

Occurrence of *Bacillus cereus* in Beef Burger Marketed in Tehran, Capital of Iran

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HIGHLIGHTS

- Totally, 31.25% beef burgers sampled from Tehran, Iran were contaminated by *Bacillus cereus*.
- The contamination rate was significantly higher in summer compared to winter.
- The beef burgers supplied in Iran markets is main source of *B. cereus*.
- More attempts must be focused on cold-chain maintenance in production, distribution, and storage of meat products.

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ABSTRACT

Background: Beef burgers made in Iran contain various compounds such as meat, cereals flour, as well as some spices which can be contaminated to *Bacillus cereus*, causing gastroenteritis in the consumer. This study is focused on occurrence of *B. cereus* in beef burgers marketed in Tehran, capital of Iran.

Methods: In this cross-sectional study, a total of 80 samples of different types of beef burgers marketed in Tehran, Iran were randomly collected based on their percentage of meat content, including 30% (n=25), 60% (n=40) as well as 90% (n=15). The samples were analyzed microbiologically by routine culture assay and biochemical tests to find *B. cereus*. Data were analyzed statistically by Microsoft Office Excel 2010.

Results: Twenty-five out of 80 (31.25%) beef burger samples were contaminated by *B. cereus*. Based on the percentage of meat content in the samples, the beef burger with 90% meat were significantly ($p<0.05$) more contaminated than the others. Also, the contamination rate was significantly ($p<0.05$) higher in summer compared to winter.

Conclusion: This survey showed that the beef burgers supplied in Iran markets is main source of *B. cereus* that can cause disease in Iranian consumers. More attempts must be focused on cold-chain maintenance in production, distribution, and storage of the meat products.

Introduction

Bacillus cereus is a facultative anaerobic and Gram-positive bacterium with wide distribution in the food and environment, namely soil, where spores persist under adverse conditions and grow when readily decomposable matter is available (Samapundo et al., 2011; Senesi and Ghelardi, 2010; Stenfors Arnesen et al., 2008). *B. cereus* is found in both vegetative and endospore form which

can induce two types of gastrointestinal diseases, including emesis and diarrhea. The bacterial spores survive during heat treatment and cooking and then germinate when the food is inadequately refrigerated, resulting in food poisoning in the consumers (Granum and Lund, 1997; Park et al., 2007; Sandra et al., 2012). The illnesses caused by *B. cereus* are often unreported, because both types of manifestations are mild in nature, self-limiting, and often do not need medical intervention. Nevertheless,

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extreme forms of the diarrheal type of *B. cereus* have been reported which include rare fatalities (Bennett et al., 2013; Jay et al., 2005).

The occurrence of *B. cereus* as a meat contaminant was reported by some investigators, not only in raw meat, but also in meat products (Rather et al., 2012; Stenfors Arnesen et al., 2008). Beef burgers made in Iran contain various compounds such as meat, cereals flour, and some spices (ISIRI, 2007) which can be contaminated to *B. cereus*, causing gastroenteritis in the consumer. Given the growing use of consumption of fast foods such as beef burgers in Iran (Mashak et al., 2015), this study is focused on occurrence of *B. cereus* in beef burgers marketed in Tehran, capital of Iran.

Materials and methods

Sampling

In this cross-sectional study, a total of 80 samples of different types of beef burgers marketed in Tehran, Iran were randomly collected based on their percentage of meat content, including 30% (n=25), 60% (n=40), and 90% (n=15). The samples were gathered from five geographical regions of the city (North, South, Central, East, and West) in two different seasons, including summer 2014 (as warm season) and winter 2014 (as cold season).

Isolation and identification of *B. cereus*

Twenty-five of each sample was suspended in 250 ml sterile normal saline to prepare an initial 1:10 dilution and inoculated on mannitol yolk polymyxin agar plate. The plates were incubated for 24-48 h at 37 °C. After 48 h, suspected colonies were stained by Gram's method. Spore forming, Gram-positive isolates were identified by the biochemical tests, including catalase production, casein hydrolysis, citrate utilization, gelatin hydrolysis, hemolysis in blood agar medium, nitrate reduction, starch hydrolysis, and penicillin susceptibility. All tests were performed based on Iranian National Standard (ISIRI, 2008) and all culture media used in this study were bought from Merck-Darmstadt (Germany) company.

