Microbiological Analysis of Street Vended Food in West Delhi

KEYWORDS

Street food, Microbial analysis, E.coli, Food hygiene

INTRODUCTION

During the last few decades, the street food sector has expanded rapidly in urban areas of low and middle-income groups, both in terms of providing access to a diversity of inexpensive foods for low-income households [1] and in offering job opportunities for many urban residents. The street food sector also contributes to the economy of an urban and pre-urban agricultural sector [2]. In India, the National Policy for Urban Street Vendors/ Hawkers stated that street vendors constitute approximately 2% of the population of a metropolis [3].

“Street foods are ready-to-eat foods and beverages, prepared and sold by vendors and hawkers on the streets, other public places, including tourist sites” [4]. Due to its low cost and convenience, an estimated 2.5 billion people worldwide consume street food each day. A study in Kolkata, India, found that an average street meal contain about 30 grams of protein, 15 grams of fat and 180 grams of carbohydrates [5].

With this positive development, there are also some public health challenges for the urban population [1] because street foods are readily contaminated from different sources and it will increase the risk of food borne diseases [6]. World Health Organization [7] stated that millions of people fall sick or die because of eating unsafe food. Food safety has emerged as an important global issue with international trade and public health implications. Food safety is “assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use” [8]. In India, the period of 1984-89, there were 721 food-borne outbreaks and 1,199 sporadic cases of food-borne disease recorded in the cities of Hyderabad and Secunderabad alone [9].

Further, WHO reports that 20% of deaths among children under five are caused by diarrheal disease [10] and UNICEF estimates that about 1,000 children below the age of five die every day in India, due to diarrhea [11]. In a national study, 37% of adults and 42% of children reported consuming fast food on one or both days of the survey [12]. Food borne bacterial pathogens commonly detected in street vended foods are Bacillus cereus causes vomiting and diarrheaa, Clostridium perfringens causes abdominal cramps and diarrheaa, Staphylococcus aureus causes vomiting, diarrheaa, loss of appetite, severe abdominal cramps and mild fever and Salmonella species causes typhoid, food poisoning and irritation and inflammation in the gastrointestinal tract [13]. Studies by [14] [15] [16] [17] have revealed frequent contamination of street food in different parts of India.

The present study was therefore undertaken to evaluate the microbial quality and safety of consumption of different street foods sold in West Delhi Markets.

MATERIALS AND METHODS

The present study was conducted at Institute of Hotel Management, Catering & Nutrition, Pusa, New Delhi, India. All media and kits that were used during this study were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Sample collection

The present study was conducted to analyze and observe the microbial load in street foods in New Delhi and the food safety aspects. It was carried with the consumer survey in which 100 subjects were taken regarding their commonly consumed street food. Five locations in West Delhi were chosen for the collection of samples, where the sale was maximum per day. Food samples (samosa, gol-gappa, burger, vegetarian momos and non-vegetarian momos) were collected twice from local street shops and franchisee's outlets at one month interval. All the samples were aseptically collected in sterile containers, stored at 4°C and analyzed within an hour of procurement.
Isolation and enumeration of microorganisms
All the samples were aseptically collected in sterile containers, stored at 4°C. Ten gram of sample was weighted under aseptic condition and properly homogenized by using a sterile pestle and mortar. Ten gram of homogenated sample was added to 90 ml of sterile 0.85% saline water in a test tube and diluted serially up to $10^{-5}$ dilution was obtained.

For bacterial isolation 0.1 ml of dilution from each tube was aseptically pipette out and plated onto different nutrient agar media using spread plate technique. The plating was done in the laminar flow to maintain aseptic conditions. All the plates were placed in an incubator at 37°C for 24 to 48 h in an inverted position. For bacterial enumeration the plates were used to determine the number of colony forming units (CFU) per gram of food sample.

