



Original Research Article

Microbial profiling of street foods of different locations at Dehradun city, India

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ABSTRACT

Keywords

Personal hygiene,
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Improper personal hygiene can facilitate the transmission of the pathogenic bacteria found in environment and on people's hands via food to humans. The present study was undertaken to investigate the microbiological quality of different food in Dehradun, India. For the microbial screening of various food samples, Pour plate technique and biochemical characterisation had been performed. Results showed the presence of considerable number of contaminating microorganisms which lead to several food borne infections. Food hawkers in India are generally unaware of food regulations and have no training in food-related matters. They also lack supportive services such as water supply of adequate quality and disposal systems, which hamper their ability to provide safe food.

Introduction

Street foods are defined as ready to eat foods and beverages sold by vendors and hawkers especially in the streets and other similar places (FAO, 2000). Existence of Street foods is because of their easy availability, variety, prevailing socio economic conditions and also the influx of unorganized labour. Though the concept of street foods was unwelcome initially, Their existence depends on certain factors such as increasing labour force and consumer demand due to rapid industrialization (Chakravarthy, 1995).

Safety of street foods is always a matter of concern as in most cases they are prepared under unsanitary conditions by the vendors

who are illiterate and do not practice hygiene. The chances of contamination of these foods increase due to the poor environmental conditions in which the preparation is done and sold (Sheth, 2005). Street foods are the cause of several types of food - borne disease. The water used for drinking and cleaning purposes is often contaminated due to unhygienic storage and handling. Moreover use of artificial colours, like metanil yellow, has led to serious health hazards. Proper garbage removal facilities are also not available, thus leading to poor environmental condition (Chakravarthy, 2003).

Chutneys are prepared from available

seasonal fruits and vegetables, herbs, which are ground to a paste or to a pulpy mash; requisite consistency is obtained by addition of water, vinegar and lime or tamarind juice. The prevalence and growth of pathogens on the raw foods especially vegetables, salads, fruits and sprouts, are used as ingredients of chutneys (Viswanathan and Kaur, 2001). The microbiological qualities of chutney sold on the street in metropolitan cities are very poor (Kakar and Udipi, 2000). Almost all samples on street vended food possess pathogenic organisms like *Salmonella species*, *Shigella species*, *Campylobacter species*; *E. coli* can contaminate the food through contact with samples. Food borne illness caused by eating microbial contaminated food is an important public health problem.

Food is contaminated by various pathogenic microorganisms which cause food infection or food intoxication. Food poisoning can be the result of either chemical or the ingestion of toxicant. Bacterial food intoxication therefore refers to food borne illness caused by the presence of a bacterial toxic formed in the food (Mensah *et al.*, 1999). This may be an attempt to make aware common people regarding microbial contamination of street vended food and health hygienic.

Despite the economic and nutritional benefits of street foods, the consumption of these roadside foods has been suggested to potentially increase the risk of food borne diseases as street foods are readily contaminated from different sources (Tambekar *et al.*, 2008). In fact, street foods have often been associated with traveller's diarrhea and other food borne diseases. Studies have revealed the frequent contamination of street food in many developing world including Nigeria.

Food borne diseases are an increasingly

recognized problem involving a wide spectrum of illnesses caused by bacterial, viral, parasitic or chemical contamination of food. Although viruses account for half of all the food borne illnesses, most hospitalizations and deaths related to food borne infections are due to bacterial agents. Diarrheal diseases are the commonest symptoms of food poisoning and in some cases, can lead to death. The diseases are caused by either toxin from the "disease-causing" microbe, or by the human body's reactions to the microbe itself (Teplitski *et al.*, 2009).

The work examined the microbiological quality of different food types such as Aloo tikki, Momo, Chowmein and Chutney, sold at three different locations of Dehradun. These locations were chosen because of the large number of commuters that patronize the spot on daily basis.

Materials and Methods

Sample Collection

In order to determine the availability of street foods, three different locations were randomly selected from city and a survey was conducted. Various food samples (Aloo tikki, Momo, Chowmein and Chutney) were collected from 3 different locations in Dehradun city.

Microbiological analysis

For the microbiological analysis of food sample, each sample was thoroughly mixed. Serial dilutions of samples were prepared for further analysis. After serial dilution pour plate technique was applied. After solidifying, Petri plates were incubated at 37°C for 24 hours in inverted position. After incubation the plates with maximum number of colonies was selected and the number of

colonies were counted on the selected plates. The isolated colonies of organism were transferred to nutrient agar slant for maintenance and further identification.