Statistical analysis

The statistical analysis of the data obtained from the present investigation was performed by Microsoft Office Excel 2010 using Chi-Square test in two periods of year (summer and winter) and three types of burgers based on the percentage of meat (30, 60, as well as 90%). Experiments were designed in full factorial design ($p < 0.05$).

Results

Twenty-five out of 80 (31.25%) beef burger samples were contaminated by *B. cereus*. Based on the percentage of meat content in the samples, the beef burgers with 90% meat content were significantly ($p < 0.05$) more contaminated than the others. Also, the contamination rate was significantly ($p < 0.05$) higher in summer compared to winter (Table 1).

Discussion

Usually, in developing countries like Iran, bacterial food-borne diseases have higher prevalence rates comparing to the developed countries (Banerjee et al., 2011; Bennett et al., 2013; Hussain et al., 2007; Jay et al., 2005; Kotiranta et al., 2000; Newell et al., 2010). In this survey, contamination rate of *B. cereus* in industrial raw beef burgers supplied in Tehran was relatively high as 31.25%. Some similar previous works carried out in this county and other regions of the world indicate some variation in prevalence rate of such bacterial contamination in meat and meat products. However, it has been proved that occurrence rate of *B. cereus* is often much higher in raw or under-cooked product compared with cooked ones because of absence of heating process in order to reduction of microbial load (Jay et al., 2005). For instance, Willayat et al. (2007) detected *B. cereus* in 30 and 15% raw and cooked meat, respectively; so, such high contamination rate found in our study could be attributed to this issue that raw Iranian burgers are prepared with no heating process. Mashak et al. (2015) reported that 56.2% refrigerated meat products of Tehran province were contaminated with *B. cereus*. According to Fang et al. (2003), 49.8% of the ready-to-eat food products sold in Taiwan were found to contain *B. cereus*. Konuma et al. (1988) stated that *B. cereus* was found in meat products (18.3%) and raw meat (6.6%) marketed in Japan. Yu et al. (2016) found that none of the 33 meat products from China were contaminated with *B. cereus*. In another survey, *B. cereus* contamination was detected in one of 350 processed meats in Australia (Eglezos et al., 2010). Also, incidence rate of *B. cereus* in raw meat and meat products from India were reported as 27.8 as well as 35%, respectively (Tewari et al., 2015) that is too similar to the results of the present study.

The higher contamination rate with *B. cereus* in beef burgers collected in warm seasons comparing to cold ones observed in the present research is somewhat predictable; because in high temperatures, cold-chain maintenance of food products is so difficult in some developing countries such as Iran where the refrigerating equipment during production, distribution, and storage of

Table 1: Occurrence of *B. cereus* in beef burgers marketed in Tehran, Iran based on their meat content percentage and sampling season

Meat content (%)	Sampling season	Sample size	No. of contamination
30	Summer	10	2
	Winter	15	3
60	Summer	15	7
	Winter	25	2
90	Summer	6	6
	Winter	9	5

foodstuffs in markets are sometimes imperfect. In the present survey, we found that according to meat content percentage used in beef burgers formulation, the burgers with 90% meat content were significantly more contaminated with *B. cereus* than the other ones; considering this finding, it is assumed that contamination of the samples were probably originated from carcasses and unhygienic conditions in Iranian slaughterhouses.

Conclusion

This survey showed that beef burgers supplied in Iran markets is main source of *B. cereus* that can cause disease in Iranian consumers. More attempts must be focused on cold-chain maintenance in production, distribution, and storage of meat products. Hygienic slaughter of animals in slaughterhouses could improve the safety of carcasses and raw meat used in beef burger formulation. Also, consumption of semi-cooked beef burgers should be avoided by the consumers.

Conflicts of interest

The authors have no conflict of interest.

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