Isolation and screening of Microorganisms from the street food From Ballari Region

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Abstract

During the past 10 years, the street food in India has expanded quickly in urban zones of low- and middle-income groups, providing a diversity of inexpensive foods for low-pay families and offering job opportunities for many local vendors. Among the various street food, panipuri or Gol gappas and Gobi are more popular. Selling of roadside foods without any protection against dust, smoke, uses of normal water without any treatment, poor handling as well as unhygienic conditions put together the street food one of the main resources of foodborne illnesses. In regard to this a total of 20 samples were collected randomly from street vendors included Panipuri, Gobi Manchurian and tested for the presence of bacteria following standard microbiological method used for isolation, enumeration and identification of bacteria. Among which *Escherichia coli* was found highest 3(60%) Pani puri and Gobi samples, then another organisms *Salmonella* spp. was found highest 3 (43%) in pani puri one in each sample and also antibiotics sensitivity disc was used to determine the antibiogram profile of the isolates. These data revealed the presence of *E. coli, K. pneumoniae* and *P. aeruginosa* from different ready to eat foods as well different reactions to certain antibiotic tested.

Key words : Street foods, *E. coli, K. pneumoniae, P. aeruginosa,* Antibiotics sensitivity test.

Food borne diseases are a great threat that involves a wide range of illnesses caused mainly through the intake of food contaminated by various agents like bacterial, viral, metals, as well as poisonous plants or chemicals[1]. Street food is one the major reason to cause food borne diseases. As Street food is defined as foods and beverages that are eatable and ordinarily made and/or sold by sellers in public settings such as streets for immediate consumption or consumption at a later time without further processing or preparation[2]. According to the Food and Agriculture Organization, street food is

characterized as 'already cooked foods and refreshments sold on streets or other open places by hawkers. The street food industry plays a very important role in meeting food requirements of commuters and urban dwellers in many cities and towns of developing countries, as it feeds thousands of people daily with a large range of foods that are relatively cheap and easily accessible. It has been reported that around 7.69% people suffer from food-borne diseases per annum globally, and around 7.5% of annual deaths (56 million deaths) are due to food-borne diseases worldwide[3]. More than 90% of foods borne diseases are caused by bacteria, including Staphylococcus spp., Salmonella spp., Clostridium spp., Campylobacter, Listeria, Vibrio, Bacillus spp., Escherichia coli, Pseudomonas spp., Proteus spp. and other species. Contamination with such pathogenic bacteria during preparation, packaging, and various handling phases can result in health hazards[4]. The traditional processing methods that are used in the preparation, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of ready to eat foods. Street food vending has become an important public health issue and a great concern to everybody[5]. This is due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety issues. In order to overcome this present study was conducted to isolate the bacteria from street food sold in Ballari City Corporation area, Karnataka.

Collection of samples :

A total of 20 PaniPuri samples were collected during the month of March (temperature 30–35°C) from the best possible places in the Ballari region. These samples were collected separately, kept in sterile tubes, and placed in a cool box with ice packs until it gets to the laboratory. Within 2–3 h, the samples were transported from the field to the laboratory, where the samples were stored at 2–4°C temperature till the sample processing started at the laboratory.

Isolation of micro-organisms :

For the isolation of pure culture of bacteria, serial dilution method was preferred and it was applied to all of the 10 samples collected from various areas of Ballari. In this method, a total of 10 sterilized tubes were dispensed with 9 ml of sterilized distilled water. Then, 1 ml of the panipuri sample was added into the 9 ml sterilized distilled water (10^{-1} dilution) and mix well. After that, 1 ml of solution was transferred into the second tube containing 9 ml of sterilized distilled water (10^{-2} dilution). This procedure was repeated for the dilution up to 10^{-8}. After completion of the serial dilutions process, 1 ml of the diluted sample from the last tube (10^{-8}) was spread into a sterile Petri dish containing media.

The media used includes Eosin methylene blue, Chromosol and Cetrimide which was prepared according to the manufacturer's instructions. The media was inoculated with the dilutions of the food substances using the pour plate method and incubated at 37°C for 24 hours. All growth media were procured from Oxoid (Oxoid, UK). Further the obtained isolates are sub cultured into nutrient agar slants for storing from which they were collected for the biochemical tests.

Microscopic and Biochemical test :

For the identification of bacteria, the culture was confirmed using Gram's staining and biochemical examinations as reported previously. The culture was streaked on MacConkey Agar and incubated at 37°C overnight. The isolated colony was picked for the Gram's stain examination. Biochemical tests such as indole test, MR test, VP test, citrate utilization test, starch hydrolysis test, catalase test, and $H_2 S$ production test were performed.

