VOLUME - 15, ISSUE - 06, JUNE - 2026 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Microbiology

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF FOOD BORNE BACTERIA ISOLATED FROM STREET VENDED STALLS

Dr Renu Pathak RDVV University, Jabalpur, Madhya Pradesh, India-482001

Mansi RDVV University, Jabalpur, Madhya Pradesh, India-482001

Dr Vartika Bajpai* DY PATIL Dental School, Lohegaon *Corresponding author

ABSTRACT Street vended foods have gained a lot of popularity in recent time owing to their appealing taste and flavor. Panipuri are one of the most popular street food in different parts of India. Consuming street food which are mostly prepared under unhygienic condition leads to food borne illness. Most common food borne bacteria include, E.coli, Klebsiella sp., Enterobacter sp., Salmonella sp. and Staphylococcus sp. In this cross-sectional study three Panipuri samples were aseptically collected from three different locations of Jabalpur, Madhya Pradesh. Isolation, Enumeration and Identification of the bacteria were carried out by following the traditional method which includes serial dilution, spread plate technique followed by microscopic, physiological and biochemical tests. The CFU counts of the samples were ranged from 5×107 to 40×107cfu/ml. Results indicates that both the isolates were belong to family Enterobactericeae and the two identified as Enterobacter sp. and Klebsiella sp. Antibiotic sensitivity test shows that Enterobacter sp. was resistant to Cefixime. All the samples were contaminated with different bacteria and on the basis of abundance in all samples two isolates were selected for identification procedure.

KEYWORDS : CFU, Panipuri, Klebsiella, Enterobacter, Street foods

INTRODUCTION:

The nutritional requirements of various microorganisms may differ appreciably but all of them require carbon, nitrogen, phosphorus and other minerals. Since foods are rich source of these compounds, they can be utilized by microorganisms also. The inability to utilize a major component of the food material will limit the microbial growth. Many food microorganisms have the ability to utilize sugars, alcohols and amino acids as sources of energy whereas few others utilize complex carbohydrates such as starch and cellulose for energy. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. Also, other nitrogenous compounds which can serve this function are proteins, peptides and nucleotides.

The term "street food" refers to a wide variety of ready- to- eat foods (RTE) and beverages sold and sometimes prepared in public places. The consumption of street food is common in many countries where unemployment is high, salaries are low, work opportunity and social programs are limited and where urbanization is taking place. Street foods are sold on public places such as school, college, universities, market, or fair often from potable food booth or food cart and are consumed on the streets itself without further preparation. Panipuri (Golgappa, Phuchka, Gupchup) is a very popular street food which in consumed by large number of population of different age groups. Panipuri is hollow puri, filled with mixture of flavoured, water chaat masala, mashed potato.[1]

Food borne illness is any illness resulting from contaminated food, it occurs when a pathogen is ingested with food and establishes itself in the human host, or when a toxigenic pathogen establishes itself in a food product and produces a toxin, which is then ingested by human host.[2] Bacterial pathogens such as Salmonella sp., Campylobacter, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Enterobacter sp., Klebsiella sp., and Pseudomonas are commonly involved in food borne diseases.[3] Ogunekumetal., 2022 analysed microbial quality in ready to eat foods and observed Staphylococcus aureus in maximum number followed by E.coli, Pseudomonas sp., Streptococcus sp., Bacillus cereus and Salmonella sp.[4]

Abdominal pain, diarrhea, vomiting, nausea, fever, respiratory difficulties are some common symptoms observed during food borne illness. These symptoms are caused by ingestion of pathogens as in case on Salmonellosis or by microbial toxin produce inside the host i.e., Clostridium perfringens; food poisoning.[5]

Sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and personal hygiene of vendors. Practices used during food preparation such as handling, cleaning, sorting and grading, packaging, storing and wrapping in low grade plastics are some of the critical factors that increase risk of inadequate food safety.

Identification of Bacteria: Identification is the process of determining to which established tax on a new isolate or unknown strain belongs. The aim of identification is to equate the properties of a pure culture with those of a well characterized and accepted species. Methods of bacterial identification can be broadly delimited to genotypic techniques based on profiling an organism's genetic material (primarily its DNA) and phenotypic techniques based on profiling either an organism's metabolic attributes or some aspect of its chemical composition.[6] Traditional way of detecting and identifying bacteria from food or other samples is based on culturing, enumeration and isolation of presumptive colonies for further identification analysis. The process relies on phenotypic technique. Morphology patterns i.e., shape, size, arrangement, staining, physiology such as growth on different selective and differential media, biochemical tests, antibiotic sensitive profile are common and less costly methods used for the identification of any given microorganism.

