Microbiological assessment of ethnic street foods of the Himalayas

Niki Kharel a, Uma Palni b, Jyoti Prakash Tamang c, *

a Food Microbiology Laboratory, Department of Botany, Sikkim Government College, Sikkim, India
b Department of Botany, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India
c Department of Microbiology, School of Life Sciences, Sikkim University, Sikkim, India

1. Introduction

Street foods are ready-to-eat foods prepared and/or sold by vendors in public places [1], and are appreciated for their unique flavor and convenience, as well as for maintaining the nutritional value of traditional foods [2]. Street foods ensure food security for low-income group urban populations [3,4]. 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 [5]. 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 campuses, and taxi stands, etc. [6]. Although there are scanty studies on street foods in India, some studies have revealed that as many as 20–30% of foods are consumed as street foods in India [7].

Gangtok, the capital of Sikkim state of India is located at a height of 5,500 feet in the Eastern Himalayas and is a popular tourist destination (Fig. 1A). Nainital is also a popular hill station in the Indian state of Uttarakhand (Fig. 1B) in the Western Himalayas [8]. It has been observed that the consumption of ethnic street foods is quite popular in both Gangtok and Nainital by local people as well as by tourists, mainly because these regions are popular tourist destinations in the country. Common ethnic street foods of Gangtok are samosa, kachori, puchkka, alu chop, vegetable momo, pork momo, alu-cheura, vegetable chowmein, jhal-muri, and sya-faley. Common ethnic street foods of Nainital are samosa, kachori, pani puri, alu tikki, vegetable momo, mutton momo, bread chop, vegetable chowmein, jhal-muri, and vegetable pakoda. Street foods in the food samples tested positive for toxin production.

Methods: A microbiological analysis was conducted to determine bacteria, including pathogenic bacteria, in the food samples. Enterotoxins were also determined.

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

Conclusion: The risks associated with street foods may be controlled by educating vendors about hygienic conditions. In conclusion, street foods are important ethnic foods sold in popular tourist spots in India for marginal local vendors.

© 2016 The Authors. Published by Elsevier B.V. on behalf of Korea Food Research Institute. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
This paper aimed to assess the microorganisms present in the ethnic street foods of Gangtok and Nainital and also to determine the presence of toxins.

2. Materials and methods

2.1. Collection of samples

Samples of street foods—samosa, kachori, puchhka, alu chop, vegetable momo, pork momo, alu-cheura, vegetable chowmein, jhal-muri, and sya-faley—were collected directly from different markets of Gangtok in Sikkim. Similarly, street foods such as samosa, kachori, panipuri, alu tikki, vegetable momo, mutton momo, bread chop, vegetable chowmein, jhal-muri, and vegetable pakoda were collected from different markets of Nainital in Uttarakhand. Samples were collected aseptically in presterile poly-bags and sterile bottles, kept in ice-boxes, and were labelled. Samples were then transferred to the laboratory and stored at 4°C until analysis. Samples were taken out from freezer and were analyzed when the temperature of the samples were at room temperature.

2.2. Reference strains

References strains used in this paper are Bacillus cereus Microbial Type Culture Collection (MTCC) 6840, Enterobacter aerogenes MTCC 111, Escherichia coli MTCC 1692, Shigella flexneri MTCC 1457, Salmonella enterica MTCC 733, Staphylococcus aureus MTCC 7443, and Staphylococcus epidermidis MTCC 3615. Originally, these reference strains were obtained from MTCC (Institute of Microbial Technology, Chandigarh, India) and were propagated in standard nutrient agar and were maintained as frozen stocks at −20°C in 15% glycerol.

Table 1
Ethnic streets foods of Gangtok.