Test for Coliform bacteria detection in food samples:
Coliform detection in food sample was enumerated by the Most Probable Number (MPN) method. Three serial dilutions (0.1, 1.0 and 10 ml) were inoculated in Lactose broth in test tubes and then incubated at 37°C for 24 to 48 h. Positive tubes (acid and gas production) were observed and plated by using spread plate technique on molten Violet Red Bile agar. E. coli culture was taken as a positive control. After solidification, incubate the plates at 37°C for 24 to 48 h. The coliform colonies were counted in sample plates. The coliform (CFU/gram) was determined by considering the countable plates by using standard protocols [18].

Test for Coliform detection in water sample:
Three serial dilutions (0.1, 1.0 and 10 ml) were inoculated in Lactose broth and incubated the tubes at 37°C for 24 to 48 h. Positive tubes (acid and gas production) were streaked on Eosin Methylene Blue Agar plates and incubated at 37°C for 24 to 48 h. Appearance of typical E. coli colonies with dark centre and metallic sheen indicated positive confirmed test. For confirmation typical E. coli colonies were transferred to tube containing lactose broth for acid and gas production and nutrient agar slant for gram reaction and cell morphology [19]. Tubes and slants were incubated at 37°C for 24 to 48 h.

Determination for Salmonella species in food samples by using “KB011 HiSalmonella™” Identification Kit
KB011 is a comprehensive test system that can be used for identification of gram-negative Salmonella species.

A. Preparation of Homogenate Sample
The food samples (1 gram each) were homogenized in a mortar pestle. One ml of homogenated sample was added to 9 ml of sterile 0.85% saline water in a test tube and diluted serially up to $10^{-5}$ dilution was obtained.

B. Preparation of Inoculums
Single well-isolated and purified colony was picked up and inoculated in a homogeneous suspension made in 2-3 ml sterile saline. The density of the suspension was adjusted to 0.1 OD at 620 nm or 0.5 McFarland’s standards. All the inoculated samples were incubated at 35-37°C for 18-27 h.

C. Inoculation of the Kit
Each of the sample was inoculated with 50 μl of the inocula prepared.

For Methyl Red Test 1-2 drops of methyl red reagent was added.
For Voges Proskauer Test 2-3 drops of barritt’s reagent A and 1 drop of barritt’s reagent B were added.
For H₂S test, Triple Sugar Iron (TSI) agar was used to observe carbohydrate utilization pattern the medium contains 1% concentration of each of lactose and sucrose and 0.1% conc. of glucose. Acid base indicator was added to detect the production of acid during carbohydrate fermentation. The data was analyzed by using various statistical tests like Mean, Standard Error and Log-10.

RESULTS AND DISCUSSION
Hundred subjects were surveyed to find the commonly consumed street food, which were taken as the main samples for the microbial analysis. The hygienic level of the food handling and processing could be determine by using the Total Plate count analysis that is widely used. Post contamination could be used to know the bacterial count which reflect the amount of heating that goes into food production [20]. All vendors were observed working without any protective cover for food, the area of preparation was not cleaned, vending cart was not protected from sun, wind and dust and sale point was not free from animals, cooked food was not protected from sun and dust, dustbin was not covered and they did not wash their hands after serving or preparing the food of performing any work etc. Results depicted in Fig 1, 2, 3, 4 revealed that the selected food sample used for study, were contaminated with E. coli, coliform and Salmonella.

The presence of E. coli, Shigella dysenteriae, Streptococcus sp, Klebsiella, and Enterobacter represent fecal contamination. Some E. coli are harmless, however Enterotoxigenic E. coli (ETEC) is associated with traveler’s diarrhea. Similarly, Shigella dysenteriae have been associated with severe bacillary dysenteriae, while Streptococcus sp, have been frequently associated with acute sore throat [21]. Due to washing of vegetables with contaminated water, it gets contaminated with Salmonella spp. Also pathogenic microorganisms are scatter through vegetables handling by infected workers, vendors and consumers in the market place. HACCP study revealed that raw vegetables themselves carried pathogens and since they were not washed they continued to be present at the time of consumption. [22]. Rather, the vendors should be sensitized and also issued some certificates so that they could be trusted and allowed to operate their business.

