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Microbiological Quality of Street-Vended Indian Chaats Sold in Bangalore

Arijit Das, G.S. Nagananda, Sourav Bhattacharya and Shilpi Bhardwaj
Department of Microbiology, Genohelix, Centre for Excellence in Biotechnology, Jain University, 127/3, Bull Temple Road, Chamarajpet, Bangalore-560019, Karnataka, India

Abstract: Microbial contamination of ready-to-eat foods and beverages sold by street vendors and hawkers has become a global health problem. Street vended chaats like panipuri and bhelpuori sold in almost all the cities throughout India are consumed by huge population of people. The present study was undertaken to investigate the microbiological quality of street foods like panipuri and bhelpuori sold in many parts of Bangalore, India. Eight different samples of these chaats were aseptically collected from four locations of Bangalore City. The samples were analyzed within an hour of procurement. Isolation, enumeration and identification of the prevalent bacteria and fungi were carried out following the standard procedures. Analysis of the food samples revealed high loads of bacterial pathogens such as Streptococcus faecalis, Escherichia coli, Staphylococcus aureus, Bacillus sp., Klebsiella sp. and Pseudomonas sp. Total viable counts of bacteria in all the samples varied between 0.4-3.0×10⁶ cfu g⁻¹, faecal coliforms between 0.03-0.14×10⁶ cfu g⁻¹ and faecal streptococci between 0.2-11×10⁴ cfu g⁻¹. Aciduric yeasts like Saccharomyces and filamentous fungi like Mucor and Rhizopus were also encountered. Salmonella and Vibrio cholerae were not detected in any of the samples. The presence of faecal streptococci and coliforms indicated faecal contamination of the processing water as well as the prevailing unhygienic conditions related to the location of the food stalls. It is suggested that regular monitoring of the quality of street foods must be practised to avoid any food-borne pathogenic outbreaks in future.

Key words: Bhelpuori, contamination, Indian chaats, panipuri, street foods

INTRODUCTION

Street foods have been reported to be contaminated with pathogens and have also been implicated in food-borne epidemics. It was found that in most localities, the street foods remain hygienically poor since bacterial loads are moderately high. Street foods like panipuri, bhelpuori and chaats are cheap, readily available and appeal to the taste of common people in India, though they may be deficient in nutritive value. Selection of street foods is based on taste, price and last on nutritional quality (Bhat and Waghray, 2000).

Raw foods, especially ready-to-eat vegetable salads, sprouts and cut fruits have been implicated in outbreaks of food borne diseases in both developed and developing countries (Kumar et al., 2006). In countries, where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian and African countries have revealed increased bacterial pathogens in the food. There have been documented outbreaks of illnesses in humans associated with the consumption of street-vended foods (Mahale et al., 2008).

In India, chaats are sold at all public places and roadside shops. However, their consumption, quick method of cleaning and handling, could often prove to be a public health threat. There are reports of food borne illnesses associated with the consumption of unhygienic foods at several places in India. Hazards and critical control points (HACCP) conducted for a selected bhelpuori vendor from urban Vadodara that involved microbial analysis of 8 ingredients of bhelpuori and 7 samples indicative of personal hygiene and environmental sanitation showed the presence of E. coli in almost all the samples and Salmonella and Shigella in knife, hand rinse, dishwater and sevpuri samples (Sheth et al., 2005). Bacteria like Salmonella sp. Shigella sp. Campylobacter sp. and E. coli can contaminate the food through contact with sewage and contaminated water (Fredlund et al., 1987; Blotstein, 1993; Bouchut, 1996; Gayler et al., 1955). Thus, the hazards and critical control points identified were high initial contamination of raw foods, poor personal hygiene and environmental sanitation.

Corresponding Author: Arijit Das, Department of Microbiology, Genohelix, Centre for Excellence in Biotechnology, Jain University, 127/3, Bull Temple Road, Chamarajpet, Bangalore-560019, India Td: +919886919207
cross-contamination between raw and cooked foods, holding of foods at ambient temperature and poor cleaning practices for stall and utensils (Sheth et al., 2005).