MATERIALS AND METHODS:

The objectives of the study were enumeration and study the growth pattern of different types of bacteria isolated from selected sampling sites; Isolation, Identification, Preservation and Maintenance of Pure culture of predominant bacteria; and to create awareness towards the hygiene and public health for street vended food.

Three different sampling stations were selected for sample collection:

1) Science College (vendor in front of the college);
2) Rani Durgawati University (outside the campus); and
3) Civil Lines (vendor in the market).

Data related to hygiene were collected by observation. Data extracted included season of sampling, site of shop, hygienic status of vendors, number of workers, their clothes condition,

hygienic condition of vending site, water source and place of food preparation.

Collection of samples: A total of three samples of Panipuri were collected during the morning hour in pre-sterile zip locked poly bags and transported aseptically to Microbiology Laboratory of Bioscience Department, Rani Durgawati University, Jabalpur, for subsequent bacteriological analysis.

Isolation and Enumeration of CFU count of bacteria: Serial dilution technique was followed to enumerate the growth of viable colonies. 0.1 ml of diluted samples (10^{-3} and 10^{-6}) were spread on to the surface of the Nutrient agar medium plates with sterile glass spreader. The plates were then incubated at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The results of total bacterial count were expressed as colony forming units per ml (CFU/ml) of Panipuri samples (Koch, 1883).[7]

$$\text{CFU/ml} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume transfered in plate}}$$

Pure culture techniques: Streak plate technique was performed to obtain single cell of the isolates and then through repeated sub-culturing Pure culture of the isolates were obtained (Koch, 1883).

Preservation and Maintenance of Pure culture: Through slant preparation and storing them at 4°C for further analysis. The culture was periodically transferred to fresh media slants to maintain its viability.

Identification and Characterization of Bacterial isolates-
Cultural Characteristics on Nutrient agar: To observe the growth pattern of the serially diluted samples isolates on to the surface of Nutrient agar medium, following criteria were selected: Elevation, Color, Size, Opacity, Texture and Margin (Hesse, 1880s).[8]

Growth on Selective and Differential media: Following the standard protocols, types of Culture medium were prepared in laboratory to study the growth characteristics of the isolates.

- MacConkey agar medium (MacConkey, 20th century).
- Eosin Methylene Blue agar medium (Harris, H. and Teague, 1916).[9]
- Xylose Lysine Deoxycholate agar medium
- Thiosulphate-Citrate-Bile Salts-Sucrose (TCBS) agar medium
- Blood agar medium [MAK: HiMedia Laboratories].

Pure colonies of two selected isolates were streaked on respective media followed by incubation at 37°C for 24-48 hours.

MORPHOLOGICAL CHARACTERIZATION:
Differential staining method: To differentiate the bacteria as gram-positive or gram-negative, Gram staining method was employed using the standard protocol devised by Hans Christian Gram in 1884.

Anthony's staining method: To identify the bacteria as virulent or non-virulent, Capsule staining method was performed according to the methodology developed by E.E Anthony in 1931.

PHYSIOLOGY OF BACTERIA:
Preparation of Bacterial Growth Curve: It was prepared at constant temperature (37°C) and pH (6.8). Isolates of *Enterobacter* sp. and *Klebsiella* sp. were studied to plot the growth curve following the Standard Turbidimetric method using vis-Spectrophotometer (MAK; Systronics) to track changes in Optical density (OD) at 600 nm. For this 0.1 ml of the bacterial suspension was inoculated into 50 ml of sterile nutrient broth. The pH of the medium was adjusted to 6.8. The flasks were incubated at 37°C (MAK; Jain Scientific). Aliquots of the culture were taken aseptically at regular intervals (1 hour) and the turbidity was measured using quartz cuvette through spectrophotometer using nutrient broth as blank. At the end of the experiment growth curve was plotted between Time in hour on X axis versus OD at 600 nm on Y axis.