![Fig.1 : Microbial load of local and branded burger](Image)

The result depicted in Fig 1 shows that the microbial load was more in branded burger then local burger because the bread of the local burger gets the heat treatment which reduce the microbial load.
In Salmonella species test methyl red, Voges Prokauer’s and citrate utilization tests were negative but the H₂S production test was positive in branded and local non-vegetarian momos.

The Salmonella is the major pathogen found in raw vegetables. Its presence can be detected by several factors such as improper handling and processing, use of contaminated water during washing, cross contamination from other rotten vegetables or the use of dirty cooking utensils [22].

CONCLUSION

Based on our findings, it was concluded that the proper facilities and training for food handling should be given to the vendors. Street foods are defined as ready-to-eat foods and beverages prepared and sold by vendors and hawkers especially in street and other similar public places. However, the unhygienic conditions in which these foods are prepared, stored and served raise a question regarding their hygiene status and microbiological quality. Past studies on street foods indicated that these foods are not meeting the microbiological standards and are contaminated with various pathogens, viz., E. coli, Vibrio, Salmonella etc. The present study was undertaken to investigate the hygiene and sanitation practices of street vendors and microbial quality of street food. The result showed that in local street foods, the initial microbial load in gol-gappa dilution was found to be $10^1 \pm 0.95$, which gradually reduced to $1.5 \pm 0.17$. No growth found in $10^2$ to $10^4$. In veg-momos, $10^1$ dilution the load was $130 \pm 2.1$, which was reduced to $34.5 \pm 1.5$ and in $10^3$ to $10^6$ no growth was observed. In non-veg momos, $10^2$ to $10^3$ the load was $325 \pm 2.5$ and $90 \pm 1.9$ but in $10^4$ to $10^6$ the load was lower than the permissible limit. In burger, the initial load in $10^1$ to $10^4$ was lower than the criteria limit, i.e. $13.5 \pm 1.13$ to $2.5 \pm 0.39$ and in $10^5, 10^6$ no growth was observed. In dilution, $10^1$ of samosa the initial load found to be $1 \pm 0$, which was lower than limit and in other dilutions no growth was found.

In franchise food products, the initial load in gol-gappa dilution $10^1$ was found to be $237.5 \pm 2.37$ and reduced to $8 \pm 0.90$. No growth was found in $10^5, 10^4$. In veg-momos, the dilution $10^1$ was $85 \pm 1.95$, which was reduced to $19 \pm 1.27, 15.5 \pm 1.19, 9.0 \pm 0.95$ and no growth was found.

It was found that the volume 10.0, 1.0 and 0.1 ml of both samples local gol-gappa and branded gol-gappa had colour change and gas was produced. It was concluded that in both the samples E. coli was present. It shows that in local burger, Coliform CFU/g was 0.5. In branded burger, veg momos, non-veg momos and local non-veg momos, the Coliform CFU/g value was 0. In local veg momos, Coliform CFU/g was 2. In local and branded samosa Coliform CFU/g value was 0.125. Positive coliform test in food sample indicate the fecal contamination and high risk of diarrhea disease.

In Salmonella detection test, it was found that the methyl red, Voges Prokauer’s and citrate utilization test were negative in all street food and franchises samples but only H₂S production test was positive in branded non-veg momos and local non-veg momos.

The preparation of food long before its consumption, storage at ambient temperature, inadequate cooling and reheating, contaminated processed food and undercooking were identified as the key factors in the food handling that contribute to food poisoning.
Therefore to ensure the food safety, producers and hawkers must maintain clean environment, minimized contact with the food sample after production and also maintain a maximum personal hygiene. Also utensils should be clean at all stages of food production and food storage time should be as less as possible.

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