The consumption of these roadside foods potentially increases the risk of food borne diseases caused by a wide variety of pathogens. There are different sources of microbial invasion of street-vendor foods. Pathogens may invade the interior surfaces of the food during peeling, slicing, handling, trimming and other processes like packaging, storing and marketing (Burro et al., 2007).

Vendors-sold foods usually make use of simple facilities like wheel barrows, trays, mats, tables and make-shift stalls, thus further increasing the risk of food contamination. Contamination from raw materials and equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens (Mahale et al., 2008). The present study was aimed at examining the microbiological quality and safety of street foods like panipuri and bhel puri sold at different parts of Bangalore city in India.

MATERIALS AND METHODS

The present study was conducted during the period from 27.10.2009 to 11.01.2010 at Genohelix, Centre for Excellence in Biotechnology, Jain University, Chamarajanagar, Bangalore, Karnataka, India.

All the media used during the course of the study were obtained from Himedia Laboratories Pvt. Limited (A- 406, Bhaveshwar Plaza, Mumbai-400086, India).

Collection of samples: Four locations in Bangalore city, India, were chosen for the collection of samples. Samples of panipuri and bhel puri were picked up from at least two shops in each zone where the sale was maximum per day. All the samples were aseptically collected in sterile containers, stored at 4°C and analyzed within an hour of procurement. Samples were removed aseptically for pH measurement using pH meter.

Isolation and enumeration of microorganisms: Isolation and enumeration of microbes were performed using serial dilution and spread plate technique. One gram of the street food sample was properly homogenized using a sterile mortar and pestle. One milliliter of the resultant homogenate was added to 9 mL of sterile 0.85% saline in a test tube and diluted serially to obtain dilutions upto $10^{-3}$. For bacterial isolation 0.1 mL of the appropriate dilution from each tube was aseptically pipetted out and plated onto different selective and differential media (Tryptone Glucose Yeast Extract agar, MacConkey agar, Deoxycholate Citrate agar, Salmonella Shigella agar, Thiouphate Citrate Bile Sucrose agar, Eosin Methylene Blue agar, Ceftriaxone agar, Baird Parker’s agar, Blood agar and Hichrome UTI agar) using the spread plate technique. All the bacterial plates were incubated in an inverted position under aerobic conditions at 37°C for 24 to 48 h. The fungal isolation was done on Potato Dextrose agar and Sabouraud Dextrose agar. The plates were incubated at 27°C for 3 to 5 days. For bacterial enumeration the plates were used to determine the number of colony forming units (cfu) per gram of food sample.

Identification and characterization of microbial isolates: Following incubation, the isolated colonies were pure cultured and Gram stained. Biochemical characterization of the isolated colonies was carried out using standard protocols (Karnan, 2002). Identification was carried out according to Bergey’s Manual. Identification of the fungal isolates was performed by lactophenol cotton blue staining and observation of macroscopic and microscopic characteristics.

RESULTS

A total of eight samples of panipuri and bhel puri were examined in this study. The samples collected aseptically in sterile containers at a temperature of 32°C, showed pH varying between 3.4 and 4.0. Among the samples tested, majority of them revealed pathogenic contamination with faecal coliforms. Bacterial enumeration revealed a high count of faecal coliforms and faecal streptococci in all the tested samples indicating poor bacteriological quality of the chauts. The significant results of bacterial enumeration have been presented in Table 1. Total viable counts of bacteria in all the samples

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>TVC (× 10^3 cfu g^-1)</th>
<th>E. coli</th>
<th>Streptococcus faecalis</th>
<th>S. aureus</th>
<th>Bacillus sp.</th>
<th>Klebsiella sp.</th>
<th>Pseudomonas sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.20</td>
<td>0.20</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>1.78</td>
<td>0.13</td>
<td>8.50</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>0.07</td>
<td>9.78</td>
<td>0.80</td>
<td>0.90</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>0.14</td>
<td>11.00</td>
<td>0.67</td>
<td>0.02</td>
<td>0.03</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>2.9</td>
<td>0.11</td>
<td>10.80</td>
<td>0.20</td>
<td>0.50</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>1.2</td>
<td>0.03</td>
<td>8.02</td>
<td>0.05</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>2.8</td>
<td>0.09</td>
<td>10.60</td>
<td>0.80</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>1.4</td>
<td>0.10</td>
<td>8.10</td>
<td>0.03</td>
<td>0.02</td>
<td>0.00</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*: Total viable counts
Fig. 1: Bacterial colonies on Tryptone Glucose Yeast Extract agar

