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MICROBIAL ANALYSIS OF FOOD SAMPLES FROM LOCAL FOOD VENDORS OF PANVEL REGION

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ABSTRACT
The aim of this study was to assess the microbiological quality of street food samples. The food samples were bought from local vendors of Panvel region. Street foods are appealing due to its taste, low cost and easy availability. People get attracted towards variety of street foods like pani-puri, ice-cream, juices, sandwiches etc. to a great extent. These foods provide adequate nutrition and energy to the consumers and thus meet their daily requirement of energy and vitamins. The increase in number of cases of food poisoning may occur due to several factors such as poor local infrastructure and lack of sanitary surveillance. Hence, there is a need for proper microbiological survey of these street foods. The study aims at analysing the microbial quality of food samples like Pani- puri, sugarcane juice, ice-cream, kulfi, Sugar Candy, Pepsi stick and Alu Tikki. The samples were brought to the laboratory and tested for the presence of coliforms, *Bacillus*, *Salmonella typhi*, *Staphylococcus aureus*, fungi and yeast. Local Ice cream samples were found to be contaminated with coliforms, *Staphylococcus aureus* and *Bacillus*. Pani-puri water sample was found to be contaminated with *Pseudomonas*, Coliforms and *Staphylococcus aureus*. Pepsi Stick and Sugar Candy did not contain bacterial contamination but yeast and fungal growth was observed. Considering the socio-economic importance of street foods, it is required that the safety and hygiene of these foods is maintained properly.

Keywords: Colony Forming Unit; coliforms; Food intoxication; Food poisoning; Pasteurization.

INTRODUCTION:

The growth and contamination of pathogenic microorganisms is one of the main causes to loss of food and spread of various diseases. Street foods are very popular as they are ready to eat, tasty and not very expensive. “Street foods are defined by the FAO as “ready-to-eat foods and beverages prepared and or sold by vendors and handlers especially in streets and other similar places for immediate consumption or consumption at a later stage without further processing or preparation” (Food and Nutrition, 1989). Street Foods are great fascination among people in Indian society. Street foods are available in every cuisine of our society. It is a large source of employment generation. Also, the convenience and low prices make street food the most favoured choice of the migrant workers in the large cities. (Manisha Choudhury, Lipi Mahanta, Jayashree Goswami, Minakshi Mazumder, Barnali Pegoo, 2010.) Hence, it is gaining much attention among the youngsters. Street foods have shown serious health concerns to the consumers. The main cause of contamination of food by pathogen is lack of food safety knowledge and not maintaining proper hygiene. Other reasons which are included are: Improper storing, handling and preparing food, improperly clean or usage of unsterilized utensils,

contamination by flies, cockroaches, insects and pest. even a single organism can multiply its number when it finds ideal growth conditions such as nutrition, pH, temperature, etc. The food safety is mainly affected by quality of raw materials, cleanliness of utensils and storage practices At many places, running water is not continuously supplied for hand and dishwashing, cooking or drinking, leading the street vendors to store water under vulnerable conditions subject to contamination. Street foods may get contaminated due to environmental conditions, such as insects, rodents, other animals and air pollution. Very few food vendors follow proper guidelines of Food safety, most of them ignore good food handling practices, exposing foods to dangerous conditions such as cross-contamination, unsafe storage and poor time-temperature conditions (Ekanem, 1998).