Identification and Biochemical characterization of Isolates

The isolated colonies were morphologically characterized e.g., colonial growth and pigmentation. In biochemical characterization, Catalase test, Amylase, Gelatine Liquefaction, IMViC test, Oxidase, Urease, Carbohydrate fermentation (lactose, sucrose, dextrose) were performed.

Biochemical tests and Staining were done in accordance to standard procedure (Aneja, 2003)

Gram staining: The gram stain is a differential stain which is used to differentiate bacteria into two groups Gram positive bacteria and Gram negative bacteria. The technique is based on the fact that Gram positive cell wall has a stronger attraction for crystal violet when iodine is applied and therefore retains the crystal violet and therefore will remain purple after decolorizing while Gram negative bacteria will be colourless after decolorizing with alcohol, counterstaining with Safranin will make them to appear pink.

Catalase test: The glass slide was held at an angle and few drops of 3% hydrogen peroxide were allowed to flow slowly over the culture. The emergence of bubbles from the organism was noted. The presence of bubble displayed a positive test indicating the presence of enzyme catalase. If no gas is produced, this is a negative reaction.

Amylase test: In this test, isolate was point inoculated on starch agar plates and incubated at 37°C for two days. After

incubation, iodine solution was poured on the agar and examined for hydrolysis of starch by the production of clear zone around the microbial growth.

Gelatin liquefaction: Gelatin is a protein produced by hydrolysis of collagen. Hydrolysis of gelatin is brought about by microorganism capable of producing a proteolytic exoenzyme known as gelatinase. The degradation of gelatin occurs in the medium by an exoenzyme, it can be detected by observing liquefaction (i.e. flooding the gelatin agar medium with mercuric chloride solution and observe the plates for clearing around the line of growth) because gelatin is also precipitated by chemicals that coagulate proteins while the end products of degradation (i.e. amino acids) are not precipitated by same chemicals.

Urease test: Urease test is performed by growing the test organisms on urea broth or agar medium containing the pH indicator phenol red (pH 6.8). During incubation, microorganisms possessing urease will produce ammonia that raises the pH of the medium/broth. As the pH becomes higher, the phenol red changes from a yellow colour (pH 6.8) to a red or deep pink colour. Failure of the development of a deep pink colour due to on ammonia production is evidence of a lack of urease production by the microorganisms.

Oxidase test: The oxidase test is a test used in microbiology to determine if a bacterium produces certain cytochrome c oxidases. It uses disks impregnated with a reagent such as N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) or N, N-dimethyl-p-phenylenediamine (DMPD), which is also a redox indicator. The reagent is a dark-blue to maroon color when oxidized, and colorless when reduced. Oxidase-positive bacteria possess

cytochrome oxidase or indophenol oxidase (an iron-containing hemoprotein). These both catalyze the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). The test reagent, TMPD dihydrochloride acts as an artificial electron donor for the enzyme oxidase. The oxidized reagent forms the colored compound indophenol blue. The cytochrome system is usually only present in aerobic organisms that are capable of using oxygen as the final hydrogen receptor. The end-product of this metabolism is either water or hydrogen peroxide (broken down by catalase). Live bacteria cultivated on trypticase soy agar plates may be prepared using sterile technique with a single-line streak inoculation. The inoculated plates are incubated at 37°C for 24–48 hours to establish colonies. Fresh bacterial preparations should be used. After colonies have grown on the medium, 2-3 drops of the reagent DMPD are added to the surface of each organism to be tested. A positive test (OX+) will result in a color change violet to purple, within 10–30 seconds. A negative test (OX-) will result in a light-pink or absence of coloration.

IMViC

Indole test: Tryptophan is an essential amino acid that can undergo oxidation by the way of enzymatic activities of bacteria and converted into metabolic products (indole, pyruvic acid and ammonia) is mediated by the enzyme tryptophanase. The presence of indole is detected by adding Kovac's reagent which produces cherry red colour. The colour is produced by the reagent which is composed of p-dimethylaminobenzaldehyde yielding the cherry red colour. Absence of red coloration demonstrates that the substrate tryptophan was not hydrolysed and indicates an indole negative.

Methyl red test: All enteric microorganisms ferment glucose and produce organic acids. The methyl red indicator which is used in this test, in the pH range of 4 will turn red, which is the indicative of a positive test. At a pH of 6, still indicating the presence of acid but with a lower hydrogen ion concentration, the indicator turns yellow and is a negative test.

Voges Proskauer test: Voges Proskauer test determines the capability of some microorganisms to produce non-acidic or neutral end products, such as acetyl methyl carbinol, from the organic acids that results from glucose metabolism. The reagent used in this test, Baritt reagent consists of mixture of alcoholic alpha-naphthol and potassium hydroxide solutions. Detection of acetyl methyl carbinol requires this end product to be oxidized to a diacetyl compound. This reaction will occur in the presence of alpha-naphthol catalyst and a guanidine group that is present in the peptone of MR-VP medium. As a result, a pink complex is formed, imparting a rose colour to the medium.