<table>
<thead>
<tr>
<th>Street foods</th>
<th>Ingredients</th>
<th>Nature of food</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alu-cheura</td>
<td>Potato, beaten rice, spices, salt</td>
<td>Cooked: fried, snack</td>
</tr>
<tr>
<td>Jhal-muri</td>
<td>Puffed rice, spices, onion, salt</td>
<td>Raw: snack</td>
</tr>
<tr>
<td>Puchhka</td>
<td>Flour, potato, tamarind, rock salt, spices</td>
<td>Mixture of fried, cooked, and raw ingredients: snack</td>
</tr>
<tr>
<td>Vegetable momo</td>
<td>Flour, cabbage, monosodium glutamate, onion, spices, salt</td>
<td>Steamed: staple</td>
</tr>
<tr>
<td>Pork/beef/chicken momo</td>
<td>Flour, pork/beef/chicken, monosodium glutamate, onion, spices, salt</td>
<td>Deep-fried: snack</td>
</tr>
<tr>
<td>Samosa</td>
<td>Flour, potato, onion, spices, oil</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Alu-faaley</td>
<td>Flour, potato, onion, spices, salt</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Sya-faaley</td>
<td>Flour, beef, onion, spices, oil</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Pyaazi</td>
<td>Gram flour, onion, chili, oil, salt</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Beef kofta</td>
<td>Gram flour, beef, onion, chili, monosodium glutamate, oil, salt</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Alu-dum</td>
<td>Potato, onion, spices, oil, salt</td>
<td>Cooked: snack</td>
</tr>
<tr>
<td>Bread chop</td>
<td>Bread, potato, chili, spices, oil, salt</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Kachori</td>
<td>Flour, gram flour, spices, oil, salt</td>
<td>Deep-fried: snack</td>
</tr>
<tr>
<td>Murai ko dalla</td>
<td>Puffed rice, jaggery, salt</td>
<td>Sweet: confectionary</td>
</tr>
<tr>
<td>Jalebi</td>
<td>Gram flour, sugar, oil</td>
<td>Fried, sweet: snack</td>
</tr>
<tr>
<td>Khurma</td>
<td>Flour, sugar, oil</td>
<td>Fried, sweet: snack</td>
</tr>
<tr>
<td>Nimki</td>
<td>Flour, salt, oil</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Vegetable chowmein</td>
<td>Flour, different types of vegetables, onion, salt, oil</td>
<td>Shallow fried: snack/staple</td>
</tr>
<tr>
<td>Chowmein</td>
<td>Flour, egg/pork/beef/chicken, vegetables, onion, salt, oil</td>
<td>Shallow fried: snack/staple</td>
</tr>
<tr>
<td>Alu chop</td>
<td>Potato, onion, spices, oil, salt</td>
<td>Fried: snack</td>
</tr>
<tr>
<td>Selroti</td>
<td>Rice batter, sugar, oil</td>
<td>Fried: confectionary</td>
</tr>
<tr>
<td>Chola</td>
<td>Water, salt, grams, potato, onion, spices, oil</td>
<td>Cooked: snack</td>
</tr>
</tbody>
</table>
2.3. Microbiological analysis

Ten grams of sample was homogenized with 90 mL of 0.85% (w/v) sterile physiological saline in a stomacher laboratory blender (400, Seward, Worthing, UK) for 1 minute. A serial dilution in the same diluent was made. Lactic acid bacteria (LAB) were isolated on de Man, Rogosa, and Sharpe agar (M641, HiMedia, Maharashtra, India) plates supplemented with 1% calcium carbonate and were incubated at 30°C under anaerobic conditions in an anaerobic gas-pack container (LE002, HiMedia) for 48–72 hours. Spore-forming bacilli were isolated on nutrient agar (MM012, HiMedia) after inactivation of vegetable cells by heating at 100°C for 2 minutes and

![Figure 2. Street foods of Gangtok. (A) Samosa. (B) Kachori. (C) Puchhka. (D) Pork/beef/chicken Momo. (E) Alu-cheura. (F) Vegetable chowmein. (G) Sya-faley.](image)
then incubated at 37°C for 24 hours. Yeasts were isolated on yeast malt agar (M424, HiMedia), supplemented with 12-μg/mL streptomycin sulfate, and were incubated aerobically at 28°C for 72 hours. Molds were isolated on potato dextrose agar (M096, HiMedia) supplemented with 10-IU/mL benzyl penicillin and incubated aerobically at 28°C for 72 hours. Total viable counts were determined in the plate count agar (M091A, HiMedia) and were incubated at 30°C for 48–72 hours. Microbiological data obtained were transformed into logarithms of the numbers of colony forming unit (cfu) per gram (g) of sample. Identified strains of microorganisms were preserved in respective media using 15% (v/v) glycerol at −20°C.

2.4. 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 carbon dioxide 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 [9]. Voges–Proskauer test, nitrate reduction, starch hydrolysis, casein hydrolysis, citrate utilization test, bile salt tolerance, and anaerobic growth were determined for characterization of *Bacillus* [10,11]. Sugar fermentation profiles of LAB and *Bacillus* were determined using commercial API 50 CHL and CHB kits (bio-Merieux, Marcy l’Etoile, France), respectively.

Violet red bile glucose agar w/o lactose (M581, HiMedia) was used for detection of enterobacteriaceae in samples [12], *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

![Fig. 3. Street foods of Nainital. (A) Kachori. (B) Alu tikka. (C) Bread chop.](image-url)
L. Listeria [13], Cetrimide agar (MM024, HiMedia) was used for the detection of *Pseudomonas* spp. [14], and Kaper’s medium (M1169, HiMedia) was used for the detection of *Aeromonas* spp. [15]. Thiocyanate citrate bile salt sucrose (M189, HiMedia) was used for the detection of *Vibrio* spp. [16]. Selective enumeration of *S. aureus* was carried out on Baird–Parker agar (MM043, HiMedia) with appropriate addition of egg yolk tellurite emulsion (FD046, HiMedia) [17].

