

# Microbiological Assessment of Street Foods of Gangtok And Nainital, Popular Hill Resorts of India

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## Abstract

Lactococcus lactis, Lactobacillus plantarum, L. brevis, Enterococcus faecium, Bacillus subtilis, B. Pumilus, B. licheniformis, B. cereus, Escherichia coli, Enterobacter aerogenes, Ent. cloacae, Salmonella enteritica, Staphylococcus aureus, S. epidermidis, and Shigella flexneri were isolated from 233 samples of different street foods collected from Gangtok and Nainital, two popular hill resorts of India. The dominant contaminant bacteria were enterobacteriaceae followed by Staphylococcus spp. and B. cereus in food samples tested. Only few street foods were tested positive for toxin production. Salmonella toxins and Staphylococcus enterotoxins were not detected in street foods tested.

**Key words:** Street foods, Nainital, Gangtok, microorganisms

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## Introduction

Street foods are ready-to-eat foods prepared and/or sold by vendors in public places (Hanashiro et al. 2005), and are appreciated for their unique flavour and convenience as well as for maintaining the nutritional value of traditional foods (Kharel and Tamang 2010). Vendors are often with no formal education, untrained in food hygiene and work under crude and unsanitary conditions and have no or very little knowledge about the cause of food borne diseases (Barro et al. 2007). Irrespective of its health effects, people consume street foods in day to day life which are sold in the streets, public places, busy market places, school areas, near college campus and taxi stands, etc (Dawson and Canet 1991). Although there are scanty studies on street foods in India, some studies have revealed that as many as 20 to 30% of the foods are consumed as street foods in India (Sudershan et al. 2009).

Gangtok, the capital of Sikkim state of India is located at a height of 5500 feet in the North East India and is a popular tourist destination. Nainital is also a popular hill station in the state of Uttarakhand. Common street foods of Gangtok are samosa, kachori, puchkka, alu chop, vegetable momo, pork momo, alu-cheura, vegetable chowmein, jhal-muri, sya-faley; and common street foods of Nainital are samosa, kachori, pani puri, alu tikki, vegetable momo, mutton momo, bread chop, vegetable chowmein, jhal-muri, and vegetable pakoda (Kharel et al. 2013). This paper is aimed to assess microorganisms present in the street foods of Gangtok and Nainital and also to determine the presence of toxins.

## Materials and Methods

**Collection of samples.** Samples of street foods of Gangtok and Nainital (Table 1 and 2) were collected aseptically in pre-sterile poly-bags and sterile bottles, kept in ice-box and were labelled. Samples were then transferred to the laboratory and stored at 4 °C until being analyzed.

**Microbiological analysis.** 10 g of sample was homogenized with 90 ml of 0.85% (w/v) sterile

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physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution in the same diluent was made. Lactic acid bacteria (LAB) were isolated on MRS agar (M641, HiMedia, India) plates supplemented with 1% calcium carbonate and were incubated at 30 °C under anaerobic condition kept in an anaerobic gas-pack container (LE002, HiMedia) for 48-72 h. Spore-forming bacilli were isolated on nutrient agar (MM012, HiMedia), after inactivation of vegetable cells by heating at 100 °C for 2 min (Tamang and Nikkuni 1996) and then incubated at 37 °C for 24 h. Presence of yeasts and moulds in samples were tested using yeast malt agar (M424, HiMedia) and potato dextrose agar (M096, HiMedia), respectively and were incubated at 28 °C for 72 h. Total viable count (TVC) was determined in the plate count agar (M091A, HiMedia) and were incubated at 30 °C for 48-72 h. Violet red bile glucose agar w/o lactose (M581, HiMedia) was used for detection of enterobacteriaceae in samples (Han et al. 2001), Salmonella-Shigella agar (M108, HiMedia) was used for the detection of Salmonella and Shigella, Listeria identification agar base (M1064, HiMedia) with Listeria selective supplement (FD061, HiMedia) for Listeria (Metaxopoulos et al. 2001), Cetrimide agar (MM024, HiMedia) was used for detection of Pseudomonas spp. (Tambekar et al. 2008). Kaper's medium (M1169, HiMedia) was used for detection of Aeromonas spp. (Popoff 1984). Thiosulphate citrate bile salt sucrose (M189, HiMedia) was used for detection of Vibrio spp. (Sankar et al. 2012). Selective enumeration of Staphylococcus aureus was carried out on Baird-Parker agar (MM043, HiMedia) with appropriate addition of egg yolk tellurite emulsion (FD046, HiMedia) (Bergdoll and Lee 2006). Total count of halo-tolerant bacteria was determined at different concentration of salt on plate count agar (Han et al. 2001). Identified strains of microorganisms were preserved in respective media using 15% (v/v) glycerol at 20 °C.