Antibiotic susceptibility :

Antibiotic resistance of bacteria has been increased worldwide due to excessive consumption of antibiotics without sufficient knowledge, which is leading to failures in the treatment of human infectious diseases. The Kirby–Bauer disk diffusion test was a very simple and effective method, to determine whether the isolated organisms are susceptible or resistant to the prescribed antibiotics. In the present study, to find the susceptibility pattern of isolates toward antibiotics, all the isolated bacterial samples were subjected to antibiotic susceptibility test. Four antibiotics, namely, ciprofloxacin (5 µg/ml), ampicillin (10 µg/ml), co-trimoxazole (25 µg/ml), and chloramphenicol (30 µg/ml) were used to detect drug resistant bacteria. A number of bacterial isolates, isolated from vendors and shops, were found to be resistant against these antibiotics. The zones of inhibition were measured and analysed by scale. A total of 168 bacterial pathogens were obtained from 180 culture positive samples from ready-to eat foods sold around **Ballari region**

Area	Collection centres	No of samples	No of Contaminated samples
Allipura	Pani puri shop	01	01
Sudha cross	Restaurant	01	01
Infantry road	Road side	01	01
Talu road	Bandi	01	01
Total	__	04	04

Sample collection:

A total of 20 PaniPuri samples were collected during the month of March (temperature 30–35°C) from the best possible places in the Ballari region. These samples were collected separately, kept in sterile tubes, and placed in a cool box with ice packs until it gets to the laboratory.

Puri samplr :

Area	Collection centres	No of samples	No of Contaminated samples
Allipura	Pani puri shop	01	01
Sudha cross	Restaurant	01	01
Infantry road	Road side	01	01
Talu road	Bandi	01	01
Total	––	04	

Gobi samples :

Area	Collection centres	No of samples	No of Contaminated samples
Allipura	Pani puri shop	01	01
Sudha cross	Restaurant	01	01
Infantry road	Road side	01	01
Talu road	Bandi	01	01
Total	––	04	04

To culture bacteria from panipuri samples:

1. *Sample collection :* Take samples from different components of the panipuri, such as pani (flavoured water), tamarind chutney, or any other ingredient. Use sterile swabs or containers to avoid contamination and ensure accurate results.

2. *Inoculation:* Transfer these samples onto appropriate culture media (like nutrient agar or selective media for specific bacteria). Spread the samples evenly on the surface of the agar plates using sterile techniques.

3. *Incubation:* Incubate the agar plates at the optimal temperature and conditions required for bacterial growth (usually around 37°C for many common bacteria). Different bacteria might require different incubation times.

4. *Observation*: After the appropriate incubation period, observe the plates for bacterial growth. Different colonies will appear, and these can be further tested or identified for specific strains or species. Testing for bacterial growth in panipuri components is often done for quality control or to ensure food safety standards are met. It's important to follow proper laboratory and safety protocols during this process to prevent any contamination

Gram's stain :

Performing a Gram stain test on panipuri or Gobi (cauliflower) is not a standard or common procedure. Gram staining is primarily used in microbiology to differentiate and categorize bacteria based on their cell wall composition. However, if you want to analyse these food items for the presence of bacteria or microbiological contaminants, a swab or sample from the surface of the food can be used to perform a Gram stain as a preliminary test
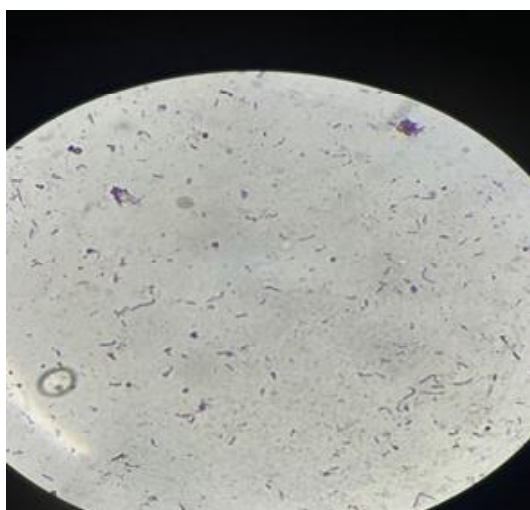
NA plate puri sample   Gram – ve Bacillus

NA plate Gobi sample Gram -ve Bacillus
[Endospore formation]

Biochemical Tests :

Citrate Test

Samples	Collection centres	Species name	Citrate Positive	Test Negative
Pani	Allipura	*E. coli*	-	-ve
Pani	Sudha cross	*E. coli*	-	-ve
Pani	Infantry road	*E. coli*	-	-ve
Pani	Talu road	*E. coli*	-	-ve
Puri	Allipura	*E. coli*	-	-ve
Puri	Sudha cross	*E. coli*	-	-ve
Puri	Talu road	*E. coli*	-	-ve
Gobi	Allipura	*E. coli*	-	-ve
Gobi	Sudha cross	*E. coli*	-	-ve
Gobi	Talu road	*E. coli*	-	-ve