Oxygen Requirement: For testing the requirement of oxygen to Enterobacter sp. and Klebsiella sp. agar stab and slant technique were performed.

Biochemical Characterization:
Biochemical analysis is the identification and classification of microorganisms that appear morphologically identical. The biochemical identification was done on the basis of different tests.

IMViC Test: This acronym stands for Indole, Methyl Red, Voges-Proskauer and Citrate test is a set of four individual tests to identify members of family Enterobacteriaceae.

Lactose broth Fermentation Test: The test was performed by following the standard methodology given in Difco & BBL Manual, 10thEd. 1984.[10] Isolates were inoculated in tubes containing Phenol Red Lactose broth and Durham's tube to detect the acid and gas production.

Nitrate reduction test: (Griess diazotization reaction by Griess, P., 1858)[11] It was done by inoculating nitrate broth with bacterial culture and incubating them for 24 hours at 37°C. The results were observed by adding 6-8 drops of each reagents i.e. sulfanilic acid and a- Napthylamine.

Catalase test: Enzyme based tests play a cruicial part in the identification of bacteria. The catalase test facilitates the detection of the enzyme catalase in bacteria. This is a differential test for gram positive genera but it also shows positive result in Enterobacteriaceae family (McLeod and Gordon, 1923).[12] The test was performed by adding 3% H_2O_2 to the bacterial culture and immediate bubble formation indicates positive result.

Urease Test: This is a diagnostic test for identification and distinguishes between Proteus and other gram-negative bacteria. Christensen's Urea agar (Christensen, 1946)[13] (MAK; HiMedia Laboratories) was used to detect urease in variety of bacteria.

Hydrogen Sulfide Production Test: The test was performed by stab inoculating the isolates in SIM media followed by incubation at 37°C for 24-48 hours. Observation of black precipitate indicates H_2S production.

Triple Sugar Iron Test: The test is used to differentiate bacteria based on their ability to ferment three sugars i.e. 1% Lactose, 1% Sucrose and 0.1%. TSI agar medium (Appendix-1.9) was developed based on Kligler's iron agar, which had been to determine lactose fermentative bacteria.

Motility Test: Motility test is used to differentiate between motile and non-motile bacteria and was performed in 0.5% agar gel.

Kirby- Bauer Disc Diffusion method: To determine the sensitivity or resistance of pathogenic facultative anaerobes, Antibiotic Sensitivity Test was performed against 12 commonly used antibiotics i.e., Azithromycin, Cefuroxime, Lincomycin, Cefpodoxime, Norfloxacin, Ofloxacin, Linezolid, Doxycyline, Ciprofloxacin, Amoxicillin, Tetracycline, Cefixime and results observed as inhibition zone were measured (in mm) (Kirby and Bauer, 1950s).[14]

Results:

This work comprehends the microbiological profile of street food Panipuri from street vendors. These foods are considered as a major reservoir of human pathogens and are found to be responsible for endemics, epidemics and pandemics from time immemorial. An analysis on three samples of Panipuri were conducted. The two isolates from Panipuri were identified through Morphological, Physiological and Biochemical characterization.

Table 1: Calculation of CFU on Nutrient agar medium

Area of sample collection	Dilution	No. of colony	Colony forming unit
	Control	No growth	-
Science College	10^{-3}	26	26×10^{4}cfu/ml
	10^{-6}	5	5×10^{7}cfu/ml
Rani Durgawati University	10^{-3}	30	30×10^{4}cfu/ml
	10^{-6}	15	15×10^{7}cfu/ml
Civil Lines	10^{-3}	TNTC	TNTC
	10^{-6}	40	40×10^{7}cfu/ml

Table 2: Identification of Cultural characteristics of the isolates

Samples	Isolates	Cultural characteristics					
		Color	Size	Margin	Elevation	Texture	Opacity
Science college (Plate 1 of 10^{-3})	S1	Yellow	Small	Entire	Raised	Smooth	Opaque
	S2	White	Punctiform	Entire	Flat	Smooth	Translucent
	S3	Greyish white	Large	Irregular	Slightly raised	Glossy	Opaque
RDVV (plate 1 of 10^{-3})	S4	Greyish white	Large	Irregular	Slightly raised	Glossy	Opaque
	S5	Yellow	Small	Entire	Raised	Smooth	Opaque
Civil Lines (plate 2 of 10^{-6}	S6	Yellow	Small	Entire	Raised	Smooth	Opaque
	S7	Greyish white	Large	Irregular	Slightly raised	Glossy	Opaque

