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Microbiological Assessment of Street Vended Panipuri Water Sample Taken from Lucknow, U. P., India

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Abstract—*The aim of this study was to assessed the microbiological quality and safety of street food vendors in the main street and markets of Lucknow. Microbial contamination of food is a major source of illness. Severe contamination is generally linked to contaminated water, but transmission could occur through contaminated foods served by the street food vendors. Owing to rapid urban population, many people live in conditions of extreme poverty and poor sanitization. This also has seen to aggravating food safety problems. Globally, death due to food borne illness mounts to 1. 8 million, to which, a significant number is contributed by India. This study aims to assess the quality of street vended panipuri water sample in terms of bacterial contamination in Lucknow city. Panipuri water sample were selected in the Lucknow city for the study.*

Keywords: *Panipuri, Water Contamination, Food borne disease, Hygiene, Street Vended food, Lucknow City.*

INTRODUCTION

Food and Agriculture Organization(FAO) defined street foods are "Ready to eat foods and beverages which are prepared and/or sold by vendors specially in streets and other similar public places"(FAO), 1989). These street foods are appreciated for characteristic flavour, variety and availability at inexpensive cost especially at crowed places such as local markets, tourist places, work places, railway stations, bus terminals, school and hospitals premises (Das *et al., 2012).* The present study was aimed at examining the bacterial quality and safety of street foods sold at different parts of Lucknow city. Panipuri is very popular street food which is consumed by large huge population of different age groups. Consuption of this type of foods potentially increases the risk of food borne diseses caused by various bacteria. Unhygienic Panipuri is harboured by potentially life-threatening bacteria like *Salmonella thypi* and *Escherichia coli(Garode and Waghode, 2012).* The spicy water of Panipuri is found to be contaminated with different bacterial pathogens like *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacilli, Staphylococcus, Lactobacillus sp.* Food-Borne and diarrheal diseses are caused by these bacterial pathogens (Tambekar *et al.* , 2011). Panipuri particularly in summer and mansoons are the season that conducive to bacterial growth in food items. Unclean locations, Unhygienic serving practice, unhygienic water, stalls present in dust and dirt area and source of transfats in Panipuri are major concerns of health issues. Contamination also from raw material and equipments, additional processing conditions, improper handling and prevalence of unhygienic conditions contribute significantly to the entry of food borne pathogens. A general lack of factual vigilance about the epidimiological significance of many street vended foods, poor knowledge of streets vendors in basic food safety criteria and inadequate public awarness of hazard posed by certain foods has severely hamperd the development of a precise. Street foods industry contributes to a significant part of total food intake of millions peoplein India. However this informal sector is unregulated and poses a health risk. People who eat street foods are at the risk of food poisoning. Most common risk factors for contamination of foods are storage of foods at inappropriate temperature conditions, poor personal hygiene and poor handling. Various worker given importance to this type of work (*Saxena and Agrwal* 2013).

Materials and Methods

The present study was conducted at Babasaheb Bhimrao Ambedkar University in the Department of Environmental Microbiology. All media(Nutrient agar, MacConkey agar, Pseudomonas Agar, Eosin Methylene Blue Agar) used during the course of assessment. For bacterial stain, staining kit contain five bacterial stain chemicals (Crystal voilet, iodine, decolorization, ethanol or Acetone, safranin) for preparing microscope slides were used for identification of Bactera. Utarathiya Chauraha and Transport Nagar is known as most crowed areas in Lucknow city where the sale was maximum per day. Two different vended water sample from Utarathiya Chauraha (Sample Code U-1 and U-2) and two water samples from different vendors (sample