There are various pathogens which can enter the food causing food spoilage and spreading various diseases in the human population. out of which the most common pathogens are: *Clostridium perfringens, Escherichia coli, Shigella spp., Proteus, Staphylococcus aureus, Salmonella spp., Bacillus cereus, Campylobacter, Enterococcus, Aerobacter, Vibrio spp., Xanthomonas campestris, Lactococcus*. The pathogens which are present in the food can cause diseases like diarrhoea, abdominal cramps, vomiting, shigellosis, gastroenteritis, Hepatitis A, Norwalk virus, rotavirus, botulism, campylobacteriosis are the common example of food infection. Food microbiology involves the study of the microorganisms which are present in the food which are either important or dangerous. By having knowledge about nutritional characteristics, biochemical and physiological characteristics of microorganisms, it becomes possible to prevent the particular food from getting contaminated and getting intoxicated by various types of toxins secreted by microorganisms. Subsequently, detection of infectious agents in the food sample can help in the treatment and cure of patients suffering from food infections and food poisoning. Various methods like High temperature storage, Low temperature storage, Radiations and Chemical agents are used for proper storage of food products. These treatments are efficient to destroy the most heat resistant of the non-spore forming pathogenic organisms *Mycobacterium tuberculosis* and *Coxiella burnetti*. It is also used to produce better starter culture in probiotic foods as *Lactobacillus* and *Streptococcus* can survive pasteurization temperatures. It is used to design effective sanitization procedure to control spoilage and pathogen problems.

Street-vended foods (SFVs) are defined as foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (Mosupye F.M. & von Holy, 1999). Street foods are said to provide a source of readily available, inexpensive, nutritional meals, while providing a source of income for the vendors, although several concerns were raised over their safety and quality (Bryan *et al.*, 1988; Mosupye and von Holy, 1999). The street food vendors are categorized into two types- those move from place to place with prepared and packaged food opted for sale on carts or bicycles, etc. and; (Almeida *et al.*, 1996) stationary vendors who have fixed stalls where food is prepared, stored and served to consumers.

Maintaining food hygiene and safety is a critical issue and every person is at risk of foodborne illnesses. World Health Organization (WHO) defines foodborne illnesses as diseases, either infectious or toxic, caused by pathogens that enter the digestive system through the ingestion of food (WHO, 2007). The consumption of street food may lead to different food borne illnesses from insanitary food vending locations. Globally, outbreaks of foodborne diseases are documented, illustrating its social and public health significance (WHO, 2002, 27 pp.). Many SFVs practice poor food handling procedures, expose food to cross-contamination and store food under inappropriate time–temperature conditions (Oteri & Ekanem, 1989). Therefore, there is a general perception that street food is unsafe as a result of poor hygiene conditions under which it is prepared, sold and consumed (Muinde & Kuria, 2005).

The Hazard Analysis and Critical Control Point (HACCP) system helps in giving complete knowledge of the food production chain and identifies major hazards and critical control points where contamination can occur. The HACCP safety principles are accepted globally as an effective tool for evaluating and controlling risk in foods and ensuring food quality and safety (Richards, Parr & Riseborough, 1993).

The aim of this study was to determine the microbial levels of street foods in Panvel area. Variety of food samples were collected from Panvel area from local vendors and its microbial load was determined.

Materials & Methods:

Following media was used during the study-
Sterile Nutrient Agar Plates
Sterile Cetrimide Agar Plates
Sterile MYP Agar Plates
Sterile Dextrose Tryptone Agar
Sterile Sabouraud's Dextrose Agar
Sterile Lauryl tryptose Broth
Sterile BGLB broth
Sterile Endo Agar Plates
Tryptophan broth
Glucose Phosphate Broth
Simmon's Citrate medium
Kovac's reagent
Methylene Blue reagent
VP reagent

The food samples collected from local vendors were-

1. *Ice-cream sample (green)*
2. *Ice-cream sample (orange)*
3. *Kulfi*
4. *Pani puri chutney*
5. *Sugar candy (Gola)*
6. *Pepsi stick*
7. *Sugarcane juice*
8. *Allu Tikki*