Table 3: Study of growth pattern of bacteria on selective and differential media

Growth media	Growth of isolates		Color/ appearance on medium	
	Enterobacter sp. (A)	*Klebsiella* sp. (B)	*Enterobacter* sp. (A)	*Klebsiella* sp. (B)
MacConkey agar medium	+	+	Pink, circular colonies	Pink coloured pointed colonies
EMB agar medium	+	+	Pink colored mucoid colonies	Dark pink,small ,circularcolonies
XLD medium	No growth	No growth	-	-
TCBS agar medium	No growth	No growth	-	-
Blood agar medium	Growth with no hemolysis	Growth with no hemolysis	Grey, flat colonies	Medium to large grey, slightly raised colonies

Table 4: Taxonomic characterization of results observed through Gram staining

Isolates	Shape	Arrangement	Gram staining character
Enterobacter sp. (A)	Rod	Single or pair or clustered	-ve
Klebsiella sp. (B)	Rod	Arranged in chain	-ve

Table 5: Observation of isolates through Anthony's staining method

Isolates	Capsule
Enterobacter sp. (A)	-
Klebsiella sp. (B)	+

Table 6: Readings of OD at constant temperature (37°C) & pH (6.8)

Enterobacter sp. (A)		*Klebsiella* sp. (B)	
Time (hours)	OD at 600 nm	Time (hours)	OD at 600 nm
0	0.026	0	0.011
1	0.049	1	0.019
2	0.143	2	0.027
3	0.230	3	0.082
4	0.285	4	0.100
5	0.300	5	0.120
6	0.287	6	0.157
7	0.274	7	0.149

Graph 3.1: Growth Curve demonstration of isolate A & B at constant temperature & pH

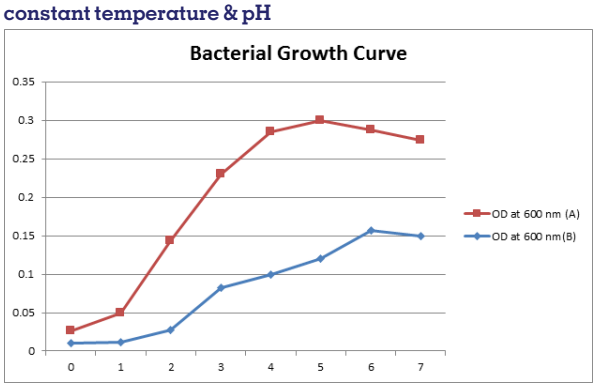

Table 7: Observation of Oxygen Requirement

Isolate	Growth on the surface of Nutrient agar slants	Growth inside 0.5% agar gel stab
Enterobacter sp. (A)	+	+
Klebsiella sp. (B)	+	+

Table 8: Interpretation of results of biochemical tests

Biochemical tests/isolates	*Enterobacter* sp. (A)	*Klebsiella* sp. (B)
Indole test	-	-
MR test	+	-
VP test	+	+
Citrate utilization test	+	+
Urease test	-	+
Nitrate reduction test	+	+
Catalase test	+	+
H_2S production	-	-
Motility test	+	-
TSI test	b/a gas	a/a gas
Lactose fermentation test	+	+

Note: + = positive, - = negative, b = basic, a = acidic

Table 9: Interpretation of results from Antibiotic Sensitivity test

Name of the Antibiotics	Isolates			
	Enterobacter sp. (A)		*Klebsiella* sp. (B)	
	Interpretation	Zone of inhibition (mm)	Interpretation	Zone of inhibition (mm)
Azithromycin	S	25	S	25

Cefuroxime	S	14	S	15
Lincomycin	S	29	S	30
Cefpodoxime	S	20	S	26
Norfloxacin	S	25	S	33
Ofloxacin	S	27	S	35
Linezolid	S	31	S	40
Doxycycline	S	22	S	33
Ciprofloxacin	S	33	S	38
Amoxycillin	S	13	S	32
Tetracycline	S	21	S	32
Cefixime	R	0	S	35

Note: S= sensitive, R= resistant

Table 10: Identification and Nomenclature of the isolates

Isolates	Identified bacteria
A	*Enterobacter* sp.
B	*Klebsiella* sp.