code TN-1 and TN-2) were aseptically collected in sterilized plastic container and stored in refrigirator and the assessment had been performed in the University Laboratory. Serial dilution is a series of sequential dilutions used to reduce the dense culture of cells to a more usable concentration. Each dilution will reduce the concentration of bacteria by a specific amount. 5 test tubes with 9 ml distilled water were taken then were autoclaved 15lbs 121°C for 15 min. After autoclaving, test tubes were allowed to be cooled down and the process for serial dilution was started. Each sample was serially diluted and then 0. 1µ sample was spread on nutrient agar, macConkey agar, EMB agar and pseudomonas agar plates respectively using spread plate technique. In this technique the media is prepared and then cooled down and then poured in the sterilized petri plates and after the media is solidified the plate is inverted and BOD incubated for 24 hr at 37°C. In the isolation of bacteria; 100 ml of Nutrient Agar , 100 ml of MacConkey Agar, 100 ml of Eosin Methylene Blue Agar , 100ml of Pseudomonas Agar were prepared and were autoclaved 15lbs 121°C for 15 min. After autoclaving the media, these media are allowed to cool down . Then they are poured into sterile petri plates. Each media is poured into 2 plates. These plates are then allowed to solidified and then inverted and incubated for 24hr in 37°C. In this technique, 0. 1µ sample is poured into the solidified plated and spread with the help of spreader. After spreading, the plates were wrapped with parafilm and kept in BOD incubator for 24hr in 37°C. The colonies observed after 24hr were mixed colonies so they were re-streaked to gain the pure culture colonies. Colony forming unit can be calculated using followig formula-"CFU/g=Numbers of colonies / weight of sample X Dilution Factor" All the media were examined for bacterial growth. Growth characteristics and other colony morphology, formation of mucoid colonies of the bacteria were carefully observed. More than five similar colonies were counted on a plate, plates were picked carefully one by one and inoculated into the nutrient broth in sterile test tube. Culture from each test tube re-inoculated on to a nutrient agar to obtain a pure growth. Bacterial identification was done using the pure culture on the nutrient agar plate. This is a method to distinguish and classify bacterial species into two large groups - Gram positive and Gram negative. Gram staining differentiates bacteria by the chemical and physical properties of their cell wall by detecting peptidoglycan, which is present in the cell wall of Gram-positive bacteria. Gram negative cells also contain peptidoglycan, but a very small layer of it that is dissolved when the alcohol is added. This is why the cell loses its initial color from the primary stain. Gram-positive bacteria retain the crystal violet stain, and thus are stained violet, while the Gram-negative bacteria do not; after washing, a counterstain is added (commonly safranin or fuchisine) that will stain these Gram-negative bacteria a pink color. Both Gram-positive bacteria and Gram-negative bacteria pick up the counter stain. The counter stain, however, is unseen on Gram-positive bacteria because of the darker crystal violet stain**.** Catalase Coagulase- Catalase is an enzyme, which is produces by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. This test was done by mixing a dense culture with two drops of H_2O_2 and looking for a bubble. Organism positive [produce bubbles] in the test were considered to be *staphylococci*, while that negative were *streptococci.* Oxidase Test- The oxidase test is used to identified bacteria that produce cytochrome C oxidase, an enzyme of the bacteria electron transport chain. When present, the cytochrome C oxidized the reagent[tetramethyl-p-phenylenediamine] to [indo phenols] purple color end products. To performed on the agar plate, by using loop to aseptically transfer a large mass of pure bacteria shown that the area of inoculation turns dark blue to black. Organism positive in the test were considered to be *pseudomonas* and *campylobacter*. Indole Test- This test demonstrated the ability of certain bacteria to decompose the amino acid *tryptophan* to *indole*. This test done by using 4ml of tryptophan broth in a sterile test tube, inoculate the tube aseptically by taking a growth from 18 to 24hrs. culture and then added 0. 5ml kovac's reagent to the broth culture to observed the presence or absence of the ring. No ring formation observed and it considered to be ***bacilli sp.*** and mostly ***klebsiella sp.***

Results and Discussion

Four samples analyzed, panipuri water sample items pathogenic bacteria were identified. In the study, higher incidence of *E. coli* and *Klebsiella* was highly observed in pani puri. Sample U-1 was free from any bacterial contamination because there was good sanitation and proper handeling has been implemented and mask and gloves was used. Panipuri sold at Transport Nagar, where the customers maximum in day, more contaminated by bacteria than the others areas.