Citrate utilization test: In the absence of fermentable glucose or lactose, some microorganisms are capable of using citrate as a carbon sources for energy. This ability depends on the presences of citrate permease that facilitates transport of citrate in the cell. During this reaction the medium becomes alkaline, the carbon dioxide that is generated combines with sodium and water to form sodium carbonate, an alkaline product. The presence of carbonate changes the Bromothymol blue indicator incorporated into the medium from green to Prussian blue. After incubation citrate positive culture are identified by the presence of growth on the surface of slants, which is accompanied by blue coloration. Citrate negative will show no growth and the medium will remain green.

Carbohydrate fermentation: Fermentation of carbohydrates (glucose, sucrose, lactose etc.) are carried out by microorganism, under anaerobic condition in which a Durham tube is placed in inverted position to trap the gas bubble formed due to production of gas. The fermentation broth contains ingredients of nutrient broth, a specific carbohydrate and a pH indicator (phenol red), which is red at neutral pH (7) and turns yellow at or below a pH of 6.8 due to the production of an organic acid.

Results and Discussion

Food borne Diseases are caused by wide spectrum of illness caused by pathogenic bacterial, viral, protozoan and chemical contamination of food which has been recognized as major problem. Major cause for the rapid increase in the occurrence of Food borne infections is the Street Foods which is easily affordable by poor people. Food poisoning can be the result of either chemical or the ingestion of toxicant. This may be an attempt to make aware common people regarding microbial contamination of street vended food and health hygienic conditions.

Isolation and Enumeration of Isolates from Food samples

The various street food samples (Aloo Tikki, Momo, Chowmein, and Chutney) were screened for the presence of pathogens responsible for causing Food borne infections by Pour plate technique at different dilutions. The no. of colonies obtained at different dilutions is compiled in Table 1 and graphical representation showing the number of colonies (CFU/ml) in different samples at different locations is presented in Figure 1.

Conclusively, improper personal hygiene

can facilitate the transmission of these pathogenic bacteria found in environment and on people's hands via food to humans (Tambekar *et al.*, 2008). The present study was undertaken to investigate the microbiological quality of different food types in Dehradun, India. Food hawkers in India are generally unaware of food regulations and have no training in food-related matters. They also lack supportive services such as water supply of adequate quality and rubbish disposal systems, which hamper their ability to provide safe food Titarmare *et al.* (2009)

The results obtained from various food samples which were screened for the presence of food borne pathogens have been discussed below. High levels of bacterial contamination at varying degrees were detected in the food types tested. Samples of Aloo tikki, Momo, Chowmein and chutney had considerable levels of contamination which can lead to various types of serious diseases.

Total of 10 bacterial isolates were obtained from the different street food types out of which 5 isolates were found to be Gram positive and 5 Gram negative. Some of cells were arranged singly, paired or in chains or clusters. Some of them are coccus in shape while others are rod in shape. They showed different morphologic patterns on agar plate as circular, opaque, round, entire, pin head colonies. They also showed varied coloration as white, cream, pink, gelatinous, mucoid. These unknown bacterial isolates were biochemically characterized by different tests. *E. coli* and other coliform bacteria could be due to inadequate hand washing by food workers and the absence of good manufacturing practices. The presence of *S. aureus* caused severe contamination through handling (Tambekar *et al.*, 2007).

When bacterial isolates were subjected to catalase test to detect the development of bubbles after the addition of hydrogen peroxide, isolates S1, S2, S3, S7, S8 showed positive result. S6, S8, S10 bacterial isolates showed positive amylase test by producing a clear zone around the colonies i.e starch hydrolysis reaction. Fermentative degradation of carbohydrates such as sucrose, lactose and dextrose was done by all the isolates by producing acid and gas. S1 and S3 isolates showed positive Indole test while others gave negative result. When subjected to MR test isolates S1, S2, S3, S7, S9 showed positive MR result and S6, S7 produced positive VP test. S2, S4 isolates were found to be Citrate utilization positive. Cultures S6, S7 showed positive result for Gelatin liquefaction and S4, S5, S10 isolates were urease positive.

In the present study, the bacteriological quality of different food samples such as Aloo tikki, Momo, Chowmein, Chutney were found to be contaminated with different bacterial pathogens like *E. coli*, *S.*

aureus, *Bacillus cereus*, *Salmonella*, *Shigella*. All these bacterial pathogens are responsible for the food borne and diarrheal diseases.