Voges-Proskauer Test [Vp]

samples	Collection centres	Species name	Voges-Proskauer Test Positive	Negative
Pani	Allipura	*E. coli*	-	-ve
Pani	Sudha cross	*E. coli*	-	-ve
Pani	Infantry road	*E. coli*	-	-ve
Pani	Talu road	*E. coli*	-	-ve
Puri	Allipura	*E. coli*	-	-ve
Puri	Sudha cross	*E. coli*	-	-ve
Puri	Talu road	*Enterobacter aerogenes*	+ve	-
Gobi	Allipura	*E. coli*	-	-ve
Gobi	Sudha cross	*Enterobacter aerogenes*	+ve	-
Gobi	Talu road	*Enterobacter aerogenes*	+ve	-

Urease test :

Samples	Collection centres	Species name	urease test Positive	Negative
Pani	Allipura	*E. coli*	-	-ve
Pani	Sudha cross	*E. coli*	-	-ve
Pani	Infantry road	*E. coli*	-	-ve
Pani	Talu road	*E. coli*	-	-ve
Puri	Allipura	*Proteus vulgaris*	+ve	-
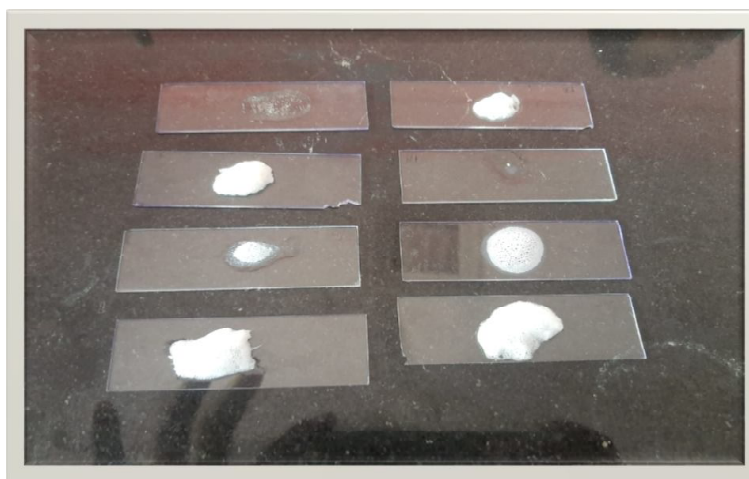
- **Note: Positive:** Copious bubbles produced, active bubbling.
- **Negative:** No or very few bubbles produced.

- The positive test is demonstrated by the immediate appearance of bubbles.
- The appearance of one or two bubbles represents a weak reaction.
- A negative test is represented by no bubbles or a few bubbles after 20 second.
  The catalase test separates staphylococci (positive) from streptococci and enterococci (negative). *Bacillus is* catalase-positive, and *Clostridium* spp. are catalase-negative.

Antibiotic Susceptibility :

Gobi Sample 1

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Gobi 01	Allipura	Amikacin	Amoxicillin- clavulanic acid	Cefepime
		Azithromycin	Ampicillin	Cefoxitin
		Aztreonam	Ampicillin-sulbactam	
		Cephazolin	Cefixime	
		Ceftazidime	Colistin	
		Chloramphenicol	Imipenem	
		Ciprofloxacin	Ticarcillin-clavulanic acid	
		Doripenem		
		Doxycycline		
		Femycin		
		Gentamycin		
		Levofloxacin		
		Meropenem		
		Minocycline		
		Nalidixic acid		
		Netilmicin		
		Nitrofurantoin		
		Norfloxacin		
		Piperacillin-Tazobactam		
		Tetracycline		
		Tobramycin		
		Trimethoprim-sulfamethoxazole		

Gobi sample 2 :

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Gobi 2	Sudha cross	Amikacin	Amoxicillin- clavulanic acid	-
		Azithromycin	Ampicillin	-
		Aztreonam	Ampicillin-sulbactam	
		Cephazolin	cefixime	
		Ceftazidime	Colistin	
		Chloramphenicol	Imipenem	
		Ciprofloxacin	Ticarcillin-clavulanic acid	
		Doripenem	cefoxitin	
		Doxycycline	cefepime	
		Femycin	ceftazidime	
		Gentamycin	cefazolin	
		Levofloxacin		
		Meropenem		
		Minocycline		
		Nalidixic acid		
		Netilmicin		
		Nitrofurantoin		
		Norfloxacin		
		Piperacillin-Tazobactam		
		Tetracycline		
		Tobramycin		
		Trimethoprim-sulfamethoxazole		