For the confirmatory tests of the following bacteria, various commercial biochemical test kits were used such as a 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 a biochemical test kit (KB007, HiMedia) for *Vibrio* spp. The confirmatory test of *Pseudomonas* spp. was done following the taxonomic keys of Sneath et al [18] and *Aeromonas* spp. was done following the taxonomic keys of Popoff [15].

2.6. Enumeration of halo-tolerant bacteria

The total count of halo-tolerant bacteria was determined at different concentrations of salt on plate count agar [12].

2.7. Hemolysis of blood agar

Nutrient agar containing 0.85% sodium chloride and 5% (v/v) defibrinated ox blood was used for a hemolytic test of *Staphylococcus* spp. [20].

2.8. Clumping factor test

Clumping factor or Protein A from *Staphylococcus* spp. isolated from street foods were tested using dry spot staphypect test kits (DR 100, Oxoid, Basingstoke, UK) [20].

2.9. Most probable number counts

The most probable number (MPN) count of coliforms was determined following the method of Cappuccino and Sherman [21]. Positive presumptive samples were further streaked on eosin methylene blue agar (M317, HiMedia) plates and incubated at 37°C for 24 hours. Water samples were tested using a biochemical test kit (KB001, HiMedia) for enterobacteriaceae.

2.10. *Staphylococcus enterotoxins*

Production of *S. aureus* enterotoxins was determined by reversed passive latex agglutination toxin detection kits (r-Biopharm, Darmstadt, Germany) [22].

2.11. *Bacillus diarrheal enterotoxin*

*Bacillus* diarrheal enterotoxin visual immunoassay was performed according to the manuals’ instruction (Tecra® Bacillus Diarrhoel Enterotoxin VIA, 3 M Centre, St. Paul, MN, USA).

2.12. Immunoassay for detection of toxins

The presence of enterotoxins and toxins of pathogenic bacteria was detected in food samples using an enzyme-linked immunosorbent assay reader (BioRad, Hercules, CA, USA) [23].

3. Results and discussion

Based on personal observations and interviews with the producers, consumers, and sellers engaged in the production of the street foods, the methods of preparation and varieties of ethnic street foods were documented [8] and are summarized in Tables 1 and 2. Various street foods are sold in Gangtok and Nainital, which include *samosa*, *kachori*, *puchkka*, *alu chop*, *momo* (vegetable, chicken, mutton, pork, beef), *alu-cheura*, *chowmein* (vegetable, chicken, mutton, pork, beef, egg), *jhal-muri*, *alu tikki*, *bread* and *chop*, and *vegetable pakoda*. It has been observed that the consumption of street foods is quite popular in both Gangtok and Nainital by local people as well as by tourists, mainly because these regions are popular tourist destinations in the country. The vendors prepare foods locally at their homes and the foods are sold in the streets, public places, busy market places, school areas, near college campuses, and taxi stands, etc. A total of 121 samples of street foods were collected from Gangtok and 112 samples of street foods were collected from Nainital and were analyzed to determine the microbial load expressed in log cfu/g and pH, respectively (Tables 3 and 4). LAB was detected up to the level of 10⁴–10⁵ cfu/g. The load of *Bacillus* was greater than 10⁶ 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*, *Lactobacillus brevis*, and *Enterococcus faecium*. Based on taxonomical keys of Slepecky and Hemphill [11], spore forming rod-shaped bacteria from street foods were identified as *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, and *Bacillus cereus*. The predominance of *Bacillus* spp. was possibly due to the presence of spores in the raw materials which may have survived cooking [24]. High loads of species of *Bacillus* up to 10⁵ cfu/g in street foods of Gangtok was similar to that reported from street foods (*panipuri*, *dahi bara*, and *chaat*) of Baripada town in Odisha state of India [16]. We found the prevalence of *S. aureus* in *kachori* of Gangtok and Nainital was 19.5% and 33%, respectively.

