



# Journal of Biological Sciences

ISSN 1727-3048

**science**  
alert

**ANSI***net*  
an open access publisher  
<http://ansinet.com>

## 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, Chamaraipet, 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 bhelpuri 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 bhelpuri 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  $0.4-3.0 \times 10^4$  cfu g<sup>-1</sup>, faecal coliforms between  $0.03-0.14 \times 10^4$  cfu g<sup>-1</sup> and faecal streptococci between  $0.2-11 \times 10^4$  cfu g<sup>-1</sup>. 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:** Bhelpuri, 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, bhelpuri 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 bhelpuri vendor from urban Vadodara that involved microbial analysis of 8 ingredients of bhelpuri 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; Blostein, 1993; Beuchat, 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,

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-vended foods. Pathogens may invade the interior surfaces of the food during peeling, slicing, handling, trimming and other processes like packaging, storing and marketing (Barro *et al.*, 2007).

Vendors-sold foods usually make use of simple facilities like wheel barrows, trays, mats, tables and makeshift 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 bhelpuri 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, Chamarajpet, 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 bhelpuri 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  $10^{-5}$ . 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, Thiosulphate Citrate Bile Sucrose agar, Eosin Methylene Blue agar, Cetrinide 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 (Kannan, 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 bhelpuri 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 chaats. The significant results of bacterial enumeration have been presented in Table 1. Total viable counts of bacteria in all the samples

**Table 1: Enumeration of pathogenic bacteria ( $\times 10^4$  cfu  $g^{-1}$ ) encountered in street-vended panipuri and bhelpuri in Bangalore city, India**

Sl. No.	TVC <sup>a</sup>	<i>E. coli</i>	<i>Streptococcus faecalis</i>	<i>S. aureus</i>	<i>Bacillus</i> sp.	<i>Klebsiella</i> sp.	<i>Pseudomonas</i> sp.
1	0.4	0.20	0.20	0.05	0.08	0.00	0.01
2	1.78	0.13	8.50	0.01	0.01	0.01	0.05
3	2.1	0.07	9.78	0.80	0.90	0.07	0.00
4	3.0	0.14	11.00	0.67	0.02	0.03	0.60
5	2.9	0.11	10.80	0.20	0.50	0.09	0.30
6	1.5	0.03	8.02	0.05	0.05	0.00	0.00
7	2.8	0.09	10.60	0.80	0.04	0.01	0.03
8	1.4	0.10	8.10	0.03	0.02	0.00	0.03