Fig. 2: Bacterial colonies on Hichrome UTI agar; E, E. coli; K, Klebsiella sp.; S, Streptococcus faecalis

varied between 0.4-3×10⁴ cfu g⁻¹, faecal coliforms between 0.03-0.14×10⁴ cfu g⁻¹ and faecal streptococci between 0.2-11×10⁴ cfu g⁻¹. Total viable counts were enumerated on Tryptone Glucose Yeast Extract agar as shown in Fig. 1.

Based on the growth on selective and differential media and biochemical tests, various bacterial isolates were identified as Escherichia coli, Klebsiella sp. and Streptococcus faecalis. Figure 2 represents the selective isolation of Escherichia coli, Klebsiella sp. and Streptococcus faecalis on Hichrome UTI agar as identified by the differential pigmentation produced by the colonies. The morphology of the faecal streptococci was studied by Gram’s staining as depicted in Fig. 3. Coagulase-positive Staphylococcus aureus and

Fig. 3: Y, yeasts; S, Streptococcus faecalis

Fig. 4: Staphylococcus sp.

Bacillus sp. were also isolated from different samples of the street-foods as revealed by the microscopic observations of the Gram stained preparations in Fig. 4 and 5, respectively. Detection of Pseudomonas sp. in the food samples also implied the insanitary quality of these street-vended foods. Salmonella and Vibrio cholerae were not encountered in any of the samples.

Colonies of acidophilic yeasts were isolated on Sabouraud Dextrose agar as depicted in Fig. 6. Based upon macroscopic and microscopic characteristics, yeast isolates obtained from the street-vended chaats were identified as Saccharomyces sp. (Fig. 6, 7). Among the filamentous fungal forms isolated on Potato Dextrose agar, Mucor sp. and Rhizopus sp. were identified as shown in Fig. 8 and 9, respectively.
**DISCUSSION**

Different opportunistic and obligate bacterial pathogens and aciduric yeasts and moulds were isolated from panipuri and bhelpuri sold by street vendors. Low pH and high temperature (above 28°C) favoured the growth of facultative acidophiles and neutrophiles, thus reducing the shelf life of street foods. In the present investigation, all the samples showed occurrence of high bacterial loads consisting of faecal coliforms and faecal streptococci. Bacterial enumeration revealed a total viable count of $0.4-3.0 \times 10^9$ cfu g$^{-1}$. The presence of these microbes in food can be linked to a number of factors such as improper handling and processing, use of
contaminated water during washing and dilution, cross contamination from rotten fruits and vegetables, or the use of dirty processing utensils like knife and trays (Bryan et al., 1992a; Khalil et al., 1994). This might also implicate the processing and rinsing water as possible sources of contamination of panipuri sold by street vendors (Nwachukwu et al., 2008).

Among the different bacterial pathogens isolated in the present study E. coli and Streptococcus faecalis showed the highest counts of 0.03–0.14×10^6 cfu g^-1 and 0.2–11×10^6 cfu g^-1, respectively, followed by Staphylococcus aureus and Bacillus sp. (Table 1). The high frequency of isolation of E. coli (Fig. 1, 2) and Streptococcus faecalis (Fig. 2, 3), which are faecal and non faecal indicators of water pollution respectively, indicated faecal contamination of the processing water resulting in poor bacteriological quality of the chaats. Our findings are in perfect correlation with the previous reports of high incidences of total faecal coliform counts and coagulase positive S. aureus encountered in street-vended fruit chaats in Patiala city (Kumar et al., 2006). Previous investigations performed by Mahale and coworkers also reported the isolation of E. coli and coagulase positive S. aureus from street-vended fruit juices from Mumbai City, India (Mahale et al., 2008).

The results of the present study are in agreement with those reported by Sheth and coworkers (Sheth et al., 2005) which revealed the presence of high Aerobic Mesophilic Colony Count (AMCC) and Staphylococcus aureus counts along with the presence of Escherichia coli in bhel puri samples from urban Vadodara. The presence of coagulase-positive Staphylococcus aureus in the chaats (Fig. 4) with a count of 0.01–0.8×10^6 cfu g^-1 might be explained by the fact that it forms the normal microflora present on/in several parts of the human body (Nester et al., 2001). This can be introduced into the street foods during handling, processing or vending.