1. Laboratory procedures for collection of food samples: Food samples were collected in sterile screw-capped wide mouthed plastic containers, at least 100 g or ml for each sample. Food samples were processed in the laboratory immediately upon receipt. However, if a laboratory analysis was postponed due to the delayed arrival of samples, those samples were refrigerated at 0–4 °C until examination but not longer than 36 hours. Microbial contamination in food samples was assessed by estimating coliforms.
2. To detect and enumerate coliforms, the violet red bile agar (VRBA) and brilliant green lactose bile (BGLB) broth were used, which are two of the conventional methods for determining coliforms in food samples (Feng *et al*., 2002; Kornacki and Johnson, 2001). Coliform counts of less than 10^2 per g or ml were considered acceptable according to the microbiological criteria of foods for infants and children as recommended by the International Commission on Microbiological Specifications for Foods [ICMSF] (ICMSF, 1986; Jay *et al.*, 2005).

Cetrimide Agar

Cetrimide is a quaternary ammonium compound with bactericidal activity against a wide range of Gram-positive and certain Gram-negative organisms.
It is a selective and differential medium used for the isolation and identification of species of *Pseudomonas.*

MYP Agar
MYP Agar is a selective and differential medium. It is used for the selective and differential enumeration of *Bacillus cereus* from the food which is mainly associated with food poisoning. It contains Mannitol, which is not fermented by *Bacillus cereus*. It also contains egg yolk (lecithin) which can be cleaved by phospholipase of B. cereus. the medium also contains polymyxin, which inhibits Gram negative bacteria.

Nutrient Agar
It can grow a variety of types of bacteria and fungi, and contain nutrients needed for the bacterial growth. Used for performing standard plate count of the food sample.

Sabouraud's Dextrose Agar
It is used for the isolation, cultivation, and maintenance of non- pathogenic and pathogenic species of fungi and yeast.

Lauryl Tryptose Broth
It is used for the most probable number test of coliforms in the sample.
It acts as a confirmation test for the lactose fermentation with gas production.

BGLB broth
Brilliant Green Bile Lactose Broth is used for confirmation of presumptive positive tubes showing gas production in lactose broth/ Lauryl sulphate broth/ Lauryl tryptose broth.

Endo Agar
It is used to confirming the detection and enumeration of coliform bacteria following presumptive test
It is also used for the detection and isolation of coliforms and fecal coliforms from milk, dairy products and food samples.

Result & Discussion:
The food samples were inoculated in various media and checked for the presence of *E. coli, Bacillus, Staphylococcus aureus, Pseudomonas, Salmonella spp.,* in the various food samples. Presence of Coliforms was detected by performing Presumptive test, Confirmed test and Completed test.

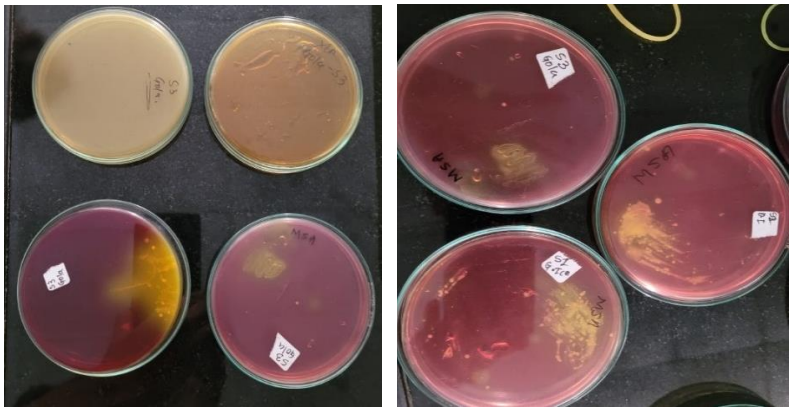
Ice-cream sample-
15 tubes of different quantities 10ml, 1ml and 0.1ml of sample was inoculated in Lauryl tryptose broth for presumptive test and kept for incubation at 37^{o}C for 24 hours. Gas production and acid production (media changes to yellow colour) and also turbidity on media was observed. The results are shown in Table No. 1.
ENDO agar- For Confirmatory test, the tubes showing positive results were streaked on ENDO agar plates and kept for incubation at 37^{o}C for 24 hours. Reddish pink colonies with metallic sheen indicated the presence of *E. coli*.
BGLB broth- For Completed test, the positive tubes (with gas and acid production) were inoculated in BGLB broth with Durham's vial and kept for incubation at 37^{o}C for 24 hours.

