Biotyping of *Bacillus cereus* from the street vended foods in Srinagar area of Kashmir, India

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Abstract

Aim: The present study was undertaken to describe the biotyping of *Bacillus cereus* isolated from different street vended mutton tikka and chutney samples.

Materials and Methods: A total of 100 street vended food samples comprising of 60 mutton tikka and 40 chutney samples were tested.

Results: The biotype 3 and biotype 4 showed the highest occurrence with, 29.63% and 25.93% isolates falling in these biotypes, respectively. The percentage occurrence of the biotypes 1, 6, 2, 5, and 7 was 14.81%, 11.11%, 7.40%, 7.40% and 3.84%, respectively. The most common found biotypes in Mutton tikka were biotypes 3(29.63%), 4(25.93%), 1(14.81%) and 6(11.11%). The *Bacillus cereus* strains isolated from chutney samples could be divided into 7 of the 9 possible biotypes. The biotypes 6 and 7 showed the highest occurrence with 38.46% and 30.76% falling in these biotypes, respectively. The biotype 5 and 2 were prevalent to the extent of 23.07%, 7.69%, respectively. The biotypes 3, 4 and 1 were absent. The mean bacterial count of 60 mutton tikka and 40 chutney samples was 4.6817 and 5.6575 $\log_{10}$ cfu/g.

Conclusion: The field isolates and the standard strains of *Bacillus cereus* had similar cultural, morphological and biochemical characteristics. The biotypes recovered from the Mutton tikka samples were biotypes 3, 4, 1 and 6 and in chutneys the biotypes recovered were 6, 7, 5 and 2. The strains of *Bacillus cereus* were highly resistant to penicillin G (92.59%).

Key words: *Bacillus cereus*, biotyping, chutney, Mutton tikka

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Introduction

Street food vending has become an important public health issue and a great concern to everybody. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues [1]. Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers on the street from pushcarts or baskets or balance pole, or from stalls or shops having fewer than four permanent Walls [2]. Street vended food types differ greatly between countries and cultures and the industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries [3]. However, food borne illnesses of microbial origin pose a major health problem with street vended foods [4, 5].

Street foods are frequently associated with diarrhoeal diseases and occur due to improper use of additives, presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices (GMPs) and good hygienic practices (GHPs). Foodborne bacterial pathogens commonly detected in street-vended foods are *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes, Escherchia coli* and *Salmonella* spp. [6,7]. *Bacillus cereus* is widely spread in the environment (soil, water and dust) from where it easily contaminates foods of both plant and animal origin such as pulses, vegetables, milk, meat and their products [8]. It possesses a diversified animal pathogencity and produces two distinct types of enterotoxins, one of which is highly heat stable, withstanding the temperature of 126°C for 90 minutes. *Bacillus cereus* is an important cause of foodborne illness in man and is

Table-1. Zone wise sampling pattern of Mutton tikka and the Chutney supplied with it.

Sample type	Season	East zone Nishat/ Shalimar	West zone Qamarwari/ Bemina	North zone Hazratbal/ Zakura	South zone Rambagh/ Jawaharnagar	Central zone Khayam/Fateh Kadal	Total
Mutton tikka	Autumn	6	6	6	6	6	30
	Winter	6	6	6	6	6	30
Chutney	Autumn	4	4	4	4	4	20
	Winter	4	4	4	4	4	20

frequently involved in a number of foodborne outbreaks worldwide [9].

Kashmir is famous for its non-vegetarian cuisines and most of these preparations are also sold in the market as vended foods. Common non-vegetarian street foods of Kashmir include Ristas, Kababs, Kantis, Roganjosh, Fried fish and Mutton tikka, the latter being most common and widely accepted street vended food of Kashmir particularly of Srinagar city. Therefore, present study was conducted to know the Biotyping of *Bacillus cereus* from the street vended mutton tikka and chutney samples in Srinagar city of Kashmir valley of Jammu and Kashmir State, India.

Materials and Methods

Sampling: Bacteriological quality of 100 street vended food samples comprising 60 Mutton *tikka* samples and 40 chutney samples collected randomly from vendors of five zones of Srinagar city viz. East, West, North, South and Central was studied in two subsequent seasons viz. autumn and winter. For this purpose, 6 samples of mutton tikka and 4 samples of chutney per zone per season were brought to veterinary public health laboratory in ice and processed within 2-3hr for determination of various *Bacillus cereus* serotypes. The nature and the zone wise sampling pattern are presented in Table-1.

Biotyping of *Bacillus cereus*:The isolates were biotyped based on their ability to ferment ammonium salt sugars viz. xylose, salicin and cellubiose as per the scheme described by [10] (Table-2). Attempts were made to locate the source of these biotypes in Mutton tikka on the basis of their previous history and available literature.

Table-2. Biotyping scheme as proposed by Jha and Narayan (1995).