Gobi Sample 3 :

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Gobi 3	Talu road	Amikacin	Amoxicillin- clavulanic acid	Femycin
		Azithromycin	Ampicillin	Ampicillin-sulbactam
		Aztreonam	cefixime	Ceftazidime
		Cefoxitin	Imipenem	Colistim
		Chloramphenicol	Ticarcillin-clavulanic acid	
		Ciprofloxacin	cefepime	
		Doripenem	ceftriaxone	
		Doxycycline	Nalidixic acid	
		Gentamycin		

Levofloxacin
Meropenem
Minocycline
Cefoxitin
Netilmicin
Nitrofurantoin
Norfloxacin
Piperacillin-Tazobactam
Tetracycline
Tobramycin
Trimethoprim-sulfamethoxazole

Puri Sample 4 :
Puri sample 5 :

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Puri 04	Allipura	Amikacin		
		Azithromycin	Ampicillin	
		Aztreonam	cefixime	
		Amoxicillin- clavulanic acid	Imipenem	
		Chloramphenicol	Ticarcillin-clavulanic acid	
		Ciprofloxacin	ceftriaxone	
		Doripenem	Nalidixic acid	
		Doxycycline	cefepime	
		Gentamycin	Colistim	
		Levofloxacin	cefixime	
		Meropenem	Ceftazidime	
		Minocycline		
		Ampicillin-sulbactam		
		Netilmicin		
		Nitrofurantoin		
		Norfloxacin		
		Piperacillin-Tazobactam		
		Tetracycline		
		Tobramycin		
		Trimethoprim-sulfamethoxazole		
		Cefazolin		
		Cefoxitin		

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Pani 05	Talu road	Amikacin Azithromycin Aztreonam Chloramphenicol Ciprofloxacin Doripenem Doxycycline Gentamycin Levofloxacin Meropenem Minocycline Ampicillin-sulbactam Netilmicin Nitrofurantoin Piperacillin-Tazobactam Tobramycin Trimethoprim-sulfamethoxazole Cefazolin Cefixime Colistim Cefoxitin Fosfomycin Norfloxacin	Ampicillin Imipenem Ceftazidime Tetracycline Amoxicillin-clavulanic acid Cefepime Ticarcillin-clavulanic acid Ceftriaxone Nalidixic acid	

Sample	Collected Center	Susceptible to these Antibiotics	Resistance to these Antibiotics	Intermediate to these antibiotics
Pani 6	Infantry Road	Amikacin Azithromycin Aztreonam Chloramphenicol Ciprofloxacin Doripenem Doxycycline Gentamycin	Ampicillin Imipenem Ceftazidime Tetracycline Amoxicillin- clavulanic acid Cefepime Ticarcillin-clavulanic acid Nalidixic acid	Colistim Cftriaxyne

Levofloxacin   Cefixime
Meropenem
Minocycline
Ampicillin-sulbactam
Netilmicin
Nitrofurantoin
Piperacillin-Tazobactam
Tobramycin
Trimethoprim-sulfamethoxazole
Cefazolin
Cefoxitin
Fosfomycin
Norfloxacin

The results of this study clearly show that the street-vended food is contaminated with different pathogenic bacteria and this is due to not following proper hygienic practices while handling the food material or utensils used for food preparation or serving. The existence of these bacteria in food can induce potential health problems for consumers. Poor personal hygiene, improper handling, and storage practice of foods and unawareness of food vendors about foodborne diseases are the responsible factors which are associated with contamination of street-vended foods in Ballari region. As street food consumption provides employment to a large group of population and street food consumption is a habit of a number of foods loving people, ways should be developed by which healthy and hygienic practices can be encouraged or the risk of microbial food contamination can be minimized. The present study results are promising in this aspect as the synthesized nanoparticles give a solution for combating commonly found food contaminants and thus pave the way for safer, more hygienic street food. The street for vendor may be sensitized with the fact of the importance of hygiene and the synthesized nanoparticles may be analysed and processed further for making their use in daily life such as in soaps, dish washes, or hand sanitizer This study results showed that, the four types of assessed street food items in Ballari City Corporation area were contaminated with at least two pathogenic bacteria. The foodborne bacteria detected in this study are also evident that street foods might pose a major problem for public health. Lack of training (orientation) on the proper handling and processing of food, poor personal hygiene of venders and unhygienic surroundings could be possible factors for observed problems in that locality. Therefore, education for vendors on food safety and hygienic practices is essential to reduce contamination rate. In addition, regular inspection on food vending practices and safety of street foods is required to improve the health standards of consumers

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