care during its storage and transportation.
The incidence and counts of *B. cereus* in flavoured milk was 16% (1.8 x 10^5 cfu/ml) and 28% (2.0 x 10^3 cfu/ml) from branded and unbranded sources respectively. Higher incidence (40%) of *B. cereus* in milks than the present study was reported by Kramer and Gilbert (1989). Out of 25 milk powder samples collected from branded and unbranded sources the incidence of *B. cereus* was 10 (40%), and 12 (48%) and the counts of *B. cereus* were 2.5 x 10^2 and 3.75 x 10^3 cfu/ml, respectively.

Similar occurrence rates for in branded milk powders have been reported in previous studies by Bedi et al. (2005) and Reyes et al. (2007), whereas lower incidence of *B. cereus* in milk powder (20% - 39%) were reported by Doll et al. (1983), Wong et al. (1988), Rangasamy et al. (1993) and Dalea and Lapusen (2008). The initial temperature used during the preparation of dried milk favours activation and germination of *B. cereus* spores and its growth on the surface of the equipment (Becker et al., 1994) and thus its presence in milk powder.

In the study, 48% and 56% samples of burfi branded and non branded sources, respectively were contaminated with *B. cereus* with counts of 4.6 x 10^3 and 5.25 x 10^3 cfu/ml. Almost similar prevalence rates and counts were reported by Bedi et al. (2005) and Sharma et al. (2003). The post preparation contamination is very common in burfi due to extensive handling.

The incidence of *B. cereus* in soil samples was 68%, 72% and 80% and its counts were 4.2 x 10^4, 3.5 x 10^5 and 4 x 10^5 cfu/ml, respectively, from college dairy farm, private organized dairy farm and small producers. Christiansson et al. (1999) reported total viable count of *B. cereus* in soil of organized dairy farm as 3.8 x 10^5 cfu/g, which is comparable with present results. The soils of most of the urban farms are contaminated with spores of *B. cereus* leading to germination under favourable conditions.

Out of 25 each water samples from college dairy farm, private organized dairy farm and small producers from villages, *B. cereus* was present in 10 (40%), 13 (52%) and 15 (60%) samples and the counts of *B. cereus* were 2.8 x 10^3, 3 x 10^4 and 4.25 x 10^5 cfu/ml, respectively. Rhodehamel and Harmon (1998) suggested that the counts of *B. cereus* in farm water should be less than 10^5 cfu/ml and the counts obtained in this study from different sources were below this count.

Out of 25 feed samples each collected from college dairy farm, private organized dairy farms and small producers from villages, the incidence of *B. cereus* was 16 (64%), 18 (72%) and 20 (80%) and the counts of *B. cereus* were 3.6 x 10^3, 5 x 10^4 and 6.2 x 10^5 cfu/ml, respectively.

*Bacillus cereus* is a spore former and widely present in grazing area, farm water, feed and fodder, which may lead to subsequent contamination of raw milk, pasteurized milk and dairy products due to improper handling during processing, storage and distribution thus posing a public health risk.

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