DISSCUSSION:

Street foods are ready-to-eat (RTE) food and beverages prepared and sold by vendors and hawkers on streets and other public places. Contamination of street vended food like Panipuri is associated with poor sanitary practices. In the present study, identification of bacteria isolated from street vended food sample Panipuri were performed on the basis of cultural characteristics, morphology, physiology and biochemical tests. Altogether three samples of Panipuri were collected from three different street vendors in Jabalpur.

The highest CFU count i.e.40×10^7cfu/ml was observed in samples from Civil Lines followed by Rani Durgawati University (15×10^7cfu/ml) & Science College (5×10^7cfu/ml), thus indicating a high rate of contamination. In a similar study by Hirani, et al., 2019, in Mumbai, reported Panipuri samples to be contaminated if the CFU counts ranged between 60×10^4 to 70×10^7cfu/ml. All the three samples were contaminated by different bacteria, and by observing the vendors, it was observed that all the vendors were serving the food with ungloved hands, no masks, and with no running water facility, which are major sources of bacterial contamination.[15] In a similar work by Khadka et al., 2018, poor personal hygiene of the vendors and improper handling were found to be key factors in contaminating the street food.[16]

On culturing, different types of bacteria were observed in different samples where two types were common i.e. present/abundance in all the three samples. These two isolates "A" i.e. Enterobacter sp. and "B" i.e. Klebsiella sp. were further analysed by cultural, morphology, physiology and biochemical tests. Growth and no growth on different selective and differential media were showed some important observations, dark pink growth on Eosin Methylene Blue agar shows the isolates are lactose fermenters as they absorb the dye and appear dark. No growth on XLD and TCBS selective media confirms that the isolates are neither Salmonella or Shigella nor any species of Vibrio. On Gram staining, both the isolates reported as rod shaped gram negative bacteria. In a similar study, by Khadka et al., 2018, in Nepal shows that Panipuri samples have high load of gram negative bacteria. On performing Anthony's staining method Klebsiella sp. shows the presence of capsule around the cell which indicating for its pathogenicity.[16]

Study of bacterial physiology was divided into two parts firstly preparation of growth curve at constant temperature and pH i.e. 37°C and 6.8, was performed by taking readings (OD) at 1 hour interval for 7 hours, sigmoidal growth curve forms which shows different growth stages of the isolates and it also shows that the Klebsiella sp. has slower growth rate than Enterobacter sp. The conditions maintained were observed to be good for both the isolates. In the second part, oxygen requirement by both the isolates were analysed by inoculating them in 0.5% agar medium, growth inside the agar presented them as facultative anaerobe.

The observations, cultural characteristics on different media, morphological characteristics (gram negative rods), results of different biochemical tests i.e. gas production, lactose fermentation identified them as Enterobacteriaceae family members, including Enterobacter sp. and Klebsiella sp. In a similar work, by Das et al. (2012), in Orissa, India, showed that Panipuri is highly contaminated by Enterobacter sp. and Klebsiella sp.[17]

On antibiotic susceptibility testing of two isolates against 12 antibiotics, Enterobacter sp. reported as resistant to Cefixime and most susceptible to Ciprofloxacin (33mm), whereas Klebsiella sp. was susceptible to all the antibiotics in the study and maximum susceptibility shown against Linezolid (40mm) followed by Ciprofloxacin (38mm).

The results of the study indicates that the street vended Panipuri sold in Jabalpur were contaminated with different pathogenic bacteria. Poor personal hygiene of the vendors and improper handling and storage practices were found to be the key factors that contributed to the contamination of Panipuri. Hence, it has been enumerated that quality of street foods must be monitored and awareness should be created. For identification of the bacterial isolates 16srRNA sequencing can be performed to identify the isolates on species level.