Ice Cream (orange) Sample

Sugar Candy sample (Gola)

Sugar candy (Gola sample) was inoculated on MYP Mannitol-Egg Yolk Polymyxin agar (MYP), Mac Conkey, agar, Mannitol Salt agar, XLD agar and Lauryl Tryptose agar. On MYP agar, pink coloured colonies were observed. On MSA agar, few golden yellow colonies were observed.

Sugarcane juice sample- The juice of sugarcane was inoculated on Sabouraud's Dextrose Agar and incubated for 48 hours at room temperature. After incubation, few colonies of yeast was observed on Sabouraud's Dextrose agar.



Gola sample

Table 1: Growth of microorganisms of different samples by using various media

S. No.	Ice-cream (orange)	Kulfi	Gola	Ice cream (green)	Sugarcane juice	Pepsi stick	Pani puri chutney	Aloo Tikki
Acid and Gas production	++	++	++	++	-	-	++	-
MSA medium	++	++	++	++	-	-	++	-
Cetrimide agar	+	-	-	-	-	-	+	-
MYP medium	++	++	-	++	-	-	++	+
Sabouraud's agar	+	+	+	+	++	+	-	+

Mac-Conkey Agar	++	++	-	++	-	-	++	-
ENDO agar	++	+++	++	-	-	-	++	-

Table 2: Viable count of organisms of different food samples on Nutrient agar media

S.No.	Food Samples	CFU/ml
1.	Ice-cream (orange)	$1.8x10^7$
2.	Sugar candy(gola)	$2.1x10^3$
3.	Pani-puri chutney	$1.2\ x10^3$
4.	Ice-cream (Green)	$2.5\ x10^8$
5.	Kulfi	$4.8x10^7$
6.	Pepsi stick	$2.4\ x10^3$
7.	Sugarcane Juice	$3.2x10^5$
8.	Aloo Tikki	$2.1\ x\ 10^2$

Pani-puri water sample
Left: MacConkey plate
Right: Nutrient Agar plate

Ice-cream sample (green)
Top: Nutrient Agar
Bottom: Mac Conkey & EMB

Kulfi sample

Table 3 – Different microbial species present in various samples

Food Samples	***Bacillus***	***E. coli***	***S. aureus***	***Yeast & Molds***	***Pseudomonas***
Ice-cream (orange)	++	++	++	-	+
Sugar candy(gola)	++	++	++	+	-
Pani-puri chutney	++	++	++	++	+
Ice-cream (Green)	++	+	+	+	-
Kulfi	++	+++	+	+	-
Pepsi stick	+	+	-	+	-
Sugarcane Juice	-	++	-	++	-
Aloo Tikki	+	-	+	+	-

SUGARCANE JUICE SAMPLE

PEPSI STICK

Conclusion: Street foods and Ready-to-eat foods may become cause of spreading disease among people. Pathogenic diseases like Typhoid, Shigellosis, *Escherichia coli* infections and Staphylococcal food poisoning can be spread if contaminated food is consumed by people. Total viable count was found to be high in sugarcane juice, Ice-cream samples and Gola samples. High microbial count in food samples exceeds the specifications set for different food groups. These findings indicate improper hygienic conditions during processing and handling, which is high health risk for consumers. It is mandatory for all the food vendors & refreshers to take proper training of Food safety and Standards before being licensed to operate. There should be implementations on national regulations of food control and ensuring that standard protocols regarding hygiene and other standards are encouraged and strictly followed. The pre-certification training should be given to local food vendors which include hazard analysis and critical control point system.

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