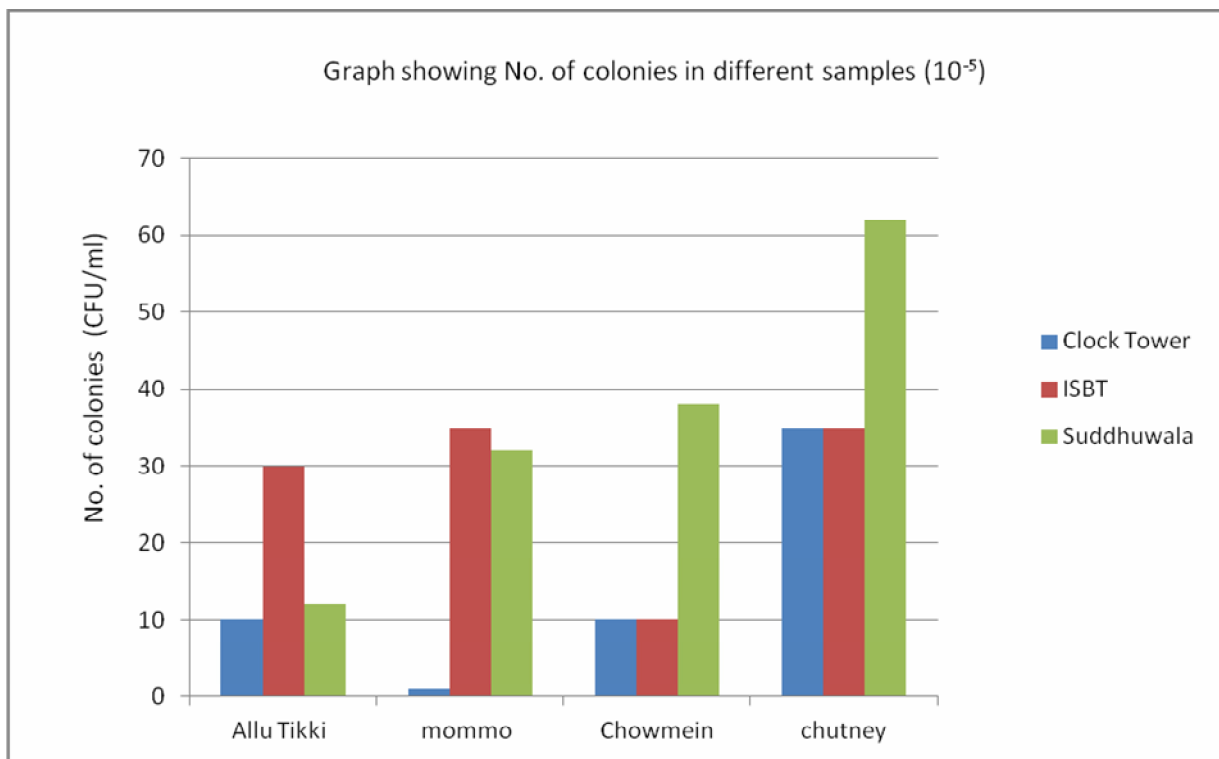
Most food pathogens are of soil or intestinal origin and are transmitted through poor food preparation, personal hygiene or public sanitation practices. Therefore to ensure the safety of the foods, producer and hawkers must maintain a clean environment, minimize contact with the food samples after production and also maintain a high level of personal hygiene. Also, utensils should be properly clean at all stages of production.

The local Government and the ministry should consider establishment of adequate facilities and utility services as well as provision of necessary information, education and training programmes for vendors and consumers. Our findings have shown that there is need for improvement of good hygiene practices (GHP) so that street food contamination can be reduced

Table.1 Microbial count in different samples at various locations

Samples	Dilution factor	Number of colonies (CFU/ml)		
		Location 1	Location 2	Location 3
Allu Tikki	10 ⁻⁵	10	30	12
	10 ⁻⁶	8	25	8
	10 ⁻⁷	7	15	7
Momo	10 ⁻⁵	Conjugated Colonies	35	32
	10 ⁻⁶	15	30	27
	10 ⁻⁷	12	22	20
Chowmein	10 ⁻⁵	10	10	38
	10 ⁻⁶	7	7	35
	10 ⁻⁷	5	5	25
Chutney	10 ⁻⁵	35	33	62
	10 ⁻⁶	28	30	58
	10 ⁻⁷	20	28	35

Fig.1 Graphical representation of microbial counting different food samples (Allu Tikki, Momo, chowmein, Chutney) at different locations in Dehradun



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