**Phenotypic characterization of microorganisms.** Cell morphology of all isolates and their motility were determined using a phase contrast microscope (Olympus CH3-BH-PC, Japan). Isolates were Gram-stained and tested for catalase production, and were preliminarily identified based on the phenotypic properties such as CO<sub>2</sub> production from glucose, ammonia production from arginine, growth at different temperatures as well as the ability to grow in different concentrations of sodium chloride and pH based on the method of (Schillinger and Lücke 1987). Voges-Proskauer test, nitrate reduction, starch hydrolysis, casein hydrolysis, citrate utilization test, bile salt tolerance and anaerobic growth were determined for characterization of Bacillus (Slepecky and Hemphill 2006). Sugar fermentation profiles of LAB and Bacillus were determined using commercial API 50 CHL and CHB kits (bioMérieux, France), respectively.

For the confirmatory tests of the following bacteria, various commercial biochemical test kits were used such as biochemical test kit (KB001, HiMedia) for

enterobacteriaceae, identification kit (KB004, HiMedia) for *S. aureus*, biochemical test kit (KB001, HiMedia) for *Salmonella* spp. and *Shigella* spp., identification kit (KB012, HiMedia) for *Listeria* spp., and biochemical test kit (KB007, HiMedia) for *Vibrio* sp. The confirmatory test of *Pseudomonas* spp. was done following the taxonomic keys of Sneath et al. (Sneath et al. 1986) and *Aeromonas* spp. was done following the taxonomic keys of Popoff (Popoff 1984).

**pH.** The pH of the sample was determined using a digital pH meter (Type 361, Systronics, India).

**Haemolysis of blood agar.** Nutrient agar containing 0.85% sodium chloride and 5% (v/v) defibrinated ox blood was used for haemolytic test of *Staphylococcus* spp. (Harrigan 1998).

**Coagulase test.** Clumping factor or Protein A from *Staphylococcus* spp. isolated from street foods were tested using dry spot staphylect test kits (DR 100, Oxoid, UK) (Harrigan 1998).

**MPN counts.** The most probable number (MPN) count of coliforms was determined following the method of Cappuccino and Sherman (Cappuccino and Sherman 1998). Positive presumptive samples were further streaked on EMB agar (M317, HiMedia) plates and incubated at 37 °C for 24 h. Water samples were tested using biochemical test kit (KB001, HiMedia) for enterobacteriaceae.

**Enterotoxin production.** Production of *Staphylococcus aureus* enterotoxin was determined by reversed passive latex agglutination (RPLA) toxin detection kits (r-Biopharm, Germany) (Chomvarin et al. 2006). *Bacillus diarrhoeal* enterotoxin visual immunoassay was performed according to the manuals' instruction (Tecra).

The presence of enterotoxins and toxins of pathogenic bacteria was detected in food samples using ELISA reader (BioRad, USA) (Boynukara et al. 2008).

## Results and Discussion

A total of 121 samples of street foods collected from Gangtok and 112 samples of street foods collected from Nainital were analysed for determination of microbial load expressed in log cfu/g and pH, respectively (Table 1 and 2). LAB was detected upto the level of 10<sup>4</sup> – 10<sup>5</sup> cfu/g. Load of *Bacillus* was >10<sup>6</sup> log cfu/g, and was the dominant microorganism in the food samples of both Gangtok and Nainital. Based on their detailed characteristics and identification profiles (data not shown), the following genera and species of LAB isolated from various street foods of Gangtok and Nainital were identified as *Lactococcus lactis*, *Lactobacillus plantarum*, *L. brevis* and *Enterococcus faecium*. Based on taxonomical keys of Slepecky and Hemphill (Slepecky and Hemphill 2006), spore forming rod-shaped bacteria from street foods were identified as *Bacillus subtilis*, *B. pumilus*, *B. licheniformis* and *B. cereus*. The predominance of *Bacillus* spp. was possibly due to the presence of spores in the raw materials

which may have survived cooking (Mosupye and Von Holy 1999). High loads of species of bacillus upto 107 cfu/g in street foods of Gangtok was similar to that reported from street foods (panipuri, dahibara and chaat) of Baripada town in Odisha state of India (Rath and Patra 2012).