On the basis of given study, following observations were made:

- By performing serial dilution technique bacteria were isolated from Panipuri samples.
- The highest CFU count i.e. 40×10^7cfu/ml were observed in samples from vendor of Civil Lines market.
- Three different types of bacteria were isolated from the samples.
- Based on abundance two types of bacterial isolates were selected and pure cultured for further analysis.
- Both the isolates studied were gram-negative rod shaped bacteria and isolate B (Klebsiella sp.) also shows capsule around its cell.
- On the basis of cultural characteristics on Eosine Methylene Blue agar and results of various biochemical tests, the isolates were identified as from the family Enterobacteriaceae, including Enterobacter sp. (A) and Klebsiella sp. (B).
- Study of bacterial physiology shows that Enterobacter sp. has fast growth rate as compare to Klebsiella sp. and both the isolates were facultative anaerobes. It was also observed that they show good growth at constant temperature and pH i.e. 37°C and 6.8.
- On Antibiotic Susceptibility Testing it was observed that Enterobacter sp. was resistant against Cefixime and most susceptible to Ciprofloxacin (33mm) whereas Klebsiella sp. was most susceptible to Linezolid (40mm) followed by Ciprofoxacin (38mm).
- Poor personal hygiene of the vendors and improper handling and storage practices were found to be the key factors that contributed to the contamination of Panipuri.

CONCLUSION:

Street food are found to be a good reservoir of pathogens which can transmit various infections into the consumers. Due to lack of awareness for hygiene and sanitation these foods serves as the growth medium for pathogens. Through present work an approach has been made to study the food bacteria. It has been enumerated that quality of street foods must be monitored and awareness should be created between consumers. Further study on molecular level need to be perform to identify them at species level.

REFERENCES:

1. Yadav N.P. and Yadav R.K. Bacterial contamination of street vended food Panipuri available in Janakpurdham, Dhanusha. Tribh Uni J Micro;2019;6(1):70-7.
2. Bintsis T. Foodborne pathogens. AIMS Micro;2017;3(3):529-63.
3. Hemalata V.B. and Virupakshaiah D.B.M. Isolation and Identification of food borne pathogens from different food sources. Inter J Cur Micro Appl Sci;2016;5(6):1017-25.
4. Omorodion N.J.P. and Ogunekum A. Microbial and physiological screening of ready to eat foods. Asian J Basic Res;2022;4(1):24-35.
5. Todd E.C.D. Foodborne Diseases: Overview of Biological Hazards and Introduction to HACCP. In: Caballero, B., Finglas, P., Toldrá, F. (eds) Encyclopedia of Food Safety;2014;3:221-42.
6. Bisen P.S., Adhyay N. and Sharma M. Modern Tools and Techniques to Understand Microbes. Springer, Cham;2012.
7. Koch R. Über die Methoden zur Erkennung, Untersuchung und Bekämpfung des Cholera-Erregers [On the methods for the detection, investigation and control of the cholera pathogen]. Arbei aus dem Kaise Gesu;1883;1:1-48.
8. Hesse F.A. Contributions to the introduction of agar-based culture media enabling observation of bacterial colony characteristics such as elevation, color, size, opacity, texture, and margin.1880.
9. Holt-Harris J. E. and Teague O. A. A new culture medium for the isolation of Bacillus typhosus from stools. J Inf Disea;1916;18(6):596-600.
10. Difco & BBL manual: Manual of microbiological culture media (10th ed.). Difco Laboratories;1984.
11. Griess P. Vorläufige Mitteilung über das Verhalten aromatischer Nitroamine gegen Nitrose Gase [Preliminary note on the behaviour of aromatic nitroamines with nitrous fumes]. Anna der Che und Pharma;1858;106(2):177-183.
12. McLeod J. W. and Gordon, J. Catalase production and sensitiveness to hydrogen peroxide amongst bacteria: With a scheme of classification based on these properties. J Pathol Bact;1923;26(4):326-31.
13. Christensen W. B. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J Bact;1946;52(4):461-6.
14. Kirby B. M. and Bauer A. W. Antibiotic susceptibility testing by a standardized single disk method. Ameri J Clin Pathol;1966;45(4):493-6.
15. Hirani D.R. Bacteriological analysis of street vended food panipuri in Mumbai metropolitan region. Inter J Curr Micro Appl Sci;2019;8(11):115-21.
16. Khadka S., Adhikari S., Rai T., Ghimire U. and Parajuli A. Bacterial contamination and risk factors associated with street-vended Panipuri sold in Bharatpur, Nepal. Inter J Food Res;2018;5:32-8.
17. Das A., Nagananda G.S., Bhattacharya S. and Bharadwaj S. Microbiological quality of street vended Indian chaats sold in Bangalore. Journal of Biological Sciences;2010;10(3):255-60.