*Puchkka* of Tadong Bazaar of Gangtok had the highest enterobacteriaceae population of 10⁴ log cfu/g followed by *samosa* of Lal Bazaar of Gangtok at 10³ log cfu/g (data not shown), which is above the acceptable levels and may prove to be hazardous to human health [25]. Similarly, some street foods of Nainital were recorded at the level of 10⁴ log cfu/g, which is also above the acceptable level [25]. The dominant pathogen during the monsoon season was *E. coli*, which may have been due to the use of affected water [14]. Food-borne bacterial pathogens commonly detected in street-vended foods are *B. cereus*, *Clostridium perfringens*, *S. aureus*, and *Salmonella* spp. [24,26–29]. *Escherichia coli* frequently contaminates food items and is often considered a good indicator of fecal pollution [30]. Presence of *Enterobacter* spp. was reported in street foods of Malaysia [31], *Escherichia coli*, *Salmonella* spp., and *S. aureus* were reported in some common street foods such as *pakoda*, *kachori*, and *samosa* of India [14]. More than 10⁵ cfu/g of *B. cereus* is considered unsatisfactory and consumption of foods with greater than or equal to 10⁵ cfu/g may even lead to food–borne illness [32]. In our findings street foods such as *samosa*, *kachori*, *puchkka*, *vegetable momo*, *pork momo*, *alu-cheura*, and *suya-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 [32].
Similarly, in food samples from Nainital only *kachori* was recorded at 10^8 cfu/g, which is at the marginal level of acceptable limits. There was low contaminant bacteria count in vegetable *chowmein* from both Gangtok and Nainital. This may be due to the use of vaccine during preparation, as similar observation was reported [33]. The presence of which can be accounted due to unhygienic working conditions, cross-contamination, or even might have resulted from the water samples used during the preparation processes [34]. Food items were generally prepared much before the time of selling and stored at room temperature, which might have provided a suitable environment for the multiplication of these pathogens [35]. Occurrence of high bacterial loads in street foods was even supported by earlier findings [35–37].

Yeast isolated from street foods of Gangtok and Nainital were identified as *Saccharomyces cerevisiae* and *Pichia burtonii*, based on taxonomical keys of Kurtzman et al [38]. Molds were not recovered in any sample examined. The lesser levels of yeast and the total absence of mold in all food samples tested could be due to the time and temperature exposure for the steaming/frying/cooking processes. The pH in street foods of Gangtok and Nainital was 5–6 except for *jhal-muri* and *sya-faley* of Gangtok which had a pH of 4.8 (Tables 3 and 4).

Only *E. coli* GS:E1 and *S. aureus* strains GS:S1, GK1:S1, GM1:S1, GB5:S1, and GB52:S1 isolated from street foods of Gangtok grew well in 10% NaCl. Similarly, *E. coli* NS:E1 and NJ1:E1 and *S. aureus* NK:S1 and NM2:S1 isolated from street foods of Nainital tolerated 10% NaCl (data not shown). This could be related to the similar findings on the study conducted on *süfua*—a Chinese fermented product where most of the enterobacteriaceae did not tolerate elevated salt levels but a considerable salt tolerance activity was recorded in *S. aureus* [12].

Strains of *S. aureus* isolated from street foods of Gangtok and Nainital showed hemolytic and coagulase activities, whereas strains of *S. epidermidis* did not show any hemolytic and coagulase activities. This could be related to the fact that *S. aureus* show positive results for hemolytic and coagulase activities, while other strains of *Staphylococcus* show negative results [39]. The level of coagulase-positive *S. aureus* is considered potentially hazardous at greater than or equal to 10^4 cfu/g, and may even lead to food-borne illnesses [33]. MPN count showed that water samples of Lal Bazaar were the most contaminated water samples of Gangtok. Similarly, water samples collected from Malhi Tall of Nainital were the most contaminated water samples. All strains tested for MPN were identified as *E. coli*.

*Escherichia 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. *Bacillus cereus* diarrheal 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 diarrhoea food poisoning [40]. Some strains of *B. cereus* may cause food poisoning with an infective dose as low as 10^3–10^6 cfu/g [41]. Some strains of *S. aureus* produce heat-stable staphylococcal enterotoxins [17]. The enterotoxin production level required for strains of *S. aureus* is greater than 10^8 cfu/g [42]. No staphylococcal enterotoxins were detected in the samples studied; this could be possibly related to some earlier studies with *S. aureus* in tempe (fermented soybean food of Indonesia) fermentation [43]. In the samples of *nham—a*
Thai fermented pork product—though samples tested positive for S. aureus, no enterotoxins were recovered [44]. The risks associated with the street foods can further be controlled and made safer if the following factors are considered. Firstly, there needs to be consumer awareness regarding the freshness, quality, and hygienic environment of the street foods. Secondly, by educating vendors about hygienic conditions, 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, if possible risk factors associated with ethnic street foods can be controlled, ethnic street foods will be totally safe for consumption and will also promote the ethnic foods in India.

Conflicts of interest

All authors have no conflicts of interest to declare.

References