<sup>a</sup>: Total viable counts

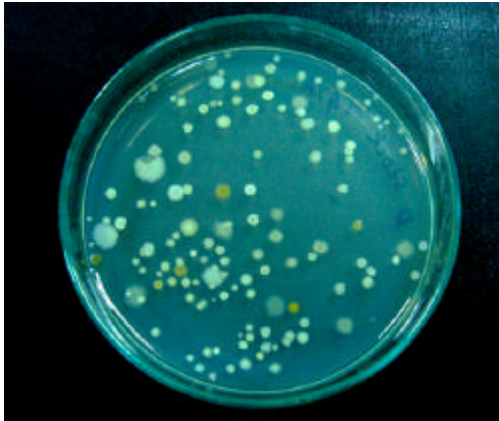


Fig. 1: Bacterial colonies on Tryptone Glucose Yeast Extract agar

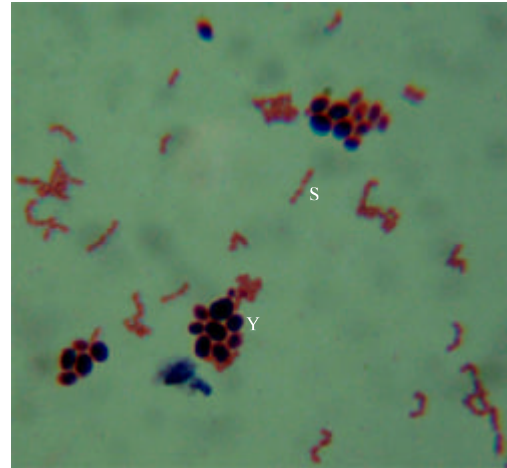


Fig. 3: Y, yeasts; S, *Streptococcus faecalis*

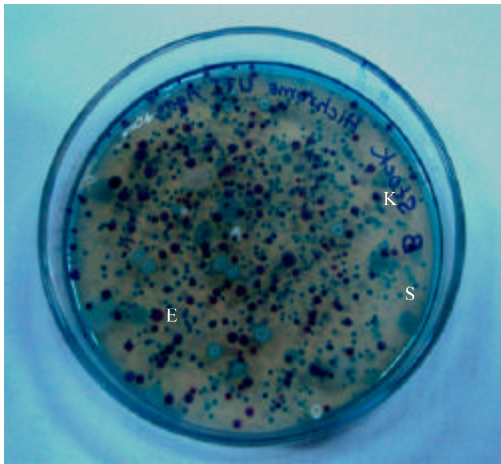


Fig. 2: Bacterial colonies on Hichrome UTI agar; E, *E. coli*; K, *Klebsiella* sp.; S, *Streptococcus faecalis*

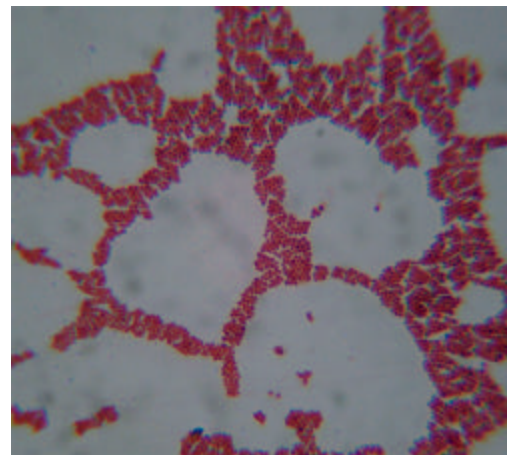


Fig. 4: *Staphylococcus* sp.

varied between  $0.43 \times 10^4$  cfu  $g^{-1}$ , faecal coliforms between  $0.03-0.14 \times 10^4$  cfu  $g^{-1}$  and faecal streptococci between  $0.2-11 \times 10^4$  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

*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.

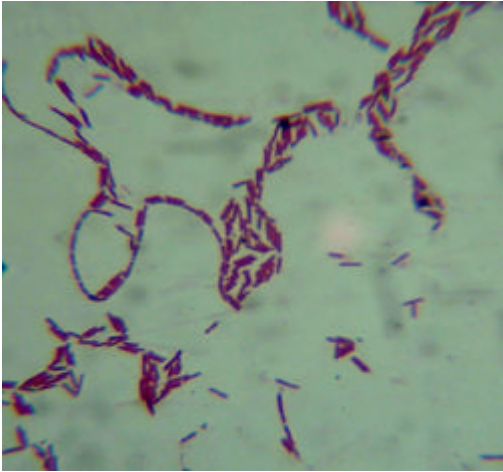


Fig. 5: Gram positive bacilli arranged in chains

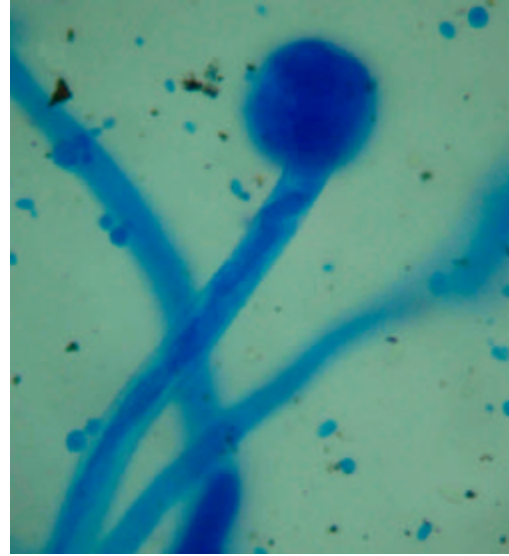


Fig. 8: *Mucor* sp.