Biotype	Xylose	Salicin	Cellubiose
1	-	-	-
2	+	-	+
3	-	+	-
4	+	-	-
5	+	+	+
6	-	-	+
7	-	+	+
8	+	+	-

Results

Biotyping: Using the biotyping scheme proposed by [10] the study was extended to the fermentation of three Ammonium salt sugars viz; xylose, salicin and cellubiose. Of the theoretically 9 possible biotypes, the *Bacillus cereus* isolates from Mutton tikka could be divided into 7 biotypes. The biotype 3 and biotype 4 showed the highest occurrence with, 29.63% and 25.93% isolates falling in these biotypes, respectively. The percentage occurrence of the biotypes 1, 6, 2, 5, and 7 was 14.81%, 11.11%, 7.40%, 7.40% and 3.84%, respectively (Table-3). The most common found biotypes in Mutton tikka were biotypes 3(29.63%), 4 (25.93%), 1(14.81%) and 6(11.11%) (Table-3). There was not much selectivity in the distribution of biotypes with regard to sampling area of Mutton tikka. However, biotype 7 could not be isolated from any zone except south zone. Biotype 2 was absent from central, north and west zone. Similarly biotype 5 was absent from north, east and west zone. Biotype 1 and 6 were absent from east and south zone.

The *Bacillus cereus* strains isolated from chutney samples could be divided into 7 of the 9 possible biotypes. The biotypes 6 and 7 showed the highest occurrence with 38.46% and 30.76% falling in these biotypes, respectively. The biotype 5 and 2 were prevalent to the extent of 23.07%, 7.69%, respectively. The biotypes 3, 4 and 1 were absent (Table 4). The distribution of biotypes from different areas did not reveal any selectivity.

However, biotype 3, 4 and 1 could not be isolated from any zone, whereas, biotype 5, 6 and 7 were absent in north and east zone. Similarly, biotype 2 was absent in all zones except central zone. The most common biotypes recovered from chutney samples were biotypes 6(38.46%), 7(30.76%), 5(23.07%) and 2(7.69%) in order. The mean viable bacterial count of mutton tikka samples in autumn season was 5.3839±0.1242 $\log_{10}$ cfu/g**.** The lowest average counts were recorded in East zone (5.6106±0.1577 $\log_{10}$ cfu/g) and the highest counts (5.6106±0.1577 $\log_{10}$ cfu/g) were recorded in the Central zone. The mean viable counts recorded in winter were 3.9795±0.12426 $\log_{10}$ cfu/g**.**

Table-3. The distribution of biotypes in Mutton tikka samples from different zones.

Zones	Biotype 1	Biotype 2	Biotype 3	Biotype 4	Biotype 5	Biotype 6	Biotype 7
Zone I (Central)	1	-	3	2	1	1	-
Zone II (North)	1	-	1	1	-	1	-
Zone III (East)	-	1	1	1	-	-	-
Zone IV (West)	1	-	2	2	-	1	-
Zone V (South)	1	1	1	1	1	-	1
Total	**4 (14.81%)**	**2 (7.40%)**	**8 (29.63%)**	**7 (25.93%)**	**2 (7.40%)**	**3 (11.11%)**	**1 (3.84%)**

Table-4. The distribution of biotypes in Chutney samples from different zones.

Zones	Biotype 1	Biotype 2	Biotype 3	Biotype 4	Biotype 5	Biotype 6	Biotype 7
Zone I (Central)	-	1	-	-	1	2	1
Zone II (North)	-	-	-	-	-	-	1
Zone III (East)	-	-	-	-	-	1	-
Zone IV (West)	-	-	-	-	1	1	1
Zone V (South)	-	-	-	-	1	1	1
Total	**0 (0%)**	**1 (7.69%)**	**0 (0%)**	**0 (0%)**	**3 (23.07%)**	**5 (38.46%)**	**4 (30.76%)**

Discussion

Biotyping has been used a simple tool to study the epidemiology of *Bacillus cereus* [10]. The scheme has been used exclusively for locating the source of contamination of different foods by *Bacillus cereus* [11, 12]. The ability of the isolates to ferment three ammonium salt sugars viz. xylose, salicin and cellibiose has been used as a basis for the biotyping of *Bacillus cereus.* In the present study, the isolates of *Bacillus cereus* were grouped into 7 of the 9 theoretically possible biotypes. The biotyping of isolates from mutton tikka revealed predominance of biotypes 3, 4, 1 and 6 with 29.63% isolates typed as biotype 3, 25.93% as biotype 4, 14.81% as biotype 1 and 11.11% as biotype 6. Biotypes 3 and 4 have been reported as most prevalent biotypes in meat and meat products by [10] and while typing heat resistant *Bacillus cereus* strains from boiled rice, found biotypes 7, 6, 5 and 1 as the most common biotypes [12]. Predominance of biotype 7, 6, and 5 from fried rice has earlier been reported by [11]. Similarly the biotyping of isolates from chutney samples revealed predominance of biotypes 6, 7, 5 and 2 with 38.46% isolates typed as biotype 6, 30.76% as biotype 7, 23.07% as biotype 5, 7.69% as biotype 2. The most prevalent biotypes recovered from the mutton tikka samples were 3 and 4, which indicates meat as the source of *Bacillus cereus* in mutton tikka samples. These findings are in the agreement with the findings of [10]. Similarly in the chutney samples the common biotypes were 6 and 7, indicating spices as the source of contamination as was also reported by earlier workers [13,14]. The mean viable counts recorded in the present study are in accordance with the findings of [15-17]. Occurrence of high bacterial load in street foods as observed in this investigation also corroborates with the earlier findings of several others [18-20].