Sample Name	Media Name	Growth
TN-1	Nutrient Agar	Yes
	EMB Agar	Yes
	MacConky agar	Yes
	Pseudomonas Agar	No
TN-2	Nutrient Agar	Yes
	EMB Agar	Yes
	MacConky Agar	Yes
	Pseudomonas Agar	No

U-1	Nutrient Agar	NO
	EMB Agar	NO
	MacConky Agar	NO
	Pseudomonas Agar	NO
U-2	Nutrient Agar	Yes
	EMB Agar	Yes
	MacConky Agar	Yes
	Pseudomonas Agar	No

Table (A) - Growth pattern on different media

Sample	**Colony Forming Unit(CFU/ml)**
TN-1	3. 7×10^3
TN-2	2. 9×10^3
U-2	3. 8×10^3

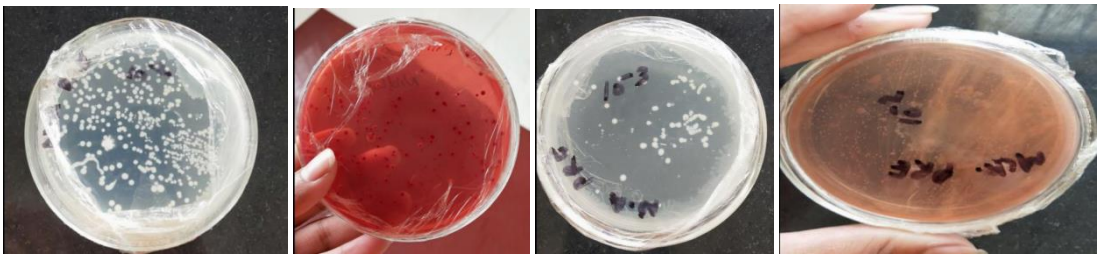
Table (B) - cfu / ml of the study sample

Serial No.	**Gram Staining**	**Indole test**	**Catalase test**	**Oxidase test**
Klebsiella	Gram-ve	-	+	-
E. coli	Gram-ve	+	+	-
Streptococcus	Gram+ve	-	+	-
Bacilli	Gram+ve	-	+	-

Table (C) - Biochemical characterization of the isolates

Sample	**Catalase Positive/Negative**
Klebsiella	Positive
Klebsiella	Negative
Lactobacillus	Negative
E. coli	Positive
Lactobacillus	Negative

Table (D) - Catalase test oxidase test and indole test of the sample



(A) (B) (C) (D)

Fig. Growth of bacteria on different media.

In the present study, the most frequent contaminating bacteria in panipuri was ***Klebsiella & E. coli.*** The presence of coliform bacteria in ready-to-eat foods indicates unhygienic conditions during processing, handling, and distribution or post processing contaminations; there are potential health risk associated with initial contamination by vendors and during preparation and handling and cross contamination as well. Pathogenic *E. coli* detected in high amount and water used by street vendors were contaminated by coliform. On previous reports, following biochemical test such as Gram staining, indole, catalase, and oxidase were used to demonstrate the presence of *Klebsiella* and *E. coli* in panipuri water samples. Food borne disease are cause by pathogenic bacteria and chemical contamination of food. The present study was undertaken to observe the bacterial quality of different street vendors in Lucknow city, India. This study is an attempt to create awareness among common people about bacterial contamination of the street foods. Improper personal hygiene of people who are preparing the foods can facilitate the transmission of pathogenic bacteria found in the environment via food to human [Tambekar et al. , 2008]. Most of the foods vendors in India are unaware of hygienic practices during food preparations and food regulations. The lack services such as good quality water supply and proper waste disposal systems. Thus, they also lack their ability to provide safe food [Titarmare et al. , 2009]. *E. coli* and *klebsiella* is one of the most common pathogenic agents of food poisoning. The presence of these pathogens evaluate the food samples as unsafe for consumption. The presence of high colony forming unit of total bacteria and coliforms indicates inadequate processing and post process recontamination due to cross-contamination with raw and dirty equipment as well as storage of foods at improper temperature which deals to rapid multiplication of pathogenic and toxigenic bacteria. The presence of *Klebsiella* in foods suggests lack of hygiene inhandling and poor water quality which were used to preparation of panipuri. Thus, proper hygiene and proper storage avoiding cross-contamination are the major process for preventing food- borne diseases. If the person handling food is infected with *E. coli* or *Klebsiella* or any other gastrointestinal illness, she/he should avoid preparing foods unless she/he takes precautions as wearing disposable gloves and following safe food handling steps This study has demonstrated that some of the most popular types of ready-to-eat foods that are sold on the streets of Lucknow city were contaminated, and do not meet the required quality and safety levels. This type contamination can be reduced by the training of preparation and cooking method, storage of food at ideal temperature, sanitation around the stalls, using of gloves and mask, proper disposal of utensils or proper cleaning of plates and utensils, common vigilency about food safety measures and free training for vendors and regular monitioring for street vendors.

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