The isolation of Bacillus sp. from panipuri and bhel puri samples (Fig. 5) implicated the ubiquitous nature of bacterial spores especially in dusty roadside locations. The mesophilic spore-formers might have been introduced into the prepared food due to the use of contaminated puffed-rice used in preparation of bhel puri. The prevalence of these mesophilic bacilli, showing a count of 0.01–0.9×10^6 cfu g^-1 in the street-vended foods, could also be explained by the fact that the ingredients such as wheat and rice flour used in preparation of these Indian chaats generally contain spores of Bacillus (Frazier and Westhoff, 2005). In general, the presence of mesophilic spore-formers Bacillus cereus in food is of great significance since this organism produces heat-sensitive (diarrheal) and heat-stable (emetic) toxins associated with food poisoning (Bryan et al., 1992b). Similar findings by Hanashiro suggested that 35% of the selected street food samples from a restricted area of São Paulo city, Brazil were considered unsuitable for consumption due to higher load of B. cereus (Hanashiro et al., 2005). Staphylococcus and Bacillus normally exhibit tolerance to a wide range of temperature and pH, which justifies the presence of these bacteria even at highly acidic conditions.

The presence of respiratory pathogen such as Klebsiella in panipuri water might be attributed to the bacterial aerosols generated due to sneezing and coughing in public places. Handling of soiled notes and currencies by the street-food vendors might also act as vector for transmission of Pseudomonas into the panipuri water.

Many a times the street foods are sold by unlicensed vendors with poor education level and untrained in food hygiene (Muinde and Kuria, 2005; Barro et al., 2006). Cross-contamination of street foods is also increased by unsanitary processing and preservation. The use of dirty utensils, as well as the open display of street foods encourages visits by flies, cockroaches, rodents and dust (Bryan et al., 1992b). Preservation of prepared foods that requires no further processing before consumption, at ambient temperatures during retail, maintenance of the food at optimum temperatures, allow the invasion by pathogenic mesophiles (Muinde and Kuria, 2005).

The organisms isolated might cause diseases that vary in severity from mild gastroenteritis to severe and sometimes chronic or opportunistic infections including food poisoning. Overall, the results of this study indicated that street foods like panipuri and bhel puri sold in many parts of Bangalore city showed contamination with faecal coliforms and faecal streptococci. One major source of contamination of foods sold by street vendors is the washing and processing water (Khalil et al., 1994). It is contended that contamination is mainly due to poor quality of water used for dilution as well as prevailing unhygienic condition related to improper washing of fruits, vegetables and utensils, inadequate storage of these at ambient temperatures in unhygienic places, maintenance of premises and personal hygiene by vendors.

In addition, the presence of surface microflora of fruits and vegetables including yeasts and moulds, use of unhygienic dusty surroundings, often swarming with flies and fruit flies, other insects and airborne dust, mixing of rotten portions with fresh stock and serving the prepared foods in filthy covers can also act as potential sources of contamination. Sweet chutney (syrup) used in the preparation of bhel puri and panipuri might act as a chief source of osmophilic yeasts such as Saccharomyces.
(Fig. 3, 6 and 7) and certain molds such as *Mucor* (Fig. 8) and *Rhizopus* (Fig. 9). Similar observations have been reported by Frazier and Westhoff (2005). The prevalence of various mold forms in Indian chaats could also be attributed to the practice of mixing inadequately washed grated fruits and vegetables as important ingredients in these foods.

The location of street-vended food stalls by the side of a dusty road with heavy vehicular traffic (airborne particles) and overcrowding (bus station and market) seems to add contamination. Such locations should be avoided for establishing the food stalls. In order to minimize the contamination level of foods, better hygiene is necessary. However, proper sanitary conditions must also be practised by the food vendors. Regular monitoring of the conditions of street-foods and better surveillance on the activities of street-food vendors must be introduced to minimize the risk of disease outbreaks associated with the consumption of street-foods like panipuri and bhelpuri.

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