The scheme to upgrade hygiene and quality of street food has also been undertaken by the Ministry of Food Processing Industries, India. Under the proposed programme, 10,000 street food vendors will be identified, and the majority of stake-holders will be upgraded in terms of quality and hygiene and efforts would be made to make it mandatory for the vendors to register with the local authorities [21].

Author's Contribution

Yasir Hafeez implement the study design. Asif iqbal and Manzoor Ahmad collected the samples, drafted the manuscript and revised manuscript. Asif iqbal and Manzoor ahmad also help in collection of samples. Yasir Hafeez and Manzoor Ahmad helped in interpretation of data. All authors read approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

References

1. Sharmila, R. (2011). Street Vended Food in Developing World: Hazard Analyses, *Indian J Microbiol.* 51(1): 100–106.
2. Food and Drug Administration (FDA). (2007). *Bacillus cereus*. In: *Bacteriological Analytical Manual*. 8th Edition, revision A, AOAC International, Gaithersburg, MD, USA, 14.01-14.08.
3. Muinde, A.M. and Kuria, E. (2005). Hygienic and

sanitary practices of vendors of street foods in Kenya. *AJFAND* online www.ajfand.net. 5: 1-13.
4. World Health Organisation (WHO) (2002). Global strategy for food safety: Safer food for better health. *WHO, Geneva Switzerland* ISBN 924154574.
5. World Health Organisation (WHO) (2009). Food safety & Food borne Illness. Fact sheet 237. World Health Organization, Geneva.
6. Bryan, F.L., Jermini, M., Schmitt, R., Chilufya, E.N., Mwanza, M., Matoba, A., Mfume, E., Chibiya, H. (1997). Hazards associated with holding and reheating foods at vending sites in a small town in Zambia. *J. Food. Prot.* 60: 391-398.
7. Umoh, V.J. and Odoba, M.B. (1999). Safety and quality evaluation of street foods sold in Zaria, Nigeria. *Food. Cont.* 10: 9-14.
8. Larsen, H.D. and Jorgensen, K. (1997). The occurrence of *Bacillus cereus* in Danish pasteurized milk. *Intern. J. Food. Micro.* 34: 179-186.
9. Lund, T., DeBuyser, M.L. and Granum, P.E. (2000). A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. *Mol. Micro.* 38: 254-61.
10. Jha, N.K. and Narayan, K.G. (1995). Biotyping of *Bacillus cereus* isolates. *J. Food. Sci. Tech.* 32: 231-32.
11. Yadava, R. (1993). Biotyping and serotyping of *Bacillus cereus* isolated from rice and chowmein. Ph.D. Thesis submitted to Birsa Agricultural University, Ranchi (Bihar), India.
12. Willayat, M.M. (1997). "Studies on *Bacillus cereus* enterotoxins and their pathogenesis". Ph.D. thesis submitted to Birsa Agricultural University, Ranchi (Bihar).
13. Shehata, T.E. and Collins, E.B. (1971). Isolation and identification of psychrophilic species of *Bacillus* from milk. *App. Micro.* 21: 466-473.
14. Shinagawa, K. (1993). Serology and characterization of toxigenic *Bacillus cereus*. *Neth. Milk and Dairy J.* 47: 89-103.
15. Sharma, C. S., Sharma, D. K., Gill, J. P. S., Aulakh, R.S., Sharma, J.K., (2003). *Bacillus cereus* from foods of animal origin in India and its public health significance. *Acta. Scand.* 44, 118.
16. Fang, T.J., Wei, Q., Liao, C., Hung, M., Wang, T., (2003). Microbiological quality of 18 °C ready-to-eat food products sold in Taiwan. *Intern. Jour. Food Micro.* 80(3), 241-250.
17. Rather, M.A. (2009). Studies on detection of virulence genes in Bacillus cereus from foods of animal origin by multiplex-PCR. Thesis submitted to GADVASU-Ludhiana.
18. Tambekar, D.H., Jaiswal, V.J., Dhanorkar, D.V., Gulhane, P.B. and Dudhane, M.N. (2009). Microbial quality & safety of street vended fruit juices: A case study of Amaravati city. *Internet J. Food Safety.* 10: 72-76.
19. Das, A., Nagananda, G.S., Bhattacharya, S. Bhardwaj, S. (2010). Microbiological quality of Street-Vended Indian Chaats sold in Bangalore. *J. Biol. Sci.* 10: 255-260.
20. Das, M., Rath, C.C. and Mohapatra, U.B. (2011). Bacteriology of a most popular street food (Panipuri) and inhibitory effect of essential oils on bacterial growth. *J. Food Sci. & Technol.* (ON Line, DOI 10. 1007/s13197 – 010 – 0202 – 2, Springer, In Press).
21. MFPOI. (2009). Upgradation of the quality of street food. http//mofpi.nic.in.

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