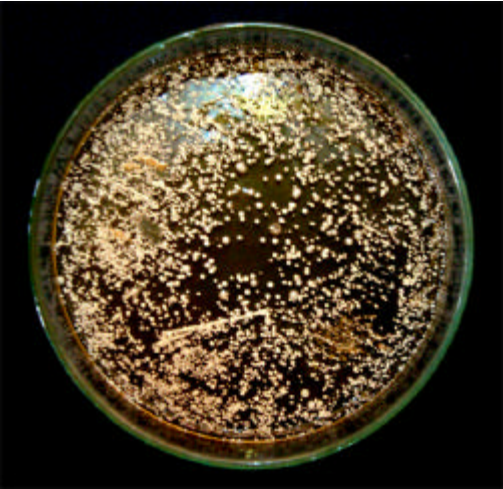


Fig. 6: Colonies of yeasts on Sabouraud dextrose agar

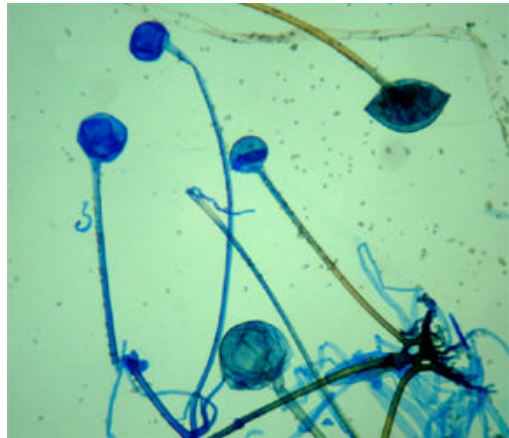


Fig. 9: *Rhizopus* sp.

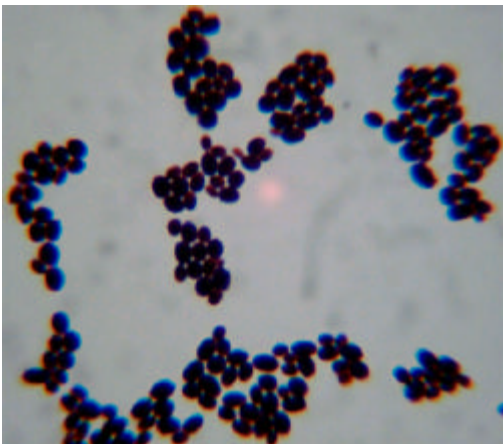


Fig. 7: Yeasts

## 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  $0.4-3.0 \times 10^4$  cfu g<sup>-1</sup>. 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  $0.03-0.14 \times 10^4$  cfu g<sup>-1</sup> and  $0.2-11 \times 10^4$  cfu g<sup>-1</sup>, 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 bhelpuri samples from urban Vadodara. The presence of coagulase-positive *Staphylococcus aureus* in the chaats (Fig. 4) with a count of  $0.01-0.8 \times 10^4$  cfu g<sup>-1</sup> 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 bhelpuri samples (Fig. 5) implicated the ubiquitous nature of bacterial spores especially in dusty road side 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 bhelpuri. The prevalence of these mesophilic bacilli, showing a count of  $0.01-0.9 \times 10^4$  cfu g<sup>-1</sup> 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 bhelpuri 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 bhelpuri 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 (air borne 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.

#### ACKNOWLEDGMENTS

Authors wish to extend their sincere gratitude to Dr. Chenraj Jain, Chairman, Jain Group of Institutions, Bangalore, for providing them with the financial and laboratory facilities required for this research work. Authors also wish to thank all the faculties and the entire supporting staff of the laboratory whose help has been invaluable for the successful completion of the research work.