Puchkka of Gangtok had the highest enterobacteriaceae population of 106 log cfu/g followed by samosa at the level of 105 log cfu/g (data not shown), which is above the acceptable levels and may prove to be hazardous to human health (Thatcher and Clark 1968). Similarly, some street foods of Nainital were recorded at the level of 104 log cfu/g, which is also above the acceptable level (Thatcher and Clark 1968). Food-borne bacterial pathogens commonly detected in street-vended foods are *B. cereus*, *Clostridium perfringens*, *S. aureus* and *Salmonella* spp. (Bryan et al. 1992). More than 104 cfu/g of *B. cereus* is considered unsatisfactory and consumption of foods at/above  $\geq 105$  cfu/g may even lead to food borne illness (Gilbert 2000). In our findings street foods such as samosa, kachori, puchkka, vegetable momo, pork momo, alu-cheura and sya-faley from Gangtok had *B. cereus* at the level of 104 cfu/g and sample of alu chop was at 105 cfu/g, which is far exceeding the acceptable levels (Gilbert 2000). Similarly, in food samples from Nainital only kachori was recorded at 104 cfu/g which is at the marginal level of acceptable limit. There was low contaminant bacteria count in vegetable chowmein from both Gangtok and Nainital. This may be due to the use of vinegar during preparation, as similar observation was reported (Entani 1998).

Yeast isolated from street foods of Gangtok and Nainital were tentatively identified as *Saccharomyces cerevisiae* and *Pichia burtonii*, based on taxonomical keys of Kurtzman and Fell (Kurtzman et al. 2011). Moulds were not recovered in any sample examined. The lesser levels of yeast and total absence of mould in all food samples tested could be due to the time and temperature exposure for steaming/frying/cooking processes. The pH in street foods of Gangtok and Nainital was 5 to 6 except for jhal-muri and sya-faley of Gangtok having pH of 4.8 (Table 1 and 2).

Strains of *S. aureus* isolated from street foods of Gangtok and Nainital showed haemolytic and coagulase activities, whereas strains of *S. epidermidis* did not show any haemolytic and coagulase activities. This could be related to the fact that *S. aureus* shows positive result for haemolytic and coagulase activities, while other strains of *Staphylococcus* shows negative result (Oranusi et al. 2006). The level of coagulase positive *S. aureus* is considered potentially hazardous above or/at the level of  $\geq 104$  cfu/g, and may even lead to food borne illness (Oranusi et al. 2006). Most probable number (MPN) count showed that water sample of Lal Bazar of Gangtok was the most contaminated water sample of Gangtok (data not shown). Similarly water sample collected from Malli Tall of Nainital was the most contaminated water sample. All strains tested for MPN were identified as *E. coli*.

*E. coli* toxin was detected from alu-cheura of Gangtok and jhal-muri of Nainital. No *Salmonella* toxins were detected from food samples collected from Gangtok and Nainital. *B. cereus* diarrhoeal enterotoxins were detected in alu-cheura and puchkka samples from Gangtok and bread chop of Nainital. The enterotoxins produced by *B. cereus* are responsible for emetic or diarrhea food poisoning (Granum 1994). The enterotoxin production level required for strains of *S. aureus* is  $>106$  cfu/g (Loir et al. 2003). No *Staphylococcal* enterotoxins was detected in the samples studied.

Risk associated with the street foods can further be controlled and made safer, if following factors are considered. Firstly, consumer awareness regarding the freshness, quality and hygienic environment of the street foods. Secondly, by educating vendors towards the hygienic condition, and lastly, the concerned Government authorities should periodically check and monitor the preparatory conditions of the shops/stalls in order to maintain the quality of the street foods. In conclusion, street foods are important ethnic foods sold in popular tourist spots in India.

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