#### REFERENCES

- Barro, N., A.R. Bello, A. Savadogo, C.A.T. Ouattara, A.J. Ilboudo and A.S. Traore, 2006. Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *Afr. J. Biotechnol.*, 5: 1107-1112.
- Barro, N., A.R. Bello, Y. Itsimbou, A. Savadogo and C.A.T. Ouattara *et al.*, 2007. Street-vended foods improvement: Contamination mechanisms and application of food safety objective strategy: Critical review. *Pak. J. Nutr.*, 6: 01-10.
- Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.*, 59: 204-216.
- Bhat, R.V. and K. Waghay, 2000. Profile of street foods sold in Asian countries. *World Rev. Nutr. Diet.*, 86: 53-99.
- Blostein, J., 1993. An outbreak of *Salmonella javiana* associated with consumption of water melon. *J. Environ. Health*, 56: 29-31.
- Bryan, F.L., P. Teufel, S. Riaz, S. Roohi, F. Qadar and Z.U.R. Malik, 1992a. Hazards and critical control points of street- vended chat, a regionally popular food in Pakistan. *J. Food Prot.*, 55: 708-713.
- Bryan, F.L., P. Teufel, S. Riaz, S. Roohi, F. Qadar and Z.U.R. Malik, 1992b. Hazards and critical control points of vending operations at a railway station and a bus station in Pakistan. *J. Food Prot.*, 55: 534-541.
- Frazier, W.C. and D.C. Westhoff, 2005. *Food Microbiology*. Tata McGraw-Hill Publishing Co. Ltd., New Delhi, ISBN: 0-07-462101-7, pp: 173-185.
- Fredlund, H., E. Back, L. Sjoberg and E. Tornquist, 1987. Water-melon as a vehicle of transmission of shigellosis. *Scandinavian J. Infec. Dis.*, 19: 219-221.
- Gayler, G.E., R.A. MacCready, J.P. Reardon and B.F. McKernan, 1955. An outbreak of salmonellosis traced to watermelon. *Public Health Rep.*, 70: 311-313.
- Hanashiro, A., M. Morita, G.R. Matté, M.H. Matté and E.A.F.S. Torres, 2005. Microbiological quality of selected street foods from a restricted area of São Paulo city, Brazil. *Food Control*, 16: 439-444.
- Kannan, N., 2002. *Laboratory Manual in General Microbiology*. Panima Publishing Corporation, New Delhi, ISBN: 81-86535-40-5, pp: 117-119.
- Khalil, K., G.B. Lindblom, K. Mazhar and B. Kaijser, 1994. Flies and water as reservoirs for bacterial enteropathogens in urban and rural areas in and around Lahore, Pakistan. *Epidemiol. Infect.*, 113: 435-444.
- Kumar, M., D. Agarwal, M. Ghosh and A. Ganguli, 2006. Microbiological safety of street vended fruit chats in Patiala city. *Indian J. Med. Microbiol.*, 24: 75-76.
- Mahale, D.P., R.G. Khade and V.K. Vaidya, 2008. Microbiological Analysis of Street Vended Fruit Juices from Mumbai City, India. *Internet J. Food Safety*, 10: 31-34.
- Muinde, O.K. and E. Kuria, 2005. Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *Afr. J. Food Agric. Nutr. Dev.*, 5: 1-15.
- Nester, E.W., D.G. Anderson, C.E. Roberts, N.N. Pearsall and M.T. Nester, 2001. *Microbiology: A Human Perspective*. 3rd Edn., McGraw-Hill, New York, ISBN: 0072318783, pp: 815-816.
- Nwachukwu, E., C.F. Ezeama and B.N. Ezeanya, 2008. Microbiology of polyethylene-packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. *Afr. J. Microbiol. Res.*, 2: 192-195.
- Sheth, M., R. Gurudasani and R. Mudbidri, 2005. Screening for pathogenic microorganisms in street-vended Bhelpuri in urban Vadodara: A HACCP approach. *J. Food Sci. Technol.*, 42: 395-399.